

## (12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(10) International Publication Number

WO 2025/207932 A1

(43) International Publication Date  
02 October 2025 (02.10.2025)

## (51) International Patent Classification:

C07K 16/28 (2006.01) C12N 7/00 (2006.01)  
A61P 25/28 (2006.01)

## (21) International Application Number:

PCT/US2025/021832

## (22) International Filing Date:

27 March 2025 (27.03.2025)

## (25) Filing Language:

English

## (26) Publication Language:

English

## (30) Priority Data:

63/570,601 27 March 2024 (27.03.2024) US

(71) Applicant: BIOGEN MA INC. [US/US]; 225 Binney Street, Cambridge, Massachusetts 02142 (US).

(72) Inventors: ARNDT, Joseph Walter; c/o Biogen MA Inc., 225 Binney Street, Cambridge, Massachusetts 02142 (US). CAMERON, Thomas Owen; c/o Biogen MA Inc., 225 Binney Street, Cambridge, Massachusetts 02142 (US). JULIAN, Mark Christopher; c/o Biogen MA Inc., 225 Binney Street, Cambridge, Massachusetts 02142 (US). MARTINO, Richard Alexander; c/o Biogen MA Inc., 225 Binney Street, Cambridge, Massachusetts 02142 (US). MEEKS, Caitlin Bryanna; c/o Biogen MA Inc., 225 Bin-

ney Street, Cambridge, Massachusetts 02142 (US). SPARROW, Junghae Suh; c/o Biogen MA Inc., 225 Binney Street, Cambridge, Massachusetts 02142 (US). YUAN, Yuan; c/o Biogen MA Inc., 225 Binney Street, Cambridge, Massachusetts 02142 (US).

(74) Agent: KAUR, Mandip et al.; CHOATE, HALL & STEWART LLP, Two International Place, Boston, Massachusetts 02110 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UY, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ,

## (54) Title: AAV CAPSID COMPRISING ANTI-TRANSFERRIN ANTIBODY DOMAINS

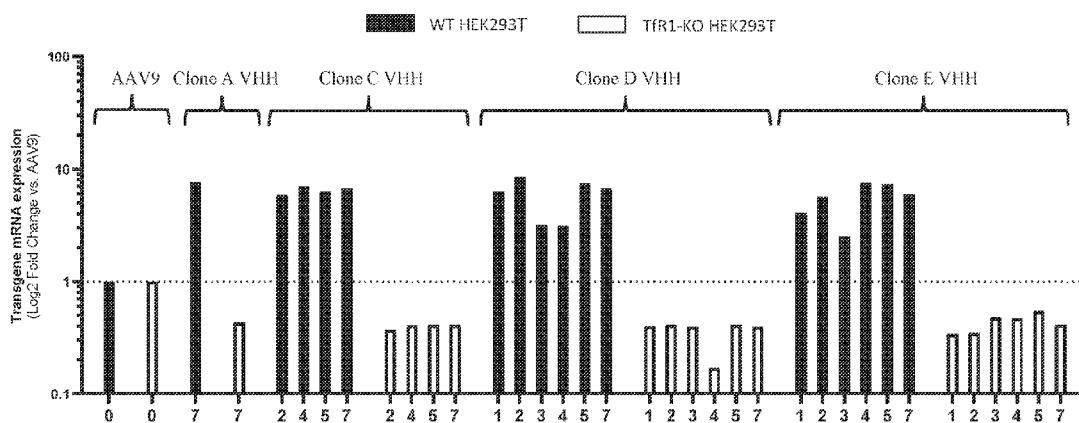


FIG. 3

(57) Abstract: The present disclosure pertains to targeting moieties, (e.g., CNS-targeting moieties) comprising single chain antibody agents which can be inserted in the capsid of a recombinant adeno- associated virus (rAAV) vector. Also disclosed herein are compositions comprising CNS-targeting moieties disclosed herein and methods of making and using the same.



RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

**Declarations under Rule 4.17:**

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

**Published:**

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

**AAV CAPSIDS COMPRISING ANTI-TRANSFERRIN ANTIBODY DOMAINS****CROSS REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims priority to and the benefit of U.S. Provisional Patent Application No. 63/570,601, filed on March 27, 2024, the entire contents of which is hereby incorporated by reference in its entirety.

**BACKGROUND**

[0002] Targeted delivery of payloads (e.g., using recombinant adeno-associated viruses) to cells or tissues for treating and/or preventing diseases remains a challenge.

**SUMMARY**

[0003] The present disclosure identifies certain challenges with existing targeted delivery of payloads. For example, the present disclosure identifies that a lack of targeting moieties that can specifically deliver payloads to a target cell or tissue with decreased non-specific delivery to other cells or tissues is a key challenge. Another challenge identified by the present disclosure is the poor potency associated with delivery vectors, e.g., recombinant adeno-associated virus vectors. In some embodiments, improving the potency of delivery vectors could be beneficial in obtaining clinically relevant outcomes.

[0004] Among other things, the present disclosure provides technologies that can address certain limitations identified in existing targeted delivery of payloads. The technologies provided herein are particularly useful for specifically delivering payloads to a target cell or tissue. In some embodiments, by specifically delivering payloads to a target cell or tissue, technologies provided herein can also increase the potency of a payload. In some embodiments, a target cell or tissue expresses one or more receptors (e.g., a human transferrin receptor (hTfR1)) recognized by a variant AAV capsid protein disclosed herein. In some embodiments, a target cell or tissue is a central nervous system (CNS) cell and/or tissue.

[0005] The present disclosure encompasses single chain antibody agents which recognize (e.g., bind to) a hTfR1 or a variant or a fragment thereof. Also disclosed herein are recombinant

adeno-associated virus (rAAV) particles comprising a variant AAV capsid protein comprising a single chain antibody agents which recognizes (e.g., binds to) a hTfR1 or a variant or a fragment thereof. In some embodiments, a targeting moiety in a variant AAV capsid protein is also referred to as a “peptide insertion.” In some embodiments, a single chain antibody agents binds to and/or recognizes a hTfR1 or a variant or a fragment thereof expressed on a cell (e.g., an endothelial cell associated with a blood brain barrier). In some embodiments, a single chain antibody agents which recognizes (e.g., binds to) a hTfR1 or a variant or a fragment thereof provides CNS-cell and/or tissue tropism. In some embodiments, rAAV particles comprising a variant capsid having a single chain antibody agents insertion disclosed herein bind to and/or recognize a target (e.g., hTfR1 or a variant or a fragment thereof) on a cell (e.g., an endothelial cell associated with a blood brain barrier). In some embodiments, binding of rAAV particles comprising a variant capsid having a single chain antibody agents insertion disclosed herein to hTfR1 or a variant or a fragment thereof on a cell, delivers the rAAV particles to said cells. In some embodiments, rAAV particles delivered to cells by binding to hTfR1 or a variant or a fragment thereof allows transcytosis of said rAAV particles across said cell (e.g., to be transported from one surface of said cell to another surface of said cell) into an organ, an interstitial space, and/or an interstitial fluid allowing for transduction of a cell and/or tissue. In some embodiments, delivery of a variant AAV capsid protein to an organ, to an interstitial space, and/or to an interstitial fluid results in transduction of a cell or tissue. Also disclosed herein are compositions comprising rAAV particles disclosed herein, and uses of the same.

**[0006]** In some embodiments, a single chain antibody agents inserted in an AAV capsid AAV capsid protein provides CNS-cell and/or tissue tropism. In some embodiments, rAAV particles comprising a variant capsid having a single chain antibody agents insertion disclosed herein bind to and/or recognize a target on a cell, e.g., a CNS cell and/or a cell in a CNS tissue, e.g., an endothelial cell associated with a blood brain barrier.

**[0007]** Without wishing to be bound by any particular theory, in some embodiments, variant AAV capsid proteins disclosed herein are particularly useful for targeting CNS cells and/or tissue expressing human Transferrin receptor (hTfR1) or a variant or a fragment thereof. In some embodiments, a variant AAV capsid protein disclosed herein binds to, e.g., specifically binds, to hTfR1 or a variant or a fragment thereof.

[0008] Further without wishing to be bound by any particular theory, in some embodiments, variant AAV capsid proteins disclosed herein can traverse a blood brain barrier (e.g., in a human subject) by binding to hTfR1 or a variant or a fragment thereof. In some embodiments, CNS cell and/or tissue tropism of a variant AAV capsid protein disclosed herein is a result of binding to hTfR1 or a variant or a fragment thereof.

[0009] Accordingly, in some embodiments, provided herein is an adeno-associated virus (rAAV) particle comprising: (a) a variant AAV capsid protein comprising a single chain antibody agent that binds to a transferrin receptor (TfR1) or a variant or a fragment thereof, wherein the single chain antibody agent is inserted in a variable region (VR) of a parental AAV capsid protein, and (b) a heterologous nucleic acid comprising a nucleotide sequence encoding a payload.

[0010] In some embodiments, a single chain antibody agent comprises one or more complementarity determining regions (CDRs). In some embodiments, a single chain antibody agent comprises a CDR1, a CDR2 and a CDR3.

[0011] In some embodiments, a single chain antibody agent is or comprises a single domain antibody (e.g., a VHH).

[0012] In some embodiments, a single chain antibody agent is or comprises a single chain Fv.

[0013] In some embodiments, a single chain antibody agent is or comprises single chain antibody agent comprises a sequence provided in **Table 1**. In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence provided in **Table 1**; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a CDR1 sequence provided in Table 1; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to a CDR1 sequence provided in **Table 1**; (ii) a CDR2 sequence provided in **Table 1**; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a CDR2 sequence provided in **Table 1**; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to a CDR2 sequence provided in **Table 1**; and/or (iii) a CDR3 sequence provided in **Table 1**; or a sequence with at least 85%, at

least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a CDR3 sequence provided in **Table 1**; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to a CDR3 sequence provided in **Table 1**.

**[0014]** In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 3; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 3; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 3; and/or (iii) a CDR3 sequence of SEQ ID NO: 4; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 4; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 4.

**[0015]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 3; and (iii) a CDR3 sequence of SEQ ID NO: 4.

**[0016]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 1, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0017]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 158, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0018]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 159, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at

least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0019]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 160, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0020]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 6; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 6; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 6; (ii) a CDR2 sequence of SEQ ID NO: 7; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 7; and/or (iii) a CDR3 sequence of SEQ ID NO: 8; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 8.

**[0021]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 6; (ii) a CDR2 sequence of SEQ ID NO: 7; and (iii) a CDR3 sequence of SEQ ID NO: 8.

**[0022]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 5, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0023]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 122, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0024] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 135, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0025] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 136, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0026] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 10; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 10; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 10; (ii) a CDR2 sequence of SEQ ID NO: 11; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 11; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 11; and/or (iii) a CDR3 sequence of SEQ ID NO: 12; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 12; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 12.

[0027] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 10; (ii) a CDR2 sequence of SEQ ID NO: 11; and (iii) a CDR3 sequence of SEQ ID NO: 12.

[0028] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 9, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0029] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 14; or a sequence with at least 85%, at least 86%, at least 87%, at least

88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 14; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 14; (ii) a CDR2 sequence of SEQ ID NO: 15; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 15; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 15; and/or (iii) a CDR3 sequence of SEQ ID NO: 16; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 16; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 16.

**[0030]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 14; (ii) a CDR2 sequence of SEQ ID NO: 15; and (iii) a CDR3 sequence of SEQ ID NO: 16.

**[0031]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 13, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0032]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 18; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 18; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 18; (ii) a CDR2 sequence of SEQ ID NO: 19; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 19; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 19; and/or (iii) a CDR3 sequence of SEQ ID NO: 20; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at

least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 20; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 20.

[0033] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 18; (ii) a CDR2 sequence of SEQ ID NO: 19; and (iii) a CDR3 sequence of SEQ ID NO: 20.

[0034] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 17, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0035] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 22; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 22; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 22; (ii) a CDR2 sequence of SEQ ID NO: 23; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 23; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 23; and/or (iii) a CDR3 sequence of SEQ ID NO: 24; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 24; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 24.

[0036] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 22; (ii) a CDR2 sequence of SEQ ID NO: 23; and (iii) a CDR3 sequence of SEQ ID NO: 24.

[0037] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 21, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0038]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 26; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 26; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 26; (ii) a CDR2 sequence of SEQ ID NO: 27; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 27; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 27; and/or (iii) a CDR3 sequence of SEQ ID NO: 28; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 28; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 28.

**[0039]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 26; (ii) a CDR2 sequence of SEQ ID NO: 27; and (iii) a CDR3 sequence of SEQ ID NO: 28.

**[0040]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 25, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0041]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 30; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 30; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 30; (ii) a CDR2 sequence of SEQ ID NO: 31; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 31; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 31; and/or (iii) a CDR3 sequence of SEQ ID NO: 32; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at

least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 32; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 32.

[0042] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 30; (ii) a CDR2 sequence of SEQ ID NO: 31; and (iii) a CDR3 sequence of SEQ ID NO: 32.

[0043] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 29, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0044] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 34; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 34; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 34; (ii) a CDR2 sequence of SEQ ID NO: 35; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 35; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 35; and/or (iii) a CDR3 sequence of SEQ ID NO: 36; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 36; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 36.

[0045] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 34; (ii) a CDR2 sequence of SEQ ID NO: 35; and (iii) a CDR3 sequence of SEQ ID NO: 36.

[0046] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 33, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0047]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 38; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 38; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 38; (ii) a CDR2 sequence of SEQ ID NO: 39; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 39; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 39; and/or (iii) a CDR3 sequence of SEQ ID NO: 40; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 40; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 40.

**[0048]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 38; (ii) a CDR2 sequence of SEQ ID NO: 39; and (iii) a CDR3 sequence of SEQ ID NO: 40.

**[0049]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 37, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0050]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 42; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 42; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 42; (ii) a CDR2 sequence of SEQ ID NO: 43; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 43; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 43; and/or (iii) a CDR3 sequence of SEQ ID NO: 44; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at

least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 44; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 44.

[0051] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 42; (ii) a CDR2 sequence of SEQ ID NO: 43; and (iii) a CDR3 sequence of SEQ ID NO: 44.

[0052] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 41, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0053] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 46; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 46; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 46; (ii) a CDR2 sequence of SEQ ID NO: 47; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 47; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 47; and/or (iii) a CDR3 sequence of SEQ ID NO: 48; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 48; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 48.

[0054] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 46; (ii) a CDR2 sequence of SEQ ID NO: 47; and (iii) a CDR3 sequence of SEQ ID NO: 48.

[0055] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 45, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0056]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 50; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 50; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 50; (ii) a CDR2 sequence of SEQ ID NO: 51; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 51; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 51; and/or (iii) a CDR3 sequence of SEQ ID NO: 52; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 52; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 52.

**[0057]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 50; (ii) a CDR2 sequence of SEQ ID NO: 51; and (iii) a CDR3 sequence of SEQ ID NO: 52.

**[0058]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 49, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0059]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 117; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 117; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 117; and/or (iii) a CDR3 sequence of SEQ ID NO: 4; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least

89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 4; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 4.

**[0060]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 117; and (iii) a CDR3 sequence of SEQ ID NO: 4.

**[0061]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 116, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0062]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 119; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 119; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 119; and/or (iii) a CDR3 sequence of SEQ ID NO: 4; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 4; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 4.

**[0063]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 119; and (iii) a CDR3 sequence of SEQ ID NO: 4.

**[0064]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 118, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0065]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 15; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 15; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 15; and/or (iii) a CDR3 sequence of SEQ ID NO: 52; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 52; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 52.

**[0066]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 15; and (iii) a CDR3 sequence of SEQ ID NO: 52.

**[0067]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 120, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0068]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 119; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 119; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 119; and/or (iii) a CDR3 sequence of SEQ ID NO: 52; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at

least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 52; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 52.

[0069] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 119; and (iii) a CDR3 sequence of SEQ ID NO: 52.

[0070] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 121, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0071] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 124; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 124; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 124; (ii) a CDR2 sequence of SEQ ID NO: 125; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 125; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 125; and/or (iii) a CDR3 sequence of SEQ ID NO: 8; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 8.

[0072] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 124; (ii) a CDR2 sequence of SEQ ID NO: 125; and (iii) a CDR3 sequence of SEQ ID NO: 8.

[0073] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 123, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0074]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 127; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 127; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 127; (ii) a CDR2 sequence of SEQ ID NO: 7; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 7; and/or (iii) a CDR3 sequence of SEQ ID NO: 128; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 128; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 128.

**[0075]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 127; (ii) a CDR2 sequence of SEQ ID NO: 7; and (iii) a CDR3 sequence of SEQ ID NO: 128.

**[0076]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 126, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0077]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 130; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 130; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 130; (ii) a CDR2 sequence of SEQ ID NO: 7; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 7; and/or (iii) a CDR3 sequence of SEQ ID NO: 131; or a sequence with at least 85%, at least 86%, at least 87%, at

least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 131; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 131.

**[0078]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 130; (ii) a CDR2 sequence of SEQ ID NO: 7; and (iii) a CDR3 sequence of SEQ ID NO: 131.

**[0079]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 129, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0080]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 6; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 6; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 6; (ii) a CDR2 sequence of SEQ ID NO: 133; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 133; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 133; and/or (iii) a CDR3 sequence of SEQ ID NO: 134; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 134; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 134.

**[0081]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 6, (ii) a CDR2 sequence of SEQ ID NO: 133; and (iii) a CDR3 sequence of SEQ ID NO: 134.

**[0082]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 132, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0083]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 6; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 6; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 6; (ii) a CDR2 sequence of SEQ ID NO: 125; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 125; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 125; and/or (iii) a CDR3 sequence of SEQ ID NO: 134; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 134; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 134.

**[0084]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 6, (ii) a CDR2 sequence of SEQ ID NO: 125; and (iii) a CDR3 sequence of SEQ ID NO: 134.

**[0085]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 137, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0086]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 130; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 130; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 130; (ii) a CDR2 sequence of SEQ ID NO: 7; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 7; and/or (iii) a CDR3 sequence of SEQ ID NO: 139; or a sequence with at least 85%, at least 86%, at least 87%, at

least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 139; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 139.

**[0087]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 130, (ii) a CDR2 sequence of SEQ ID NO: 7; and (iii) a CDR3 sequence of SEQ ID NO: 139.

**[0088]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 138, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0089]** In some embodiments, a single chain antibody agent binds to a human TfR1 (hTfR1), or a cyno TfR1. In some embodiments, a single chain antibody agent binds to a human TfR1. In some embodiments, a single chain antibody agent binds to cyno TfR1.

**[0090]** In some embodiments, a single chain antibody agent binds to hTfR1 with an affinity ( $K_D$ ) of about 10 nM to about 2500 nM, e.g., when fused to an Fc domain or when inserted in a variable region (VR) of a parental AAV capsid protein.

**[0091]** In some embodiments, a single chain antibody agent comprises one or more linkers. In some embodiments, one or more linkers are situated on the N-terminus of the single chain antibody agent and/or the C-terminus of the single chain antibody agent.

**[0092]** In some embodiments, one or more linkers comprise one or more (GGGGS) repeats. In some embodiments, one or more linkers comprise one or more (GGGGS) repeats on the N-terminus of the single chain antibody agent and/or the C-terminus of the single chain antibody agent.

**[0093]** In some embodiments, one or more linkers comprise a coiled-coil alpha helix domain or a fragment thereof.

**[0094]** In some embodiments, a single chain antibody agent comprises one or more coiled-coil alpha helix domains on the N-terminus of the single chain antibody agent and/or the C-terminus of the single chain antibody agent.

[0095] In some embodiments, a coiled-coil alpha helix domain comprises a leading coil sequence according to SEQ ID NO: 53, or a sequence with at least 90% identity thereto.

[0096] In some embodiments, a coiled-coil alpha helix domain comprises a returning coil sequence according to SEQ ID NO: 54, or a sequence with at least 90% identity thereto.

[0097] In some embodiments, a single chain antibody agent comprises a (GGGGS)<sub>5</sub> linker according to SEQ ID NO: 59 at the N-terminus and a (GGGGS)<sub>1</sub> linker according to SEQ ID NO: 55 at the C-terminus.

[0098] In some embodiments, a single chain antibody agent comprises a (GGGGS)<sub>4</sub> linker according to SEQ ID NO: 58 at the N-terminus.

[0099] In some embodiments, a single chain antibody agent comprises: (i) a leading coiled-coil alpha helix domain and a (GGGGS)<sub>5</sub> linker according to SEQ ID NO: 60 at the N-terminus; and (ii) a (GGGGS)<sub>1</sub> linker and a returning coiled-coil alpha helix domain according to SEQ ID NO: 61 at the C-terminus.

[0100] In some embodiments, a single chain antibody agent comprises a linker according to SEQ ID NO: 65, e.g., at the N terminus or the C terminus.

[0101] In some embodiments, a single chain antibody agent insertion site is located between two adjacent amino acids in the variable region of the parental AAV capsid protein.

[0102] In some embodiments, a single chain antibody agent insertion site is located between two non-adjacent amino acids in the variable region of the parental AAV capsid protein.

[0103] In some embodiments, a single chain antibody agent insertion site is in a VP1 of the parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion site is not in a VP2 and/or VP3 of the parental AAV capsid protein.

[0104] In some embodiments, a single chain antibody agent insertion site is in a VP1, VP2 and/or VP3 of the parental AAV capsid protein.

[0105] In some embodiments, insertion of the single chain antibody agent replaces a contiguous stretch of amino acids of the parental AAV capsid protein.

[0106] In some embodiments, insertion of the single chain antibody agent does not replace a contiguous stretch of amino acids of the parental AAV capsid protein.

[0107] In some embodiments, a single chain antibody agent insertion is in VR-I, VR-II, VR-III, VR-IV, VR-V, VR-VI, VR-VII, VR-VIII or VR-IX of the parental AAV capsid protein.

[0108] In some embodiments, a single chain antibody agent insertion is in VR-IV of the parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion site is in a VP1 of the parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion site is not in a VP2 and/or VP3 of the parental AAV capsid protein.

[0109] In some embodiments, a parental AAV capsid protein is an AAV9 capsid protein and VR-IV comprises amino acids 452-460 of VP1, VP2, or VP3 of the AAV9 capsid protein.

[0110] In some embodiments, a single chain antibody agent insertion site is located between amino acids: (1) 454 and 461 of VP1 of the AAV9 capsid protein, (2) 454 and 457 of VP1 of the AAV9 capsid protein; or (3) 451 and 460 of VP1 of the AAV9 capsid protein.

[0111] In some embodiments, a single chain antibody agent insertion is in VR-VIII of the parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion site is in a VP1 of the parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion site is not in a VP2 and/or VP3 of the parental AAV capsid protein.

[0112] In some embodiments, a parental AAV capsid protein is an AAV9 capsid protein and VR-VIII comprises amino acids 580 to 601 of VP1, VP2, or VP3 of the AAV9 capsid protein.

[0113] In some embodiments, a single chain antibody agent insertion site is located between amino acids 588 and 590 of VP1 of an AAV9 capsid protein or the corresponding position in VP1 of another parental AAV capsid protein.

[0114] This disclosure further provides a composition comprising a rAAV particle disclosed herein and a pharmaceutically acceptable excipient.

[0115] In some embodiments, provided herein is an isolated cell transduced with a rAAV particle disclosed herein.

[0116] Further disclosed herein is an isolated nucleic acid comprising a nucleotide sequence encoding a variant AAV capsid protein, wherein the variant AAV capsid protein comprises a single chain antibody agent that binds to a transferrin receptor (TfR) or a variant or a

fragment thereof, and wherein the single chain antibody agent is inserted in a variable region (VR) of a parental AAV capsid protein.

[0117] In some embodiments, a single chain antibody agent insertion is in VR-I, VR-II, VR-III, VR-IV, VR-V, VR-VI, VR-VII, VR-VIII or VR-IX of the parental AAV capsid protein.

[0118] In some embodiments, a single chain antibody agent insertion is in VR-I of the parental AAV capsid protein.

[0119] In some embodiments, a single chain antibody agent insertion is in VR-II of the parental AAV capsid protein.

[0120] In some embodiments, a single chain antibody agent insertion is in VR-III of the parental AAV capsid protein.

[0121] In some embodiments, a single chain antibody agent insertion is in VR-IV of the parental AAV capsid protein.

[0122] In some embodiments, a parental AAV capsid protein is an AAV9 capsid protein and VR-IV comprises amino acids 452 to 460 of VP1, VP2, or VP3 of the AAV9 capsid protein.

[0123] In some embodiments, a single chain antibody agent insertion is in VR-V of the parental AAV capsid protein.

[0124] In some embodiments, a single chain antibody agent insertion is in VR-VI of the parental AAV capsid protein.

[0125] In some embodiments, a single chain antibody agent insertion is in VR-VII of the parental AAV capsid protein.

[0126] In some embodiments, a single chain antibody agent insertion is in VR-VIII of the parental AAV capsid protein.

[0127] In some embodiments, a parental AAV capsid protein is an AAV9 capsid protein and VR-VIII comprises amino acids 580 to 601 of VP1, VP2, or VP3 of the AAV9 capsid protein.

[0128] In some embodiments, provided herein is an isolated cell comprising a nucleic acid disclosed herein.

[0129] This disclosure provides a variant AAV capsid protein, wherein the variant AAV capsid protein comprises a single chain antibody agent that binds to a transferrin receptor (TfR) or a variant or a fragment thereof, and wherein the single chain antibody agent is inserted in a variable region (VR) of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR-I, VR-II, VR-III, VR-IV, VR-V, VR-VI, VR-VII, VR-VIII or VR-IX of the parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR-IV of the parental AAV capsid protein. In some embodiments, a parental AAV capsid protein is an AAV9 capsid protein and VR-IV comprises amino acids 452 to 460 of VP1, VP2, or VP3 of the AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is in VR-VIII of the parental AAV capsid protein. In some embodiments, a parental AAV capsid protein is an AAV9 capsid protein and VR-VIII comprises amino acids 580 to 601 of VP1, VP2, or VP3 of the AAV9 capsid protein.

[0130] Also provided herein is a method of delivering a payload to a CNS cell and/or tissue, comprising administering a pharmaceutical composition disclosed herein to a CNS cell and/or tissue.

[0131] Further provided herein is a composition comprising a rAAV particle disclosed herein for use in delivering a payload to a CNS cell and/or tissue. In some embodiments, delivering comprises administering a composition to a CNS cell and/or tissue.

[0132] This disclosure provides use of a composition comprising a rAAV particle disclosed herein in the manufacture of a medicament for delivering a payload to a CNS cell and/or tissue. In some embodiments, delivering comprises administering a composition to a CNS cell and/or tissue.

[0133] In some embodiments, a CNS cell and/or tissue is *in vitro*.

[0134] In some embodiments, a CNS cell and/or tissue is *in vivo*.

[0135] In some embodiments, a CNS cell and/or tissue is from a subject that has, or has been determined to have, a CNS disorder.

[0136] In some embodiments, a CNS cell or tissue is chosen from: a CNS epithelial cell, an endothelial cell associated with a blood brain barrier, a nerve cell, a CNS connective tissue cell, a stem cell, a progenitor cell, a CNS immune cell, a spinal cord cell, a cell that lines one or

more brain ventricles, a nerve support cell, a glial cell, a fat cell, a meninges cell, or any combination thereof.

**[0137]** In some embodiments, a CNS cell comprises an endothelial cell associated with a blood brain barrier. In some embodiments, an endothelial cell associated with a blood brain barrier is part of, or forms a blood brain barrier. In some embodiments, an endothelial cell associated with a blood brain barrier is or comprises a brain capillary endothelial cell (BCEC). In some embodiments, an endothelial cell associated with a blood brain barrier is or comprises a brain microvascular endothelial cell (BMEC). In some embodiments, an endothelial cell associated with a blood brain barrier expresses a hTfR1 or a fragment or variant thereof.

**[0138]** In some embodiments, a CNS cell comprises a CNS epithelial cell. In some embodiments, a CNS epithelial cell comprises a cell that lines one or more brain ventricles.

**[0139]** In some embodiments, a CNS cell comprises a nerve cell (neuron). In some embodiments, a neuron comprises a unipolar neuron, a bipolar neuron, a pseudounipolar neuron, a multipolar neuron, or combinations thereof. In some embodiments, a neuron is a motor neuron, a sensory neuron, an interneuron, an excitatory neuron, an inhibitor neuron, a sympathetic neuron, a parasympathetic neuron, or combinations thereof. In some embodiments, a neuron comprises a pyramidal neuron, a dopaminergic neuron, a cholinergic neurons, an adrenergic neuron, a GABAergic neuron, a glutamatergic neuron, a serotonergic neuron, a purinergic neuron, a histaminergic neuron, a lower motor neuron, or combinations thereof.

**[0140]** In some embodiments, a nerve cell (neuron) comprises nerve support cells. In some embodiments, nerve support cells comprise glial cells. In some embodiments, glial cells comprise astrocytes, microglial cells, ependymal cells, oligodendrocytes, Schwann cells, or combinations thereof.

**[0141]** In some embodiments, a CNS cell comprises a CNS connective tissue cell. In some embodiments, a CNS connective tissue cell comprises fat cells or meninges cells, or both.

**[0142]** In some embodiments, a CNS cell comprises a stem cell or progenitor cell. In some embodiments, a stem cell comprises a neural stem cell.

**[0143]** In some embodiments, a CNS cell comprises a cell that lines one or more brain ventricles.

- [0144] In some embodiments, a CNS cell comprises a meninges cell.
- [0145] In some embodiments, a CNS cell comprises a fat cell.
- [0146] In some embodiments, a CNS tissue comprises tissue found in: cortex, thalamus, hypothalamus, striatum, putamen, caudate nucleus, hippocampus, entorhinal cortex, basal ganglia, deep cerebellar nuclei, or other parts of the brain and/or spinal cord.
- [0147] In some embodiments, CNS tissue comprises tissue found in a frontal cortex, a parietal cortex, an occipital cortex, a temporal cortex or combinations thereof.
- [0148] In some embodiments of any of the AAV particles comprising a variant AAV capsid protein, compositions comprising a rAAV particle, or methods of using the same disclosed herein, a variant AAV capsid protein confers increased infectivity and/or transduction of a central nervous system (CNS) cell and/or tissue compared to the infectivity and/or transduction of the CNS cell and/or tissue by a control AAV particle comprising a corresponding parental AAV capsid protein.
- [0149] In some embodiments, a rAAV particle comprising a variant AAV capsid protein confers at least 1.5-fold, at least 2-fold, at least 2.5-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 30-fold, at least 40-fold, at least 50-fold, at least 60-fold, at least 70-fold, at least 80-fold, at least 90-fold, at least 100-fold, at least 120-fold, at least 140-fold, at least 160-fold, at least 180-fold, at least 200-fold, at least 250-fold, at least 300-fold, at least 400-fold, at least 500-fold, at least 600-fold, at least 700-fold, at least 800-fold, at least 900-fold, at least 1000-fold, at least 1500-fold, at least 2000-fold, at least 4000-fold increased infectivity and/or transduction of a CNS cell compared to: (1) infectivity and/or transduction of a CNS cell by a control AAV particle comprising a corresponding parental AAV capsid protein; or (2) infectivity and/or transduction of a CNS cell which does not express a receptor recognized by a variant AAV capsid protein (e.g., hTfR1) with variant AAV capsid protein.
- [0150] In some embodiments, a rAAV particle comprising a variant AAV capsid protein confers about 1.5-fold, about 2-fold, about 2.5-fold, about 3-fold, about 4-fold, about 5-fold, about 6-fold, about 7-fold, about 8-fold, about 9-fold, about 10-fold, about 15-fold, about 20-

fold, about 25-fold, about 30-fold, about 40-fold, about 50-fold, about 60-fold, about 70-fold, about 80-fold, about 90-fold, about 100-fold, about 120-fold, about 140-fold, about 160-fold, about 180-fold, about 200-fold, about 250-fold, about 300-fold, about 400-fold, about 500-fold, about 600-fold, about 700-fold, about 800-fold, about 900-fold, about 1000-fold, about 1500-fold, about 2000-fold, about 4000-fold, increased infectivity and/or transduction of a CNS cell or tissue compared to: (1) infectivity and/or transduction of a CNS cell by a control AAV particle comprising a corresponding parental AAV capsid protein; or (2) infectivity and/or transduction of a CNS cell which does not express a receptor recognized by a variant AAV capsid protein (e.g., hTfR1) with variant AAV capsid protein.

**[0151]** In some embodiments, a rAAV particle comprising a variant AAV capsid protein confers 1.5-fold, 2-fold, 2.5-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 15-fold, 20-fold, 25-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 120-fold, 140-fold, 160-fold, 180-fold, 200-fold, 250-fold, 300-fold, 400-fold, 500-fold, 600-fold, 700-fold, 800-fold, 900-fold, 1000-fold, 1500-fold, 2000-fold, 4000-fold, increased infectivity and/or transduction of a CNS cell or tissue compared to: (1) infectivity and/or transduction of a CNS cell by a control AAV particle comprising a corresponding parental AAV capsid protein; or (2) infectivity and/or transduction of a CNS cell which does not express a receptor recognized by a variant AAV capsid protein (e.g., hTfR1) with variant AAV capsid protein.

**[0152]** In some embodiments of any of the variant AAV capsid proteins, AAV particles comprising a variant AAV capsid protein, compositions comprising a variant AAV capsid protein, or methods of using the same, a variant AAV capsid protein comprises one or more modifications to an amino acid sequence flanking a single chain antibody agent insertion. In some embodiments, one or more modifications are within about 10 amino acids upstream or downstream of the location of a single chain antibody agent insertion site, e.g., within about 5 amino acids upstream or downstream of the location of the single chain antibody agent insertion site.

**[0153]** In some embodiments, one or more modifications comprises an insertion, deletion, mutation, or combinations thereof.

[0154] In some embodiments, a deletion is a deletion of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9 or at least 10 amino acids. In some embodiments, a deletion is a deletion of about 1 to about 10 amino acids.

[0155] In some embodiments, when the insertion is in VR-IV of a parental AAV capsid protein the deletion comprises a deletion of amino acids 455 to 460 inclusive of endpoints of VP1 of the AAV9 capsid protein. In some embodiments, when the insertion is in VR-IV of a parental AAV capsid protein the deletion comprises a deletion of amino acids 455 and 456 of VP1 of the AAV9 capsid protein. In some embodiments, when the insertion is in VR-IV of a parental AAV capsid protein the deletion comprises a deletion of amino acids 452 to 459 inclusive of endpoints of VP1 of the AAV9 capsid protein.

[0156] In some embodiments, the single chain antibody agent insertion is in VR-IV of the parental AAV capsid protein and (a) the single chain antibody agent insertion site is located between amino acids 454 and 461 of VP1 of the AAV9 capsid protein; and (b) the deletion is a deletion of amino acids 455 to 460 inclusive of endpoints of VP1 of the AAV9 capsid protein.

[0157] In some embodiments, the single chain antibody agent insertion is in VR-IV of the parental AAV capsid protein and (a) the single chain antibody agent insertion site is located between amino acids 454 and 457 of VP1 of the AAV9 capsid protein; and (b) the deletion is a deletion of amino acids 455 and 456 of VP1 of the AAV9 capsid protein.

[0158] In some embodiments, the single chain antibody agent insertion is in VR-IV of the parental AAV capsid protein and (a) the single chain antibody agent insertion site is located between amino acids 451 and 460 in of VP1 of the AAV9 capsid protein; and (b) the deletion is a deletion of amino acids 452 to 459 inclusive of endpoints of VP1 of the AAV9 capsid protein.

[0159] In some embodiments, when the insertion is in VR-VIII of the parental AAV capsid protein the deletion comprises a deletion of amino acid 589 of VP1 of the AAV9 capsid protein.

[0160] In some embodiments, the single chain antibody agent insertion is in VR-IV of the parental AAV capsid protein and (a) the single chain antibody agent insertion site is located between amino acids 588 and 590 of VP1 of the AAV9 capsid protein; and (b) the deletion is a deletion of amino acid 588 of VP1 of the AAV9 capsid protein.

[0161] In some embodiments of a rAAV particle, a variant AAV capsid protein, a composition, or a method disclosed herein, one or more modifications are located in a variable region of parental AAV capsid protein. In some embodiments, a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, one or more modifications are located in an AAV9 capsid protein variable region, e.g., VR-I, VR-II, VR-III, VR-IV, VR-V, VR-VI, VR-VII, VR-VIII or VR-IX, or any combination thereof.

[0162] In some embodiments, one or more modifications are located in: VR-VIII of a VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, one or more modifications are located in: VR-VIII of a VP1, but not in a VP2 and/or VP3 of an AAV9 capsid protein. In some embodiments, one or more modifications are located in: VR-VIII of a VP2 and/or VP3 of an AAV9 capsid protein but not in a VP1.

[0163] In some embodiments, one or more modifications are located in: VR-IV of a VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, one or more modifications are located in: VR- IV of a VP1, but not in a VP2 and/or VP3 of an AAV9 capsid protein. In some embodiments, one or more modifications are located in: VR- IV of a VP2 and/or VP3 of an AAV9 capsid protein but not in a VP1.

[0164] In some embodiments, a variant AAV capsid protein further comprises one or more modifications to an amino acid sequence that is at or near a glycan binding region. In some embodiments, one or more modifications reduces glycan binding. In some embodiments, a glycan is galactose. By way of example, glycan binding residues of an AAV9 capsid protein include, but are not limited to: (a) amino acids 271 and 272 of a VP1, VP2 or VP3; (b) amino acid 446 of a VP1, VP2 or VP3; (c) amino acid 470 of a VP1, VP2 or VP3; (d) amino acids 501 to 505 (e.g., at any one or all or a combination of residues 501, 502, 503, 504 or 505) of a VP1, VP2 or VP3; or (e) amino acids 489 and 545 of a VP1, VP2 or VP3. Based on the exemplary glycan binding domains and/or residues provided herein for an AAV9 capsid protein, those with knowledge in the pertinent field would be able to readily ascertain the corresponding glycan binding domain and/or residues in a different parental AAV capsid protein.

[0165] In some embodiments of a rAAV particle, a variant AAV capsid protein, a composition, or a method disclosed herein, one or more modifications is at a glycan binding domain of a parental AAV capsid protein. In some embodiments, a parental AAV capsid protein

is an AAV9, AAV1, AAV2, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV10, AAV11, AAV12, AAV13, AAVhu68, or AAVrh10 capsid protein. In some embodiments, a parental AAV capsid protein is an AAV9 capsid protein.

**[0166]** In some embodiments, one or more modifications is at or between amino acids: (a) 271 and 272 of a VP1, VP2, or VP3 of an AAV9 capsid protein or a corresponding position in a capsid protein of another parental AAV capsid protein; (b) 446 of a VP1, VP2, or VP3 of an AAV9 capsid protein or a corresponding position in a capsid protein of another parental AAV capsid protein; (c) 470 of a VP1, VP2, or VP3 of an AAV9 capsid protein or a corresponding position in a capsid protein of another parental AAV capsid protein; (d) 501 and 505 (e.g., at any one or all or a combination of residues 501, 502, 503, 504 or 505) of a VP1, VP2, or VP3 of an AAV9 capsid protein or a corresponding position in a capsid protein of another parental AAV capsid protein; (e) 489 and 545 of a VP1, VP2, or VP3 of an AAV9 capsid protein or a corresponding position in a capsid protein of another parental AAV capsid protein; (f) 591 and 621 of a VP1, VP2, or VP3 of an AAV9 capsid protein or a corresponding position in a capsid protein of another parental AAV capsid protein; or (g) any combination or all of (a)-(f).

**[0167]** In some embodiments of a rAAV particle, a variant AAV capsid protein, a composition, or a method disclosed herein, one or more modifications is at or between amino acids: (a) 271 and 272 of a VP1, VP2 or VP3 of an AAV9 capsid protein; (b) 446 of a VP1, VP2 or VP3 of an AAV9 capsid protein; (c) 470 of a VP1, VP2 or VP3 of an AAV9 capsid protein; (d) 501 and 505 (e.g., at any one or all or a combination of residues 501, 502, 503, 504 or 505) of a VP1, VP2 or VP3 of an AAV9 capsid protein; (e) 489 and 545 of VP1of a VP1, VP2 or VP3 of an AAV9 capsid protein; (f) 591 and 621 of a VP1, VP2 or VP3 of an AAV9 capsid protein; or (g) any combination or all of (a)-(f).

**[0168]** In some embodiments of any of the variant AAV capsid proteins, AAV particles comprising a variant AAV capsid protein, compositions comprising a variant AAV capsid protein, or methods of using the same, a variant AAV capsid protein has at least 90%, at least 91%, at least 92%, at least 93%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity relative to a parental AAV capsid protein.

[0169] In some embodiments, percent identity is determined by comparing the sequence of a variant AAV capsid protein without a single chain antibody agent insertion, with a parental AAV capsid protein.

[0170] In some embodiments, a variant AAV capsid protein and a parental AAV capsid protein have 100% identity when: (a) a single chain antibody agent insertion in a variant AAV capsid protein is not taken into account in the sequence comparison; and (b) a variant AAV capsid protein does not have one or more modifications other than a single chain antibody agent insertion.

[0171] In some embodiments, a variant AAV capsid protein and a parental AAV capsid protein have less than 100% identity when: (a) a single chain antibody agent insertion in a variant AAV capsid protein is not taken into account in the sequence comparison; and (b) a variant AAV capsid protein comprises one or more modifications other than a single chain antibody agent insertion.

[0172] In some embodiments, a parental AAV capsid protein is an AAV9 capsid protein of SEQ ID NO: 2001.

[0173] In some embodiments, a parental AAV capsid protein is an AAV1 capsid protein of SEQ ID NO: 2002.

[0174] In some embodiments, a parental AAV capsid protein is an AAV2 capsid protein of SEQ ID NO: 2003.

[0175] In some embodiments, a parental AAV capsid protein is an AAV3B capsid protein of SEQ ID NO: 2007.

[0176] In some embodiments, a parental AAV capsid protein is an AAV4 capsid protein of SEQ ID NO: 2008.

[0177] In some embodiments, a parental AAV capsid protein is an AAV5 capsid protein of SEQ ID NO: 2004.

[0178] In some embodiments, a parental AAV capsid protein is an AAV6 capsid protein of SEQ ID NO: 2005.

[0179] In some embodiments, a parental AAV capsid protein is an AAV8 capsid protein of SEQ ID NO: 2006.

[0180] In some embodiments, a parental AAV capsid protein is an AAV7 capsid protein of SEQ ID NO: 2009.

[0181] In some embodiments, a parental AAV capsid protein is an AAV10 capsid protein of SEQ ID NO: 2010.

[0182] In some embodiments, a parental AAV capsid protein is an AAV11 capsid protein of SEQ ID NO: 2011.

[0183] In some embodiments, a parental AAV capsid protein is an AAV12 capsid protein of SEQ ID NO: 2012.

[0184] In some embodiments, a parental AAV capsid protein is an AAV13 capsid protein of SEQ ID NO: 2013.

[0185] In some embodiments, a parental AAV capsid protein is an AAVu68 capsid protein of SEQ ID NO: 2014.

[0186] In some embodiments, a parental AAV capsid protein is an AA Vrh10 capsid protein of SEQ ID NO: 2015.

[0187] In some embodiments of any of the AAV particles comprising a variant AAV capsid protein, compositions comprising a rAAV particle, or methods of using the same disclosed herein, a payload is or comprises a polypeptide that is encoded by a nucleic acid sequence within a rAAV particle.

[0188] In some embodiments, a polypeptide is or comprises a CRISPR-Cas protein, or a variant or fragment thereof. In some embodiments, a CRISPR-Cas protein is chosen from : a Type II, Type V or Type VI CRISPR-Cas protein (e.g., a Cas9 protein), a Cas12a protein, a Cas12b protein, a Cas12c protein, a Cas12d protein, a Cas12e protein, a Cas12f protein, a Cas12g protein, a Cas12h protein, a Cas12i protein, a Cas13a protein, a Cas13b protein or a variant or fragment of any of the foregoing. In some embodiments, the payload also comprises a guide RNA, gRNA, sgRNA, or crRNA/tracrRNA that interacts with the CRISPR-Cas protein. In some embodiments a CRISPR-Cas protein is fused with one or more domains, e.g., an activator

domain and/or a repressor domain. In some embodiments a CRISPR-Cas protein is a nuclease. In some embodiment a CRISPR-Cas protein is a nickase and only cleaves one strand of a target nucleic acid molecule. In some embodiment a CRISPR-Cas protein is inactivated and binds to but does not cleave a target nucleic acid molecule.

**[0189]** In some embodiments, a polypeptide is or comprises a Zinc finger protein, or a variant or fragment thereof. In some embodiments, a Zinc finger protein is chosen from: a Zinc finger nuclease, an artificial restriction enzyme fusion protein, a sequence-targeted zinc-finger DNA-binding unit optionally fused with a nuclease domain (e.g., Fok1 nuclease domain), or a variant or fragment or any combination of any of the foregoing. In some embodiments a Zinc finger protein is fused with one or more domains, e.g., an activator domain and/or a repressor domain.

**[0190]** In some embodiments, a polypeptide is or comprises a Transcription Activator-Like Effector (TAL) protein, or a variant or fragment thereof. In some embodiments, a TAL comprises: a TAL effector DNA binding domain (e.g., a TAL effector DNA binding domain isolated from *Xanthomonas* spp.), a Transcription Activator-Like Effector Nuclease (TALEN), e.g., a TAL effector DNA binding domain fused with a nuclease domain (e.g., Fok1 nuclease domain), or a variant or fragment or any combination of any of the foregoing. In some embodiments a TAL protein is fused with one or more domains, e.g., an activator domain and/or a repressor domain.

**[0191]** In some embodiments, a polypeptide is or comprises a base editor, or a variant or fragment thereof. In some embodiments, a base editor comprises a deaminase, an adenosine deaminase enzyme (ABE), a cytosine deaminase enzyme (CBE), an APOBEC1, an APOBEC3A, an APOBEC3G, an evoAPOBEC, a BE4-YE1, a CDA1, an activation-induced cytidine deaminase (AID), a mutant TadA, an adenosine deaminases (TadA\*), an *E. coli* tRNA-specific adenosine deaminase (TadA), a deaminase associated with a DNA binding domain monomer, a base editing enzyme that is RNA guided, a DNA glycosylase inhibitor, one or more DNA glycosylase inhibitor domains, a 5-methylcytosine deaminase, a cytidine deaminase domain, an adenine deaminase domain, an adenosine base editor (ABE), a Target-ACEmax, a synchronous programmable adenine and cytosine editor (SPACE), an A&C-Bemax., a circularly permuted base editor, an adenosine deaminase enzyme (ADAR), a RNA editing for programmable

adenosine to inosine replacement (REPAIR), a leveraging endogenous ADAR for programmable editing of RNA (LEAPER) or a variant or fragment or combination of any of the foregoing. In some embodiments, the payload also comprises a guide RNA, gRNA, sgRNA, or crRNA/tracrRNA that interacts with the base editor.

**[0192]** In some embodiments, a polypeptide is or comprises a prime editor, or a variant or fragment thereof, or a system comprising the same. In some embodiments, a prime editor and/or system comprising the same comprises: a reverse transcriptase, a prime editing enzyme, an editing enzyme that includes a reverse transcriptase domain, an Avian Myeloblastosis Virus (AMV) Reverse Transcriptase, a Murine Leukemia Virus (MLV) Reverse Transcriptase, a HIV-1 reverse transcriptase, a bacterial reverse transcriptase, a reverse transcriptase associated with a DNA binding domain and/or protein, a reverse transcriptase fused to a DNA binding domain that is a catalytically impaired nuclease domain (e.g., a nickase), a prime editing 1 system (PE1), a prime editing 2 system (PE2), a prime editing 3 system (PE3), a prime editing 3b system (PE3b) or a variant or fragment or any combination of any of the foregoing. In some embodiments, the payload also comprises a prime editing gRNA (pegRNA) or an extended sgRNA that interacts with the prime editor.

**[0193]** In some embodiments, a polypeptide is or comprises a meganuclease, or a variant or fragment thereof. In some embodiments, a meganuclease is chosen from: a homing endonuclease, a LAGLIDADG family meganuclease, a GIYYIG family meganuclease, a His-Cyst box family meganuclease, or HNH family endonuclease, an I-SceI, an I-CeuI, a PI-PspI, a PI-SceI, an I-SceIV, an I-CsmI, an I-PanI, an I-SceII, an I-PpoI, an I-SceIII, an I-CreI, an I-TevI, an I-TevII an I-TevIII or a variant or fragment or any combinations of any of the foregoing.

**[0194]** In some embodiments, a polypeptide is associated with a CNS disorder.

**[0195]** In some embodiments, a CNS disorder is a result of a genetic abnormality.

**[0196]** In some embodiments, a CNS disorder is not a result of a genetic abnormality.

**[0197]** In some embodiments, a CNS disorder is chosen from: Friedreich's Ataxia, Dravet Syndrome, Spinocerebellar Ataxia Type 3, Niemann-Pick Type C, Huntington's Disease, Pompe Disease, Myotonic Dystrophy Type 1, Glut1 Deficiency Syndrome (De Vivo Syndrome), Tay-Sachs, Spinal Muscular Atrophy, Alzheimer's disease, Amyotrophic lateral

sclerosis (ALS), Danon disease, Rett Syndrome, Angelman Syndrome, Parkinson's disease, tauopathies, genetic epilepsies (e.g., a STXBP1 genetic epilepsy, or a CDKL5 genetic epilepsy), or combinations thereof.

- [0198] In some embodiments, a polypeptide is an enzyme.
- [0199] In some embodiments, a polypeptide is an antibody.
- [0200] In some embodiments, a polypeptide is a secreted protein.
- [0201] In some embodiments of any of the rAAV particles comprising a variant AAV capsid protein, compositions comprising a rAAV particle, or methods of using the same disclosed herein, a payload is or comprises an RNA molecule that is encoded by a nucleic acid sequence within a rAAV particle. In some embodiments, an RNA molecule is or comprises an siRNA, a miRNA, a gRNA, an antisense RNA, a circular RNA, an snRNA or an aptamer.
- [0202] In some embodiments, an RNA molecule targets a nucleic acid molecule encoding a polypeptide associated with a CNS disorder.
- [0203] In some embodiments of any of the rAAV particles comprising a variant AAV capsid protein, compositions comprising a rAAV particle, or methods of using the same disclosed herein, a payload is or comprises a DNA molecule. In some embodiments, a DNA molecule is or comprises a nucleic acid sequence of up to about 5,100 nt in length, e.g., up to about 5,000 nt, up to about 4,900, up to about 4,800, up to about 4,700, up to about 4,600, up to about 4,500, up to about 4,400, etc.
- [0204] In some embodiments of any of the rAAV particles comprising a variant AAV capsid protein, compositions comprising a rAAV particle, or methods of using the same disclosed herein, a nucleotide sequence encoding a payload is operably linked to a promoter.
- [0205] In some embodiments, a promoter is a CNS promoter or a variant or a fragment thereof. In some embodiments, a CNS promoter is chosen from: a GFAP promoter, a SYN1 promoter, a NSE/RU5' promoter, a neuroactive peptide cholecystokinin (CCK) promoter, a myelin basic promoter (MBP), a human myelin associated glycoprotein promoter, a phosphate-activated glutaminase (PAG) promoter, a vesicular glutamate transporter (vGLUT) promoter, a glutamic acid decarboxylase (GAD) promoter, Camk2a promoter, TH (tyrosine hydroxylase)

promoter, Hb9 promoter, CNP promoter, NES (nestin) promoter, Tub1a promoter, SST (somatostatin) promoter, MeCP2 promoter, or combinations thereof.

[0206] In some embodiments, a promoter is or comprises a chicken beta actin hybrid (CBh) promoter or a variant or a fragment thereof.

[0207] In some embodiments, a promoter is or comprises a hSyn1 promoter or a variant or a fragment thereof.

[0208] In some embodiments, a promoter is or comprises a GFAP promoter or a variant or a fragment thereof.

[0209] In some embodiments of any of the methods of using a pharmaceutical composition comprising a rAAV particle comprising a variant AAV capsid protein disclosed herein, a pharmaceutical composition is administered via a route of administration chosen from: intravenous, intraarterial, intracoronary, intraparenchymal, subpial, intrathecal, intraocular, intracerebroventricular (ICV), intracisternal magna (ICM), or intramuscular.

[0210] In some embodiments, a subject is a human.

[0211] Also provided herein is a method of treating a subject having a CNS disorder and/or ameliorating a symptom of a CNS disorder in a subject, the method comprising administering to the subject a pharmaceutical composition disclosed herein.

[0212] In some embodiments, a CNS disorder is chosen from Friedreich's Ataxia, Dravet Syndrome, Spinocerebellar Ataxia Type 3, Niemann-Pick Type C, Huntington's Disease, Pompe Disease, Myotonic Dystrophy Type 1, Glut1 Deficiency Syndrome (De Vivo Syndrome), Tay-Sachs, Spinal Muscular Atrophy, Alzheimer's disease, Amyotrophic lateral sclerosis (ALS), Danon disease, Rett Syndrome, Angelman Syndrome, Parkinson's disease, tauopathies, genetic epilepsies (e.g., a STXBP1 genetic epilepsy, or a CDKL5 genetic epilepsy), or combinations thereof.

[0213] Other features, objects, and advantages of the present invention are apparent in the detailed description that follows. It should be understood, however, that the detailed description, while indicating embodiments of the present invention, is given by way of illustration only, not limitation. Various changes and modifications within the scope of the invention will become apparent to those skilled in the art from the detailed description.

## BRIEF DESCRIPTION OF THE DRAWING

- [0214] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings(s) will be provided by the Office upon request and payment of the necessary fee.
- [0215] The Figures described below, which together make up the Drawing, are for illustration purposes only, not for limitation.
- [0216] **FIGS. 1A-1C** show schematics depicting single variant and barcoded library production schemes for AAV particles displaying VHHS in the capsid (also referred to herein as “AAV-VHH”). **FIG. 1A** shows a schematic detailing the standard triple transfection method for AAV single capsid production. **FIG. 1B** shows a schematic detailing the quadruple transfection method for AAV-VHH production. **FIG. 1C** shows a schematic detailing the transfection method for barcoded AAV-VHH library production, with each capsid packaging a mixture of cis-plasmids consisting of two CAG-driven and two hSyn1-driven H2B-GFP transgenes with unique 16-nt barcodes embedded in the 3'-UTR.
- [0217] **FIGS. 2A-2B** show an illustration of designs for inserting an exemplary VHH in an AAV9 VP1 subunit. \* indicates ablation of VP2 or VP3 start codons. Bars with a dash represent GGGGS linker. Crossed bars represent VHH sequences. Bars with a circle represent 18-amino acid alpha helix sequences that are predicted to form a coiled-coil structure. Blank bars represent wild-type AAV9 VP1 sequence.
- [0218] **FIG. 3** shows a bar graph depicting transduction of AAV-VHH library variants in WT and TfR1 KO HEK293T cells. Each bar represents a unique capsid, with linker designs designated by numbers on the x-axis, with data grouped by VHH. Y-axis represents Log10 fold enhancement in CAG-driven transgene expression of a AAV capsid displaying an exemplary VHH over AAV9 in WT HEK293T cells (closed bars), and TfR1 KO HEK293T cells (open bars).
- [0219] **FIG. 4** shows an illustration of TfR1 constructs used with *in vivo* testing of AAV-VHHs. Human, cyno, and murine TfR1 domains are represented as hashed, black, and white regions, respectively.
- [0220] **FIGS. 5A-5B** show graphs depicting relative AAV-VHH capsid transduction over AAV9 in WT and hTfR1-KI<sup>+/+</sup> mouse models, as determined by barcode expression driven by

CAG or hSyn1 promoter normalized by barcode fractions in the input AAV library. Each symbol represents a unique capsid in the barcoded AAV9-VHH library. Plots show brain transduction enhancement over AAV9 for capsid variants in (**FIG. 5A**) WT mice, and (**FIG. 5B**) hTfR1-KI<sup>+/+</sup> model. Linker designs are shown in **FIGS. 6A-6B**.

[0221] **FIGS. 6A-6B** show bar graphs depicting AAV-VHH brain transduction fold enhancement over AAV9 based on CAG-driven barcode expression in (**FIG. 6A**) WT, and (**FIG. 6B**) hTfR1-KI<sup>+/+</sup> models. X axis indicates linker design, and unique anti-TfR VHHs are grouped.

[0222] **FIGS. 7A-7B** depict relative AAV-VHH capsid brain transduction over AAV9 in cynomolgus macaque. **FIG. 7A:** Each symbol represents a unique capsid in the barcoded AAV9-VHH library. X- and y-axes represent CAG- and hSyn1-driven barcode expression enhancement over AAV9 in the brain, respectively. Linker designs are shown in **FIG. 7B**. **FIG. 7B** shows AAV-VHH brain transduction fold enhancement over AAV9 based on CAG-driven barcode expression in the cyno model. X axis indicates linker design, and unique anti-TfR VHHs are grouped.

[0223] **FIGS. 8A-8B** show transgene expression and vector genome biodistribution mediated by Clone A VHH-D2 (Design 2), Clone A VHH-D7 (Design 7) and AAV9 in WT mice. Each symbol represents a biological replicate. **FIG. 8A** represents normalized mCherry mRNA fold enhancement over AAV9 in different tissue types. **FIG. 8B** represents vector genome copies per cell in different tissue types.

[0224] **FIGS. 9A-9B** show mCherry immunohistochemistry images on sagittal brain sections of AAV9, Clone A VHH-D2, or Clone A VHH-D7 treated WT mice. Scale bars represent 5 mm. **FIG. 9A** is in color and **FIG. 9B** is in black and white.

[0225] **FIGS. 10A-10B** show mCherry immunohistochemistry images in brain cortex of AAV9, Clone A VHH-D2, or Clone A VHH-D7 treated WT mice. Scale bars represent 200 microns. **FIG. 10A** is in color and **FIG. 10B** is in black and white.

[0226] **FIGS. 11A-11B** show mCherry immunohistochemistry images in hippocampus of AAV9, Clone A VHH-D2, or Clone A VHH-D7 treated WT mice. Scale bars represent 1 mm. **FIG. 11A** is in color and **FIG. 11B** is in black and white.

[0227] **FIGS. 12A-12B** show mCherry immunohistochemistry images in cerebellum of AAV9, Clone A VHH-D2, or Clone A VHH-D7 treated WT mice. Scale bars represent 200 microns. **FIG. 12A** is in color and **FIG. 12B** is in black and white.

[0228] **FIGS. 13A-13B** show normalized mCherry mRNA fold enhancement and vector genome copies per cell for different AAV-VHH clones over AAV9 in brain, spinal cord, heart, quad, liver, and spleen (for vector genome only) hTfR1-KI<sup>+/+</sup> mice. **FIG. 13A** shows normalized mCherry mRNA fold enhancement and **FIG. 13B** shows vector genome copies per cell. Each dot represents a biological replicate.

[0229] **FIGS. 14A-14B** shows mCherry immunohistochemistry on sagittal brain sections of AAV9, Clone A VHH-D2, Clone A VHH-D7, or Clone B VHH-D1 treated hTfR1-KI<sup>+/+</sup> mice. Scale bars represent 5 mm. **FIG. 14A** is in color and **FIG. 14B** is in black and white.

[0230] **FIGS. 15A-15B** shows mCherry immunohistochemistry images in cortical regions of AAV9, Clone A VHH-D2, Clone A VHH-D7, or Clone B VHH-D1 treated hTfR1-KI<sup>+/+</sup> mice. Scale bars represent 250 microns. **FIG. 15A** is in color and **FIG. 15B** is in black and white.

[0231] **FIGS. 16A-16B** show mCherry immunohistochemistry images in hippocampus of AAV9, Clone A VHH-D2, Clone A VHH-D7, or Clone B VHH-D1 treated hTfR1-KI<sup>+/+</sup> mice. Scale bars represent 1 mm. **FIG. 16A** is in color and **FIG. 16B** is in black and white.

[0232] **FIGS. 17A-17B** show mCherry immunohistochemistry images in cerebellar cortex of AAV9, Clone A VHH-D2, Clone A VHH-D7, or Clone B VHH-D1 treated hTfR1-KI<sup>+/+</sup> mice. Scale bars represent 500 microns. **FIG. 17A** is in color and **FIG. 17B** is in black and white.

[0233] **FIGS. 18A-18B** show schematics depicting cryo-EM structures of human TfR1 in complex with Clone A VHH and human holo transferrin. **FIG. 18A** shows a schematic depicting a cryo-EM density map of human TfR1 in complex with Clone A VHH and transferrin at 2.4 Å resolution. **FIG. 18B** shows a schematic depicting an atomic model of a human TfR1/Clone A VHH/transferrin complex.

[0234] **FIG. 19** shows a schematic depicting an atomic model of Clone A VHH highlighting paratope residues. O atoms are in white, and N atoms are in black.

[0235] **FIG. 20** shows a partial alignment of TfR1 apical and helical domain sequences encompassing a predicted Clone A VHH epitope. Amino acid residues within 4 Å of Clone A

VHH, as observed in Clone A VHH-huTfR1 ECD cryo-EM structures, are highlighted in black, with a subset of residues from the other TfR1 protomer (monomer) underlined. The two residues divergent between cyno and human TfR1 within the Clone A VHH epitope are in italics. Strictly conserved residues are denoted by asterisk, strongly conserved residues by colon, and moderately conserved residues by period. Sources of sequences: Human (*Homo sapiens*, Uniprot P02786.2); Cynomolgus (*Macaca fascicularis*, NCBI Reference Sequence: XP\_045243212.1); Mouse (*Mus Musculus*, Uniprot Q62351).

[0236] **FIG. 21** shows a schematic depicting an atomic model of a Clone A VHH-human TfR1 ECD complex, highlighting amino acid residues divergent between cyno and human TfR1 ECD (R325, G724) as well as Clone A VHH residues in close contact (D53, Q99, V101).

[0237] **FIGS. 22A-22B** show schematics depicting a cryo-EM structure of human TfR1 in complex with Clone B VHH and human holo transferrin. **FIG. 22A** shows a schematic depicting a cryo-EM density map of transferrin receptor in complex with Clone B VHH and transferrin at 2.7 Å resolution. **FIG. 22B** shows a schematic depicting an atomic model of TfR1/Clone B VHH/transferrin complex.

[0238] **FIG. 23** shows a partial alignment of TfR1 apical and helical domain sequences encompassing a predicted Clone B VHH epitope. The residues within 4 Å of Clone B VHH, as observed in Clone B VHH-huTfR1 ECD cryo-EM structures, are highlighted in black, with a subset of residues from the other TfR1 protomer (monomer) underlined. One residue divergent between cyno and human TfR1 within a Clone B VHH epitope is in italics. Strictly conserved residues are denoted by asterisk, strongly conserved residues by colon, and moderately conserved residues by period. Sources of sequences: Human (*Homo sapiens*, Uniprot P02786.2); Cynomolgus (*Macaca fascicularis*, NCBI Reference Sequence: XP\_045243212.1); Mouse (*Mus Musculus*, Uniprot Q62351).

[0239] **FIGS. 24A-24D** depict plots demonstrating monovalent VHH-Fc fusion binding to CHO cells stably expressing the human (circle), cyno (square), murine (triangle), or TfR1 knock-out (diamond) cell lines. EC50 values for (**FIG. 24A**) Clone A VHH, (**FIG. 24B**) Clone B VHH, and (**FIG. 24C**) Clone D VHH binding were determined by a 3-parameter logarithmic fit in the GraphPad Prism software and are summarized in **Table 7**. **FIG. 24D** shows a schematic depicting the structure of a monovalent VHH-Fc fusion.

[0240] **FIG. 25** shows plots depicting kinetic curves for monovalent VHH-Fc binding to surface-immobilized, His-tagged human, cyno, and mouse TfR1 ectodomain. KD values were determined using global fits with a 1:1 kinetics model or with a steady state analysis model (**Table 7**). Monovalent VHH-Fc concentrations decrease from top to bottom in a 4-fold dilution. Top concentrations are 4000 nM for Clone A VHH and Clone B VHH, 800 nM for Clone D VHH.

[0241] **FIG. 26** shows a graph depicting correlation between monovalent VHH-TfR1 affinity and AAV-VHH neuronal transduction. Each symbol represents an AAV9 capsid displaying an exemplary VHH. X-axis represents monovalent K<sub>Ds</sub> of the corresponding VHHS against human (circles), cyno (squares), or murine (triangles) TfR1 homologs. Y-axis represents hSyn1-driven transgene expression fold enhancement of exemplary AAV-VHH over AAV9 in the brains of corresponding animal models.

[0242] **FIG. 27** are graphs showing the results of bio-layer interferometry (BLI) binding analysis to assess binding of exemplary AAV-VHH capsids to recombinant human or cyno TfR1 ectodomain (ECD). Each curve represents an increasing concentration of human or cyno TfR1 ECD (2.7, 8.2, 24.7, 74.1, 222, 667, 2000 nM) in solution. The constructs used were: Clone A-VHH-D2, Clone B-VHH-D1, Clone F-VHH-D2 and Clone M VHH-D2. Data was fit with a 1:2 bivalent analyte model.

[0243] **FIG. 28** is a correlation plot comparing exemplary VHH-Fc SPR affinity (K<sub>D</sub>) to human or cynoTfR1 vs. exemplary AAV-VHH BLI affinity (K<sub>D</sub>) to human or cynoTfR1. Data is presented on a log-log scale with an x=y dashed line. In both the VHH and AAV context, the clones tested were Clones A, B, D and H and in the context of AAV-VHH the construct designs are similar to those in **FIG. 27**. Binding affinity to cynoTfR1 is represented as squares and affinity to human TfR1 is represented as circles.

[0244] **FIG. 29** is a graph showing transduction by exemplary AAV-VHHS in TfR1 knock-out CHO cells expressing human, cyno or murine TfR1 homologs. Fold transduction in the y-axis is shown as compared to transduction with AAV9.

[0245] **FIG. 30** is a graph showing transduction by exemplary AAV-VHHS in the brain of B-hTfR1 mice. Transgene expression was driven by either the CAG promoter or hSyn1 promoter. Fold transduction in the y-axis is shown as compared to transduction with AAV9.

[0246] **FIG. 31** is a graph showing transduction by exemplary AAV-VHHs in the brain of WT mice. Transgene expression was driven by either the CAG promoter or hSyn1 promoter. Fold transduction in the y-axis is shown as compared to transduction with AAV9.

[0247] **FIG. 32** is a graph showing vector genome biodistribution in B-hTfR1 mouse brain transduced with exemplary AAV-VHHs.

[0248] **FIG. 33** is a graph showing RNA levels of an exemplary transgene in the brain of B-hTfR1 animals administered exemplary AAV-VHHs. Exemplary transgene expression levels in the y-axis are shown in comparison to expression levels of a control RNA, mRPP30, and normalized to expression of the exemplary transgene in the liver of animals administered AAV particles having Clone B-VHH-D1.

[0249] **FIG. 34** is a graph showing the percentage of cortical neurons positive for expression of an exemplary transgene delivered with exemplary AAV-VHHs in B-hTfR1 mouse brain.

[0250] **FIG. 35** are panels of representative images showing RNAscope *in situ* hybridization (ISH) and NeuN antibody immunofluorescent staining in the brain of B-hTfR1 mice administered AAV particles comprising Clone B VHH-D1. The images show AAV-VHH mediated transgene expression in B-hTfR1 mouse brain. The brain slices were stained with an ISH probe recognizing WPRE (as a surrogate for AAV vector; red), a NeuN antibody to label neurons (teal), and DAPI for nuclear labeling; blue. The middle row shows WPRE signal, the top row shows a merge of WPRE ISH and NeuN and DAPI immunofluorescence signals, and the bottom row highlights zoomed-in regions of the cortex outlined in the middle row. Scale bar is 1 mm.

[0251] **FIG. 36** is a graph showing vector genome biodistribution in the spinal cord of B-hTfR1 mice administered exemplary AAV-VHHs.

[0252] **FIG. 37** is a graph showing RNA levels of an exemplary transgene in the spinal cord of B-hTfR1 animals administered exemplary AAV-VHHs. Exemplary transgene expression levels in the y-axis are shown in comparison to expression levels of a control RNA, murine RPP30 (mRPP30), and normalized to expression of the exemplary transgene in the liver of animals administered AAV particles having Clone B-VHH-D1.

[0253] **FIG. 38** is a graph showing vector genome biodistribution in the liver of B-hTfR1 mice administered exemplary AAV-VHHs.

[0254] **FIG. 39** is a graph showing RNA levels of an exemplary transgene in the liver of B-hTfR1 animals administered exemplary AAV-VHHs. Exemplary transgene expression levels in the y-axis are shown in comparison to expression levels of a control RNA, mRPP30, and normalized to expression of the exemplary transgene in the liver of animals administered AAV particles having Clone B-VHH-D1.

[0255] **FIG. 40** is a graph showing vector genome biodistribution in various tissues of NHPs administered exemplary AAV-VHHs or AAV9 intravenously. The exemplary AAV-VHHs comprised Clone F VHH-D2 or Clone M VHH-D2. Vector genome distribution from AAV9 (without VHH insertion) is shown as a reference. AAV9 was administered to 10-month old NHPs; and AAV-VHHs were administered to 3+ years old NHPs.

[0256] **FIG. 41** is a graph showing vector genome biodistribution in various tissues of NHPs administered an exemplary AAV-VHH having Clone B VHH-D1 dosed at two different doses:  $3.3 \times 10(13)$  vg/kg (open squares) or  $1.1 \times 10(14)$  vg/kg (open triangles). Data from animals administered AAV9 (without VHH insertion) is shown as a reference. AAV9 was administered to 10-month old NHPs; and AAV-VHH was administered to 3+ years old NHPs.

[0257] **FIG. 42** is a graph showing RNA levels of the WPRE element as a surrogate for transgene expression in NHP administered exemplary AAV-VHHs having Clone F VHH-D2 or Clone M VHH-D2. WPRE RNA expression in various tissue from NHPs administered exemplary AAV-VHHs is compared to WPRE RNA expression in the prefrontal cortex in NHPs administered AAV particles comprising an AAV9 capsid (without VHH) and normalized to expression of a control RNA, hRPP30. AAV9 was administered to 10-month old NHPs; and AAV-VHHs were administered to 3+ years old NHPs.

[0258] **FIG. 43** is a graph showing RNA levels of the WPRE element as a surrogate for transgene expression in NHP administered exemplary AAV-VHHs having Clone B VHH-D1 in NHPs at two doses:  $3.3 \times 10(13)$  vg/kg (open squares) or  $1.1 \times 10(14)$  vg/kg (open triangles). WPRE RNA expression in various tissue from NHPs administered exemplary AAV-VHHs is compared to WPRE RNA expression in the prefrontal cortex in NHPs administered AAV particles comprising an AAV9 capsid (without VHH) and normalized to expression of a control

RNA, hRPP30. AAV9 was administered to 10-month old NHPs; and AAV-VHH was administered to 3+ years old NHPs.

## DEFINITIONS

**[0259]** In this application, unless otherwise clear from context, (i) the term “a” may be understood to mean “at least one”; (ii) the term “or” may be understood to mean “and/or”; (iii) the terms “comprising” and “including” may be understood to encompass itemized components or steps whether presented by themselves or together with one or more additional components or steps; (iv) the terms “about” and “approximately” may be understood to permit standard variation as would be understood by those of ordinary skill in the art; and (v) where ranges are provided, endpoints are included.

**[0260]** **5' and 3':** The terms “5’” and “3’” are relative terms to define a spatial relationship or directionality between two or more segment of a nucleic acid sequence. Thus, 3' of a nucleic acid indicates a segment of the nucleic acid that is downstream of another segment, while 5' indicates a segment of the nucleic acid that is upstream of another segment. For example, 3' may indicate that a segment is in the 3' half of the nucleic acid sequence or even at the 3' end of the nucleic acid sequence. Similarly, 5' may indicate that a segment is in the 5' half of the nucleic acid sequence or even at the 5' end of the nucleic acid sequence. Unless indicated otherwise, the directionality of a nucleic acid will be in the 5' to 3' direction of translation.

**[0261]** **About or approximately:** As used herein, the terms “approximately” or “about” in reference to a number are generally taken to include numbers that fall within a range of 5%, 10%, 15%, or 20% in either direction (greater than or less than) of the number unless otherwise stated or otherwise evident from the context (except where such number would be less than 0% or exceed 100% of a possible value).

**[0262]** **Adeno-associated virus (AAV):** As used herein, the terms “Adeno-associated virus” and “AAV” refer to viral particles, in whole or in part, of the family *Parvoviridae* and the genus *Dependoparvovirus*. AAV is a small replication-defective, nonenveloped virus. AAV includes, but is not limited to, AAV serotype 1, AAV serotype 2, AAV serotype 3 (including serotypes 3A and 3B), AAV serotype 4, AAV serotype 5, AAV serotype 6, AAV serotype 7, AAV serotype 8, AAV serotype 9, AAV serotype 10, AAV serotype 11, AAV serotype 12, AAV

serotype 13, snake AAV, avian AAV, bovine AAV, canine AAV, equine AAV, ovine AAV, goat AAV, shrimp AAV, non-human primate AAV, e.g., from rhesus monkeys, and any variant of any of the foregoing. Wild-type AAV is replication deficient and requires co-infection of cells by a helper virus, e.g., adenovirus, herpes, or vaccinia virus, e.g., an Ad2 or Ad5 virus, or supplementation of helper viral genes, in order to replicate.

[0263] ***Ad2 helper:*** As used herein, the term “Ad2 helper” refers to the Adenovirus serotype 2 (Ad2) helper virus (e.g., wildtype or recombinantly engineered Ad2 helper virus) and various Ad2 helper genes and/or Ad2 helper polypeptides or nucleic acids, including, but not limited, to E1a, E1b, E2a, E4Orf6, VA RNA, and any variant or fragment of any of the foregoing. In some embodiments, an Ad2 helper vector (e.g., plasmid) encodes Ad2 helper polypeptides or nucleic acids (e.g., one, two, three, or four of E1 (e.g., E1a and/or E1b), E2a, E4, or VA RNA) necessary to generate functional rAAV particles. In certain embodiments, the Ad2 helper vector is transfected into an E1 complementing cell line (e.g., HEK293). The nucleotide sequence of an Ad2 helper vector and Ad2 helper virus genes can be derived from the Adenovirus 2 genome (GenBank Accession No. J01917.1).

[0264] ***Ad5 helper:*** As used herein, the term “Ad5 helper” refers to the Adenovirus serotype 5 (Ad5) helper virus (e.g., wildtype or recombinantly engineered Ad5 helper virus) and various Ad5 helper genes and/or Ad5 helper polypeptides or nucleic acids, including, but not limited, to E1a, E1b, E2a, E4Orf6, and/or VA RNA. In some embodiments, an Ad5 helper vector (e.g., plasmid) encodes Ad5 helper polypeptides or nucleic acids (e.g., one, two, three, or four of E1 (e.g., E1a and/or E1b), E2a, E4, or VA RNA) necessary to generation functional rAAV particles. In certain embodiments, the Ad5 helper vector is transfected into an E1 complementing cell line (e.g., HEK293). The nucleotide sequence of an Ad5 helper vector and Ad5 helper genes can be derived from the Adenovirus 5 genome (GenBank Accession No. AY601635).

[0265] ***Administration:*** As used herein, the term “administration” refers to the administration of a composition comprising rAAV particles as described herein to a subject. Administration may be by any appropriate route. For example, in some embodiments, administration may be local or systemic administration (e.g., to a mammal, e.g., to a human, e.g., a patient). A composition of the disclosure may be administered by injection or infusion by any route. For example, a composition may be administered by retinal, subretinal, intravitreal,

suprachoroidal, intraspinal, intracisternal magna, or intrathecal injection or infusion. Additional exemplary routes of administration may include, but are not limited to, bronchial (e.g., bronchial instillation), buccal, enteral, interdermal, intra-arterial, intradermal, intragastric, intramedullary, intramuscular, intranasal, intraperitoneal, intrathecal, intravenous, intraventricular, mucosal, nasal, oral, rectal, subcutaneous, sublingual, topical, tracheal (e.g., intratracheal instillation), transdermal, vaginal, and vitreal.

[0266] **Bioreactor:** The term “bioreactor,” as used herein, refers to any vessel used for the growth of a cell culture (e.g., a mammalian cell culture). The bioreactor can be of any size and/or any shape so long as it is useful for culturing a cell culture (e.g., a mammalian cell culture).

[0267] **Cap polypeptide:** As used herein, the term “Cap polypeptide” refers to the structural proteins that form a functional AAV capsid, which can in turn package DNA and infect or transduce a target cell. In some embodiments, a Cap polypeptide comprises a variant AAV capsid protein as disclosed herein. In some embodiments, Cap polypeptides will comprise all of the AAV capsid subunits, but less than all of the capsid subunits may be present as long as a functional capsid is produced. In some embodiments, the nucleic acid sequence encoding Cap polypeptides will be present on a single vector (e.g., plasmid). In some embodiments, the nucleic acid sequence encoding Cap polypeptides will be present on more than one vector (e.g., plasmid), e.g., VP1 encoded by a nucleic acid sequence on a first vector, and VP2 and VP3 encoded by a nucleic acid on a second vector. In some embodiments, the Cap polypeptide comprises an AAV1, AAV2, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAVhu68, or AAVrh10 Cap polypeptide, or a variant of any of the foregoing. AAV capsid genes and proteins have been described in, e.g., Knipe DM. et al., (2001) *Fields Virology* 6(1), which is hereby incorporated by reference in its entirety.

[0268] **Cell Density:** As used herein, the term “cell density” refers to that number of cells present in a given volume of medium or the number of cells present in a given surface area. For example, cell density may be represented as viable cells (vc)/cm<sup>2</sup> of culture medium or vc/mL.

[0269] **Corresponding to:** As used herein, the term “corresponding to” may be used to designate the position or identity of a structural element in a compound or composition through comparison with an appropriate reference compound or composition. For example, in some embodiments, a monomeric residue in a polymer (e.g., an amino acid residue in a polypeptide or

a nucleic acid residue in a polynucleotide) may be identified as “corresponding to” a residue in an appropriate reference polymer. For example, those of skill in the art appreciate that residues in a provided polypeptide or polynucleotide sequence are often designated (*e.g.*, numbered or labeled) according to the scheme of a related reference sequence (even if, *e.g.*, such designation does not reflect literal numbering of the provided sequence). By way of illustration, if a reference sequence includes a particular amino acid motif at positions 100-110, and a second related sequence includes the same motif at positions 110-120, the motif positions of the second related sequence can be said to “correspond to” positions 100-110 of the reference sequence. Those of skill in the art appreciate that corresponding positions can be readily identified, *e.g.*, by alignment of sequences, and that such alignment is commonly accomplished by any of a variety of known tools, strategies, and/or algorithms, including without limitation software programs such as, for example, BLAST, CS-BLAST, CUDASW++, DIAMOND, FASTA, GGSEARCH/GLSEARCH, Genoole, HMMER, Hhpred/Hhsearch, IDF, Infernal, KLAST, USEARCH, parasail, PSI-BLAST, PSI-Search, ScalaBLAST, Sequilab, SAM, SSEARCH, SWAPHI, SWAPHI-LS, SWIMM, or SWIPE. Two sequences can be identified as corresponding if they are identical or if they share substantial identity (*e.g.*, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity) over a length of (*e.g.*, at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 200, at least 300, at least 400, at least 500 or more) units (*e.g.*, nucleotides or amino acids).

[0270] **Culture:** As used herein, the terms “culture” and “cell culture” refer to a cell population (*e.g.*, a eukaryotic cell population) that is suspended in or covered by a medium under conditions suitable to survival and/or growth of the cell population. As will be clear to those of ordinary skill in the art, these terms can also refer to the combination comprising the cell population and the medium.

[0271] **Fragment:** As used herein, the terms “fragment” or “portion” refers to a structure that includes a discrete portion of the whole, but lacks one or more moieties found in the whole structure. In some embodiments, a fragment consists of such a discrete portion. In some embodiments, a fragment consists of or comprises a characteristic structural element or moiety found in the whole. In some embodiments, a nucleotide fragment comprises or consists of at

least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, or more monomeric units (e.g., nucleic acids) as found in the whole nucleotide. In some embodiments, a nucleotide fragment comprises or consists of at least about 5%, 10%, 15%, 20%, 25%, 30%, 25%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more of the monomeric units (e.g., residues) found in the whole nucleotide. The whole material or entity may in some embodiments be referred to as the “parent” of the whole.

[0272] **Gene:** As used herein, the term “gene” refers to a DNA sequence that codes for a product (e.g., an RNA product and/or a polypeptide product). In some embodiments, a gene includes a coding sequence (i.e., a sequence that encodes a particular product). In some embodiments, a gene includes a non-coding sequence. In some particular embodiments, a gene may include both coding (e.g., exonic) and non-coding (e.g., intronic) sequences. In some embodiments, a gene may include one or more regulatory elements that, for example, may control or effect one or more aspects of gene expression (e.g., inducible expression, etc.).

[0273] **Gene therapy:** As used herein, the term “gene therapy” refers to delivery of a payload with a vector, e.g., a recombinant AAV particle. In some embodiments, gene therapy comprises delivery and/or expression of a payload (e.g., a therapeutic product) to treat or prevent a disorder or condition for which such therapy is sought. In some embodiments, gene therapy comprises: insertion, deletion, or editing (e.g., by mutation, by duplication, by demethylation, by methylation, by upregulation, by downregulation, etc.) of specific genomic DNA sequences to treat or prevent a disorder or condition for which such therapy is sought. In some embodiments, the insertion or deletion of genomic DNA sequences occurs in specific cells (e.g., target cells). Target cells may be from a mammal and/or may be cells in a mammalian subject. Mammals include but are not limited to humans, dogs, cats, cows, sheep, pigs, llamas, etc. In some embodiments, heterologous DNA is transferred to target cells. The heterologous DNA may be introduced into the selected target cells in a manner such that the heterologous DNA is expressed and a therapeutic product encoded thereby is produced. In some embodiments, a therapeutic product is a polypeptide encoded by the heterologous DNA. In some embodiments, a therapeutic product is an RNA encoded by the heterologous DNA. In some embodiments, a therapeutic product is a polypeptide and an RNA encoded by the heterologous DNA. Additionally or

alternatively, the heterologous DNA may in some manner mediate expression of DNA that encodes the therapeutic product, or it may encode a product, such as a polypeptide or RNA that in some manner mediates or modulates, directly or indirectly, expression of a therapeutic product. Genetic therapy may also be used to deliver nucleic acid encoding a gene product that replaces a defective gene or supplements a gene product produced by the mammal or the cell in which it is introduced. The heterologous DNA encoding the therapeutic product may be modified prior to introduction into the cells of the afflicted host in order to enhance or otherwise alter the product or expression thereof. Genetic therapy may also involve delivery of an inhibitor or repressor or other modulator of gene expression. Such an inhibitor or repressor or other modulator can be a polypeptide, peptide, or nucleic acid (e.g., DNA or RNA). Gene therapy may include *in vivo* or *ex vivo* techniques. In some embodiments, viral and non-viral based gene transfer methods can be used to introduce a nucleic acid encoding a polypeptide of interest or to introduce a therapeutic nucleic acid into mammalian cells or target tissues. Non-viral vector delivery systems include DNA plasmids, naked nucleic acid, and nucleic acid complexed with a delivery vehicle, such as poloxamers or liposomes. Viral vector delivery systems include DNA and RNA viruses, which have either episomal or integrated genomes after delivery to the cell. For a review of gene therapy procedures, see Anderson WF., (1992) *Science* 256(5058): pp. 808-813; Miller AD., (1992) *Nature* 357(6378): pp. 455-460; Feuerbach FJ. Et al., (1996) *Kidney Int.* 49(6) : pp. 1791-1794 ; Urnov FD. Et al., (2010) *Nat. Rev Genet.* 11(9): pp. 636-646; and Collins M. et al., (2015) *Proc Biol Sci.* 282(1821), each of which is hereby incorporated by reference in its entirety.

[0274] **Host Cell:** As used herein, the term “host cell” refers to a cell into which exogenous DNA (recombinant or otherwise) has been introduced. Persons of skill upon reading this disclosure will understand that such terms refer not only to the particular subject cell, but also to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term “host cell” as used herein. In some embodiments, host cells include prokaryotic and eukaryotic cells selected from any of the Kingdoms of life that are suitable for expressing an exogenous DNA (e.g., a recombinant nucleic acid sequence).

[0275] ***Identity:*** As used herein, the term “identity” refers to the overall relatedness between polymeric molecules, e.g., between nucleic acid molecules (e.g., DNA molecules and/or RNA molecules) and/or between polypeptide molecules. In some embodiments, polymeric molecules are considered to be “substantially identical” to one another if their sequences are at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identical. Calculation of the percent identity of two nucleic acid or polypeptide sequences, for example, can be performed by aligning the two sequences for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second sequences for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In certain embodiments, the length of a sequence aligned for comparison purposes is at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or substantially 100% of the length of a reference sequence. The nucleotides at corresponding positions are then compared. When a position in the first sequence is occupied by the same residue (e.g., nucleotide or amino acid) as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which needs to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. For example, the percent identity between two nucleotide sequences can be determined using the algorithm of Meyers and Miller (CABIOS, 1989, 4: 11-17), which has been incorporated into the ALIGN program (version 2.0). In some exemplary embodiments, nucleic acid sequence comparisons made with the ALIGN program use a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. The percent identity between two nucleotide sequences can, alternatively, be determined using the GAP program in the GCG software package using an NWSgapdna.CMP matrix.

[0276] ***Improve, increase, inhibit, or reduce:*** As used herein the terms “improve”, “increase,” “inhibit,” “reduce,” or grammatical equivalents thereof, indicate values that are relative to a baseline or other reference measurement. In some embodiments, an appropriate reference measurement may be or comprise a measurement in a particular system (e.g., in a single sample, e.g., of a culture medium) under otherwise comparable conditions absent presence

of (e.g., prior to and/or after) a particular agent or treatment, or in presence of an appropriate comparable reference agent. In some embodiments, an appropriate reference measurement may be or comprise a measurement in a comparable system known or expected to respond in a particular way, in presence of the relevant agent or treatment.

[0277] **Medium:** As used herein, the terms “medium,” “culture medium,” and “growth medium” refer to a solution comprising nutrients to nourish cells (e.g., growing cells, e.g., eukaryotic cells). Typically, these solutions provide essential and non-essential amino acids, vitamins, energy sources, lipids, and trace elements required by the cell for survival and/or minimal growth. The solution can also comprise components that enhance survival and/or growth above the minimal rate, including hormones and growth factors. The solution can be formulated to a pH and concentration of one or more salts that are optimal for cellular survival and/or proliferation. For example, the medium can also be a “defined medium” or “chemically defined medium,” e.g., a serum-free medium that contains no proteins, hydrolysates, or components of unknown composition. Defined media are free of animal-derived components and all components have a known chemical structure. One of skill in the art understands a defined medium can comprise recombinant polypeptides, for example, but not limited to, hormones, cytokines, interleukins, and/or other signaling molecules.

[0278] **CNS targeting moiety:** The phrase “CNS targeting moiety” as used herein refers to a single chain antibody agent which is effective in targeting a central nervous system (CNS) cell and/or tissue, e.g., an endothelial cell associated with a blood brain barrier, a cell or tissue that is present in the brain, spinal cord, or CNS system. In some embodiments, a CNS-targeting moiety can target a CNS cell or tissue by: (i) contacting a CNS cell or tissue (e.g., binding to one or more receptors expressed on a CNS cell or tissue); (ii) contacting a cell in contact with a CNS cell or tissue (e.g., binding to one or more receptors expressed on a cell in contact with a CNS cell or tissue); (iii) delivering a payload to a CNS cell or tissue; or (iv) any combination of (i)-(iii). In some embodiments, a CNS cell or tissue comprises: a CNS epithelial cell, an endothelial cell associated with a blood brain barrier, a nerve cell, a CNS connective tissue cell, a stem cell, a progenitor cell, a CNS immune cell, a spinal cord cell, a cell that lines one or more brain ventricles, a nerve support cell, a glial cell, a fat cell, a meninges cell, or a combination thereof. In some embodiments, a CNS tissue comprises a tissue found in a: cortex, thalamus, hypothalamus, striatum, putamen, caudate nucleus, hippocampus, entorhinal cortex, basal

ganglia, deep cerebellar nuclei, or other parts of a brain and/or spinal cord. In some embodiments, a CNS targeting moiety can be conjugated or fused to a payload. In some embodiments, a CNS targeting moiety can be incorporated into a vector, e.g., a viral vector or a non-viral vector. In some embodiments, a CNS targeting moiety can be inserted in an AAV capsid to form a variant AAV capsid protein as disclosed.

[0279] **Nucleic acid:** The term “nucleic acid” includes any nucleotides, analogs thereof, and polymers thereof. The term “polynucleotide” as used herein refer to a polymeric form of nucleotides of any length, either ribonucleotides (RNA) or deoxyribonucleotides (DNA). These terms refer to the primary structure of the molecules and, thus, include double- and single-stranded DNA, and double- and single-stranded RNA. These terms include, as equivalents, analogs of either RNA or DNA made from nucleotide analogs and modified polynucleotides such as, though not limited to, methylated, protected and/or capped nucleotides or polynucleotides. The terms encompass poly- or oligo-ribonucleotides (RNA) and poly- or oligo-deoxyribonucleotides (DNA); RNA or DNA derived from N-glycosides or C-glycosides of nucleobases and/or modified nucleobases; nucleic acids derived from sugars and/or modified sugars; and nucleic acids derived from phosphate bridges and/or modified phosphorus-atom bridges (also referred to herein as “internucleotide linkages”). The term encompasses nucleic acids containing any combinations of nucleobases, modified nucleobases, sugars, modified sugars, phosphate bridges or modified phosphorus atom bridges. Examples include, and are not limited to, nucleic acids containing ribose moieties, the nucleic acids containing deoxy-ribose moieties, nucleic acids containing both ribose and deoxyribose moieties, nucleic acids containing ribose and modified ribose moieties. In some embodiments, the prefix poly- refers to a nucleic acid containing 2 to about 10,000, 2 to about 50,000, or 2 to about 100,000 nucleotide monomer units. In some embodiments, the prefix oligo- refers to a nucleic acid containing 2 to about 200 nucleotide monomer units. In accordance with the methods and compositions described herein, in some embodiments, an RNA comprises a short hairpin RNA (shRNA), small interfering RNA (siRNA), mRNA, snRNA, CRISPR/Cas guide RNA, microRNA (miRNA), and/or a precursor thereof.

[0280] **Payload:** As used herein, the term “payload” refers to a nucleic acid sequence of interest (e.g., comprising a sequence that encodes a target payload, such as a target polypeptide or RNA) that is desired to be introduced into a cell, tissue, organ, organism, and/or system

comprising cells. A target payload can be a heterologous protein with a therapeutic purpose, e.g., an enzyme or antibody. The target payload can be a heterologous nucleic acid with a therapeutic purpose, e.g., an miRNA, siRNA, shRNA, mRNA, snRNA, or CRISPR/Cas guide RNA, or a precursor thereof. One of skill in the art will recognize that the target payload can be selected from any heterologous protein or nucleic acid of interest. As used herein, “encode” or “encodes” means directs the expression of or processed into. For example, as used herein, a nucleic acid encodes a polypeptide sequence if it directs the expression of that polypeptide sequence. As another example, as used herein, a nucleic acid precursor (e.g., a pri-miRNA or pre-miRNA) encodes a further processed version of the nucleic acid (e.g., mature miRNA) if it is processed into the further processed version.

[0281] ***Pharmaceutical composition:*** As used herein, the term “pharmaceutical composition” refers to a composition comprising rAAV particles that is suitable for administration to a human or animal subject. In some embodiments, a pharmaceutical composition comprises an active agent formulated together with one or more pharmaceutically acceptable carriers. In some embodiments, the active agent is present in a unit dose amount appropriate for administration in a therapeutic regimen. In some embodiments, a therapeutic regimen comprises one or more doses administered according to a schedule that has been determined to achieve a desired therapeutic effect when administered to a subject or population in need thereof (e.g., by a statistically significant probability). A pharmaceutical composition may be specially formulated for administration in solid or liquid form. In some embodiments, a pharmaceutical composition is formulated for administration by parenteral administration, such as by subcutaneous, intramuscular, intravenous or epidural injection. In some embodiments, a pharmaceutical composition is formulated as a sterile solution or suspension, e.g., in a sustained-release formulation. Pharmaceutical compositions of the disclosure may be formulated for administration by injection or infusion (e.g., subcutaneous, intramuscular, intravenous or epidural injection or infusion). For example, compositions may be formulated for administration by retinal, subretinal, intravitreal, suprachoroidal, intraspinal intracisternal magna, or intrathecal injection or infusion. In some embodiments, a pharmaceutical composition is intended and suitable for administration to a human subject. In some embodiments, a pharmaceutical composition is substantially free of contaminants (e.g., sterile and substantially pyrogen-free). Formulations of the pharmaceutical compositions may include, but are not limited to,

formulations for oral administration, such as drenches (aqueous or non-aqueous solutions or suspensions), tablets (e.g., targeted for buccal, sublingual, and systemic absorption), boluses, powders, granules, pastes for application to the tongue; topical application, such as a cream, ointment, or a controlled-release patch or spray applied to the skin, lungs, or oral cavity; intravaginally or intrarectally, for example, as a pessary, cream, or foam; sublingually; ocularly; transdermally; or nasally, pulmonary, and to other mucosal surfaces.

[0282] ***Polypeptide:*** The term “polypeptide”, as used herein, generally has its art-recognized meaning of a polymer of at least three amino acids. Those of ordinary skill in the art will appreciate that the term “polypeptide” is intended to be sufficiently general as to encompass not only polypeptides having a complete sequence recited herein, but also to encompass polypeptides that represent functional fragments (e.g., fragments retaining at least one activity) of such complete polypeptides. Moreover, those of ordinary skill in the art understand that protein sequences generally tolerate some substitution without destroying activity. Thus, any polypeptide that retains activity and shares at least about 30-40% overall sequence identity, often greater than about 50%, 60%, 70%, or 80%, and further usually including at least one region of much higher identity, often greater than 90% or even 95%, 96%, 97%, 98%, or 99% in one or more highly conserved regions, usually encompassing at least 3-4 and often up to 20 or more amino acids, with another polypeptide of the same class, is encompassed within the relevant term “polypeptide” as used herein. Polypeptides may contain L-amino acids, D-amino acids, or both and may contain any of a variety of amino acid modifications or analogs known in the art. Useful modifications include, e.g., terminal acetylation, amidation, methylation, etc. In some embodiments, proteins may comprise natural amino acids, non-natural amino acids, synthetic amino acids, and combinations thereof. The term “peptide” is generally used to refer to a polypeptide having a length of less than about 100 amino acids, less than about 50 amino acids, less than 20 amino acids, or less than 10 amino acids.

[0283] ***Recombinant:*** As used herein, the term “recombinant” is intended to refer to polypeptides that are designed, engineered, prepared, expressed, created, manufactured, and/or isolated by recombinant means, such as polypeptides expressed using a recombinant expression vector transfected into a host cell; polypeptides isolated from a recombinant, combinatorial human polypeptide library; polypeptides isolated from an animal (e.g., a mouse, rabbit, sheep, fish, etc.) that is transgenic for or otherwise has been manipulated to express a gene or genes, or

gene components that encode and/or direct expression of the polypeptide or one or more component(s), portion(s), element(s), or domain(s) thereof; and/or polypeptides prepared, expressed, created or isolated by any other means that involves splicing or ligating selected nucleic acid sequence elements to one another, chemically synthesizing selected sequence elements, and/or otherwise generating a nucleic acid that encodes and/or directs expression of the polypeptide or one or more component(s), portion(s), element(s), or domain(s) thereof. In some embodiments, one or more of such selected sequence elements is found in nature. In some embodiments, one or more of such selected sequence elements is designed *in silico*. In some embodiments, one or more such selected sequence elements results from mutagenesis (e.g., *in vivo* or *in vitro*) of a known sequence element, e.g., from a natural or synthetic source such as, for example, in the germline of a source organism of interest (e.g., of a human, a mouse, etc.).

[0284] **Recombinant AAV (rAAV) particle:** A “recombinant AAV particle”, or “rAAV particle,” as used herein, refers to a transduction-competent, replication-defective viral particle comprising an AAV protein shell (e.g., comprising a capsid as described herein) encapsulating a payload that is flanked on both sides by ITRs. An AAV particle is produced in a suitable host cell (e.g., a HEK293 cell). For example, the host cell is transfected with at least one vector encoding one or more helper polypeptides and nucleic acids (e.g., Ad2 helper polypeptides and nucleic acids), at least one Rep polypeptide, at least one Cap polypeptide, and at least one payload (e.g., for polypeptide expression or a therapeutic nucleic acid), such that the host cell is capable of producing the Rep and Cap polypeptides necessary for packing the rAAV particle. rAAV particles may be used for subsequent gene delivery.

[0285] **Rep polypeptide:** The term “Rep polypeptide”, as used herein, refers to the AAV non-structural proteins that mediate AAV replication for the production of AAV particles. The AAV replication genes and proteins have been described in, e.g., Knipe 2001, which is hereby incorporated by reference in its entirety.

[0286] **Seeding:** The term “seeding” as used herein refers to the process of providing a cell culture to a vessel (e.g., a bioreactor or culture flask). For example, the process of providing a cell culture may include propagation of the cells in another bioreactor or vessel before providing to the bioreactor or other vessel. The cells have been frozen and thawed immediately prior to providing them to the bioreactor or vessel. The term “seeding” refers to providing any number of cells, including a single cell.

[0287] ***Single chain antibody agent:*** The term “single chain antibody agent” as used herein refers to a polypeptide that comprises at least one variable domain that includes at least two complementarity determining regions (CDRs) and structural elements recognized by those skilled in the art as an immunoglobulin variable domain. In some embodiments, a single chain antibody agent includes three CDRs. In some embodiments, a single chain antibody agent comprises a polypeptide whose amino acid sequence includes at least one CDR (e.g., at least one heavy chain CDR and/or at least one light chain CDR) that is substantially identical to one found in a reference antibody. In some embodiments an included CDR is substantially identical to a reference CDR in that it is either identical in sequence or contains between 1-5 amino acid substitutions as compared with the reference CDR. In some embodiments an included CDR is substantially identical to a reference CDR in that it shows at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with the reference CDR. In some embodiments, a single chain antibody agent is a single domain antibody. In some embodiments, a single domain antibody is a camelid single domain antibody derived from a camelid heavy-chain antibody (HcAb). In some embodiments, a camelid single domain antibody is also referred to as a VH. In some embodiments, a single domain antibody is a shark single domain antibody derived from a shark heavy-chain antibody (HcAb). In some embodiments, a shark single domain antibody is also referred to as a VNAR. In some embodiments, a single chain antibody agent is a single chain fragment variable (scFv) which is a single protein chain in which the V<sub>H</sub> and V<sub>L</sub> regions pair to form monovalent molecules see e.g., Bird et al. (1988) Science 242:423-426; and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883). In some embodiments, a single chain antibody agent may include one or more sequence elements that are humanized, primatized, chimeric, etc, as is known in the art. In some embodiments, an antibody may lack a covalent modification (e.g., attachment of a glycan) that it would have if produced naturally. In some embodiments, an antibody may contain a covalent modification (e.g., attachment of a glycan, a payload [e.g., a detectable moiety, a therapeutic moiety, a catalytic moiety, etc], or other pendant group [e.g., poly-ethylene glycol, etc.]).

[0288] ***Specifically binds:*** The term “specifically binds” as used herein refers to an antibody that interacts more frequently, more rapidly, with greater duration, with greater affinity, or with some combination of the above to a particular antigen, epitope, protein, or target molecule than with alternative substances. An antibody that specifically binds an antigen can be

identified, for example, by immunoassays, ELISAs, surface plasmon resonance (SPR), or other techniques known to those of skill in the art. In some embodiments, an antibody that specifically binds an antigen (e.g., human TfR1) can bind related antigens (e.g., cyno TfR1). An antibody that specifically binds an antigen can bind the target antigen at a higher affinity than its affinity for a different antigen. The different antigen can be a related antigen. In some embodiments, an antibody that specifically binds an antigen can bind the target antigen with an affinity that is at least 20 times greater, at least 30 times greater, at least 40 times greater, at least 50 times greater, at least 60 times greater, at least 70 times greater, at least 80 times greater, at least 90 times greater, or at least 100 times greater, than its affinity for a different antigen. In some embodiments, an antibody that specifically binds a particular antigen binds a different antigen at such a low affinity that binding cannot be detected using an assay described herein or otherwise known in the art. In some embodiments, affinity is measured using SPR technology in a Biacore system as described herein or as known to those of skill in the art.

[0289] **Subject:** As used herein, the term “subject” refers to an organism, for example, a mammal (e.g., a human, a non-human mammal, a non-human primate, a primate, a laboratory animal, a mouse, a rat, a hamster, a gerbil, a cat, a dog). In some embodiments, a human subject is an adult, adolescent, or pediatric subject. In some embodiments, a subject is suffering from a disease, disorder or condition, e.g., a disease, disorder or condition that can be treated as provided herein, e.g., a neurological disease or disorder or a cancer or a tumor listed herein. In some embodiments, a subject is susceptible to a disease, disorder, or condition; in some embodiments, a susceptible subject is predisposed to and/or shows an increased risk (as compared to the average risk observed in a reference subject or population) of developing the disease, disorder or condition. In some embodiments, a subject displays one or more symptoms of a disease, disorder or condition. In some embodiments, a subject does not display a particular symptom (e.g., clinical manifestation of disease) or characteristic of a disease, disorder, or condition. In some embodiments, a subject does not display any symptom or characteristic of a disease, disorder, or condition. In some embodiments, a subject is a patient. In some embodiments, a subject is an individual to whom diagnosis and/or therapy is and/or has been administered.

[0290] **Titer:** As used herein, the term “titer” refers to the quantity of virus in a given volume. Titer, for example, can be expressed as viral genome copies (vg) per given volume or

plaque forming units (pfu) per given volume. In some embodiments, titer can be expressed as number of capsids per given volume.

[0291] ***Transfection:*** As used herein, the term “transfection” refers to the introduction of nucleic acid molecules, such as DNA or RNA (e.g., mRNA) molecules, into cells, such as eukaryotic cells (e.g., mammalian cells). For example, transfection can include vector-based transfection, viral-based transfection, electroporation, lipofection (e.g., with cationic lipids and/or liposomes), calcium phosphate precipitation, nanoparticle-based transfection, and/or transfection based on cationic polymers (e.g., DEAE-dextran or polyethylenimine). In some embodiments, viral-based transfection is also referred to herein as transduction.

[0292] ***Treating:*** As used herein, the term “treating” refers to providing treatment, e.g., providing any type of medical or surgical management of a subject. The treatment can be provided in order to reverse, alleviate, inhibit the progression of, prevent or reduce the likelihood of a disease, disorder, or condition, or in order to reverse, alleviate, inhibit or prevent the progression of, prevent or reduce the likelihood of one or more symptoms or manifestations of a disease, disorder or condition. “Prevent” refers to causing a disease, disorder, condition, or symptom or manifestation of such not to occur for at least a period of time in at least some individuals. Treating can include administering an agent to the subject following the development of one or more symptoms or manifestations indicative of a condition, disease, or disorder, e.g., in order to reverse, alleviate, reduce the severity of, and/or inhibit or prevent the progression of the condition and/or to reverse, alleviate, reduce the severity of, and/or inhibit or one or more symptoms or manifestations of the condition. A composition comprising rAAV particles of the disclosure can be administered to a subject who has developed a disorder or is at increased risk of developing such a disorder relative to a member of the general population. A composition of the disclosure can be administered prophylactically or before development of any symptom or manifestation of the condition. Typically, in this case, the subject will be at risk of developing the condition.

[0293] ***Variant:*** As used herein in the context of molecules, e.g., nucleic acids or polypeptides, the term “variant” refers to a molecule that shows significant structural identity with a reference molecule but differs structurally from the reference molecule, e.g., in the presence or absence or in the level of one or more chemical moieties as compared to the reference entity. In some embodiments, a variant also differs functionally from its reference

molecule. In general, whether a particular molecule is properly considered to be a “variant” of a reference molecule is based on its degree of structural identity with the reference molecule. As will be appreciated by those skilled in the art, any biological or chemical reference molecule has certain characteristic structural elements. A variant, by definition, is a distinct molecule that shares one or more such characteristic structural elements but differs in at least one aspect from the reference molecule. To give but a few examples, a polypeptide may have a characteristic sequence element that comprises a plurality of amino acids having designated positions relative to one another in linear or three-dimensional space and/or contributing to a particular structural motif and/or biological function; a nucleic acid may have a characteristic sequence element that comprises a plurality of nucleotide residues having designated positions relative to one another in linear or three-dimensional space. In some embodiments, a variant polypeptide or nucleic acid may differ from a reference polypeptide or nucleic acid as a result of one or more differences in amino acid or nucleotide sequence and/or one or more differences in chemical moieties (e.g., carbohydrates, lipids, phosphate groups) that are covalently components of the polypeptide or nucleic acid (e.g., that are attached to the polypeptide or nucleic acid backbone). In some embodiments, a variant polypeptide or nucleic acid shows an overall sequence identity with a reference polypeptide or nucleic acid that is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99%. In some embodiments, a variant polypeptide or nucleic acid does not share at least one characteristic sequence element with a reference polypeptide or nucleic acid. In some embodiments, a reference polypeptide or nucleic acid has one or more biological activities. In some embodiments, a variant polypeptide or nucleic acid shares one or more of the biological activities of the reference polypeptide or nucleic acid. In some embodiments, a variant polypeptide or nucleic acid lacks one or more of the biological activities of the reference polypeptide or nucleic acid. In some embodiments, a variant polypeptide or nucleic acid shows a reduced level of one or more biological activities as compared to the reference polypeptide or nucleic acid. In some embodiments, a polypeptide or nucleic acid of interest is considered to be a “variant” of a reference polypeptide or nucleic acid if it has an amino acid or nucleotide sequence that is identical to that of the reference but for a small number of sequence alterations at particular positions. Typically, fewer than about 20%, about 15%, about 10%, about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, or about 2% of the residues in a variant are substituted, inserted, or deleted, as compared to

the reference. In some embodiments, a variant polypeptide or nucleic acid comprises about 10, about 9, about 8, about 7, about 6, about 5, about 4, about 3, about 2, or about 1 substituted residues as compared to a reference. Often, a variant polypeptide or nucleic acid comprises a very small number (e.g., fewer than about 5, about 4, about 3, about 2, or about 1) number of substituted, inserted, or deleted, functional residues (i.e., residues that participate in a particular biological activity) relative to the reference. In some embodiments, a variant polypeptide or nucleic acid comprises not more than about 5, about 4, about 3, about 2, or about 1 addition or deletion, and, in some embodiments, comprises no additions or deletions, as compared to the reference. In some embodiments, a variant polypeptide or nucleic acid comprises fewer than about 25, about 20, about 19, about 18, about 17, about 16, about 15, about 14, about 13, about 10, about 9, about 8, about 7, about 6, and commonly fewer than about 5, about 4, about 3, or about 2 additions or deletions as compared to the reference. In some embodiments, a reference polypeptide or nucleic acid is one found in nature.

**[0294]** *Vector:* As used herein, the term “vector” refers to a molecule comprising a nucleic acid molecule, where the vector is capable of transporting the nucleic acid molecule into a cell. By way of non-limiting example, one type of vector is a “plasmid,” which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be packaged into a viral capsid and can be transferred into another cell and/or organism. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as “expression vectors.”

**[0295]** Standard techniques may be used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer’s specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures may be generally performed according to conventional methods known in the art and as described in various general and more specific references that are cited

and discussed throughout the present specification. See, e.g., Sambrook J. et al., (1989) Molecular Cloning. A Laboratory Manual, *Cold Spring Harbor Laboratory Press* 2<sup>nd</sup> edition, which is incorporated herein by reference in its entirety.

[0296] **VP:** As used herein, the term “VP” refers to an AAV VP1 capsid protein, an AAV VP2 capsid protein, an AAV VP3 capsid protein, or variants or fragments or combinations of any of the foregoing. The term “capsid protein” is used interchangeably herein with VP. The numbering used herein in describing exemplary locations of single chain antibody agent insertions in VP1, VP2 or VP3 are used relative to AAV VP1 numbering. For example VP1, VP2 and VP3 of the AAV9 capsid protein correspond to amino acids 1 to 736 of VP1, amino acids 138 to 736 of VP1 and amino acids 203 to 736 of VP1, respectively. Thus, reference to a single chain antibody agent insertion between positions 588 and 589 in an AAV capsid variant refers to positions 588 and 589 in VP1, VP2 or VP3 relative to VP1 numbering. Those with knowledge in the pertinent field would be able to readily ascertain the corresponding position in VP2 and VP3, e.g., by comparing the sequences of VP1, VP2 and VP3 of the parental AAV capsid proteins using methods known in the field such as sequence alignment. In some embodiments, a VP capsid protein is a VP1 capsid protein. In some embodiments, a VP capsid protein is a VP2 capsid protein. In some embodiments, a VP capsid protein is a VP3 capsid protein. In some embodiments, a VP protein comprises a single chain antibody agent insertion disclosed herein.

[0297] **Variant AAV capsid protein:** As used herein, the term “variant AAV capsid protein” refers to a VP capsid protein (e.g., a VP1, VP2, or VP3) comprising an insertion of a single chain antibody agent relative to a corresponding parental AAV capsid protein (e.g., a parental VP1, VP2, or VP3).

## DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

[0298] The present disclosure provides, *inter alia*, improved recombinant adeno-associated virus (rAAV) particles that can be used for targeting cells or tissue, e.g., CNS cells and/or tissue. Safe and efficient therapeutic payload delivery to a CNS cell and/or tissue remains a major challenge in gene therapy. Recombinant adeno-associated viruses (rAAVs) have emerged as some of the most promising vectors for *in vivo* gene therapy, and are currently under clinical evaluation for a number of disorders including CNS disorders. However, naturally

occurring AAV capsids sub-optimally target CNS cells or tissue, and require extremely high doses to achieve minimum effective transgene expression. This poses daunting manufacturing challenges as well as safety concerns.

**[0299]** The present disclosure is based, in part, on the discovery that AAV tropism to certain cells and/or tissue can be obtained by inserting a single chain antibody agent into an AAV capsid to generate a variant AAV capsid protein and direct said variant AAV capsid protein to certain cells and/or tissue. Without wishing to be bound by any particular theory, in some embodiments, AAV tropism to certain cells and/or tissue can be obtained with a variant AAV capsid protein which binds to one or more cell surface receptors such as hTfR1 or a variant or a fragment thereof.

**[0300]** Further without wishing to be bound by theory, binding of a variant AAV capsid protein (e.g., in an AAV particle) to hTfR1 or a variant or a fragment thereof, delivers a variant AAV capsid protein to said cell and allows for a variant AAV capsid protein (e.g., in an AAV particle) to transcytose said cell (e.g., to be transported from one surface of said cell to another surface of said cell). In some embodiments, transcytosis of a variant AAV capsid protein (e.g., in an AAV particle) across a cell by binding to a hTfR1 delivers a variant AAV capsid protein (e.g., in an AAV particle) to an organ, to an intersitial space, and/or to an interstitial fluid allowing for transduction of a cell and/or tissue. In some embodiments, delivery of a variant AAV capsid (e.g., in an AAV particle) protein to an organ, to an intersitial space, and/or to an interstitial fluid results in transduction of a cell or tissue.

**[0301]** For example, when a variant AAV capsid protein (e.g., in an AAV particle) is directed to an endothelial cell which expresses a hTfR1 or a variant or a fragment thereof, said variant AAV capsid protein (e.g., in an AAV particle) can bind to and transcytose the endothelial cell and/or transduce the endothelial cell. In some embodiments, a variant AAV capsid protein (e.g., in an AAV particle) that transcytoses an endothelial cell can be delivered to an organ, an intersitial space, and/or an interstitial fluid.

**[0302]** As another example example, when a variant AAV capsid protein (e.g., in an AAV particle) is directed to an endothelial cell which is part of, or forms a blood brain barrier (e.g., by binding to a hTfR1 or a fragment or variant thereof), said variant AAV capsid protein (e.g., in an AAV particle) can bind to and transcytose the endothelial cell and be delivered to the brain. In some embodiments, a variant AAV capsid protein (e.g., in an AAV particle) delivered

to a brain transduces one or more cells in a brain. In some embodiments, a variant AAV capsid protein (e.g., in an AAV particle) directed to an endothelial cell transduces an endothelial cell. In some embodiments, transduction of an endothelial cell by a variant AAV capsid protein (e.g., in an AAV particle) results in expression of a payload. In some embodiments, an AAV particle comprises a variant AAV capsid and a heterologous nucleic acid comprising a nucleotide sequence encoding a payload, e.g., as described herein.

[0303] Additionally, this disclosure provides the discovery that AAV CNS cell and/or tissue tropism can be obtained by inserting a single chain antibody agent (e.g., a single domain antibody (e.g., a VH<sub>H</sub> or a single chain Fv) into an AAV capsid to direct said AAV capsid to a CNS cell and/or tissue. In some embodiments, a variant AAV capsid protein can be directed to a CNS cell and/or tissue (e.g., an endothelial cell associated with a blood brain barrier) by binding to one or more receptors expressed on endothelial cells).

[0304] Accordingly, disclosed herein are technologies for identifying variant AAV capsid proteins with CNS cell and/or tissue tropism by, e.g., binding to hTfR1 or variants or fragments thereof. Also disclosed herein are novel variant AAV capsid proteins that have enhanced CNS cell and/or tissue tropism for example by binding to hTfR1 or variants or fragments thereof.

[0305] In some embodiments, rAAV particles comprising a variant AAV capsid having a single chain antibody agent insertion disclosed herein bind to and/or recognize a target on a CNS cell and/or tissue. Without wishing to be bound by any particular theory, in some embodiments, rAAV particles comprising a variant AAV capsid comprising a single chain antibody agent insertion disclosed herein can enhance vector attachment, internalization, transcytosis, and/or payload expression in a CNS cell and/or tissue.

[0306] This disclosure further provides the surprising insight that binding affinity of a single chain antibody agent described herein (e.g., in an AAV particle comprising a single chain antibody agent inserted in a variable region (VR) of a parental AAV capsid protein) to TfR1 (e.g., human, cyno and/or mouse TfR1) is inversely correlated with the transduction level and/or efficiency of one or more CNS cells and/or tissue by an AAV particle comprising a single chain antibody agent inserted in VR of a parental AAV capsid protein. For example, as shown in **Example 1** and **FIG. 26**, exemplary single chain antibody agents that have higher binding affinity (e.g., lower K<sub>d</sub>) to human TfR1 (e.g., fused to an Fc domain or in a VR of a parental

AAV capsid protein) showed generally lower transduction of neurons as observed in animals administered an AAV particle comprising a single chain antibody agent inserted in a VR of a parental AAV capsid protein. Without wishing to be bound by any particular theory, it is believed that inserting a single domain antibody agent in a VR of a parental AAV capsid protein can alter the ability of the single domain antibody agent to recognize and/or bind to TfR1, e.g., human, cyno and/or mouse TfR1. Accordingly, the present disclosure provides technologies and methods of identifying single chain antibody agents that bind to TfR1 and are useful for insertion in a variable region of a parental AAV capsid protein, e.g., to transduce CNS cells and/or tissue. Also provided herein are exemplary single chain antibody agents that bind to TfR1 and can be inserted in a variable region of a parental AAV capsid protein, e.g., to transduce CNS cells and/or tissue.

### **Transferrin receptor (hTfR1) binders**

**[0307]** Transferrin receptor (TfR), also known as CD71, is a transmembrane glycoprotein expressed in various sites of the human body at differing levels, whose function is to mediate cellular uptake of iron from a plasma glycoprotein, transferrin. Iron uptake from transferrin involves the binding of transferrin to the transferrin receptor, internalization of transferrin within an endocytic vesicle by receptor-mediated endocytosis and the release of iron from the protein by a decrease in endosomal pH. Ponka P, Lok CN.. Int J Biochem Cell Biol. 1999 Oct;31(10):1111-37 and Xiaopeng Mo, in Brain Targeted Drug Delivery System, 2019. Apotransferrin (i.e., non-iron conjugate) binds to TfR when bound to two Fe 3+ ions to form holotransferrin (i.e., iron conjugate). The complex of TfR and holotransferrin is translocated into the cell by receptor-mediated endocytosis. TfR and transferrin dissociate in an endosomal environment, and transferrin moves into the cell while TfR is recycled to the cell membrane. Transferrin is thought to translocate into cells by binding to TfR and dissociation from TfR.

**[0308]** In humans and cynomolgus monkeys, two transferrin receptors, TfR1 and TfR2 have been characterized. TfR1 is a high affinity ubiquitously expressed receptor while expression of TfR2 is restricted to certain cell types and is unaffected by intracellular iron concentrations. TfR2 binds to transferrin with a 25-30 fold lower affinity than TfR1. The single chain antibody agents disclosed herein specifically bind to TfR1 (e.g., human and/or cyno TfR1).

[0309] Human TfR1 is (hTfR1) is a homodimeric type II transmembrane protein composed of a cytoplasmic domain, a single-pass transmembrane region, and an extracellular domain. See e.g., Montemiglio, L.C., Testi, C., Ceci, P. et al. *Cryo-EM structure of the human ferritin-transferrin receptor 1 complex*. Nat Commun 10, 1121 (2019), the entire contents of which are hereby incorporated by reference. Each monomer of the extracellular domain comprises: (1) a protease-like domain which can be in contact with a cell membrane, (2) a helical domain comprising dimer contact regions, and (3) an apical domain (e.g., as described in FIG. 1A in Montemiglio 2019).

[0310] The sequences for human TfR1, cyno TfR1, and mouse TfR1 are as follows:

**Human TfR1 (UniProt No. P02786.2; SEQ ID NO:2016)**

```
MMDQARSAFSNLFGGEPLSYTRFLARQVDGDNSHVEMKLA VDEEENADNNTKANVTKP KRC
SGSICYGTIAVIVFFLIGFMIGYLGYCKGVEPKTECERLAGTESPVREEPGEDFPAARRLYWDDLK
RKLSEKLDSTDFTGTLKLLNENSYVPREAGSQKDENLALYVENQFREFKLSKVWRDQHFVKIQV
KDSAQNSVIIVDKNGRLVYL VENPGGYVAYS KAATVTGKLVHANFGTKKFEDLYTPVNGSIVI
VRAGKITFAEKVANAESLNAIGVLIYMDQT KFPIVNAEL SFFGH AHLGTGDPYTPGFP SFNHTQFP
PSRSSGLPNIPVQTISRAAAEKLFGNMEGDCPSDWKT DSCRMVTSE SKNVKLT VSNVLKEIKILN
IFGV IKGFV EPDHYVVVG AQRDAWGP GAAKSGVGTALLKLAQM FSDMV LKDGFQPSRSIIFAS
WSAGDFGSVGATEWLEG YLSSLHLKAFTYINLDKAVL GTSNFKVSASPLL YTLIEKTMQNVKHP
VTGQFLYQDSNWASKVEK LTL DNAAFPFLAYSGIPAVSFCFCEDTDYPYL GTTMDTYKELIERIP
ELNKVARAAA EVAGQFVIK LTHDVELNLDYERYNSQLLSFVRDLNQYRADIKEMGLSLQWLYS
ARGDFFRATSRLTTDFGNAEKDRFVMKKLNDRVMRVEYHFLSPYVSPKESPFRHVFWGSGSH
LPALLENLKLRKQNN GA FNETLFRNQLALATWTI QGAANALSGDVWDIDNEF
```

**Cyno TfR1 (UniProt No. G8F602; SEQ ID NO:2017)**

```
MMDQARSAFSNLFGGEPLSYTRFLARQVDGDNSHVEMKLA VDEEENADNNTKANGTKPKRC
GGNICYGTIAVIIFFLIGFMIGYLGYCKGVEPKTECERLAGTESPAREEPEEDFPAAPR LYWDDLK
RKLSEKLDTTDFTSTIKLLNENLYVPREAGSQKDENLALYIENQFREFKLSKVWRDQHFVKIQV
DSAQNSVIIVDKNGGLVYL VENPGGYVAYS KAATVTGKLVHANFGTKKFEDLDSPVNGSIVIV
RAGKITFAEKVANAESLNAIGVLIYMDQT KFPIVKADLSFFGH AHLGTGDPYTPGFP SFNHTQFPP
SQSSGLPNIPVQTISRAAAEKLFGNMEGDCPSDWKT DSCRMVTSENKS VKLT VSNVLKETKILN
IFGV IKGFV EPDHYVVVG AQRDAWGP GAAKSSVGTALLKLAQM FSDMV LKDGFQPSRSIIFAS
WSAGDFGSVGATEWLEG YLSSLHLKAFTYINLDKAVL GTSNFKVSASPLL YTLIEKTMQDVKHP
VTGRSLYQDSNWASKVEK LTL DNAAFPFLAYSGIPAVSFCFCEDTDYPYL GTTMDTYKELVERIP
ELNKVARAAA EVAGQFVIK LTHDTEL NLDYERYNSQLLFLRDLNQYRADVKEMGLSLQWLYS
ARGDFFRATSRLTTDFRNAEKDRFVMKKLNDRVMRVEYYFLSPYVSPKESPFRHVFWGSGSH
TLSALLESLKLRQNN SAFNETLFRNQLALATWTI QGAANALSGDVWDIDNEF
```

**Mouse TfR1 (Genbank No. NP\_035768.1; SEQ ID NO: 2018)**

```
MMDQARSAFSNLFGGEPLSYTRFLARQVDGDNSHVEMKLA ADEEENADNNMKASVR
KPKRFNGRLCFAAIALVIFFLIGFMMSGYLG YCKRVEQKEECVKLAETEETDKSETMETED
VPTSSRLYWADLKTLSEKLN SIEFADTIKQLSQNTYTPREAGSQKDESLAYYIENQFHEF
KFSK VWRDEHYVKIQVKSSIGQNMVTIVQSNGNLDPVESPEGYVAFSKPTEVSGKLVHA
```

NFGTKKDFEELSYSVNGSLVIVRAGEITFAEKVANAQSFNAIGVLIYMDKNKFPVVEAD  
 LALFGHAHLGTGDPYTPGFPFSNHTQFPPSQSSGLPNIPVQTISRAAAEKLFGKMEGSCPA  
 RWNIDSSCKLELSQNQNVKLIVKNVLKERRILNIFGVIKGYEEPDRYVVVGAQRDALGA  
 GVAAKSSVGTGLLLKLAQVFSDMISKDGFRPSRSIIFASWTAGDFGAVGATEWLEGYS  
 SLHLKAFTYINLDKVVLGTSNFKVSASPLLYTLMGKIMQDVKHPVDGKSLYRDSNWISK  
 VEKLSFDNAAYPFLAYS GIPAVSFCFCEDADYPYLGTRLDTYEALTQKVPQLNQMVRTA  
 AEVAGQLIILTHDVELNDYEMYNSKLLSFMKDLNQFKTDIRDMLGLSLQWLYSARGD  
 YFRATSRLTTDFHNAEKTNRFVMREINDRIMKVEYHFLSPYVSPRESPFRHIFWGSGSHT  
 LSALVENLKLQRQKNITAFNETLFRNQLALATWTIQGVANALSGDIWNIDNEF

**[0314]** In some embodiments, an rAAV particle comprises a variant AAV capsid protein comprising a single chain antibody agent that binds to a transferrin receptor (TfR1) or a variant or a fragment thereof, wherein the single chain antibody agent is inserted in a variable region (VR) of a parental AAV capsid protein. In some embodiments, a single chain antibody agent is or comprises a single domain antibody (e.g., a VHH).

**[0315]** In some embodiments, a rAAV particle disclosed herein comprises: (1) a variant AAV (e.g., AAV9) capsid protein comprising a single chain antibody agent disclosed herein; and (2) one or more sequences of a VP (e.g., VP1, VP2, and/or VP3) of an AAV9 capsid protein. In some embodiments, a single chain antibody agent inserted in a variant AAV capsid protein disclosed herein comprises a single chain antibody agent sequence provided in **Table 1**.

**Table 1:** Sequence for exemplary single chain antibody agents

Clone	Feature	SEQ ID NO	Sequence
A	VHH	1	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQ APGKEREFAWVGDAAGNTAYADSVRGRFTISRDNAKNT AYLQMNSLKPEDTGVYYCKSDQSVWGQGTLVTVSS
	CDR1	2	GSIPPISVMY
	CDR2	3	WVGDAGNTAYADSVRG
	CDR3	4	KSDQSV
B	VHH	5	QVQLVESGGGLVQAGGSMRLSCLNSGRPFSNYAMGWF RQAPGKEREFAAISRTGGSSNYANSVKGRFTITKSDAK TTVYLMQNSLKPEDTAVYYCAAEETFGITWYGSHEEDFR RSWGQGTLVTVSS
	CDR1	6	GRPFNSNYAMG
	CDR2	7	AISRTGGSSNYANSVKKG
	CDR3	8	AAEETFGITWYGSHEEDFRS
C	VHH	9	DVQLQESGGGLVQPGGSLRLSCVASGSITPASVMYWYR QAPGKEREFAWVSSHENTAYADSVRGRFTISRDDAKN TAYLQMNSLKPEDTGVYYCKSDQSPWGQGTQTVSS

Clone	Feature	SEQ ID NO	Sequence
	CDR1	10	GSITPASVMY
	CDR2	11	WVSSHENTAYADSVRG
	CDR3	12	KSDQSP
D	VHH	13	DVQLQESGGPVQPGGSLRLSCVASGSITPINVMYWYRQ APGKGREFVAWVGSAAGNTAYADSVRGRFTVSRDDAKN TAYLQMNSLKSEDTGVYFCRTDQSPWGQGTQTVSS
	CDR1	14	GSITPINVMY
	CDR2	15	WVGSAGNTAYADSVRG
	CDR3	16	RTDQSP
E	VHH	17	DVQLQESGGPVQPGGSLRLSCVASGSITPINVMYWYRQ APGKGREFVAWVGSHGNTAYADSVRGRFTVSRDNVGN TAYLQMDSLKSEDTGVYYCKTDQSAWGQGTQTVSS
	CDR1	18	GSITPINVMY
	CDR2	19	WVGSHGNTAYADSVRG
	CDR3	20	KTDQSA
F	VHH	21	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQ APGKEREFAWVGsAGNTAYADSVRGRFTISRDNAKNT AYLQMNSLKPEDTGYYYCKSDQSVWGQGTLVTVSS
	CDR1	22	GSIPPISVMY
	CDR2	23	WVGSAGNTAYADSVRG
	CDR3	24	KSDQSV
G	VHH	25	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQ APGKEREFAWVGdGNTAYADSVRGRFTISRDNAKNT AYLQMNSLKPEDTGYYYCKSDQSVWGQGTLVTVSS
	CDR1	26	GSIPPISVMY
	CDR2	27	WVGDGGBTAYADSVRG
	CDR3	28	KSDQSV
H	VHH	29	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQ APGKEREFAWVGDAAGNTAYADSVRGRFTISRDNAKNT AYLQMNSLKPEDTGYYYCKSDQSSWGQGTLVTVSS
	CDR1	30	GSIPPISVMY
	CDR2	31	WVGDAAGNTAYADSVRG
	CDR3	32	KSDQSS
I	VHH	33	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQ APGKEREFAWVGDAAGNTAYADSVRGRFTISRDNAKNT AYLQMNSLKPEDTGYYYCKSDQSAWGQGTLVTVSS
	CDR1	34	GSIPPISVMY
	CDR2	35	WVGDAAGNTAYADSVRG
	CDR3	36	KSDQSA
J	VHH	37	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQ APGKEREFAWVGDAAGNTAYADSVRGRFTISRDNAKNT AYLQMNSLKPEDTGYYYCKSDQSPWGQGTLVTVSS

Clone	Feature	SEQ ID NO	Sequence
	CDR1	38	GSIPPISVMY
	CDR2	39	WVGDAGNTAYADSVRG
	CDR3	40	KSDQSP
K	VHH	41	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQ APGKEREFAWVGSAGNTAYADSVRGRFTISRDNAKNT AYLQMNSLKPEDTGVYYCKSDQSAWGQGTLVTVSS
	CDR1	42	GSIPPISVMY
	CDR2	43	WVGSAGNTAYADSVRG
	CDR3	44	KSDQSA
	VHH	45	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQ APGKEREFAWVGDAGNTAYADSVRGRFTISRDNAKNT AYLQMNSLKPEDTGVYYCKSDSSVWGQGTLVTVSS
L	CDR1	46	GSIPPISVMY
	CDR2	47	WVGDAGNTAYADSVRG
	CDR3	48	KSDSSV
M	VHH	49	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQ APGKEREFAWVGDAGNTAYADSVRGRFTISRDNAKNT AYLQMNSLKPEDTGVYYCKSDGSVWGQGTLVTVSS
	CDR1	50	GSIPPISVMY
	CDR2	51	WVGDAGNTAYADSVRG
	CDR3	52	KSDGSV
	VHH	158	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQ APGKEREFAWVGDAGNTAYADSVRGRFTISRDNAKNT AYLQMNSLKPEDTGVYYCKSDQSVWGCGTLVTVSS
N	CDR1	2	GSIPPISVMY
	CDR2	3	WVGDAGNTAYADSVRG
	CDR3	4	KSDQSV
	VHH	159	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQ APGKEREFAWVGDAGNTAYADSVRGRFTISRDNAKNT AYLQMNSLKPEDTGVYYCKSDQSVWGCGTLVTVPP
O	CDR1	2	GSIPPISVMY
	CDR2	3	WVGDAGNTAYADSVRG
	CDR3	4	KSDQSV
	VHH	160	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQ APGKEREFAWVGDAGNTAYADSVRGRFTISRDNAKNT AYLQMNSLKPEDTGVYYCKSDQSVWGQGTLVTVPP
P	CDR1	2	GSIPPISVMY
	CDR2	3	WVGDAGNTAYADSVRG
	CDR3	4	KSDQSV
	VHH	116	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQ APGKEREFAWVGSGGNTAYADSVRGRFTISRDNAKNT AYLQMNSLKPEDTGVYYCKSDQSVWGQGTLVTVSS

Clone	Feature	SEQ ID NO	Sequence
	CDR1	2	GSIPPISVMY
	CDR2	117	WVGSGGNTAYADSVRG
	CDR3	4	KSDQSV
R	VHH	118	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQ APGKEREFAWVGAAGNTAYADSVRGRFTISRDNAKNT AYLQMNSLKPEDTGVYYCKSDQSVWGQGTLTVSS
	CDR1	2	GSIPPISVMY
	CDR2	119	WVGAAGNTAYADSVRG
	CDR3	4	KSDQSV
	VHH	120	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQ APGKEREFAWVGSAGNTAYADSVRGRFTISRDNAKNT AYLQMNSLKPEDTGVYYCKSDGSVWGQGTLTVSS
S	CDR1	2	GSIPPISVMY
	CDR2	15	WVGSAGNTAYADSVRG
	CDR3	52	KSDGSV
T	VHH	121	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQ APGKEREFAWVGAAGNTAYADSVRGRFTISRDNAKNT AYLQMNSLKPEDTGVYYCKSDGSVWGQGTLTVSS
	CDR1	2	GSIPPISVMY
	CDR2	119	WVGAAGNTAYADSVRG
	CDR3	52	KSDGSV
	VHH	122	QVQLVESGGGLVQAGGSLRLSCANSRPFNSNYAMGWFR QAPGKEREFAAISRTGGSSNYANSVKGRFTITKSDAKTT VYLQMNSLKPEDTAVYYCAAEETFGITWYGSHEEDFRS WGQGTLTVSS
U	CDR1	6	GRPFNSNYAMG
	CDR2	7	AISRTGGSSNYANSVKKG
	CDR3	8	AAEETFGITWYGSHEEDFRS
V	VHH	123	QVQLVESGGGLVQAGGSMRLSCLNSRPFNSNYAMCWFR QAPGKEREFACISRTGGSSNYANSVKGRFTITKSDAKTT VYLQMNSLKPEDTAVYYCAAEETFGITWYGSHEEDFRS WGQGTLTVSS
	CDR1	124	GRPFNSNYAMC
	CDR2	125	CISRTGGSSNYANSVKKG
	CDR3	8	AAEETFGITWYGSHEEDFRS
	VHH	126	QVQLVESGGGLVQAGGSMRLSCLNSRPFNSNCAMGWFR QAPGKEREFAAISRTGGSSNYANSVKGRFTITKSDAKTT VYLQMNSLKPEDTAVYYCACEETFGITWYGSHEEDFRS WGQGTLTVSS
W	CDR1	127	GRPFNSNCAMG
	CDR2	7	AISRTGGSSNYANSVKKG
	CDR3	128	ACEETFGITWYGSHEEDFRS

Clone	Feature	SEQ ID NO	Sequence
X	VHH	129	QVQLVESGGGLVQAGGSMRLSCLNSGRPFSNYCMGWFR QAPGKEREVAAISRTGGSSNYANSVKGRFTITKSDAKTT VYLMQMNSLKPEDTAVYYCAAEETFGITWYCSHEEDFRS WGQGTLTVSS
	CDR1	130	GRPFNSNYCMG
	CDR2	7	AISRTGGSSNYANSVKG
	CDR3	131	AAEETFGITWYCSHEEDFRS
Y	VHH	132	QVQLVESGGGLVQAGGSMRLSCLNSGRPFSNYAMGWF RQAPGKEREVAAISRTGGSSCYANSVKGRFTITKSDAKT TVYLMQMNSLKPEDTAVYYCAAEETFGITWCGSHEEDFRS WGQGTLTVSS
	CDR1	6	GRPFNSNYAMG
	CDR2	133	AISRTGGSSCYANSVKG
	CDR3	134	AAEETFGITWCGSHEEDFRS
Z	VHH	135	QVQLVESGGGLVQAGGSLRLSCLNSGRPFSNYAMGWF RQAPGKEREVAAISRTGGSSNYANSVKGRFTITKSDAKTT VYLMQMNSLKPEDTAVYYCAAEETFGITWYGSHEEDFRS WGQGTLTVSS
	CDR1	6	GRPFNSNYAMG
	CDR2	7	AISRTGGSSNYANSVKG
	CDR3	8	AAEETFGITWYGSHEEDFRS
AA	VHH	136	QVQLVESGGGLVQAGGSMRLSCANSGRPFSNYAMGWF RQAPGKEREVAAISRTGGSSNYANSVKGRFTITKSDAK TT VYLMQMNSLKPEDTAVYYCAAEETFGITWYGSHEEDF RSWGQGTLTVSS
	CDR1	6	GRPFNSNYAMG
	CDR2	7	AISRTGGSSNYANSVKG
	CDR3	8	AAEETFGITWYGSHEEDFRS
BB	VHH	137	QVQLVESGGGLVQAGGSMRLSCLNSGRPFSNYAMGWF RQAPGKEREVACISRTGGSSNYANSVKGRFTITKSDAKT TVYLMQMNSLKPEDTAVYYCAAEETFGITWCGSHEEDFRS WGQGTLTVSS
	CDR1	6	GRPFNSNYAMG
	CDR2	125	CISRTGGSSNYANSVKG
	CDR3	134	AAEETFGITWCGSHEEDFRS
CC	VHH	138	QVQLVESGGGLVQAGGSMRLSCLNSGRPFSNYCMGWFR QAPGKEREVAAISRTGGSSNYANSVKGRFTITKSDAKTT VYLMQMNSLKPEDTAVYYCAAEECFGITWYGSHEEDFRS WGQGTLTVSS
	CDR1	130	GRPFNSNYCMG
	CDR2	7	AISRTGGSSNYANSVKG
	CDR3	139	AAEECFGITWYGSHEEDFRS

[0316] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence provided in **Table 1**; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a CDR1 sequence provided in Table 1; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to a CDR1 sequence provided in **Table 1**; (ii) a CDR2 sequence provided in **Table 1**; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a CDR2 sequence provided in **Table 1**; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to a CDR2 sequence provided in **Table 1**; and/or (iii) a CDR3 sequence provided in **Table 1**; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a CDR3 sequence provided in **Table 1**; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to a CDR3 sequence provided in **Table 1**.

[0317] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 3; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 3; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 3; and/or (iii) a CDR3 sequence of SEQ ID NO: 4; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 4; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 4.

[0318] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 3; and (iii) a CDR3 sequence of SEQ ID NO: 4.

[0319] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 1, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0320] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 158, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0321] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 159, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0322] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 160, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0323] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 6; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 6; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 6; (ii) a CDR2 sequence of SEQ ID NO: 7; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 7; and/or (iii) a CDR3 sequence of SEQ ID NO: 8; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at

least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 8.

**[0324]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 6; (ii) a CDR2 sequence of SEQ ID NO: 7; and (iii) a CDR3 sequence of SEQ ID NO: 8.

**[0325]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 5, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0326]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 122, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0327]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 135, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0328]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 136, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0329]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 10; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 10; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 10; (ii) a CDR2 sequence of SEQ ID NO: 11; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 11; or a sequence

having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 11; and/or (iii) a CDR3 sequence of SEQ ID NO: 12; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 12; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 12.

**[0330]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 10; (ii) a CDR2 sequence of SEQ ID NO: 11; and (iii) a CDR3 sequence of SEQ ID NO: 12.

**[0331]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 9, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0332]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 14; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 14; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 14; (ii) a CDR2 sequence of SEQ ID NO: 15; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 15; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 15; and/or (iii) a CDR3 sequence of SEQ ID NO: 16; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 16; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 16.

**[0333]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 14; (ii) a CDR2 sequence of SEQ ID NO: 15; and (iii) a CDR3 sequence of SEQ ID NO: 16.

**[0334]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 13, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at

least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0335]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 18; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 18; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 18; (ii) a CDR2 sequence of SEQ ID NO: 19; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 19; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 19; and/or (iii) a CDR3 sequence of SEQ ID NO: 20; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 20; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 20.

**[0336]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 18; (ii) a CDR2 sequence of SEQ ID NO: 19; and (iii) a CDR3 sequence of SEQ ID NO: 20.

**[0337]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 17, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0338]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 22; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 22; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 22; (ii) a CDR2 sequence of SEQ ID NO: 23; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 23; or a sequence

having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 23; and/or (iii) a CDR3 sequence of SEQ ID NO: 24; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 24; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 24.

**[0339]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 22; (ii) a CDR2 sequence of SEQ ID NO: 23; and (iii) a CDR3 sequence of SEQ ID NO: 24.

**[0340]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 21, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0341]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 26; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 26; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 26; (ii) a CDR2 sequence of SEQ ID NO: 27; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 27; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 27; and/or (iii) a CDR3 sequence of SEQ ID NO: 28; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 28; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 28.

**[0342]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 26; (ii) a CDR2 sequence of SEQ ID NO: 27; and (iii) a CDR3 sequence of SEQ ID NO: 28.

**[0343]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 25, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at

least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0344]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 30; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 30; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 30; (ii) a CDR2 sequence of SEQ ID NO: 31; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 31; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 31; and/or (iii) a CDR3 sequence of SEQ ID NO: 32; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 32; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 32.

**[0345]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 30; (ii) a CDR2 sequence of SEQ ID NO: 31; and (iii) a CDR3 sequence of SEQ ID NO: 32.

**[0346]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 29, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0347]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 34; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 34; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 34; (ii) a CDR2 sequence of SEQ ID NO: 35; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 35; or a sequence

having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 35; and/or (iii) a CDR3 sequence of SEQ ID NO: 36; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 36; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 36.

**[0348]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 34; (ii) a CDR2 sequence of SEQ ID NO: 35; and (iii) a CDR3 sequence of SEQ ID NO: 36.

**[0349]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 33, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0350]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 38; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 38; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 38; (ii) a CDR2 sequence of SEQ ID NO: 39; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 39; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 39; and/or (iii) a CDR3 sequence of SEQ ID NO: 40; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 40; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 40.

**[0351]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 38; (ii) a CDR2 sequence of SEQ ID NO: 39; and (iii) a CDR3 sequence of SEQ ID NO: 40.

**[0352]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 37, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at

least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0353]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 42; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 42; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 42; (ii) a CDR2 sequence of SEQ ID NO: 43; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 43; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 43; and/or (iii) a CDR3 sequence of SEQ ID NO: 44; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 44; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 44.

**[0354]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 42; (ii) a CDR2 sequence of SEQ ID NO: 43; and (iii) a CDR3 sequence of SEQ ID NO: 44.

**[0355]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 41, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0356]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 46; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 46; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 46; (ii) a CDR2 sequence of SEQ ID NO: 47; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 47; or a sequence

having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 47; and/or (iii) a CDR3 sequence of SEQ ID NO: 48; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 48; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 48.

[0357] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 46; (ii) a CDR2 sequence of SEQ ID NO: 47; and (iii) a CDR3 sequence of SEQ ID NO: 48.

[0358] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 45, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0359] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 50; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 50; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 50; (ii) a CDR2 sequence of SEQ ID NO: 51; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 51; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 51; and/or (iii) a CDR3 sequence of SEQ ID NO: 52; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 52; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 52.

[0360] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 50; (ii) a CDR2 sequence of SEQ ID NO: 51; and (iii) a CDR3 sequence of SEQ ID NO: 52.

[0361] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 49, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at

least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0362]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 117; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 117; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 117; and/or (iii) a CDR3 sequence of SEQ ID NO: 4; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 4; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 4.

**[0363]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 117; and (iii) a CDR3 sequence of SEQ ID NO: 4.

**[0364]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 116, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0365]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 119; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 119; or a sequence

having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 119; and/or (iii) a CDR3 sequence of SEQ ID NO: 4; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 4; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 4.

[0366] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 119; and (iii) a CDR3 sequence of SEQ ID NO: 4.

[0367] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 118, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0368] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 15; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 15; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 15; and/or (iii) a CDR3 sequence of SEQ ID NO: 52; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 52; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 52.

[0369] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 15; and (iii) a CDR3 sequence of SEQ ID NO: 52.

[0370] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 120, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at

least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0371]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 119; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 119; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 119; and/or (iii) a CDR3 sequence of SEQ ID NO: 52; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 52; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 52.

**[0372]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 119; and (iii) a CDR3 sequence of SEQ ID NO: 52.

**[0373]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 121, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0374]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 124; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 124; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 124; (ii) a CDR2 sequence of SEQ ID NO: 125; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 125;

or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 125; and/or (iii) a CDR3 sequence of SEQ ID NO: 8; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 8.

[0375] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 124; (ii) a CDR2 sequence of SEQ ID NO: 125; and (iii) a CDR3 sequence of SEQ ID NO: 8.

[0376] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 123, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0377] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 127; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 127; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 127; (ii) a CDR2 sequence of SEQ ID NO: 7; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 7; and/or (iii) a CDR3 sequence of SEQ ID NO: 128; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 128; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 128.

[0378] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 127; (ii) a CDR2 sequence of SEQ ID NO: 7; and (iii) a CDR3 sequence of SEQ ID NO: 128.

[0379] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 126, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at

least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0380]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 130; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 130; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 130; (ii) a CDR2 sequence of SEQ ID NO: 7; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 7; and/or (iii) a CDR3 sequence of SEQ ID NO: 131; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 131; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 131.

**[0381]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 130; (ii) a CDR2 sequence of SEQ ID NO: 7; and (iii) a CDR3 sequence of SEQ ID NO: 131.

**[0382]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 129, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0383]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 6; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 6; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 6; (ii) a CDR2 sequence of SEQ ID NO: 133; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 133; or a sequence

having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 133; and/or (iii) a CDR3 sequence of SEQ ID NO: 134; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 134; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 134.

**[0384]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 6, (ii) a CDR2 sequence of SEQ ID NO: 133; and (iii) a CDR3 sequence of SEQ ID NO: 134.

**[0385]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 132, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0386]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 6; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 6; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 6; (ii) a CDR2 sequence of SEQ ID NO: 125; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 125; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 125; and/or (iii) a CDR3 sequence of SEQ ID NO: 134; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 134; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 134.

**[0387]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 6, (ii) a CDR2 sequence of SEQ ID NO: 125; and (iii) a CDR3 sequence of SEQ ID NO: 134.

**[0388]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 137, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at

least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0389]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 130; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 130; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 130; (ii) a CDR2 sequence of SEQ ID NO: 7; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 7; and/or (iii) a CDR3 sequence of SEQ ID NO: 139; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 139; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 139.

**[0390]** In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 130, (ii) a CDR2 sequence of SEQ ID NO: 7; and (iii) a CDR3 sequence of SEQ ID NO: 139.

**[0391]** In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 138, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[0392]** In some embodiments, a single chain antibody agent binds to hTfR1 with an affinity ( $K_D$ ) of about 10 nM to about 2500 nM, e.g., when fused to an Fc domain or when inserted in a VR region of a parental AAV capsid protein. In some embodiments, binding affinity of a single chain antibody agent to hTfR1 is about 10 nM to about 2500 nM, about 20 nM to about 2500 nM, about 30 nM to about 2500 nM, about 40 nM to about 2500 nM, about 50 nM to about 2500 nM, about 60 nM to about 2500 nM, about 70 nM to about 2500 nM, about 80 nM to about 2500 nM, about 90 nM to about 2500 nM, about 100 nM to about 2500 nM, about 200 nM to about 2500 nM, about 300 nM to about 2500 nM, about 400 nM to about 2500 nM, about 500

nM to about 2500 nM, about 600 nM to about 2500 nM, about 700 nM to about 2500 nM, about 800 nM to about 2500 nM, about 900 nM to about 2500 nM, about 1000 nM to about 2500 nM, about 1500 nM to about 2500 nM, about 2000 nM to about 2500 nM, about 10 nM to about 2500 nM, about 10 nM to about 2000 nM, about 10 nM to about 1500 nM, about 10 nM to about 1000 nM, about 10 nM to about 900 nM, about 10 nM to about 800 nM, about 10 nM to about 700 nM, about 10 nM to about 600 nM, about 10 nM to about 500 nM, about 10 nM to about 400 nM, about 10 nM to about 300 nM, about 10 nM to about 200 nM, about 10 nM to about 100 nM, about 10 nM to about 90 nM, about 10 nM to about 80 nM, about 10 nM to about 70 nM, about 10 nM to about 60 nM, about 10 nM to about 50 nM, about 10 nM to about 40 nM, about 10 nM to about 30 nM, about 10 nM to about 20 nM, e.g., when fused to an Fc domain or when inserted in a VR region of a parental AAV capsid protein.

**[0393]** In some embodiments, binding affinity of a single chain antibody agent to hTfR1 is about 10 nM, about 20 nM, about 30 nM, about 40 nM, about 50 nM, about 60 nM, about 70 nM, about 80 nM, about 90 nM, about 100 nM, about 200 nM, about 300 nM, about 400 nM, about 500 nM, about 600 nM, about 700 nM, about 800 nM, about 900 nM, about 1000 nM, about 1500 nM, about 2000 nM or about 2500 nM, e.g., when fused to an Fc domain or when inserted in a VR region of a parental AAV capsid protein.

**[0394]** In some embodiments, binding affinity of a single chain antibody agent to hTfR1 is 10 nM, 20 nM, 30 nM, 40 nM, 50 nM, 60 nM, 70 nM, 80 nM, 90 nM, 100 nM, 200 nM, 300 nM, 400 nM, 500 nM, 600 nM, 700 nM, 800 nM, 900 nM, 1000 nM, 1500 nM, 2000 nM or 2500 nM, e.g., when fused to an Fc domain or when inserted in a VR region of a parental AAV capsid protein.

**[0395]** In some embodiments, a single chain antibody agent binds to cyno TfR1 with an affinity ( $K_D$ ) of about 10 nM to about 2500 nM, e.g., when fused to an Fc domain or when inserted in a VR region of a parental AAV capsid protein. In some embodiments, binding affinity of a single chain antibody agent to cyno TfR1 is about 10 nM to about 2500 nM, about 20 nM to about 2500 nM, about 30 nM to about 2500 nM, about 40 nM to about 2500 nM, about 50 nM to about 2500 nM, about 60 nM to about 2500 nM, about 70 nM to about 2500 nM, about 80 nM to about 2500 nM, about 90 nM to about 2500 nM, about 100 nM to about 2500 nM, about 200 nM to about 2500 nM, about 300 nM to about 2500 nM, about 400 nM to about 2500 nM, about 500

nM to about 2500 nM, about 600 nM to about 2500 nM, about 700 nM to about 2500 nM, about 800 nM to about 2500 nM, about 900 nM to about 2500 nM, about 1000 nM to about 2500 nM, about 1500 nM to about 2500 nM, about 2000 nM to about 2500 nM, about 10 nM to about 2500 nM, about 10 nM to about 2000 nM, about 10 nM to about 1500 nM, about 10 nM to about 1000 nM, about 10 nM to about 900 nM, about 10 nM to about 800 nM, about 10 nM to about 700 nM, about 10 nM to about 600 nM, about 10 nM to about 500 nM, about 10 nM to about 400 nM, about 10 nM to about 300 nM, about 10 nM to about 200 nM, about 10 nM to about 100 nM, about 10 nM to about 90 nM, about 10 nM to about 80 nM, about 10 nM to about 70 nM, about 10 nM to about 60 nM, about 10 nM to about 50 nM, about 10 nM to about 40 nM, about 10 nM to about 30 nM, about 10 nM to about 20 nM, e.g., when fused to an Fc domain or when inserted in a VR region of a parental AAV capsid protein. In some embodiments, binding affinity of a single chain antibody agent to cyno TfR1 is about 10 nM, about 20 nM, about 30 nM, about 40 nM, about 50 nM, about 60 nM, about 70 nM, about 80 nM, about 90 nM, about 100 nM, about 200 nM, about 300 nM, about 400 nM, about 500 nM, about 600 nM, about 700 nM, about 800 nM, about 900 nM, about 1000 nM, about 1500 nM, about 2000 nM or about 2500 nM, e.g., when fused to an Fc domain or when inserted in a VR region of a parental AAV capsid protein.

**[0396]** In some embodiments, binding affinity of a single chain antibody agent to cyno TfR1 is 10 nM, 20 nM, 30 nM, 40 nM, 50 nM, 60 nM, 70 nM, 80 nM, 90 nM, 100 nM, 200 nM, 300 nM, 400 nM, 500 nM, 600 nM, 700 nM, 800 nM, 900 nM, 1000 nM, 1500 nM, 2000 nM or 2500 nM, e.g., when fused to an Fc domain or when inserted in a VR region of a parental AAV capsid protein.

**[0397]** In some embodiments, a single chain antibody agent is fused to a C terminus of an Fc domain. In some embodiments, a single chain antibody agent is fused to a N terminus of an Fc domain.

**[0398]** In some embodiments, binding affinity of a single chain antibody agent described herein (e.g., fused to an Fc domain or in an AAV particle comprising a single chain antibody agent inserted in a variable region (VR) of a parental AAV capsid protein) to hTfR1 does not directly correlate with the transduction level and/or efficiency of one or more CNS cells and/or tissue by an AAV particle comprising a single chain antibody agent inserted in VR of a parental AAV capsid protein.

*Exemplary TfR1 epitopes*

[0399] TfR1 binding single domain antibody agents described herein can bind to one or more epitopes on TfR1 (e.g., human TfR1, cyno TfR1, and/or mouse TfR1). In some embodiments, a TfR1 binding single domain antibody agent described herein binds to one or more epitopes on human TfR1. In some embodiments, a TfR1 binding single domain antibody agent described herein binds to one or more epitopes on cyno TfR1.

[0400] In some embodiments, an epitope on TfR1 (e.g., human TfR1, cyno TfR1, and/or mouse TfR1) bound by a single domain antibody agent described herein is a linear epitope.

[0401] In some embodiments, an epitope on TfR1 (e.g., human TfR1, cyno TfR1, and/or mouse TfR1) bound by a single domain antibody agent described herein is a conformational epitope.

[0402] In some embodiments, an epitope on TfR1 (e.g., human TfR1, cyno TfR1, and/or mouse TfR1) bound by a single domain antibody agent described herein is a discontinuous epitope.

[0403] In some embodiments, an epitope on TfR1 (e.g., human TfR1, cyno TfR1, and/or mouse TfR1) bound by a single domain antibody agent described herein comprises one or more domains of TfR1 (e.g., human TfR1, cyno TfR1, and/or mouse TfR1). In some embodiments, one or more domains of TfR1 (e.g., human TfR1, cyno TfR1, and/or mouse TfR1) is or comprises an extracellular domain of TfR1 (e.g., human TfR1, cyno TfR1, and/or mouse TfR1) or a fragment thereof. In some embodiments, one or more domains of TfR1 (e.g., human TfR1, cyno TfR1, and/or mouse TfR1) is or comprises an apical domain, a helical domain, a protease-like domain of TfR1 (e.g., human TfR1, cyno TfR1, and/or mouse TfR1), and/or fragments of one or more of the foregoing domains.

[0404] In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises an extracellular domain of human TfR1 or a fragment thereof. In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises: (1) an apical domain of human TfR1 or a fragment thereof, (2) a helical domain of human TfR1 or a fragment thereof, or (3) an apical domain of human TfR1 or a fragment thereof, and a helical domain of human TfR1 or a fragment thereof.

**[0405]** In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises: (1) one or more residues K189, Y309, F321, P322, P323, S324, R325, L329, or L381 from an apical domain of human TfR1, and/or (2) one or more residues E634, M635, G636, R719, N722, N723, G724, A725, F726, N727, E728, T729 or R732 from a helical domain of human TfR1.

**[0406]** In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises one or more residues K189, Y309, F321, P322, P323, S324, R325, L329, or L381 from an apical domain of human TfR1. In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises all of residues K189, Y309, F321, P322, P323, S324, R325, L329, and L381 from an apical domain of human TfR1.

**[0407]** In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises one or more residues E634, M635, G636, R719, N722, N723, G724, A725, F726, N727, E728, T729 or R732 from a helical domain of human TfR1. In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises all of residues E634, M635, G636, R719, N722, N723, G724, A725, F726, N727, E728, T729 and R732 from a helical domain of human TfR1.

**[0408]** In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises all of residues K189, Y309, F321, P322, P323, S324, R325, L329, and L381 from an apical domain of human TfR1, and one or more residues E634, M635, G636, R719, N722, N723, G724, A725, F726, N727, E728, T729 or R732 from a helical domain of human TfR1. In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises one or more residues K189, Y309, F321, P322, P323, S324, R325, L329, or L381 from an apical domain of human TfR1, and all of residues E634, M635, G636, R719, N722, N723, G724, A725, F726, N727, E728, T729 and R732 from a helical domain of human TfR1. In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises all of residues K189, Y309, F321, P322, P323, S324, R325, L329, and L381 from an apical domain of human TfR1, and all of residues E634, M635, G636, R719, N722, N723, G724, A725, F726, N727, E728, T729 and R732 from a helical domain of human TfR1.

[0409] In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises: (1) one or more residues K189, P323, S324, R325, L381, or E383 from an apical domain of human TfR1, and/or (2) one or more residues E634, M635, R719, N723, N727, E728, T729, or R732 from a helical domain of human TfR1.

[0410] In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises one or more residues K189, P323, S324, R325, L381, or E383 from an apical domain of human TfR1. In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises all of residues K189, P323, S324, R325, L381, and E383 from an apical domain of human TfR1.

[0411] In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises one or more residues E634, M635, R719, N723, N727, E728, T729, or R732 from a helical domain of human TfR1. In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises all of residues E634, M635, R719, N723, N727, E728, T729, and R732 from a helical domain of human TfR1.

[0412] In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises all of residues K189, P323, S324, R325, L381, and E383 from an apical domain of human TfR1 and one or more residues E634, M635, R719, N723, N727, E728, T729, or R732 from a helical domain of human TfR1. In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises one or more residues K189, P323, S324, R325, L381, or E383 from an apical domain of human TfR1 and all of residues E634, M635, R719, N723, N727, E728, T729, and R732 from a helical domain of human TfR1. In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises all of residues K189, P323, S324, R325, L381, and E383 from an apical domain of human TfR1, and all of residues E634, M635, R719, N723, N727, E728, T729, and R732 from a helical domain of human TfR1.

[0413] In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises: (1) one or more residues K189, P323, S324, R325, or L381 from an apical domain of human TfR1, and/or (2) one or more residues E634, M635, R719, N723, N727, E728, T729, or R732 from a helical domain of human TfR1.

[0414] In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises one or more residues K189, P323, S324, R325, or L381 from an apical domain of human TfR1. In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises all of residues K189, P323, S324, R325, and L381 from an apical domain of human TfR1.

[0415] In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises one or more residues E634, M635, R719, N723, N727, E728, T729, or R732 from a helical domain of human TfR1. In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises all of residues E634, M635, R719, N723, N727, E728, T729, and R732 from a helical domain of human TfR1.

[0416] In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises all of residues K189, P323, S324, R325, and L381 from an apical domain of human TfR1 and one or more residues E634, M635, R719, N723, N727, E728, T729, or R732 from a helical domain of human TfR1. In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises one or more residues K189, P323, S324, R325, or L381 from an apical domain of human TfR1 and all of residues E634, M635, R719, N723, N727, E728, T729, and R732 from a helical domain of human TfR1. In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises all of residues K189, P323, S324, R325, and L381 from an apical domain of human TfR1 and all of residues E634, M635, R719, N723, N727, E728, T729, and R732 from a helical domain of human TfR1.

[0417] In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises an extracellular domain of cyno TfR1. In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises an apical domain, a helical domain, or an apical domain and a helical domain of cyno TfR1.

[0418] In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises: (1) one or more residues K189, Y309, F321, P322, P323, S324, Q325, L329, or L381 from an apical domain of cyno TfR1, and/or (2) one or more

residues E634, M635, G636, R719, N722, N723, S724, A725, F726, N727, E728, T729, or R732 from a helical domain of cyno TfR1.

**[0419]** In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises one or more residues K189, Y309, F321, P322, P323, S324, Q325, L329, or L381 from an apical domain of cyno TfR1. In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises all of residues K189, Y309, F321, P322, P323, S324, Q325, L329, and L381 from an apical domain of cyno TfR1.

**[0420]** In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises one or more residues E634, M635, G636, R719, N722, N723, G724, A725, F726, N727, E728, T729, or R732 from a helical domain of cyno TfR1. In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises all of residues E634, M635, G636, R719, N722, N723, G724, A725, F726, N727, E728, T729, and R732 from a helical domain of cyno TfR1.

**[0421]** In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises all of residues K189, Y309, F321, P322, P323, S324, Q325, L329, and L381 from an apical domain of cyno TfR1, and one or more residues E634, M635, G636, R719, N722, N723, G724, A725, F726, N727, E728, T729, or R732 from a helical domain of cyno TfR1. In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises one or more residues K189, Y309, F321, P322, P323, S324, Q325, L329, or L381 from an apical domain of cyno TfR1, and all of residues E634, M635, G636, R719, N722, N723, G724, A725, F726, N727, E728, T729, and R732 from a helical domain of cyno TfR1. In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises all of residues K189, Y309, F321, P322, P323, S324, Q325, L329, and L381 from an apical domain of cyno TfR1, and all of residues E634, M635, G636, R719, N722, N723, G724, A725, F726, N727, E728, T729, and R732 from a helical domain of cyno TfR1.

**[0422]** In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises: (1) one or more residues K189, P323, S324, Q325,

L381, or E383 from an apical domain of cyno TfR1, and/or (2) one or more residues E634, M635, R719, N723, N727, E728, T729, or R732 from a helical domain of cyno TfR1.

**[0423]** In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises one or more residues K189, P323, S324, Q325, L381, or E383 from an apical domain of cyno TfR1. In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises all of residues K189, P323, S324, Q325, L381, and E383 from an apical domain of cyno TfR1.

**[0424]** In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises one or more residues E634, M635, R719, N723, N727, E728, T729, or R732 from a helical domain of cyno TfR1. In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises all of residues E634, M635, R719, N723, N727, E728, T729, and R732 from a helical domain of cyno TfR1.

**[0425]** In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises all of residues K189, P323, S324, Q325, L381, and E383 from an apical domain of cyno TfR1 and one or more residues E634, M635, R719, N723, N727, E728, T729, or R732 from a helical domain of cyno TfR1. In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises one or more residues K189, P323, S324, Q325, L381, or E383 from an apical domain of cyno TfR1 and all of residues E634, M635, R719, N723, N727, E728, T729, and R732 from a helical domain of cyno TfR1. In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises all of residues K189, P323, S324, Q325, L381, and E383 from an apical domain of cyno TfR1, and all of residues E634, M635, R719, N723, N727, E728, T729, and R732 from a helical domain of cyno TfR1.

### **Adeno-associated viruses (AAVs)**

**[0426]** Adeno-associated viruses (AAVs) are small, nonenveloped, single-stranded DNA (ssDNA) viruses that belong to the *Parvoviridae* family. At least twelve distinct AAV serotypes have been identified from human and nonhuman primate sources (see DiMatta M.A. et al.,

(2012) *J. Virology* 86(12): pp. 6947-6958, the entire contents of which is hereby incorporated by reference)(hereinafter “DiMattia 2012”). AAV9 is one of the human AAV serotypes that has enhanced transduction efficiency in cardiac and skeletal muscle, liver tissue, pancreatic tissue, and the eye compared to other serotypes (DiMattia 2012).

**[0427]** The AAV wild-type genome contains at least three genes, *rep*, *cap* and *X* (Büning H. et al., (2019) *Molecular Therapy: Methods & Clinical Development* vol. 12 pages 248-265). The *cap* gene encodes for viral proteins VP1, VP2, and VP3, and assembly-activating protein (AAP). All three VPs (i.e., viral proteins) are capsid monomers.

**[0428]** Transcription of the *cap* gene results in two messenger RNA: a messenger RNA which encodes VP1 and a messenger RNA which encodes VP2 and VP3 (as described in Warrington KH et al., (2004) *Journal of Virology* volume 78(12) pages 6595-6609). VP1, VP2, and VP3 are present at ratios of 1:1:10, respectively. The VP3 region is observed in all capsid structures of AAV serotypes that have been studied (DiMattia 2012).

**[0429]** VP proteins comprise beta strands, alpha helical regions, and structurally variable regions (VRs) in the surface loops which connect the beta strands. Without wishing to be bound by any particular theory, it is believed that differences in sequence and/or conformations of VRs contribute to the variability in cellular tropism, differences in tissue transduction efficiency, and/or antigenic reactivity among different AAV serotypes. In some embodiments, differences in VR sequence and/or structure among different AAV serotypes allow for differential recognition of cell surface glycans and/or tissue specific protein or lipid receptor interaction for internalization.

**[0430]** Wild type AAV9 (WT AAV9) has nine variable regions VR-I, VR-II, VR-III, VR-IV, VR-V, VR-VI, VR-VII, VR-VIII and VR-IX (DiMattia 2012, see also Table 3 therein). AAV9 VR-I encompasses amino acid positions 262-269. AAV VR-II encompasses amino acid positions 327-332 and has a role, e.g., in genome packaging. AAV9 VR-III encompasses amino acid positions 382-386. AAV9 VR-IV encompasses amino acid positions 452-460 and has a role, e.g., in liver transduction and/or a delayed blood clearance phenotype. AAV9 VR-V encompasses amino acid positions 488-505 and has a role, e.g., in LamR receptor binding, liver and/or muscle-specific transduction, and/or a delayed blood clearance phenotype. AAV9 VR-VI encompasses amino acid positions 527-539 and has a role, e.g., in LamR receptor binding, and/or

a delayed blood clearance phenotype. AAV9 VR-VII encompasses amino acid positions 545-558 and has a role, e.g., in liver transduction and/or delayed blood clearance phenotype. AAV9 VR-VIII encompasses amino acid positions 581-593 and has a role, e.g., in LamR receptor binding and/or transduction. AAV9 VR-IX encompasses amino acid positions 704-714 and has a role, e.g., in heart tropism, melanoma tropism and/or altered tropism.

**[0431]** In some embodiments, a rAAV particle disclosed herein is a recombinant AAV (rAAV) particle. In some embodiments, a rAAV particle comprises a variant AAV9 capsid protein comprising a single chain antibody agent insertion disclosed herein. In some embodiments, a single chain antibody agent insertion is in any one or all or a combination of VR-I, VR-II, VR-III, VR-IV, VR-V, VR-VI, VR-VII, VR-VIII and VR-IX of a parental AAV capsid protein.

**[0432]** In some embodiments, a parental AAV capsid protein comprises the sequence of a wildtype AAV capsid protein, or a sequence having at least 95% identity to the sequence of a wildtype AAV capsid protein, or a sequence having no more than 20 mutations (e.g., substitutions) as compared to the sequence of a wildtype AAV capsid protein. In some embodiments, a parental AAV capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to the sequence of a wildtype AAV capsid protein. In some embodiments, a parental AAV capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to the sequence of a wildtype AAV capsid protein.

**[0433]** In some embodiments, a parental AAV capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to the sequence of a wildtype AAV capsid protein and one or more mutations, e.g., as disclosed herein.

**[0434]** In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding

profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3, or any combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

**[0435]** In some embodiments, a parental AAV capsid protein is other than an AAV9 capsid protein and comprises one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) at a position of VP1, VP2 or VP3 corresponding to position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein.

**[0436]** In some embodiments, one or more mutations comprises a mutation to an amino acid sequence that is at or near a glycan binding region. In some embodiments, one or more mutations reduces glycan binding. In some embodiments, a glycan is galactose.

**[0437]** In some embodiments, one or more modifications is at or between amino acids: (a) 271 and 272 of a VP1, VP2, or VP3 of an AAV9 capsid protein or a corresponding position in a capsid protein of another parental AAV capsid protein; (b) 446 of a VP1, VP2, or VP3 of an AAV9 capsid protein or a corresponding position in a capsid protein of another parental AAV capsid protein; (c) 470 of a VP1, VP2, or VP3 of an AAV9 capsid protein or a corresponding position in a capsid protein of another parental AAV capsid protein; (d) 501 and 505 (e.g., at any one or all or a combination of residues 501, 502, 503, 504 or 505) of a VP1, VP2, or VP3 of an AAV9 capsid protein or a corresponding position in a capsid protein of another parental AAV capsid protein; (e) 489 and 545 of a VP1, VP2, or VP3 of an AAV9 capsid protein or a corresponding position in a capsid protein of another parental AAV capsid protein; (f) 591 and 621 of a VP1, VP2, or VP3 of an AAV9 capsid protein or a corresponding position in a capsid protein of another parental AAV capsid protein; or (g) any combination or all of (a)-(f).

**[0438]** In some embodiments, one or more mutations comprises a mutation at positions: (a) 271 and 272 of a VP1, VP2 or VP3 of an AAV9 capsid protein; (b) 446 of a VP1, VP2 or VP3 of an AAV9 capsid protein; (c) 470 of a VP1, VP2 or VP3 of an AAV9 capsid protein; (d) 501 and 505 (e.g., at any one or all or a combination of residues 501, 502, 503, 504 or 505) of a VP1, VP2 or VP3 of an AAV9 capsid protein; (e) 489 and 545 of a VP1, VP2, or VP3 of an

AAV9 capsid protein; (f) 591 and 621 of a VP1, VP2 or VP3 of an AAV9 capsid protein; or (g) any combination or all of (a)-(f).

**[0439]** In some embodiments, a parental AAV capsid protein comprises an AAV9 capsid protein, an AAV1 capsid protein, an AAV2 capsid protein, an AAV3B capsid protein, an AAV4 capsid protein, an AAV5 capsid protein, an AAV6 capsid protein, an AAV7 capsid protein, an AAV8 capsid protein, an AAV10 capsid protein, an AAV11 capsid protein, an AAV12 capsid protein, an AAV13 capsid protein, an AAVhu68 capsid protein, or an AAVrh10 capsid protein.

**[0440]** In some embodiments, a parental AAV capsid protein comprises: an AAV9 capsid protein. In some embodiments, an AAV9 capsid protein comprises: the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001.

**[0441]** In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein.

**[0442]** In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. Exemplary mutations including liver de-targeting mutations are disclosed in Pulicherla N. et al., (2011) *Molecular Therapy* volume 19, pages 1070-1078, the entire contents of which are hereby incorporated by reference.

[0443] In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3, or any combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, e.g., a W503R mutation. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 595, e.g., a W595C mutation. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 457, e.g., a N457H mutation. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 574, e.g., a T574S mutation. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 592, e.g., a Q592L mutation. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 498, e.g., a N498Y or an N498I mutation. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 602, e.g., a L602F mutation. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 468, e.g., a P468T mutation. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 500, e.g., a E500D mutation.

[0444] In some embodiments, one or more mutations comprises a mutation to an amino acid sequence that is at or near a glycan binding region. In some embodiments, one or more mutations reduces glycan binding. In some embodiments, a glycan is galactose.

[0445] In some embodiments, one or more modifications is at or between amino acids: (a) 271 and 272 of a VP1, VP2, or VP3 of an AAV9 capsid protein or a corresponding position in a capsid protein of another parental AAV capsid protein; (b) 446 of a VP1, VP2, or VP3 of an AAV9 capsid protein or a corresponding position in a capsid protein of another parental AAV

capsid protein; (c) 470 of a VP1, VP2, or VP3 of an AAV9 capsid protein or a corresponding position in a capsid protein of another parental AAV capsid protein; (d) 501 and 505 (e.g., at any one or all or a combination of residues 501, 502, 503, 504 or 505) of a VP1, VP2, or VP3 of an AAV9 capsid protein or a corresponding position in a capsid protein of another parental AAV capsid protein; (e) 489 and 545 of a VP1, VP2, or VP3 of an AAV9 capsid protein or a corresponding position in a capsid protein of another parental AAV capsid protein; (f) 591 and 621 of a VP1, VP2, or VP3 of an AAV9 capsid protein or a corresponding position in a capsid protein of another parental AAV capsid protein; or (g) any combination or all of (a)-(f).

**[0446]** In some embodiments, one or more mutations comprises a mutation at positions: (a) 271 and 272 of a VP1, VP2 or VP3 of an AAV9 capsid protein; (b) 446 of a VP1, VP2 or VP3 of an AAV9 capsid protein; (c) 470 of a VP1, VP2 or VP3 of an AAV9 capsid protein; (d) 501 and 505 (e.g., at any one or all or a combination of residues 501, 502, 503, 504 or 505) of a VP1, VP2 or VP3 of an AAV9 capsid protein; (e) 489 and 545 of a VP1, VP2 or VP3 of an AAV9 capsid protein; (f) 591 and 621 of a VP1, VP2 or VP3 of an AAV9 capsid protein; or (g) any combination or all of (a)-(f).

**[0447]** In some embodiments, a parental AAV capsid protein comprises: an AAV1 capsid protein. In some embodiments, an AAV1 capsid protein sequence is provided in SEQ ID NO: 2002.

**[0448]** In some embodiments, a parental AAV capsid protein comprises: an AAV2 capsid protein. In some embodiments, an AAV2 capsid protein sequence is provided in SEQ ID NO: 2003.

**[0449]** In some embodiments, a parental AAV capsid protein comprises: an AAV3B capsid protein. In some embodiments, an AAV3B capsid protein sequence is provided in SEQ ID NO: 2007.

**[0450]** In some embodiments, a parental AAV capsid protein comprises an AAV4 capsid protein. In some embodiments, an AAV4 capsid protein sequence is provided in SEQ ID NO: 2008.

[0451] In some embodiments, a parental AAV capsid protein comprises: an AAV5 capsid protein. In some embodiments, an AAV5 capsid protein sequence is provided in SEQ ID NO: 2004.

[0452] In some embodiments, a parental AAV capsid protein comprises: an AAV6 capsid protein. In some embodiments, an AAV6 capsid protein sequence is provided in SEQ ID NO: 2005.

[0453] In some embodiments, a parental AAV capsid protein comprises an AAV7 capsid protein. In some embodiments, an AAV7 capsid protein sequence is provided in SEQ ID NO: 2009.

[0454] In some embodiments, a parental AAV capsid protein comprises: an AAV8 capsid protein. In some embodiments, an AAV8 capsid protein sequence is provided in SEQ ID NO: 2006.

[0455] In some embodiments, a parental AAV capsid protein comprises an AAV10 capsid protein. In some embodiments, an AAV10 capsid protein sequence is provided in SEQ ID NO: 2010.

[0456] In some embodiments, a parental AAV capsid protein comprises an AAV11 capsid protein. In some embodiments, an AAV11 capsid protein sequence is provided in SEQ ID NO: 2011.

[0457] In some embodiments, a parental AAV capsid protein comprises an AAV12 capsid protein. In some embodiments, an AAV12 capsid protein sequence is provided in SEQ ID NO: 2012.

[0458] In some embodiments, a parental AAV capsid protein comprises an AAV13 capsid protein. In some embodiments, an AAV13 capsid protein sequence is provided in SEQ ID NO: 2013.

[0459] In some embodiments, a parental AAV capsid protein comprises an AA Vhu68 capsid protein. In some embodiments, an AA Vhu68 capsid protein sequence is provided in SEQ ID NO: 2014.

[0460] In some embodiments, a parental AAV capsid protein comprises an AAVrh10 capsid protein. In some embodiments, an AAVrh10 capsid protein sequence is provided in SEQ ID NO: 2015.

[0461] In some embodiments, a single chain antibody agent insertion is in VR-I of a parental AAV capsid protein, e.g., AAV9, AAV1, AAV2, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV10, AAV11, AAV12, AAV13, AAvh68, or AAVrh10.

[0462] In some embodiments, a single chain antibody agent insertion is in VR-II of a parental AAV capsid protein, e.g., AAV9, AAV1, AAV2, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV10, AAV11, AAV12, AAV13, AAvh68, or AAVrh10.

[0463] In some embodiments, a single chain antibody agent insertion is in VR-III of a parental AAV capsid protein, e.g., AAV9, AAV1, AAV2, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV10, AAV11, AAV12, AAV13, AAvh68, or AAVrh10.

[0464] In some embodiments, a single chain antibody agent insertion is in VR-IV of a parental AAV capsid protein, e.g., AAV9, AAV1, AAV2, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV10, AAV11, AAV12, AAV13, AAvh68, or AAVrh10.

[0465] In some embodiments, a single chain antibody agent insertion is in VR-V of a parental AAV capsid protein, e.g., AAV9, AAV1, AAV2, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV10, AAV11, AAV12, AAV13, AAvh68, or AAVrh10.

[0466] In some embodiments, a single chain antibody agent insertion is in VR-VI of a parental AAV capsid protein, e.g., AAV9, AAV1, AAV2, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV10, AAV11, AAV12, AAV13, AAvh68, or AAVrh10.

[0467] In some embodiments, a single chain antibody agent insertion is in VR-VII of a parental AAV capsid protein, e.g., AAV9, AAV1, AAV2, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV10, AAV11, AAV12, AAV13, AAvh68, or AAVrh10.

[0468] In some embodiments, a single chain antibody agent insertion is in VR-VIII of a parental AAV capsid protein, e.g., AAV9, AAV1, AAV2, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV10, AAV11, AAV12, AAV13, AAvh68, or AAVrh10.

[0469] In some embodiments, a single chain antibody agent insertion is in VR-IX of a parental AAV capsid protein, e.g., AAV9, AAV1, AAV2, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV10, AAV11, AAV12, AAV13, AAVhu68, or AAVrh10.

[0470] In some embodiments, a parental AAV capsid protein is chosen from an AAV9, AAV1, AAV2, AAV3B, AAV5, AAV6 or AAV8 capsid protein, and VR-VIII comprises amino acids 580 to 601 of a VP1, VP2 or VP3 of an AAV9 capsid protein or the corresponding positions in the capsid proteins of another parental AAV capsid protein, e.g., an AAV1, AAV2, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV10, AAV11, AAV12, AAV13, AAVhu68, or AAVrh10 capsid protein.

[0471] In some embodiments, a parental AAV capsid protein is an AAV9 capsid protein, and VR-VIII comprises amino acids 580 to 601 of a VP1, VP2 or VP3 of an AAV9 capsid protein.

[0472] In some embodiments, a single chain antibody agent insertion is in a VP (e.g., VP1, VP2, and/or VP3) of a parental AAV capsid protein.

[0473] In some embodiments, a single chain antibody agent insertion site is located between amino acids 588 and 589 of a VP1, VP2 or VP3 of a parental AAV capsid protein (e.g., AAV9, AAV1, AAV2, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV10, AAV11, AAV12, AAV13, AAVhu68, or AAVrh10).

[0474] In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of a VP1, VP2 or VP3 of an AAV9 capsid protein.

[0475] In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of a VP1 and VP2 of an AAV9 capsid protein.

[0476] In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of a VP1 and VP3 of an AAV9 capsid protein.

[0477] In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of a VP2 and VP3 of an AAV9 capsid protein.

[0478] In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of a VP1, VP2 and VP3 of an AAV9 capsid protein.

[0479] In some embodiments, a single chain antibody agent insertion is located between amino acids 454 and 461 of a VP1, VP2 or VP3 of an AAV9 capsid protein.

[0480] In some embodiments, a single chain antibody agent insertion is located between amino acids 454 and 461 of a VP1 and VP2 of an AAV9 capsid protein.

[0481] In some embodiments, a single chain antibody agent insertion is located between amino acids 454 and 461 of a VP1 and VP3 of an AAV9 capsid protein.

[0482] In some embodiments, a single chain antibody agent insertion is located between amino acids 454 and 461 of a VP2 and VP3 of an AAV9 capsid protein.

[0483] In some embodiments, a single chain antibody agent insertion is located between amino acids 454 and 461 of a VP1, VP2 and VP3 of an AAV9 capsid protein.

[0484] In some embodiments, a single chain antibody agent insertion is located between amino acids 454 and 457 of a VP1, VP2 or VP3 of an AAV9 capsid protein.

[0485] In some embodiments, a single chain antibody agent insertion is located between amino acids 454 and 457 of a VP1 and VP2 of an AAV9 capsid protein.

[0486] In some embodiments, a single chain antibody agent insertion is located between amino acids 454 and 457 of a VP1 and VP3 of an AAV9 capsid protein.

[0487] In some embodiments, a single chain antibody agent insertion is located between amino acids 454 and 457 of a VP2 and VP3 of an AAV9 capsid protein.

[0488] In some embodiments, a single chain antibody agent insertion is located between amino acids 454 and 457 of a VP1, VP2 and VP3 of an AAV9 capsid protein.

[0489] In some embodiments, a single chain antibody agent insertion is located between amino acids 451 and 460 of a VP1, VP2 or VP3 of an AAV9 capsid protein.

[0490] In some embodiments, a single chain antibody agent insertion is located between amino acids 451 and 460 of a VP1 and VP2 of an AAV9 capsid protein.

[0491] In some embodiments, a single chain antibody agent insertion is located between amino acids 451 and 460 of a VP1 and VP3 of an AAV9 capsid protein.

[0492] In some embodiments, a single chain antibody agent insertion is located between amino acids 451 and 460 a VP2 and VP3 of an AAV9 capsid protein.

[0493] In some embodiments, a single chain antibody agent insertion is located between amino acids 451 and 460 of a VP1, VP2 and VP3 of an AAV9 capsid protein.

[0494] In some embodiments, a single chain antibody agent insertion site is located between two adjacent amino acids in VR-VIII of a parental AAV capsid protein.

[0495] In some embodiments, a single chain antibody agent insertion site is located between two non-adjacent amino acids in VR-VIII of a parental AAV capsid protein.

[0496] In some embodiments, insertion of a heterologous peptide (e.g., a single chain antibody agent, e.g., a VHH) replaces a contiguous stretch of amino acids of a parental AAV capsid protein.

[0497] In some embodiments, insertion of a heterologous peptide (e.g., a single chain antibody agent, e.g., a VHH) does not replace a contiguous stretch of amino acids of a parental AAV capsid protein.

[0498] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV9 capsid protein, e.g., as compared to a WT AAV9 capsid protein. In some embodiments, an AAV9 WT capsid protein sequence is provided in SEQ ID NO: 2001. In some embodiments, a variant AAV9 capsid protein has at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity relative to a WT AAV9 capsid protein.

[0499] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV1 capsid protein, e.g., as compared to a WT AAV1 capsid protein. In some embodiments, an AAV1 WT capsid protein is provided in SEQ ID NO: 2002. In some embodiments, a variant AAV1 capsid protein has at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity relative to a WT AAV1 capsid.

[0500] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV2 capsid protein, e.g., as compared to a WT AAV2 capsid protein. In some embodiments, an AAV2 WT capsid protein is provided in SEQ ID NO: 2003. In some embodiments, a variant

AAV2 capsid protein has at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity relative to a WT AAV2 capsid protein.

[0501] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV3B capsid protein, e.g., as compared to a WT AAV3B capsid protein. In some embodiments, an AAV3B WT capsid protein is provided in SEQ ID NO: 2007. In some embodiments, a variant AAV3B capsid protein has at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity relative to a WT AAV3B capsid protein.

[0502] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV5 capsid protein, e.g., as compared to a WT AAV5 capsid protein. In some embodiments, an AAV5 WT capsid protein is provided in SEQ ID NO: 2004. In some embodiments, a variant AAV5 capsid protein has at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity relative to a WT AAV5 capsid protein.

[0503] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV6 capsid protein, e.g., as compared to a WT AAV6 capsid protein. In some embodiments, an AAV6 WT capsid protein is provided in SEQ ID NO: 2005. In some embodiments, a variant AAV6 capsid protein has at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity relative to a WT AAV6 capsid protein.

[0504] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV8 capsid protein, e.g., as compared to a WT AAV8 capsid protein. In some embodiments, an AAV8 WT capsid protein is provided in SEQ ID NO: 2006. In some embodiments, a variant AAV8 capsid protein has at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity relative to a WT AAV8 capsid protein.

[0505] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV4 capsid protein, e.g., as compared to a WT AAV4 capsid protein. In some embodiments, an AAV4 WT capsid protein is provided in SEQ ID NO: 2008. In some embodiments, a variant

AAV4 capsid protein has at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity relative to a WT AAV4 capsid protein.

**[0506]** In some embodiments, a rAAV particle disclosed herein comprises a variant AAV7 capsid protein, e.g., as compared to a WT AAV7 capsid protein. In some embodiments, an AAV7 WT capsid protein is provided in SEQ ID NO: 2009. In some embodiments, a variant AAV7 capsid protein has at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity relative to a WT AAV7 capsid protein.

**[0507]** In some embodiments, a rAAV particle disclosed herein comprises a variant AAV10 capsid protein, e.g., as compared to a WT AAV10 capsid protein. In some embodiments, an AAV10 WT capsid protein is provided in SEQ ID NO: 2010. In some embodiments, a variant AAV10 capsid protein has at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity relative to a WT AAV10 capsid protein.

**[0508]** In some embodiments, a rAAV particle disclosed herein comprises a variant AAV11 capsid protein, e.g., as compared to a WT AAV11 capsid protein. In some embodiments, an AAV11 WT capsid protein is provided in SEQ ID NO: 2011. In some embodiments, a variant AAV11 capsid protein has at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity relative to a WT AAV11 capsid protein.

**[0509]** In some embodiments, a rAAV particle disclosed herein comprises a variant AAV12 capsid protein, e.g., as compared to a WT AAV12 capsid protein. In some embodiments, an AAV12 WT capsid protein is provided in SEQ ID NO: 2012. In some embodiments, a variant AAV12 capsid protein has at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity relative to a WT AAV12 capsid protein.

**[0510]** In some embodiments, a rAAV particle disclosed herein comprises a variant AAV13 capsid protein, e.g., as compared to a WT AAV13 capsid protein. In some embodiments, an AAV13 WT capsid protein is provided in SEQ ID NO: 2013. In some embodiments, a

variant AAV13 capsid protein has at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity relative to a WT AAV13 capsid protein.

[0511] In some embodiments, a rAAV particle disclosed herein comprises a variant AAVhu68 capsid protein, e.g., as compared to a WT AAVhu68 capsid protein. In some embodiments, an AAVhu68 WT capsid protein is provided in SEQ ID NO: 2014. In some embodiments, a variant AAVhu68 capsid protein has at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity relative to a WT AAVhu68 capsid protein.

[0512] In some embodiments, a rAAV particle disclosed herein comprises a variant AAVrh10 capsid protein, e.g., as compared to a WT AAVrh10 capsid protein. In some embodiments, an AAVrh10 WT capsid protein is provided in SEQ ID NO: 2015. In some embodiments, a variant AAVrh10 capsid protein has at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity relative to a WT AAVrh10 capsid protein.

[0513] Additional modifications to an AAV9 capsid protein (not including single chain antibody agent insertions disclosed herein) are possible including, for example, variants disclosed in International Patent Application WO 2003/052052 filed on November 12, 2002, the entire contents of which are hereby incorporated by reference. In some embodiments, a rAAV particle disclosed herein comprises a variant AAV9 capsid comprising a single chain antibody agent insertion disclosed herein and one or more AAV9 capsid modifications disclosed in WO 2003/052052.

[0514] Several other reports disclose modifications to an AAV9 capsid protein, including: Pulicherla N. et al., (2011) *Mol Ther.* 19(6): pp. 1070–1078; Wang D. et al., (2018) *Mol Ther Methods Clin Dev.* (9): pp. 234-246; Adachi K. et al., (2014) *Nat. Comm.* (5): art. 3075; or Bell CL. Et al., (2012) *J Virol.* 86(13): pp. 7326–7333, the entire contents each of which are hereby incorporated by reference. In some embodiments, a rAAV particle disclosed herein comprises a variant AAV9 capsid comprising a single chain antibody agent insertion disclosed herein and one or more AAV9 capsid modifications disclosed in any of the reports referenced herein.

*Variant AAV capsid proteins with insertion of single chain antibody agents*

[0515] Among other things, disclosed herein, are AAV capsid protein variants, e.g., AAV9 capsid protein variants having one or more single chain antibody agent insertions, e.g., as disclosed herein.

[0516] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein, e.g., a variant AAV9 capsid protein comprising a single chain antibody agent insertion disclosed herein.

[0517] In some embodiments, a single chain antibody agent insertion is in any one or all or a combination of VR-I, VR-II, VR-III, VR-IV, VR-V, VR-VI, VR-VII, VR-VIII and VR-IX of an AAV capsid protein, e.g., an AAV9 capsid protein.

[0518] In some embodiments, a single chain antibody agent insertion is in VR-I of an AAV capsid protein, e.g., an AAV9 capsid protein.

[0519] In some embodiments, a single chain antibody agent insertion is in VR-II of an AAV capsid protein, e.g., an AAV9 capsid protein.

[0520] In some embodiments, a single chain antibody agent insertion is in VR-III of an AAV capsid protein, e.g., an AAV9 capsid protein.

[0521] In some embodiments, a single chain antibody agent insertion is in VR-IV of an AAV capsid protein, e.g., an AAV9 capsid protein.

[0522] In some embodiments, VR-IV of an AAV9 capsid protein comprises amino acids 452 to 460 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion site is located between amino acids 452 to 460 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion site is located between amino acids 452-455, amino acids 452-450, or amino acids 455-460 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion site is located between amino acids 452-453, 452-454, 452-455, 452-456, 452-457, 452-458, 452-459, 452-460, 455-460, 455-456, 452-459, of VP1, VP2, or VP3 of an AAV9 capsid protein.

[0523] In some embodiments, a single chain antibody agent insertion is located between amino acids 454 and 461 of a VP1 of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 and 461 of a VP2 of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 and 461 of a VP3 of an AAV9 capsid protein.

[0524] In some embodiments, a single chain antibody agent insertion is located between amino acids 454 and 461 of a VP1 and VP2 of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 and 461 of a VP2 and VP3 of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 and 461 of a VP1 and VP3 of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 and 461 of a VP1, VP2 and VP3 of an AAV9 capsid protein.

[0525] In some embodiments, a single chain antibody agent insertion is located between amino acids 454 and 457 of a VP1 of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 and 457 of a VP2 of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 and 457 of a VP3 of an AAV9 capsid protein.

[0526] In some embodiments, a single chain antibody agent insertion is located between amino acids 454 and 457 of a VP1 and VP2 of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 and 457 of a VP2 and VP3 of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 and 457 of a VP1 and VP3 of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 and 457 of a VP1, VP2 and VP3 of an AAV9 capsid protein.

[0527] In some embodiments, a single chain antibody agent insertion is located between amino acids 451 and 460 of a VP1 of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 and 460 of a VP2 of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 and 460 of a VP3 of an AAV9 capsid protein.

[0528] In some embodiments, a single chain antibody agent insertion is located between amino acids 451 and 460 of a VP1 and VP2 of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 and 460 of a VP2 and VP3 of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 and 460 of a VP1 and VP3 of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 and 460 of a VP1, VP2 and VP3 of an AAV9 capsid protein.

[0529] In some embodiments, a single chain antibody agent insertion site is located between two adjacent amino acids in VR-VIII of an AAV capsid protein, e.g., an AAV9 capsid protein.

[0530] In some embodiments, a single chain antibody agent insertion site is located between two non-adjacent amino acids in VR-VIII of an AAV capsid protein, e.g., an AAV9 capsid protein.

[0531] In some embodiments, a single chain antibody agent insertion is in VR-V of an AAV capsid protein, e.g., an AAV9 capsid protein.

[0532] In some embodiments, a single chain antibody agent insertion is in VR-VI of an AAV capsid protein, e.g., an AAV9 capsid protein.

[0533] In some embodiments, a single chain antibody agent insertion is in VR-VII of an AAV capsid protein, e.g., an AAV9 capsid protein.

[0534] In some embodiments, a single chain antibody agent insertion is in VR-VIII of an AAV capsid protein, e.g., an AAV9 capsid protein.

[0535] In some embodiments, VR-VIII of an AAV9 capsid protein comprises amino acids 580 to 601 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion site is located between amino acids 580 to 601 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion site is located between amino acids 580-585, amino acids 585-590, amino acids 590-595, or amino acids 595-601 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion site is located between amino acids 580-581, between amino acids 581-582, between amino acids 582-583, between amino acids 583-

584, between amino acids 584-585, between amino acids 585-586, between amino acids 586-587, between amino acids 587-588, between amino acids 588-589, between amino acids 589-590, between amino acids 590-591, between amino acids 591-592, between amino acids 592-593, between amino acids 593-594, between amino acids 594-595, between amino acids 595-596, between amino acids 596-597, between amino acids 597-598, between amino acids 598-599, between amino acids 599-600, or between amino acids 600-601 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion site is located between amino acids 588 and 590 of VP1, VP2, or VP3 of an AAV9 capsid protein.

[0536] In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of a VP1 of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of a VP2 of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of a VP3 of an AAV9 capsid protein.

[0537] In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of a VP1 and VP2 of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of a VP2 and VP3 of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of a VP1 and VP3 of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of a VP1, VP2 and VP3 of an AAV9 capsid protein.

[0538] In some embodiments, a single chain antibody agent insertion site is located between two adjacent amino acids in VR-VIII of an AAV capsid protein, e.g., an AAV9 capsid protein.

[0539] In some embodiments, a single chain antibody agent insertion site is located between two non-adjacent amino acids in VR-VIII of an AAV capsid protein, e.g., an AAV9 capsid protein.

[0540] In some embodiments, a single chain antibody agent insertion is in VR-IX of an AAV capsid protein, e.g., an AAV9 capsid protein.

[0541] In some embodiments, a single chain antibody agent insertion is in VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion site is located between amino acids 588 and 590 of VP1, VP2, or VP3 of an AAV9 capsid protein.

[0542] In some embodiments, insertion of a heterologous peptide (e.g., a single chain antibody agent, e.g., a VHH) replaces a contiguous stretch of amino acids of a parental AAV capsid protein, e.g., an AAV9 parental capsid protein.

[0543] In some embodiments, insertion of a heterologous peptide(e.g., a single chain antibody agent, e.g., a VHH) does not replace a contiguous stretch of amino acids of a parental AAV capsid protein, e.g., an AAV9 parental capsid protein.

[0544] In some embodiments, a recombinant AAV particle (rAAV) disclosed herein comprises a variant AAV capsid protein, e.g., a variant AAV9 capsid protein, comprising a single chain antibody agent insertion disclosed herein, and one or more sequences of VP1, VP2, or VP3 of an AAV9 capsid protein.

[0545] In some embodiments, a recombinant AAV particle (rAAV) disclosed herein comprises a variant AAV capsid protein, e.g., a variant AAV9 capsid protein, comprising a single chain antibody agent insertion disclosed herein. In some embodiments, a single chain antibody agent insertion disclosed herein comprises one or more linkers. In some embodiments, one or more linkers are situated on the N-terminus of a single chain antibody agent and/or the C-terminus of a single chain antibody agent. In some embodiments, one or more linkers are situated on the N-terminus of a single chain antibody agent. In some embodiments, one or more linkers are situated on the C-terminus of a single chain antibody agent. In some embodiments, one or more linkers are situated on the N-terminus and on the C-terminus of a single chain antibody agent.

[0546] In some embodiments, one or more linkers comprise one or more GGGGS repeats. In some embodiments, a single chain antibody agent comprises one GGGGS linker provided in SEQ ID NO: 55 on the N-terminus and/or the C-terminus of a single chain antibody agent. In some embodiments, a single chain antibody agent comprises two GGGGS linkers provided in SEQ ID NO: 56 on the N-terminus and/or the C-terminus of a single chain antibody agent. In some embodiments, a single chain antibody agent comprises three GGGGS linkers

provided in SEQ ID NO: 57 on the N-terminus and/or the C-terminus of a single chain antibody agent. In some embodiments, a single chain antibody agent comprises four GGGGS linkers provided in SEQ ID NO: 58 on the N-terminus and/or the C-terminus of a single chain antibody agent. In some embodiments, a single chain antibody agent comprises five GGGGS linkers provided in SEQ ID NO: 59 on the N-terminus and/or the C-terminus of a single chain antibody agent.

[0547] In some embodiments, one or more linkers comprise a coiled-coil alpha helix domain or a fragment thereof. In some embodiments, a single chain antibody agent comprises one or more coiled-coil alpha helix domains on the N-terminus of the single chain antibody agent and/or the C-terminus of the single chain antibody agent. In some embodiments, one or more linkers comprise a coiled-coil alpha helix domain that is or comprises a leading coil according to SEQ ID NO: 53 or a sequence with at least 90% identity thereto. In some embodiments, one or more linkers comprise a coiled-coil alpha helix domain that is or comprises a returning coil according to SEQ ID NO: 54 or a sequence with at least 90% identity thereto.

[0548] In some embodiments, a single chain antibody agent comprises one or more GGGGS linkers as described herein and one or more coiled-coil alpha helix domains as described herein on the N-terminus of the single chain antibody agent. In some embodiments, a single chain antibody agent comprises one or more GGGGS linkers as described herein and one or more coiled-coil alpha helix domains as described herein on the C-terminus of the single chain antibody agent. In some embodiments, a single chain antibody agent comprises one or more GGGGS linkers as described herein and one or more coiled-coil alpha helix domains as described herein on the N-terminus and on the C-terminus of the single chain antibody agent.

[0549] In some embodiments, a single chain antibody agent comprises a (GGGGS)<sub>5</sub> linker according to SEQ ID NO: 59 at the N-terminus and a (GGGGS)<sub>1</sub> linker according to SEQ ID NO: 55 at the C-terminus.

[0550] In some embodiments, a single chain antibody agent comprises a (GGGGS)<sub>4</sub> linker according to SEQ ID NO: 58 at the N-terminus.

[0551] In some embodiments, a single chain antibody agent comprises (i) a leading coiled-coil alpha helix domain and a (GGGGS)<sub>5</sub> linker according to SEQ ID NO: 60 at the N-

terminus; and (ii) a (GGGGS)<sub>1</sub> linker and a returning coiled-coil alpha helix domain according to SEQ ID NO: 61 at the C-terminus.

[0552] Exemplary linker sequences described herein are provided in **Table 2**.

**Table 2: Exemplary linker sequences**

SEQ ID NO	Feature	Sequence
53	Alpha helix leading coil	GGSGAKLAALKAKLAALK
54	Alpha helix returning coil	ELAALEAELAALEAGGSG
55	(GGGGS) <sub>1</sub>	GGGGS
56	(GGGGS) <sub>2</sub>	GGGGSGGGGS
57	(GGGGS) <sub>3</sub>	GGGGSGGGGGSGGGGS
58	(GGGGS) <sub>4</sub>	GGGGSGGGGGSGGGGGSGGGGS
59	(GGGGS) <sub>5</sub>	GGGGSGGGGGSGGGGSGGGGSGGGGS
60	Leading coil and (GGGGS) <sub>5</sub>	GGSGAKLAALKAKLAALKGGGGSGGGGGSGGGSG GGSGGGGS
61	(GGGGS) <sub>1</sub> and returning coil	GGGGSELAALEAELAALEAGGSG
65	(GGGS) <sub>3</sub> with Proline and Serine	GGGGSGGGGSGGGSPPCGGSGG

[0553] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV (e.g., AAV9) capsid protein comprising a single chain antibody agent provided in **Table 1**.

[0554] In some embodiments, a rAAV particle disclosed herein comprises: (1) a variant AAV (e.g., AAV9) capsid protein comprising any one of the single chain antibody agents provided in **Table 1**; and (2) one or more sequences of a VP (e.g., VP1, VP2, and/or VP3) of an AAV (e.g., AAV9) capsid protein.

[0555] In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein.

[0556] In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein.

[0557] In some embodiments, the single chain antibody agent is inserted in a VP1, VP2 and/or VP3 of a parental AAV capsid protein.

[0558] In some embodiments, a rAAV particle disclosed herein comprising a variant AAV (e.g., AAV9) capsid protein comprising a single chain antibody agent provided in **Table 1** has an at least 1.5 fold enhanced CNS transduction compared to a rAAV particle comprising a corresponding parental AAV (e.g., AAV9) capsid protein, e.g., without a single chain antibody agent insertion.

[0559] In some embodiments, a rAAV particle disclosed herein comprising a variant AAV (e.g., AAV9) capsid protein comprising a single chain antibody agent provided in **Table 1** can be useful for CNS-targeting, e.g., targeting a rAAV particle comprising a variant AAV (e.g., AAV9) capsid protein to a CNS cell and/or tissue.

[0560] In some embodiments, a single chain antibody agent insertion in a variant AAV capsid protein disclosed herein does not comprise an additional sequence N-terminal of a single chain antibody agent sequence provided in **Table 1**.

[0561] In some embodiments, a single chain antibody agent insertion in a variant AAV capsid protein disclosed herein does not comprise an additional sequence C-terminal of a single chain antibody agent sequence provided in **Table 1**.

[0562] In some embodiments, a single chain antibody agent insertion in a variant AAV capsid protein disclosed herein does not comprise an additional sequence N-terminal and C-terminal of a single chain antibody agent sequence provided in **Table 1**.

#### **Exemplary capsid variants with single chain antibody agent insertions in VR-VIII**

[0563] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises any one of the single chain antibody agent insertions disclosed herein; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-VIII or VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein (e.g., a sequence between the insertion site of a single

chain antibody agent is deleted in a parental AAV9 capsid protein); and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein.

[0564] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **Table 1**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-VIII of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR-VIII of an AAV9 capsid protein. In some embodiments, a VR-VIII of an AAV9 capsid protein comprises amino acids 580 to 601 of VP1, VP2 or VP3. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 589 is deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at

least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0565] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **Table 1**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 461 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 to 460 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at

least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0566] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **Table 1**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 457 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a

single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 and 456 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0567] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **Table 1**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein.

In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 to 460 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 452 to 459 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0568] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 1**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-VIII of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR-VIII of an AAV9 capsid protein. In some embodiments, a VR-VIII of an AAV9 capsid protein comprises amino acids 580 to 601 of VP1, VP2 or VP3. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 589 is deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in **SEQ ID NO: 2001**; or a sequence having at least 95% identity to **SEQ ID NO: 2001**; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises the sequence provided in **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to **SEQ ID NO: 2001**, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces

binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0569] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 1**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 461 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 to 460 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9

capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0570] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 1**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 457 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 and 456 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an

AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0571] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 1**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 to 460 of VP1, VP2, or

VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 452 to 459 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0572] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 5**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-VIII of a parental AAV capsid protein; (4) at least one amino acid is deleted in a

parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR-VIII of an AAV9 capsid protein. In some embodiments, a VR-VIII of an AAV9 capsid protein comprises amino acids 580 to 601 of VP1, VP2 or VP3. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 589 is deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a

binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0573] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 5**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 461 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 to 460 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that

alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0574] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 5**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 457 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 and 456 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid

protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0575] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 5**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 to 460 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 452 to 459 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20

mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0576] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 9**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-VIII of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR-VIII of an AAV9 capsid protein. In some embodiments, a VR-

VIII of an AAV9 capsid protein comprises amino acids 580 to 601 of VP1, VP2 or VP3. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 589 is deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0577] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 9**; (2) a single chain antibody

agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 461 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 to 460 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a

binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0578] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 9**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 457 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 and 456 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that

alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0579] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 9**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 to 460 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 452 to 459 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid

protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0580] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 13**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-VIII of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR-VIII of an AAV9 capsid protein. In some embodiments, a VR-VIII of an AAV9 capsid protein comprises amino acids 580 to 601 of VP1, VP2 or VP3. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 589 is deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a

sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

**[0581]** In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 13**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain

antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 461 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 to 460 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0582] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 13**; (2) a single chain antibody

agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 457 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 and 456 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a

binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0583] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 13**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 to 460 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 452 to 459 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that

alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0584] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 17**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-VIII of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR-VIII of an AAV9 capsid protein. In some embodiments, a VR-VIII of an AAV9 capsid protein comprises amino acids 580 to 601 of VP1, VP2 or VP3. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 589 is deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in **SEQ ID NO: 2001**; or a sequence having at least 95% identity to **SEQ ID NO: 2001**; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations

(e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

**[0585]** In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 17**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 461 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 to 460 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence

having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

**[0586]** In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 17**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain

antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 457 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 and 456 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0587] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 17**; (2) a single chain antibody

agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 to 460 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 452 to 459 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a

binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0588] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 21**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-VIII of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR-VIII of an AAV9 capsid protein. In some embodiments, a VR-VIII of an AAV9 capsid protein comprises amino acids 580 to 601 of VP1, VP2 or VP3. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 589 is deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid

protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0589] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 21**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 461 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 to 460 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations

(e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0590] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 21**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 457 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 and 456 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence

having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0591] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 21**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain

antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 to 460 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 452 to 459 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0592] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 25**; (2) a single chain antibody

agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-VIII of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR-VIII of an AAV9 capsid protein. In some embodiments, a VR-VIII of an AAV9 capsid protein comprises amino acids 580 to 601 of VP1, VP2 or VP3. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 589 is deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in

a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0593] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 25**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 461 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 to 460 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid

protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0594] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 25**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 457 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 and 456 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations

(e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

**[0595]** In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 25**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 to 460 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 452 to 459 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence

having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

**[0596]** In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 29**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-VIII of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain

antibody agent insertion is in VR-VIII of an AAV9 capsid protein. In some embodiments, a VR-VIII of an AAV9 capsid protein comprises amino acids 580 to 601 of VP1, VP2 or VP3. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 589 is deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0597] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a

single chain antibody agent insertion provided in **SEQ ID NO: 29**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 461 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 to 460 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in **SEQ ID NO: 2001**; or a sequence having at least 95% identity to **SEQ ID NO: 2001**; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises the sequence provided in **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to **SEQ ID NO: 2001**, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in

a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

**[0598]** In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 29**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 457 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 and 456 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid

protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0599] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 29**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 to 460 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 452 to 459 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations

(e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

**[0600]** In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 33**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-VIII of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR-VIII of an AAV9 capsid protein. In some embodiments, a VR-VIII of an AAV9 capsid protein comprises amino acids 580 to 601 of VP1, VP2 or VP3. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 589 is deleted. In some embodiments, an AAV9 capsid protein

comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

**[0601]** In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 33**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a

VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 461 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 to 460 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0602] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a

single chain antibody agent insertion provided in **SEQ ID NO: 33**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 457 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 and 456 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in

a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

**[0603]** In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 33**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 to 460 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 452 to 459 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid

protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

**[0604]** In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 37**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-VIII of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR-VIII of an AAV9 capsid protein. In some embodiments, a VR-VIII of an AAV9 capsid protein comprises amino acids 580 to 601 of VP1, VP2 or VP3. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 589 is deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in **SEQ ID NO: 2001**; or a sequence having at least 95% identity to **SEQ ID NO: 2001**; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises a sequence having no

more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0605] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 37**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 461 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 to 460 are deleted. In some embodiments, an AAV9 capsid protein comprises the

sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0606] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 37**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a

VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 457 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 and 456 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0607] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a

single chain antibody agent insertion provided in **SEQ ID NO: 37**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 to 460 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 452 to 459 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in **SEQ ID NO: 2001**; or a sequence having at least 95% identity to **SEQ ID NO: 2001**; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises the sequence provided in **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to **SEQ ID NO: 2001**, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in

a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

**[0608]** In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 41**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-VIII of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR-VIII of an AAV9 capsid protein. In some embodiments, a VR-VIII of an AAV9 capsid protein comprises amino acids 580 to 601 of VP1, VP2 or VP3. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 589 is deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in **SEQ ID NO: 2001**; or a sequence having at least 95% identity to **SEQ ID NO: 2001**; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises the sequence provided in **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to **SEQ ID NO: 2001**, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or

more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

**[0609]** In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 41**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 461 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 to 460 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no

more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0610] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 41**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 457 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 and 456 are deleted. In some embodiments, an AAV9 capsid protein comprises the

sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0611] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 41**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a

VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 to 460 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 452 to 459 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0612] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a

single chain antibody agent insertion provided in **SEQ ID NO: 45**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-VIII of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR-VIII of an AAV9 capsid protein. In some embodiments, a VR-VIII of an AAV9 capsid protein comprises amino acids 580 to 601 of VP1, VP2 or VP3. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 589 is deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in **SEQ ID NO: 2001**; or a sequence having at least 95% identity to **SEQ ID NO: 2001**; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises the sequence provided in **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to **SEQ ID NO: 2001**, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574,

592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0613] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 45**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 461 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 to 460 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or

more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

**[0614]** In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 45**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 457 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 and 456 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no

more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0615] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 45**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 to 460 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 452 to 459 are deleted. In some embodiments, an AAV9 capsid protein comprises the

sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0616] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 49**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-VIII of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a

VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR-VIII of an AAV9 capsid protein. In some embodiments, a VR-VIII of an AAV9 capsid protein comprises amino acids 580 to 601 of VP1, VP2 or VP3. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 589 is deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0617] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV

capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 49**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 461 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 to 460 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574,

592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0618] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 49**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 457 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 and 456 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or

more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

**[0619]** In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 49**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 to 460 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 452 to 459 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no

more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0620] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 158**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-VIII of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR-VIII of an AAV9 capsid protein. In some embodiments, a VR-VIII of an AAV9 capsid protein comprises amino acids 580 to 601 of VP1, VP2 or VP3. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid

protein, e.g., amino acids 589 is deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0621] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 158**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental

AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 461 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 to 460 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0622] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV

capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 158**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 457 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 and 456 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574,

592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0623] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 158**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 to 460 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 452 to 459 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or

more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

**[0624]** In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 159**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-VIII of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR-VIII of an AAV9 capsid protein. In some embodiments, a VR-VIII of an AAV9 capsid protein comprises amino acids 580 to 601 of VP1, VP2 or VP3. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 589 is deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in **SEQ ID NO: 2001**; or a sequence having at least 95% identity to **SEQ ID NO: 2001**; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to **SEQ**

ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0625] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 159**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 461 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino

acids 455 to 460 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0626] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 159**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental

AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 457 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 and 456 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0627] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV

capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 159**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 to 460 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 452 to 459 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574,

592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0628] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 160**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-VIII of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR-VIII of an AAV9 capsid protein. In some embodiments, a VR-VIII of an AAV9 capsid protein comprises amino acids 580 to 601 of VP1, VP2 or VP3. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 589 is deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ

ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0629] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 160**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 461 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 to 460 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ

ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0630] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 160**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 457 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino

acids 455 and 456 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0631] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 160**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental

AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 to 460 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 452 to 459 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0632] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV

capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 116**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-VIII of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR-VIII of an AAV9 capsid protein. In some embodiments, a VR-VIII of an AAV9 capsid protein comprises amino acids 580 to 601 of VP1, VP2 or VP3. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 589 is deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in **SEQ ID NO: 2001**; or a sequence having at least 95% identity to **SEQ ID NO: 2001**; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises the sequence provided in **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to **SEQ ID NO: 2001**, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid

protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0633] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 116**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 461 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 to 460 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ

ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0634] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 116**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 457 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 and 456 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ

ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0635] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 116**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 to 460 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino

acids 452 to 459 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0636] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 118**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-VIII of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental

AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR-VIII of an AAV9 capsid protein. In some embodiments, a VR-VIII of an AAV9 capsid protein comprises amino acids 580 to 601 of VP1, VP2 or VP3. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 589 is deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0637] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 118**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 461 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 to 460 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some

embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0638] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 118**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 457 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 and 456 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at

least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0639] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 118**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 to 460 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 452 to 459 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at

least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0640] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 120**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-VIII of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR-VIII of an AAV9 capsid protein. In some embodiments, a VR-VIII of an AAV9 capsid protein comprises amino acids 580 to 601 of VP1, VP2 or VP3. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and

590 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 589 is deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0641] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 120**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a

parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 461 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 to 460 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0642] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 120**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 457 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 and 456 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some

embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0643] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 120**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 to 460 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 452 to 459 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at

least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

**[0644]** In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 121**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-VIII of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR-VIII of an AAV9 capsid protein. In some embodiments, a VR-VIII of an AAV9 capsid protein comprises amino acids 580 to 601 of VP1, VP2 or VP3. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 589 is deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an

AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0645] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 121**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 461 of VP1, VP2, or

VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 to 460 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0646] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 121**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a

parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 457 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 and 456 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0647] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 121**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 to 460 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 452 to 459 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some

embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0648] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 122**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-VIII of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR-VIII of an AAV9 capsid protein. In some embodiments, a VR-VIII of an AAV9 capsid protein comprises amino acids 580 to 601 of VP1, VP2 or VP3. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 589 is deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9

capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0649] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 122**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 461 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 to 460 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an

AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0650] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 122**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 457 of VP1, VP2, or

VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 and 456 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0651] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 122**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a

parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 to 460 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 452 to 459 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0652] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 123**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-VIII of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR-VIII of an AAV9 capsid protein. In some embodiments, a VR-VIII of an AAV9 capsid protein comprises amino acids 580 to 601 of VP1, VP2 or VP3. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 589 is deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in **SEQ ID NO: 2001**; or a sequence having at least 95% identity to **SEQ ID NO: 2001**; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises the sequence provided in **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to **SEQ ID NO: 2001**, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces

binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0653] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 123**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 461 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 to 460 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9

capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0654] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 123**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 457 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 and 456 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an

AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0655] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 123**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 to 460 of VP1, VP2, or

VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 452 to 459 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0656] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 126**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-VIII of a parental AAV capsid protein; (4) at least one amino acid is deleted in a

parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR-VIII of an AAV9 capsid protein. In some embodiments, a VR-VIII of an AAV9 capsid protein comprises amino acids 580 to 601 of VP1, VP2 or VP3. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 589 is deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a

binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0657] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 126**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 461 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 to 460 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that

alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0658] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 126**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 457 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 and 456 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid

protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0659] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 126**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 to 460 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 452 to 459 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20

mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0660] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 129**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-VIII of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR-VIII of an AAV9 capsid protein. In some embodiments, a VR-

VIII of an AAV9 capsid protein comprises amino acids 580 to 601 of VP1, VP2 or VP3. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 589 is deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0661] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 129**; (2) a single chain antibody

agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 461 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 to 460 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a

binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0662] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 129**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 457 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 and 456 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that

alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0663] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 129**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 to 460 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 452 to 459 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid

protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0664] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 132**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-VIII of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR-VIII of an AAV9 capsid protein. In some embodiments, a VR-VIII of an AAV9 capsid protein comprises amino acids 580 to 601 of VP1, VP2 or VP3. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 589 is deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a

sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

**[0665]** In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 132**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain

antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 461 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 to 460 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0666] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 132**; (2) a single chain antibody

agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 457 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 and 456 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a

binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0667] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 132**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 to 460 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 452 to 459 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that

alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0668] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 135**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-VIII of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR-VIII of an AAV9 capsid protein. In some embodiments, a VR-VIII of an AAV9 capsid protein comprises amino acids 580 to 601 of VP1, VP2 or VP3. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 589 is deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in **SEQ ID NO: 2001**; or a sequence having at least 95% identity to **SEQ ID NO: 2001**; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations

(e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

**[0669]** In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 135**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 461 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 to 460 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence

having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

**[0670]** In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 135**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain

antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 457 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 and 456 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0671] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 135**; (2) a single chain antibody

agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 to 460 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 452 to 459 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a

binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0672] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 136**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-VIII of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR-VIII of an AAV9 capsid protein. In some embodiments, a VR-VIII of an AAV9 capsid protein comprises amino acids 580 to 601 of VP1, VP2 or VP3. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 589 is deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid

protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0673] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 136**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 461 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 to 460 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations

(e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

**[0674]** In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 136**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 457 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 and 456 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence

having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0675] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 136**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain

antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 to 460 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 452 to 459 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0676] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 137**; (2) a single chain antibody

agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-VIII of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR-VIII of an AAV9 capsid protein. In some embodiments, a VR-VIII of an AAV9 capsid protein comprises amino acids 580 to 601 of VP1, VP2 or VP3. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 589 is deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in

a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0677] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 137**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 461 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 to 460 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid

protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0678] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 137**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 457 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 and 456 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations

(e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

**[0679]** In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 137**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 to 460 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 452 to 459 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence

having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

**[0680]** In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 138**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-VIII of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain

antibody agent insertion is in VR-VIII of an AAV9 capsid protein. In some embodiments, a VR-VIII of an AAV9 capsid protein comprises amino acids 580 to 601 of VP1, VP2 or VP3. In some embodiments, a single chain antibody agent insertion is located between amino acids 588 and 590 of VP1, VP2, or VP3 of an AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 589 is deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

**[0681]** In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a

single chain antibody agent insertion provided in **SEQ ID NO: 138**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 461 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 to 460 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in **SEQ ID NO: 2001**; or a sequence having at least 95% identity to **SEQ ID NO: 2001**; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises the sequence provided in **SEQ ID NO: 2001**. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to **SEQ ID NO: 2001**, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in

a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

**[0682]** In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 138**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 454 to 457 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 455 and 456 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid

protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

[0683] In some embodiments, a rAAV particle disclosed herein comprises a variant AAV capsid protein comprising a single chain antibody agent insertion relative to a parental AAV capsid protein. In some embodiments, (1) a single chain antibody agent insertion comprises a single chain antibody agent insertion provided in **SEQ ID NO: 138**; (2) a single chain antibody agent comprises one or more linkers disclosed herein; (3) a single chain antibody agent insertion site is in a VR-IV of a parental AAV capsid protein; (4) at least one amino acid is deleted in a parental AAV capsid protein; and (5) a parental AAV capsid protein comprises an AAV9 capsid protein. In some embodiments, the single chain antibody agent is inserted in a VP1 of a parental AAV capsid protein. In some embodiments, the single chain antibody agent is not inserted in a VP2 and/or VP3 of a parental AAV capsid protein. In some embodiments, a single chain antibody agent insertion is in VR- IV of an AAV9 capsid protein. In some embodiments, a single chain antibody agent insertion is located between amino acids 451 to 460 of VP1, VP2, or VP3 of a parental AAV9 capsid protein. In some embodiments, a sequence between the insertion site of a single chain antibody agent is deleted in a parental AAV9 capsid protein, e.g., amino acids 452 to 459 are deleted. In some embodiments, an AAV9 capsid protein comprises the sequence of a wild-type AAV9 capsid protein provided in SEQ ID NO: 2001; or a sequence having at least 95% identity to SEQ ID NO: 2001; or a sequence having no more than 20 mutations (e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having no more than 1, no more than 2, no more than 5, no more than 10, or no more than 20 mutations

(e.g., substitutions) as compared to SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises the sequence provided in SEQ ID NO: 2001. In some embodiments, an AAV9 capsid protein comprises a sequence having at least 95% identity (e.g., at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 99.5% identity) to SEQ ID NO: 2001, and one or more mutations, e.g., as disclosed herein. In some embodiments, one or more mutations comprises a mutation that alters a binding profile of a parental AAV capsid protein (e.g., binding to one or more tissues). In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein comprises a mutation that reduces binding to one or more tissues such as liver tissue, e.g., a liver-detargeting mutation. In some embodiments, one or more mutations that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a mutation at position 503, 595, 457, 574, 592, 498, 602, 468, or 500 of a VP1 of an AAV9 capsid protein or the corresponding position in a VP2 or VP3 protein or a combination thereof. In some embodiments, a mutation that alters a binding profile of a parental AAV capsid protein (e.g., a liver de-targeting mutation) comprises a W503R mutation.

### **Characterization of variant AAV capsid proteins**

**[0684]** A variant AAV capsid protein disclosed herein can have enhanced CNS-tropism. In some embodiments, a variant AAV capsid protein can be present in a rAAV particle. In some embodiments, a rAAV particle comprising a variant AAV capsid protein disclosed herein is characterized in that when administered to a cell or tissue or subject, a variant AAV capsid protein confers increased infectivity and/or transduction of a CNS cell or tissue compared to: (1) infectivity and/or transduction of a CNS cell by a control AAV particle comprising a corresponding parental AAV capsid protein; or (2) infectivity and/or transduction of a CNS cell which does not express a receptor recognized by a variant AAV capsid protein (e.g., hTfR1) with variant AAV capsid protein.

**[0685]** In some embodiments, a rAAV particle comprising a variant AAV capsid protein confers at least 1.5-fold, at least 2-fold, at least 2.5-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 30-fold, at least 40-fold, at least 50-fold, at least 60-fold,

at least 70-fold, at least 80-fold, at least 90-fold, at least 100-fold, at least 120-fold, at least 140-fold, at least 160-fold, at least 180-fold, at least 200-fold, at least 250-fold, at least 300-fold, at least 400-fold, at least 500-fold, at least 600-fold, at least 700-fold, at least 800-fold, at least 900-fold, at least 1000-fold, at least 1500-fold, at least 2000-fold, at least 4000-fold increased infectivity and/or transduction of a CNS cell or tissue compared to: (1) infectivity and/or transduction of a CNS cell by a control AAV particle comprising a corresponding parental AAV capsid protein; or (2) infectivity and/or transduction of a CNS cell which does not express a receptor recognized by a variant AAV capsid protein (e.g., hTfR1) with variant AAV capsid protein.

**[0686]** In some embodiments, a rAAV particle comprising a variant AAV capsid protein confers about 1.5-fold, about 2-fold, about 2.5-fold, about 3-fold, about 4-fold, about 5-fold, about 6-fold, about 7-fold, about 8-fold, about 9-fold, about 10-fold, about 15-fold, about 20-fold, about 25-fold, about 30-fold, about 40-fold, about 50-fold, about 60-fold, about 70-fold, about 80-fold, about 90-fold, about 100-fold, about 120-fold, about 140-fold, about 160-fold, about 180-fold, about 200-fold, about 250-fold, about 300-fold, about 400-fold, about 500-fold, about 600-fold, about 700-fold, about 800-fold, about 900-fold, about 1000-fold, about 1500-fold, about 2000-fold, about 4000-fold, increased infectivity and/or transduction of a CNS cell or tissue compared to: (1) infectivity and/or transduction of a CNS cell by a control AAV particle comprising a corresponding parental AAV capsid protein; or (2) infectivity and/or transduction of a CNS cell which does not express a receptor recognized by a variant AAV capsid protein (e.g., hTfR1) with variant AAV capsid protein.

**[0687]** In some embodiments, a rAAV particle comprising a variant AAV capsid protein confers 1.5-fold, 2-fold, 2.5-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 15-fold, 20-fold, 25-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 120-fold, 140-fold, 160-fold, 180-fold, 200-fold, 250-fold, 300-fold, 400-fold, 500-fold, 600-fold, 700-fold, 800-fold, 900-fold, 1000-fold, 1500-fold, 2000-fold, 4000-fold, increased infectivity and/or transduction of a CNS cell or tissue compared to: (1) infectivity and/or transduction of a CNS cell by a control AAV particle comprising a corresponding parental AAV capsid protein; or (2) infectivity and/or transduction of a CNS cell which does not express a receptor recognized by a variant AAV capsid protein (e.g., hTfR1) with variant AAV capsid protein.

[0688] In some embodiments, a variant AAV capsid protein is an AAV9 variant capsid protein.

[0689] In some embodiments, a rAAV particle comprising a variant AAV capsid protein disclosed herein and a heterologous nucleic acid comprising a nucleotide sequence encoding a payload is characterized in that when administered to a cell or tissue or subject, delivery of a payload is enhanced to a CNS cell or tissue as compared to delivery of a similar payload with an otherwise similar AAV particle without a variant AAV capsid protein disclosed herein.

[0690] In some embodiments, a rAAV particle comprising a variant AAV capsid protein disclosed herein is characterized in that when administered to a cell or tissue or subject, increased expression of a payload is observed compared to expression of a similar payload with an otherwise similar AAV particle without a variant AAV capsid protein disclosed herein. In some embodiments, expression of a payload is increased by at least 1.5-fold, at least 2-fold, at least 2.5-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, at least 10-fold, at least 15-fold, at least 20-fold, at least 30-fold, at least 40-fold, at least 50-fold, at-least 60-fold, at-least 70-fold, at-least 80-fold, at-least 90-fold, or at-least 100-fold.

[0691] In some embodiments, expression of a payload is increased by about 1.5-fold, about 2-fold, about 2.5-fold, about 3-fold, about 4-fold, about 5-fold, about 6-fold, about 7-fold, about 8-fold, about 9-fold, about 10-fold, about 15-fold, about 20-fold, about 30-fold, about 40-fold, about 50-fold, about 60-fold, about 70-fold, about 80-fold, about 90-fold, or about 100-fold.

[0692] In some embodiments, expression of a payload is increased by 1.5-fold, 2-fold, 2.5-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, or 100-fold.

[0693] In some embodiments, expression of a payload is increased by about 1.5-fold to about 100-fold, about 1.5-fold to about 90-fold, about 1.5-fold to about 80-fold, about 1.5-fold to about 70-fold, about 1.5-fold to about 60-fold, about 1.5-fold to about 50-fold, about 1.5-fold to about 40-fold, about 1.5-fold to about 30-fold, about 1.5-fold to about 20-fold, about 1.5-fold to about 15-fold, about 1.5-fold to about 10-fold, about 1.5-fold to about 9-fold, about 1.5-fold to about 8-fold, about 1.5-fold to about 7-fold, about 1.5-fold to about 6-fold, about 1.5-fold to about 5-fold, about 1.5-fold to about 4-fold, about 1.5-fold to about 3-fold, about 1.5-fold to

about 2-fold, about 2-fold to about 100-fold, about 3-fold to about 100-fold, about 4-fold to about 100-fold, about 5-fold to about 100-fold, about 6-fold to about 100-fold, about 7-fold to about 100-fold, about 8-fold to about 100-fold, about 9-fold to about 100-fold, about 10-fold to about 100-fold, about 15-fold to about 100-fold, about 20-fold to about 100-fold, about 30-fold to about 100-fold, about 40-fold to about 100-fold, about 50-fold to about 100-fold, about 60-fold to about 100-fold, about 70-fold to about 100-fold, about 80-fold to about 100-fold, about 90-fold to about 100-fold.

### **CNS cells and/or tissues for delivering AAV particles comprising variant AAV capsid proteins**

**[0694]** Disclosed herein are variant AAV capsid proteins which have enhanced tropism to CNS cells and/or tissue. In some embodiments, variant AAV capsid proteins disclosed herein can be used for targeting CNS cells and/or tissue. In some embodiments, a CNS cell or tissue is chosen from: a CNS epithelial cell, an endothelial cell associated with a blood brain barrier, a nerve cell, a CNS connective tissue cell, a stem cell, a progenitor cell, a CNS immune cell, a spinal cord cell, a cell that lines one or more brain ventricles, a nerve support cell, a glial cell, a fat cell, a meninges cell, or combinations thereof.

**[0695]** In some embodiments, a CNS cell comprises an endothelial cell associated with a blood brain barrier. In some embodiments, an endothelial cell associated with a blood brain barrier is part of, or forms a blood brain barrier. In some embodiments, an endothelial cell associated with a blood brain barrier is or comprises a brain capillary endothelial cell (BCEC). In some embodiments, an endothelial cell associated with a blood brain barrier is or comprises a brain microvascular endothelial cells (BMEC). In some embodiments, an endothelial cell associated with a blood brain barrier expresses a hTfR1 or a fragment or variant thereof.

**[0696]** In some embodiments, a CNS cell comprises a CNS epithelial cell. In some embodiments, a CNS epithelial cell comprises a cell that lines one or more brain ventricles.

**[0697]** In some embodiments, a CNS cell comprises a nerve cell (neuron). In some embodiments, a neuron comprises a unipolar neuron, a bipolar neuron, a pseudounipolar neuron, a multipolar neuron, or combinations thereof. In some embodiments, a neuron is a motor neuron,

a sensory neuron, an interneuron, an excitatory neuron, an inhibitor neuron, a sympathetic neuron, a parasympathetic neuron, or combinations thereof. In some embodiments, a neuron comprises a pyramidal neuron, a dopaminergic neuron, a cholinergic neurons, an adrenergic neuron, a GABAergic neuron, a glutamatergic neuron, a serotonergic neuron, a purinergic neuron, a histaminergic neuron, a lower motor neuron, or combinations thereof.

[0698] In some embodiments, a nerve cell (neuron) comprises nerve support cells. In some embodiments, nerve support cells comprise glial cells. In some embodiments, glial cells comprise astrocytes, microglial cells, ependymal cells, oligodendrocytes, Schwann cells, or combinations thereof.

[0699] In some embodiments, a CNS cell comprises a CNS connective tissue cell. In some embodiments, a CNS connective tissue cell comprises fat cells or meninges cells, or both.

[0700] In some embodiments, a CNS cell comprises a stem cell or progenitor cell. In some embodiments, a stem cell comprises a neural stem cell.

[0701] In some embodiments, a CNS cell comprises a cell that lines one or more brain ventricles.

[0702] In some embodiments, a CNS cell comprises a meninges cell.

[0703] In some embodiments, a CNS cell comprises a fat cell.

[0704] In some embodiments, a CNS tissue comprises tissue found in: cortex, thalamus, hypothalamus, striatum, putamen, caudate nucleus, hippocampus, entorhinal cortex, basal ganglia, deep cerebellar nuclei, or other parts of the brain and/or spinal cord.

[0705] In some embodiments, CNS tissue comprises tissue found in a frontal cortex, a parietal cortex, an occipital cortex, a temporal cortex or combinations thereof.

#### **Payloads for use in AAV particles comprising a variant AAV capsid protein**

[0706] A rAAV particle comprising a variant AAV capsid protein disclosed herein can also comprise a heterologous nucleic acid sequence comprising a nucleotide sequence encoding a payload.

[0707] In some embodiments, a payload is or comprises a polypeptide, e.g., encoded by a nucleic acid sequence within an rAAV particle. In some embodiments, a payload polypeptide is chosen from: a CRISPR-Cas protein, a Zinc finger protein, a TAL, a base editor, a prime editor, a meganuclease, or any combination thereof.

[0708] In some embodiments, a polypeptide is or comprises a CRISPR-Cas protein. In some embodiments, a CRISPR-Cas protein is chosen from: a Type II, Type V or Type VI CRISPR-Cas protein, e.g., a Cas9 protein, a Cas12a protein, a Cas12b protein, a Cas12c protein, a Cas12d protein, a Cas12e protein, a Cas12f protein, a Cas12g protein, a Cas12h protein, a Cas12i protein, a Cas13a protein, a Cas13b protein or a variant or fragment thereof of any of the foregoing. In some embodiments a CRISPR-Cas protein is fused with one or more domains, e.g., an activator domain and/or a repressor domain. In some embodiments a CRISPR-Cas protein is a nuclease. In some embodiment a CRISPR-Cas protein is a nickase and only cleaves one strand of a target nucleic acid molecule. In some embodiment a CRISPR-Cas protein is inactivated and binds to but does not cleave a target nucleic acid molecule.

[0709] In some embodiments, a polypeptide is or comprises a Zinc finger protein, or a variant or fragment thereof. In some embodiments, a Zinc finger protein is chosen from: a Zinc finger nuclease, an artificial restriction enzyme fusion protein, a sequence-targeted zinc-finger DNA-binding unit optionally fused with a nuclease domain (e.g., Fok1 nuclease domain), or a variant or fragment or combination of any of the foregoing. In some embodiments a Zinc finger protein is fused with one or more domains, e.g., an activator domain and/or a repressor domain.

[0710] In some embodiments, a polypeptide is or comprises a Transcription Activator-Like Effector (TAL) protein, or a variant or fragment thereof. In some embodiments, a TAL comprises: a TAL effector DNA binding domain (e.g., a TAL effector DNA binding domain isolated from *Xanthomonas* spp.), a Transcription Activator-Like Effector Nuclease (TALEN), e.g., a TAL effector DNA binding domain fused with a nuclease domain (e.g., Fok1 nuclease domain), or a variant or fragment or combination of any of the foregoing. In some embodiments a TAL protein is fused with one or more domains, e.g., an activator domain and/or a repressor domain.

[0711] In some embodiments, a polypeptide is or comprises a base editor, or a variant or fragment thereof. In some embodiments, a base editor comprises a deaminase, an adenosine

deaminase enzyme (ABE), a cytosine deaminase enzyme (CBE), an APOBEC1, an APOBEC3A, an APOBEC3G, an evoAPOBEC, a BE4-YE1, a CDA1, an activation-induced cytidine deaminase (AID), a mutant TadA, an adenosine deaminases (TadA\*), an E. coli tRNA-specific adenosine deaminase (TadA), a deaminase associated with a DNA binding domain monomer, a base editing enzyme that is RNA guided, a DNA glycosylase inhibitor, one or more DNA glycosylase inhibitor domains, a 5-methylcytosine deaminase, a cytidine deaminase domain, an adenine deaminase domain, an adenosine base editor (ABE), a Target-ACEmax, a synchronous programmable adenine and cytosine editor (SPACE), an A&C-Bemax., a circularly permuted base editor, an adenosine deaminase enzyme (ADAR), a RNA editing for programmable adenosine to inosine replacement (REPAIR), a leveraging endogenous ADAR for programmable editing of RNA (LEAPER) or a variant or fragment or combination of any of the foregoing. In some embodiments, the payload also comprises a guide RNA, gRNA, sgRNA, or crRNA/tracrRNA that interacts with the base editor.

**[0712]** In some embodiments, a polypeptide is or comprises a prime editor, or a variant or fragment thereof, or a system comprising the same. In some embodiments, a prime editor and/or system comprising the same comprises: a reverse transcriptase, a prime editing enzyme, an editing enzyme that includes a reverse transcriptase domain, an Avian Myeloblastosis Virus (AMV) Reverse Transcriptase, a Murine Leukemia Virus (MLV) Reverse Transcriptase, a HIV-1 reverse transcriptase, a bacterial reverse transcriptase, a reverse transcriptase associated with a DNA binding domain and/or protein, a reverse transcriptase fused to a DNA binding domain that is a catalytically impaired nuclease domain (e.g., a nickase), a prime editing 1 system (PE1), a prime editing 2 system (PE2), a prime editing 3 system (PE3), a prime editing 3b system (PE3b) or a variant or fragment or combination of any of the foregoing. In some embodiments, the payload also comprises a prime editing gRNA (pegRNA) or an extended sgRNA that interacts with the prime editor.

**[0713]** In some embodiments, a polypeptide is or comprises a meganuclease, or a variant or fragment thereof. In some embodiments, a meganuclease is chosen from: a homing endonuclease, a LAGLIDADG family meganuclease, a GIYYIG family meganuclease, a His-Cyst box family meganuclease, or HNH family endonuclease, an I-SceI, an I-CeuI, a PI-PspI, a

PI-SceI, an I-SceIV, an I-CsmI, an I-PanI, an I-SceII, an I-PpoI, an I-SceIII, an I-CreI, an I-TevI, an I-TevII, an I-TevIII or a variant or fragment or combination of any of the foregoing

[0714] In some embodiments, a polypeptide is associated with a CNS disorder. In some embodiments, a CNS disorder is a CNS disorder disclosed herein. In some embodiments, a CNS disorder is chosen from Friedreich's Ataxia, Dravet Syndrome, Spinocerebellar Ataxia Type 3, Niemann-Pick Type C, Huntington's Disease, Pompe Disease, Myotonic Dystrophy Type 1, Glut1 Deficiency Syndrome (De Vivo Syndrome), Tay-Sachs, Spinal Muscular Atrophy, Alzheimer's disease, Amyotrophic lateral sclerosis (ALS), Danon disease, Rett Syndrome, Angelman Syndrome, Parkinson's disease, tauopathies, genetic epilepsies (e.g., a STXBP1 genetic epilepsy, or a CDKL5 genetic epilepsy), or any combination thereof.

[0715] In some embodiments, a polypeptide is an enzyme. In some embodiments, an enzyme is a lysosomal enzyme or an adenosine deaminase enzyme.

[0716] In some embodiments, a polypeptide is an antibody.

[0717] In some embodiments, a polypeptide is a secreted protein.

[0718] In some embodiments, a payload is or comprises an RNA molecule. In some embodiments, an RNA molecule is an siRNA, a miRNA, a gRNA, antisense RNA, circular RNA, an snRNA or an aptamer, or combinations thereof.

[0719] In some embodiments, a payload is or comprises a DNA molecule. In some embodiments, a DNA molecule comprises a nucleic acid sequence of up to 5,100 nt in length, e.g., up to about 5,000 nt, up to about 4,900, up to about 4,800, up to about 4,700, up to about 4,600, up to about 4,500, up to about 4,400, etc.

[0720] In some embodiments, an RNA molecule targets a nucleic acid molecule encoding a polypeptide associated with a CNS disorder. In some embodiments, a CNS disorder is a CNS disorder disclosed herein. In some embodiments, a CNS disorder is chosen from Friedreich's Ataxia, Dravet Syndrome, Spinocerebellar Ataxia Type 3, Niemann-Pick Type C, Huntington's Disease, Pompe Disease, Myotonic Dystrophy Type 1, Glut1 Deficiency Syndrome (De Vivo Syndrome), Tay-Sachs, Spinal Muscular Atrophy, Alzheimer's disease, Amyotrophic lateral sclerosis (ALS), Danon disease, Rett Syndrome, Angelman Syndrome, genetic epilepsies (e.g., a STXBP1 genetic epilepsy, or a CDKL5 genetic epilepsy), or combinations thereof.

**Promoters for use in AAV particles comprising a variant AAV capsid protein**

[0721] A rAAV particle comprising a variant AAV capsid protein disclosed herein can comprise a heterologous a nucleotide sequence encoding a payload which is operably linked to a promoter, or a variant or a fragment thereof.

[0722] In some embodiments, a promoter is a CNS cell or tissue-specific promoter, or a variant or a fragment thereof. In some embodiments, a CNS promoter is chosen from: a Glial Fibrillary Acidic Protein (GFAP) promoter or a variant or a fragment thereof, a synapsin-1 (SYN1) promoter or a variant or a fragment thereof, a neuron-specific enolase/RU5' (NSE/RU5') promoter or a variant or a fragment thereof, a neuroactive peptide cholecystokinin (CCK) promoter or a variant or a fragment thereof, a myelin basic promoter (MBP) or a variant or a fragment thereof, a human myelin associated glycoprotein promoter or a variant or a fragment thereof, a phosphate-activated glutaminase (PAG) promoter or a variant or a fragment thereof, a vesicular glutamate transporter (vGLUT) promoter or a variant or a fragment thereof, a glutamic acid decarboxylase (GAD) promoter or a variant or a fragment thereof, or any combination thereof.

[0723] In some embodiments, a CNS promoter is a human SYN1 (hSYN1) promoter or a variant or a fragment thereof. An exemplary hSYN1 promoter sequence is provided as SEQ ID NO: 62.

[0724] AGTGCAAGTGGTTTAGGACCAGGATGAGGCGGGTGGGGTGCCT  
ACCTGACGACCGACCCCGACCCACTGGACAAGCACCCAACCCCCATTCCCCAAATT  
GCGCATCCCCTATCAGAGAGGGGGAGGGAAACAGGATGCGCGAGGCGCGTGC  
CACTGCCAGCTTCAGCACCGCGAACAGTGCCTCGCCCCGCTGGCGCGCGCC  
ACCGCCGCCTCAGCACTGAAGGCGCGTGCAGTCACTCGCCGGCCCCGCAAAC  
CCCTTCCCGGCCACCTGGTCGCGTCCGCGCCGCCGGCCAGCCGGACCGCACC  
ACGCGAGGCGCGAGATAAGGGGGCACGGCGCGACCATCTGCCTGCGGCGCCGG  
CGACTCAGCGCTGCCTCAGTCTGCCTGGCAGCGGAGGAGTCGTGTCGTGCCTGA  
GAGCGCAG (SEQ ID NO: 62)

[0725] In some embodiments, a CNS promoter is a GFAP promoter or a variant or a fragment thereof. An exemplary GFAP promoter sequence is provided as SEQ ID NO: 63.

[0726] AACATATCCTGGTGTGGAGTAGGGGACGCTGCTCTGACAGAGGCTCGG  
GGGCCTGAGCTGGCTCTGTGAGCTGGGAGGAGGCAGACAGCCAGGCCTGTCTGC  
AAGCAGACCTGGCAGCATTGGCTGGCCGCCCCCAGGGCCTCCTCTCATGCCAG  
TGAATGACTCACCTTGGCACAGACACAATGTTGGGTGGCACAGTGCCTGCTTCC  
CGCCGCACCCCAGCCCCCTCAAATGCCTTCCGAGAAGCCCATTGAGCAGGGGCTT  
GCATTGCACCCCAGCCTGACAGCCTGGCATCTTGGATAAAAGCAGCACAGCCCC  
TAGGGGCTGCCCTGCTGTGGGCCACCGGCGTGGAGAACAAAGGCTCTATTCA  
CCTGTGCCAGGAAAGGGATCAGGGATGCCAGGCATGGACAGTGGGTGGCAGG  
GGGGAGAGGAGGGCTGTCTGCTCCCAGAAGTCCAAGGACACAAATGGTGAGGG  
GAGAGCTCTCCCCATAGCTGGCTGCCACCCAGGCATGCCAGTCTAGCCCAC  
GGGTGTTGCCAGGGCACCCGGGCATGCCAGTCTAGCCCACCTTCATAAAGCC  
CTCGCATCCCAGGAGCGAGCAGAGCCAGAGCAGGGTTGGAGAGGAGACGCATCAC  
CCGCTGCTCGC (SEQ ID NO: 63)

[0727] In some embodiments, a CNS promoter is a chicken beta actin hybrid (CBh) promoter or a variant or a fragment thereof. An exemplary CBh promoter sequence is provided as SEQ ID NO: 64.

[0728] CGTTACATAACTTACGGTAATGGCCCGCCTGGCTGACCGCCCAACGA  
CCCCCGCCCATTGACGTCAATAGTAACGCCAATAGGGACTTCCATTGACGTCAATG  
GGTGGAGTATTACGGTAAACTGCCACTTGGCAGTACATCAAGTGTATCATATGCC  
AAAGTACGCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTGTGCCA  
GTACATGACCTTATGGACTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCT  
ATTACCATGGTCGAGGTGAGCCCCACGTTCTGCTCACTCTCCCATCTCCCCCCC  
CCCCACCCCCAATTGTATTATTATTAAATTATTTGTGCAGCGATGGGGC  
GGGGGGGGGGGGGGGGCGCGCCAGGCAGGGCGGGCGGGCGAGGGCGGG  
CGGGCGAGGCAGGAGAGGTGCGCGGCAGCCAATCAGAGCGCGCGCTCCGAAAG  
TTCCCTTTATGGCGAGGCAGGCAGGCAGGCAGGCAGGCAGGCAGGCAGGC  
CGGGCGGGAGTCGCTGCGACGCTGCCTCGCCCCGTGCCCGCTCCGCCGCC  
CGCGCCGCCGCCGCCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGG

GACGGCCCTTCTCCTCCGGGCTGTAATTAGCTGAGCAAGAGGTAAGGGTTAAGGG  
ATGGTTGGTGGTGGGGTATTAATGTTAACCTGGAGCACCTGCCTGAAATCAC  
TTTTTTTCAG (SEQ ID NO: 64)

### Uses of AAV particles comprising a variant AAV capsid protein

[0729] The present disclosure, among other things, provides methods of delivering a composition (e.g., a pharmaceutical composition) comprising a plurality of rAAV particles as described herein to a cell or tissue, e.g., a CNS cell or tissue. The present disclosure also provides methods of treating a subject with a composition (e.g., a pharmaceutical composition) comprising a plurality of rAAV particles produced using a method or system described herein.

[0730] In some embodiments, disclosed herein is a method for treating a CNS disorder in a subject comprising administering a composition (e.g., a pharmaceutical composition) comprising a plurality of rAAV particles described herein.

[0731] In some embodiments, also disclosed herein is a method for amelioration a symptom of a CNS disorder in a subject comprising administering a composition (e.g., a pharmaceutical composition) comprising a plurality of rAAV particles described herein.

[0732] In some embodiments, a CNS disorder is a result of a genetic abnormality.

[0733] In some embodiments, a CNS disorder is not a result of a genetic abnormality.

[0734] In some embodiments, a CNS disorder is a CNS disorder disclosed herein.

[0735] Exemplary CNS disorders are disclosed in International Patent Application PCT/US2019/054345 filed on October 2, 2019, the entire contents of which is hereby incorporated by reference.

[0736] In some embodiments, a CNS disorder is chosen from one or more of: Friedreich's Ataxia, Dravet Syndrome, Spinocerebellar Ataxia Type 3, Niemann-Pick Type C, Huntington's Disease, Pompe Disease, Myotonic Dystrophy Type 1, Glut1 Deficiency Syndrome (De Vivo Syndrome), Tay-Sachs, Spinal Muscular Atrophy, Alzheimer's disease, Amyotrophic lateral sclerosis (ALS), Danon disease, Rett Syndrome, Angelman Syndrome, Parkinson's disease, tauopathies or genetic epilepsies (e.g., a STXBP1 genetic epilepsy, or a CDKL5 genetic

epilepsy). In some embodiments, a CNS disorder is chosen from one or more of: (a) neuro-muscular disease, e.g., Amyotrophic lateral sclerosis (ALS) or Huntington's disease, or a Myotonic Dystrophy; (b) Alzheimer's disease; (c) an expanded repeat disease, e.g., Huntington's disease or a Myotonic Dystrophy, or (d) a combination of any one of or all of (a)-(c).

[0737] In some embodiments, a composition (e.g., a pharmaceutical composition) comprising a plurality of rAAV particles as described herein is administered to a subject suffering from or at risk of a disease, disorder, or condition. In some embodiments, a composition (e.g., a pharmaceutical composition) comprising a plurality of rAAV particles as described herein is administered in combination with one or more additional therapeutics agents to a subject. In some embodiments, a composition (e.g., a pharmaceutical composition) comprising a plurality of rAAV particles as described herein is contacted with an organ, tissue, or cells *ex vivo*. The organ, tissue, or cells can be introduced into a subject and can be protected from damage that would otherwise be caused by the recipient's immune system.

[0738] In some embodiments, methods and kits of the present invention may be used for the evaluation and/or monitoring of gene therapy. In some embodiments, gene therapy comprises administration of a composition (e.g., a pharmaceutical composition) comprising a plurality of rAAV particles described herein. In some embodiments, samples for evaluating and/or monitoring gene therapy may be obtained prior to the initiation of gene therapy. In some embodiments, samples are obtained after a first gene therapy treatment or dose. In some embodiments, samples are obtained after the conclusion of gene therapy. In some embodiments, samples are obtained at specific time points, intervals, or any other metric of time before, during, or after gene therapy is performed.

### **Methods of Making Anti-TfR1 Antibodies**

[0739] The anti-TfR1 antibodies described herein can be produced by any suitable method known in the art. Such methods range from direct protein synthesis methods to constructing a DNA sequence encoding polypeptide sequences and expressing those sequences in a suitable host. In some embodiments, a DNA sequence is constructed using recombinant technology by isolating or synthesizing a DNA sequence encoding a wild-type protein of interest. Optionally, the sequence can be mutagenized by site-specific mutagenesis to provide

functional variants thereof. In some embodiments, a DNA sequence encoding a polypeptide of interest is constructed by chemical synthesis using an oligonucleotide synthesizer.

Oligonucleotides can be designed based on the amino acid sequence of the desired polypeptide and selecting those codons that are favored in the host cell in which the recombinant polypeptide of interest will be produced. Standard methods can be applied to synthesize a polynucleotide sequence encoding an isolated polypeptide of interest. For example, a complete amino acid sequence can be used to construct a back-translated gene. Further, a DNA oligomer containing a nucleotide sequence coding for the particular isolated polypeptide can be synthesized. For example, several small oligonucleotides coding for portions of the desired polypeptide can be synthesized and then ligated. The individual oligonucleotides typically contain 5' or 3' overhangs for complementary assembly.

[0740] Once assembled (by synthesis, site-directed mutagenesis, or another method), a polynucleotide sequence encoding a particular polypeptide of interest can be inserted into an expression vector and operatively linked to an expression control sequence appropriate for expression of the protein in a desired host. Proper assembly can be confirmed by nucleotide sequencing, restriction enzyme mapping, and/or expression of a biologically active polypeptide in a suitable host. As is well-known in the art, in order to obtain high expression levels of a transfected gene in a host, the gene must be operatively linked to transcriptional and translational expression control sequences that are functional in the chosen expression host.

[0741] In some embodiments, a recombinant expression vector is used to amplify and express DNA encoding an antibody against human TfR1. For example, a recombinant expression vector can be a replicable DNA construct that includes synthetic or cDNA-derived DNA fragments encoding a polypeptide chain of an anti-TfR1 antibody operatively linked to suitable transcriptional and/or translational regulatory elements derived from mammalian, microbial, viral or insect genes. A transcriptional unit generally comprises an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, transcriptional promoters or enhancers, (2) a structural or coding sequence that is transcribed into mRNA and translated into protein, and (3) appropriate transcription and translation initiation and termination sequences. Regulatory elements can include an operator sequence to control transcription. The ability to replicate in a host, usually conferred by an origin of replication, and

a selection gene to facilitate recognition of transformants can also be included. DNA regions are “operatively linked” when they are functionally related to each other. For example, DNA for a signal peptide (secretory leader) is operatively linked to DNA for a polypeptide if it is expressed as a precursor that participates in the secretion of the polypeptide; a promoter is operatively linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operatively linked to a coding sequence if it is positioned so as to permit translation. In some embodiments, structural elements intended for use in yeast expression systems include a leader sequence enabling extracellular secretion of translated protein by a host cell. In some embodiments, in situations where recombinant protein is expressed without a leader or transport sequence, a polypeptide may include an N-terminal methionine residue. This residue can optionally be subsequently cleaved from the expressed recombinant protein to provide a final product.

**[0742]** The choice of an expression control sequence and an expression vector generally depends upon the choice of host. A wide variety of expression host/vector combinations can be employed. Useful expression vectors for eukaryotic hosts include, for example, vectors comprising expression control sequences from SV40, bovine papilloma virus, adenovirus, and cytomegalovirus. Useful expression vectors for bacterial hosts include known bacterial plasmids, such as plasmids from *E. coli*, including pCR1, pBR322, pMB9 and their derivatives, and wider host range plasmids, such as M13 and other filamentous single-stranded DNA phages.

**[0743]** In some embodiments, an anti-TfR1 antibody of the present disclosure is expressed from one or more vectors. In some embodiments, a heavy chain polypeptide is expressed by a vector. Thus, the present disclosure provides vectors encoding an anti-TfR1 antibody (e.g., a single chain antibody agent, e.g., a VHH) described herein. In one embodiment, the vector encodes a heavy chain polypeptide of an anti-TfR1 antibody described herein. In one embodiment, the vector encodes a single chain anti-TfR1 antibody described herein, e.g., a VHH.

**[0744]** Suitable host cells for expression of an anti-TfR1 antibody or a TfR1 protein or fragment thereof to use as an antigen or immunogen include prokaryotes, yeast cells, insect cells, or higher eukaryotic cells under the control of appropriate promoters. Prokaryotes include gram-negative or gram-positive organisms, for example *E. coli* or *Bacillus*. Higher eukaryotic cells

include established cell lines of mammalian origin as described herein. Cell-free translation systems may also be employed. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts, as well as methods of protein production, including antibody production are well-known in the art.

[0745] Various mammalian culture systems may be used to express recombinant polypeptides. Expression of recombinant proteins in mammalian cells may be desirable because these proteins are generally correctly folded, appropriately modified, and biologically functional. Examples of suitable mammalian host cell lines include, but are not limited to, COS-7 (monkey kidney-derived), L-929 (murine fibroblast-derived), C127 (murine mammary tumor-derived), 3T3 (murine fibroblast-derived), CHO (Chinese hamster ovary-derived), HeLa (human cervical cancer-derived), BHK (hamster kidney fibroblast-derived), HEK-293 (human embryonic kidney-derived) cell lines and variants thereof. Mammalian expression vectors can comprise non-transcribed elements such as an origin of replication, a suitable promoter and enhancer linked to the gene to be expressed, and other 5' or 3' flanking non-transcribed sequences, and 5' or 3' non-translated sequences, such as necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, and transcriptional termination sequences.

[0746] Expression of recombinant proteins in insect cell culture systems (*e.g.*, baculovirus) also offers a robust method for producing correctly folded and biologically functional proteins. Baculovirus systems for production of heterologous proteins in insect cells are well-known to those of skill in the art.

[0747] Thus, the present disclosure provides cells comprising the anti-TfR1 antibody described herein. The present disclosure also provides cells comprising one or more polynucleotides encoding an anti-TfR1 antibody described herein or one or more vectors encoding anti-TfR1 antibody described herein. In one embodiment, the cell comprises a polynucleotide encoding an anti-TfR1 antibody described herein.. In one embodiment, the cell comprises a polynucleotide encoding a heavy chain of an anti-TfR1 antibody described herein. In one embodiment, the cell comprises a vector encoding a an anti-TfR1 antibody described herein. In one embodiment, the cell comprises a vector encoding a heavy chain of an anti-TfR1 antibody described herein. In some embodiments, the cells produce the anti-TfR1 antibodies described herein. In some embodiments, the cells produce an antibody. In some embodiments, the cells

produce an antibody that binds human TfR1. In some embodiments, the cells produce an antibody that binds cyno TfR1. In some embodiments, the cells produce an antibody that binds human TfR1 and cyno TfR1. In some embodiments, the cell is a prokaryotic cell (e.g., *E. coli*). In some embodiments, the cell is a eukaryotic cell. In some embodiments, the cell is a mammalian cell. In some embodiments, the cell is a hybridoma cell.

**[0748]** Proteins produced by a host cell can be purified according to any suitable method. Standard methods include chromatography (e.g., ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for protein purification. Affinity tags such as hexa-histidine maltose binding domain, influenza coat sequence, and glutathione-S-transferase can be attached to the protein to allow easy purification by passage over an appropriate affinity column. Affinity chromatography used for purifying immunoglobulins include, but are not limited to, Protein A, Protein G, and Protein L chromatography. Isolated proteins can be physically characterized using techniques known to those of skill in the art, including but not limited to, proteolysis, size exclusion chromatography (SEC), mass spectrometry (MS), nuclear magnetic resonance (NMR), isoelectric focusing (IEF), high performance liquid chromatography (HPLC), and x-ray crystallography. The purity of isolated proteins can be determined using techniques known to those of skill in the art, including but not limited to, SDS-PAGE, SEC, capillary gel electrophoresis, IEF, and capillary isoelectric focusing (cIEF).

**[0749]** In some embodiments, supernatants from expression systems that secrete recombinant protein into culture media are first concentrated using a commercially available protein concentration filter, for example, an Amicon® or Millipore Pellicon® ultrafiltration unit. Following the concentration step, the concentrate can be applied to a suitable purification matrix. In some embodiments, an anion exchange resin is employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose, or other types commonly employed in protein purification. In some embodiments, a cation exchange step is employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. In some embodiments, a hydroxyapatite media is employed, including but not limited to, ceramic hydroxyapatite (CHT). In some embodiments, one or more reverse-phase HPLC steps employing hydrophobic RP-

HPLC media, *e.g.*, silica gel having pendant methyl or other aliphatic groups, are employed to further purify a recombinant protein. In some embodiments, hydrophobic interaction chromatography (HIC) is used to separate recombinant proteins based on their hydrophobicity. HIC is a useful separation technique for purifying proteins while maintaining biological activity due to the use of conditions and matrices that operate under less denaturing conditions than some other techniques. Some or all of the foregoing purification steps, in various combinations, can be employed to provide a homogeneous recombinant protein.

#### **Method of transfecting host cells using AAV particles comprising a variant AAV capsid protein**

[0750] The present disclosure, among other things, provides methods for transfection of a host cell comprising: combining nucleic acids with a transfection reagent and introducing the mix to host cells under conditions that lead to transfection of the host cells with the nucleic acids.

[0751] In some embodiments, nucleic acids used in a method disclosed herein comprise one or more vectors. In some embodiments, nucleic acids disclosed herein comprise one or more vectors encoding: (i) at least one payload flanked by an AAV inverted terminal repeat (ITR) on either side of the at least one payload, (ii) at least one AAV Rep polypeptide, (iii) at least one AAV Cap polypeptide, and/or (iv) at least one Adenoviral helper polypeptide.

[0752] A host cell (*e.g.*, a mammalian host cell, *e.g.*, a HEK293) can be transfected with: at least one helper polypeptide or nucleic acid (*e.g.*, at least one Ad2 helper polypeptide or nucleic acid), at least one Rep polypeptide or a fragment thereof, at least one Cap polypeptide or a fragment thereof, and at least one payload (*e.g.*, for polypeptide expression or an inhibitory or guide nucleic acid).

[0753] In some embodiments, a transfection method disclosed herein is or comprises transient transfection. In some embodiments, a transient transfection method is a suspension transient transfection (sTT). In some embodiments, a transient transfection method is an adherent transient transfection.

[0754] In some embodiments, the disclosure provides transfected host cells comprising two, three, or four vectors as described herein.

[0755] In some embodiments, the method comprises transfecting a host cell with three vectors. In some embodiments, the three vectors comprise: (i) a first vector encoding at least one payload flanked by an AAV ITR on either side of the at least one payload, (ii) a second vector encoding at least one AAV Rep polypeptide and at least one AAV Cap polypeptide, and (iii) a third vector encoding at least one Adenoviral helper polypeptide.

[0756] In some embodiments, the method comprises transfecting a host cell with two vectors. In some embodiments, the two vectors comprises (i) a first vector encoding at least one AAV Cap polypeptide and at least one payload flanked by an AAV ITR on either side of the at least one payload; and (ii) a second vector encoding at least one Adenoviral helper polypeptide and at least one AAV Rep polypeptide.

[0757] Transfection methods disclosed herein comprise transfection of nucleic acids (e.g., comprising one or more vector) with any transfection reagent known to a skilled person for introducing nucleic acid molecules into host cells (e.g., mammalian cells, such as HEK293). In some embodiments, a transfection reagent comprises a lipid, a polymer, or a combination thereof. In some embodiments, a transfection reagent is a reagent that can form a complex with the nucleic acids.

[0758] In some embodiments, a transfection reagent comprise a polymer, a lipid, or both a polymer and a lipid. In some embodiments, a transfection reagent is or comprises a polymer. In some embodiments, a transfection reagent is or comprises lipid. In some embodiments, a transfection reagent comprises a polymer and a lipid.

[0759] In some embodiments, a transfection reagent is or comprises a polymer, e.g., a cationic polymer. In some embodiments, a transfection reagent comprises polyethyleneimine (PEI), FectoVIR, TransIT-VirusGEN, or a combination thereof. In some embodiments, a transfection reagent is or comprises polyethyleneimine (PEI).

[0760] In some embodiments, host cells are transfected with PEI. In some embodiments, host cells are transfected with a weight (wt.) ratio of DNA to transfection reagent (e.g., PEI) of about 1:1 to about 1:2, about 1:1 to about 1:5, or about 1:1 to about 1:10, e.g., about 1:0.05, about 1:1, about 1:1.25, about 1:1.5, about 1:2, about 1:2.5, about 1:3, about 1:3.5, about 1:4, about 1:4.5, about 1:5, about 1:6, about 1:7, about 1:8, about 1:9, or about 1:10. In some

embodiments, a wt. ratio of DNA to transfection reagent is dependent on cell culture density (e.g., of adherent or suspension host cells).

**[0761]** In some embodiments, a vector mass ratio of: (i) a first vector encoding at least one payload to (ii) a second vector encoding at least one Rep polypeptide and/or at least one Cap polypeptide to (iii) a third vector encoding at least one helper polypeptide is used in a method of transfection disclosed herein. In some embodiments, a vector mass ratio of: (i) a first vector encoding at least one payload to (ii) a second vector encoding at least one Rep polypeptide and/or at least one Cap polypeptide to (iii) a third vector encoding at least one helper polypeptide is about 1:1:1. In some embodiments, a vector mass ratio of: (i) a first vector encoding at least one payload to (ii) a second vector encoding at least one Rep polypeptide and/or at least one Cap polypeptide to (iii) a third vector encoding at least one helper polypeptide is not about 1:1:1.

**[0762]** In some embodiments, a vector mass ratio of: (i) a first vector encoding at least one payload to (ii) a second vector encoding at least one Rep polypeptide and/or at least one Cap polypeptide to (iii) a third vector encoding at least one helper polypeptide is about 1:0.5:1, about 1:1:2, about 1:1:3, about 1:1:4, about 1:1:5, about 1:1:6, about 1:1:7, about 1:1:8, about 1:1:9, about 1:1:10, about 5:10:1, about 1:0.5:2, about 1:0.5:10, about 1:0.5:5, about 0.5:5:1, about 1:10:20, about 1:2:1, about 1:3:1, about 1:4:1, about 1:5:1, about 1:6:1, about 1:7:1, about 1:8:1, about 1:9:1, about 1:10:1, about 10:1:1, about 9:1:1, about 8:1:1, about 7:1:1, about 6:1:1, about 6:1:1, about 4:1:1, about 3:1:1, about 2:1:1, or about 1:0.5:5.

**[0763]** In some embodiments, a vector mass ratio of: (i) a first vector encoding at least one payload to (ii) a second vector encoding at least one Rep polypeptide and/or at least one Cap polypeptide to (iii) a third vector encoding at least one helper polypeptide is about 1:0.5:1 to about 1:0.5:10; about 1:1:1 to about 1:1:10; about 0.5:1:1 to about 5:1:1; about 1:1:1 to about 1:10:1; or about 1:1:1 to about 10:1:1.

## Host Cells

**[0764]** The present disclosure, among other things, provides host cells for transfection with at least one vector as described herein for production of rAAV particles. A host cell includes a progeny cell of an original cell transfected with at least one vector described herein.

A progeny cell of a parental cell may not be substantially identical in morphology or genomic content as a parent cell due to natural, accidental, or deliberate mutation.

[0765] Components for a host cell to produce rAAV particles may be provided in trans or at least one vector. A stable host cell may comprise at least one polypeptide to produce rAAV particles using methods known to those of skill in the art. In some embodiments, a stable host cell comprises at least one polypeptide under control of an inducible promoter. In other embodiments, a stable host cell comprises at least one polypeptide under control of a constitutive promoter. For example, a stable host cell (e.g., a HEK293 cell) may comprise a nucleic acid encoding an E1 helper polypeptide under the control of a constitutive promoter. Other stable host cells may be generated by one of skill in the art using routine methods.

[0766] Exemplary host cells include prokaryotes or eukaryotes (single-cell or multiple-cell), bacterial cells (e.g., strains of *E. coli*, *Bacillus spp.*, *Streptomyces spp.*), mycobacteria cells, fungal cells, yeast cells (e.g., *S. cerevisiae*, *S. pombe*, *P. pastoris*, *P. methanolica*), plant cells, insect cells (e.g., SF-9, SF-21, baculovirus-infected insect cells, or *Trichoplusia ni*), non-human animal cells, human cells, or cell fusions, such as hybridomas or quadromas. In some embodiments the host cell is a mammalian cell. In some embodiments, the host cell is a human, monkey, ape, hamster, rat, or mouse cell.

[0767] In some embodiments, the host cell is selected from a kidney cell (e.g., HEK293, 293 EBNA, MSR 293, MDCK, HaK, or BHK), CHO cell (e.g., CHO K1, DXB-1 1 CHO, or Veggie-CHO), COS cell (e.g., COS-7), retinal cell, Vero cell, CV1 cell, HepG2 cell, WI38 cell, MRC 5 cell, Colo205 cell, HB 8065 cell, HL-60 cell (e.g., BHK21), Jurkat cell, Daudi cell, A431 cell (epidermal), CV-1 cell, U937 cell, 3T3 cell, L cell, C127 cell, SP2/0 cell, NS-0 cell, MMT 060562 cell, Sertoli cell, BRL 3 A cell, HT1080 cell, myeloma cell, tumor cell, or a cell line derived from an aforementioned cell.

[0768] In some embodiments, the host cell comprises a kidney cell (e.g., HEK293, 293 EBNA, MSR 293, MDCK, HaK, or BHK). In certain embodiments, the host cell comprises a HEK293 cell. In some embodiments, the host cell (e.g., a HEK 293 cell) comprises or expresses an E1 polypeptide. In some embodiments, the host cell does not comprise or express an E1 polypeptide. In some embodiments, the host cell comprises a CHO cell (e.g., CHO-K, DXB-1 1 CHO, or Veggie-CHO). In certain embodiments, the host cell comprises a CHO-K cell.

[0769] In some embodiments, host cells are or comprise suspension cells. In some embodiments, at least 10% +/- 15%, at least 15 +/- 15%, at least 20 +/- 15%, at least 25 +/- 15%, at least 30 +/- 15%, at least 35 +/- 15%, at least 40 +/- 15%, at least 45 +/- 15%, at least 50 +/- 15%, at least 55 +/- 15%, at least 60 +/- 15%, at least 65 +/- 15%, at least 70 +/- 15%, at least 75 +/- 15%, at least 80 +/- 15%, at least 85 +/- 15%, at least 90 +/- 15%, at least 95 +/- 15%, at least 99 +/- 15%, or more host cells in culture are suspended.

[0770] In some embodiments, prior to transfection, host cells (e.g., adherent or suspended host cells) are seeded at a certain density. In some embodiments, prior to transfection, host cells (e.g., adherent host cells) are seeded at a density of at least about  $1.0 \times 10^4$  viable cells (vc)/cm<sup>2</sup>, e.g., at a density of about  $1.0 \times 10^4$  vc/cm<sup>2</sup> to about  $2.0 \times 10^4$  vc/cm<sup>2</sup>, e.g., about  $1.0 \times 10^4$  vc/cm<sup>2</sup>, about  $1.1 \times 10^4$  vc/cm<sup>2</sup>, about  $1.2 \times 10^4$  vc/cm<sup>2</sup>, about  $1.3 \times 10^4$  vc/cm<sup>2</sup>, about  $1.4 \times 10^4$  vc/cm<sup>2</sup>, about  $1.5 \times 10^4$  vc/cm<sup>2</sup>, about  $1.6 \times 10^4$  vc/cm<sup>2</sup>, about  $1.7 \times 10^4$  vc/cm<sup>2</sup>, about  $1.8 \times 10^4$  vc/cm<sup>2</sup>, about  $1.9 \times 10^4$  vc/cm<sup>2</sup>, or about  $2.0 \times 10^4$  vc/cm<sup>2</sup>. In some embodiments, prior to transfection, host cells (e.g., suspended host cells) are seeded at a density of at least  $1.0 \times 10^6$  vc/cm<sup>2</sup> +/- 15%, e.g., at a density of  $1.0 \times 10^6$  vc/cm<sup>2</sup> +/- 15% to  $2.0 \times 10^6$  vc/cm<sup>2</sup> +/- 15%, e.g.,  $1.0 \times 10^6$  vc/cm<sup>2</sup> +/- 15%,  $1.1 \times 10^6$  vc/cm<sup>2</sup> +/- 15%,  $1.2 \times 10^6$  vc/cm<sup>2</sup> +/- 15%,  $1.3 \times 10^6$  vc/cm<sup>2</sup> +/- 15%,  $1.4 \times 10^6$  vc/cm<sup>2</sup> +/- 15%,  $1.5 \times 10^6$  vc/cm<sup>2</sup> +/- 15%,  $1.6 \times 10^6$  vc/cm<sup>2</sup> +/- 15%,  $1.7 \times 10^6$  vc/cm<sup>2</sup> +/- 15%,  $1.8 \times 10^6$  vc/cm<sup>2</sup> +/- 15%,  $1.9 \times 10^6$  vc/cm<sup>2</sup> +/- 15%, or  $2.0 \times 10^6$  vc/cm<sup>2</sup> +/- 15%.

## Vectors

[0771] Many forms of vectors can be used in methods of producing rAAV particles described herein. Non-limiting examples of vectors include plasmids, bacteriophage vectors, cosmids, phagemids, artificial chromosomes, and viral vectors (e.g., vectors suitable for gene therapy). A vector genetic element may be delivered by any suitable method known in the art, e.g., to those with skill in nucleic acid manipulation and include genetic engineering, recombinant engineering, and synthetic techniques (See, e.g., Sambrook 1989 as referenced herein).

[0772] In some embodiments, a vector encodes at least one helper polypeptide. In some embodiments, a vector encodes at least one Rep polypeptide and/or at least one Cap polypeptide. In some embodiments, a vector encodes at least one payload (e.g., for expression of polypeptide or as an inhibitory or guide nucleic acid). In some embodiments, a vector encodes at least one

helper polypeptide and at least one Rep polypeptide. In some embodiments, a vector encodes at least one Cap polypeptide and at least one payload.

[0773] A vector can include conventional control elements operably linked to a nucleic acid encoding any polypeptide or payload described herein, in a manner that permits transcription, translation and/or expression in a cell transfected with a vector described herein. Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals, such as splicing and polyadenylation (polyA) signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (e.g., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance secretion of the encoded product. A number of expression control sequences, including promoters that are native, constitutive, inducible, and/or tissue-specific, are known in the art and may be included in a vector described herein.

[0774] Examples of constitutive promoters include, but are not limited to, a retroviral Rous sarcoma virus (RSV) LTR promoter (optionally with the RSV enhancer), a cytomegalovirus (CMV) promoter (optionally with CMV enhancer), an SV40 promoter, and an dihydrofolate reductase promoter.

[0775] Inducible promoters allow regulation of gene expression and can be regulated by exogenously supplied compounds, environmental factors, such as temperature, or the presence of a specific physiological state (e.g., acute phase, a particular differentiation state of the cell, or in replicating cells only). Inducible promoters and inducible systems are available from a variety of commercial sources, including, without limitation, Invitrogen, Clontech and Ariad. Many other systems have been described and can be readily selected by one of skill in the art. Examples of inducible promoters regulated by exogenously supplied promoters include a zinc-inducible sheep metallothionein (MT) promoter, a dexamethasone (Dex)-inducible mouse mammary tumor virus (MMTV) promoter, a T7 polymerase promoter system, an ecdysone insect promoter, a tetracycline-repressible system, a tetracycline-inducible system, a RU486-inducible system, and an rapamycin-inducible system. Still other types of inducible promoters that may be useful are regulated by a specific physiological state, such as temperature, acute phase, a particular differentiation state of the cell, or in replicating cells only.

[0776] In another embodiment, a native promoter or fragment thereof for a nucleic acid encoding any polypeptide or payload described herein may be used. In some embodiments,

other native expression control elements, such as enhancer elements, polyadenylation sites, or Kozak consensus sequences, may also be used to mimic native expression.

### ***Vector encoding Helper Polypeptides***

[0777] The present disclosure, among other things, provides vectors (e.g., plasmids) encoding at least one helper polypeptide. AAV is a helper-dependent DNA parvovirus, which belongs to the genus *Dependovirus*. Production of recombination AAV requires co-infection with a related virus (e.g., adenovirus, herpes, or vaccinia virus) or a helper vector encoding helper polypeptides, such as structural proteins and proteins for viral genome replication.

[0778] A helper vector can comprise nucleotide sequences for non-AAV derived viral and/or cellular functions upon which AAV is dependent for replication, which may include, but are not limited to, activation of gene transcription, stage specific mRNA splicing, DNA replication, synthesis of at least one Cap polypeptide, and/or capsid assembly. Viral-based helper polypeptides can be derived from any known helper viruses such as adenovirus, herpesvirus, vaccinia virus, or a combination thereof. Thus, a helper vector (e.g., a plasmid) for culturing of the host cell can comprise sufficient helper polypeptides to permit packaging of the recombinant AAV vector into the AAV capsid polypeptides.

[0779] In some embodiments, a helper vector comprises an Ad2 helper vector. In certain embodiments, a nucleic acid sequence of an Ad2 helper vector is derived from an Adenovirus 2 genome (GenBank Accession No. J01917.1). In some embodiments, a helper vector comprises an Ad5 helper vector. In certain embodiments, a nucleic acid sequence of an Ad5 helper vector is derived from an Adenovirus 5 genome (GenBank Accession No. AY601635).

[0780] Helper polypeptides and nucleic acids can comprise at least one, two, three, or four of E1, E2a, E4, or VA RNA. In some embodiments, E1 comprises E1a and/or E1b. In some embodiments, one or both of E2a and VA RNA increase stability and/or efficiency of AAV mRNA translation, such as for *cap* gene transcripts. In some embodiments, E4 facilitates DNA replication. In some embodiments, E1a comprises a transactivator (e.g., regulating activity of at least one Ad gene, AAV *rep* gene, and/or AAV *cap* gene). In some embodiments, E1b comprises a viral mRNA transport. Helper polypeptides are described in further detail in Coura

Rdos. and Nardi BN., (2008) *Genetics and Molecular Biology* 31(1): pp. 1-11, which is hereby incorporated by reference in its entirety.

[0781] In some embodiments, a helper vector comprises a selection marker. Exemplary selection markers include, but are not limited to, antibiotic resistance genes. In some embodiments, an antibiotic resistance gene is not a gene encoding penicillin. In some embodiments, an antibiotic resistance gene is not a gene encoding a penicillin-derivative. In some embodiments, an antibiotic resistance gene comprises an antibiotic resistance gene chosen from kanamycin, puromycin, neomycin, hygromycin, blasticidin, gentamycin, Gr18, or zeocin. In certain embodiments, an antibiotic resistance gene comprises an antibiotic resistance gene for kanamycin.

[0782] In some embodiments, nucleic acids encoding helper polypeptides are oriented in the same direction (e.g., 5' to 3') on a helper vector. In some embodiments, nucleic acids encoding helper polypeptides are transcribed in the same direction from a helper vector. In certain embodiments, helper polypeptides and nucleic acids comprise VA RNA and E4 oriented in the same direction on a helper vector. In certain embodiments, helper polypeptides comprise E4 and E2a oriented in the same direction on a helper vector. In certain embodiments, helper polypeptides and nucleic acids comprise VA RNA, E4, and E2a oriented from 5' to 3' in direction on a helper vector. In some embodiments, a helper vector does not comprise a nucleic acid sequence encoding a Fiber protein or a fragment thereof (e.g., does not comprise a nucleic acid sequence of GenBank Accession No. AP\_000226.1 or a fragment thereof).

#### *Vector encoding Rep and/or Cap Polypeptides*

[0783] The present disclosure, among other things, provides vectors (e.g., plasmids) encoding at least one Rep polypeptide and/or at least one Cap polypeptide (e.g., a variant Cap disclosed herein). Production of rAAV particles can include culturing of a host cell with at least one Rep polypeptide and at least one Cap polypeptide (e.g., a variant Cap disclosed herein). Rep proteins (e.g., one, two, three, or four Rep78, Rep68, Rep52, and Rep40) are involved in viral DNA replication, resolution of replicative intermediates, and generation of single-stranded genomes. In some embodiments, a vector comprises a nucleic acid sequence encoding one, two, three, or four of Rep78, Rep68, Rep52, or Rep40, or a variant of any of the foregoing.

[0784] In some embodiments, a Rep polypeptide comprises a nucleic acid sequence derived from an AAV2 serotype. For example, a nucleic acid sequence encoding a Rep polypeptide may be derived from the AAV2 genome (as found in Accession No. NC\_001401). In some embodiments, a Rep polypeptide comprises an AAV2 Rep polypeptide operably linked to a p5 and/or p19 promotor (as found in Accession No. NC\_001401). In some embodiments, a Rep polypeptide comprises an amino acid sequence of YP\_680422.1 or a fragment thereof. In some embodiments, a promoter is operably linked to a nucleic acid sequence encoding at least one Rep polypeptide. In certain embodiments, a promoter operably linked to a nucleic acid sequence encoding at least one Rep polypeptide comprises a p5 and/or p19 promoter. In some embodiments, a wildtype promoter of AAV2 or a variant thereof is operably linked to a nucleic acid sequence encoding at least one Rep polypeptide. In some embodiments, a promoter (e.g., a p5 promoter) regulating expression of at least one Rep polypeptide is located in a different location on a vector than a wildtype promoter of AAV2 or a variant thereof. In certain embodiments, a promoter (e.g., a p5 promoter) is located 3' of a nucleic acid encoding at least one Rep polypeptide. In certain embodiments, a promoter (e.g., a p5 promoter) is located 5' of a nucleic acid encoding at least one Rep polypeptide.

[0785] Cap polypeptides (e.g., VP1, VP2, and VP3) are structural proteins comprising a Capsid. In some embodiments, a vector comprises a nucleic acid sequence encoding one, two, or three of VP1, VP2, and VP3, e.g., comprising a variant AAV capsid protein disclosed herein. In some embodiments, a vector comprises a nucleic acid sequence encoding at least one Cap polypeptide (e.g., a variant AAV capsid polypeptide disclosed herein) and at least one Rep polypeptide. In other embodiments, a vector encodes at least one Cap polypeptide (e.g., a variant AAV capsid polypeptide disclosed herein) and a separate vector encodes at least one Rep polypeptide.

[0786] For example, a nucleic acid sequence encoding a Cap polypeptide comprising an AAV variant capsid protein disclosed herein may further comprise a nucleic acid sequence derived from a known AAV genome sequence including, but not limited to: AAV9/hu14 provided as SEQ ID NO: 123 in U.S. Patent 7,906,111; AAV1 Accession No. NC\_002077 or AF063497; AAV2 Accession No. NC\_001401; AAV5 Accession No. Y18065 or AF085716; Accession No. AAV6 NC\_001862; or AAV8 Accession No NC\_006261.1. In certain embodiments, a nucleic acid sequence encoding a Cap polypeptide (e.g., a variant AAV capsid

polypeptide disclosed herein) is derived from an AAV genome sequence or a variant thereof as described in US Patent No. 7,906,111, which is hereby incorporated by reference in its entirety. In certain embodiments, a nucleic acid sequence encoding a Cap polypeptide (e.g., a variant AAV capsid polypeptide disclosed herein) is derived from an AAV genome sequence or a variant thereof as described in International Publication No. WO 2018/160582, which is hereby incorporated by reference in its entirety.

[0787] In certain embodiments, a Cap polypeptide comprising an AAV variant capsid protein disclosed herein further comprises a nucleic acid sequence derived from an AAV2 serotype, or a variant thereof. In certain embodiments, a Cap polypeptide comprising an AAV variant capsid protein disclosed herein further comprises a nucleic acid sequence derived from an AAV1 serotype, or a variant thereof. In certain embodiments, a Cap polypeptide comprising an AAV variant capsid protein disclosed herein further comprises a nucleic acid sequence derived from an AAV2 serotype, or a variant thereof. In certain embodiments, a Cap polypeptide comprising an AAV variant capsid protein disclosed herein further comprises a nucleic acid sequence derived from an AAV3B serotype, or a variant thereof. In certain embodiments, a Cap polypeptide comprising an AAV variant capsid protein disclosed herein further comprises a nucleic acid sequence derived from an AAV5 serotype, or a variant thereof. In certain embodiments, a Cap polypeptide comprising an AAV variant capsid protein disclosed herein further comprises a nucleic acid sequence derived from an AAV8 serotype, or a variant thereof. In certain embodiments, a Cap polypeptide comprising an AAV variant capsid protein disclosed herein further comprises a nucleic acid sequence derived from an AAV9 serotype, or a variant thereof. In certain embodiments, a Cap polypeptide comprising an AAV variant capsid protein disclosed herein further comprises a nucleic acid sequence derived from an AAV4 serotype, or a variant thereof. In certain embodiments, a Cap polypeptide comprising an AAV variant capsid protein disclosed herein further comprises a nucleic acid sequence derived from an AAV7 serotype, or a variant thereof. In certain embodiments, a Cap polypeptide comprising an AAV variant capsid protein disclosed herein further comprises a nucleic acid sequence derived from an AAV10 serotype, or a variant thereof. In certain embodiments, a Cap polypeptide comprising an AAV variant capsid protein disclosed herein further comprises a nucleic acid sequence derived from an AAV11 serotype, or a variant thereof. In certain embodiments, a Cap polypeptide comprising an AAV variant capsid protein disclosed herein further comprises a nucleic acid

sequence derived from an AAV12 serotype, or a variant thereof. In certain embodiments, a Cap polypeptide comprising an AAV variant capsid protein disclosed herein further comprises a nucleic acid sequence derived from an AAV13 serotype, or a variant thereof. In certain embodiments, a Cap polypeptide comprising an AAV variant capsid protein disclosed herein further comprises a nucleic acid sequence derived from an AAVu68 serotype, or a variant thereof. In certain embodiments, a Cap polypeptide comprising an AAV variant capsid protein disclosed herein further comprises a nucleic acid sequence derived from an AA Vrh10 serotype, or a variant thereof.

**[0788]** In some embodiments, a promoter is operably linked to a nucleic acid sequence encoding at least one Cap polypeptide comprising an AAV variant capsid protein disclosed herein. In some embodiments, a wildtype promoter of AAV2, AAV5, AAV8, AAV9, is operably linked to a nucleic acid sequence encoding at least one Rep polypeptide. In certain embodiments, a p40 promoter is operably linked to a nucleic acid sequence encoding at least one Cap polypeptide comprising an AAV variant capsid protein disclosed herein further comprises.

#### *Vector encoding Payload*

**[0789]** The present disclosure, among other things, provides vectors (e.g., plasmids) encoding at least one payload. A payload sequence is generally a sequence of interest that is desired to be introduced into a cell, tissue, organ, or organism.

**[0790]** In some embodiments, a payload is flanked by inverted terminal repeats (ITRs). The AAV sequences of a rAAV vector typically comprise cis-acting 5' and 3' inverted terminal repeat (ITR) sequences (See, e.g., Carter B.J., (1990) *Handbook of Parvoviruses* (I): pp. 155-168, which is hereby incorporated by reference in its entirety). ITR sequences are typically about 145 nt in length. In some embodiments, one or both of a 5'ITR or a 3' ITR nucleic acid sequence are modified relative to a known ITR nucleic acid sequence. Modification of ITR nucleic acid sequences is within one of skill in the art (See, e.g., Sambrook J. 1989; and Fisher K. et al., (1996) *J Virol* 70: pp. 520-532, each of which is hereby incorporated by reference in its entirety). AAV ITR sequences may be obtained from any known AAV, including mammalian AAV types.

**[0791]** In some embodiments, a payload is a heterologous protein with a therapeutic purpose, e.g., an enzyme, cytokine, antibody, receptor, fusion protein, or chimeric polypeptide.

In some embodiments, a payload is linked to a secretion signal sequence for secretion of an expressed polypeptide from a host cell. In some embodiments, a payload is a heterologous nucleic acid with a therapeutic purpose, e.g., an miRNA, siRNA, shRNA, mRNA, snRNA, or CRISPR/Cas guide RNA, or a precursor thereof. One of skill in the art will recognize that a payload can be selected from any heterologous protein or nucleic acid of interest. In some embodiments, a payload sequence comprises one or more aptamer-binding domains or polypeptide-binding domains (e.g., transcription factor binding domains). A vector will also typically include other regulatory elements (e.g., promoters, introns, and/or enhancers) to regulate expression or amount of a payload in a cell or tissue.

**[0792]** In accordance with various embodiments, a payload sequence can be of any length, e.g., between 2 and 10,000 nucleotides in length or any integer value there between. In some embodiments, a nucleic acid sequence encoding a payload comprises at least 20 nucleotides, at least 50 nucleotides, at least 75 nucleotides, at least 100 nucleotides, at least 150 nucleotides, at least 200 nucleotides, at least 250 nucleotides, at least 300 nucleotides, at least 350 nucleotides, at least 400 nucleotides, at least 450 nucleotides, at least 500 nucleotides, at least 550 nucleotides, at least 600 nucleotides, at least 650 nucleotides, at least 700 nucleotides, at least 750 nucleotides, at least 800 nucleotides, at least 850 nucleotides, at least 900 nucleotides, at least 950 nucleotides, at least 1000 nucleotides, at least 1100 nucleotides, at least 1200 nucleotides, at least 1300 nucleotides, at least 1400 nucleotides, at least 1500 nucleotides, at least 1600 nucleotides, at least 1700 nucleotides, at least 1800 nucleotides, at least 2000 nucleotides, at least 2500 nucleotides, at least 3000 nucleotides, at least 4000 nucleotides, at least 5000 nucleotides, at least 6000 nucleotides, at least 7000 nucleotides, at least 8000 nucleotides, at least 9000 nucleotides. In some embodiments, a nucleic acid sequence encoding a payload comprises between 50 and 25,000 nucleotides in length, between 100 and 20,000 nucleotides in length, between 500 and 10,000 nucleotides in length, between 1,000 and 8,000 nucleotides in length, and/or between 2,000 and 5,000 nucleotides in length.

### Culture vessels and culturing parameters

**[0793]** The present disclosure, among other things, provides methods for culturing of a host cell with at least one vector described herein for production of rAAV particles. A wide variety of growth media (e.g., mammalian growth media) may be used in accordance with the

present invention. In certain embodiments, cells may be grown in one of a variety of chemically defined media, wherein the components of the media are both known and controlled. In certain embodiments, cells may be grown in a complex medium, in which not all components of the medium are known and/or controlled.

[0794] A culture of host cells can be prepared in any medium suitable for a particular cell type being cultured. In some embodiments, a host cell medium comprises, e.g., inorganic salts, carbohydrates (e.g., sugars, such as glucose, galactose, maltose, or fructose), amino acids, vitamins (e.g., B group vitamins (e.g., B12)), vitamin A, vitamin E, riboflavin, thiamine, or biotin), fatty acids (e.g., cholesterol or steroids), proteins (e.g., albumin, transferrin, fibronectin, or fetuin), serum (e.g., albumins, growth factors, or growth inhibitors, such as, fetal bovine serum, newborn calf serum, or horse serum), trace elements (e.g., zinc, copper, selenium, or tricarboxylic acid intermediates), hydrolysates (e.g., derived from plant or animal sources), or combinations thereof.

[0795] Commercially available media can be used for culturing host cells described herein. Exemplary media can include, but is not limited to, Dulbecco's Modified Eagle's Medium ([DMEM], Sigma), FreeStyle<sup>TM</sup> F17 Expression Medium (ThermoFisher), DMEM/F12 medium (Invitrogen), CD OptiCHO<sup>TM</sup> medium (Invitrogen), CD EfficientFeed<sup>TM</sup> media (Invitrogen), Cell Boost (HyClone<sup>TM</sup>) media (GE Life Sciences), BalanCD<sup>TM</sup> CHO Feed (Irvine Scientific), BD Recharge<sup>TM</sup> (Becton Dickinson), Cellvento Feed<sup>TM</sup> (EMD Millipore), Ex-cell CHOZN Feed<sup>TM</sup> (Sigma-Aldrich), CHO Feed Bioreactor Supplement (Sigma-Aldrich), SheffCHO<sup>TM</sup> (Kerry), Zap-CHO<sup>TM</sup> (Invitria), ActiCHO<sup>TM</sup> (PAA/GE Healthcare), Minimal Essential Medium (Sigma), or RPMI-1640 (Sigma). Media can be supplemented as necessary with hormones and/or other growth factors (e.g., insulin, transferrin, or epidermal growth factor), salts (e.g., sodium chloride, calcium, magnesium, or phosphate), buffers (e.g., HEPES), nucleosides (e.g., adenosine or thymidine), antibiotics (e.g., kanamycin, puromycin, neomycin, hygromycin, blasticidin, gentamycin, Gr18, or zeocin), trace elements, lipids (e.g., linoleic or other fatty acids), or glucose or an equivalent energy source. In some embodiments, the media for culturing host cells comprises glutamine or a glutamine dipeptide. In some embodiments, the media for culturing host cells comprises a surfactant. In some embodiments, the nutrient media is serum-free media, a protein-free media, or a chemically defined media. Any other necessary

supplements can also be included at appropriate concentrations that would be known to those skilled in the art.

[0796] After culturing of host cells as described herein, a plurality of rAAV particles are recovered. In some embodiments, rAAV particles are recovered by lysing host cells and recovering rAAV particles from lysate, e.g., after centrifugation. In some embodiments, rAAV particles are recovered from culture supernatant. In some embodiments, a lysis solution for host cells comprises chemical reagents, e.g., detergents (e.g., sodium dodecyl sulfate (SDS), ethyl trimethyl ammonium bromide, Triton X-100, bile salts, such as cholate, or zwitterionic detergents, such as CHAPS). In some embodiments, a lysis solution for host cells comprises a salt (e.g., NaCl) and a high pH (e.g., a pH of greater than about 7). In some embodiments, rAAV particles are purified using purification methods, such as chromatography (e.g., affinity chromatography or ion-exchange chromatography (e.g., cation exchange chromatography)) or filtration (e.g., UF/DF filtration)).

[0797] In some embodiments, a plurality of rAAV particles are produced in a large-scale preparation. In some embodiments, a large-scale preparation of host cells (e.g., suspension host cells) is at least 3 liters +/- 15% of culture media, 10 liters +/- 15% of culture media, e.g., between 50 liters +/- 15% to 1000 liters +/- 15% of culture media or between 50 liters +/- 15% to 2000 liters +/- 15% of culture media, e.g., at least 20 liters +/- 15%, 30 liters +/- 15%, 40 liters +/- 15%, 50 liters +/- 15%, 55 liters +/- 15%, 60 liters +/- 15%, 65 liters +/- 15%, 70 liters +/- 15%, 75 liters +/- 15%, 80 liters +/- 15%, 85 liters +/- 15%, 90 liters +/- 15%, 95 liters +/- 15%, 100 liters +/- 15%, 200 liters +/- 15%, 300 liters +/- 15%, 400 liters +/- 15%, 500 liters +/- 15%, 600 liters +/- 15%, 700 liters +/- 15%, 800 liters +/- 15%, 900 liters +/- 15%, 1,000 liters +/- 15%, 1,250 liters +/- 15%, 1,500 liters +/- 15%, 1,750 liters +/- 15%, 2,000 liters +/- 15%, or more of culture media.

[0798] In some embodiments, a large-scale preparation of host cells (e.g., adherent host cells) is at least 5 m<sup>2</sup> +/- 15% of culture media, e.g., between 5 m<sup>2</sup> +/- 15% to 500 m<sup>2</sup> +/- 15% of culture media, e.g., at least 5 m<sup>2</sup> +/- 15%, 10 m<sup>2</sup> +/- 15%, 15 m<sup>2</sup> +/- 15%, 20 m<sup>2</sup> +/- 15%, 25 m<sup>2</sup> +/- 15%, 20 m<sup>2</sup> +/- 15%, 35 m<sup>2</sup> +/- 15%, 40 m<sup>2</sup> +/- 15%, 45 m<sup>2</sup> +/- 15%, 50 m<sup>2</sup> +/- 15%, 55 m<sup>2</sup> +/- 15%, 60 m<sup>2</sup> +/- 15%, 65 m<sup>2</sup> +/- 15%, 75 m<sup>2</sup> +/- 15%, 80 m<sup>2</sup> +/- 15%, 85 m<sup>2</sup> +/- 15%, 90 m<sup>2</sup> +/- 15%, 95 m<sup>2</sup> +/- 15%, 100 m<sup>2</sup> +/- 15%, 150 m<sup>2</sup> +/- 15%, 175 m<sup>2</sup> +/- 15%, 200 m<sup>2</sup> +/- 15%, 225 m<sup>2</sup> +/- 15%, 250 m<sup>2</sup> +/- 15%, 275 m<sup>2</sup> +/- 15%, 300 m<sup>2</sup> +/- 15%, 325 m<sup>2</sup> +/- 15%, 330 m<sup>2</sup> +/- 15%,

340 m<sup>2</sup> +/- 15%, 350 m<sup>2</sup> +/- 15%, 375 m<sup>2</sup> +/- 15%, 400 m<sup>2</sup> +/- 15%, 425 m<sup>2</sup> +/- 15%, 450 m<sup>2</sup> +/- 15%, 475 m<sup>2</sup> +/- 15%, 500 m<sup>2</sup> +/- 15%, 600 m<sup>2</sup> +/- 15%, 700 m<sup>2</sup> +/- 15%, 800 m<sup>2</sup> +/- 15%, 900 m<sup>2</sup> +/- 15%, 1000 m<sup>2</sup> +/- 15%, 1100 m<sup>2</sup> +/- 15%, 1200 m<sup>2</sup> +/- 15%, 1300 m<sup>2</sup> +/- 15%, 1400 m<sup>2</sup> +/- 15%, 1500 m<sup>2</sup> +/- 15%, 1600 m<sup>2</sup> +/- 15%, 1700 m<sup>2</sup> +/- 15%, 1800 m<sup>2</sup> +/- 15%, 1900 m<sup>2</sup> +/- 15%, 2000 m<sup>2</sup> +/- 15%, 2500 m<sup>2</sup> +/- 15%, 3000 m<sup>2</sup> +/- 15%, 3500 m<sup>2</sup> +/- 15%, 4000 m<sup>2</sup> +/- 15%, 4500 m<sup>2</sup> +/- 15%, 5000 m<sup>2</sup> +/- 15%, 5500 m<sup>2</sup> +/- 15%, 6000 m<sup>2</sup> +/- 15%, 6500 m<sup>2</sup> +/- 15%, 7000 m<sup>2</sup> +/- 15%, 7500 m<sup>2</sup> +/- 15%, 8000 m<sup>2</sup> +/- 15%, 8500 m<sup>2</sup> +/- 15%, 9000 m<sup>2</sup> +/- 15%, 9500 m<sup>2</sup> +/- 15%, 10,000 m<sup>2</sup> +/- 15%, 11,000 m<sup>2</sup> +/- 15%, 12,000 m<sup>2</sup> +/- 15%, 13,000 m<sup>2</sup> +/- 15%, 14,000 m<sup>2</sup> +/- 15%, 15,000 m<sup>2</sup> +/- 15%, or more of culture media.

**[0799]** A host cell can be cultured in a cell culture vessel or a bioreactor. In some embodiments, a cell culture vessel is suitable for/used for culturing adherent cells. In other embodiments, a cell culture vessel is suitable for/used for culturing suspension cells. Exemplary cell culture vessels include 35mm, 60mm, 100mm, or 150mm dishes, multi-well plates (e.g., 6-well, 12-well, 24-well, 48-well, or 96 well plates), or flasks (e.g., T-flasks, e.g., T-25, T-75, or T-160 flasks), or shaker flasks.

**[0800]** In some embodiments, a host cell is cultured in a bioreactor. In some embodiments, a bioreactor is suitable for/used for culturing adherent cells. In some embodiments, a bioreactor is suitable for/used for culturing suspension cells. A bioreactor can be, e.g., a continuous flow batch bioreactor, a perfusion bioreactor, a batch process bioreactor, or a fed batch bioreactor. An exemplary bioreactor is a fixed bed bioreactor, e.g., an iCELLis bioreactor (used for culturing adherent cells). A bioreactor can be maintained under conditions sufficient to produce rAAV particles. Culture conditions can be modulated to optimize yield, purity, or structure of rAAV particles.

**[0801]** In some embodiments, a bioreactor comprises a plurality of host cells. In some embodiments, host cells in a bioreactor comprise viable cells (vc).

**[0802]** In some embodiments, a bioreactor comprises at least about 1 x 10<sup>6</sup>, about 1 x 10<sup>7</sup>, about 1 x 10<sup>8</sup>, about 1 x 10<sup>9</sup>, about 1 x 10<sup>10</sup>, about 1 x 10<sup>11</sup>, about 1 x 10<sup>12</sup>, about 1 x 10<sup>13</sup>, or about 1 x 10<sup>14</sup> host cells (e.g., viable host cells). In some embodiments, a bioreactor comprises between 1 x 10<sup>6</sup> to 1 x 10<sup>14</sup> host cells; between 1 x 10<sup>6</sup> to 0.5 x 10<sup>14</sup> host cells; between 1 x 10<sup>6</sup> to 1 x 10<sup>13</sup> host cells; between 1 x 10<sup>6</sup> to 0.5 x 10<sup>13</sup> host cells; between 1 x 10<sup>6</sup> to 1 x 10<sup>12</sup> host cells; between 1 x 10<sup>6</sup> to 0.5 x 10<sup>12</sup> host cells; between 1 x 10<sup>6</sup> to 1 x 10<sup>11</sup> host cells; between 1 x 10<sup>6</sup> to

$0.5 \times 10^{11}$  host cells; between  $1 \times 10^6$  to  $1 \times 10^{10}$  host cells; between  $1 \times 10^6$  to  $0.5 \times 10^{10}$  host cells; between  $1 \times 10^6$  to  $1 \times 10^9$  host cells; between  $1 \times 10^6$  to  $0.5 \times 10^9$  host cells; between  $1 \times 10^6$  to  $1 \times 10^8$  host cells; between  $1 \times 10^6$  to  $0.5 \times 10^8$  host cells; between  $1 \times 10^6$  to  $1 \times 10^7$  host cells; between  $1 \times 10^6$  to  $0.5 \times 10^7$  host cells; between  $0.5 \times 10^7$  to  $1 \times 10^{14}$  host cells; between  $1 \times 10^8$  to  $1 \times 10^{14}$  host cells; between  $0.5 \times 10^9$  to  $1 \times 10^{14}$  host cells; between  $1 \times 10^9$  to  $1 \times 10^{14}$  host cells; between  $0.5 \times 10^{10}$  to  $1 \times 10^{14}$  host cells; between  $1 \times 10^{10}$  to  $1 \times 10^{14}$  host cells; between  $0.5 \times 10^{11}$  to  $1 \times 10^{14}$  host cells; between  $1 \times 10^{11}$  to  $1 \times 10^{14}$  host cells; between  $0.5 \times 10^{12}$  to  $1 \times 10^{14}$  host cells; between  $1 \times 10^{12}$  to  $1 \times 10^{14}$  host cells; between  $0.5 \times 10^{13}$  to  $1 \times 10^{14}$  host cells; between  $1 \times 10^{13}$  to  $1 \times 10^{14}$  host cells; or between  $0.5 \times 10^{13}$  to  $1 \times 10^{14}$  host cells.

**[0803]** In some embodiments, a bioreactor comprises about 0.5 million host cells/mL, about 1 million host cells/mL, about 1.5 million host cells/mL, about 2 million host cells/mL, about 2.5 million host cells/mL, about 3 million host cells/mL, about 3.5 million host cells/mL, about 4 million host cells/mL, about 4.5 million host cells/mL, about 5 million host cells/mL, about 5.5 million host cells/mL, about 6 million host cells/mL, about 7 million host cells/mL, about 8 million host cells/mL, about 9 million host cells/mL, about 10 million host cells/mL. In some embodiments, host cells in a bioreactor comprise viable cells (vc).

**[0804]** In some embodiments, a bioreactor comprises at least about 1 liter, about 2 liters, about 3 liters, about 10 liters, about 20 liters, about 30 liters, about 40 liters, about 50 liters, about 55 liters, about 60 liters, about 65 liters, about 70 liters, about 75 liters, about 80 liters, about 85 liters, about 90 liters, about 95 liters, about 100 liters, about 200 liters, about 300 liters, about 400 liters, about 500 liters, about 600 liters, about 700 liters, about 800 liters, about 900 liters, about 1000 liters, about 1500 liters, about 2000 liters or about 3000 liters of culture media.

**[0805]** In an embodiment, a bioreactor is maintained under conditions that promote growth of a host cell, e.g., at a temperature (e.g., 37°C) and gas concentration (e.g., 5% - 10% CO<sub>2</sub>) that is permissive for growth of the host cell. For example, a bioreactor can perform one or more of the following: feeding of nutrients and/or carbon sources, injection of suitable gas (e.g., oxygen), inlet and outlet flow of fermentation or cell culture medium, separation of gas and liquid phases, maintenance of temperature, maintenance of oxygen and CO<sub>2</sub> levels, maintenance of pH level, agitation (e.g., stirring), cleaning, and/or sterilization. Exemplary bioreactor units, may contain multiple reactors within a unit, e.g., a unit can comprise 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, or 100, or more bioreactors. Any suitable bioreactor diameter

and/or shape can be used. In some embodiments, suitable reactors can be round, e.g., cylindrical. In some embodiments, suitable reactors can be square, e.g., rectangular.

### rAAV Particle Production

**[0806]** The present disclosure, among other things, rAAV particles produced using methods described herein. Generally, rAAV particles produced using methods described herein may be of any AAV serotype. AAV serotypes generally have different tropisms to infect different tissues. In some embodiments, an AAV serotype is selected based on a tropism.

**[0807]** In some embodiments, a rAAV particle may comprise or be based on a serotype selected from any of the following serotypes, and variants thereof, including, but not limited to: AAV1, AAV2, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAVu68, or AA Vrh10.

**[0808]** In certain embodiments, a rAAV particle comprising an AAV variant capsid protein disclosed herein comprises an AAV1 serotype or a variant thereof. In certain embodiments, a rAAV particle comprising an AAV variant capsid protein disclosed herein comprises an AAV2 serotype or a variant thereof. In certain embodiments, a rAAV particle comprising an AAV variant capsid protein disclosed herein comprises an AAV3B serotype or a variant thereof. In certain embodiments, a rAAV particle comprising an AAV variant capsid protein disclosed herein comprises an AAV5 serotype or a variant thereof. In certain embodiments, a rAAV particle comprising an AAV variant capsid protein disclosed herein comprises an AAV8 serotype or a variant thereof. In certain embodiments, a rAAV particle comprising an AAV variant capsid protein disclosed herein comprises an AAV9 serotype or a variant thereof. In certain embodiments, a rAAV particle comprising an AAV variant capsid protein disclosed herein comprises an AAV4 serotype or a variant thereof. In certain embodiments, a rAAV particle comprising an AAV variant capsid protein disclosed herein comprises an AAV7 serotype or a variant thereof. In certain embodiments, a rAAV particle comprising an AAV variant capsid protein disclosed herein comprises an AAV10 serotype or a variant thereof. In certain embodiments, a rAAV particle comprising an AAV variant capsid protein disclosed herein comprises an AAV11 serotype or a variant thereof. In certain embodiments, a rAAV particle comprising an AAV variant capsid protein disclosed herein comprises an AAV12 serotype or a variant thereof. In certain embodiments, a rAAV particle

comprising an AAV variant capsid protein disclosed herein comprises an AAV13 serotype or a variant thereof. In certain embodiments, a rAAV particle comprising an AAV variant capsid protein disclosed herein comprises AAVu68 or a variant thereof. In certain embodiments, a rAAV particle comprising an AAV variant capsid protein disclosed herein comprises AA Vrh10 or a variant thereof.

**[0809]** In some embodiment, a plurality of rAAV particles are produced with methods described herein at a higher titer, e.g., such there is improved rAAV particle production. In some embodiments, the improved production comprises a higher yield of the plurality of rAAV particles relative to a plurality of rAAV particles produced with a helper vector comprising a nucleic acid sequence of an antibiotic resistance gene other than KanR (e.g., an Ampicillin resistance gene). In some embodiments, a high titer is relative to AAV particles produced from a reference helper vector (e.g., an Ad5 vector, e.g., an Ad5 vector described herein), e.g., under otherwise identical conditions.

**[0810]** In some embodiments, a high titer is greater than  $7.0 \times 10^9$  vg/mL +/- 15%, e.g., when cultured in suspension. In some embodiments, a high titer is greater than about  $7.0 \times 10^9$  vg/mL, e.g., greater than about  $7.5 \times 10^9$  vg/mL,  $8.0 \times 10^9$  vg/mL,  $8.5 \times 10^9$  vg/mL,  $9.0 \times 10^9$  vg/mL,  $1.0 \times 10^{10}$  vg/mL,  $1.5 \times 10^{10}$  vg/mL,  $2.0 \times 10^{10}$  vg/mL,  $2.5 \times 10^{10}$  vg/mL,  $3.0 \times 10^{10}$  vg/mL,  $3.5 \times 10^{10}$  vg/mL,  $4.0 \times 10^{10}$  vg/mL,  $4.5 \times 10^{10}$  vg/mL,  $5.0 \times 10^{10}$  vg/mL,  $5.5 \times 10^{10}$  vg/mL,  $6.0 \times 10^{10}$  vg/mL,  $6.5 \times 10^{10}$  vg/mL,  $7.0 \times 10^{10}$  vg/mL,  $7.5 \times 10^{10}$  vg/mL,  $8.0 \times 10^{10}$  vg/mL,  $8.5 \times 10^{10}$  vg/mL,  $9.0 \times 10^{10}$  vg/mL,  $9.5 \times 10^{10}$  vg/mL,  $1.0 \times 10^{11}$  vg/mL,  $1.5 \times 10^{11}$  vg/mL,  $2.0 \times 10^{11}$  vg/mL, or higher, e.g., when cultured in suspension.

**[0811]** In some embodiments, a high titer of rAAV particles is at least about  $7.0 \times 10^9$  vg/cm<sup>2</sup>, about  $7.5 \times 10^9$  vg/cm<sup>2</sup>, about  $8.0 \times 10^9$  vg/cm<sup>2</sup>, about  $8.5 \times 10^9$  vg/cm<sup>2</sup>, about  $9.0 \times 10^9$  vg/cm<sup>2</sup>, about  $9.5 \times 10^9$  vg/cm<sup>2</sup>, about  $1.0 \times 10^{10}$  vg/cm<sup>2</sup>, about  $1.5 \times 10^{10}$  vg/cm<sup>2</sup>, or higher, e.g., when cultured in a bioreactor, e.g., a fixed bed bioreactor.

**[0812]** In some embodiments, a high titer of rAAV particles is greater than  $5.0 \times 10^{13}$  vg/m<sup>2</sup> +/- 15%, e.g., greater than  $6.0 \times 10^{13}$  vg/m<sup>2</sup> +/- 15,  $7.0 \times 10^{13}$  vg/m<sup>2</sup> +/- 15,  $8.0 \times 10^{13}$  vg/m<sup>2</sup> +/- 15,  $9.0 \times 10^{13}$  vg/m<sup>2</sup> +/- 15,  $1.0 \times 10^{14}$  vg/m<sup>2</sup> +/- 15,  $2.0 \times 10^{14}$  vg/m<sup>2</sup> +/- 15,  $3.0 \times 10^{14}$  vg/m<sup>2</sup> +/- 15,  $4.0 \times 10^{14}$  vg/m<sup>2</sup> +/- 15,  $5.0 \times 10^{14}$  vg/m<sup>2</sup> +/- 15,  $6.0 \times 10^{14}$  vg/m<sup>2</sup> +/- 15,  $7.0 \times 10^{14}$  vg/m<sup>2</sup> +/- 15,  $8.0 \times 10^{14}$  vg/m<sup>2</sup> +/- 15,  $9.0 \times 10^{14}$  vg/m<sup>2</sup> +/- 15, or more.

**[0813]** In some embodiments, a plurality of rAAV particles described herein is harvested after at least 3 days of culturing. In some embodiments, a plurality of rAAV particles described herein is harvested after at least about 3 days to about 10 days of culturing, e.g., about 3 days to about 7 days, about 3 days to about 5 days, about 4 days to about 9 days, about 4 days to about 8 days, or about 4 days to about 6 days of culturing, e.g., after at least about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, about 10 days, or longer after culturing. In some embodiments, a plurality of rAAV particles produced with methods described herein is substantially free of one or both of a helper adenovirus or a herpes virus. In some embodiments, a plurality of rAAV particles is substantially free of one or both of a helper adenovirus or a herpes virus, e.g., a purity of at least about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more free of one or both of a helper adenovirus or a herpes virus.

**[0814]** The foregoing methods for producing recombinant vectors are not meant to be limiting, and other suitable methods will be apparent to the skilled artisan.

### rAAV Particle Compositions

**[0815]** The present disclosure, among other things, provides a composition comprising a plurality of rAAV particles formed by methods described herein and/or using systems described herein. In some embodiments, a composition comprises a pharmaceutical composition comprising at least one pharmaceutically acceptable component (e.g., a pharmaceutically acceptable carrier, diluent, or excipient). Such pharmaceutical compositions are useful for, among other things, administration to a subject *in vivo* or *ex vivo*.

**[0816]** In some embodiments, pharmaceutical compositions also contain a pharmaceutically acceptable carrier, excipient, or diluent. Such excipients include any pharmaceutical agent, e.g., a pharmaceutical agent that does not itself induce an immune response harmful to the individual receiving the composition, and which may be administered without undue toxicity. Pharmaceutically acceptable excipients include, but are not limited to, liquids, such as water, saline, glycerol, sugars, and ethanol. Pharmaceutically acceptable salts can also be included therein, for example, mineral acid salts, such as hydrochlorides, hydrobromides, phosphates, or sulfates; and the salts of organic acids, such as acetates,

propionates, malonates, or benzoates. Additionally, auxiliary substances, such as wetting or emulsifying agents or pH buffering substances, may be present in such vehicles.

[0817] Pharmaceutical compositions may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, or succinic. Salts tend to be more soluble in aqueous or other protonic solvents than corresponding free base forms. In some embodiments, a pharmaceutical composition may be a lyophilized powder.

[0818] Pharmaceutical compositions can include solvents (aqueous or non-aqueous), solutions (aqueous or non-aqueous), emulsions (e.g., oil-in-water or water-in-oil), suspensions, syrups, elixirs, dispersion and suspension media, coatings, and isotonic and absorption promoting or delaying agents, compatible with pharmaceutical administration or *in vivo* contact or delivery. Aqueous and non-aqueous solvents, solutions, and suspensions may include suspending agents and thickening agents. Such pharmaceutically acceptable carriers include tablets (coated or uncoated), capsules (hard or soft), microbeads, powder, granules, and crystals. Supplementary active compounds (e.g., preservatives, antibacterial, antiviral, and antifungal agents) can also be incorporated into the compositions.

[0819] Pharmaceutical compositions can be formulated to be compatible with a particular route of administration or delivery, as set forth herein or known to one of skill in the art. Thus, pharmaceutical compositions include carriers, diluents, or excipients suitable for administration by various routes.

[0820] Compositions suitable for parenteral administration can comprise aqueous and non-aqueous solutions, suspensions or emulsions of the active compound, which preparations are typically sterile and can be isotonic with the blood of the intended recipient. Non-limiting illustrative examples include water, buffered saline, Hanks' solution, Ringer's solution, dextrose, fructose, ethanol, animal, vegetable, or synthetic oils. Aqueous injection suspensions may contain substances that increase the viscosity of a suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions may be prepared as appropriate oil injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils, such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Optionally,

the suspension may also contain suitable stabilizers or agents that increase solubility to allow for preparation of highly concentrated solutions.

**[0821]** Cosolvents and adjuvants may be added to the formulation. Non-limiting examples of cosolvents contain hydroxyl groups or other polar groups, for example, alcohols, such as isopropyl alcohol; glycols, such as propylene glycol, polyethyleneglycol, polypropylene glycol, glycol ether; glycerol; polyoxyethylene alcohols and polyoxyethylene fatty acid esters. Adjuvants include, for example, surfactants such as, soya lecithin and oleic acid; sorbitan esters such as sorbitan trioleate; and polyvinylpyrrolidone.

**[0822]** After pharmaceutical compositions have been prepared, they may be placed in an appropriate container and labeled for treatment. Such labeling can include amount, frequency, and method of administration.

**[0823]** Pharmaceutical compositions and delivery systems appropriate for the compositions, methods and uses of the disclosure are known in the art (see, e.g., Fox LM., (2006) *Am J Pharm Educ.* 70(3): p. 71).

## **Administration**

**[0824]** The present disclosure, among other things, provides methods of administering a composition (e.g., a pharmaceutical composition) comprising a plurality of rAAV particles formed by methods described herein and/or produced using systems described herein. Compositions (e.g., pharmaceutical compositions) comprising rAAVs produced with the methods described herein or using systems described herein can be used to treat a CNS disorder, e.g., administered to subjects suffering from or susceptible to a CNS disorder described herein.

**[0825]** In some embodiments, a composition (e.g., a pharmaceutical composition) comprising a plurality of rAAV particles described herein which is administered to a subject systemically (e.g., via intravenous delivery, etc.) can provide tropism to CNS cell and/or tissue. Without wishing to be bound by any particular theory, a composition comprising a plurality of rAAV particles described herein administered systemically to a subject provides CNS cell and/or tissue tropism (e.g., targeting to CNS cell and/or tissue) by binding to a receptor (e.g., a human Transferrin receptor (hTfR1)) which allows receptor-mediated transcytosis (RMT) across the

blood brain barrier. In some embodiments, rAAV particles described herein comprise variant AAV capsid proteins which can bind to hTfR1 and transcytose across a blood brain barrier. In some embodiments, rAAV particles described herein and compositions comprising the same can be targeted to CNS cell and/or tissue by systemic delivery, e.g., intravenous delivery.

**[0826]** In some embodiments, the route and/or mode of administration can vary depending upon the desired results. One with skill in the art (e.g., a physician), is aware that dosage regimens can be adjusted to provide the desired response, e.g., a therapeutic response. Methods of administration include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, oral, sublingual, intracerebral, intrathecal, intravaginal, transdermal, rectal, by inhalation, or topical, particularly to the ears, nose, eyes, or skin. Mode of administration is left to discretion of a practitioner.

**[0827]** For example, a composition may be administered by retinal, subretinal, intravitreal, intracameral or suprachoroidal injection or infusion. Additional exemplary routes of administration may include, but are not limited to, bronchial (e.g., bronchial instillation), buccal, enteral, interdermal, intra-arterial, intracisterna magna (ICM), intradermal, intragastric, intramedullary, intramuscular, intranasal, intra-parenchymal (e.g., intra-thalamic), intraperitoneal, intrathecal, intravenous, intraventricular, mucosal, nasal, oral, rectal, intraspinal, spinal sub-pial, subcutaneous, sublingual, topical, tracheal (e.g., intratracheal instillation), transdermal, vaginal, and vitreal administration.

**[0828]** Methods and uses disclosed herein include delivery and administration systemically, regionally or locally, or by any route, for example, by injection or infusion. A composition (e.g., a pharmaceutical composition) comprising a plurality of rAAV particles formed by methods described herein may be administered by injection or infusion by any route.

**[0829]** Delivery of a pharmaceutical composition *in vivo* may generally be accomplished via injection using a conventional syringe, although other delivery methods such as convection-enhanced delivery can also be used. For example, compositions may be delivered subcutaneously, epidermally, intradermally, intrathecally, intraorbitally, intramucosally, intraperitoneally, intravenously, intra-pleurally, intraarterially, intracoronarily, orally, intrahepatically, via the portal vein, or intramuscularly. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal applications. A clinician

specializing in treatment of patients with certain diseases or disorders may determine the optimal route for administration of vectors described herein.

**[0830]** Additionally, a pharmaceutical composition disclosed herein may also be administered by perfusion, e.g., by limb perfusion.

**[0831]** The disclosure provides methods for introducing a composition (e.g., a pharmaceutical composition) comprising rAAV particles described herein into a cell, a tissue, or an animal. In some embodiments, such methods comprise contacting a cell, a tissue, or an animal with a composition comprising rAAV particles described herein, such that at least one payload is expressed or present in the cell, tissue, or animal.

**[0832]** The disclosure also provides methods for administering a composition (e.g., a pharmaceutical composition) comprising rAAV particles described herein to a subject. In some embodiments, such methods include administering to a subject (e.g., a mammal), a composition comprising rAAV particles described herein, such that at least one payload is expressed or present in the subject (e.g., in a cell or tissue of a subject). In some embodiments, a method includes providing cells of a subject (e.g., a mammal) with a composition (e.g., a pharmaceutical composition) comprising rAAV particles described herein, such that at least one payload is expressed or present in the subject.

**[0833]** A composition (e.g., a pharmaceutical composition) comprising rAAV particles described herein can be administered in a sufficient or effective amount to a subject in need thereof. Doses can vary and depend upon a type, onset, progression, severity, frequency, duration, or probability of disease to which treatment is directed, the clinical endpoint desired, previous or simultaneous treatments, the general health, age, gender, race or immunological competency of the subject, and other factors that will be appreciated by a skilled artisan. Dose amount, number, frequency, or duration may be proportionally increased or reduced, as indicated by any adverse side effects, complications, or other risk factors of treatment and status of the subject. A skilled artisan will appreciate the factors that may influence the dosage and timing required to provide an amount sufficient for providing a therapeutic or prophylactic benefit.

**[0834]** A dose to achieve a therapeutic effect will vary based on several factors including, but not limited to: route of administration, level of payload or payload expression required to achieve a therapeutic effect, specific disease treated, any host immune response, and stability of

payload or payload expression. One skilled in the art can determine a dose range to treat a patient having a particular disease or disorder based on the aforementioned factors, as well as other factors.

**[0835]** An effective amount or a sufficient amount can (but need not) be provided in a single administration, may require multiple administrations, and, can (but need not) be, administered alone or in combination with another composition. For example, an amount may be proportionally increased as indicated by need of a subject, type, status, and severity of disease treated or side effects (if any) of treatment. Amounts considered effective also include amounts that result in a reduction of use of another treatment, therapeutic regimen, or protocol.

**[0836]** Accordingly, pharmaceutical compositions include compositions comprising rAAV particles in an effective amount to achieve an intended therapeutic purpose. Determining a therapeutically effective dose is well within the capability of a skilled medical practitioner using techniques and guidance provided herein. Therapeutic doses can depend on, among other factors, age and general condition of a subject, severity of a disease or disorder, and payload amount or expression in a subject. Thus, a therapeutically effective amount in humans will fall in a relatively broad range that may be determined by a medical practitioner based on response of an individual patient to rAAV-based treatment. Pharmaceutical compositions may be delivered to a subject so as to allow production of a payload described herein *in vivo* by gene- and or cell-based therapies or by *ex vivo* modification of a patient's or donor's cells.

**[0837]** In some embodiments, a composition (e.g., a pharmaceutical composition) comprising rAAV particles described herein may be administered to a subject once daily, weekly, every 2, 3, or 4 weeks, or even at longer intervals. In some embodiments, a composition (e.g., a pharmaceutical composition) comprising rAAV particles described herein may be administered according to a dosing regimen that includes (i) an initial administration that is once daily, weekly, every 2, 3, or 4 weeks, or even at longer intervals; followed by (ii) a period of no administration of, e.g., 1, 2, 3, 4, 5, 6, 8, or 10 months, or 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 years. In some embodiments, a composition (e.g., a pharmaceutical composition) comprising rAAV particles described herein may be administered (i) one or more times during an initial time period of up to 2, 4, or 6 weeks or less; followed by (ii) a period of no administration of, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 years. In some embodiments, a subject is monitored before and/or following

treatment with a composition (e.g., a pharmaceutical composition) comprising rAAV particles described herein.

*Immunosuppressive regimens*

[0838] Methods disclosed herein may also comprise administration of an immunosuppressive regimen in combination with an rAAV particle or a composition comprising the same.

[0839] In some embodiments, an immunosuppressive regimen comprises: (i) dexamethasone or prednisolone, and (ii) a calcineurin inhibitor. In some embodiments, a calcineurin inhibitor comprises a macrolide. In some embodiments, a calcineurin inhibitor comprises tacrolimus.

[0840] In some embodiments, an immunosuppressive regimen is administered intraosseously, intrathecally, intravenously, and/or orally. In some embodiments, an immunosuppressive regimen is administered intraosseously. In some embodiments, dexamethasone is administered intrathecally.

[0841] In some embodiments, an immunosuppressive regimen is administered daily.

[0842] In some embodiments, administration of an immunosuppressive regimen is initiated prior to administration of a rAAV particle or a composition comprising the same.

[0843] In some embodiments, an immunosuppressive regimen is administered (i) on each of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more days prior to administration of a rAAV particle or a composition comprising the same, (ii) on a same day as administration of a rAAV particle or a composition comprising the same, and/or (iii) on each day following administration of a rAAV particle or a composition comprising the same for about 1 week, 2 weeks, 3 weeks, 4 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, or longer.

[0844] In some embodiments, prednisolone is administered at a dose of about 0.3 mg/kg to about 10mg/kg. In some embodiments, prednisolone is administered at a dose of about 0.3 mg/kg, about 1mg/kg, about 2 mg/kg, about 3 mg/kg, about 4 mg/kg, about 5 mg/kg, about 6 mg/kg, about 7 mg/kg, about 8 mg/kg, about 9 mg/kg or about 10 mg/kg. In some embodiments, prednisolone is administered at a dose of about 3 mg/kg. In some embodiments, prednisolone is administered at a dose of 3 mg/kg.

[0845] In some embodiments, dexamethasone is administered at a dose of about 0.1 mg/kg to about 1 mg/kg. In some embodiments, dexamethasone is administered at a dose of about 0.1 mg/kg, about 0.2 mg/kg, about 0.3 mg/kg, about 0.4 mg/kg, about 0.5 mg/kg, about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, about 0.9 mg/kg, or about 1 mg/kg. In some embodiments, dexamethasone is administered at a dose of about 0.5 mg/kg.

[0846] In some embodiments, a calcineurin inhibitor is tacrolimus. In some embodiments, tacrolimus is administered at a dose of about 0.5 mg/kg to about 5 mg/kg. In some embodiments, tacrolimus is administered at a dose of about 0.5 mg/kg, about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, about 0.9 mg/kg, about 1 mg/kg, about 2 mg/kg, about 3 mg/kg, about 4 mg/kg or about 5 mg/kg.

[0847] In some embodiments, tacrolimus is administered at a dose of about 1 mg/kg.

[0848] In some embodiments, an immunosuppressive regimen comprises prednisolone and tacrolimus. In some embodiments, prednisolone is administered at a dose of about 3mg/kg and tacrolimus is administered at a dose of about 1mg/kg. In some embodiments, an immunosuppressive regimen is administered daily from 2 days prior to administration of a dose of a rAAV particle or a composition comprising the same to 20 days after administration of a dose of a rAAV particle or a composition comprising the same. In some embodiments, an immunosuppressive regimen is dosed orally. In some embodiments, a rAAV particle or a composition comprising the same is dosed intravenously.

[0849] In some embodiments, an immunosuppressive regimen comprises dexamethasone and tacrolimus. In some embodiments, dexamethasone is administered at a dose of about 0.5mg/kg and tacrolimus is administered at a dose of about 1mg/kg. In some embodiments, an immunosuppressive regimen is administered daily from 2 days prior to administration of a dose of a rAAV particle or a composition comprising the same to 20 days after administration of a dose of a rAAV particle or a composition comprising the same. In some embodiments, an immunosuppressive regimen is dosed orally. In some embodiments, a rAAV particle or a composition comprising the same is dosed intravenously.

#### **AAV capsid reference sequences**

## [0850] SEQ ID NO: 2001: AAV9 VP1 capsid reference sequence

1 MAADGYLPDW LEDNLSEGIR EWWALKPGAP QPKANQQHQD NARGLVLPGY KYLGPGNGLD  
 61 KGE PVNAADA AALEHDKAYD QQLKAGDNPY LKYNHADAEF QERLKEDTSF GGNLGRAVFO  
 121 AKKRILLEPLG LVEAAKTAP GKKRPVEQSP QEPDSSAGIG KSGAQPAKCR LNFGQTGDTE  
 181 SVPDPQPIGE PPAAPSGVGS LTMASGGAP VADNNEGADG VGSSSGNWHC DSQWLGDRCI  
 241 TTSTRTWALP TYNHHLYKQI SNSTSGGSSN DNAYFGYSTP WGYZDFNRFH CHFSPRDWQR  
 301 LINNNWGFRP KRLNFKLFNI QVKEVTDNNNG VKTIANNLTS TVQVFTDSY QLPYVLGSAH  
 361 EGCLPPFPAD VFMI PQYGYL TLNDGSQAVG RSSFYCLEYF PSQMLRTGNN FQFSYEFEV  
 421 PFHSSYAHQS SLDRLMNPLI DQYLYYLSKT INGSGQNQQT LKFSVAGPSN MAVQGRNYIP  
 481 GPSYRQQRVS TTVTQNNNSE FAWPGASSWA LNGRNSLMNP GPAMASHKEG EDRFFPLSGS  
 541 LIFGKQGTGR DNVDADKVMI TNEEEIKTTN PVATESYGVV ATNHQSAQAO AQTGWVQNQG  
 601 ILPGMVWQDR DVYLQGPIWA KIPHTDGFH PSPLMGGFGM KHPPPQILIK NTPVPADPPT  
 661 AFNKDKLNSF ITQYSTGQVS VEIEWELQKE NSKRWNPEIQ YTSNYYKSNN VEFVNTEGV  
 721 YSEPRPIGTR YLTRNL

## [0851] SEQ ID NO: 2002: AAV1 VP1 capsid reference sequence

1 MAADGYLPDW LEDNLSEGIR EWWDLKPGAP KPKANQQKQD DGRGLVLPGY KYLGPFNGLD  
 61 KGE PVNAADA AALEHDKAYD QQLKAGDNPY LRYNHADAEF QERLQEDTSF GGNLGRAVFO  
 121 AKKRVLEPLG LVEEGAKTAP GKKRPVEQSP QEPDSSSGIG KTGQQPAKCR LNFGQTGDSE  
 181 SVPDPQPLGE PPATPAAVGP TTMASGGAP MADNNNEGADG VGNASGNWHC DSTWLGDRCI  
 241 TTSTRTWALP TYNHHLYKQI SSASTGASND NHYFGYSTPW GYDFNRFHCH HFSPRDWQR  
 301 INNNWGFRPK RLNFKLFNIQ VKEVTTNDGV TTIANNLTST VQVFTDSEYQ LPYVLGSAH  
 361 GCLPPFPADV FMI PQYGYLT LNNGSQAVGR SFYCLEYFP SQMLRTGNNF TFSYTTEEVP  
 421 FHSSYAHQS SLDRLMNPLID QYLYYLNRTQ NQSGSAQNKD LLFSRGSPAG MSVQPKNWLP  
 481 GPCYRQQRVS KTKTDNNNSN FTWTGASKYN LNGRESIINP GTAMASHKDD EDKFFPMMSGV  
 541 MIFGKESAGA SNTALDNVMI TDEEEIKATN PVATERFGTV AVNFQSSSTD PATGDVHAM  
 601 ALPGMVWQDR DVYLQGPIWA KIPHTDGFH PSPLMGGFGM KNPPPQILIK NTPVPANPPA  
 661 EFSATKFASF ITQYSTGQVS VEIEWELQKE NSKRWNPEVQ YTSNYAKSAN VDFTVDNNGL  
 721 YTEPRPIGTR YLTRPL

## [0852] SEQ ID NO: 2003: AAV2 VP1 capsid reference sequence

1 MAADGYLPDW LEDTLSEGIR QWWKLKPGPP PPKPAERHKD DSRGLVLPGY KYLGPFNGLD  
 61 KGE PVNEADA AALEHDKAYD RQLDSDGNPY LKYNHADAEF QERLQEDTSF GGNLGRAVFO  
 121 AKKRVLEPLG LVEPVKTAP GKKRPVEHSP VEPDSSSGTG KAGQQPARKR LNFGQTGDAD  
 181 SVPDPQPLGQ PPAAPSLGT NTMATGSGAP MADNNNEGADG VGNSSGNWHC DSTWMGDRCI  
 241 TTSTRTWALP TYNHHLYKQI SSQSGASNDN HYFGYSTPW GYDFNRFHCH FSPRDWQR  
 301 NNNWGFRPKR LNFKLFNIQV KEVTQNDGTT TIANNLTSTV QVFTDSEYQL PYVLGSAH  
 361 CLPPFPADV MWPQYGYLTL NNNGSQAVGRS SFYCLEYFPS QMLRTGNNFT FSYTfedvPF  
 421 HSSYAHQS SLDRLMNPLID QYLYYLNRTNT PSGTTTQSRL QFSQAGASDI RDQSRNWLP  
 481 PCYRQQRVSK TSADNNNSEY SWTGATKYHL NGRDSLNVPG PAMASHKDD EKFFPQSGV  
 541 IFGKQGSEKT NVDIEKVMIT DEEEIRTTNP VATEQYGSVS TNLQRGNRQA ATADVNTQGV  
 601 LPGMVWQDRD VYLYQGPIWAK IIPHTDGFHPS SPLMGGFGM HPPPQILIKN TPVPANPST  
 661 FSAAKFASF TQYSTGQSV EIEWELQKEN SKRWNPEIQQ TSNYNKSVNV DFTVDTNGVY  
 721 SEPRPIGTRY LTRNL

## [0853] SEQ ID NO: 2007: AAV3B VP1 capsid reference sequence

1 MAADGYLPDW LEDNLSEGIR EWWALKPGVP QPKANQQHQD NRRGLVLPGY KYLGPGNGLD  
 61 KGE PVNEADA AALEHDKAYD QQLKAGDNPY LKYNHADAEF QERLQEDTSF GGNLGRAVFO  
 121 AKKRILEPLG LVEAAKTAP GKKRPVDQSP QEPDSSSGVG KSGKQPAKCR LNFGQTGDSE  
 181 SVPDPQPLGE PPAAPTSLGS NTMASGGAP MADNNNEGADG VGNSSGNWHC DSQWLGDRCI  
 241 TTSTRTWALP TYNHHLYKQI SSQSGASNDN HYFGYSTPW GYDFNRFHCH FSPRDWQR  
 301 NNNWGFRPKK LSFKLFNIQV KEVTQNDGTT TIANNLTSTV QVFTDSEYQL PYVLGSAH  
 361 CLPPFPADV MWPQYGYLTL NNNGSQAVGRS SFYCLEYFPS QMLRTGNNFQ FSYTfedvPF

421 HSSYAHSQL DRLMNPLIDQ YLYYLNRTOG TTSGTTNQSR LLFSQAGPQS MSLQARNWLP  
 481 GPCYRQQRLS KTANDNNNSN FPWTAASKYH LNGRDSLVP GPAMASHKDD EEKFFPMHGN  
 541 LIFGKEGT TA SNAELDNVMI TDEEEIRTTN PVATEQYGTV ANNLQSSNTA PTTRTVNDQG  
 601 ALPGMVWQDR DVYLGQPIWA KIPHTDGHFH PSPLMGGFGL KHPPQIMIK NTPVPANPPT  
 661 TFSPAKFASF ITQYSTGQVS VEIEWELQE NSKRWNPEIQ YTSNYNKSBN VDFTVDTNGV  
 721 YSEPRPIGTR YLTRNL

**[0854] SEQ ID NO: 2004: AAV5 VP1 capsid reference sequence**

1 MSFVDHPPDW LEEVGEGLRE FLGLEAGPPK PKPNQQHQDQ ARGLVLPGYN YLGPNGGLDR  
 61 GE PVNRADEV AREHDISYNE QLEAGDNPYL KYNHADAEFQ EKLADDTSFG GN LGKA FQ A  
 121 KKRVLEPFGL VEEGAKTAPT GKRIDDHPK RKKARTEEDS KPSTSSDAEA GPSGSQQLQI  
 181 PAQPASSLGA DTMSAGGGP LGDNNQGADG VGNASGDWHC DSTWMGDRVV TKSTRTWVLP  
 241 SYNNHQYREI KSGSDGSNA NAYFGYSTPW GFDFNRFHHS WSPRDWQRL INNYWGFRPR  
 301 SLRVKIFNIQ VKEVTVQDST TTIANNLTST VQVFTDDDYQ LPYVVNGNTE GCLPAFPQV  
 361 FTLPQYGYAT LNRDNTENPT ERSSFFCLEY FPSKMLRTGN NFEFTYNFEE VPFHSSFAPS  
 421 QNLFKLANPL VDQYLYRFVS TNNTGGVQFN KNLAGRYANT YKNWFPGPMG RTQGWNLGSG  
 481 VNRASVSAFA TTNRMELEGA SYQVPPQPNG MTNNLQGSNT YA LENTMIFN SQPANPGTTA  
 541 TYLEGNMLIT SESETQPVNR VAYNVGGQMA TNQSSSTAP ATGTYNLQEI VPGSVWMERD  
 601 VYLGQPIWAK IPETGAHFHP SPAMGGFGLK HPPPMLIKN TPVPGNITSF SDVPVSSFIT  
 661 QYSTGQVTVE MEWE LKKENS KRWNPEIQT NNYNDPQFVD FAPDSTGEYR TTRPIGTRYL  
 721 TRPL

**[0855] SEQ ID NO: 2005: AAV6 VP1 capsid reference sequence**

1 MAADGYLPDW LEDNLSEGIR EWWDLKPGAP KPKANQQKQD DGRGLVLPGY KYLGPFNGLD  
 61 KGE PVNAADA AA LEHD KAYD QQLKAGDNPY LRYNHADAEF QERLQEDTSF GGNLGRAVFO  
 121 AKKRVLEPFG LVEGAKTAP GKKRPVEQSP QEPDSSSGIG KTGQQPAKCR LNFGQTGDSE  
 181 SVDPDPQPLGE PPATPAAVGP TTMASGGAP MADNNEGADG VGNASGNWHC DSTWLGDRVI  
 241 TTSTRTWALP TYNNHLYKQI SSASTGASND NHYFGYSTPW GFDFNRFHHC HFSPRDWQRL  
 301 INNNWGFRPK RLNFKLFNIQ VKEVTTNDGV TTIANNLTST VQVFS DSEYQ LFYVLGSAHQ  
 361 GCLPPFPADV FMI PQYGYLT LNNGSQAVGR SSFYCLEYFP SQMLRTGNNF TFSYTFEDVP  
 421 FHSSYAHSQL LDRLMNPLID QYLYYLNRTO NQSGSAQNKD LLFSRGSPAG MSVQPKNWLP  
 481 GPCYRQQRVS KTKTDNNNSN FTWTGASKYN LNGRESIINP GTAMASHKDD KDKFFPMMSGV  
 541 MIFGKESAGA SNTALDNVMI TDEEEIKATN PVATERFGTV AVNLQSSSTD PATGDVHVMG  
 601 ALPGMVWQDR DVYLGQPIWA KIPHTDGHFH PSPLMGGFGL KHPPQILIK NTPVPANPPA  
 661 EFSATKFASF ITQYSTGQVS VEIEWELQE NSKRWNPEVQ YTSNYAKSAN VDFTVDDNNGL  
 721 YTEPRPIGTR YLTRPL

**[0856] SEQ ID NO: 2006: AAV8 VP1 capsid reference sequence**

1 MAADGYLPDW LEDNLSEGIR EWWALKPGAP KPKANQQKQD DGRGLVLPGY KYLGPFNGLD  
 61 KGE PVNAADA AA LEHD KAYD QQLQAGDNPY LRYNHADAEF QERLQEDTSF GGNLGRAVFO  
 121 AKKRVLEPLG LVEGAKTAP GKKRPVEPSP QRSPDSSTGI GKKGQQPARK RLNFQGTGDS  
 181 ESVPDPQPLG EPPAAPSGVG PNTMAAGGGP PMADNNEGAD GVGS SSGNWH CDSTWLGDRV  
 241 ITTSTRTWAL PTYNNHLYKQ ISNGTSGGAT NDNTYFGYST PWGYFD FNRF HCHFS PRDWQ  
 301 RLINNNWGFR PKRLSFKLFN IQVKEVTQNE GTKTIANNLT STIQVFTDSE YQLPYVLGSA  
 361 HQGCLPPFP DA VFMI PQYGY LT LNNGSQAV GRSSFYCLEY FPSQMLRTGN NFQFTYTFED  
 421 VPFHSSYAHSQL DRLMNPL IDQYLYLSR TQTTGGTANT QTLGF SQGGP NTMANQAKNW  
 481 LPGPCYRQQR VSTTTGQNNN SNFAWTAGTK YHLNGRNSLA NPGIAMATHK DDEERFFPSN  
 541 GILIFGKQNA ARDNADYSVD MLTSEEIKT TNPVATEEYD IVADNLQQQN TAPQIGTVNS  
 601 QGALPGMVWQ NRDVYLGQPI WAKIPHTDGN FHPSPLMGGF GLKH PPPQIL IKNTPVPADP  
 661 PTTFNQSKLN SFITQYSTGQ VSVEIEWELQ KENS KRWNPE IQYTSNYYKS TSVDFAVNTE  
 721 GVYSEPRPIG TRYLTRNL

## [0857] SEQ ID NO: 2008: AAV4 VP1 capsid reference sequence

1 MTDGYLPDW EDNLSEGVR EWWALQPGAPK PKANQQHQDN ARGLVLPGYK YLGPGNGLDK  
 61 GEPVNAADAA ALEHDKAYDQ QLKAGDNPYL KYNHADAEFQ QRLQEDTSFG GNLGRAVFQ  
 121 KKRVLEPLG LVEEGAKTAP GKKRPLESPQ QPDSSSTGIGK KGKQPAKKL VFEDETGAGD  
 181 GPPEGSTSGA MSSDSEMRAA AGGAAVEGGQ GADGVGNASG DWHDSTWSE GHVTTTSTR  
 241 WVLPTYNNHL YKRLGESLQS NTYNGFSTPW GFDFNRFH HFSPRDWQRL INNNWGMRP  
 301 AMRVKIFNIQ VKEVTTSNGE TTVANNLTST VQIFADSSYE LPYVMDAGQE GSLPPFPNDV  
 361 FMVPQYGYCG LVTGNTSQQQ TDRNAFYCLE YFPSQMLRTG NNFETYSFE KVPFHSMYAH  
 421 SQSLDRLMNP LIDQYLWGLQ STTTGTTLNA GTATTNFTKL RPTNFSNFKK NWLPGPSIKQ  
 481 QGFSKTANQN YKIPATGSDS LIKYETHSTL DGRWSALTPG PPMATAGPAD SKFSNSQLIF  
 541 AGPKQNGNTA TVPGTLIFTS EEELAATNAT DTMWGNLPG GDQSNSNLPT VDRLTALGAV  
 601 PGMVWQRDI YYQGPWAKI PHTDGHFHP PLIGGFGLKH PPPQIFIKNT PVPANPATTF  
 661 SSTPVNSFIT QYSTGQVSQ IDWEIQKERS KRWNPEVQFT SNYGQONSLL WAPDAAGKYT  
 721 EPRAIGTRYL THHL

## [0858] SEQ ID NO: 2009: AAV7 VP1 capsid reference sequence

1 MAADGYLPDW LEDNLSEGIR EWWDLKPGAP KPKANQQKQD NGRGLVLPGY KYLGPFNGLD  
 61 KGE PVNAADA AALEHDKAYD QQLKAGDNPY LRYNHADAEF QERLQEDTSF GGNLGRAVFQ  
 121 AKKRVLEPLG LVEEGAKTAP GKKRVEPSP QRSPDSSTGI GKKGQQPARK RLNGQQTGDS  
 181 ESVDPDQPLG EPPAAPSSVG SGTVAAGGGA PMADNNEGAD GVGNASGNWH CDSTWLGD  
 241 ITTSTRTWAL PTYNNHLYKQ ISSETAGSTN DNTPYFGYSTP WGYFDFNRFH CHFSPRDWQ  
 301 LINNNWGRFP KKLRFKLFNI QVKEVTTNDG VTTIANNLTS TIQVFSDSEY QLPYVLGS  
 361 QGCLPPFPAD VFMI PQYGYL TLNNNGSQVG RSSFYCLEYF PSQMLRTGNN FEFSYSFEDV  
 421 PFHSSYAHQS SLDRLMNPLI DQYLYYLART QSNPGGTAGN RELQFYQGGP STMAEQAKNW  
 481 LPGPCFRQQR VSXTLDQNNN SNFAWTGATK YHNGRNSLV NPGVAMATHK DDEDRFFPSS  
 541 GVLFKGKTA TNKTTLENVL MTNEEEIRPT NPVATEEYGI VSSNLQAANT AAQTVVVNNQ  
 601 GALPGMVWQ RDVYLQGPIW AKIPHTDGNF HPSPLMGFFG LKHPPPQILI KNTPVPANPP  
 661 EVFTPAKFAS FITQYSTGQV SVEIEWELQ ENSKRWNPEI QYTSNFEKQT GVDFAVDSQG  
 721 VYSEPRPIGT RYLTRNL

## [0859] SEQ ID NO: 2010: AAV10 VP1 capsid reference sequence

1 MAADGYLPDW LEDNLSEGIR EWWDLKPGAP KPKANQQKQD DGRGLVLPGY KYLGPFNGLD  
 61 KGE PVNAADA AALEHDKAYD QQLKAGDNPY LRYNHADAEF QERLQEDTSF GGNLGRAVFQ  
 121 AKKRVLEPLG LVEEAAKTAP GKKRVEPSP QRSPDSSTGI GKKGQQPAKK RLNGQQTG  
 181 ESVDPDQPIG EPPAGPSGLG SGTMAAGGGA PMADNNEGAD GVGSSSGNWH CDSTWLGD  
 241 ITTSTRTWAL PTYNNHLYKQ ISNGTSGGST NDNTYFGYST PWGYFDFNRFH HCHFSPRDWQ  
 301 RLINNNWGRFP KRLSFKLFN IQVKEVTQNE GTKTIANNLT STIQVFTDSE YQLPYVLGS  
 361 HQGCLPPFPAD DVFMIPQYGY LTLNNGSQAV GRSSFYCLEY FPSQMLRTGNN NEFSYTFED  
 421 VPFHSSYAHQS QSLDRLMNPL IDQYLYYLSR TQSTGGTQGT QQLLFSQAGP ANMSAQAKNW  
 481 LPGPCYRQQR VSTTLSQNNN SNFAWTGATK YHNGRDSLV NPGVAMATHK DDEERFFPSS  
 541 GVLMFGKQGA GRDNVDYSSV MLTSEEIKT TNPVATEQYQ VVADNLQQAN TGPIVGNVNS  
 601 QGALPGMVWQ RDVYLQGPI WAKIPHTDGN FHPSPLMGGF GLKHPPPQIL IKNTPVPADP  
 661 PTTFSQAKLA SFITQYSTGQ VSVEIEWELQ KENSKRWNPE IGYTSNYYKS TNVDFAVNTE  
 721 GTYSEPRPIG TRYLTRNL

## [0860] SEQ ID NO: 2011: AAV11 VP1 capsid reference sequence

1 MAADGYLPDW LEDNLSEGIR EWWDLKPGAP KPKANQQKQD DGRGLVLPGY KYLGPFNGLD  
 61 KGE PVNAADA AALEHDKAYD QQLKAGDNPY LRYNHADAEF QERLQEDTSF GGNLGRAVFQ  
 121 AKKRVLEPLG LVEEGAKTAP GKKRPLESPQ EPDSSSGIGK KGKQPAKRL NFEEDTGAGD  
 181 GPPEGSDTSA MSSDIEMRAA PGGNAVDAGQ GSDGVGNASG DWHDSTWSE GKVTTTSTR

241 WVLPTYNHNL YLRLGTTSSS NTYNGFSTPW GYFDNFRCFC HFSPRDWQRL INNNWGLRPK  
 301 AMRVKIFNIQ VKEVTTSNGE TTVANNLTST VQIFADSSYE LPYVMDAGQE GSLPPFPNDV  
 361 FMVPQYGYCG IVTGENQNQT DRNAFYCLEY FPSQMLRTGN NFEMAYNFEK VPFHSMYAH  
 421 QSLDRLMNPL LDQYLWHLQS TTSGETLNQG NAATTFGKIR SGDFAFYRKN WLPGPCVKQ  
 481 RFSKTASQNY KIPASGGNAL LKYDTHYTLN NRWSNIAPGP PMATAGPSDG DFSNAQLIFP  
 541 GPSVTGNTTT SANNLLFTSE EEIAATNPRD TDMFGQIADN NQNATTAPIT GNVTAMGVLP  
 601 GMVWQNRDIY YQGPIWAKIP HADGHFHPSP LIGGFGLKHP PPQIFIKNTP VPANPATTFT  
 661 AARVDSFITQ YSTGQAVAVQI EWEIEKERSK RWNPEVQFTS NYGNQSSMLW APDTTGKYTE  
 721 PRVIGSRYLT NHL

**[0861] SEQ ID NO: 2012: AAV12 VP1 capsid reference sequence**

1 MAADGYLPDW LEDNLSEGIR EWWALKPGAP QPKANQQHQD NGRGLVLPGY KYLGPGNGLD  
 61 KGE PVNEADA AALEHDKAYD KQLEQGDNPY LKYNHADAEF QQR LATDTSF GGNLGRAVFO  
 121 AKKRILEPLG LVEGVKTAP GKKRPLEKTP NRPTNPDSKG APAKKKQKDG EPADSARRTL  
 181 DFEDSGAGDG PPEGSSSGEM SHDAEMRAAP GGNAVEAGQQ ADGVGNASGD WHCDSTWSEG  
 241 RVTTTSTRTW VLPTYNHLY LRIGTTANSN TYNGFSTPWG YFDFNRFHCH FSPRDWQRLI  
 301 NNNWGLRPKS MRVKIFNIQV KEVTTNSGET TVANNLTSTV QIFADSTYEL PYVMDAGQEG  
 361 SFPPFPNDVF MVPQYGYCGV VTGKNQNQTD RNAFYCLEYF PSQMLRTGNN FEVSYQFEKV  
 421 PFHSMYAHQS SLDRMMNPLL DQYLWHLQST TTGNSLNQGT ATTTYGKITT GDFAYYYRKNW  
 481 LPGACIKQQK FSKNANQNYK IPASGGDALL KYDTHTTLNG RWSNMAPGPP MATAGAGDSD  
 541 FSNSQLIFAG PNPSGNTTTS SNNLLFTSEE EIATTNPRDT DMFGQIADNN QNATTAPHIA  
 601 NLDAMGIVPG MVWQNRDIY YQGPIWAKVPH TDGHFHPSP LMGGFGLKHPP PQIFIKNTPV  
 661 PANPNNTFSA ARINSFLTQY STGQAVQID WEIQKEHSKR WNPEVQFTSN YGTQNSMLWA  
 721 PDNAGNYHEL RAIGSRFLTH HL

**[0862] SEQ ID NO: 2013: AAV13 VP1 capsid reference sequence**

1 MTDGYLPDW EDNLSEGVR WWALQPGAPK PKANQQHQDN ARGLVLPGYK YLGPGNGLDK  
 61 GEPVNAADAA ALEHDKAYD QLKAGDNPYL KYNHADAEFQ ERLQEDTSFG GNLGRAVFO  
 121 KKRILEPLGL VEEAAKTAPG KKRPVEQSPA EPDSSSGIGK SQQQPARKRL NFGQTGDTE  
 181 VPDPQPLGQP PAAPSGVGST TMASGGGAPM ADNNEGADGV GNSSGNWHCD SQWLGDRVIT  
 241 TSTRTRWALPT YNNHLYKQIS SQSGATNDNH YFGYSTPWGY FDFNRFHCH SPRDWQRLIN  
 301 NNWGFRRPKRL NFKLFNQIVKQ EVTQNDGTTT IANNLTSTVQ VFTDSEYQLP YVLGSAHQGC  
 361 LPPFPADVM VPQYGYLTLN NGSQAVGRSS FYCLEYFPSQ MLRTGNNFQF SYTFEDVPFH  
 421 SSYAHQSLSRQ RLMNPLIDQY LYLYNRTQTA SGTQOSRLLF SQAGPTSMSL QAKNWLPGPC  
 481 YRQQRSLSKQA NDNNNSNFPW TGATKYHLNG RDSSLVNP GPA MASHKDDKEK FFPMHGTLIF  
 541 GKEGTNANNA DLENVMITDE EEIRTTNPVA TEQYGTVSNN LQNSNAGPTT GTVNHQGALP  
 601 GMVWQDRDVY LQGPIWAKIP HTDGHFHPSP LMGGFGLKHPP PPQIMIKNTP VPANPPTNFS  
 661 AAKFASFITQ YSTGQVSVEI EWELQENSK RWNPEIQYTS NYNKSJVNVDF TVDTNGVYSE  
 721 PRPIGTRYLT RNL

**[0863] SEQ ID NO: 2014: AA Vhu68 VP1 capsid reference sequence**

1 MAADGYLPDW LEDNLSEGIR EWWALKPGAP QPKANQQHQD NARGLVLPGY KYLGPGNGLD  
 61 KGE PVNEADA AALEHDKAYD QQLKAGDNPY LKYNHADAEF QERLKEDTSF GGNLGRAVFO  
 121 AKKRILLEPLG LVEEAAKTAP GKKRVEQSP QEPDSSVGIG KSGAQPAKCR LNFGQTGDTE  
 181 SVDPDPQPIGE PPAAPSGVGS LTMASGGGAP VADNNEGADG VGSSSGNWHC DSQWLGDRVI  
 241 TTSTRTRWALP TYNHLYKQI SNSTSGGSSN DNAYFGYSTP WGYFDFNRFH CHFSPRDWQR  
 301 LINNNWGLFRP KRLNFKLFNI QVKEVTDNNNG VKTIANNLTS TVQVFTDSDY QLPYVLSA  
 361 EGCLPPFPAD VFMIPQYGYL TLNDGSQAVG RSSFYCLEYF PSQMLRTGNN FQFSYEFENV  
 421 PFHSSYAHQS SLDRLMNPLI DQYLYYLSKT INGSGQNQQT LKFSVAGPSN MAVQGRNYIP  
 481 GPSYRQQRVS TTVTQNNNSE FAWPGASSWA LNGRNSLMNP GPAMASHKEG EDRFFPLSGS  
 541 LIFGKQGTGR DNVDADKVM TNEEEIKTTN PVATESYQGV ATNHQSAQAO AQTGWVQNZQ

601 ILPGMVWQDR DVYLQGPIWA KIPHTDGNFH PSPLMGGFGM KHPPPQILIK NTPVPADPPT  
661 AFNKDKLNSF ITQYSTGQVS VEIEWELQKE NSKRWNPEI Q YTSNYYKSNN VEFAVNTEGV  
721 YSEPRPIGTR YLTRNL

[0864] SEQ ID NO: 2014: AAVrh10 VP1 capsid reference sequence

1 MAADGYLPDW LEDNLSEGIR EWWDLKPGAP KPKANQQKQD DGRGLVLPGY KYLGPFNGLD  
61 KGEPVNAADA AALEHDKAYD QQLKAGDNPY LRYNHADAEF QERLQEDTSF GGNLGRAVFO  
121 AKKRVLEPLG LVEGAKTAP GKKRPVEPSP QRSPDSSTGI GKKGQQPAKK RLNFQQTGDS  
181 ESVPDQPPIG EPPAGPSGLG SGTMAAGGGGA PMADNNEGAD GVGSSSGNWH CDSTWLGDRV  
241 ITTSTRTWAL PTYNHHLYKQ ISNGTSGGST NDNTYFGYST PWGYFDFNRF HCHFSPRDWQ  
301 RLINNNWGFR PKRLNFKLFN IQVKEVQTNE GTKTIANNLT STIQVFTDSE YQLPYVLGSA  
361 HQGCLPPFFPA DVFMIPQYGY LTLNNGSQAV GRSSFYCLEY FPSQMLRTGN NFEFSYQFED  
421 VPFHSSYAHQS QSLDRLMNPL IDQYLYYLSR TQSTGGTAGT QQLLFSQAGP NNMSAQAKNW  
481 LPGPCYRQQR VSTTLSQNNN SNFAWTGATK YHLNGRDSLV NPGVAMATHK DDEERFFPSS  
541 GVLMFGKQGA GKDNVDYSSV MLTSEEIKT TNPVATEQYG VVADNLQQQN AAPIVGAVNS  
601 QGALPGMVWQ NRDVYLQGPI WAKIPHTDGN FHPSPLMGGF GLKHPPPQIL IKNTPVPADP  
661 PTTFSQAKLA SFITQYSTGQ VSVEIEWELQ KENSKRWNP E IQYTSNYYKS TNVDFAVNTD  
721 GTYSEPRPIG TRYLTRNL

[0865] The disclosure is further illustrated by the following example. An example is provided for illustrative purposes only. It is not to be construed as limiting the scope or content of the disclosure in any way.

[0866] All publications, patent applications, patents, and other references mentioned herein, including GenBank Accession Numbers, are incorporated by reference in their entirety. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described herein.

## EXAMPLES

[0867] The following example is put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present disclosure, and is not intended to limit the scope of what the inventors regard as their discovery nor are they intended to represent that the experiments below are all or the only experiments performed.

**Example 1: Identification of capsids with enhanced CNS cell or tissue tropism**

[0868] This Example describes production of rAAV particles displaying exemplary anti-TfR1 VHHS inserted in the AAV capsid for targeting AAV capsids to central nervous system or to muscle cells. In some embodiments, CNS- and/or muscle-targeting AAV capsids are produced by inserting an anti-TfR1 VHH in in a VP1 protein of an AAV capsid.

**Materials and Methods**

[0869] *VHH Immunization Campaign Reagent Production.* For VHH discovery, DNA, protein and cell line antigens were generated as follows: DNA comprised of plasmid vectors encoding full length human, murine or cyno TfR1 driven by a mammalian promoter. Protein antigens comprised of His-tagged TfR1 ectodomains with human, cyno, mouse or alpaca sequences. Cell line antigens comprised of alpaca skin stromal cells (ASSCs) engineered to stably express full-length human, cyno or mouse TfR1.

[0870] *VHH antibody production.* Antibody VHH reagents were generated in bivalent format on human IgG1 (N297Q) Fc or monovalent format where VHH was fused to human IgG1(N297Q) Fc with knob mutation and paired with IgG1(N297Q) Fc with hole mutations to facilitate generation of the asymmetric monovalent VHH-Fc fusion. An N-terminal signal sequence preceded the antibody gene in all expression constructs to facilitate secretion extracellularly. Constructs were transiently transfected into Chinese Hamster Ovary-S (CHO-S) cells for expression and antibodies were purified from culture supernatant once cells were harvested. Proteins were purified by loading supernatant on HiTrap MabSelect SuRe columns (Cytiva), eluting with 25 mM sodium phosphate, 100 mM sodium chloride, pH 2.8, then neutralizing 1:60 (v:v) with 0.5 M sodium phosphate, pH 8.6. Secondary purification to remove mis-paired homodimers was performed by passing neutralized eluates over MonoRab Anti-Camelid VHH affinity resin (GenScript), eluting with a stepwise pH gradient of 0.1 M glycine at pH 4.5, 4.0, and 3.5, then neutralizing with 1 M Tris-HCl, pH 8.0. Aggregate content was assessed, and if >5% of total protein, antibodies were further purified by size exclusion chromatography on a Superdex 75 column (Cytiva) in PBS.

[0871] *Stable cell line production.* For antibody screening and affinity measurements, stable cell lines were generated where endogenous hamster TfR1 was knocked out of Chinese Hamster Ovary-S (CHO-S) cells by CRISPR- Cas9 manipulation and the engineered cells were

transduced with plasmids encoding full length human, cyno, or mouse TfR1. Cell surface expression of TfR1 in all cell lines was verified by staining cells either with Alexa647-labeled human transferrin or appropriate antibodies against TfR1 followed by analysis by flow cytometry.

**[0872]** *Production of human, cyno, and murine TfR1 Ectodomain reagent:* To support binding analysis, recombinant human TfR1 ectodomain (huTfR1 ECD) (aa89-760) and cynomolgous macaque TfR1 ectodomain (cyTfR1 ECD) (aa89-760), and murine TfR1 Ectodomain (muTfR1 ECD) (aa89-763) with N-terminal 8xHis-Gly tags, were transiently expressed in CHO-S. TfR1 protein was purified using nickel Sepharose excel resin (Cytiva) eluting in 50 mM Tris, 400 mM NaCl, 10 mM MgCl<sub>2</sub>, 500 mM imidazole, pH 7.8, followed by size exclusion chromatography on a superdex 200 26/600 column equilibrated in PBS + 0.2 M NaCl. Eluate was aliquoted and stored at -80C with the addition of 10% glycerol to prevent protein aggregation upon freeze-thaw.

**[0873]** *Human TfR1-Tf binary complex formation:* To form and enrich the human TfR1 ectodomain / holo-transferrin binary complex (huTfR1-Tf), 1 mg holo-transferrin (Sigma; T0665) was incubated with 0.5 mg human TfR1 ECD before size selection using a Superdex 200 column with 10 mM HEPES (pH 7.5) + 100mM NaCl as running buffer. Peaks corresponding to the binary complex were isolated and flash frozen. Presence of both human TfR1 ECD and holo-transferrin in the complex was verified using SDS-PAGE.

**[0874]** *Alpaca immunization and VHH discovery:* To generate single-domain antibodies against the TfR1 ectodomain (ECD), Alpacas were immunized (Capralogics, Inc.) using two immunization schemes. In scheme 1, two alpacas were immunized with 2 mg of murine TfR1 DNA via Dermojet intradermal injection on day 0. Four additional boosts on day 14, 28, 42 and 56 were performed with 2 mg of alternating murine human or cyno TfR1 DNA in an identical manner. Three weeks following the DNA boosts, 50 million ASSC cells over-expressing human or mouse TfR1 were injected on days 77 and 91 via subcutaneous delivery with incomplete Freund's adjuvant administered in tandem. Following the DNA or ASSC cellular boosts, 300 mL whole blood collections were taken on days 63 and 98. In scheme 2, one alpaca was immunized with 1 mg of KLH conjugated His-tagged TfR1 ectodomain containing human, cyno, mouse or alpaca sequences on day 0, and again on day 7 via subcutaneous injection. Three additional

injections were performed on days 17, 31 and 45 with 0.4 mg of protein. Two final injections consisting of 1 mg of protein were performed on days 45 and 52. All immunizations in scheme 2 were pre-mixed with GERBU adjuvant prior to administration. A 300 mL whole blood collection was taken following the final boost on days 56 and 62.

**[0875]** Circulating PBMCs were isolated from the whole blood collections from both immunization schemes by diluting with PBS at a 1:1 ratio and proceeding with a density gradient centrifugation method using Ficoll-Paque Plus (GE Healthcare, 17-1440-02). Isolated PBMCs were frozen at -80 in RNAlater (ThermoFisher, AM7021). The VHH libraries were generated from the PBMCs using methods described previously (Pardon, *Nature Protocols*, 2019, vol.9 issue 3). Total RNA was purified from PBMCs (Qiagen, 75142) and cDNA was generated via RT-PCR (ThermoFisher, 18091050). VHH repertoires were PCR amplified from total cDNA using a forward primer specific to the VHH leader sequence 5'-CTGGGTGGTCCTGGCTGC-3' (SEQ ID NO: 2019) and reverse primers specific to the IgG2/3 hinge regions 5'-GGAGCTGGGTCTCGCTGTGGTGC-3', 5'-TGGTTGTGGTTTG GTCTTGGGTT-3' (SEQ ID NO: 2020) (Maass et al., 2007). A second round of PCR was performed with primers specific to the VHH FW1 V-gene and FW4 J-gene regions found in the IMGT database, and the resulting amplicons were transformed into yeast via homologous recombination using published methods (Rappazzo, *Science*, 2021).

**[0876]** Yeast display VHH libraries were screened in accordance with the methods disclosed in US Patent Publications 20100056386 and 20090181855 as fusions to human IgG1 Fc. Iterative rounds of selection were performed against recombinant HIS tagged human and cyno TfR1, as well as human TfR1 pre-blocked with recombinant human holo-transferrin (Sigma; T0665). Colonies were subsequently sequenced to identify unique clones, using techniques known in the art. Following this campaign, antibodies were expressed from yeast and purified on protein A resin. Yeast-expressed antibodies were tested for binding to human TfR1 ECD recombinant protein using Bio-Layer Interferometry (BLI). Antibody binding was similarly performed against human TfR1 after pre-blocking of human TfR1 ECD with recombinant human holo-transferrin. BLI was performed on an Octet RED384 and Octet HTX instruments (ForteBio) according to standard procedures. Yeast-expressed antibodies were also tested for binding to CHO cells over-expressing full-length human, cyno and mouse TfR1. Cells were

incubated with antibodies at a single 200 nM concentrations for 2 hours on ice, washed twice with isotonic buffer, then incubated with a fluorescently labeled anti-Fc secondary antibody (Jackson; 109-546-008), fixed in 1% PFA and analyzed on a flow cytometer.

**[0877]** *DNA Constructs for AAV-VHH production:* DNA constructs were designed to facilitate the production of rAAV particles displaying an exemplary anti-TfR1 VHH inserted in the AAV capsid (AAV-VHH) using a quadruple transfection procedure like that described in Eichhoff et al. 2019, with modifications to enable VHH incorporation into an AAV9 backbone. The VP1 plasmid consists of the Rep2 and AAV9 virus protein 1 (VP1) coding sequence with VHH inserted at a specific location, and a linker sequence flanking the insertion site. Exemplary linker sequences used to produce AAV-VHH particles described herein are described in Table 2. The VP1 plasmid also contains an ACG to ACC mutation at the non-canonical translation initiation start site of VP2, and an ATG to TTG mutation at the translation initiation start site of VP3, which combine to ablate VP2 and VP3 expression, respectively. The VP2/3 plasmid consists of the Rep2 and AAV9 VP1 coding sequence, with an ATG to AAG mutation at the translation initiation start site of VP1, which ablates only VP1 expression from this plasmid.

**[0878]** Barcode cis-plasmids for library production consist of AAV2 inverted terminal repeats (ITR2)-flanked sequence containing either a ubiquitous CAG promoter or neuronal-tropic hSyn1 promoter, an open reading frame encoding histone H2B with C-terminal enhanced green fluorescent protein (H2B-EGFP), 16bp specific molecular barcode, and bovine growth hormone (bGH) polyadenylation signal.

**[0879]** Cis-plasmid (pSS305) for single vector production carries an ITR2-flanked vector genome composed of a CAG promoter, sequence encoding mCherry with C-terminal 3xFLAG, WPRE, and human growth hormone (hGH) polyadenylation signal.

**[0880]** *AAV9 and AAV-VHH Individual Particle Production:* AAV9 control particles and AAV9 particles displaying a Clone A VHH with linker design 2 inserted in the AAV capsid (Clone A VHH-D2), a Clone A VHH with linker design 7 (Clone A VHH-D7), and Clone B VHH with linker design 1 (Clone B VHH-D1) were produced. To produce AAV9 control particles, Viral Production Cells 2.0 (VPC2) were transfected using PEI Max with 1) an Ad helper plasmid, 2) a Rep/Cap plasmid, and 3) a plasmid carrying an AAV expression cassette of either CAG-mCherry-WPRE or hSyn1-transgene X-WPRE using a triple transfection protocol

(Xiao Xiao et al. 1998) using a 2:1.5:1 mass ratio (**FIG. 1A**). Transgene X is an exemplary transgene that is useful for delivery to CNS tissue. To produce AAV-VHH particles displaying an exemplary anti-TfR1 VHH, Viral Production Cells 2.0 (VPC2) were transfected using PEI Max with 1) an Ad helper plasmid, 2) the VP2/3 plasmid, 3) a corresponding VP1 plasmid, and 4) a gene of interest (GOI) plasmid (CAGGS.mCherry or hSyn1.Transgene X)using a quadruple transfection protocol (Warrington et al. 2004, Judd et al. 2012, Eichoff et al. 2019) using a 2:1.2:1.2:1 mass ratio (**FIG. 1B**). At 72h post transfection, AAV was released from the cells by Triton x-100-based chemical lysis, followed by clarification via centrifugation or filtration. Particles were then purified through either 2-step chromatography using Avipure AAV9 affinity resin followed by anion exchange polishing or harvest tangential flow filtration (TFF) followed by iodixanol ultracentrifugation. Purified viruses were then buffer exchanged into phosphate-buffered saline (pH 7.4) with 0.001% Pluronic F-68 or an IV buffer (20 mM Tris pH 8, 200 mM NaCl, 1 mM MgCl<sub>2</sub>, 50 ppm Pluronic F-68), Analytical characterization included genome titer quantification (digital droplet PCR (ddPCR)-based), purity analysis (SYPRO-Ruby staining of PAGE gels or CE-SDS), aggregation assessment (DLS), packaging efficiency profile (mass photometry), bioburden (negative microbial growth in permissive medium), and endotoxin measurement (Endosafe LAL).

**[0881]** *AAV-VHH Barcode Library Production:* VHH-insertion AAV variants along with parental AAV9, were selected for the barcode AAV library production. Particle production was performed by transfecting HEK293T cells using PEI Max with a mixture of the following plasmids, 1) an Ad helper plasmid, 2) the VP2/3 plasmid, 3) a specific VP1 plasmid, and 4) equal mixture of 4 unique barcoded cis plasmids, with two barcodes driven by a CAG promoter and two barcodes driven by an hSyn1 promoter. (**FIG. 1C**). Separate transfections were performed for individual AAV particles. Crude lysates for all library variants were harvested three days post-transfection and pooled together for iodixanol density gradient purification. Particle preparations were buffer exchanged into phosphate-buffered saline (pH 7.4) with 0.001% Pluronic F-68, and subsequently subjected to digital droplet PCR (ddPCR)-based titration, purity analysis by SYPRO-Ruby staining of PAGE gels, and endotoxin measurement.

**[0882]** *VHH Affinity Assessment via Flow Cytometry:* Monovalent VHH-Fc fusions were incubated with stable cell lines at the indicated concentrations for 1-2 hours on ice, washed 3

times with isotonic buffer, incubated with PE-conjugated anti-hFc for 1 hour on ice, fixed with 1% paraformaldehyde for 15 min at RT, then analyzed by flow cytometry for detection of antibody signal by PE. Mean fluorescence intensity (MFI) of the PE fluorophore was calculated at each concentration and EC<sub>50</sub> values were determined by a 3-parameter logarithmic fit in the GraphPad Prism software.

**[0883]** *VHH Affinity Assessment via Surface Plasmon Resonance (SPR).* SPR binding analysis was performed on a Biacore 8K(+) instrument at room temperature in 10mM HEPES, 150mM NaCl, 3mM EDTA, 0.05%BSA, 0.005% P20, pH 7.4, using a CM5 chip with an anti-His capture kit (Cytiva). His-tagged human, cynomolgus monkey, or murine TfR1 ectodomain was captured by anti-His capture at 40-80pg/mm<sup>2</sup> on the sensor surface, then monovalent VHH-Fc fusion reagents were injected at concentrations ranging from 781 pM to 4000 nM. 10 mM glycine (pH 1.5) was used for chip regeneration between binding cycles. Binding sensorgrams were measured and analyzed with a 1:1 binding model using the Biacore Insight Evaluation software to produce monovalent affinity values (equilibrium dissociation constants, K<sub>D</sub>). In cases where the 1:1 binding model did not fit the sensorgram data, a steady state analysis was performed to determine K<sub>D</sub>.

**[0884]** *AAV-VHH Affinity Assessment via Bio-Layer Interferometry (BLI).* BLI binding analysis was performed on an Octet HTX instrument at room temperature in 10mM HEPES, 150mM NaCl, 1%BSA, 0.05% P20, pH 7.4, using streptavidin biosensors (Sartorius) coated with CaptureSelect biotin anti-AAVX (ThermoFisher). Capsids at 1.0E+11 GC/mL were captured by AAVX on the sensor surface, followed by addition of human or cynomolgus monkey TfR1 ectodomain at concentrations ranging from 2.7 nM to 2000 nM. Binding sensorgrams were trimmed to bias toward initial off-rate and analyzed with a 1:2 bivalent analyte model using the ForteBio Data Analysis software to produce affinity measurements (K<sub>D</sub>).

**[0885]** *Grid preparation and data collection for cryo-electron microscopy studies:* TfR1-Tf-VHH complex was prepared by adding 15 uLs of VHH monovalent Fc (2 mg/mL) to 60 uLs of huTfR1-Tf binary complex (0.4 mg/mL) immediately before electron microscopy grid preparation. 3.5 uLs of this complex was applied to glow discharged Quantifoil R1.2/1.3 grids, blotted for 5 seconds, then plunge frozen into liquid ethane using a Vitrobot Mark IV. Data collection was performed on a Glacios cryo-transmission electron microscope (Cryo-TEM)

operating at 200 kV equipped with a K3 direct electron detector. ~6000 movies were collected at 36,000x magnification with a total electron dose of 43 e-/Å<sup>2</sup> over 50 frames.

**[0886]** *CryoEM data processing:* Raw movies were motion corrected in RELION using MotionCor2 and CTF values were estimated using GCTF. Using an initial subset of 500 random micrographs, Topaz picking was performed using the general model. Particles were classified and good picks were used to train a Topaz model. The train model was then used to pick particles over the entire dataset. Pick particles were extracted and imported into cryoSPARC for 2D classification. A random subset of 100k particles was used in ab-initio to generate initial 3D references and then all particles were classified using heterogenous refinement. The best class was refined using homogeneous refinement and then particles were imported into RELION. Particles underwent iterative CTF refinements followed by Bayesian polishing. Particles were sorted using fixed angle 3D classification and best class was further refined to obtain best cryoEM density map at 2.4Å resolution.

**[0887]** *Atomic model building and refinement:* The crystal structure of huTfR1-Tf (PDB: 3S9N) and an AlphaFold model of VHH was docked into the cryoEM in Chimera then rigid body docking was performed in Phenix. Model was rebuilt manually using Coot followed by real space refinement in Phenix.

**[0888]** *Animals.* Adult human TfR1 knock-in mice (hTfR1-KI) mice were purchased from Biocytogen (Catalog# 110861; also referred to herein as B-hTfR1-KI), and genotype was confirmed by Transnetyx. Homozygous hTfR1-KI<sup>+/+</sup> mice were housed at Biocytogen throughout the study. Adult males were used for AAV library screening. Six to eight week-old hTfR1-KI male and female mice were used for capsid validation with the hSyn1-Transgene X-WPRE transgene. A 30-month-old and a 41-month-old male cynomolgus macaque of Vietnamese origin were included in the barcode AAV library screening study. Both animals were pre-screened for lack of anti-AAV9 neutralizing antibodies before inclusion in the study.

**[0889]** *Barcode AAV library screening in vitro:* WT and TfR1-knockout HEK293T cells were seeded in 24-well plates at 5e5 cells per well. Barcode AAV library was added to cells at 100,000 multiplicity of infection (MOI). Cells were incubated with vector library for 48 hours, and subsequently washed and lysed for RNA extraction. Extracted RNA was treated with DNase, reverse transcribed, and PCR-amplified for NGS analysis.

[0890] *Barcoded AAV library screening in vivo:* AAV libraries were I.V. injected at 1E12 vg per animal to 4 WT and 3 hTfR1-KI<sup>+/+</sup> mice in C57BL/6 background. Necropsies were performed 21 days post-injection, and brains and livers were collected for RNA isolation, reverse transcription, and amplicon-seq on transgene barcodes. For library screening in NHP, AAV library was I.V. injected into two 30-41 month-old cynomolgus macaques. Necropsies were performed 21 days post-injection, with brains, livers, and additional tissues collected for molecular analysis. RNA was isolated from collected tissues using TRIzol following manufacturer's protocol. To enrich capsid-specific mRNA, hybridization-based mRNA capture was performed by incubating total RNA with biotinylated antisense oligos complementary to AAV library-derived mRNA, followed by affinity capture on streptavidin paramagnetic beads. Captured RNA was then reverse transcribed, and cDNA PCR-amplified for NGS analysis.

[0891] *NGS and bioinformatics analysis:* A custom AAV amplicon-sequencing pipeline was developed to process the NGS raw data. Briefly, paired-end reads were merged, followed by sequential trimming on constant regions allowing a maximum of a 10% error rate. Variable and flanking regions were quality filtered with minimum Phred score of 20. Sequences matching the distinctive 16bp molecular barcode were retained for further analysis. Variant fraction in a given AAV or tissue amplicon library is normalized to reads per million (rpm) plus a pseudo count of 0.1.

[0892] For Variant i, log-transformed enrichment score =

[0893]  $\text{Log2}(\text{Vi\_tissue} * \text{Vaav9\_inputAAV} / (\text{Vi\_inputAAV} * \text{Vaav9\_tissue}))$

[0894] False discovery rate (FDR) was calculated by performing student's t test using log-transformed enrichments scores from all biological and technical replicates and adjusting p values using Benjamini-Hochberg method.

[0895] *Individual AAV-VHH particle Evaluation in Humanized Mice:* For AAV vectors packaging the CAG-mCherry-WPRE transgene, each vector was administered at dose of 5E13 vg/kg to three WT (C57BL/6) mice and three 8- to 10-week-old hTfR1-KI mice in C57BL/6 background. For vectors packaging the hSyn1-TransgeneX-WPRE cargo, each vector was administered a dose of 1.1 E13 vg/kg, 3.3 E13 vg/kg, or 11 E13 vg/kg to 6-8 week-old hTfR1-KI mice in C57BL/6 background. Necropsies were performed 21 days post-injection. Left brain hemispheres were fixed in 10% formalin for mCherry immunohistochemistry (IHC) or WPRE

RNAscope in situ hybridization (ISH). Right brain hemispheres, spinal cord, liver, heart, quadriceps, and spleen were flash frozen for vector genome biodistribution and transgene mRNA quantification analyses as described below.

**[0896]** For animals dosed with CAG-mCherry-WPRE packaging AAV vectors, tissues were homogenized in Qiagen Buffer RLT Plus using Genogrinder. Tissue homogenates were aliquoted for DNA extraction using Qiagen DNeasy kit, and RNA extraction using TRIzol LS reagent following the manufacturer's protocol. For animals dosed with hSyn1-Transgene X-WPRE packaging AAV vectors, separate tissue samples were used for DNA and RNA extraction. DNA extraction was performed as described above, and RNA was extracted using MagMAX-96 total RNA isolation kit with Kingfisher Flex Purification system according manufacturer's instructions. Vector genome biodistribution was determined via duplex qPCR using primer-probe sets targeting mCherry for the CAG-mCherry-WPRE cargo or Transgene X for the hSyn1- Transgene X-WPRE cargo. Results were normalized by Rpp30 copy numbers quantified in a separate channel. Transgene mRNA quantification was performed via RT-qPCR, using primer-probe sets against mCherry or Transgene X, as well as the endogenous Rpp30 as control. Transgene mRNA level was normalized against the endogenous Rpp30 mRNA level per sample for cross-sample comparison.

**[0897]** *Individual AAV-VHH particle Evaluation in NHPs.* Two separate NHP studies were conducted to evaluate CNS transduction of AAV particles with an AAV9 capsid and AAV particles with exemplary AAV-VHH capsids. In **Study A**, AAV particles with an AAV9 capsid packaging a hSyn1-Transgene X-WPRE transgene were dosed intravenously to two 10-month-old cynomolgus macaques at 1.1E14 vg/kg with no immunosuppression regimen. In **Study B**, AAV particles having exemplary VHHs inserted in the capsid (Clone B VHH-D1, Clone F VHH-D2, or Clone M VHH-D2) was each intravenously dosed into 3 cynomolgus macaques at a dose of 1.1E14 vg/kg. In addition, AAV particles having Clone B VHH-D1 were also dosed at a lower dose of 3.3E13 vg/kg in three additional animals. Cynomolgus macaques around 3 years of age were used for this study. Animals were orally dosed daily with immunosuppressants dexamethasone (0.5mg/kg) and Tacrolimus (1mg/kg), from 3 days prior to AAV dosing to 43 days post-AAV injection. In Studies A and B, animals were euthanized 6 weeks post-injection, and tissues were collected for analysis. DNA and RNA were isolated from NHP tissues with

protocols described above for mouse studies with hSyn1-Transgene X-WPRE transgene. Vector genome biodistribution was determined via duplex qPCR using primer-probe sets against the WPRE element and RPP30 genomic DNA. Transgene mRNA quantification was performed via RT-qPCR, using primer-probe sets against the WPRE element, as well as the endogenous RPP30 as control. Transgene mRNA level was normalized against the endogenous RPP30 mRNA level per sample for cross-sample comparison.

**[0898]** *mCherry Immunohistochemistry:* Tissues were drop-fixed in 10% neutral-buffer formalin for 2 days, washed in PBS, and paraffin blocked. For immunohistochemical (IHC) staining of mCherry, staining was conducted on the Leica Bond RXm platform using standard chromogenic methods. For antigen retrieval (HIER), slides were heated in a pH9 EDTA-based buffer for 25 minutes at 94 °C, followed by a 30-minute anti-mCherry antibody incubation (1:6000, Abcam ab167453). Antibody binding was detected using an HRP-conjugated secondary polymer (Biocare, MACH 2 goat anti-rabbit, Ref# RHRP520MM, ready to use at room temperature), followed by chromogenic visualization with diaminobenzidine (DAB). A hematoxylin counterstain was used to visualize nuclei.

**[0899]** *WPRE RNAscope in situ hybridization (ISH) and NeuN antibody immunofluorescent staining.* Tissues were drop-fixed in 10% neutral-buffered formalin for 48 hours at room temperature and subsequently washed in PBS. Tissues were then dehydrated through a series of graded ethanol solutions, cleared with xylene, and blocked in paraffin. For fluorescent WPRE RNAscope and NeuN antibody-based tissue staining, samples were processed on the Leica BOND RX platform using a combinatorial FISH+antibody staining protocol. For the Leica BOND RX platform, tissues were processed as follows:(1) baking and dewaxing, (2) HIER using Advanced Cell Diagnostics (ACD) ER2 buffer for 15 minutes at 95 °C, (3) ACD protease treatment for 15 minutes, (4) RNAscope probe application using ACD RNAscope LS Multiplex Fluorescent Reagent Kit (ACD 322800) and WPRE RNAscope 2.5 LS Probe (ACD 450268), (5) Opal 570 application (1:1000, Akoya Biosciences NEL830001KT), (6) tissue blocking using Akoya Biosciences Opal anti-rabbit Auto-IHC kit (Akoya Biosciences NEL830001KT), (7) NeuN primary antibody application (1:250, Millipore ABN90P), (8) anti-guinea pig secondary (1:250, Abcam ab102356) and anti-rabbit HRP tertiary antibody

application (1:5, Akoya Biosciences NEL830001KT), (9) and Opal 690 application (1:500, Akoya Biosciences NEL830001KT). A DAPI counterstain was used to visualize nuclei.

### **Results**

**[0900]** *Design of AAV-VHH particles and barcode AAV library.* First, VHH domains that were cross-reactive to mouse, cyno and human TfR1 were identified by immunizing alpacas either with DNA expressing murine, cyno, and human homologs of TfR1 followed by cell-based booster, or with recombinant alpaca TfR1 ectodomains containing human, cyno and mouse TfR1 sequences. VHH sequences from both immunization schemes were isolated from PBMCs and re-cloned for expression in a yeast display format, where colonies were subjected to additional rounds of selection on recombinantly expressed human and cyno TfR1 ectodomain reagents. A set of unique VHH sequences with confirmed binding to human TfR1 were selected for testing as AAV-VHH fusions (**Table 1**).

**[0901]** To preserve conformation and functionality of the VP1 subunit with inserted VHH domains, structural biology was leveraged to identify a combination of select VP1 variable regions as VHH insertion sites and peptide linkers that are predicted to be favorable in maintaining VP1 and VHH functionalities (**FIGS. 2A-2B**). AAV9-VHH capsids were designed with combinations of anti-TfR1 VHHs and linker designs as shown in **Table 3**. To facilitate testing of the numerous capsids in parallel, a barcoded library including the 34 AAV9-VHH particle designs and AAV9 control (**FIG. 1C**) was built. Each AAV capsid displaying an exemplary VHH was packaged with a mixture of cis-plasmids consisting of two CAG-driven and two hSyn1-driven H2B-GFP transgenes with unique 16-nt barcodes embedded in the 3'-UTR. A lookup table (LUT) was generated where each capsid variant corresponds to four unique barcodes, with two driven by the CAG promoter, and two by the hSyn1 promoter.

**Table 3: Exemplary Linker designs and single chain antibody agent insertions**

<b>Linker Design</b>	<b>Insertion VR</b>	<b>Deleted WT AA Location</b>	<b>N-Terminal Linker Design</b>	<b>C-Terminal Linker Design</b>
D1	VR-IV	455-460	GGGGS x5	GGGGS
D2	VR-IV	455-456	GGGGS x4	None
D3	VR-IV	452-459	GGGGS x4	None
D4	VR-IV	455-460	leading coil + (GGGGS x5)	GGGGS + returning coil

D5	VR-VIII	589	GGGGS x5	GGGGS
D7	VR-VIII	589	leading coil + (GGGGS x5)	GGGGS + returning coil
D8	VR-IV	455-456	GGGGSGGGGSG GGGSPPCGGSG G (SEQ ID NO: 65)	None
D10	VR-IV	455-456	GGGGS x3	None

**[0902]** *Anti-TfR1 AAV9-VHHs show TfR1-dependent transduction in vitro.* A subset of the barcoded library was screened in vitro to evaluate whether AAV-VHH particles target TfR1 while maintaining transduction activity. WT or TfR1-knockout HEK293T cells were incubated with a subset of the barcoded library at 100,000 MOI for two days before lysis and isolation of cellular RNA for transgene barcode amplicon-seq. VHH insertion designs influence AAV capsid activities, as evidenced by varying AAV capsid transduction efficiencies in WT HEK293T cells within the same VHH insertion group (**FIG. 3**). While all AAV-VHH particles showed similar or lower transduction compared to AAV9 in TfR1-knockout HEK293T cells, AAV-VHH capsids inserted with 6 out of the 9 tested VHHs showed >2-fold improved transduction efficiency in WT HEK293T cells compared to AAV9, suggesting that transduction enhancement is dependent on the presence of VHH on capsids as well as TfR1 expression in target cells (**FIG. 3**). This result indicates that at least a subset of tested VHHs were successfully incorporated into the capsid and confer in-context affinity to TfR1.

*Library screening in mouse models identified human- and murine-TfR1 targeted capsids with enhanced CNS transduction*

**[0903]** To evaluate CNS transduction efficiency of individual library variants, the barcoded AAV library was tested in wild type (WT) mice and a humanized mouse model. A hTfR1 knock-in (KI) mouse model, hTfR1-KI<sup>+/+</sup>, which expresses a fully human TfR1 ectodomain with murine transmembrane and intracellular regions was used (**FIG. 4**). The barcoded AAV library was I.V. dosed in WT mice at 1e12 vg per mouse and the KI mouse model at 5e13 vg/kg. Following a 21-day in-life period, brain tissue was collected and capsid variant transduction was analyzed via amplicon-seq on expressed transgene barcodes (**FIGs. 5A-5B, Table 4**). As shown in **FIGs. 5A-5B**, a high correlation between CAG- and hSyn1-driven

transgene barcode enrichment from the same AAV-VH-VHH particles was observed, which suggests that retargeting AAV to hTfR1 enables neuronal transduction in the CNS. AAV9 particles displaying a Clone A VH-VHH demonstrated the highest CNS transduction among all library variants in a WT model, showing a 10.1-fold enhancement in CAG-driven barcode expression over AAV9 (see triangles in **FIG. 5A, Table 4**). In hTfR1-KI mice, while AAV9 particles displaying a Clone A VH-VHH maintained superior CNS transduction over AAV9, AAV9 particles displaying a Clone B VH-VHH outperformed the AAV capsids displaying a Clone A VH-VHH, showing a 13.1-fold enhancement over AAV9 based on CAG-driven barcode enrichment (see squares in **FIG. 5B, Table 4**). Linker designs influenced CNS transduction by AAV-VHH particles, although optimal linker pairs are VH-VHH-dependent (**FIGS. 6A-6B**). Interestingly, rankings of linker designs for exemplary VH-VHH insertions were consistent across the two mouse models. The present Example demonstrates that CNS-targeting activities of AAV9-VHHs are VH-VHH- and linker design-dependent.

**Table 4: Transgene enrichment of exemplary AAV-VHH**

Exemplary AAV-VHH	WT murine TfR brain - CAG FC to AAV9	WT murine TfR brain - hSyn1 FC to AAV9	hTfR1-KI brain - CAG FC to AAV9	hTfR1-KI brain - hSyn1 FC to AAV9	cyno brain - CAG FC to AAV9	cyno brain - hSyn1 FC to AAV9
Clone B VHH-D1	B	A	C	C	C	B
Clone B VHH-D2	A	A	B	B	B	B
Clone B VHH-D3	A	A	B	B	B	B
Clone B VHH-D4	A	A	B	B	B	B
Clone B VHH-D5	A	A	B	B	B	B
Clone B VHH-D7	A	A	A	A	A	A
Clone D VHH-D1	A	A	B	B	B	B
Clone D VHH-D2	A	A	C	C	B	B

Exemplary AAV-VHH	WT murine TfR brain - CAG FC to AAV9	WT murine TfR brain - hSyn1 FC to AAV9	hTfR1-KI brain - CAG FC to AAV9	hTfR1-KI brain - hSyn1 FC to AAV9	cyno brain - CAG FC to AAV9	cyno brain - hSyn1 FC to AAV9
Clone D VHH-D3	A	A	B	B	B	B
Clone D VHH-D4	A	B	B	B	B	B
Clone D VHH-D5	A	A	B	B	B	B
Clone D VHH-D7	B	B	B	B	B	B
Clone E VHH-D1	A	A	B	B	A	A
Clone E VHH-D2	A	A	B	B	A	B
Clone E VHH-D3	A	A	A	A	A	A
Clone E VHH-D4	A	A	B	B	A	B
Clone E VHH-D5	A	A	B	B	A	A
Clone E VHH-D7	A	A	A	A	A	A
Clone C VHH-D2	B	A	B	B	A	A
Clone C VHH-D4	B	B	B	B	A	B
Clone C VHH-D5	A	A	B	B	A	B
Clone C VHH-D7	B	A	B	B	A	B
Clone A VHH-D2	B	B	B	B	C	C
Clone A VHH-D7	B	B	B	B	C	C

[0904] For Table 4, a designation of “A” indicates a fold change of less than 2, a designation of “B” indicates a fold change of more than 2 and less than 10, and a designation of “C” indicates a fold change of more than 10.

[0905] *Barcoded AAV library screening in cynomolgus macaques identified anti-TfR1 AAV9-VHHS with enhanced brain transduction.* To evaluate performance of AAV-VHH particles in non-human primates (NHPs), the barcoded AAV library was screened in two cynomolgus monkeys. Monkeys were I.V. dosed and tissues were collected at 21 days post-injection. By analyzing capsid variant transduction in brain via amplicon-seq on transgene barcodes, 22 capsid variants were identified that showed enhanced CNS transduction in the NHP brain compared to parental wild-type AAV9, with both CAG promoter-driven and hSyn1 promoter-driven brain transduction fold-change to AAV9 greater than 1 (**Table 4**). AAV9 particles comprising a Clone A VHH insertion exhibited ~34-fold CNS transduction enhancement compared to AAV9 (**FIG. 7A, Table 4**). Notably, both CAG-driven CNS transduction and also hSyn1-driven CNS transduction was observed. In some embodiments, this shows that AAV particles displaying an exemplary VHH insertion as described herein can transverse the blood brain barrier (BBB) and transduce neurons. Among all tested AAVs displaying an exemplary VHH insertion as described herein, AAV capsids displaying a Clone A VHH, Clone B VHH, or Clone D VHH insertion displayed the highest CNS transduction in cynomolgus macaques. Linker designs influenced CNS transduction by AAV-VHH particles in the cyno model, although optimal linker pairs were VHH-dependent, as observed in the murine models (**FIG 7B**).

[0906] *AAV-VHH particles enhanced CNS transduction in WT, and hTfR1-KI mice.* Since Clone A VHH recognizes murine TfR1 and enhances AAV-VHH uptake in the murine brain (**FIGs. 5A-5B**), VHH-dependent particle retargeting was assessed in WT mice. Clone A VHH-D2, Clone A VHH-D7 or AAV9 packaged with a CAG-driven mCherry transgene was intravenously injected into WT mice at 1E12 vg per animal. Indeed, both CloneA VHH-D2 and Clone A VHH-D7 particles showed > 7-fold higher CNS biodistribution, and mediated >10-fold higher brain and spinal cord transduction compared to AAV9 in WT mice, indicating that both Clone A VHH-inserted particles are capable of transducing the murine CNS (**FIGs. 8A-8B**). mCherry IHC revealed that Clone A VHH-D2 and Clone A VHH-D7 mediated widespread neuron and astrocyte transduction in WT mice across various brain regions including cortex, hippocampus, and cerebellum (**FIGS. 9A-9B, 10A-10B, 11A-11B and 12A-12B**).

[0907] hTfR1-KI mice were I.V. dosed with an AAV9-VHH or AAV9 control packaging a CAG-driven mCherry transgene at 5E13 vg/kg, and vector genome biodistribution and transgene expression was analyzed 3 weeks post-injection. While genome biodistribution in certain peripheral organs, including quadriceps, heart, and spleen, were comparable between AAV9-VHHS and AAV9, all tested AAV9-VHHS showed lower liver and enhanced brain biodistribution in hTfR1-KI<sup>+/+</sup> mice (**FIGS. 13A-13B**). Analysis on transgene mRNA levels revealed that Clone B VHH-D1 transduced brain and spinal cord ~85-fold and ~69-fold better over AAV9, respectively, while Clone A VHH-D2 and Clone A VHH-D7 showed modestly higher CNS transduction than AAV9 (**FIG. 13A**). Interestingly, the three AAV9-VHHS were comparable or weaker in peripheral organ transduction compared to AAV9, with the exception that Clone B VHH-D1 showed a slightly higher heart transduction than AAV9 (**FIG. 13A**). Consistent with mCherry mRNA quantification, robust and widespread mCherry protein expression in hTfR1-KI brain was mediated by Clone B VHH-D1 (**FIGS. 14A-14B, 15A-15B, 16A-16B, and 17A-17B**). Cortical and CA2 neurons as well as Purkinje cells were highly transduced in examined brain regions, indicating strong neuronal tropism of Clone B VHH-D1. In addition, mild-to-modest neuron and astrocyte transduction by Clone A VHH-D2 and Clone A VHH-D7 was observed. The present Example demonstrates that anti-TfR1 AAV9-VHHS are capable of efficient CNS-targeting and transduction *in vivo*.

[0908] *Characterization of Clone A VHH binding site on human TfR1 ectodomain via cryo-electron microscopy.* To identify the molecular basis for antigen recognition of anti-TfR1 Clone A VHH, a cryo-EM structure was generated on the ternary complex of Clone A VHH, human TfR1 ECD, and holo human transferrin to a resolution of 2.4 Å. An atomic model of the complex was obtained by fitting a homology model of Clone A VHH and available TfR1-Tf crystal structures into the cryo-EM density map followed by manual rebuilding (**FIGs. 18A-18B**). The model revealed that Clone A VHH binds to an epitope spanning both peptide chains of the TfR1 homodimer in the helical and apical domains. There are 22 TfR1 residues and 23 Clone A VHH residues that are within 4 Å of the binding partner. The predicted discontinuous epitope is formed by residues K189, Y309, F321, P322, P323, S324, R325, L329, and L381 from the apical domain and E634, M635, G636, R719, N722, N723, G724, A725, F726, N727, E728, T729, and R732 from the helical domain of human TfR1. The antigen-binding site of Clone A VHH is formed by all three CDRs and is composed of P29, P30, S32, V33, Y35, W50, D53,

A54, G55, N56, T57, D61, D98, Q99, S100, and V101 plus additional framework contacts at Y37, E44, R45, E46, F47, K96, and W102 (**FIG. 19**) (**Table 5**).

**Table 5: Structure of anti-TfR1 Clone A VHH complex with hTfR and Tf**

CDR	Contact residue (<4Å)	CDR sequence ( <b>bold positions indicate contact residues</b> )
CDR H1	P29, P30, S32, V33, Y35	<b>GSI<del>PPI</del>SVMY</b> (SEQ ID NO: 2)
CDR H2	W50, D53, A54, G55, N56, T57, D61	<b>WVGDA<u>GNTAYA</u>DSVRG</b> (SEQ ID NO: 3)
CDR H3	D98, Q99, S100, V101	<b>KSD<u>QSV</u></b> (SEQ ID NO: 4)
Framework	Y37, E44, R45, E46, F47, K96, W102	

[0909] Since Clone A VHH recognizes human and cynomolgus macaque homologs of TfR1, cyno and human sequence conservation in TfR1 epitope was evaluated. The predicted TfR1 epitope spanning both the apical and helical domains is highly conserved between human and cyno homologs, with only two amino acid residue substitutions at R325Q and G724S (hu>cyno TfR1) (**FIGS. 20-21**), consistent with the observed cyno-human cross-reactivity of Clone A VHH.

[0910] Based on the cryo-EM structure of Clone A VHH-huTfR1, a cohort of Clone A VHH mutants was designed with amino acid residue substitutions in Clone A VHH paratope to modulate TfR1 affinity for optimal CNS-targeting, and to potentially minimize affinity gap to cyno and human TfR1. Three sites in a Clone A VHH sequence, i.e. D53, Q99, and V101, were identified as residues interacting with cyno-human divergent epitope regions, amino acid substitutions were introduced, D53S (e.g., Clone F VHH), Q99G (e.g., Clone M VHH), and V101S/A/P (e.g., Clone H VHH, Clone I VHH, Clone J VHH, respectively), that were predicted to strengthen hydrogen bonds and Van der Waals forces between VHH and cyno TfR1 (**Table 4**). Amino acid frequencies were referenced from available antibody variable regions in the NCBI Protein database to enhance the chance that the substituting amino acids are tolerated in CDRs.

[0911] *Characterization of Clone B VHH binding site on human TfR1 ectodomain via cryo-electron microscopy.* To identify the molecular basis for antigen recognition of anti-TfR1

Clone B VHH, a cryo-EM structure was generated on the ternary complex of Clone B VHH, human TfR1 ECD, and holo human transferrin (Sigma T0665) to a resolution of 6.5 Å (lower resolution) or 2.7 Å (higher resolution). Using the higher resolution of 2.7 Å, an atomic model of the complex was obtained by rigid body fitting an IgFold prediction of Clone B VHH and available TfR1-Tf crystal structures into the cryo-EM density map (**FIGS. 22A-22B**). The final model revealed that Clone B VHH binds to a similar epitope to that of Clone A VHH, spanning both peptide chains of the TfR1 homodimer in the helical and apical domains. Fourteen TfR1 residues and 15 Clone B VHH residues are within 4 Å of the binding partner. The discontinuous epitope is formed by residues K189, P323, S324, R325, L381, and E383 from the apical domain, and E634, M635, R719, N723, N727, E728, T729, and R732 from the helical domain of human TfR1. The antigen-binding site of Clone B VHH is formed by all three CDRs and is composed of R27, P28, F29, S30, N31, R53, T54, G55, G56, F102, I104, and T105, plus additional framework contacts at D74, A75, and K76 (**Table 6**). Based on the cryoEM structure obtained with the 2.7 Å resolution, the TfR1 epitope for Clone B VHH is highly conserved between human and cyno homologs, with one amino acid residue substitution at R325Q(hu>cyno TfR1) (**FIG. 23**), supporting the observation that Clone B VHH is cross-reactive against cyno and human TfR1.

**Table 6: Structure of anti-TfR1 Clone B VHH complex with hTfR and Tf**

CDR	Contact residue (<5Å)	CDR sequence ( <b>bold</b> positions indicate contact residues)
CDR H1	R27, P28, F29, S30, N31	<b>G</b> RPFSNYAMG (SEQ ID NO: 6)
CDR H2	R53, T54, G55, G66	AIS <b>R</b> TGGSSNYANSVKG (SEQ ID NO: 7)
CDR H3	F102, I104, T105,	AAEET <b>F</b> G <b>I</b> TWYGSHEEDFRS (SEQ ID NO: 8)
Framework	D74, A75, K76,	

[0912] *VHH-TfR1 affinities correlate with CNS transduction activities of AAV-VHHS.* It has been shown that brain uptake of anti-TfR1 antibodies can be boosted by reducing antibody affinities to TfR1 (see, e.g., Yu YJ et al, “Boosting Brain Uptake of a Therapeutic Antibody by Reducing Its Affinity for a Transcytosis Target”. *Science Translational Medicine*. 2011, the

entire contents of which is hereby incorporated by reference). To test if this affinity-activity relationship is relevant for TfR1-mediated AAV transcytosis, monovalent VHH affinities to TfR1 homologs was measured. Clone A and its variants Clones F-L, Clone B, Clone C, Clone D, and Clone E VHHs were prepared as monovalent Fc fusion reagents and tested for binding to TfR1 homologs. A flow cytometry-based assay, and a surface plasmon resonance (SPR) assay were used (**Table 7**). The flow cytometry assay provides a receptor-saturation based EC<sub>50</sub> value as a measurement of binding affinity. The SPR assay provides a kinetics-based or steady state analysis-based K<sub>D</sub> value determination as a measurement of binding affinity. Binding curves for Clone A VHH, Clone B VHH, and Clone D VHH, the molecules with the highest BBB penetration activity in hTfR1-KI<sup>+/+</sup> and cynomolgus macaque models, are shown in **FIGS. 24A-24D** and **FIG. 25**. All tested VHHs bind to TfR1 ectodomains from multiple species with different affinities. Without wishing to be bound to any theory, in some embodiments, this could impact tissue-targeting activities of AAV-VHH particles in different animal models. A proportional relationship between VHH-TfR1 K<sub>D</sub> and AAV-VHH neuronal transduction activity (log<sub>2</sub>) in library screening was observed, in that VHHs with close-to-micromolar K<sub>Ds</sub> to TfR1 impart higher neuronal transduction activities to AAV vectors than those with low nanomolar affinities (**FIG. 26**).

**Table 7: Summary of EC50 and KD values for monovalent VH<sub>H</sub>-Fc fusion / TfR binding**

Exemplary VH <sub>H</sub>	Human TfR Surface Plasmon Resonance	Human TfR Flow Cytometry	Cyto TfR Surface Plasmon Resonance	Cyto TfR Flow Cytometry	Murine TfR Surface Plasmon Resonance	Murine TfR Flow Cytometry	h/m KI TfR Flow Cytometry		
ka (M-1s-1)	kd (s-1)	KD (nM)	ka (M-1s-1)	kd (s-1)	KD (nM)	ka (M-1s-1)	kd (s-1)	KD (nM)	EC50 (nM)
Clone B	Steady state analysis	1200	180	Steady state analysis	66	32	No binding	470	>1000
Clone D	3.2E+04	1.1E-02	330	55	1.3E+05	3.3E-03	25	14	7E+04
Clone E	6.3E+04	1.0E-03	16	14	1.2E+05	4.9E-04	4.1	13	9E+04
Clone C	5.2E+04	3.4E-03	66	6.6	6.4E+04	5.9E-04	9.3	9.2	7E+04
Clone A	3.1E+04	3.5E-03	110	27	3.4E+04	1.8E-02	540	97	2E+04
Clone G	3.8E+04	1.2E-02	330	46	4.8E+04	5.7E-03	120	29	n.d.
Clone F	1.8E+04	1.8E-02	1030	93	7.2E+04	1.5E-02	210	48	n.d.
Clone K	2.1E+04	1.7E-02	830	81	8.1E+04	5.8E-03	72	21	n.d.
Clone M	4.2E+04	1.4E-02	330	60	4.7E+04	3.7E-02	790	180	n.d.
Clone L	6.4E+04	1.1E-03	17	8.8	4.7E+04	1.1E-02	240	30	n.d.
Clone I	4.0E+04	3.7E-03	91	22	3.5E+04	7.9E-03	230	43	n.d.
Clone J	4.1E+04	3.6E-03	87	21	3.2E+04	7.8E-03	250	41	n.d.

Clone H	3.3E+04	5.4E-03	130	34	2.8E+04	1.0E-02	370	89	n.d.	n.d.	n.d.	89	n.d.
---------	---------	---------	-----	----	---------	---------	-----	----	------	------	------	----	------

**[0913]** The correlation between in-context AAV-TfR1 affinities and VHH-TfR1 affinities was examined using AAV particles having AAV9 capsids with the following VHH construct designs: Clone A VHH-D2, Clone B VHH-D1, Clone F VHH-D2, and Clone M VHH-D2. Clones F and M VHHS each differs from Clone A by a single amino acid residue. Therefore, the linker design for Clone A, D2, was used for the display of Clones F and M. A bio-layer interferometry (BLI) binding assay was performed to determine binding affinity of the capsids to TfR1 recombinant protein. As showin in **FIGs. 27-28** and **Table 7**, a linear correlation was observed between VHH-TfR1 SPR affinities and in-context AAV-TfR1 BLI affinities. This data suggest that the VHH monovalent affinity remains consistent when inserted into an AAV capsid and packaged as an AAV particle.

**Table 8:** Summary of KD values for in-context AAV-TfR1 binding as measured by biolayer interferometry

Capsid	huTfR KD (nM)	cynoTfR KD (nM)
Clone A VHH-D2	54	218
Clone B VHH-D1	> 2000	270
Clone F VHH-D2	320	147
Clone M VHH-D2	218	878

**[0914]** *Wave 2 AAV-VHH library design and in vitro characterization.* Based on the observed relationship between VHH-TfR1 affinity and CNS transduction in hTfR1-KI (B-hTfR1-KI) mice with VHHS described above (also referred to as Wave 1 herein), Clone A VHH variants with single or double amino acid residue substitutions were evaluated. Six affinity variants of Clone A (Clones F, G, H, I, J, K) were incorporated into AAV9 with Design 2. In addition, Clone A VHH variants Clone N, O or P was displayed on AAV9 capsid with linkers of higher rigidity via designs D8 and 10 (**Table 3**), where proline residues (D8 and 10) and/or paired cysteines were introduced (D8). CDR sequences for Clones N, O and P remain identical to that of Clone A VHH. Wave 2 barcoded libraries were produced as described above, with AAV9, Clone A VHH-D2, Clone A VHH-D7, and Clone B VHH-D1 as internal comparators.

**[0915]** All Wave 2 AAV-VHH capsids showed human, cyno, and murine TfR1-dependent transduction in TfR1 knock-out CHO cells expressing human, cyno, or murine TfR1 homologs (**FIG. 29**), demonstrating functional presentation of anti-TfR1 VHHS when packaged

in AAV particles with VHHS inserted in the capsid. All AAV-VHHS showed reduced transduction compared to AAV9 in TfR1 knockout CHO cells, suggesting that VHH presentation on capsid dampens basal transduction activities that are TfR1-independent.

**[0916]** *VHH-AAV variants characterized through in vivo screening in human TfR1 knock-in mice.* CNS transduction of Wave 2 variants was evaluated by screening the barcoded library in WT and hTfR1-KI mice. Similar to Wave 1 library screening (as described above), a high concordance was observed between CAG promoter and hSyn1- promoter driven transgene fold enhancement over AAV9 for all library variants, suggesting successful transversal of brain endothelium by AAV particles having a capsid with the specified VHH constructs as compared to AAV particles with an AAV9 capsid (**FIG. 30**). Clone F VHH-D2 and Clone K VHH-D2 demonstrated the highest brain transduction enhancement over AAV9 in hTfR1-KI (B-hTfR1) mice, exceeding the transduction observed with the parental variant Clone A VHH-D2 by about 30% (**FIG. 30**). This observation aligns with the affinity-activity relationship derived from the Wave 1 library (**FIG. 26**), as Clone F VHH-D2 and Clone K VHH-D2 have the lowest affinities to human TfR1 among tested variants ( $K_D$  1030 and 830 nM, respectively). It was further observed that Clone B VHH-D1 showed slightly lower CNS transduction enhancement over AAV9 in Wave 2 compared to Wave 1 library screening. Without wishing to be bound by any particular theory, this observed difference is likely due to differential competition among distinct sets of library variants for human TfR1. All Wave 2 variants also demonstrated enhanced CNS transduction compared to AAV9 in WT mice, likely due to cross-reactivity with murine TfR1 (**FIG. 31**). Clones F and A variants exhibited the highest brain transduction enhancement over AAV9 in WT mice. A summary of this data is also shown in **Table 9**.

**Table 9:** Wave 2 library screening in WT and B-hTfR1 mice. Numbers indicate linear fold enhancement of transgene expression over AAV9 driven by respective promoters.

	WT mouse brain		B-hTfR1 mouse brain	
	CAG-driven fold over AAV9	hSyn1-driven fold over AAV9	CAG-driven fold over AAV9	hSyn1-driven fold over AAV9
Clone F VHH-D2	C	C	C	B
Clone A VHH-D2	C	C	B	B
Clone N VHH-D8	C	C	C	B
Clone G VHH-D2	C	C	B	B

Clone A VHH-D7	C	B	B	B
Clone O VHH-D8	B	B	B	A
Clone J VHH-D2	B	B	B	A
Clone P VHH-D10	B	A	B	A
Clone K VHH-D2	A	A	C	B
Clone H VHH-D2	A	A	B	B
Clone B VHH-D1	A	A	B	B
Clone I VHH-D2	A	A	B	B

[0917] For **Table 9**, a designation of “A” indicates a fold change of less than 10, a designation of “B” indicates a fold change of more than 10 and less than 20, a designation of “C” indicates a fold change of more than 20.

[0918] *Exemplary VHH-AAVs show robust and dose-dependent CNS transduction in B-hTfR1 mice.* Two top capsids from Wave 1 and 2 library screening in hTfR1-KI (B-hTfR1) mice, Clone B VHH-D1 and Clone F VHH-D2 were selected for further *in vivo* validation. In addition, Clone M VHH was selected as a candidate for AAV display due to its mid-range affinities to cyno and human TfR1 (**FIG. 27, Table 7**).

[0919] AAV particles with capsids having and displaying Clone B VHH-D1, Clone F VHH-D2, or Clone M VHH-D2 and further packaging a hSyn1-driven exemplary cargo (Transgene X) were administered to B-hTfR1 mice. The AAV particles were administered intravenously at doses of 1.1 E13 vg/kg, 3.3 E13 vg/kg, or 11 E13 vg/kg. Transduction and vector genome biodistribution were analyzed 3 weeks post-injection. All three capsids demonstrated robust vector genome biodistribution and transduction in CNS tissues of B-hTfR1 mice, particularly at 3.3 and 11E13 vg/kg doses (**FIGs. 32-37**). Specifically, all three capsids exhibited similar vector genome biodistribution in brain and spinal cord, with approximately 1 vg/dg at the low dose and >5 vg/dg at the high dose (**FIGs. 32 and 36**). Interestingly, despite similar CNS biodistribution, Clone B VHH-D1 mediated the highest CNS transgene mRNA expression of the three candidates, showing approximately 4-fold higher transgene expression in brain and 1.5 to 2-fold higher transgene expression in spinal cord compared to Clone F VHH-D2 and Clone M VHH-D2 (**FIGs. 33 and 37**). All three AAV-VHHS exhibited biodistribution in the liver (**FIG. 38**) and when combined with the neuronal promoter hSyn1, liver transgene expression was significantly lower than that observed in CNS tissues (compare transgene expression in **FIG. 33** (brain) and **FIG. 37** (spinal cord) with **FIG. 39** (liver)).

[0920] Cortical neuronal transduction efficiency was evaluated via combinatorial fluorescent RNAScope and antibody tissue staining using an RNAscope probe against WPRE (as a surrogate for the transgene) and an antibody against the neuronal marker NeuN (**FIGs. 34-35**). Administration of AAV particles comprising capsids displaying all three VHHS demonstrated dose-dependent and robust cortical neuron transduction. At a dose of 3.3E14 vg/kg, Clone B VHH-D1, Clone F VHH-D2, and Clone M VHH-D2 led to 73%, 23%, and 28% transgene-positive cortical neurons, respectively. At the higher 1.1E14 vg/kg dose, these values increased to 95%, 65%, and 73%, respectively.

[0921] *Exemplary VHH-AAVs demonstrate enhanced CNS transduction in NHPs compared to AAV9.* Two separate NHP studies were conducted to evaluate CNS transduction of AAV particles having an AAV9 capsid and AAV particles having AAV9 capsids displaying exemplary AAV-VHHS. In Study A, AAV particles having an AAV9 capsid and further packaging the hSyn1-Transgene X-WPRE transgene was intravenously dosed to two 10-month-old-cynomolgus macaques at 11 E13 vg/kg with no immunosuppression. In Study B, AAV particles having AAV9 capsids displaying Clone B VHH-D1, Clone F VHH-D2, or Clone M VHH-D2 intravenously dosed into 3 cynomolgus macaques at 11 E13 vg/kg. In addition, Clone B VHH-D1 was evaluated at a lower dose of 3.3 E13 vg/kg in three additional animals. Cynomolgus macaques around 3 years of age were used for this study. Animals were orally dosed daily with immunosuppressants from 3 days prior to AAV dosing to 43 days post-AAV injection. In Studies A and B, animals were euthanized 6 weeks post-injection, and tissues were collected for analysis.

[0922] Compared to AAV particles having AAV9 capsids dosed at 11 E13 vg/kg in juvenile animals, vector genome biodistribution for AAV particles having AAV9 capsids displaying each of the three VHHS was higher in brain and spinal cord, comparable or lower in tested skeletal muscles, and significantly lower in liver (**FIGs. 40-41**). Transgene expression showed a similar trend. At 11 E13 vg/kg, all three AAV-VHHS drove significantly higher transgene expression across widespread brain and spinal cord regions compared to AAV9, while expression in skeletal muscle and liver was comparable or lower (**FIGs. 42-43**). Clone B VHH-D1 dosed at 1.1E14 vg/kg mediated 1.6 to 12.9-fold higher transgene mRNA expression in CNS

regions, and 3.1 to 40-fold higher transgene mRNA expression in peripheral organs compared to the 3.3E13 vg/kg dose (**FIG. 43**).

### ***Discussion***

**[0923]** The present Example demonstrates insertion of a variety of anti-TfR1 VHH domains with multiple linker designs into the AAV9 VP1 subunit, which yielded AAV capsids exhibiting gain of TfR1-dependent BBB transcytosis function *in vivo*. Several AAV-VHH particle designs were identified that led to higher CNS transduction in NHP brain, WT mouse brain, and hTfR1-KI mouse brain relative to a parental AAV9. These AAV-VHH particles achieved significantly enhanced brain transduction over parental AAV9 particles.

**[0924]** Among other things, it is an insight of the present disclosure that incorporation of anti-TfR1 VHHS in AAV capsids may yield novel AAV capsids with enhanced muscle delivery. For examples, it has been shown that targeting tissues with anti-TfR1 antibodies demonstrate improved delivery of the ASO modality to skeletal muscle (Dyne Therapeutics, April 27, 2021), suggesting it is possible to use anti-TfR1 targeting to enhance muscle delivery. Accoridngly, the present disclosure provides AAV-VHH particles that can be used for targeting AAVs to muscle cells and/or tissue.

**[0925]** Without wishing to be bound to any theory, VHH insertion strategies described herein may offer advantages for tuning and optimizing AAV activity. Because an inserted receptor binding activity is known a-priori, a targeting domain can be assessed for receptor binding affinity across species such as mouse, cynomolgus macaques, and humans using standard biochemical binding assays. Among the advantages of VHH insertion strategies described herein, is that receptor binding is self-contained within a modular protein insertion domain. Candidate targeting domains can therefore be easily inserted between different AAV serotypes to influence cell-type tropism. This property also enabled structural studies on a VHH / huTfR1 ECD complex which would have been more challenging to implement using a larger AAV-VHH particle.

**[0926]** Accordingly, the present Example demonstrates use of a VHH insertion strategy and exemplary linker designs for targeting AAV particles to central nervous system cells and/or tissue. AAV-VHH particles described herein can also be used to target AAV particles to other hTfR1 expressing cells and/or tissue such as muscle cells and/or tissue.

[0927] The present Example also describes production of rAAV particles displaying an exemplary anti-TfR1 VHH inserted in the AAV capsid for targeting AAV capsids to central nervous system. In particular, the present Example demonstrates that rAAV particles displaying an exemplary anti-TfR1 VHH inserted in the AAV capsid lead to higher CNS transduction in NHP brain, WT mouse brain, and hTfR1-KI mouse brain relative to a parental AAV9. Taken together, the data provided herein supports the development of novel anti-hTfR1 VHHs for insertion in AAV capsids to direct AAV particles to hTfR1 expressing cells and/or tissue.

## NUMBERED EMBODIMENTS

[0928] Embodiment 1. A recombinant adeno-associated virus (rAAV) particle comprising:

[0929] (a) a variant AAV capsid protein comprising a single chain antibody agent that binds to a transferrin receptor (TfR1) or a variant or a fragment thereof, wherein the single chain antibody agent is inserted in a variable region (VR) of a parental AAV capsid protein, and

[0930] (b) a heterologous nucleic acid comprising a nucleotide sequence encoding a payload.

[0931] Embodiment 2. The rAAV particle of embodiment 1, wherein the single chain antibody agent comprises one or more complementarity determining regions (CDRs).

[0932] Embodiment 3. The rAAV particle of embodiment 2, wherein the single chain antibody agent comprises a CDR1, a CDR2 and a CDR3.

[0933] Embodiment 4. The rAAV particle of any one of the preceding embodiments, wherein the single chain antibody agent is or comprises: (i) a single domain antibody (e.g., a VHH); or (ii) a single chain Fv.

[0934] Embodiment 5. The rAAV particle of any one of the preceding embodiments, wherein the single chain antibody agent comprises a sequence provided in **Table 1**.

[0935] Embodiment 6. The rAAV particle of any one of the preceding embodiments, wherein the single chain antibody agent comprises:

[0936] (i) a CDR1 sequence provided in **Table 1**; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at

least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a CDR1 sequence provided in Table 1; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to a CDR1 sequence provided in **Table 1**;

[0937] (ii) a CDR2 sequence provided in **Table 1**; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a CDR2 sequence provided in **Table 1**; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to a CDR2 sequence provided in **Table 1**; and/or

[0938] (iii) a CDR3 sequence provided in **Table 1**; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a CDR3 sequence provided in **Table 1**; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to a CDR3 sequence provided in **Table 1**.

[0939] Embodiment 7. The rAAV particle of any one of embodiments 1-6, wherein the single chain antibody agent comprises:

[0940] (i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2;

[0941] (ii) a CDR2 sequence of SEQ ID NO: 3; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 3; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 3; and/or

[0942] (iii) a CDR3 sequence of SEQ ID NO: 4; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 4; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 4.

[0943] Embodiment 8. The rAAV particle of embodiment 7, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 3; and (iii) a CDR3 sequence of SEQ ID NO: 4.

[0944] Embodiment 9. The rAAV particle of embodiment 7 or 8, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 1, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0945] Embodiment 10. The rAAV particle of embodiment 7 or 8, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 158, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0946] Embodiment 11. The rAAV particle of embodiment 7 or 8, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 159, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0947] Embodiment 12. The rAAV particle of embodiment 7 or 8, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 160, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0948] Embodiment 13. The rAAV particle of any one of embodiments 1-6, wherein the single chain antibody agent comprises:

[0949] (i) a CDR1 sequence of SEQ ID NO: 6; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 6; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 6;

[0950] (ii) a CDR2 sequence of SEQ ID NO: 7; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 7; and/or

[0951] (iii) a CDR3 sequence of SEQ ID NO: 8; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 8.

[0952] Embodiment 13A. The rAAV particle of embodiment 13, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 6; (ii) a CDR2 sequence of SEQ ID NO: 7; and (iii) a CDR3 sequence of SEQ ID NO: 8.

[0953] Embodiment 14. The rAAV particle of embodiment 13 or 13A, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 5, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0954] Embodiment 15. The rAAV particle of embodiment 13 or 13A, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 122, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0955] Embodiment 16. The rAAV particle of embodiment 13 or 13A, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 135, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0956] Embodiment 17. The rAAV particle of embodiment 13 or 13A, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 136, or a sequence having at least

85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0957] Embodiment 18. The rAAV particle of any one of embodiments 1-6, wherein the single chain antibody agent comprises:

[0958] (i) a CDR1 sequence of SEQ ID NO: 10; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 10; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 10;

[0959] (ii) a CDR2 sequence of SEQ ID NO: 11; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 11; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 11; and/or

[0960] (iii) a CDR3 sequence of SEQ ID NO: 12; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 12; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 12.

[0961] Embodiment 19. The rAAV particle of embodiment 18, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 10; (ii) a CDR2 sequence of SEQ ID NO: 11; and (iii) a CDR3 sequence of SEQ ID NO: 12.

[0962] Embodiment 20. The rAAV particle of embodiment 18 or 19, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 9, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0963] Embodiment 21. The rAAV particle of any one of embodiments 1-6, wherein the single chain antibody agent comprises:

[0964] (i) a CDR1 sequence of SEQ ID NO: 14; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 14; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 14;

[0965] (ii) a CDR2 sequence of SEQ ID NO: 15; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 15; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 15; and/or

[0966] (iii) a CDR3 sequence of SEQ ID NO: 16; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 16; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 16.

[0967] Embodiment 22. The rAAV particle of embodiment 21, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 14; (ii) a CDR2 sequence of SEQ ID NO: 15; and (iii) a CDR3 sequence of SEQ ID NO: 16.

[0968] Embodiment 23. The rAAV particle of embodiment 21 or 22, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 13, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0969] Embodiment 24. The rAAV particle of any one of embodiments 1-6, wherein the single chain antibody agent comprises:

[0970] (i) a CDR1 sequence of SEQ ID NO: 18; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 18; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 18;

[0971] (ii) a CDR2 sequence of SEQ ID NO: 19; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 19; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 19; and/or

[0972] (iii) a CDR3 sequence of SEQ ID NO: 20; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 20; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 20.

[0973] Embodiment 25. The rAAV particle of embodiment 24, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 18; (ii) a CDR2 sequence of SEQ ID NO: 19; and (iii) a CDR3 sequence of SEQ ID NO: 20.

[0974] Embodiment 26. The rAAV particle of embodiment 25 or 26, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 17, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0975] Embodiment 27. The rAAV particle of any one of embodiments 1-6, wherein the single chain antibody agent comprises:

[0976] (i) a CDR1 sequence of SEQ ID NO: 22; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 22; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 22;

[0977] (ii) a CDR2 sequence of SEQ ID NO: 23; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 23; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 23; and/or

[0978] (iii) a CDR3 sequence of SEQ ID NO: 24; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 24; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 24.

[0979] Embodiment 28. The rAAV particle of embodiment 27, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 22; (ii) a CDR2 sequence of SEQ ID NO: 23; and (iii) a CDR3 sequence of SEQ ID NO: 24.

[0980] Embodiment 29. The rAAV particle of embodiment 27 or 28, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 21, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0981] Embodiment 30. The rAAV particle of any one of embodiments 1-6, wherein the single chain antibody agent comprises:

[0982] (i) a CDR1 sequence of SEQ ID NO: 26; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 26; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 26;

[0983] (ii) a CDR2 sequence of SEQ ID NO: 27; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 27; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 27; and/or

[0984] (iii) a CDR3 sequence of SEQ ID NO: 28; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 28; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 28.

[0985] Embodiment 31. The rAAV particle of embodiment 30, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 26; (ii) a CDR2 sequence of SEQ ID NO: 27; and (iii) a CDR3 sequence of SEQ ID NO: 28.

[0986] Embodiment 32. The rAAV particle of embodiment 30 or 31, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 25, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0987] Embodiment 33. The rAAV particle of any one of embodiments 1-6, wherein the single chain antibody agent comprises:

[0988] (i) a CDR1 sequence of SEQ ID NO: 30; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 30; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 30;

[0989] (ii) a CDR2 sequence of SEQ ID NO: 31; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 31; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 31; and/or

[0990] (iii) a CDR3 sequence of SEQ ID NO: 32; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 32; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 32.

[0991] Embodiment 34. The rAAV particle of embodiment 33, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 30; (ii) a CDR2 sequence of SEQ ID NO: 31; and (iii) a CDR3 sequence of SEQ ID NO: 32.

[0992] Embodiment 35. The rAAV particle of embodiment 33 or 34, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 29, or a sequence having at least

85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0993] Embodiment 36. The rAAV particle of any one of embodiments 1-6, wherein the single chain antibody agent comprises:

[0994] (i) a CDR1 sequence of SEQ ID NO: 34; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 34; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 34;

[0995] (ii) a CDR2 sequence of SEQ ID NO: 35; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 35; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 35; and/or

[0996] (iii) a CDR3 sequence of SEQ ID NO: 36; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 36; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 36.

[0997] Embodiment 37. The rAAV particle of embodiment 36, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 34; (ii) a CDR2 sequence of SEQ ID NO: 35; and (iii) a CDR3 sequence of SEQ ID NO: 36.

[0998] Embodiment 38. The rAAV particle of embodiment 36 or 37, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 33, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0999] Embodiment 39. The rAAV particle of any one of embodiments 1-6, wherein the single chain antibody agent comprises:

[1000] (i) a CDR1 sequence of SEQ ID NO: 38; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 38; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 38;

[1001] (ii) a CDR2 sequence of SEQ ID NO: 39; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 39; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 39; and/or

[1002] (iii) a CDR3 sequence of SEQ ID NO: 40; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 40; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 40.

[1003] Embodiment 40. The rAAV particle of embodiment 39, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 38; (ii) a CDR2 sequence of SEQ ID NO: 39; and (iii) a CDR3 sequence of SEQ ID NO: 40.

[1004] Embodiment 41. The rAAV particle of embodiment 39 or 40, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 37, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[1005] Embodiment 42. The rAAV particle of any one of embodiments 1-6, wherein the single chain antibody agent comprises:

[1006] (i) a CDR1 sequence of SEQ ID NO: 42; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 42; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 42;

[1007] (ii) a CDR2 sequence of SEQ ID NO: 43; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 43; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 43; and/or

[1008] (iii) a CDR3 sequence of SEQ ID NO: 44; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 44; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 44.

[1009] Embodiment 43. The rAAV particle of embodiment 42, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 42; (ii) a CDR2 sequence of SEQ ID NO: 43; and (iii) a CDR3 sequence of SEQ ID NO: 44.

[1010] Embodiment 44. The rAAV particle of embodiment 42 or 43, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 41, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[1011] Embodiment 45. The rAAV particle of any one of embodiments 1-6, wherein the single chain antibody agent comprises:

[1012] (i) a CDR1 sequence of SEQ ID NO: 46; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 46; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 46;

[1013] (ii) a CDR2 sequence of SEQ ID NO: 47; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 47; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 47; and/or

[1014] (iii) a CDR3 sequence of SEQ ID NO: 48; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 48; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 48.

[1015] Embodiment 46. The rAAV particle of embodiment 45, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 46; (ii) a CDR2 sequence of SEQ ID NO: 47; and (iii) a CDR3 sequence of SEQ ID NO: 48.

[1016] Embodiment 47. The rAAV particle of embodiment 45 or 46, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 45, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[1017] Embodiment 48. The rAAV particle of any one of embodiments 1-6, wherein the single chain antibody agent comprises:

[1018] (i) a CDR1 sequence of SEQ ID NO: 50; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 50; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 50;

[1019] (ii) a CDR2 sequence of SEQ ID NO: 51; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 51; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 51; and/or

[1020] (iii) a CDR3 sequence of SEQ ID NO: 52; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 52; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 52.

[1021] Embodiment 49. The rAAV particle of embodiment 48, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 50; (ii) a CDR2 sequence of SEQ ID NO: 51; and (iii) a CDR3 sequence of SEQ ID NO: 52.

[1022] Embodiment 50. The rAAV particle of embodiment 48 or 49, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 49, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[1023] Embodiment 51. The rAAV particle of any one of embodiments 1-6, wherein the single chain antibody agent comprises:

[1024] (i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2;

[1025] (ii) a CDR2 sequence of SEQ ID NO: 117; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 117; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 117; and/or

[1026] (iii) a CDR3 sequence of SEQ ID NO: 4; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 4; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 4.

[1027] Embodiment 52. The rAAV particle of embodiment 51, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 117; and (iii) a CDR3 sequence of SEQ ID NO: 4.

[1028] Embodiment 53. The rAAV particle of embodiment 51 or 52, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 116, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least

92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[1029] Embodiment 54. The rAAV particle of any one of embodiments 1-6, wherein the single chain antibody agent comprises:

[1030] (i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2;

[1031] (ii) a CDR2 sequence of SEQ ID NO: 119; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 119; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 119; and/or

[1032] (iii) a CDR3 sequence of SEQ ID NO: 4; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 4; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 4.

[1033] Embodiment 55. The rAAV particle of embodiment 54, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 119; and (iii) a CDR3 sequence of SEQ ID NO: 4.

[1034] Embodiment 56. The rAAV particle of embodiment 54 or 55, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 118, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[1035] Embodiment 57. The rAAV particle of any one of embodiments 1-6, wherein the single chain antibody agent comprises:

[1036] (i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least

93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2;

[1037] (ii) a CDR2 sequence of SEQ ID NO: 15; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 15; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 15; and/or

[1038] (iii) a CDR3 sequence of SEQ ID NO: 52; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 52; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 52.

[1039] Embodiment 58. The rAAV particle of embodiment 57, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 15; and (iii) a CDR3 sequence of SEQ ID NO: 52.

[1040] Embodiment 59. The rAAV particle of embodiment 57 or 58, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 120, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[1041] Embodiment 60. The rAAV particle of any one of embodiments 1-6, wherein the single chain antibody agent comprises:

[1042] (i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2;

[1043] (ii) a CDR2 sequence of SEQ ID NO: 119; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%

identity to SEQ ID NO: 119; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 119; and/or

[1044] (iii) a CDR3 sequence of SEQ ID NO: 52; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 52; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 52.

[1045] Embodiment 61. The rAAV particle of embodiment 60, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 119; and (iii) a CDR3 sequence of SEQ ID NO: 52.

[1046] Embodiment 62. The rAAV particle of embodiment 60 or 61, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 121, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[1047] Embodiment 63. The rAAV particle of any one of embodiments 1-6, wherein the single chain antibody agent comprises:

[1048] (i) a CDR1 sequence of SEQ ID NO: 124; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 124; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 124;

[1049] (ii) a CDR2 sequence of SEQ ID NO: 125; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 125; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 125; and/or

[1050] (iii) a CDR3 sequence of SEQ ID NO: 8; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least

93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 8.

**[1051]** Embodiment 64. The rAAV particle of embodiment 63, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 124; (ii) a CDR2 sequence of SEQ ID NO: 125; and (iii) a CDR3 sequence of SEQ ID NO: 8.

**[1052]** Embodiment 65. The rAAV particle of embodiment 63 or 64, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 123, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

**[1053]** Embodiment 66. The rAAV particle of any one of embodiments 1-6, wherein the single chain antibody agent comprises:

**[1054]** (i) a CDR1 sequence of SEQ ID NO: 127; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 127; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 127;

**[1055]** (ii) a CDR2 sequence of SEQ ID NO: 7; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 7; and/or

**[1056]** (iii) a CDR3 sequence of SEQ ID NO: 128; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 128; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO:128.

[1057] Embodiment 67. The rAAV particle of embodiment 66, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 127; (ii) a CDR2 sequence of SEQ ID NO: 7; and (iii) a CDR3 sequence of SEQ ID NO: 128.

[1058] Embodiment 68. The rAAV particle of embodiment 66 or 67, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 126, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[1059] Embodiment 69. The rAAV particle of any one of embodiments 1-6, wherein the single chain antibody agent comprises:

[1060] (i) a CDR1 sequence of SEQ ID NO: 130; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 130; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 130;

[1061] (ii) a CDR2 sequence of SEQ ID NO: 7; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 7; and/or

[1062] (iii) a CDR3 sequence of SEQ ID NO: 131; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 131; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 131.

[1063] Embodiment 70. The rAAV particle of embodiment 69, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 130; (ii) a CDR2 sequence of SEQ ID NO: 7; and (iii) a CDR3 sequence of SEQ ID NO: 131.

[1064] Embodiment 71. The rAAV particle of embodiment 69-70, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 129, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[1065] Embodiment 72. The rAAV particle of any one of embodiments 1-6, wherein the single chain antibody agent comprises:

[1066] (i) a CDR1 sequence of SEQ ID NO: 6; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 6; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 6;

[1067] (ii) a CDR2 sequence of SEQ ID NO: 133; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 133; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 133; and/or

[1068] (iii) a CDR3 sequence of SEQ ID NO: 134; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 134; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 134.

[1069] Embodiment 73. The rAAV particle of embodiment 72, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 6; (ii) a CDR2 sequence of SEQ ID NO: 133; and (iii) a CDR3 sequence of SEQ ID NO: 134.

[1070] Embodiment 74. The rAAV particle of embodiment 72 or 73, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 132, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[1071] Embodiment 75. The rAAV particle of any one of embodiments 1-6, wherein the single chain antibody agent comprises:

[1072] (i) a CDR1 sequence of SEQ ID NO: 6; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 6; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 6;

[1073] (ii) a CDR2 sequence of SEQ ID NO: 125; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 125; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 125; and/or

[1074] (iii) a CDR3 sequence of SEQ ID NO: 134; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 134; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 134.

[1075] Embodiment 76. The rAAV particle of embodiment 75, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 6; (ii) a CDR2 sequence of SEQ ID NO: 125; and (iii) a CDR3 sequence of SEQ ID NO: 134.

[1076] Embodiment 77. The rAAV particle of embodiment 75 or 76, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 137, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[1077] Embodiment 78. The rAAV particle of any one of embodiments 1-6, wherein the single chain antibody agent comprises:

[1078] (i) a CDR1 sequence of SEQ ID NO: 130; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%

identity to SEQ ID NO: 130; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 130;

[1079] (ii) a CDR2 sequence of SEQ ID NO: 7; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 7; and/or

[1080] (iii) a CDR3 sequence of SEQ ID NO: 139; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 139; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 139.

[1081] Embodiment 79. The rAAV particle of embodiment 78, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 130; (ii) a CDR2 sequence of SEQ ID NO: 7; and (iii) a CDR3 sequence of SEQ ID NO: 139.

[1082] Embodiment 80. The rAAV particle of embodiment 78 or 79, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 138, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[1083] Embodiment 81. The rAAV particle of any one of the preceding embodiments, wherein the single chain antibody agent binds to a human TfR (hTfR).

[1084] Embodiment 82. The rAAV particle of any one of the preceding embodiments, wherein the single chain antibody agent binds to hTfR1 with an affinity ( $K_D$ ) of:

[1085] (i) about 10 nM to about 2500 nM, about 20 nM to about 2500 nM , about 30 nM to about 2500 nM, about 40 nM to about 2500 nM, about 50 nM to about 2500 nM, about 60 nM to about 2500 nM, about 70 nM to about 2500 nM, about 80 nM to about 2500 nM, about 90 nM to about 2500 nM, about 100 nM to about 2500 nM, about 200 nM to about 2500 nM, about 300 nM to about 2500 nM, about 400 nM to about 2500 nM, about 500 nM to about 2500 nM, about

600 nM to about 2500 nM, about 700 nM to about 2500 nM, about 800 nM to about 2500 nM, about 900 nM to about 2500 nM, about 1000 nM to about 2500 nM, about 1500 nM to about 2500 nM, about 2000 nM to about 2500 nM, about 10 nM to about 2500 nM, about 10 nM to about 2000 nM, about 10 nM to about 1500 nM, about 10 nM to about 1000 nM, about 10 nM to about 900 nM, about 10 nM to about 800 nM, about 10 nM to about 700 nM, about 10 nM to about 60 nM, about 10 nM to about 500 nM, about 10 nM to about 400 nM, about 10 nM to about 300 nM, about 10 nM to about 200 nM, about 10 nM to about 100 nM, about 10 nM to about 90 nM, about 10 nM to about 80 nM, about 10 nM to about 70 nM, about 10 nM to about 60 nM, about 10 nM to about 50 nM, about 10 nM to about 40 nM, about 10 nM to about 30 nM, about 10 nM to about 20 nM ;

**[1086]** (ii) about 10 nM, about 20 nM, about 30 nM, about 40 nM, about 50 nM, about 60 nM, about 70 nM, about 80 nM, about 90 nM, about 100 nM, about 200 nM, about 300 nM, about 400 nM, about 500 nM, about 600 nM, about 700 nM, about 800 nM, about 900 nM, about 1000 nM, about 1500 nM, about 2000 nM or about 2500 nM; or

**[1087]** (iii) 10 nM, 20 nM, 30 nM, 40 nM, 50 nM, 60 nM, 70 nM, 80 nM, 90 nM, 100 nM, 200 nM, 300 nM, 400 nM, 500 nM, 600 nM, 700 nM, 800 nM, 900 nM, 1000 nM, 1500 nM, 2000 nM or 2500 nM,

**[1088]** wherein affinity is measured when the single chain antibody agent is fused to an Fc domain or when inserted in a VR region of a parental AAV capsid protein.

**[1089]** Embodiment 83. The rAAV particle of any one of embodiments 1-80, wherein the single chain antibody agent binds to a cyano TfR.

**[1090]** Embodiment 84. The rAAV particle of embodiment 83, wherein the single chain antibody agent binds to cyano TfR1 with an affinity ( $K_D$ ) of:

**[1091]** (i) about 10 nM to about 2500 nM, about 20 nM to about 2500 nM , about 30 nM to about 2500 nM, about 40 nM to about 2500 nM, about 50 nM to about 2500 nM, about 60 nM to about 2500 nM, about 70 nM to about 2500 nM, about 80 nM to about 2500 nM, about 90 nM to about 2500 nM, about 100 nM to about 2500 nM, about 200 nM to about 2500 nM, about 300 nM to about 2500 nM, about 400 nM to about 2500 nM, about 500 nM to about 2500 nM, about 600 nM to about 2500 nM, about 700 nM to about 2500 nM, about 800 nM to about 2500 nM,

about 900 nM to about 2500 nM, about 1000 nM to about 2500 nM, about 1500 nM to about 2500 nM, about 2000 nM to about 2500 nM, about 10 nM to about 2500 nM, about 10 nM to about 2000 nM, about 10 nM to about 1500 nM, about 10 nM to about 1000 nM, about 10 nM to about 900 nM, about 10 nM to about 800 nM, about 10 nM to about 700 nM, about 10 nM to about 60 nM, about 10 nM to about 500 nM, about 10 nM to about 400 nM, about 10 nM to about 300 nM, about 10 nM to about 200 nM, about 10 nM to about 100 nM, about 10 nM to about 90 nM, about 10 nM to about 80 nM, about 10 nM to about 70 nM, about 10 nM to about 60 nM, about 10 nM to about 50 nM, about 10 nM to about 40 nM, about 10 nM to about 30 nM, about 10 nM to about 20 nM ;

[1092] (ii) about 10 nM, about 20 nM, about 30 nM, about 40 nM, about 50 nM, about 60 nM, about 70 nM, about 80 nM, about 90 nM, about 100 nM, about 200 nM, about 300 nM, about 400 nM, about 500 nM, about 600 nM, about 700 nM, about 800 nM, about 900 nM, about 1000 nM, about 1500 nM, about 2000 nM or about 2500 nM; or

[1093] (iii) 10 nM, 20 nM, 30 nM, 40 nM, 50 nM, 60 nM, 70 nM, 80 nM, 90 nM, 100 nM, 200 nM, 300 nM, 400 nM, 500 nM, 600 nM, 700 nM, 800 nM, 900 nM, 1000 nM, 1500 nM, 2000 nM or 2500 nM,

[1094] wherein affinity is measured when the single chain antibody agent is fused to an Fc domain or when inserted in a VR region of a parental AAV capsid protein.

[1095] Embodiment 85. The rAAV particle of any one of the preceding embodiments, wherein the single chain antibody agent binds to one or more epitopes on TfR1 (e.g., human TfR1, cyno TfR1, and/or mouse TfR1).

[1096] Embodiment 86. The rAAV particle of embodiment 85, wherein the single chain antibody agent binds to one or more epitopes on human TfR1.

[1097] Embodiment 87. The rAAV particle of embodiment 85, wherein the single chain antibody agent binds to one or more epitopes on cyno TfR1.

[1098] Embodiment 88. The rAAV particle of any one of embodiments 85-87, wherein the epitope is a linear epitope.

[1099] Embodiment 89. The rAAV particle of any one of embodiments 85-87, wherein the epitope is a conformational epitope.

[1100] Embodiment 90. The rAAV particle of any one of embodiments 85-87, wherein the epitope is a discontinuous epitope.

[1101] Embodiment 91. The rAAV particle of any one of embodiments 85-90, wherein the epitope comprises one or more domains of TfR1 (e.g., human TfR1, cyno TfR1, and/or mouse TfR1).

[1102] Embodiment 92. The rAAV particle of any one of embodiments 85-91, wherein the one or more domains comprises an extracellular domain.

[1103] Embodiment 93. The rAAV particle of any one of embodiments 85-92, wherein the one or more domains comprises: (1) an apical domain of human TfR1 or a fragment thereof, (2) a helical domain of human TfR1 or a fragment thereof, or (3) an apical domain of human TfR1 or a fragment thereof, and a helical domain of human TfR1 or a fragment thereof.

[1104] Embodiment 94. The rAAV particle of any one of embodiments 85-93, wherein the epitope comprises: (1) one or more residues K189, Y309, F321, P322, P323, S324, R325, L329, or L381 from an apical domain of human TfR1, and/or (2) one or more residues E634, M635, G636, R719, N722, N723, G724, A725, F726, N727, E728, T729, or R732 from a helical domain of human TfR1.

[1105] Embodiment 95. The rAAV particle of any one of embodiments 85-93, wherein the epitope comprises: (1) one or more residues K189, P323, S324, R325, L381, or E383 from an apical domain of human TfR1, and/or (2) one or more residues E634, M635, R719, N723, N727, E728, T729, or R732 from a helical domain of human TfR1.

[1106] Embodiment 96. The rAAV particle of any one of embodiments 85-93, wherein the epitope comprises: (1) one or more residues K189, P323, S324, R325, or L381 from an apical domain of human TfR1, and/or (2) one or more residues E634, M635, R719, N723, N727, E728, T729, or R732 from a helical domain of human TfR1.

[1107] Embodiment 97. The rAAV particle of any one of the preceding embodiments, wherein the single chain antibody agent comprises one or more linkers.

[1108] Embodiment 98. The rAAV particle of embodiment 97, wherein the one or more linkers are situated on the N-terminus of the single chain antibody agent and/or the C-terminus of the single chain antibody agent.

[1109] Embodiment 99. The rAAV particle of embodiment 97 or 98, wherein the one or more linkers comprise one or more (GGGGS) repeats.

[1110] Embodiment 100. The rAAV particle of embodiment 99, wherein the one or more linkers comprise one or more (GGGGS) repeats on the N-terminus of the single chain antibody agent and/or the C-terminus of the single chain antibody agent.

[1111] Embodiment 101. The rAAV particle of any one of embodiments 97-100, wherein the one or more linkers comprise a coiled-coil alpha helix domain or a fragment thereof.

[1112] Embodiment 102. The rAAV particle of embodiment 101, wherein the single chain antibody agent comprises one or more coiled-coil alpha helix domains on the N-terminus of the single chain antibody agent and/or the C-terminus of the single chain antibody agent.

[1113] Embodiment 103. The rAAV particle of embodiment 101 or 102, wherein the coiled-coil alpha helix domain comprises a leading coil sequence according to SEQ ID NO: 53, or a sequence with at least 90% identity thereto.

[1114] Embodiment 104. The rAAV particle of embodiment 101 or 102, wherein the coiled-coil alpha helix domain comprises a returning coil sequence according to SEQ ID NO: 54, or a sequence with at least 90% identity thereto.

[1115] Embodiment 105. The rAAV particle of embodiment 100, wherein the single chain antibody agent comprises a (GGGGS)<sub>5</sub> linker according to SEQ ID NO: 59 at the N-terminus and a (GGGGS)<sub>1</sub> linker according to SEQ ID NO: 55 at the C-terminus.

[1116] Embodiment 106. The rAAV particle of embodiment 100, wherein the single chain antibody agent comprises a (GGGGS)<sub>4</sub> linker according to SEQ ID NO: 58 at the N-terminus.

[1117] Embodiment 107. The rAAV particle of embodiment 100, wherein the single chain antibody agent comprises: (i) a leading coiled-coil alpha helix domain and a (GGGGS)<sub>5</sub> linker according to SEQ ID NO: 60 at the N-terminus; and (ii) a (GGGGS)<sub>1</sub> linker and a returning coiled-coil alpha helix domain according to SEQ ID NO: 61 at the C-terminus.

[1118] Embodiment 108. The rAAV particle of embodiment 98 or 99, wherein the one or more linkers comprise a linker according to SEQ ID NO: 65 at the N terminus or the C terminus.

[1119] Embodiment 109. The rAAV particle of any one of the preceding embodiments, wherein the single chain antibody agent insertion site is located between two adjacent amino acids in the variable region of the parental AAV capsid protein.

[1120] Embodiment 110. The rAAV particle of any one of the preceding embodiments, wherein the single chain antibody agent insertion site is located between two non-adjacent amino acids in the variable region of the parental AAV capsid protein.

[1121] Embodiment 111. The rAAV particle of any one of the proceeding embodiments, wherein the single chain antibody agent insertion site is in a VP1 of the parental AAV capsid protein.

[1122] Embodiment 112. The rAAV particle of any one of the proceeding embodiments, wherein the single chain antibody agent insertion site is not in a VP2 and/or VP3 of the parental AAV capsid protein.

[1123] Embodiment 113. The rAAV particle of any one of the preceding embodiments, wherein the insertion of the single chain antibody agent replaces a contiguous stretch of amino acids of the parental AAV capsid protein.

[1124] Embodiment 114. The rAAV particle of any one of embodiments 1-112, wherein the insertion of the single chain antibody agent does not replace a contiguous stretch of amino acids of the parental AAV capsid protein.

[1125] Embodiment 115. The rAAV particle of any one of the preceding embodiments, wherein the single chain antibody agent insertion is in VR-I, VR-II, VR-III, VR-IV, VR-V, VR-VI, VR-VII, VR-VIII or VR-IX of the parental AAV capsid protein.

[1126] Embodiment 116. The rAAV particle of any one of the preceding embodiments, wherein the single chain antibody agent insertion is in VR-I of the parental AAV capsid protein.

[1127] Embodiment 117. The rAAV particle of any one of embodiments 1-115, wherein the single chain antibody agent insertion is in VR-II of the parental AAV capsid protein.

[1128] Embodiment 118. The rAAV particle of any one of embodiments 1-115, wherein the single chain antibody agent insertion is in VR-III of the parental AAV capsid protein.

[1129] Embodiment 119. The rAAV particle of any one of embodiments 1-115, wherein the single chain antibody agent insertion is in VR-IV of the parental AAV capsid protein.

[1130] Embodiment 120. The rAAV particle of embodiment 119, wherein the parental AAV capsid protein is an AAV9 capsid protein and VR-IV comprises amino acids 452-460 of VP1, VP2, or VP3 of the AAV9 capsid protein.

[1131] Embodiment 121. The rAAV particle of embodiment 120, wherein the single chain antibody agent insertion site is located between amino acids: (1) 454 and 461 of VP1 of the AAV9 capsid protein, (2) 454 and 457 of VP1 of the AAV9 capsid protein; or (3) 451 and 460 of VP1 of the AAV9 capsid protein.

[1132] Embodiment 122. The rAAV particle of any one of embodiments 1-115, wherein the single chain antibody agent insertion is in VR-V of the parental AAV capsid protein.

[1133] Embodiment 123. The rAAV particle of any one of embodiments 1-115, wherein the single chain antibody agent insertion is in VR-VI of the parental AAV capsid protein.

[1134] Embodiment 124. The rAAV particle of any one of embodiments 1-115, wherein the single chain antibody agent insertion is in VR-VII of the parental AAV capsid protein.

[1135] Embodiment 125. The rAAV particle of embodiment 124, wherein the single chain antibody agent insertion is in VR-VIII of the parental AAV capsid protein.

[1136] Embodiment 126. The rAAV particle of embodiment 125, wherein the parental AAV capsid protein is an AAV9 capsid protein and VR-VIII comprises amino acids 580 to 601 of VP1, VP2, or VP3 of the AAV9 capsid protein.

[1137] Embodiment 127. The rAAV particle of embodiment 126, wherein the single chain antibody agent insertion site is located between amino acids 588 and 590 of VP1 of an

AAV9 capsid protein or the corresponding position in VP1 of another parental AAV capsid protein.

**[1138]** Embodiment 128. The rAAV particle of any one of the preceding embodiments, wherein the variant AAV capsid protein confers at least 1.5-fold increased infectivity and/or transduction of a central nervous system (CNS) cell compared to infectivity and/or transduction of: (i) the CNS cell by a control AAV particle comprising the corresponding parental AAV capsid protein without a single antibody agent inserted in a VR region in the AAV capsid protein; or (ii) a CNS cell which does not express a receptor recognized by the variant AAV capsid protein (e.g., hTfR) by the variant AAV capsid protein.

**[1139]** Embodiment 129. The rAAV particle of embodiment 128, wherein the CNS cell comprises a CNS epithelial cell, an endothelial cell associated with a blood brain barrier, a nerve cell, a CNS connective tissue cell, a stem cell or progenitor cell, a CNS immune cell, a spinal cord cell, or combinations thereof.

**[1140]** Embodiment 130. The rAAV particle of embodiment 129, wherein the CNS epithelial cell comprises a cell that lines one or more brain ventricles.

**[1141]** Embodiment 131. The rAAV particle of embodiment 129, wherein the nerve cell comprises nerve support cells, e.g., astrocytes, glial cells, and Schwann cells.

**[1142]** Embodiment 132. The rAAV particle of embodiment 129, wherein the CNS connective tissue cell comprises fat cells, meninges cells, or both.

**[1143]** Embodiment 133. The rAAV particle of any one of the preceding embodiments, wherein the variant AAV capsid protein further comprises one or more modifications to an amino acid sequence flanking the single chain antibody agent insertion site.

**[1144]** Embodiment 134. The rAAV particle embodiment 133, wherein the one or more modifications are: (a) between the two amino acids flanking the insertion site; (b) within about 10 amino acids upstream or downstream of the location of the single chain antibody agent insertion site, e.g., within about 5 amino acids upstream or downstream of the location of the single chain antibody agent insertion site.

**[1145]** Embodiment 135. The rAAV particle of 133 or 134, wherein the one or more modifications comprises an insertion, deletion, mutation, or combinations thereof.

[1146] Embodiment 136. The rAAV particle of any one of embodiments 133-135, wherein the one or more modifications is a deletion.

[1147] Embodiment 137. The rAAV particle of embodiment 136, wherein the deletion is between two amino acids flanking the insertion site.

[1148] Embodiment 138. The rAAV particle of embodiment 137, wherein the deletion is a deletion of at least 1 amino acid.

[1149] Embodiment 139. The rAAV particle of embodiment 137, wherein the deletion is a deletion of about 1 to 10 amino acids.

[1150] Embodiment 140. The rAAV particle of any one of embodiments 135-139, wherein when the insertion is in VR-IV of the parental AAV capsid protein the deletion comprises: (1) a deletion of amino acids 455 to 460 inclusive of endpoints of VP1 of the AAV9 capsid protein; (2) a deletion of amino acids 455 and 456 of VP1 of the AAV9 capsid protein; or (3) a deletion of amino acids 452 to 459 inclusive of endpoints of VP1 of the AAV9 capsid protein.

[1151] Embodiment 141. The rAAV particle of any one of embodiments 135-139, wherein the single chain antibody agent insertion is in VR-IV of the parental AAV capsid protein and: (a) the single chain antibody agent insertion site is located between amino acids 454 and 461 of VP1 of the AAV9 capsid protein; and (b) the deletion is a deletion of amino acids 455 to 460 inclusive of endpoints of VP1 of the AAV9 capsid protein.

[1152] Embodiment 142. The rAAV particle of any one of embodiments 135-139, wherein the single chain antibody agent insertion is in VR-IV of the parental AAV capsid protein and: (a) the single chain antibody agent insertion site is located between amino acids 454 and 457 of VP1 of the AAV9 capsid protein; and (b) the deletion is a deletion of amino acids 455 and 456 of VP1 of the AAV9 capsid protein.

[1153] Embodiment 143. The rAAV particle of any one of embodiments 135-139, wherein the single chain antibody agent insertion is in VR-IV of the parental AAV capsid protein and: (a) the single chain antibody agent insertion site is located between amino acids 451 and 460 in of VP1 of the AAV9 capsid protein; and (b) the deletion is a deletion of amino acids 452 to 459 inclusive of endpoints of VP1 of the AAV9 capsid protein.

[1154] Embodiment 144. The rAAV particle of any one of embodiments 135-139, wherein when the insertion is in VR-VIII of the parental AAV capsid protein the deletion comprises a deletion of amino acid 589 of VP1 of the AAV9 capsid protein.

[1155] Embodiment 145. The rAAV particle of embodiment 144, wherein the single chain antibody agent insertion is in VR-IV of the parental AAV capsid protein and: (a) the single chain antibody agent insertion site is located between amino acids 588 and 590 of VP1 of the AAV9 capsid protein; and (b) the deletion is a deletion of amino acid 588 of VP1 of the AAV9 capsid protein.

[1156] Embodiment 146. The rAAV particle of any one of the preceding embodiments wherein the variant AAV capsid protein further comprises one or more modifications to an amino acid sequence that is at or near a glycan binding region.

[1157] Embodiment 147. The rAAV particle of embodiment 146, wherein the one or more modifications reduces glycan binding.

[1158] Embodiment 148. The rAAV particle of embodiment 146 or 147, wherein the glycan is galactose.

[1159] Embodiment 149. The rAAV particle of any one of embodiments 146-148, wherein the one or more modifications is at or between amino acids: (a) 271 and 272 of VP1 of the AAV9 capsid protein or the corresponding position in the capsid protein of another AAV serotype; (b) 446 of VP1 of the AAV9 capsid protein or the corresponding position in the capsid protein of another AAV serotype; (c) 470 of VP1 of the AAV9 capsid protein or the corresponding position in the capsid protein of another AAV serotype; (d) 501 and 505 (e.g., at any one or all or a combination of residues 501, 502, 503, 504 or 505) of VP1 of the AAV9 capsid protein or the corresponding position in the capsid protein of another AAV serotype; (e) 489 and 545 of VP1 of the AAV9 capsid protein or the corresponding position in the capsid protein of another AAV serotype; (f) 591 and 621 of VP1 of the AAV9 capsid protein or the corresponding position in the capsid protein of another AAV serotype; or (g) any combination, or all, of (a)-(f).

[1160] Embodiment 150. The rAAV particle of any one of the preceding embodiments, wherein the variant AAV capsid protein has at least 90% identity relative to a

parental AAV capsid protein, wherein percent identity is determined by comparing the sequence of the variant AAV capsid protein without the single chain antibody agent insertion, with the parental AAV capsid protein.

**[1161]** Embodiment 151. The rAAV particle of embodiment 150, wherein the variant AAV capsid protein and the parental AAV capsid protein have 100% identity when: (a) the single chain antibody agent insertion in the variant AAV capsid protein is not taken into account in the sequence comparison; and (b) the variant AAV capsid protein does not have one or more modifications other than the single chain antibody agent insertion.

**[1162]** Embodiment 152. The rAAV particle of embodiment 150, wherein the variant AAV capsid protein and the parental AAV capsid protein have less than 100% identity when: (a) the single chain antibody agent insertion in the variant AAV capsid protein is not taken into account in the sequence comparison; and (b) the variant AAV capsid protein comprises one or more modifications other than the single chain antibody agent insertion.

**[1163]** Embodiment 153. The rAAV particle of any one of the preceding embodiments, wherein the parental AAV capsid protein is an AAV9 capsid protein of SEQ ID NO: 2001.

**[1164]** Embodiment 154. The rAAV particle of any one of embodiments 1-153, wherein the parental AAV capsid protein is an AAV1 capsid protein of SEQ ID NO: 2002.

**[1165]** Embodiment 155. The rAAV particle of any one of embodiments 1-153, wherein the parental AAV capsid protein is an AAV2 capsid protein of SEQ ID NO: 2003.

**[1166]** Embodiment 156. The rAAV particle of any one of embodiments 1-153, wherein the parental AAV capsid protein is an AAV3B capsid protein of SEQ ID NO: 2007.

**[1167]** Embodiment 157. The rAAV particle of any one of embodiments 1-153, wherein the parental AAV capsid protein is an AAV4 capsid protein of SEQ ID NO: 2008.

**[1168]** Embodiment 158. The rAAV particle of any one of embodiments 1-153, wherein the parental AAV capsid protein is an AAV5 capsid protein of SEQ ID NO: 2004.

**[1169]** Embodiment 159. The rAAV particle of any one of embodiments 1-153, wherein the parental AAV capsid protein is an AAV6 capsid protein of SEQ ID NO: 2005.

[1170] Embodiment 160. The rAAV particle of any one of embodiments 1-153, wherein the parental AAV capsid protein is an AAV7 capsid protein of SEQ ID NO: 2009

[1171] Embodiment 161. The rAAV particle of any one of embodiments 1-153, wherein the parental AAV capsid protein is an AAV8 capsid protein of SEQ ID NO: 2006.

[1172] Embodiment 162. The rAAV particle of any one of embodiments 1-153, wherein the parental AAV capsid protein is an AAV10 capsid protein of SEQ ID NO: 2010.

[1173] Embodiment 163. The rAAV particle of any one of embodiments 1-153, wherein the parental AAV capsid protein is an AAV11 capsid protein of SEQ ID NO: 2011.

[1174] Embodiment 164. The rAAV particle of any one of embodiments 1-153, wherein the parental AAV capsid protein is an AAV12 capsid protein of SEQ ID NO: 2012.

[1175] Embodiment 165. The rAAV particle of any one of embodiments 1-153, wherein the parental AAV capsid protein is an AAV13 capsid protein of SEQ ID NO: 2013.

[1176] Embodiment 166. The rAAV particle of any one of embodiments 1-153, wherein the parental AAV capsid protein is an AAVhu68 capsid protein of SEQ ID NO: 2014.

[1177] Embodiment 167. The rAAV particle of any one of embodiments 1-153, wherein the parental AAV capsid protein is an AAVrh10 capsid protein of SEQ ID NO: 2015.

[1178] Embodiment 168. The rAAV particle of any one of the preceding embodiments, wherein the payload is or comprises a polypeptide.

[1179] Embodiment 169. The rAAV particle of embodiment 168, wherein the polypeptide is or comprises: (i) a CRISPR-Cas protein, or a variant or a fragment thereof; (ii) a Zinc finger protein, or a variant or a fragment thereof; (iii) a TALEN protein, or a variant or a fragment thereof; (iv) a base editor, or a variant or a fragment thereof; (v) a prime editor, or a variant or a fragment thereof; and/or (vi) a meganuclease or a variant or a fragment thereof.

[1180] Embodiment 170. The rAAV particle of embodiment 169, wherein the CRISPR-Cas protein is a Type II, Type V or Type VI CRISPR-Cas protein, e.g., a Cas9 protein, a Cas12a protein, a Cas12b protein, a Cas12c protein, a Cas12d protein, a Cas12e protein, a Cas12f protein, a Cas12g protein, a Cas12h protein, a Cas12i protein, a Cas13a protein, a Cas13b protein or a variant or fragment of any of the foregoing.

[1181] Embodiment 171. The rAAV particle of any one of embodiments 168-170, wherein the polypeptide is associated with a CNS disorder.

[1182] Embodiment 172. The rAAV particle of embodiment 171, wherein the CNS disorder is a result of a genetic abnormality.

[1183] Embodiment 173. The rAAV particle of embodiment 171, wherein the CNS disorder is not a result of a genetic abnormality.

[1184] Embodiment 174. The rAAV particle of any one of embodiments 171-173, wherein the CNS disorder is chosen from Friedreich's Ataxia, Dravet Syndrome, Spinocerebellar Ataxia Type 3, Niemann-Pick Type C, Huntington's Disease, Pompe Disease, Myotonic Dystrophy Type 1, Glut1 Deficiency Syndrome (De Vivo Syndrome), Tay-Sachs, Spinal Muscular Atrophy, Alzheimer's disease, Amyotrophic lateral sclerosis (ALS), Danon disease, Rett Syndrome, Angelman Syndrome, Parkinson's disease, tauopathies, genetic epilepsies, or combinations thereof.

[1185] Embodiment 175. The rAAV particle of embodiment 168, wherein the polypeptide is an enzyme.

[1186] Embodiment 176. The rAAV particle of embodiment 168, wherein the polypeptide is an antibody.

[1187] Embodiment 177. The rAAV particle of embodiment 168, wherein the polypeptide is a secreted protein.

[1188] Embodiment 178. The rAAV particle of any one of embodiments 1-167, wherein the payload is or comprises an RNA molecule.

[1189] Embodiment 179. The rAAV particle of embodiment 178, wherein the RNA molecule is a mRNA, an siRNA, a miRNA, a gRNA, an antisense RNA, a circular RNA, an snRNA, or an aptamer.

[1190] Embodiment 180. The rAAV particle of embodiment 179, wherein the RNA molecule targets a nucleic acid molecule encoding a polypeptide associated with a CNS disorder.

[1191] Embodiment 181. The rAAV particle of embodiment 180, wherein the CNS disorder is chosen from Friedreich's Ataxia, Dravet Syndrome, Spinocerebellar Ataxia Type 3,

Niemann-Pick Type C, Huntington's Disease, Pompe Disease, Myotonic Dystrophy Type 1, Glut1 Deficiency Syndrome (De Vivo Syndrome), Tay-Sachs, Spinal Muscular Atrophy, Alzheimer's disease, Amyotrophic lateral sclerosis (ALS), Danon disease, Rett Syndrome, Angelman Syndrome, Parkinson's disease, tauopathies, genetic epilepsies, or combinations thereof.

**[1192]** Embodiment 182. The rAAV particle of any one of embodiments 1-167, wherein the payload is or comprises a DNA molecule.

**[1193]** Embodiment 183. The rAAV particle of any one of the preceding embodiments, wherein the nucleotide sequence encoding a payload is operably linked to a promoter.

**[1194]** Embodiment 184. The rAAV particle of embodiment 183, wherein the promoter is a CNS promoter.

**[1195]** Embodiment 185. The rAAV particle of embodiment 184, wherein the CNS promoter is chosen from: a GFAP promoter, a SYN1 promoter, a NSE/RU5' promoter, a neuroactive peptide cholecystokinin (CCK) promoter, a myelin basic promoter (MBP), a human myelin associated glycoprotein promoter, a phosphate-activated glutaminase (PAG) promoter, a vesicular glutamate transporter (vGLUT) promoter, a glutamic acid decarboxylase (GAD) promoter, Camk2a promoter, TH (tyrosine hydroxylase) promoter, Hb9 promoter, CNP promoter, NES (nestin) promoter, Tub1a promoter, SST (somatostatin) promoter, MeCP2 promoter, or combinations thereof.

**[1196]** Embodiment 186. A pharmaceutical composition comprising: (a) a rAAV particle of any one of the preceding embodiments; and (b) a pharmaceutically acceptable excipient.

**[1197]** Embodiment 187. A method of delivering a payload to a CNS cell, comprising administering the pharmaceutical composition of embodiment 186 to the CNS cell.

**[1198]** Embodiment 188. The method of embodiment 187, wherein the CNS cell is *in vitro*.

**[1199]** Embodiment 189. The method of embodiment 187, wherein the CNS cell is *in vivo*.

[1200] Embodiment 190. The method of embodiment 189, wherein the CNS cell is from a subject that has, or has been determined to have, a CNS disorder.

[1201] Embodiment 191. A method of treating a subject having a CNS disorder and/or ameliorating a symptom of a CNS disorder in a subject, the method comprising administering to the subject the pharmaceutical composition of embodiment 186.

[1202] Embodiment 192. The method of embodiment 190 or 191, wherein the CNS disorder is chosen from Friedreich's Ataxia, Dravet Syndrome, Spinocerebellar Ataxia Type 3, Niemann-Pick Type C, Huntington's Disease, Pompe Disease, Myotonic Dystrophy Type 1, Glut1 Deficiency Syndrome (De Vivo Syndrome), Tay-Sachs, Spinal Muscular Atrophy, Alzheimer's disease, Amyotrophic lateral sclerosis (ALS), Danon disease, Rett Syndrome, Angelman Syndrome, Parkinson's disease, tauopathies, genetic epilepsies, or combinations thereof.

[1203] Embodiment 193. The method of any one of embodiments 187-192, wherein the pharmaceutical composition is administered via a route of administration chosen from: intravenous, intraarterial, intracoronoary, intraparenchymal, subpial, intrathecal, intraocular, intracerebroventricular (ICV), intracisternal magna (ICM), or intramuscular.

[1204] Embodiment 194. The method of any one of embodiments 187-193, wherein the subject is a human.

[1205] Embodiment 195. An isolated cell transduced with the rAAV particle of any one of embodiments 1-185.

[1206] Embodiment 196. An isolated nucleic acid comprising a nucleotide sequence encoding a variant AAV capsid protein, wherein the variant AAV capsid protein comprises a single chain antibody agent that binds to a transferrin receptor (TfR) or a variant or a fragment thereof, and wherein the single chain antibody agent is inserted in a variable region (VR) of a parental AAV capsid protein.

[1207] Embodiment 197. The isolated nucleic acid of embodiment 196, wherein the single chain antibody agent insertion is in VR-I, VR-II, VR-III, VR-IV, VR-V, VR-VI, VR-VII, VR-VIII or VR-IX of the parental AAV capsid protein.

[1208] Embodiment 198. The isolated nucleic acid of embodiment 196 or 197, wherein the single chain antibody agent insertion is in VR-IV of the parental AAV capsid protein.

[1209] Embodiment 199. The isolated nucleic acid of embodiment 198, wherein the parental AAV capsid protein is an AAV9 capsid protein and VR-IV comprises amino acids 452 to 460 of VP1, VP2, or VP3 of the AAV9 capsid protein.

[1210] Embodiment 200. The isolated nucleic acid of embodiment 198 or 199, wherein the single chain antibody agent insertion is in VR-VIII of the parental AAV capsid protein.

[1211] Embodiment 201. The isolated nucleic acid of embodiment 200, wherein the parental AAV capsid protein is an AAV9 capsid protein and VR-VIII comprises amino acids 580 to 601 of VP1, VP2, or VP3 of the AAV9 capsid protein.

[1212] Embodiment 202. An isolated cell comprising the nucleic acid of any one of embodiments 196-201.

[1213] Embodiment 203. A variant AAV capsid protein, wherein the variant AAV capsid protein comprises a single chain antibody agent that binds to a transferrin receptor (TfR) or a variant or a fragment thereof, and wherein the single chain antibody agent is inserted in a variable region (VR) of a parental AAV capsid protein.

[1214] Embodiment 204. The variant AAV capsid protein of embodiment 203, wherein the single chain antibody agent insertion is in VR-I, VR-II, VR-III, VR-IV, VR-V, VR-VI, VR-VII, VR-VIII or VR-IX of the parental AAV capsid protein.

[1215] Embodiment 205. The variant AAV capsid protein of embodiment 203 or 204, wherein the single chain antibody agent insertion is in VR-IV of the parental AAV capsid protein.

[1216] Embodiment 206. The variant AAV capsid protein of embodiment 205, wherein the parental AAV capsid protein is an AAV9 capsid protein and VR-IV comprises amino acids 452 to 460 of VP1, VP2, or VP3 of the AAV9 capsid protein.

[1217] Embodiment 207. The variant AAV capsid protein of embodiment 205 or 206, wherein the single chain antibody agent insertion is in VR-VIII of the parental AAV capsid protein.

[1218] Embodiment 208. The variant AAV capsid protein of embodiment 207, wherein the parental AAV capsid protein is an AAV9 capsid protein and VR-VIII comprises amino acids 580 to 601 of VP1, VP2, or VP3 of the AAV9 capsid protein.

[1219] Embodiment 209. A composition comprising the pharmaceutical composition of embodiment 186, for use in a method of delivering a payload to a CNS cell.

[1220] Embodiment 210. Use of the pharmaceutical composition of embodiment 186, in the manufacture of a medicament for delivering a payload to a CNS cell.

[1221] Embodiment 211. The composition for use of embodiment 209, or the use of embodiment 210, comprising administering the pharmaceutical composition to a CNS cell.

[1222] Embodiment 212. The composition for use of embodiment 211, or the use of embodiment 211, wherein the CNS cell is *in vitro*.

[1223] Embodiment 213. The composition for use of embodiment 211, or the use of embodiment 211, wherein the CNS cell is *in vivo*.

[1224] Embodiment 214. The composition for use of embodiment 211, or the use of embodiment 211, wherein the CNS cell is from a subject that has, or has been determined to have, a CNS disorder.

[1225] Embodiment 215. A composition comprising the pharmaceutical composition of embodiment 186, for use in a method of treating a subject having a CNS disorder and/or ameliorating a symptom of a CNS disorder in a subject.

[1226] Embodiment 216. Use of the pharmaceutical composition of embodiment 186, in the manufacture of a medicament for treating a subject having a CNS disorder and/or ameliorating a symptom of a CNS disorder in a subject.

[1227] Embodiment 217. The composition for use of embodiment 251, or the use of embodiment 216, comprising administering the pharmaceutical composition to a subject.

[1228] Embodiment 218. The composition for use of embodiment 215 or 217, or the use of embodiment 216 or 217, wherein the CNS disorder is chosen from Friedreich's Ataxia, Dravet Syndrome, Spinocerebellar Ataxia Type 3, Niemann-Pick Type C, Huntington's Disease, Pompe Disease, Myotonic Dystrophy Type 1, Glut1 Deficiency Syndrome (De Vivo Syndrome), Tay-Sachs, Spinal Muscular Atrophy, Alzheimer's disease, Amyotrophic lateral sclerosis (ALS), Danon disease, Rett Syndrome, Angelman Syndrome, Parkinson's disease, tauopathies, genetic epilepsies, or combinations thereof.

[1229] Embodiment 219. The composition for use of any one of embodiments 215 or 217-218, or the use of any one of embodiments 216-218, wherein the pharmaceutical composition is administered via a route of administration chosen from: intravenous, intraarterial, intracoronoary, intraparenchymal, subpial, intrathecal, intraocular, intracerebroventricular (ICV), intracisternal magna (ICM), or intramuscular.

[1230] Embodiment 220. The composition for use of any one of embodiments 215 or 217-219, or the use of any one of embodiments 216-219, wherein the subject is a human.

[1231] Embodiment 221. A method of delivering the rAAV particle of any one of embodiments 1-185, the variant AAV capsid of any one of embodiments 203-208, or the composition of embodiment 186, to a cell, tissue or subject.

[1232] Embodiment 222. Use of the rAAV particle of any one of embodiments 1-185, the variant AAV capsid of any one of embodiments 203-208, or the composition of embodiment 186, in the manufacture of a medicament for delivering to a cell, tissue or subject.

[1233] Embodiment 223. A composition comprising the rAAV particle of any one of embodiments 1-185, the variant AAV capsid of any one of embodiments 203-208, or the composition of embodiment 186, for use in delivering to a cell, tissue or subject.

[1234] Embodiment 224. The method of embodiment 221, the use of embodiment 222 or the composition for use of embodiment 223, wherein delivering comprises administering, e.g., using an administration method described herein.

[1235] Embodiment 225. The method of embodiment 221 or 224, the use of embodiment 222 or 224, or the composition for use of embodiment 223 or 224, wherein the subject is a human.

[1236] Embodiment 226. The method of any one of embodiments 187-194 or 221 or 224-225, the use of any one of embodiments 216-220, 222 or 224-225, or the composition for use of any one of embodiments 209, 211-215, 217-220, or 223-225, wherein the rAAV particle or composition is characterized in that when delivered to a cell, tissue, or subject, increased infectivity and/or transduction of a central nervous system (CNS) cell is observed compared to infectivity and/or transduction of: (i) the CNS cell by a control AAV particle comprising the corresponding parental AAV capsid protein; or (ii) a CNS cell which does not express a receptor recognized by the variant AAV capsid protein (e.g., hTfR) by the variant AAV capsid protein.

[1237] Embodiment 227. The method of any one of embodiments 187-194 or 221 or 224-225, the use of any one of embodiments 216-220, 222 or 224-225, or the composition for use of any one of embodiments 209, 211-215, 217-220, or 223-225, wherein the rAAV particle or composition is characterized in that when delivered to a cell, tissue, or subject, increased expression of a payload is observed compared to expression of a similar payload in a cell, tissue or subject administered a control AAV particle comprising the corresponding parental AAV capsid protein without a single antibody agent inserted in a VR region in the AAV capsid protein.

[1238] Embodiment 228. The method of any one of embodiments 187-194 or 221 or 224-227, the use of any one of embodiments 216-220, 222 or 224-227, or the composition for use of any one of embodiments 209, 211-215, 217-220, or 223-227,, comprising administering the pharmaceutical composition in combination with an immunosuppressive regimen.

[1239] Embodiment 229. The method of any one of embodiments 187-194 or 221 or 224-228, the use of any one of embodiments 216-220, 222 or 224-228, or the composition for use of any one of embodiments 209, 211-215, 217-220, or 223-228, wherein the immunosuppressive regimen comprises: (i) dexamethasone or prednisolone, and (ii) a calcineurin inhibitor.

[1240] Embodiment 230. The composition for use, or the use of, embodiment 229, wherein prednisolone is administered at a dose of about 3 mg/kg, e.g., +/- 10% of 3 mg/kg.

[1241] Embodiment 231. The composition for use, or the use of, embodiment 229, wherein dexamethasone is administered at a dose of about 0.5 mg/kg, e.g., +/- 10% of 0.5 mg/kg.

[1242] Embodiment 232. The composition for use, or the use of, embodiment 229, wherein the calcineurin inhibitor is tacrolimus.

[1243] Embodiment 233. The composition for use, or the use of, embodiment 232, wherein tacrolimus is administered at a dose of about 1 mg/kg, e.g., +/- 10% of 1 mg/kg.

[1244] Embodiment 234. The method, composition for use, or the use of, any one of embodiments 229-233, wherein the immunosuppressive regimen is administered daily.

[1245] Embodiment 235. The method, composition for use, or the use of, any one of embodiments 229-234, wherein the administration of the immunosuppressive regimen is initiated prior to administration of the rAAV particle or a composition comprising the same.

[1246] Embodiment 236. The method, composition for use, or the use of, any one of embodiments 229-235, wherein the immunosuppressive regimen is administered: (i) on each of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more days prior to administration of the rAAV particle or a composition comprising the same; (ii) on a same day as administration of the rAAV particle or a composition comprising the same; and/or (iii) on each day following administration of the rAAV particle or a composition comprising the same for about 1 week, 2 weeks, 3 weeks.

## EQUIVALENTS

[1247] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. The scope of the present invention is not intended to be limited to the above Description, but rather is as set forth in the following claims

## CLAIMS

*We claim:*

1. A recombinant adeno-associated virus (rAAV) particle comprising:
  - (a) a variant AAV capsid protein comprising a single chain antibody agent that binds to a transferrin receptor (TfR1) or a variant or a fragment thereof, wherein the single chain antibody agent is inserted in a variable region (VR) of a parental AAV capsid protein, and
  - (b) a heterologous nucleic acid comprising a nucleotide sequence encoding a payload.
2. The rAAV particle of claim 1, wherein the single chain antibody agent comprises one or more complementarity determining regions (CDRs), optionally wherein the single chain antibody agent comprises a CDR1, a CDR2 and a CDR3.
3. The rAAV particle of claim 1 or 2, wherein the single chain antibody agent is or comprises:
  - (i) a single domain antibody (e.g., a VHH); or
  - (ii) a single chain Fv.
4. The rAAV particle of any one of the preceding claims, wherein the single chain antibody agent comprises a sequence provided in **Table 1**.
5. The rAAV particle of any one of the preceding claims, wherein the single chain antibody agent comprises:
  - (i) a CDR1 sequence provided in **Table 1**; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a CDR1 sequence provided in Table 1; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to a CDR1 sequence provided in **Table 1**;
  - (ii) a CDR2 sequence provided in **Table 1**; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a

CDR2 sequence provided in **Table 1**; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to a CDR2 sequence provided in **Table 1**; and/or

(iii) a CDR3 sequence provided in **Table 1**; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a CDR3 sequence provided in **Table 1**; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to a CDR3 sequence provided in **Table 1**.

6. The rAAV particle of any one of claims 1-5, wherein the single chain antibody agent comprises:

(i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2;

(ii) a CDR2 sequence of SEQ ID NO: 3; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 3; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 3; and/or

(iii) a CDR3 sequence of SEQ ID NO: 4; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 4; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 4.

7. The rAAV particle of claim 6, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 1, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

8. The rAAV particle of any one of claims 1-5, wherein the single chain antibody agent comprises:

(i) a CDR1 sequence of SEQ ID NO: 6; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 6; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 6;

(ii) a CDR2 sequence of SEQ ID NO: 7; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 7; and/or

(iii) a CDR3 sequence of SEQ ID NO: 8; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 8.

9. The rAAV particle of claim 8, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 5, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

10. The rAAV particle of any one of claims 1-5, wherein the single chain antibody agent comprises:

(i) a CDR1 sequence of SEQ ID NO: 22; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 22; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 22;

(ii) a CDR2 sequence of SEQ ID NO: 23; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 23; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 23; and/or

(iii) a CDR3 sequence of SEQ ID NO: 24; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at

least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 24; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 24.

11. The rAAV particle of claim 10, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 21, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

12. The rAAV particle of any one of claims 1-5, wherein the single chain antibody agent comprises:

(i) a CDR1 sequence of SEQ ID NO: 50; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 50; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 50;

(ii) a CDR2 sequence of SEQ ID NO: 51; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 51; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 51; and/or

(iii) a CDR3 sequence of SEQ ID NO: 52; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 52; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 52.

13. The rAAV particle of claim 12, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 49, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

14. The rAAV particle of any one of the preceding claims, wherein the single chain antibody agent binds to a human TfR (hTfR), or a cyno TfR.

15. The rAAV particle of any one of the preceding claims, wherein the single chain antibody agent binds to hTfR1 with an affinity ( $K_D$ ) of about 10 nM to about 25000 nM.
16. The rAAV particle of any one of the preceding claims, wherein the single chain antibody agent comprises one or more linkers.
17. The rAAV particle of claim 16, wherein the one or more linkers are situated on the N-terminus of the single chain antibody agent and/or the C-terminus of the single chain antibody agent.
18. The rAAV particle of claim 16 or 17, wherein the one or more linkers comprise:
  - (i) one or more (GGGS) repeats, and/or
  - (ii) a coiled-coil alpha helix domain or a fragment thereof, optionally wherein the coiled-coil alpha helix domain comprises a leading coil sequence according to SEQ ID NO: 53, or a sequence with at least 90% identity thereto, and/or the coiled-coil alpha helix domain comprises a returning coil sequence according to SEQ ID NO: 54, or a sequence with at least 90% identity thereto.
19. The rAAV particle of any one of the preceding claims, wherein the single chain antibody agent insertion site is located between two adjacent amino acids in the variable region of the parental AAV capsid protein.
20. The rAAV particle of any one of claims 1-18, wherein the single chain antibody agent insertion site is located between two non-adjacent amino acids in the variable region of the parental AAV capsid protein.
21. The rAAV particle of any one of the proceeding claims, wherein the single chain antibody agent insertion site is in a VP1 of the parental AAV capsid protein.
22. The rAAV particle of any one of the proceeding claims, wherein the single chain antibody agent insertion site is not in a VP2 and/or VP3 of the parental AAV capsid protein.

23. The rAAV particle of any one of the preceding claims, wherein the insertion of the single chain antibody agent replaces a contiguous stretch of amino acids of the parental AAV capsid protein.
24. The rAAV particle of any one of claims 1-22, wherein the insertion of the single chain antibody agent does not replace a contiguous stretch of amino acids of the parental AAV capsid protein.
25. The rAAV particle of any one of the preceding claims, wherein the single chain antibody agent insertion is in VR-I, VR-II, VR-III, VR-IV, VR-V, VR-VI, VR-VII, VR-VIII or VR-IX of the parental AAV capsid protein.
26. The rAAV particle of any one of the preceding claims, wherein the single chain antibody agent insertion is in VR-IV of the parental AAV capsid protein.
27. The rAAV particle of claim 26, wherein the parental AAV capsid protein is an AAV9 capsid protein and VR-IV comprises amino acids 452-460 of VP1, VP2, or VP3 of the AAV9 capsid protein.
28. The rAAV particle of claim 27, wherein the single chain antibody agent insertion site is located between amino acids:
  - (1) 454 and 461 of VP1 of the AAV9 capsid protein,
  - (2) 454 and 457 of VP1 of the AAV9 capsid protein; or
  - (3) 451 and 460 of VP1 of the AAV9 capsid protein.
29. The rAAV particle of any one of claims 1-25, wherein the single chain antibody agent insertion is in VR-VIII of the parental AAV capsid protein.

30. The rAAV particle of claim 29, wherein the parental AAV capsid protein is an AAV9 capsid protein and VR-VIII comprises amino acids 580 to 601 of VP1, VP2, or VP3 of the AAV9 capsid protein.

31. The rAAV particle of claim 30, wherein the single chain antibody agent insertion site is located between amino acids 588 and 590 of VP1 of an AAV9 capsid protein or the corresponding position in VP1 of another parental AAV capsid protein.

32. The rAAV particle of any one of the preceding claims, wherein the variant AAV capsid protein confers at least 1.5-fold increased infectivity and/or transduction of a central nervous system (CNS) cell compared to infectivity and/or transduction of:

(i) the CNS cell by a control AAV particle comprising the corresponding parental AAV capsid protein without an insertion of a single chain antibody agent in the AAV capsid protein; or

(ii) a CNS cell which does not express a receptor recognized by the variant AAV capsid protein (e.g., hTfR) by the variant AAV capsid protein.

33. The rAAV particle of claim 32, wherein the CNS cell comprises a CNS epithelial cell, an endothelial cell associated with a blood brain barrier, a nerve cell, a CNS connective tissue cell, a stem cell or progenitor cell, a CNS immune cell, a spinal cord cell, or combinations thereof.

34. The rAAV particle of claim 33, wherein:

- (i) the CNS epithelial cell comprises a cell that lines one or more brain ventricles,
- (ii) the nerve cell comprises nerve support cells, and/or
- (iii) the CNS connective tissue cell comprises fat cells, meninges cells, or both.

35. The rAAV particle of any one of the preceding claims, wherein the variant AAV capsid protein further comprises one or more modifications to an amino acid sequence flanking the single chain antibody agent insertion site.

36. The rAAV particle of claim 35, wherein the one or more modifications are:

(a) between the two amino acids flanking the insertion site;  
(b) within about 10 amino acids upstream or downstream of the location of the single chain antibody agent insertion site, e.g., within about 5 amino acids upstream or downstream of the location of the single chain antibody agent insertion site.

37. The rAAV particle of 35 or 36, wherein the one or more modifications comprises an insertion, deletion, mutation, or combinations thereof.

38. The rAAV particle of claim 37, wherein the deletion is between two amino acids flanking the insertion site, optionally wherein the deletion is a deletion of:

- (i) at least 1 amino acid, or
- (ii) about 1 to 10 amino acids.

39. The rAAV particle of claim 37 or 38, wherein when the insertion is in VR-IV of the parental AAV capsid protein the deletion comprises:

- (1) a deletion of amino acids 455 to 460 inclusive of endpoints of VP1 of the AAV9 capsid protein;
- (2) a deletion of amino acids 455 and 456 of VP1 of the AAV9 capsid protein; or
- (3) a deletion of amino acids 452 to 459 inclusive of endpoints of VP1 of the AAV9 capsid protein.

40. The rAAV particle of claim 37 or 38, wherein when the insertion is in VR-VIII of the parental AAV capsid protein the deletion comprises a deletion of amino acid 589 of VP1 of the AAV9 capsid protein.

41. The rAAV particle of any one of the preceding claims wherein the variant AAV capsid protein further comprises one or more modifications to an amino acid sequence that is at or near a glycan binding region, optionally wherein the one or more modifications reduces glycan binding.

42. The rAAV particle of any one of claims 35-41, wherein the one or more modifications is at or between amino acids:

- (a) 271 and 272 of VP1 of the AAV9 capsid protein or the corresponding position in the capsid protein of another AAV serotype;
- (b) 446 of VP1 of the AAV9 capsid protein or the corresponding position in the capsid protein of another AAV serotype;
- (c) 470 of VP1 of the AAV9 capsid protein or the corresponding position in the capsid protein of another AAV serotype;
- (d) 501 and 505 (e.g., at any one or all or a combination of residues 501, 502, 503, 504 or 505) of VP1 of the AAV9 capsid protein or the corresponding position in the capsid protein of another AAV serotype;
- (e) 489 and 545 of VP1 of the AAV9 capsid protein or the corresponding position in the capsid protein of another AAV serotype;
- (f) 591 and 621 of VP1 of the AAV9 capsid protein or the corresponding position in the capsid protein of another AAV serotype; or
- (g) any combination, or all, of (a)-(f).

43. The rAAV particle of any one of the preceding claims, wherein the variant AAV capsid protein has at least 90% identity relative to a parental AAV capsid protein, wherein percent identity is determined by comparing the sequence of the variant AAV capsid protein without the single chain antibody agent insertion, with the parental AAV capsid protein.

44. The rAAV particle of any one of the preceding claims, wherein the parental AAV capsid protein is an AAV9 capsid protein of SEQ ID NO: 2001.

45. The rAAV particle of any one of the preceding claims, wherein the payload is or comprises a polypeptide.

46. The rAAV particle of claim 45, wherein the polypeptide is or comprises:

- (i) a CRISPR-Cas protein, optionally, wherein the CRISPR-Cas protein is a Type II, Type V or Type VI CRISPR-Cas protein, e.g., a Cas9 protein, a Cas12a protein, a Cas12b protein, a

Cas12c protein, a Cas12d protein, a Cas12e protein, a Cas12f protein, a Cas12g protein, a Cas12h protein, a Cas12i protein, a Cas13a protein, a Cas13b protein or a variant or fragment of any of the foregoing;

- (ii) a Zinc finger protein, or a variant or a fragment thereof;
- (iii) a TALEN protein, or a variant or a fragment thereof;
- (iv) a base editor, or a variant or a fragment thereof;
- (v) a prime editor, or a variant or a fragment thereof;
- (vi) a meganuclease or a variant or a fragment thereof;
- (vii) an enzyme;
- (viii) an antibody; and/or
- (ix) a secreted protein.

47. The rAAV particle of claim 45 or 46, wherein the polypeptide is associated with a CNS disorder.

48. The rAAV particle of any one of claims 1-44, wherein the payload is or comprises an RNA molecule, optionally wherein the RNA molecule is a mRNA, an siRNA, a miRNA, a gRNA, an antisense RNA, a circular RNA, an snRNA, or an aptamer.

49. The rAAV particle of claim 48, wherein the RNA molecule targets a nucleic acid molecule encoding a polypeptide associated with a CNS disorder.

50. The rAAV particle of claim 47 or 49, wherein the CNS disorder is chosen from Friedreich's Ataxia, Dravet Syndrome, Spinocerebellar Ataxia Type 3, Niemann-Pick Type C, Huntington's Disease, Pompe Disease, Myotonic Dystrophy Type 1, Glut1 Deficiency Syndrome (De Vivo Syndrome), Tay-Sachs, Spinal Muscular Atrophy, Alzheimer's disease, Amyotrophic lateral sclerosis (ALS), Danon disease, Rett Syndrome, Angelman Syndrome, Parkinson's disease, tauopathies, genetic epilepsies, or combinations thereof.

51. The rAAV particle of any one of claims 1-44, wherein the payload is or comprises a DNA molecule.

52. The rAAV particle of any one of the preceding claims, wherein the nucleotide sequence encoding a payload is operably linked to a promoter, optionally wherein the promoter is a CNS promoter.

53. The rAAV particle of claim 52, wherein the CNS promoter is chosen from: a GFAP promoter, a SYN1 promoter, a NSE/RU5' promoter, a neuroactive peptide cholecystokinin (CCK) promoter, a myelin basic promoter (MBP), a human myelin associated glycoprotein promoter, a phosphate-activated glutaminase (PAG) promoter, a vesicular glutamate transporter (vGLUT) promoter, a glutamic acid decarboxylase (GAD) promoter, Camk2a promoter, TH (tyrosine hydroxylase) promoter, Hb9 promoter, CNP promoter, NES (nestin) promoter, Tub1a promoter, SST (somatostatin) promoter, MeCP2 promoter, or any combination thereof.

54. A pharmaceutical composition comprising:

- (a) a rAAV particle of any one of the preceding claims; and
- (b) a pharmaceutically acceptable excipient.

55. A method of delivering a payload to a CNS cell, comprising administering the pharmaceutical composition of claim 54 to the CNS cell.

56. A method of treating a subject having a CNS disorder and/or ameliorating a symptom of a CNS disorder in a subject, the method comprising  
administering to the subject the pharmaceutical composition of claim 54.

57. The method of claim 56, wherein the CNS disorder is chosen from Friedreich's Ataxia, Dravet Syndrome, Spinocerebellar Ataxia Type 3, Niemann-Pick Type C, Huntington's Disease, Pompe Disease, Myotonic Dystrophy Type 1, Glut1 Deficiency Syndrome (De Vivo Syndrome), Tay-Sachs, Spinal Muscular Atrophy, Alzheimer's disease, Amyotrophic lateral sclerosis (ALS), Danon disease, Rett Syndrome, Angelman Syndrome, Parkinson's disease, tauopathies, genetic epilepsies, or any combination thereof.

58. The method of any one of claims 55-57, wherein the pharmaceutical composition is administered via a route of administration chosen from: intravenous, intraarterial, intracoronary, intraparenchymal, subpial, intrathecal, intraocular, intracerebroventricular (ICV), intracisternal magna (ICM), or intramuscular.
59. The method of any one of claims 55-58, wherein the subject is a human.
60. An isolated cell transduced with the rAAV particle of any one of claims 1-53.
61. An isolated nucleic acid comprising a nucleotide sequence encoding a variant AAV capsid protein,  
wherein the variant AAV capsid protein comprises a single chain antibody agent that binds to a transferrin receptor (TfR) or a variant or a fragment thereof, and  
wherein the single chain antibody agent is inserted in a variable region (VR) of a parental AAV capsid protein.
62. An isolated cell comprising the nucleic acid of claim 61.
63. A variant AAV capsid protein,  
wherein the variant AAV capsid protein comprises a single chain antibody agent that binds to a transferrin receptor (TfR) or a variant or a fragment thereof, and  
wherein the single chain antibody agent is inserted in a variable region (VR) of a parental AAV capsid protein.

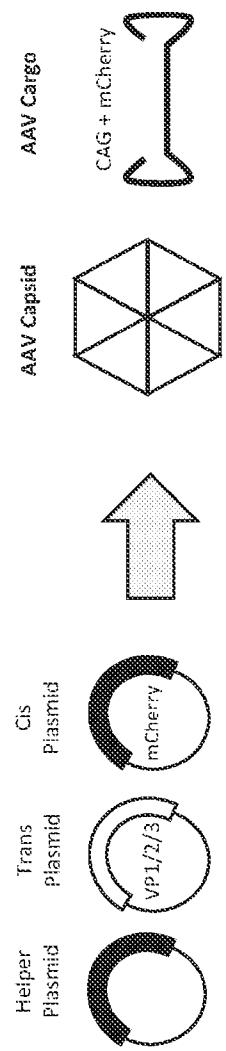


FIG. 1A

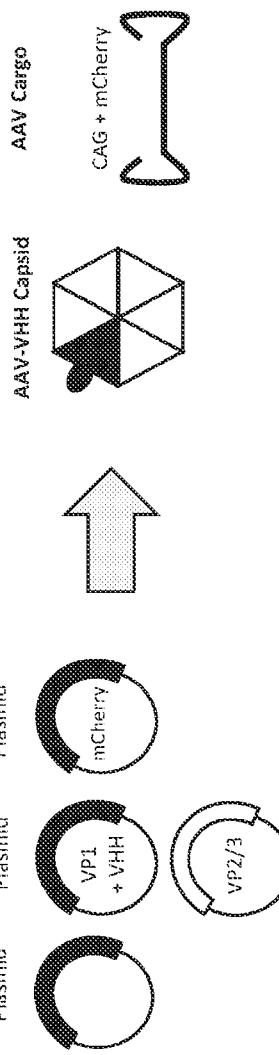


FIG. 1B

2/59

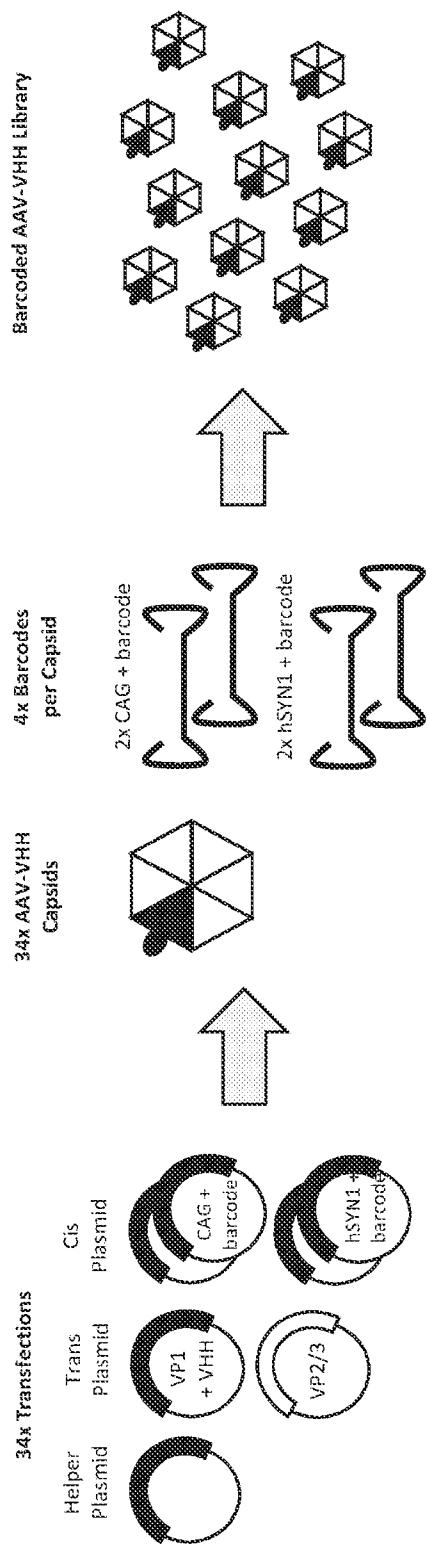


FIG. 1C

3/59

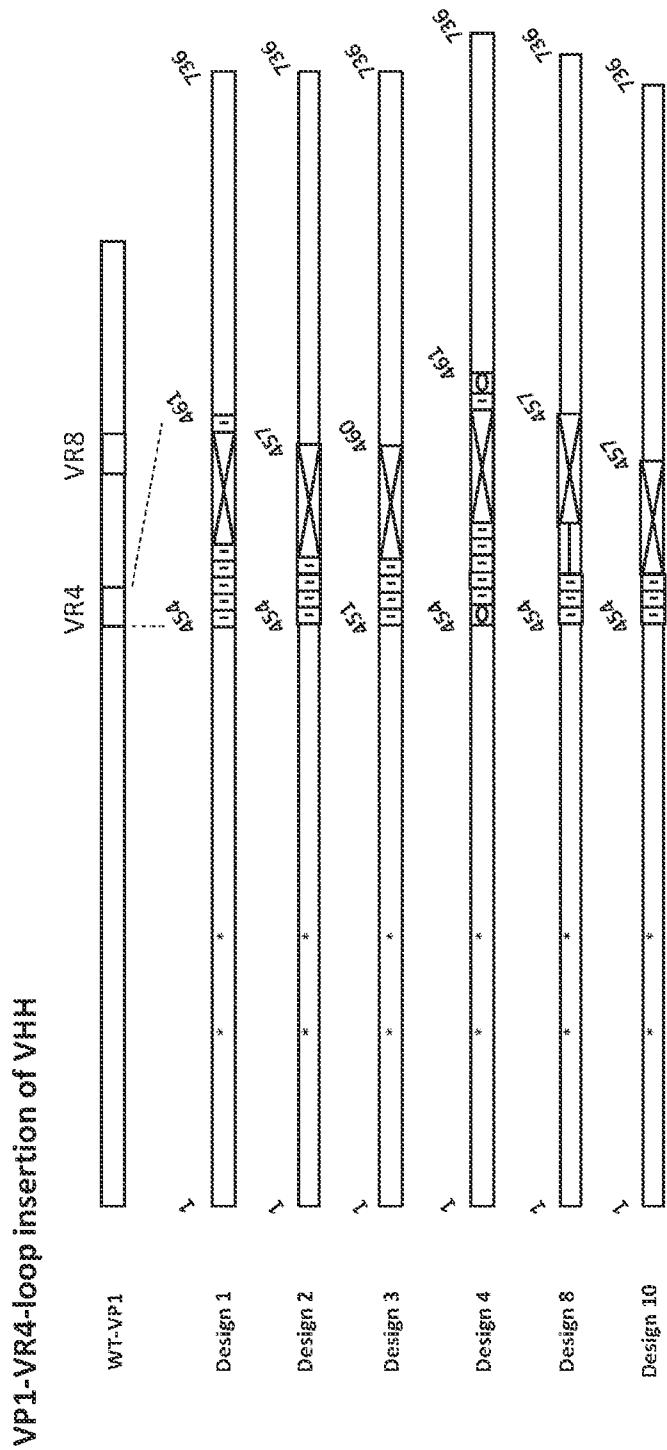


FIG. 2A

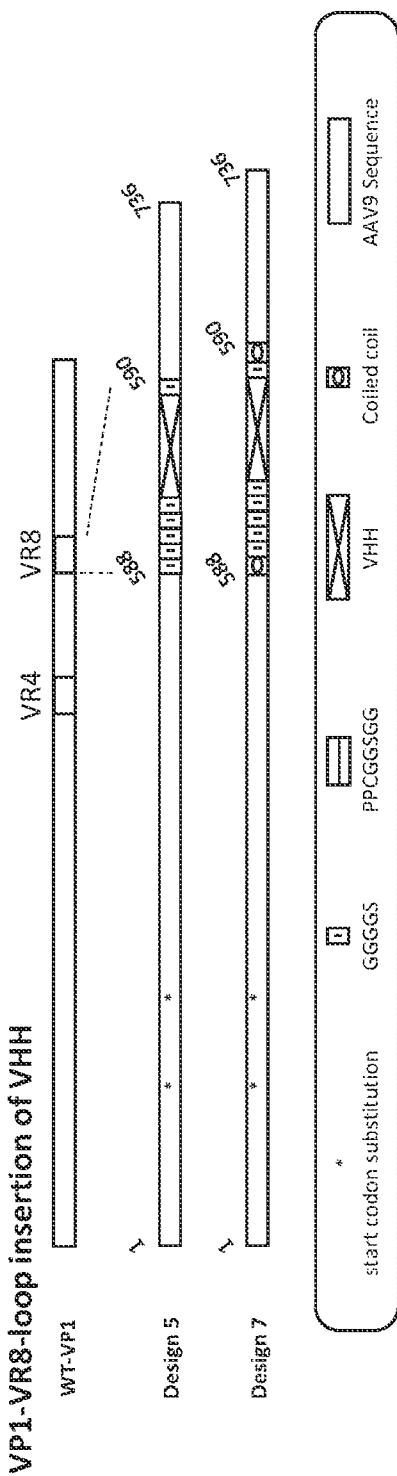


FIG. 2B

5/59

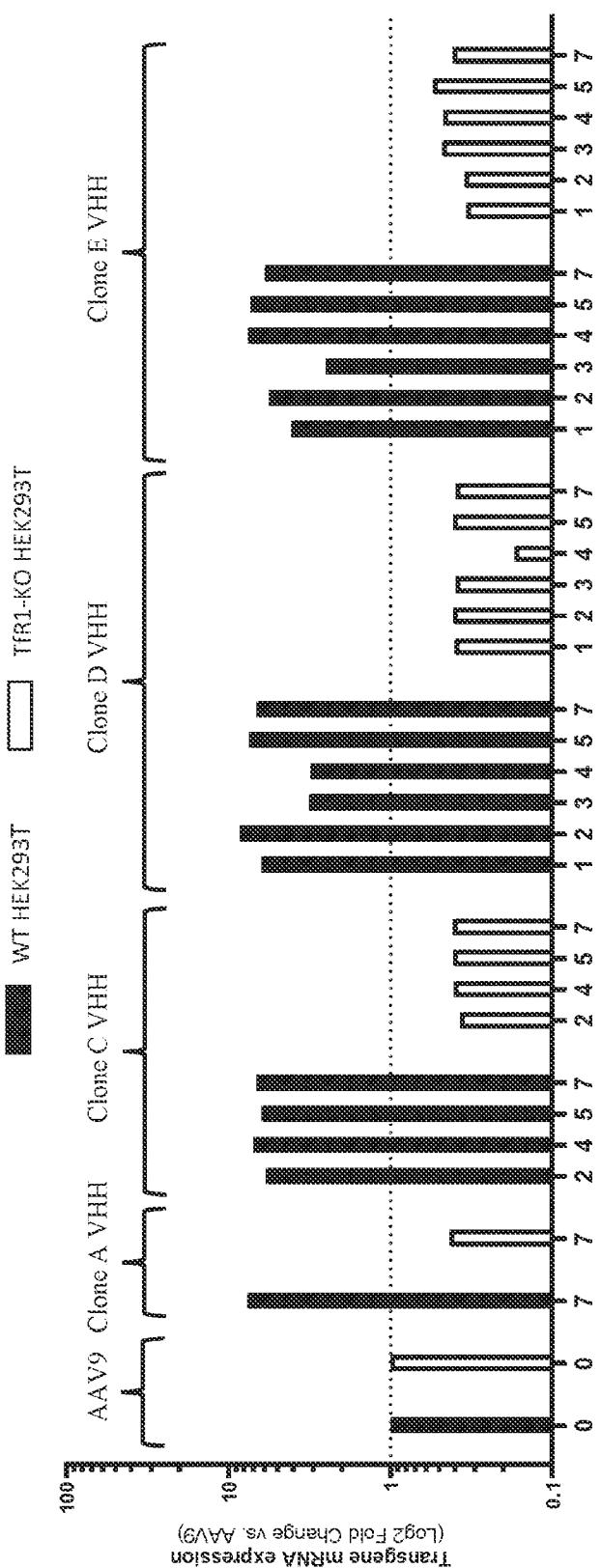


FIG. 3

6/59

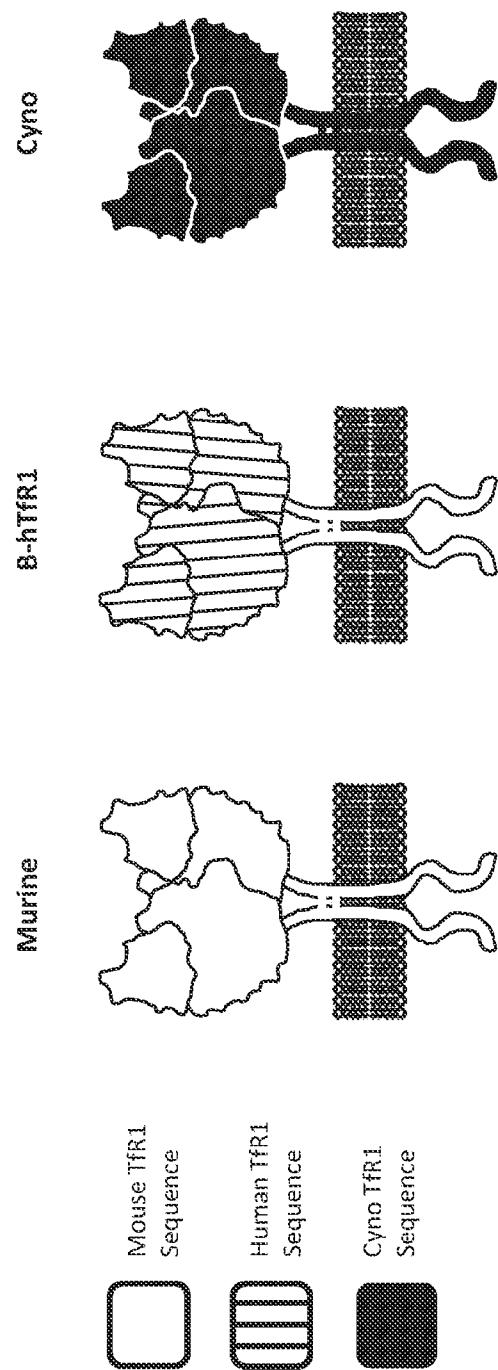


FIG. 4

7/59

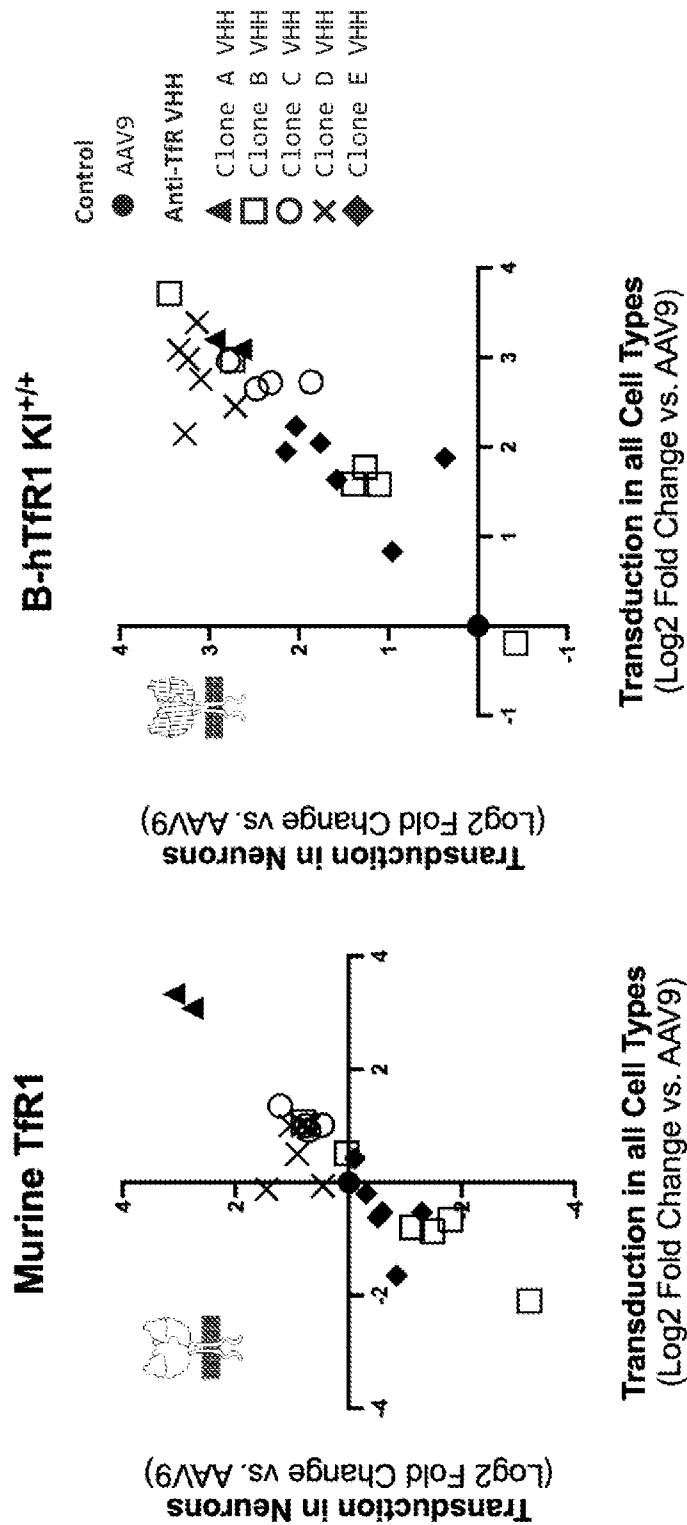


FIG. 5B

FIG. 5A

8/59

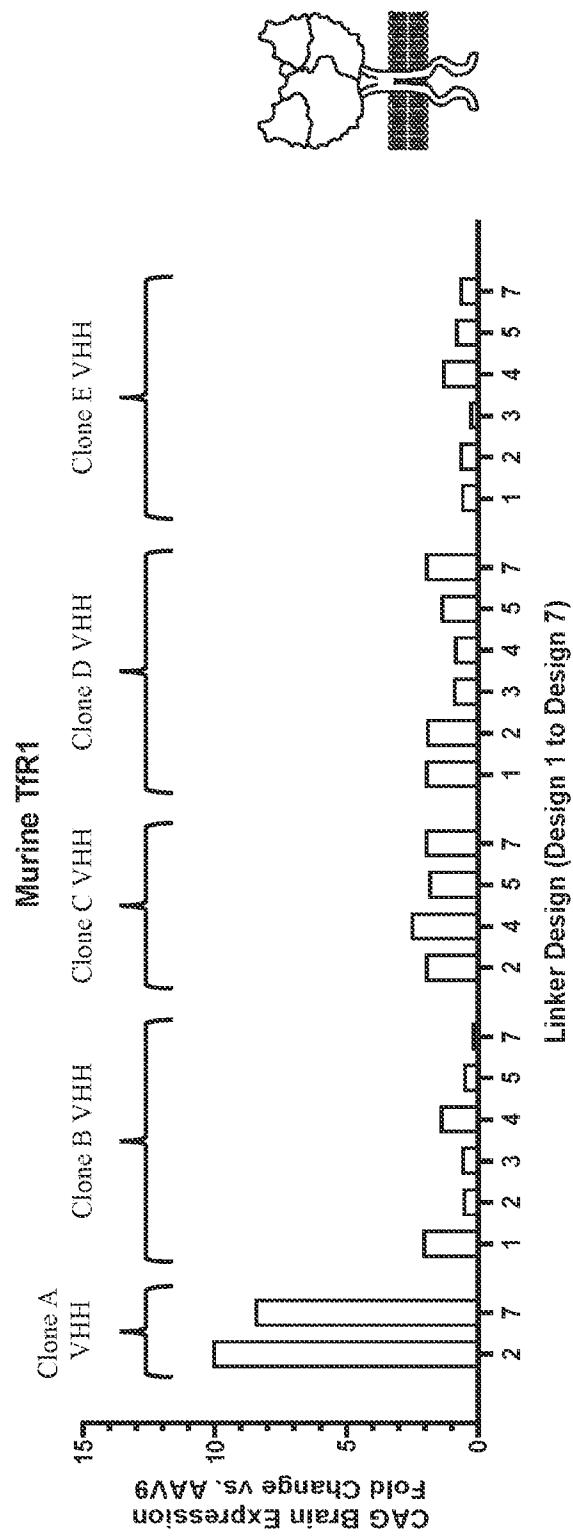


FIG. 6A

9/59

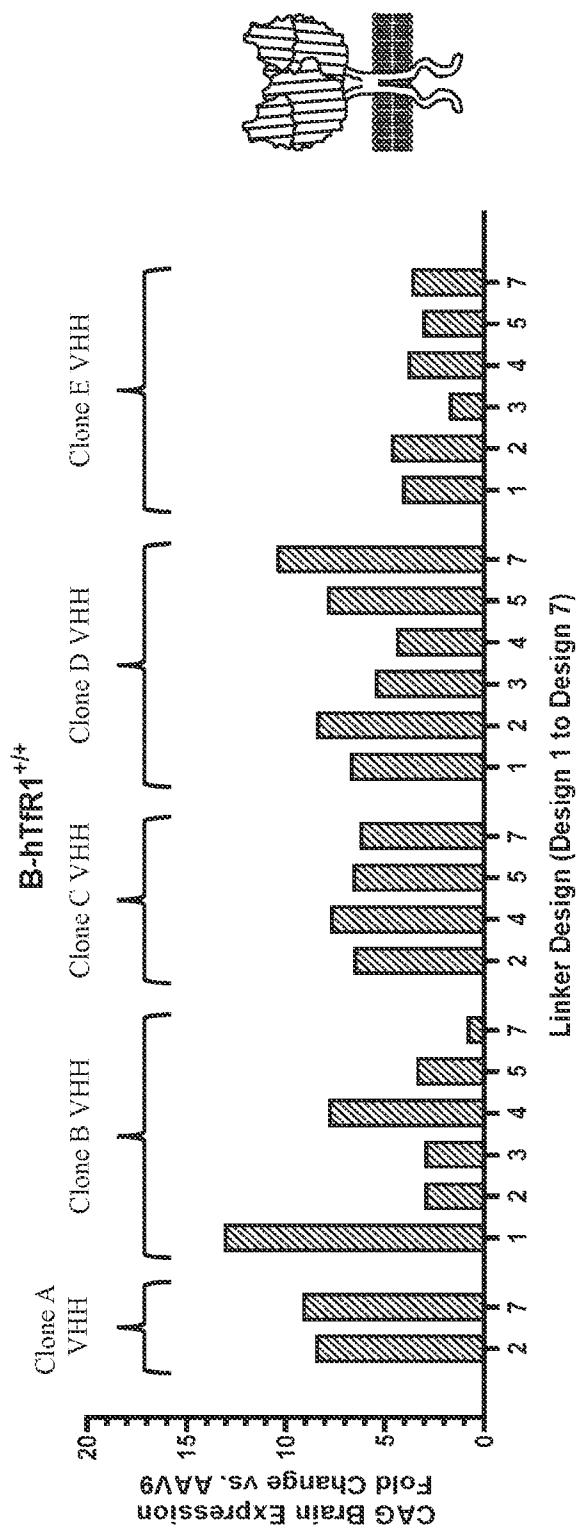
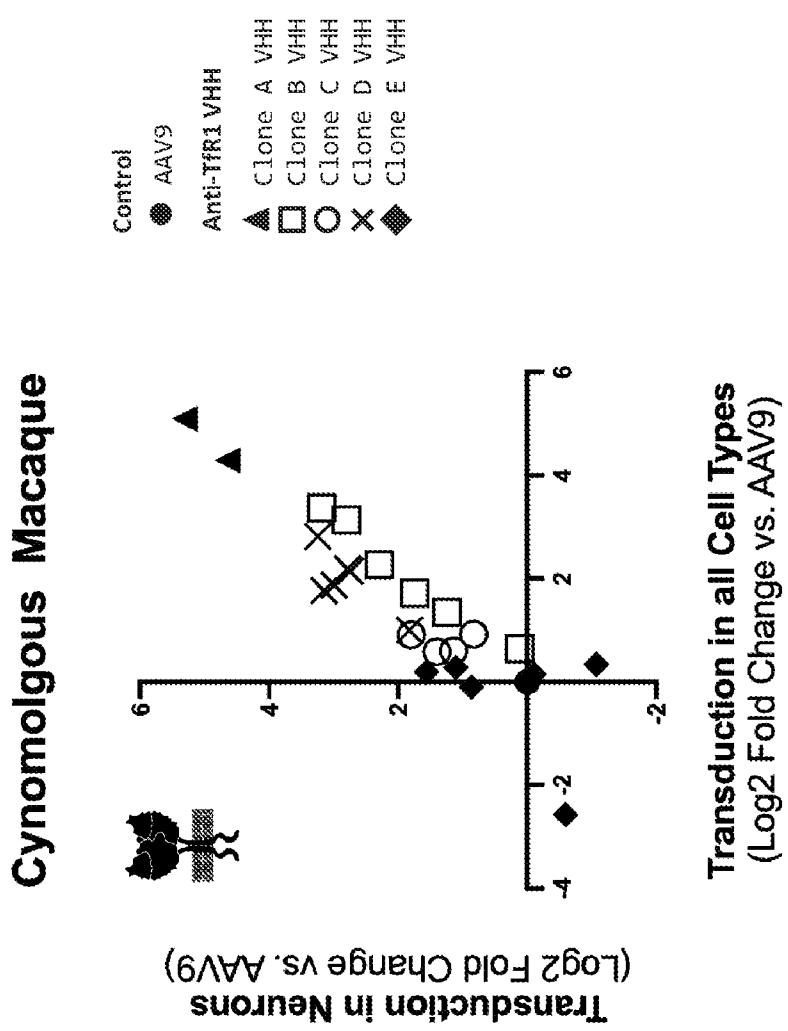


FIG. 6B

10/59



11/59

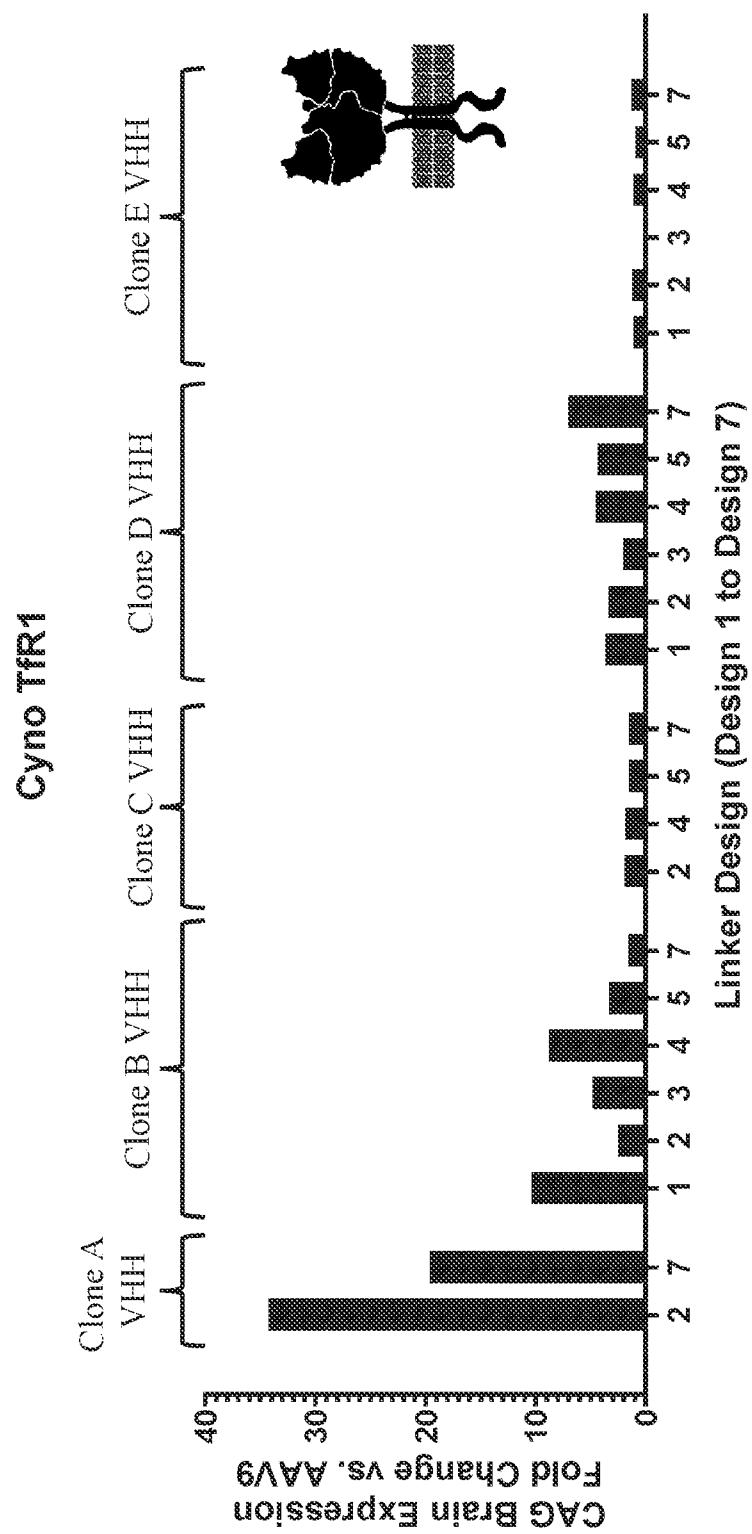


FIG. 7B

12/59

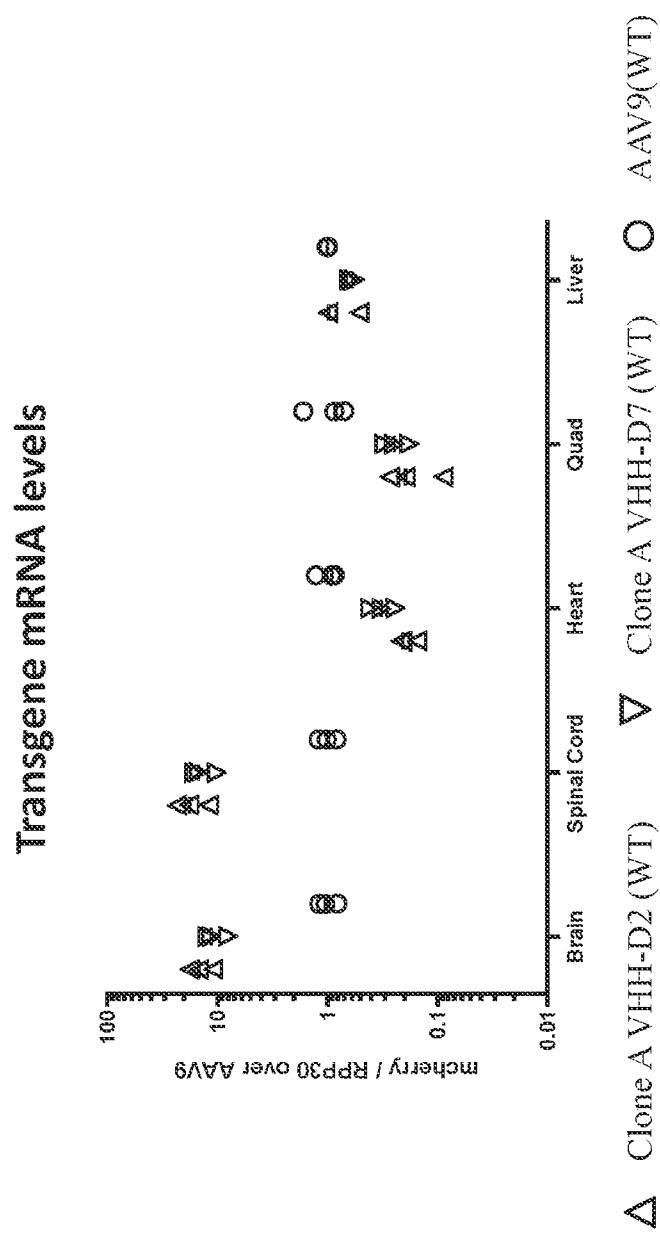


FIG. 8A

13/59

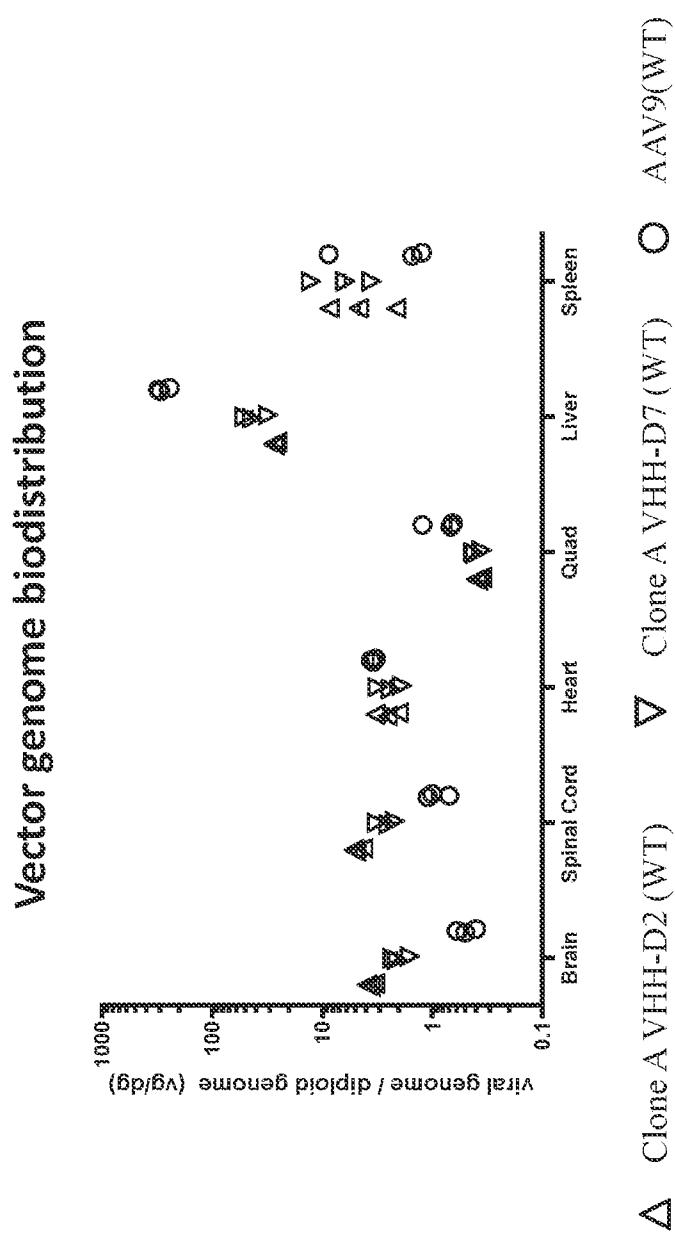


FIG. 8B

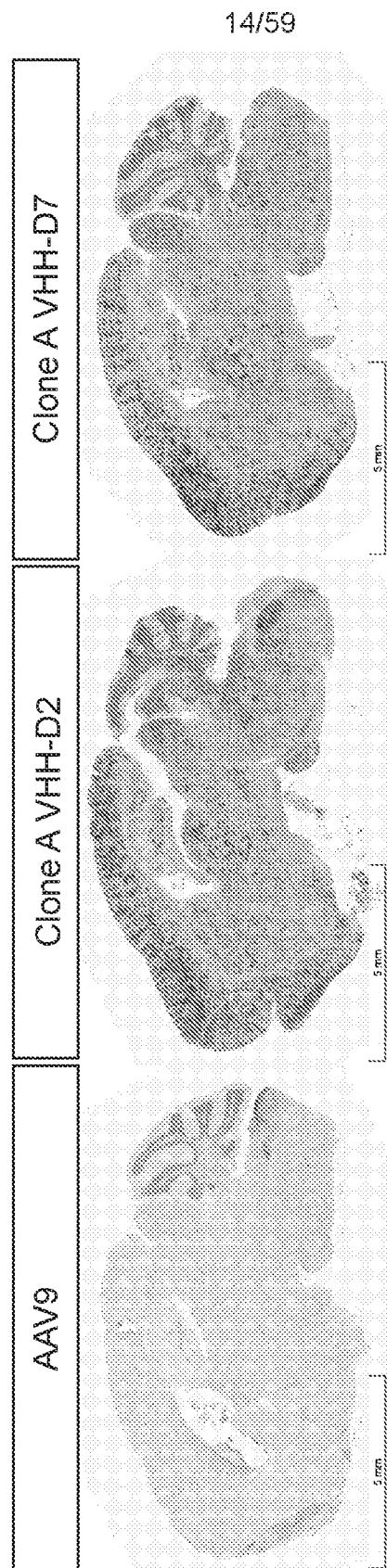


FIG. 9A

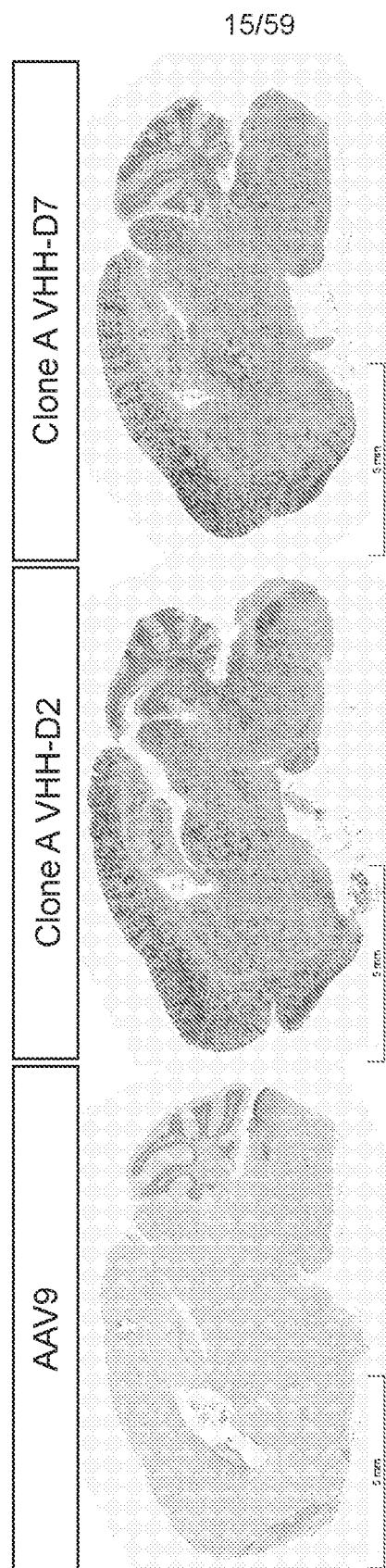


FIG. 9B

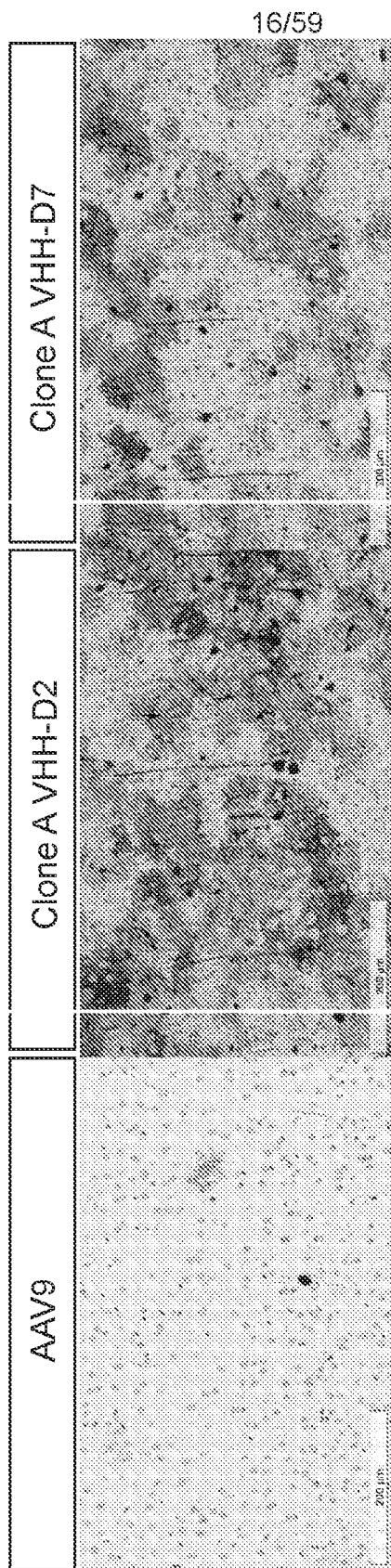


FIG. 10A

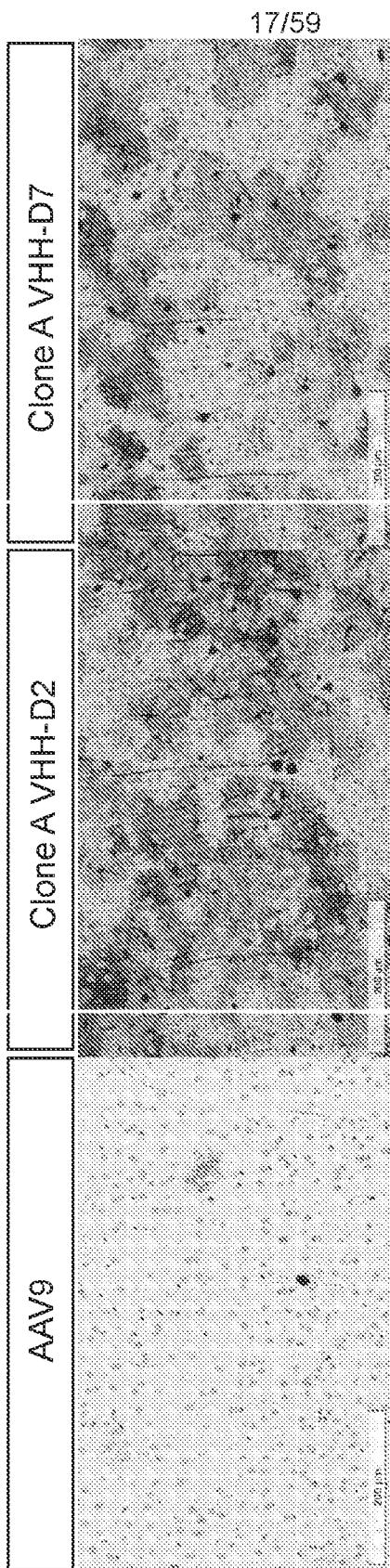


FIG. 10B

18/59

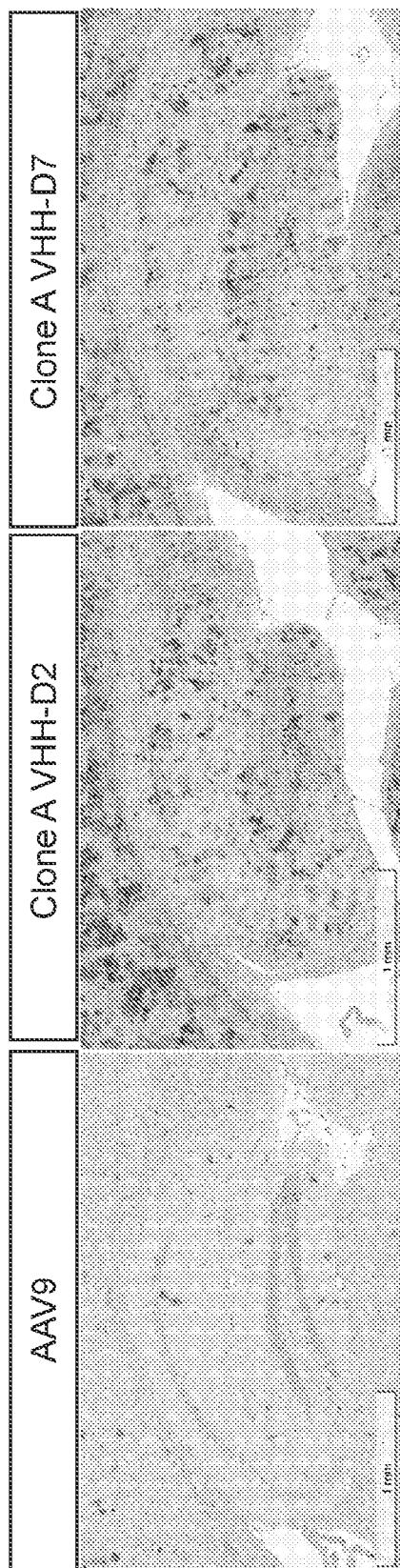


FIG. 11A

19/59

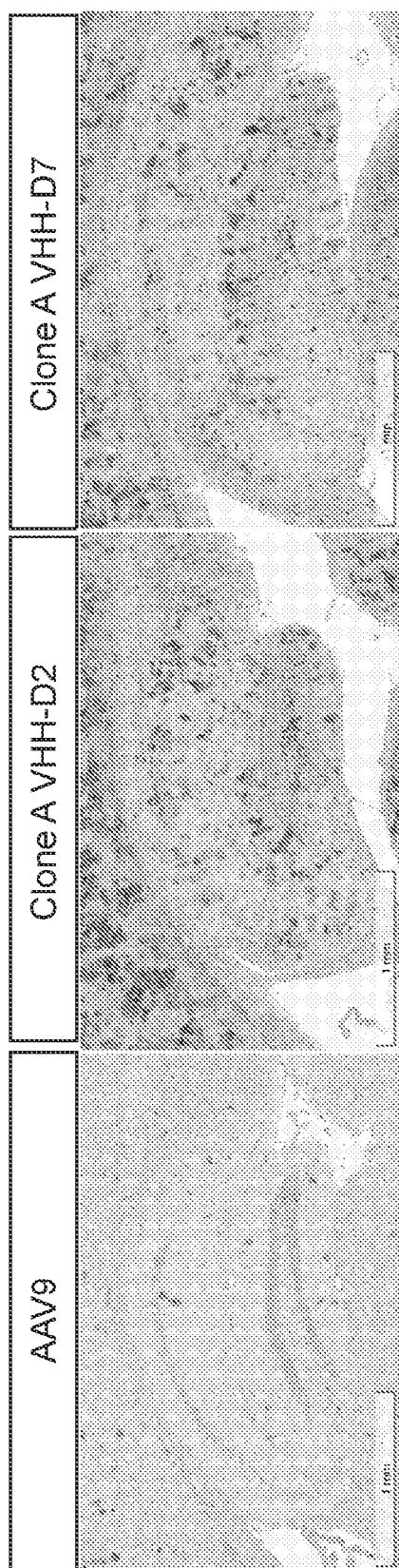


FIG. 11B

20/59



FIG. 12A

21/59

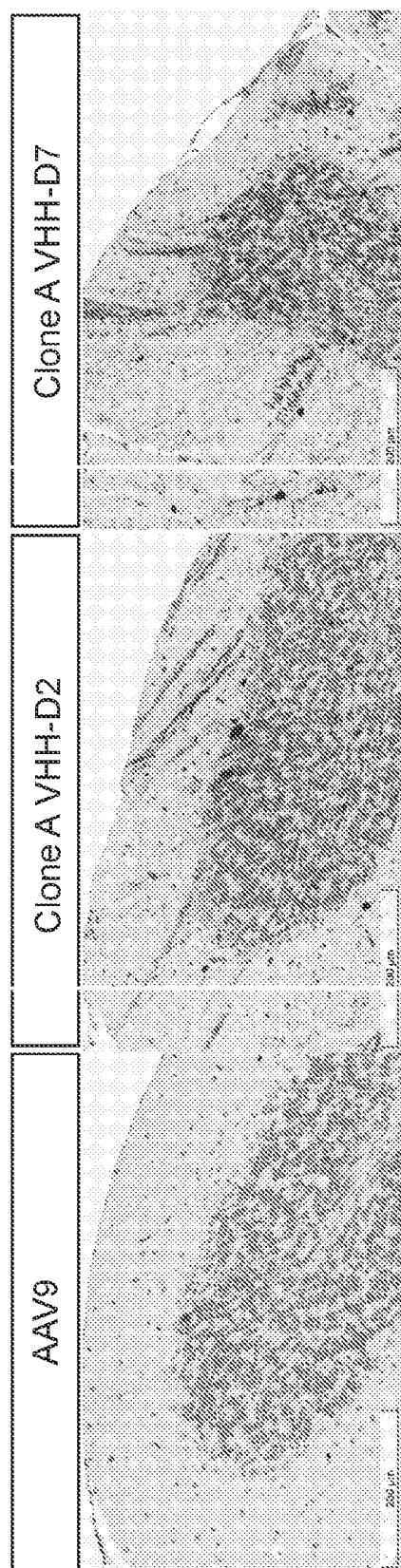


FIG. 12B

22/59

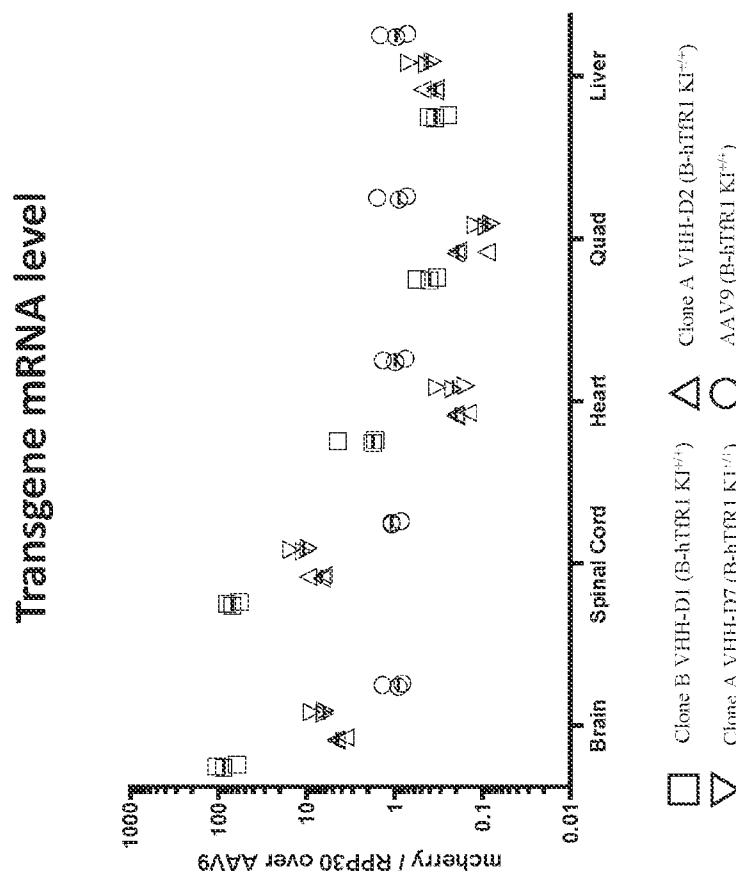


FIG. 13A

23/59

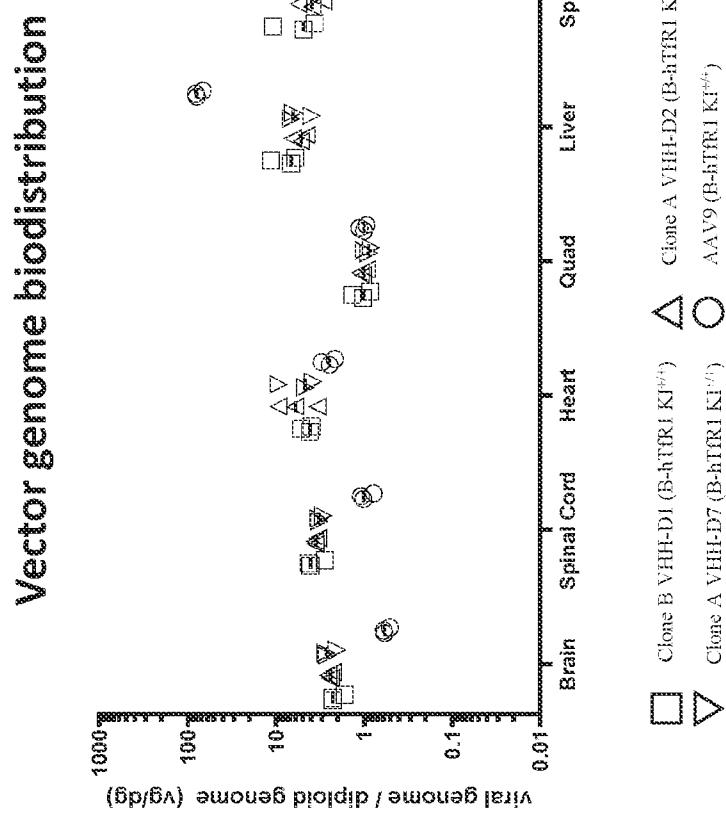


FIG. 13B

24/59

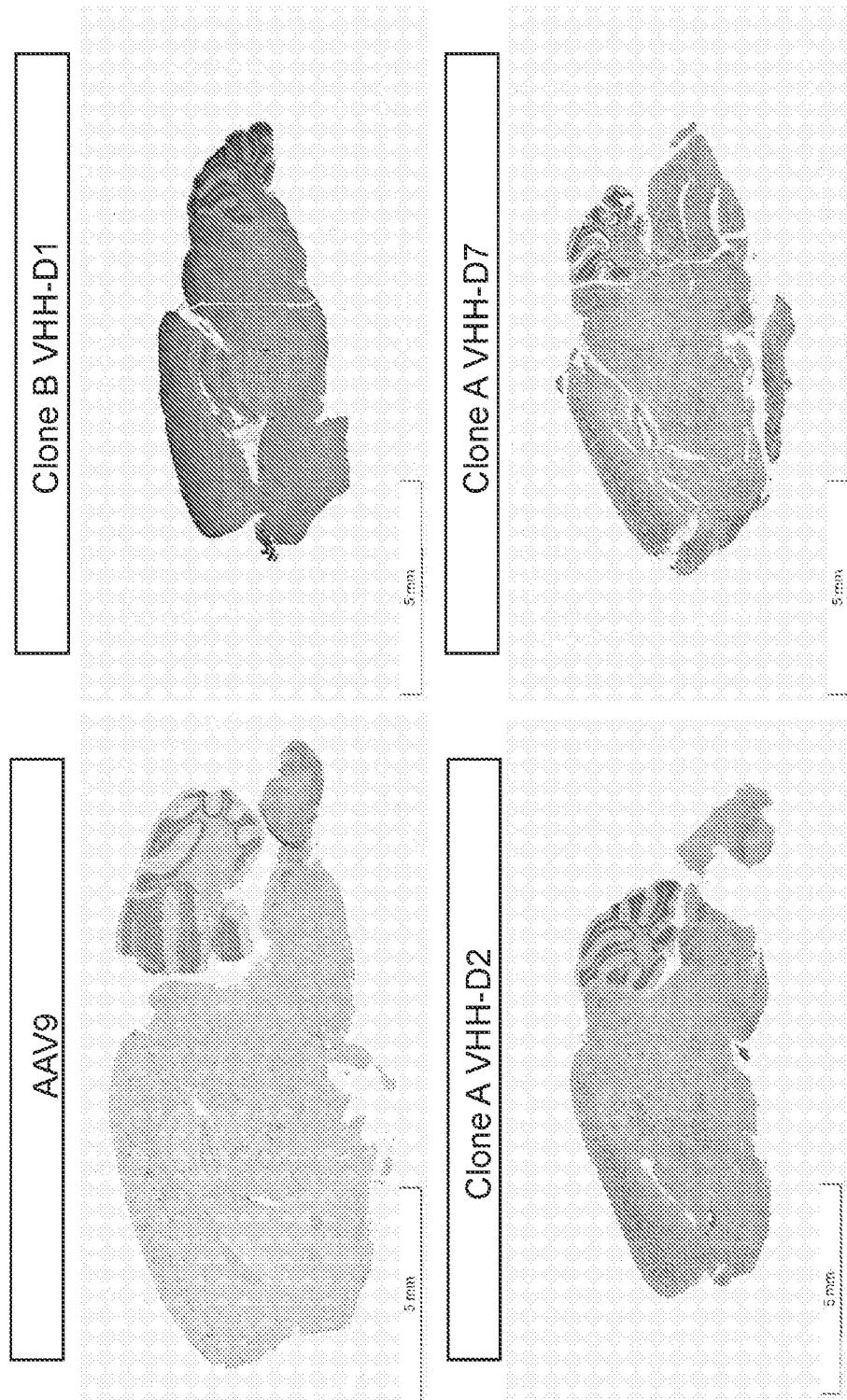


FIG. 14A

25/59

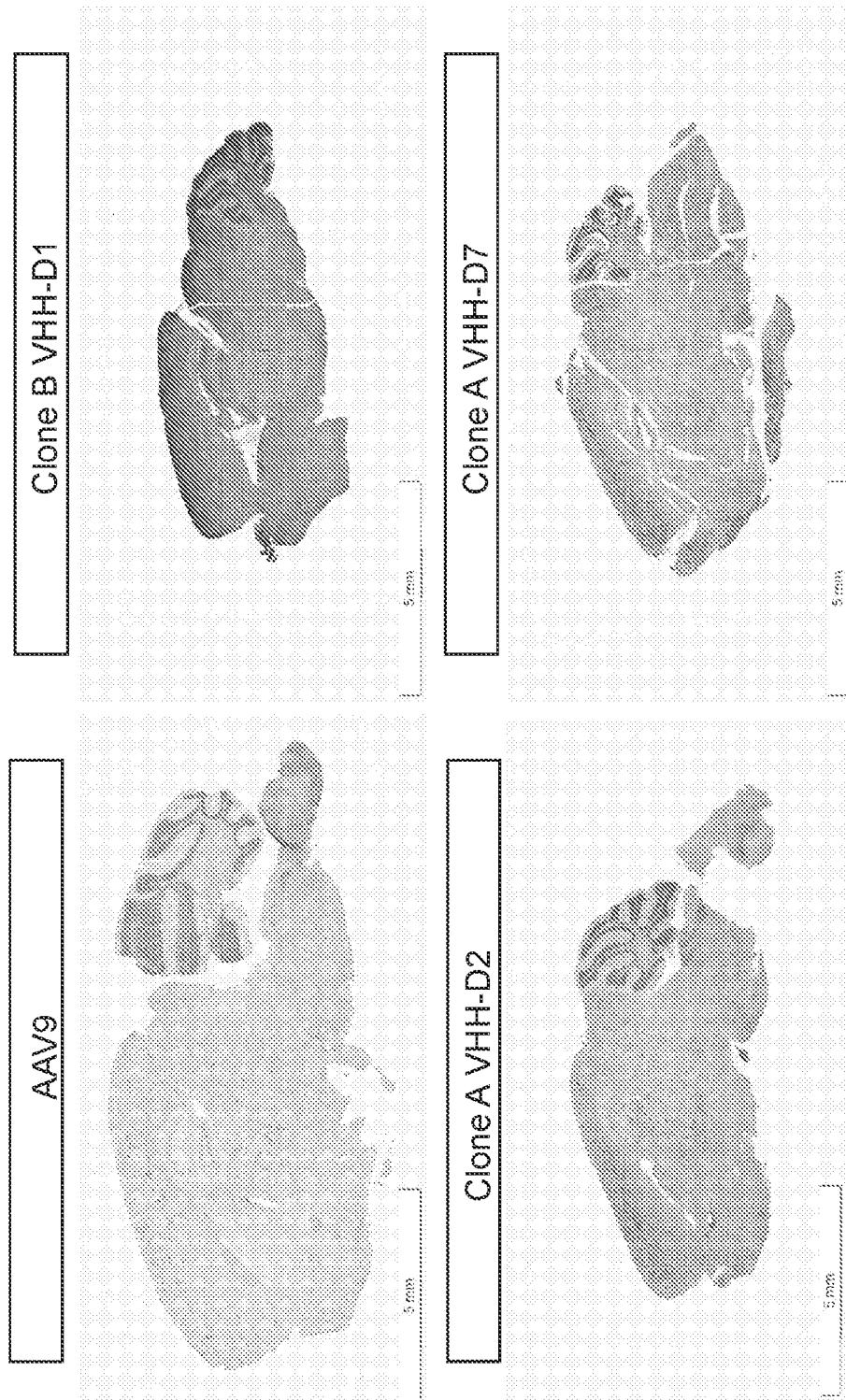


FIG. 14B

26/59

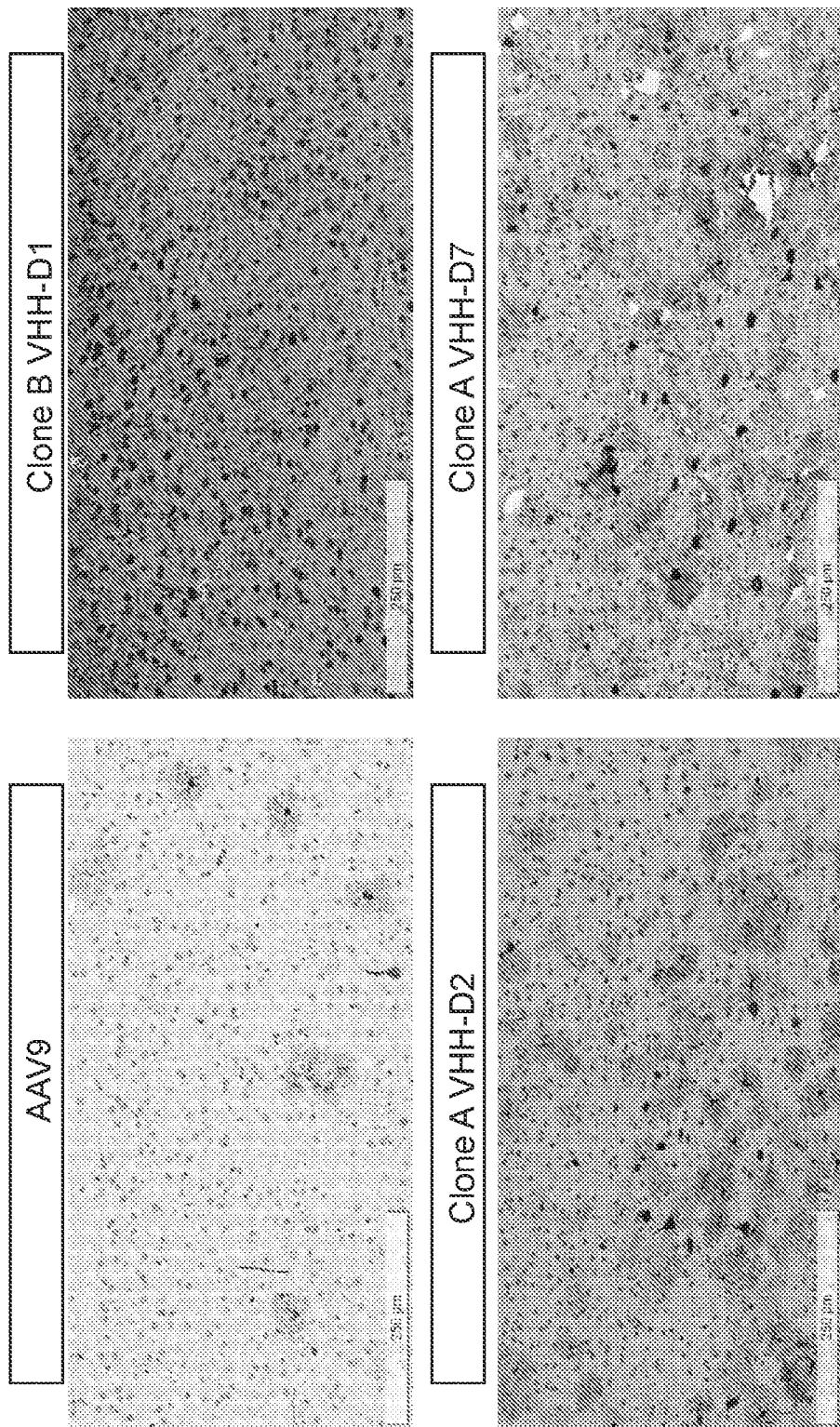


FIG. 15A

27/59

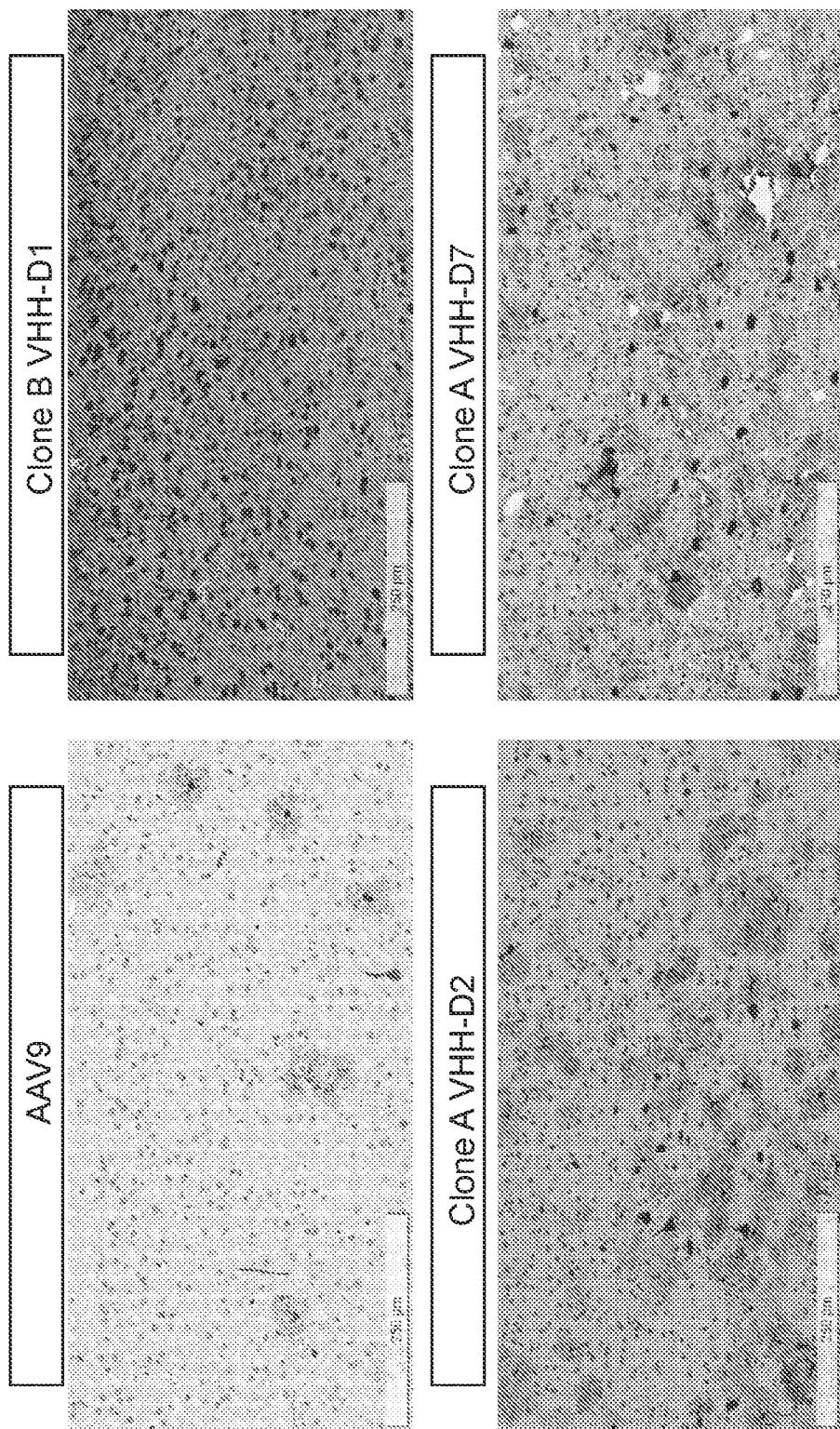


FIG. 15B

28/59

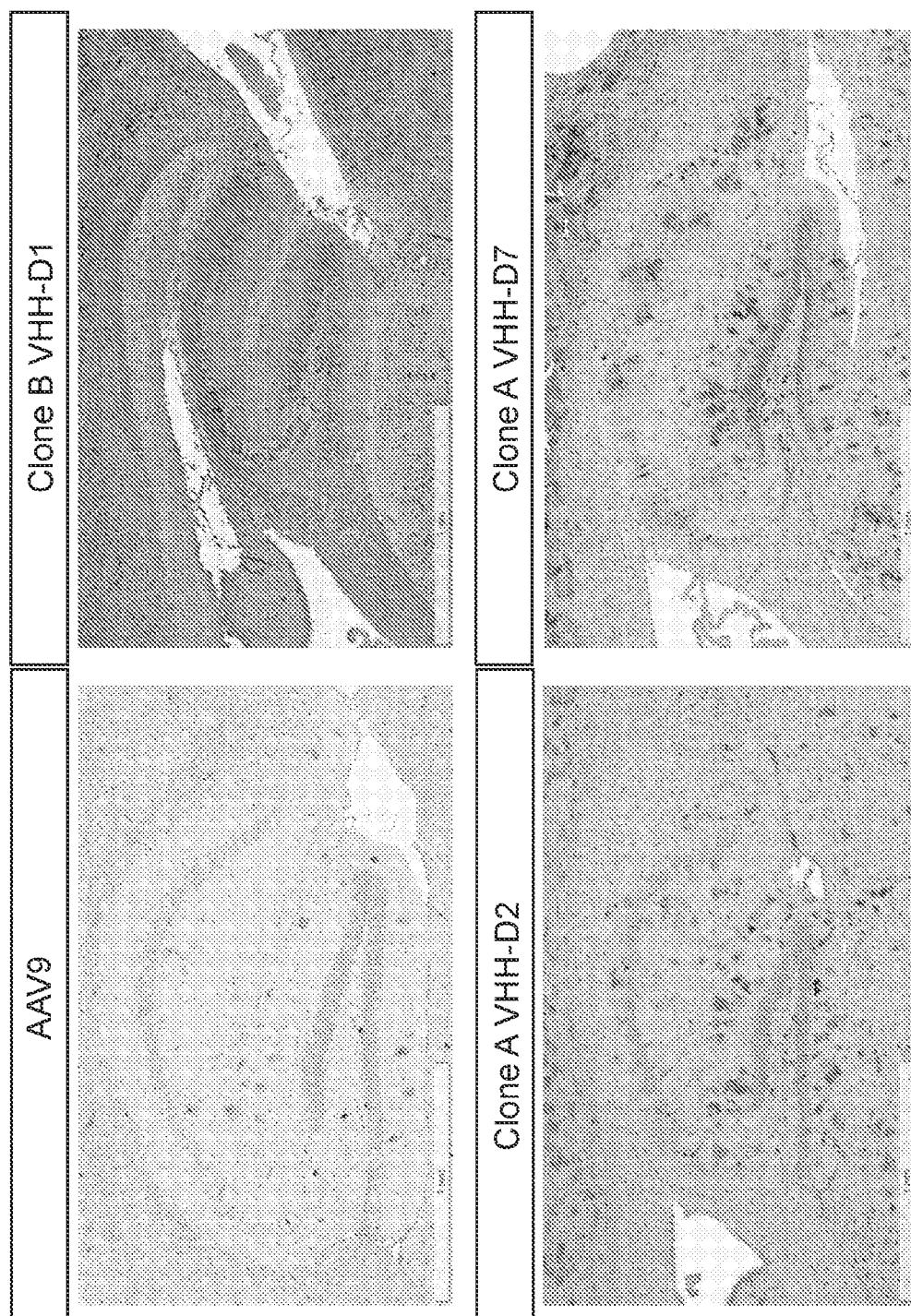


FIG. 16A

29/59

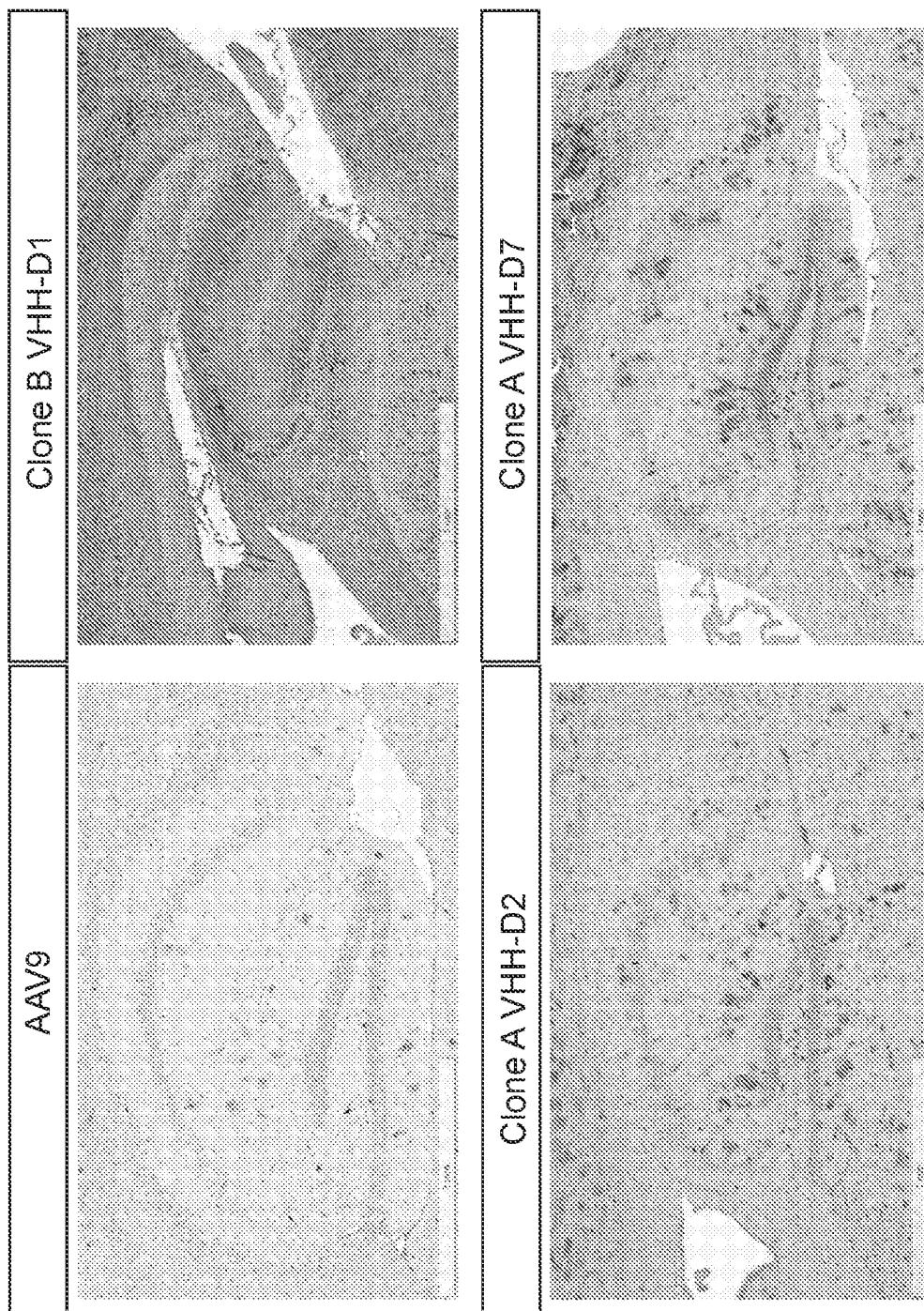


FIG. 16B

30/59

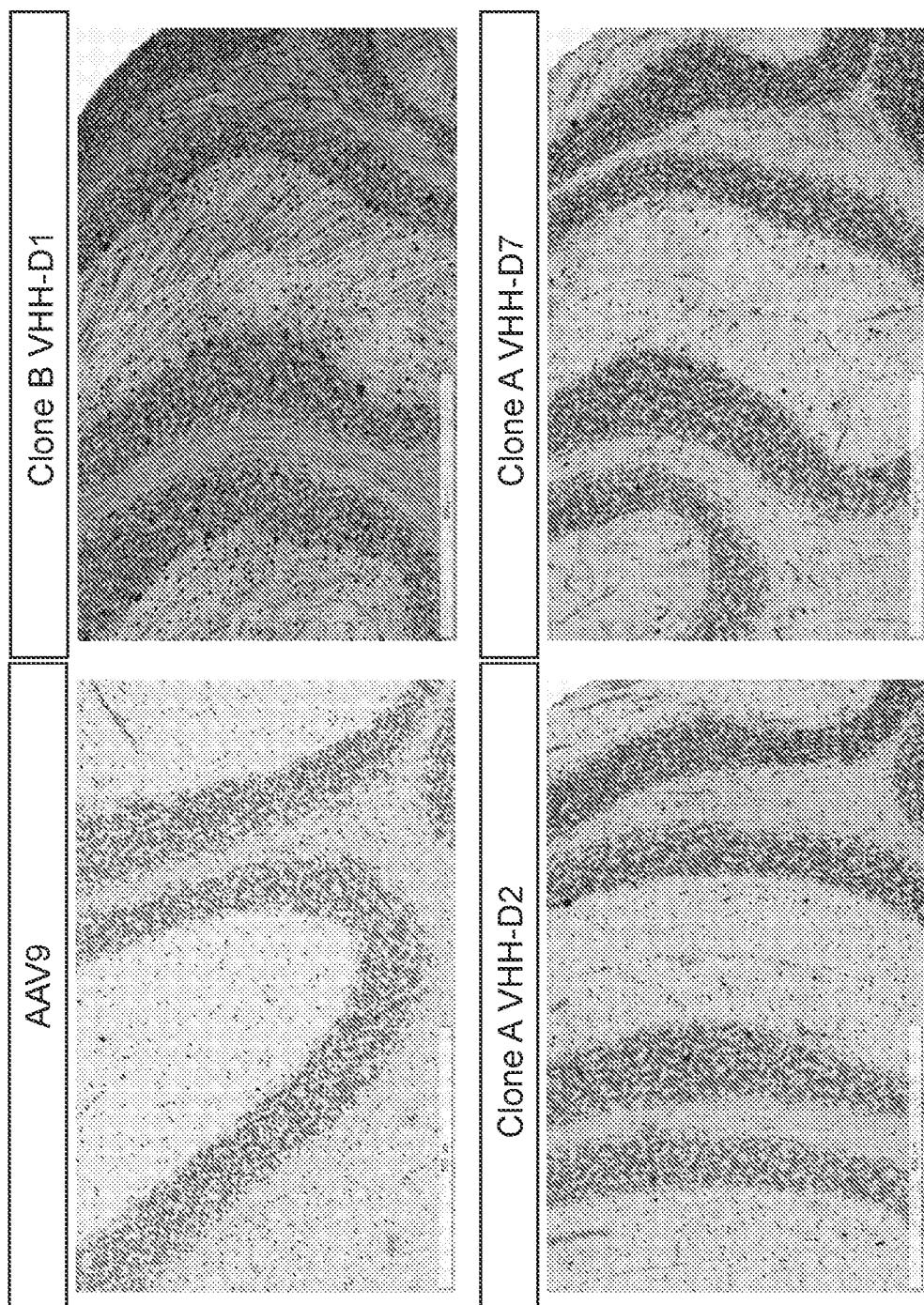


FIG. 17A

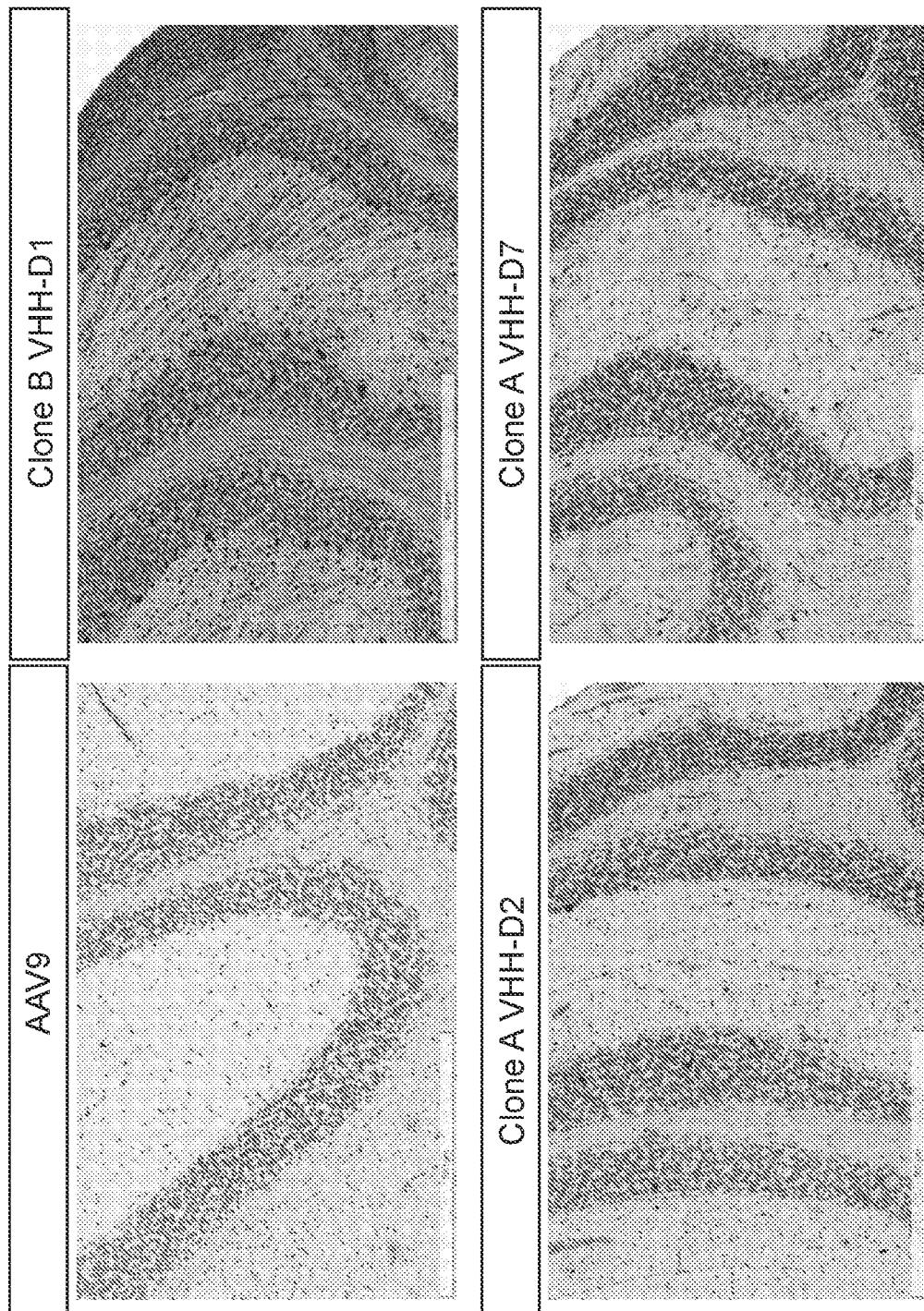


FIG. 17B

32/59

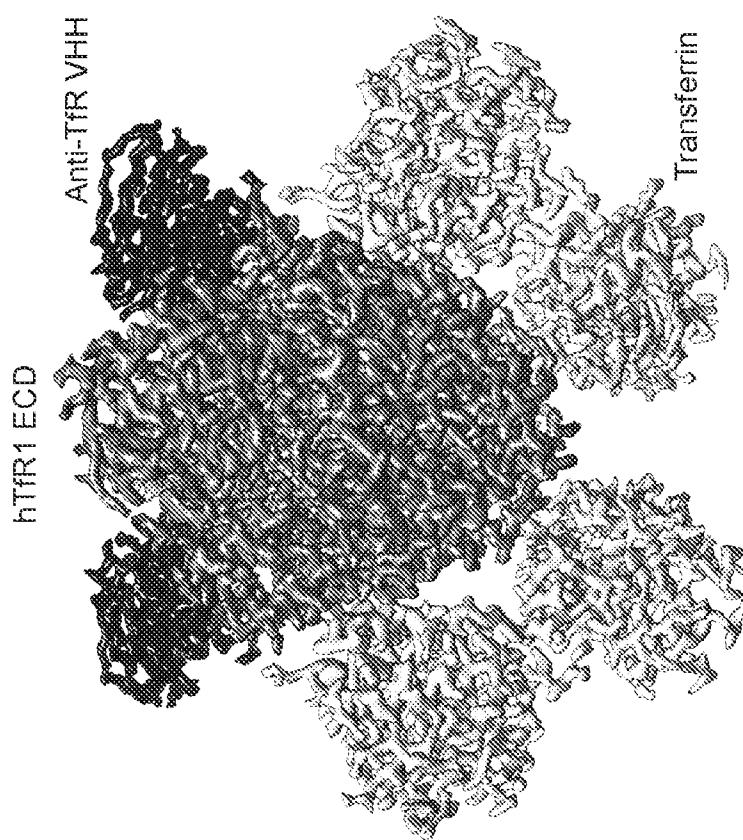


FIG. 18A

33/59

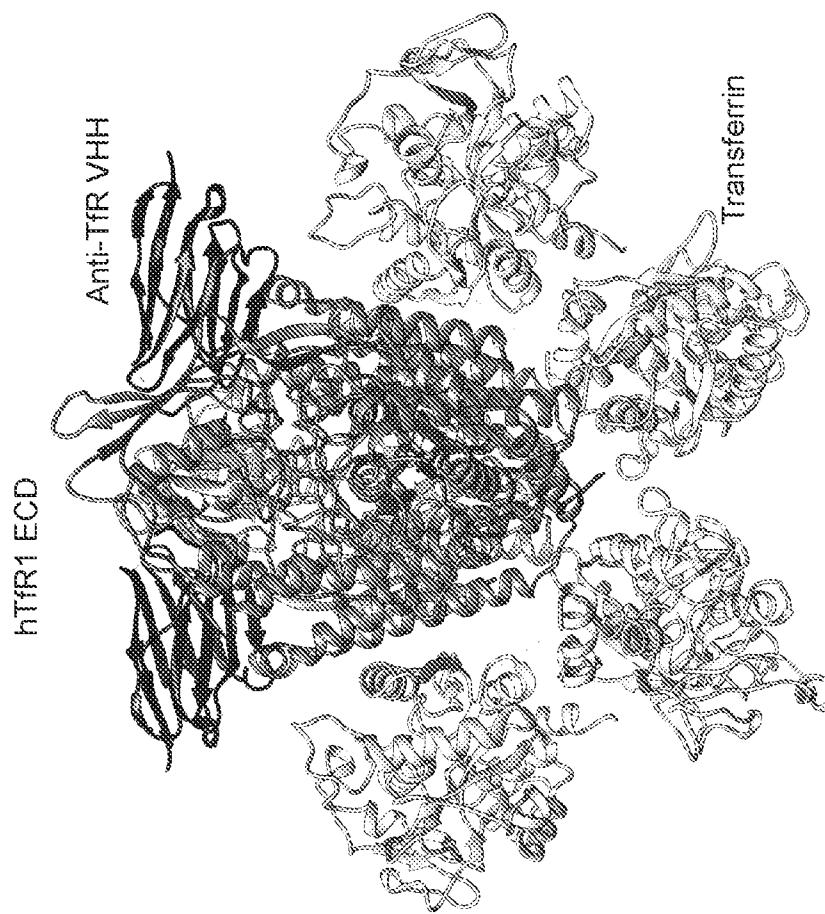


FIG. 18B

34/59

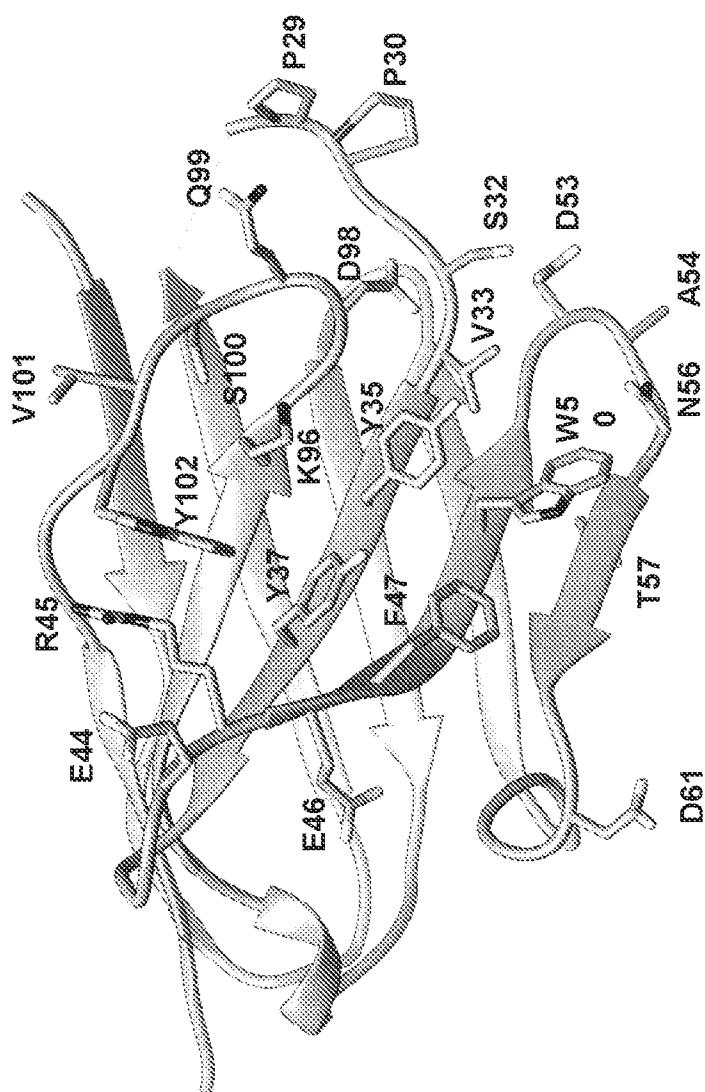


FIG. 19

35/59

LOG 20

36/59

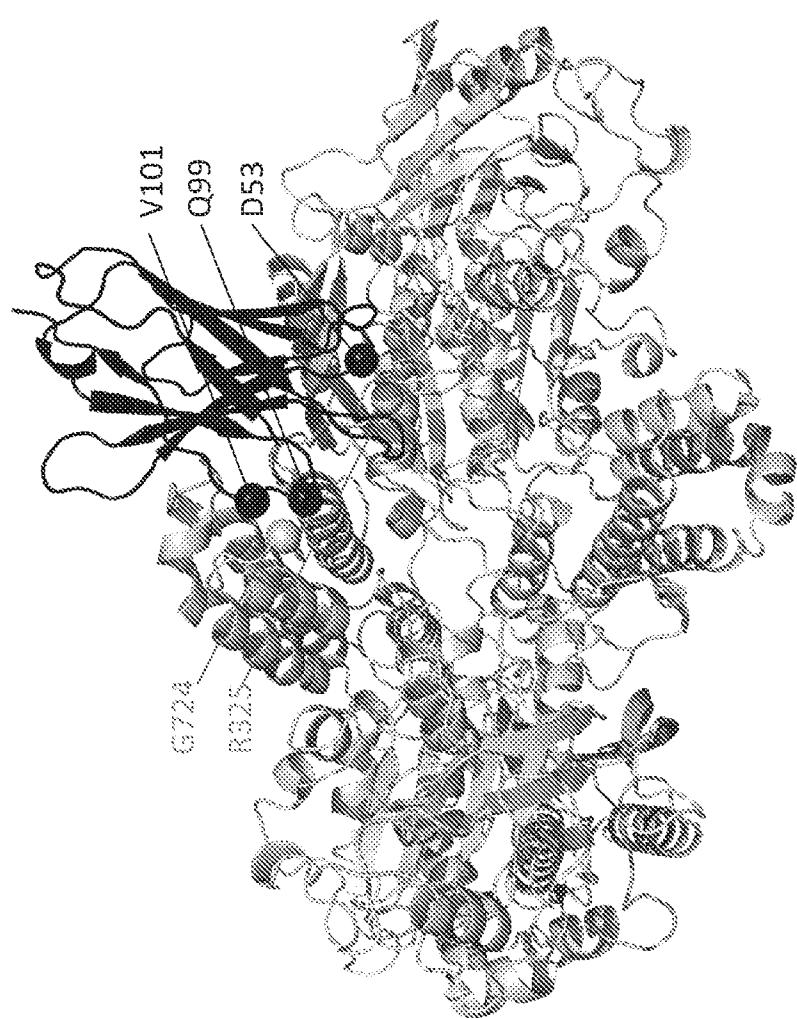


FIG. 21

37/59

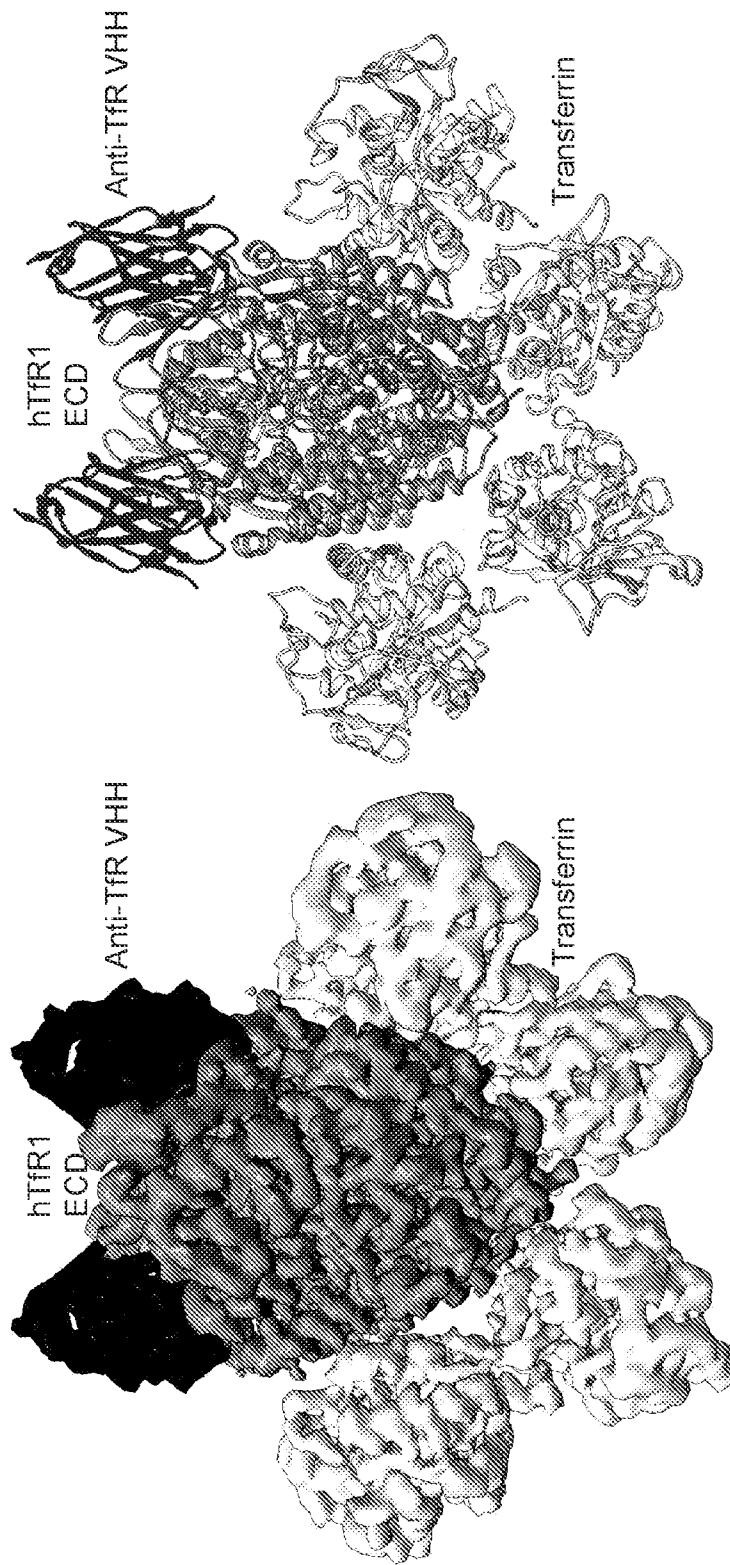


FIG. 22B

FIG. 22A

38/59

三  
二  
一  
七

39/59

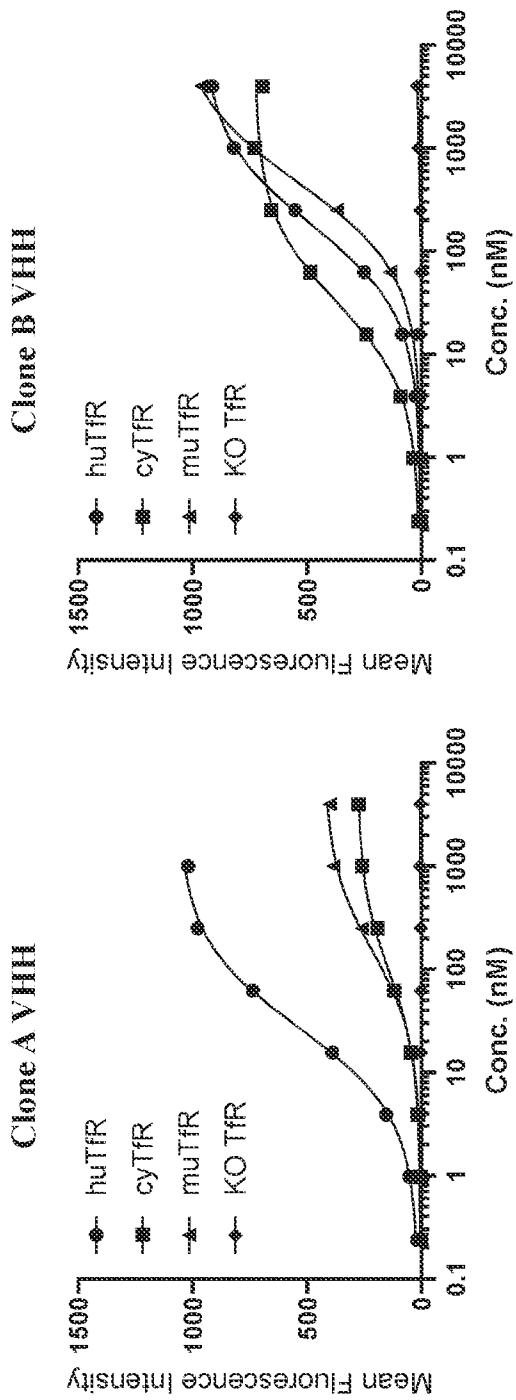


FIG. 24A

FIG. 24B

40/59

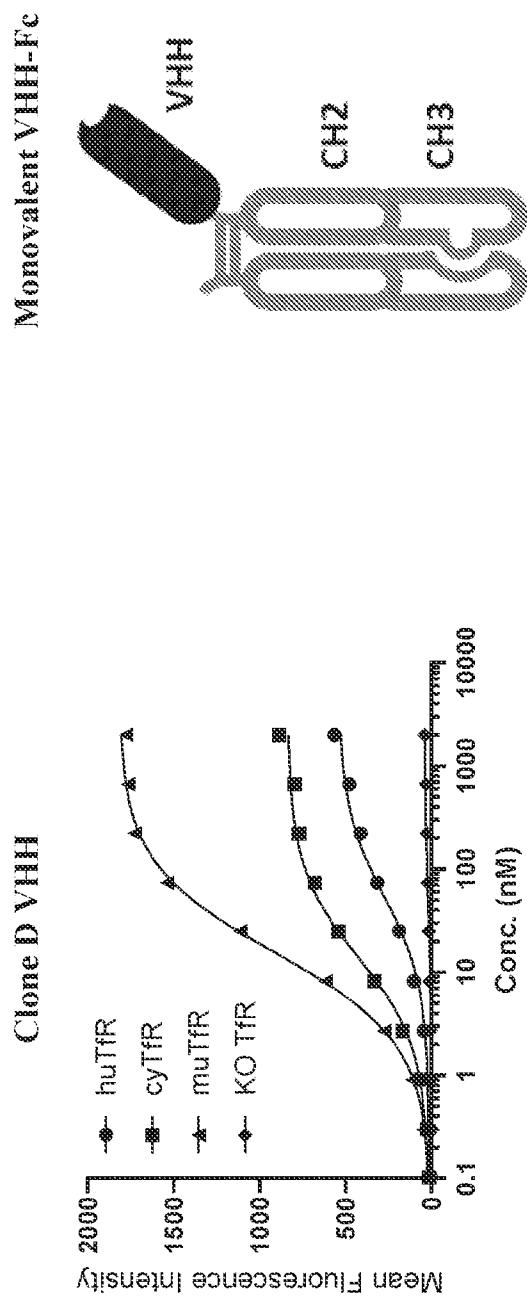


FIG. 24D

FIG. 24C

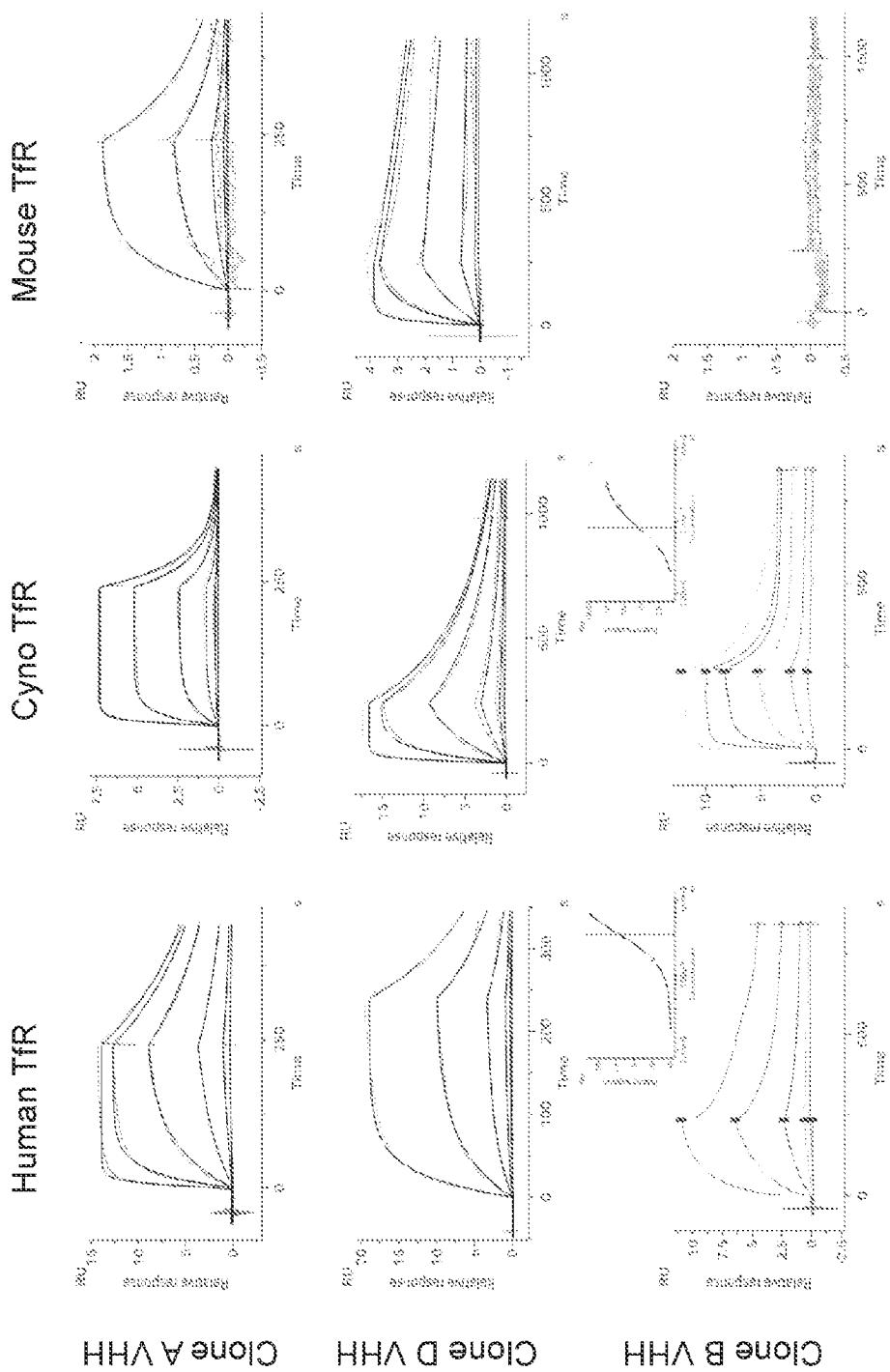


FIG. 25

42/59

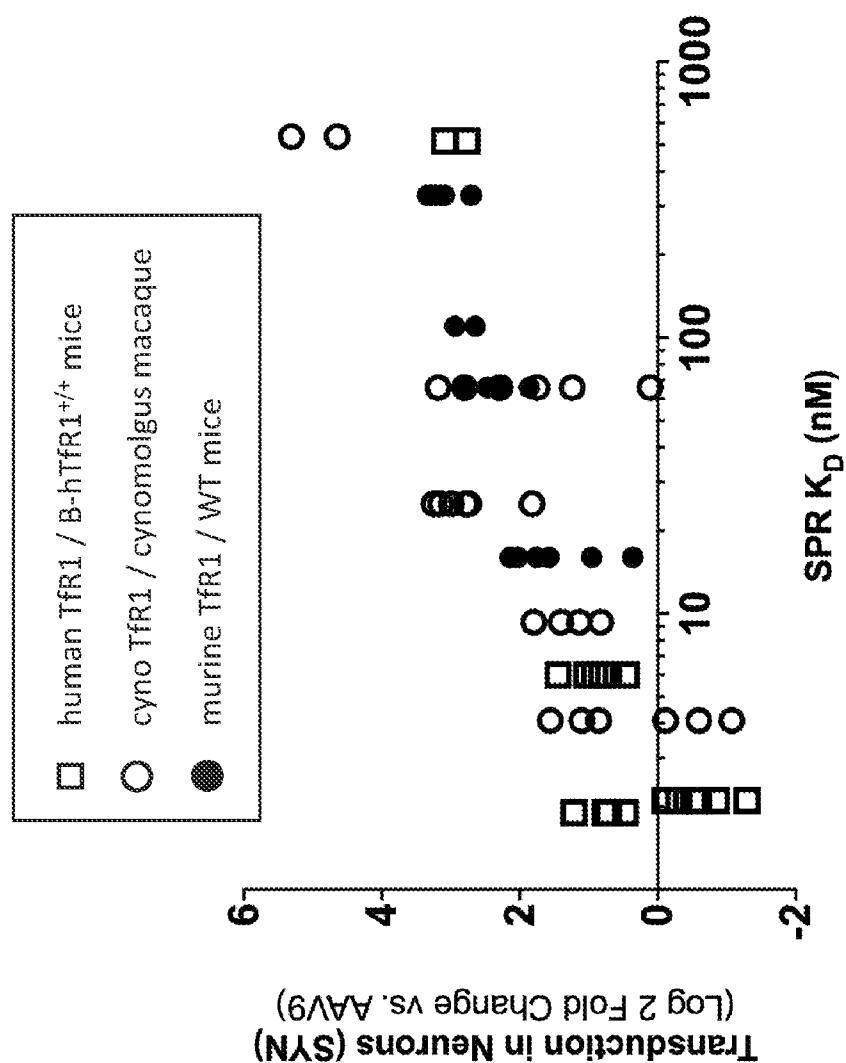


FIG. 26

43/59

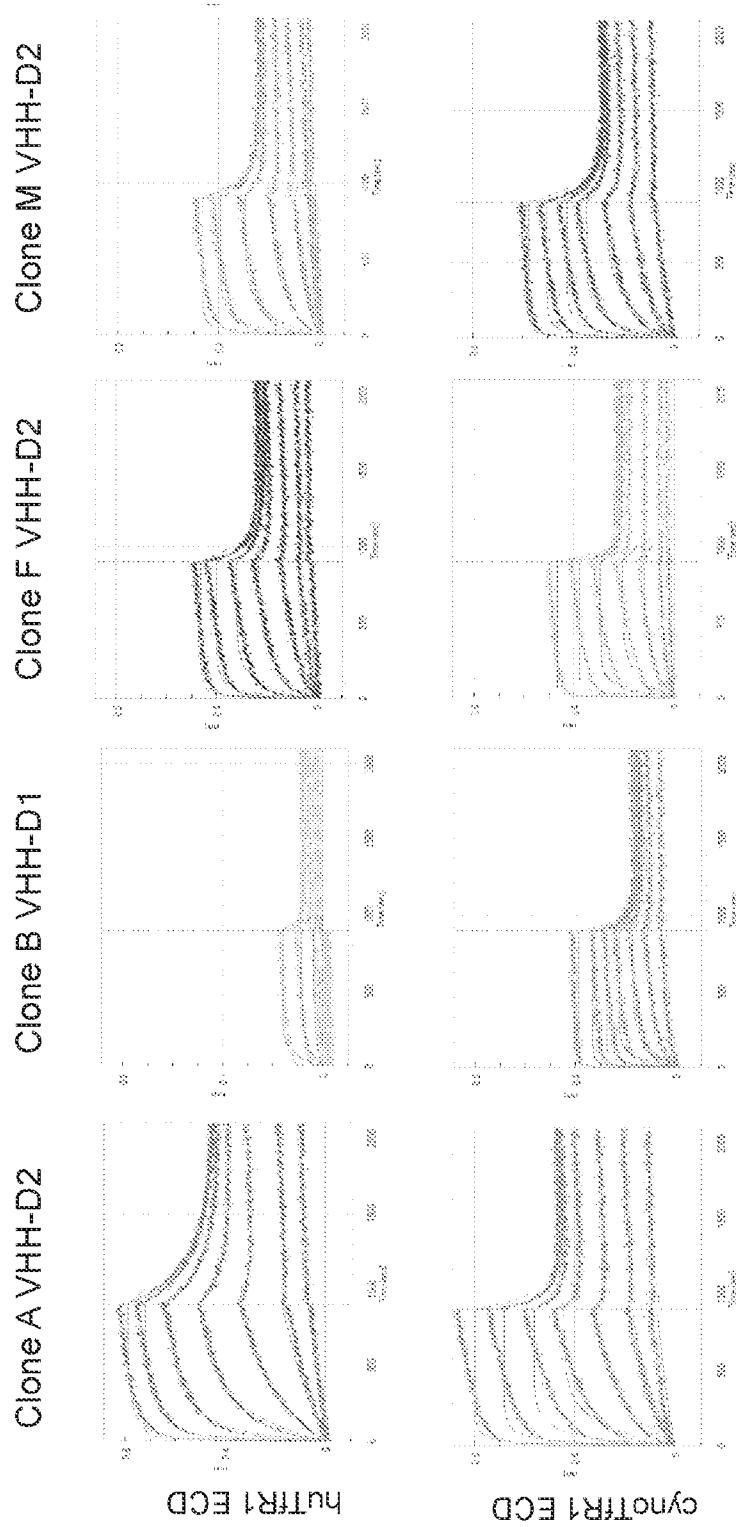


FIG. 27

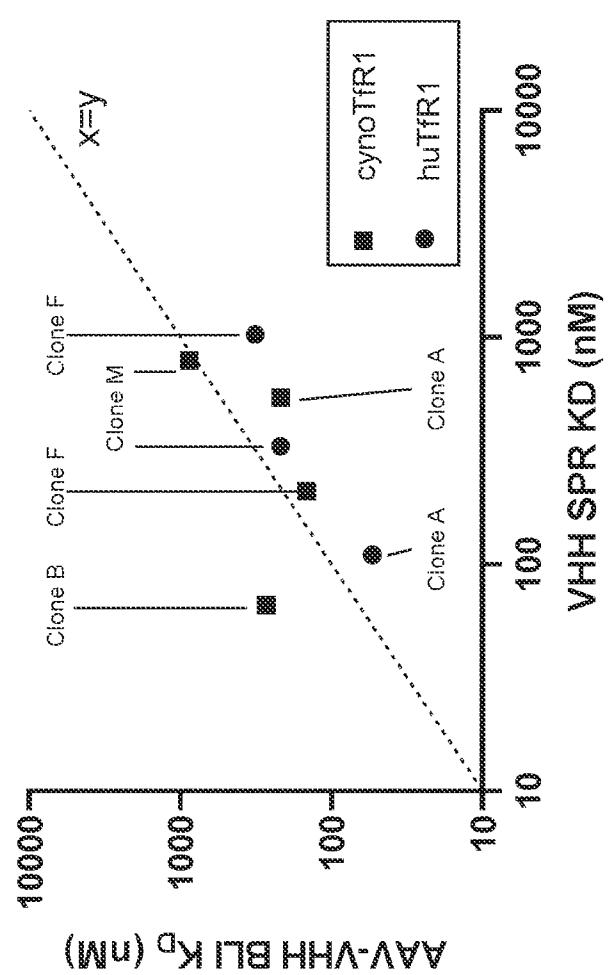
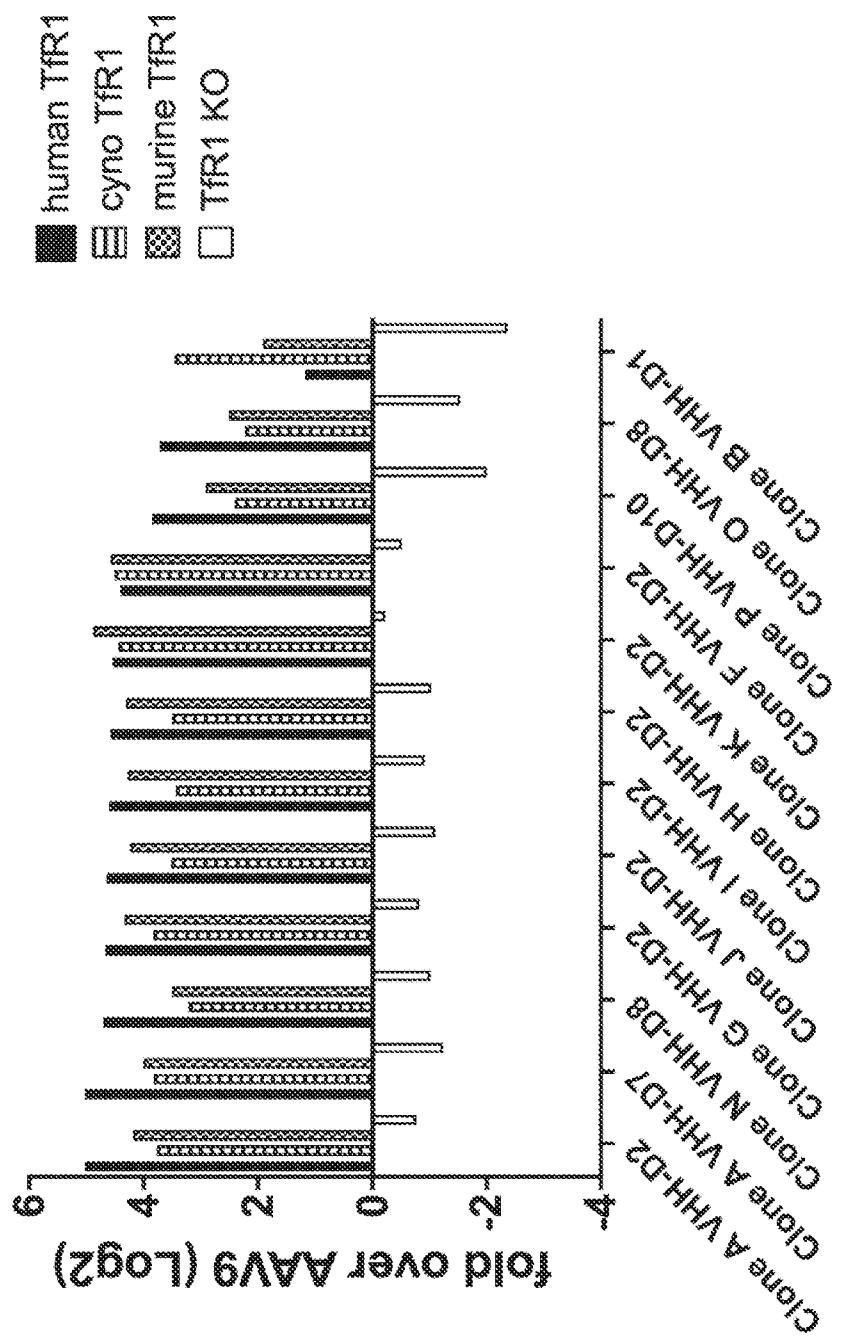


FIG. 28

45/59



46/59

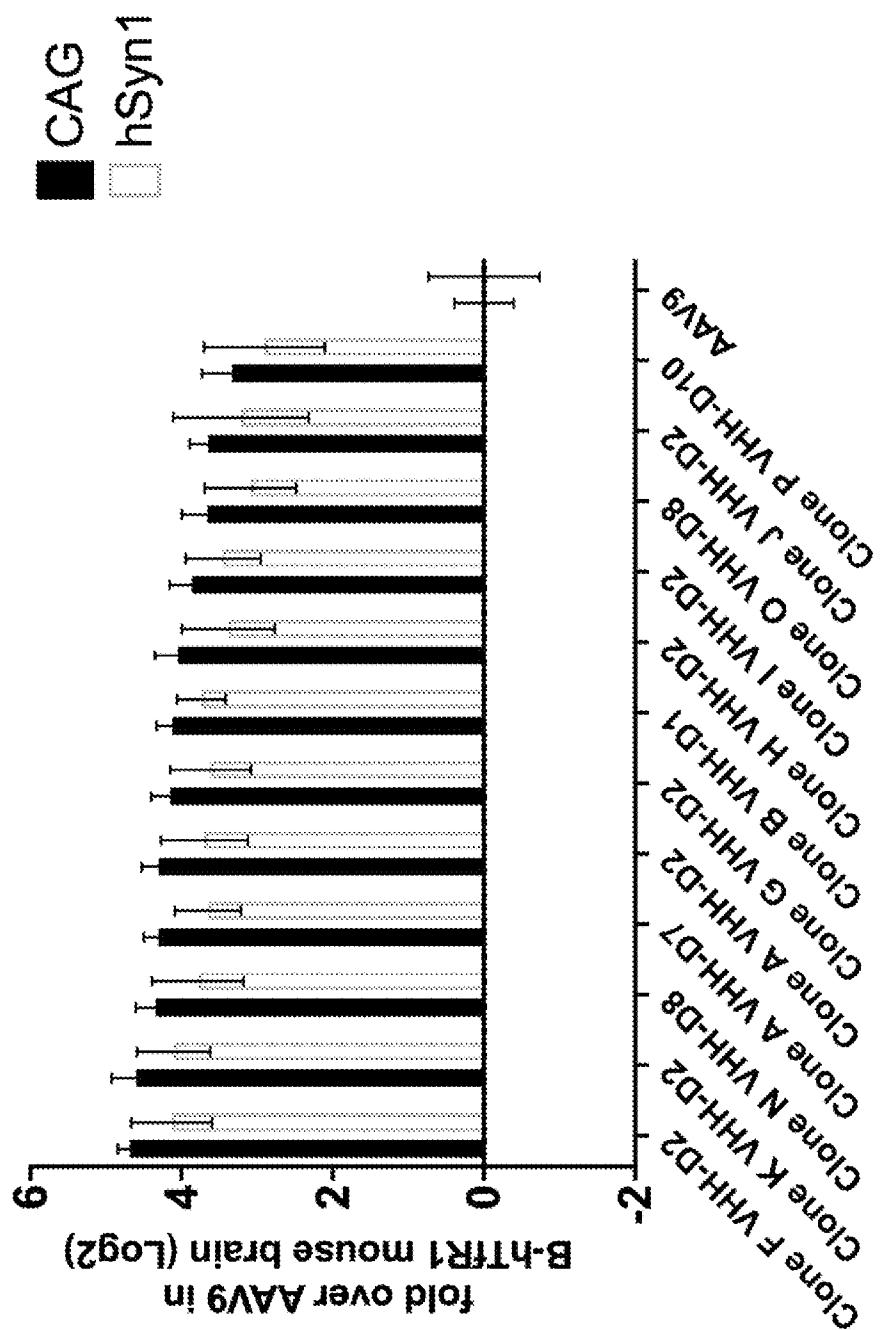


FIG. 30

47/59

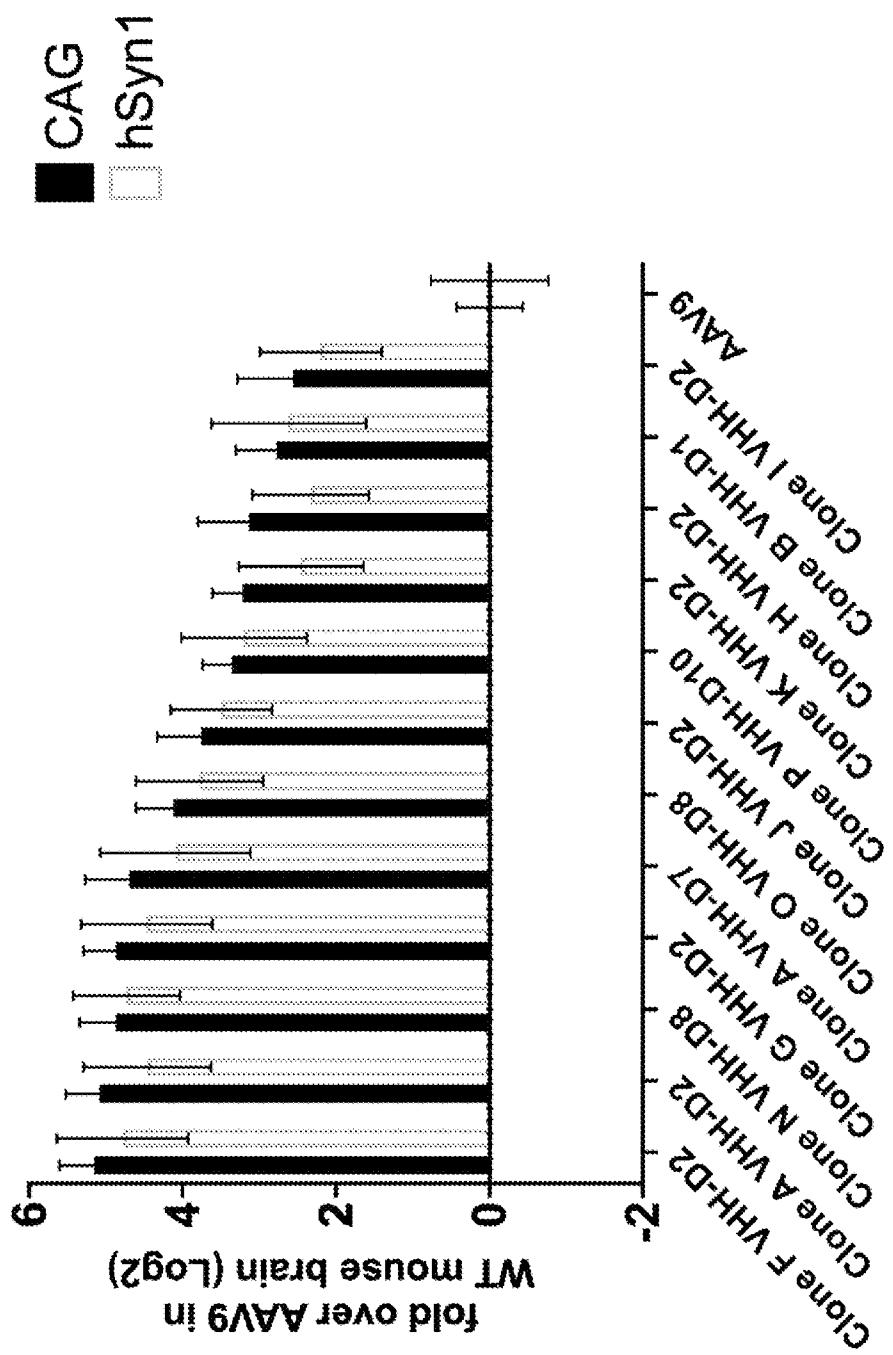


FIG. 31

48/59

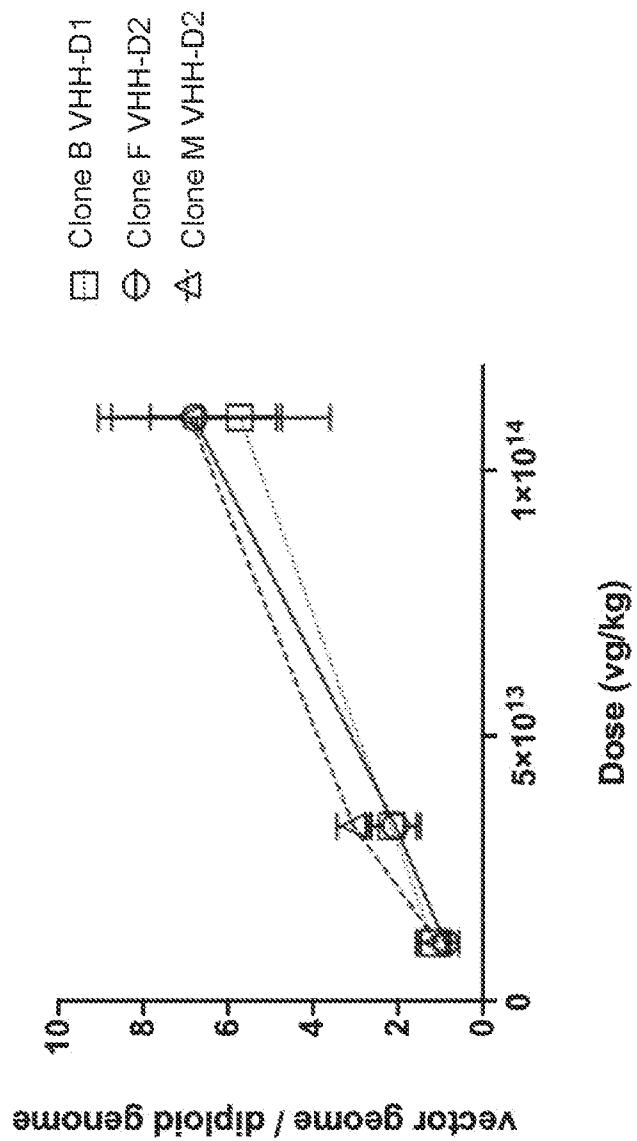


FIG. 32

49/59

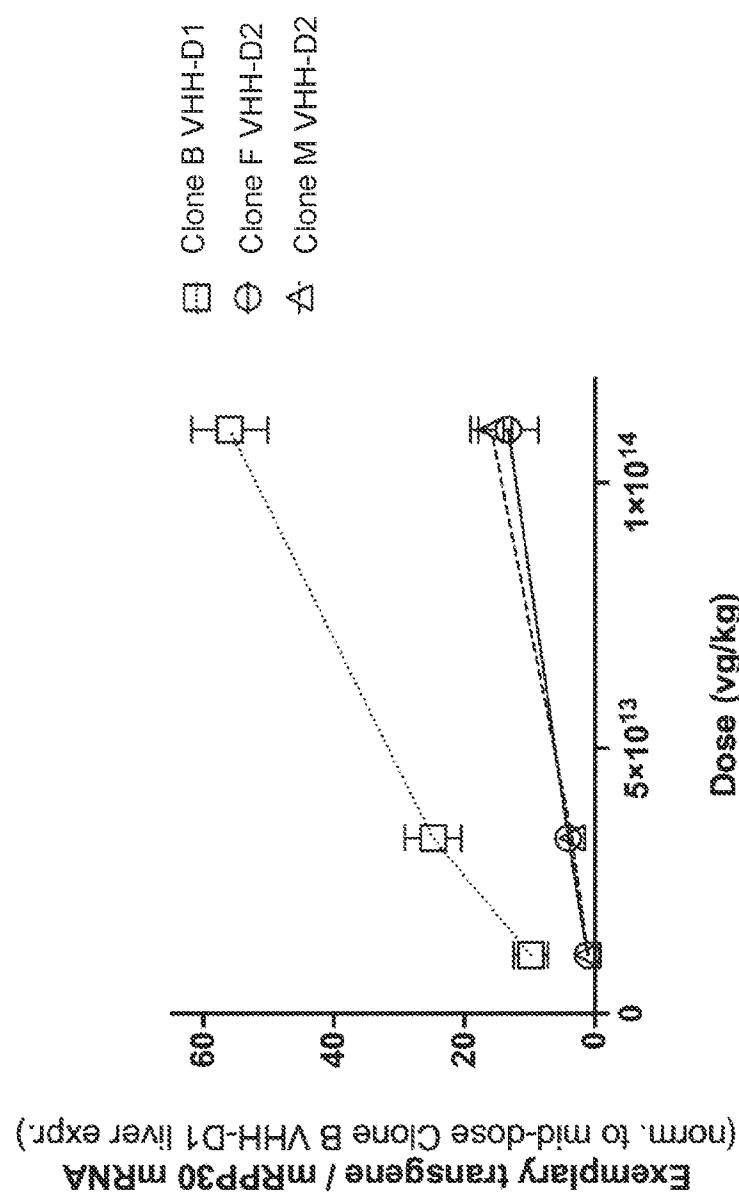


FIG. 33

50/59

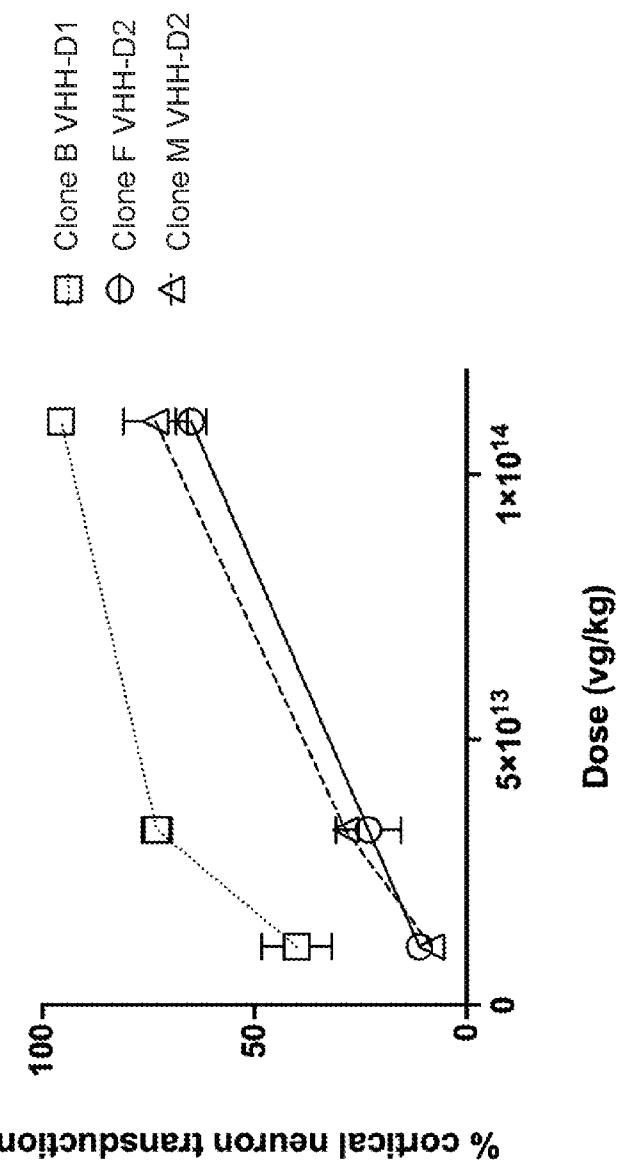


FIG. 34

51/59

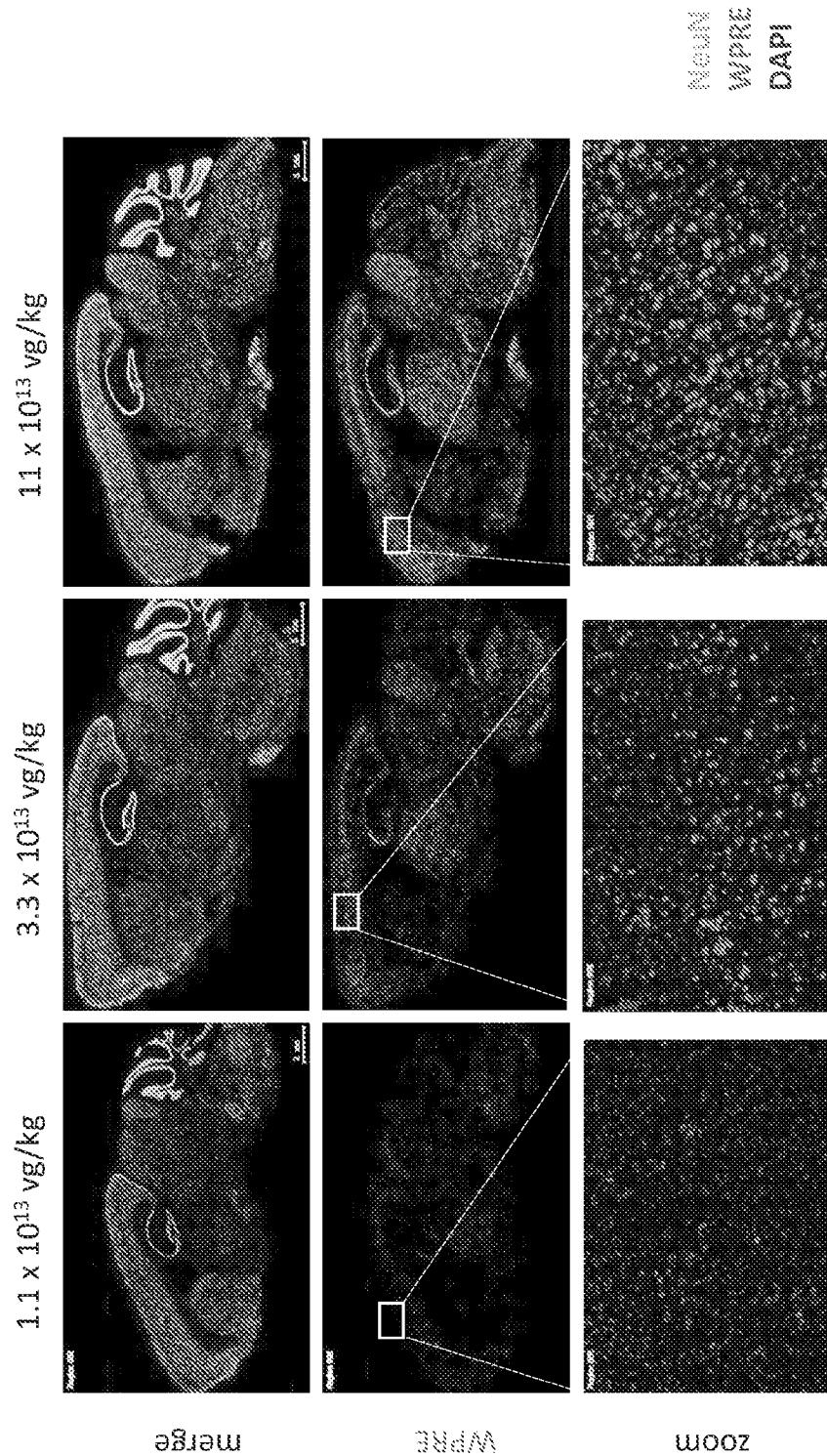
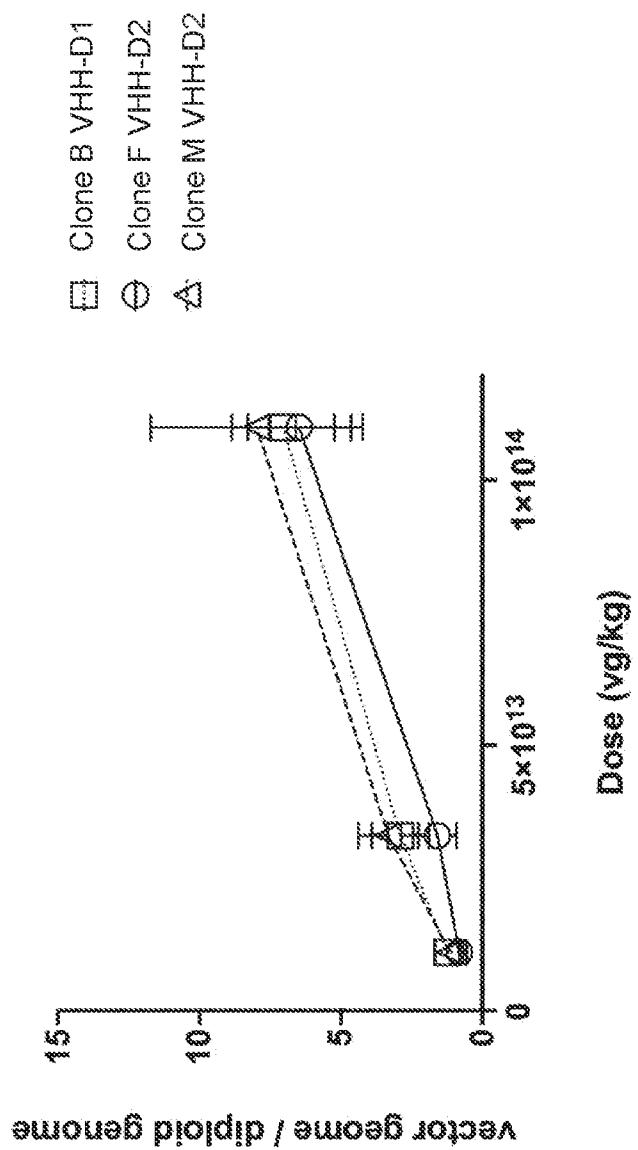


FIG. 35

52/59



53/59

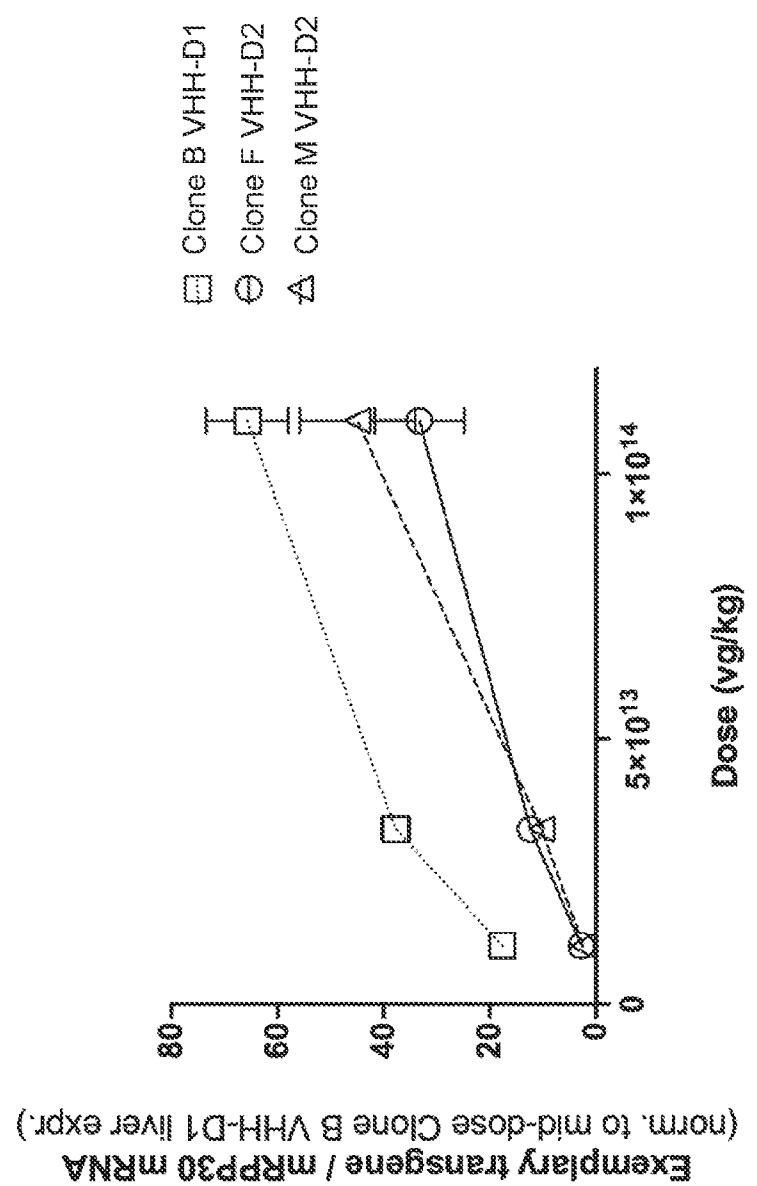


FIG. 37

54/59

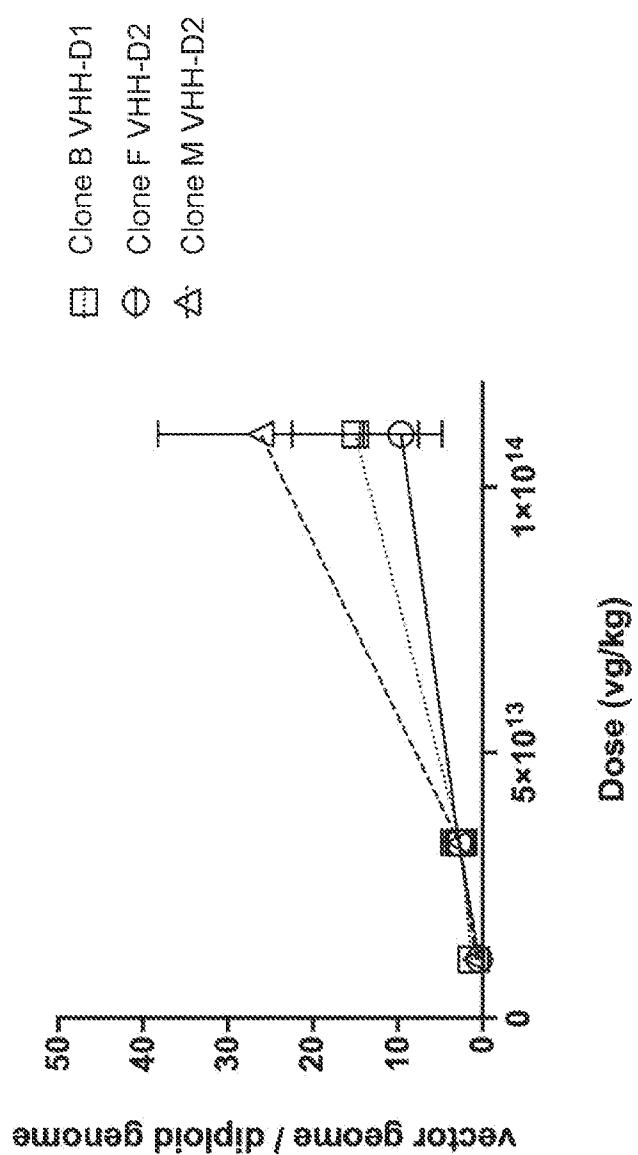


FIG. 38

55/59

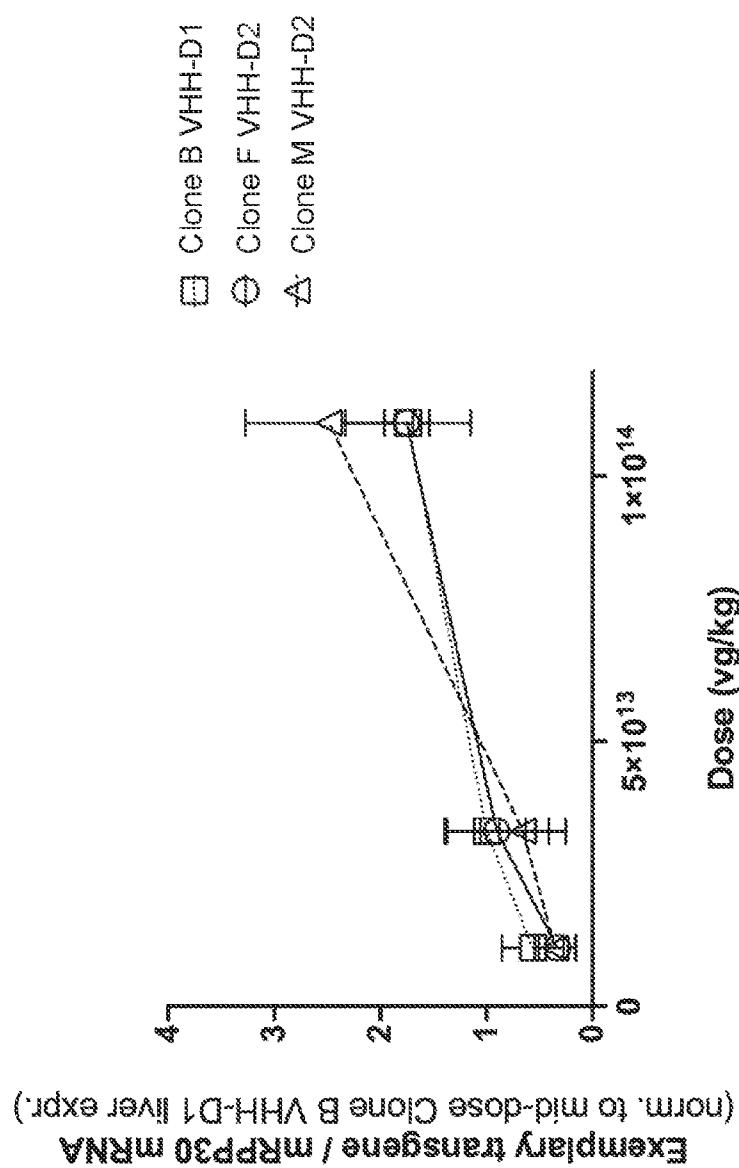


FIG. 39

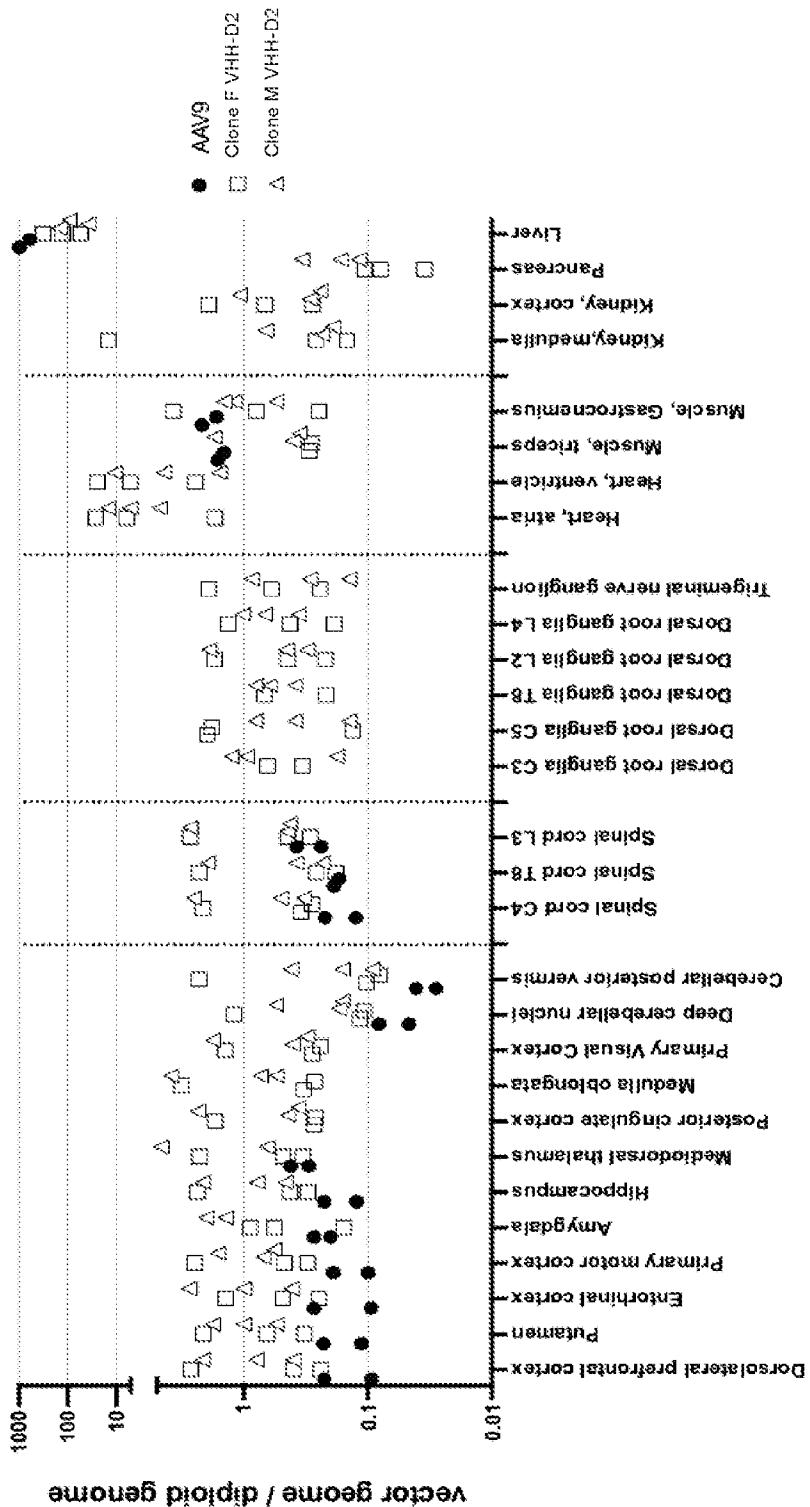


FIG. 40

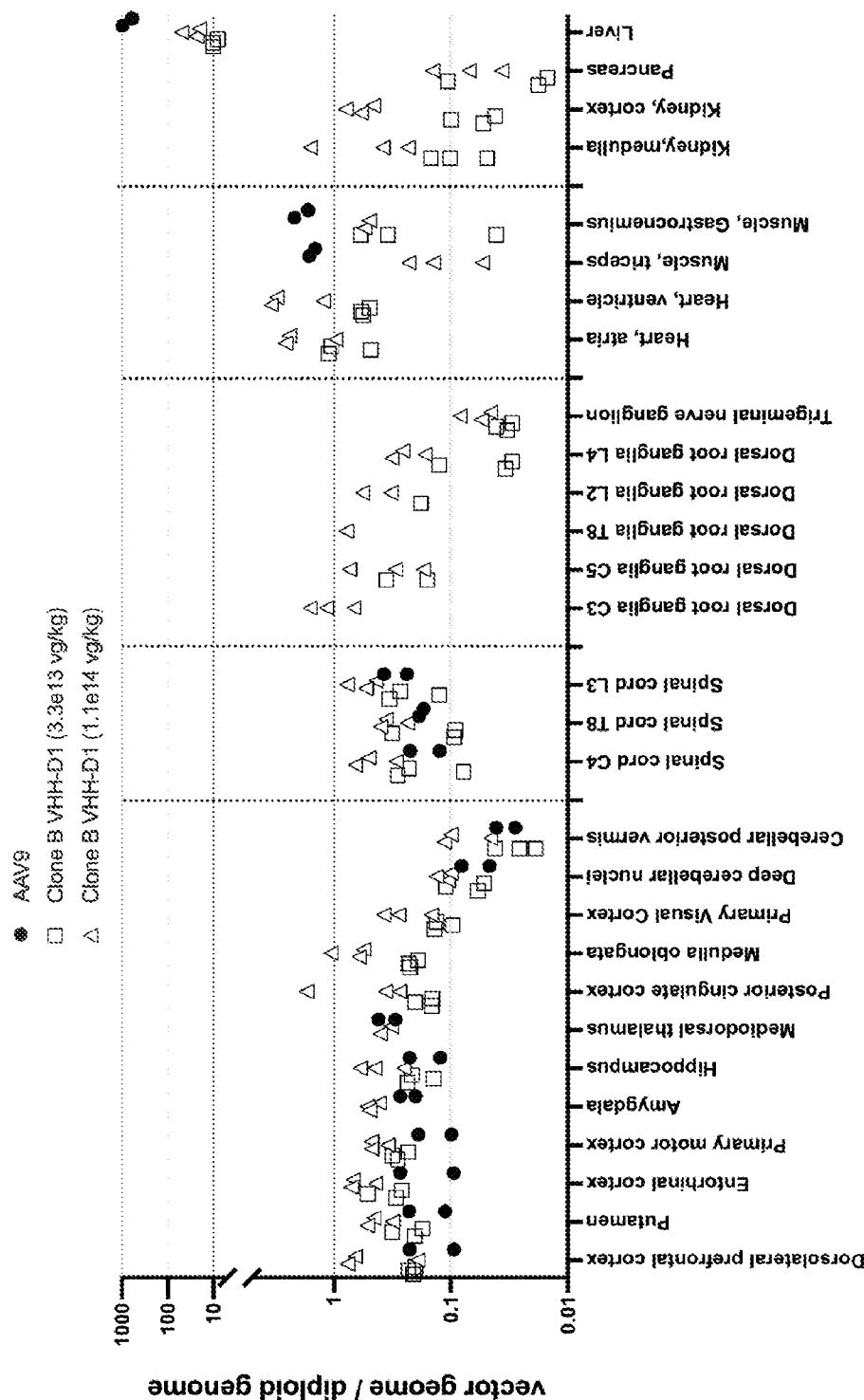


FIG. 41

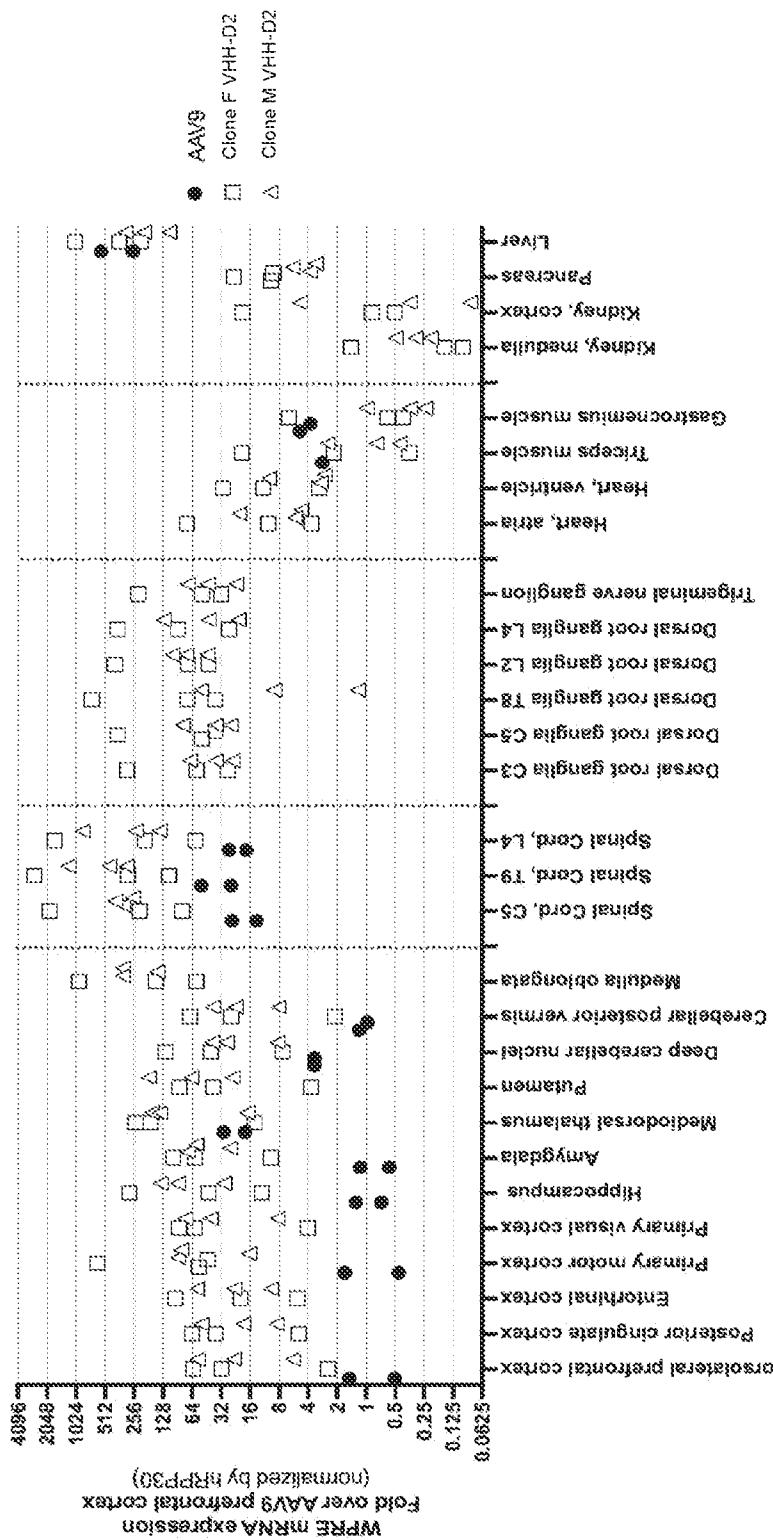


FIG. 42

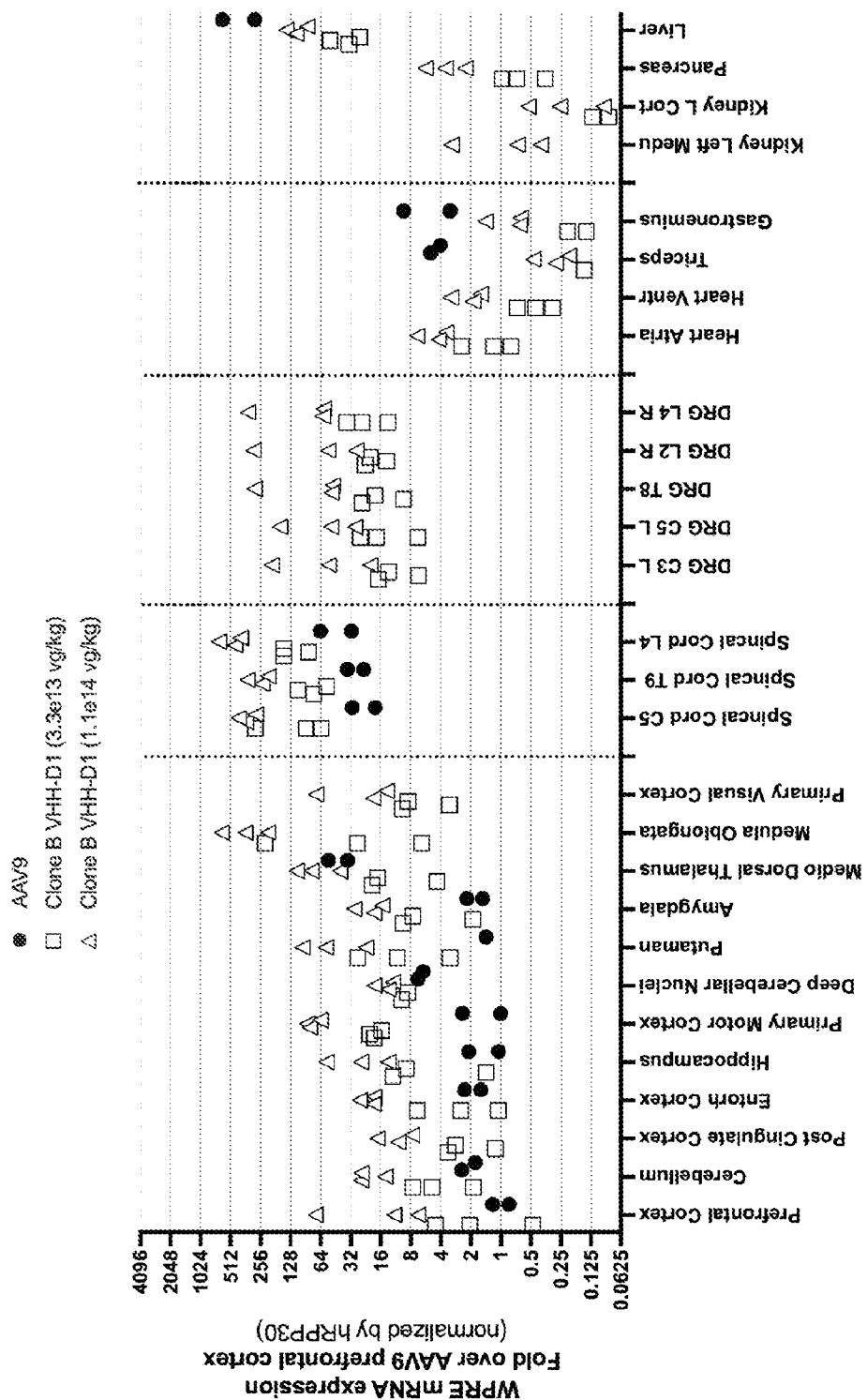


FIG. 43

# INTERNATIONAL SEARCH REPORT

International application No PCT/US2025/021832
---

**A. CLASSIFICATION OF SUBJECT MATTER**

INV. C07K16/28 A61P25/28 C12N7/00

ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

C07K A61P C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO - Internal

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>WO 2023/187728 A1 (TAKEDA PHARMACEUTICALS CO [JP]) 5 October 2023 (2023-10-05) Page 43, page 47</p> <p>-----</p> <p>WO 2024/016003 A2 (BROAD INST INC [US] ; BARRY ANDREW [US] ET AL.) 18 January 2024 (2024-01-18) Page 2, page 116</p> <p>-----</p> <p>- / -</p>	1 - 63
Y		1 - 63

Further documents are listed in the continuation of Box C.

See patent family annex.

\* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

16 July 2025

28/07/2025

Name and mailing address of the ISA/  
European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040,  
Fax: (+31-70) 340-3016

Authorized officer

Sitch, David

## INTERNATIONAL SEARCH REPORT

International application No PCT/US2025/021832
---

## C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>EICHHOFF ANNA MAREI ET AL: "Nanobody-Enhanced Targeting of AAV Gene Therapy Vectors", MOLECULAR THERAPY- METHODS &amp; CLINICAL DEVELOPMENT, vol. 15, 1 December 2019 (2019-12-01), pages 211-220, XP055878345, GB ISSN: 2329-0501, DOI: 10.1016/j.omtm.2019.09.003 Abstract, page 212, page 213 -----</p>	1 - 63

**INTERNATIONAL SEARCH REPORT****Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
  - a.  forming part of the international application as filed.
  - b.  furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).  
 accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2.  With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International application No  
PCT/US2025/021832

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 2023187728	A1	05-10-2023	CN 119301262 A EP 4504950 A1 JP 2025514631 A US 2025213730 A1 WO 2023187728 A1	10-01-2025 12-02-2025 09-05-2025 03-07-2025 05-10-2023
WO 2024016003	A2	18-01-2024	AU 2023307223 A1 CN 120225541 A EP 4554961 A2 KR 20250051026 A TW 202428311 A WO 2024016003 A2	20-02-2025 27-06-2025 21-05-2025 16-04-2025 16-07-2024 18-01-2024