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(54) Title: COMPOSITIONS USEFUL IN TREATMENT OF CDKL5 DEFICIENCY DISORDER (CDD)

(57) Abstract: Provided is a recombinant adeno-associated virus (rAAV) having an AAV capsid and a vector genome which comprises a nucleic acid sequence encoding a functional CDKL5 (hCDKL5). Also provided are a production system useful for producing the rAAV, a pharmaceutical composition comprising the rAAV, and a method of treating a subject having CDD, or ameliorating symptoms of CDD, or delaying progression of CDD via administrating an effective amount of the rAAV to a subject in need thereof.

COMPOSITIONS USEFUL IN TREATMENT OF CDKL5 DEFICIENCY DISORDER (CDD)

5 BACKGROUND OF THE INVENTION

CDKL5 Deficiency Disorder (CDD) is a serious neurodevelopmental disorder affecting young children. The underlying cause is lack of CDKL5 protein expression due to mutations in the X-linked Cyclin-Dependent Kinase-Like 5 gene, CDKL5 (Mendelian Inheritance in Man, MIM: 300203; previously known as STK9), resulting in a range of phenotypes, including EIEE2 (MIM: 300672), a form of early infantile epileptic encephalopathy [Bahi-Buisson, N. et al. Key clinical features to identify girls with CDKL5 mutations. *Brain* 131, 2647-2661, doi:10.1093/brain/awn197 (2008)], and infantile spasms [Fehr, S. et al. *Eur J Hum Genet* 21, 266-273, doi:10.1038/ejhg.2012.156 (2013); Kalscheuer, V. M. et al., *American Journal of Human Genetics*, 72, 1401-1411, doi:10.1086/375538 (2003); Tao, J. et al. *American Journal of Human Genetics* 75, 1149-1154, doi:10.1086/426460 (2004). Weaving, L. S. et al. *American Journal of Human Genetics* 75, 1079-1093, doi:10.1086/426462 (2004)]. In addition to the characteristic early-onset seizures, the phenotype may also include a number of other features, such as stereotypic hand movements, severe psychomotor retardation and general hypotonia. The early postnatal onset of symptoms indicates that CDKL5 plays a crucial role in brain development. CDKL5 is also expressed within the mature adult nervous system. CDKL5 is expressed throughout the cell, including the nucleus and the cytoplasm of the cell soma and dendrites.

CDKL5 gene mutations are the cause of most cases of CDD, a progressive neurologic developmental disorder and one of the most common causes of cognitive disability in females. Males who have the genetic mutation that causes CDD are affected in devastating ways. Most of them die before birth or in early infancy. See, e.g., ninds.nih.gov/Disorders/Patient-Caregiver-Education/Fact-Sheets/Rett-Syndrome-Fact-Sheet and omim.org/entry/312750.

Currently, there is no cure for CDD, and treatment focuses on alleviating disease symptoms. In many cases seizures are poorly controlled, thus there is an urgent medical need to find novel therapeutic approaches.

SUMMARY OF THE INVENTION

Provided herein is a recombinant adeno-associated virus (rAAV) which is useful for treating CDKL5 Deficiency Disorder (CDD) in a subject in need thereof. The rAAV carries a vector genome comprising inverted terminal repeats (ITR) and a novel nucleic acid

- 5 sequence encoding a functional human CDKL5 protein under the control of regulatory sequences which direct the hCDKL5 expression in a target cell.

In certain embodiments, a recombinant adeno-associated virus (rAAV) useful for treating CDD is provided. The rAAV comprises: (a) an AAV capsid; and (b) a vector genome packaged in the AAV capsid of (a), wherein the vector genome comprises inverted 10 terminal repeats (ITR) and a nucleic acid sequence encoding a functional human CDKL5 (hCDKL5) under control of regulatory sequences which direct the hCDKL5 expression in central nervous system cells, and wherein the hCDKL5-coding sequence is nt 699 to 3581 of SEQ ID NO: 3 (or SEQ ID NO: 22) or a sequence at least about 95% identical to nt 699 to 3581 of SEQ ID NO: 3 (or SEQ ID NO: 22). In certain embodiments, the functional 15 hCDKL5 has an amino acid sequence of SEQ ID NO: 2. In certain embodiments, the regulatory sequences comprise Ubiquitin C (UbC) promoter. In some embodiments, the regulatory sequences comprise WPRE element.

In certain embodiments, a recombinant adeno-associated virus (rAAV) useful for treating CDD is provided in which the functional human CDKL5 (hCDKL5) is hCDKL5 20 isoform 2. In certain embodiments, a recombinant adeno-associated virus (rAAV) useful for treating CDD is provided in which the functional human CDKL5 (hCDKL5) is hCDKL5 isoform 3. In certain embodiments, a recombinant adeno-associated virus (rAAV) useful for treating CDD is provided in which the functional human CDKL5 (hCDKL5) is hCDKL5 isoform 4.

25 In certain embodiments, the rAAV useful for treating CDD comprises an AAV9 capsid. In some embodiments, the rAAV useful for treating CDD comprises an AAVhu68 capsid. In other embodiments, the rAAV useful for treating CDD comprises an AAVrh91 capsid.

30 In certain embodiments, the CDKL5-2GS coding sequence is SEQ ID NO: 24 or a sequence at least 95% identical thereto which encodes SEQ ID NO: 6. In certain embodiments, the CDKL5-3GS coding sequence is SEQ ID NO: 25 or a sequence at least 95% identical thereto which encodes SEQ ID NO: 8. In certain embodiments, the CDKL5-

4GS coding sequence is SEQ ID NO: 26 or a sequence at least 95% identical thereto which encodes SEQ ID NO: 10.

In certain embodiments, the vector genome comprises: at least two tandem repeats of dorsal root ganglion (drg)-specific miRNA target sequences in the 3' untranslated region for 5 the hCDKL5, wherein the at least two tandem repeats comprise at least a first miRNA target sequence and at least a second miRNA target sequence which may be the same or different, and target miR183 or miR182.

In certain embodiments, a vector other than rAAV is used for delivery of an expression cassette as described herein. The vector may be a recombinant parvovirus, a 10 recombinant lentivirus, a recombinant retrovirus, or a recombinant adenovirus; or a non-viral vector selected from naked DNA, naked RNA, an inorganic particle, a lipid particle, a polymer-based vector, or a chitosan-based formulation.

In certain embodiment, a composition comprising a stock of rAAV or other viral vector as described herein and an aqueous suspension media is provided.

15 In certain embodiments, a method of treating CDD is provided which comprises administrating an effective amount of the rAAV or other vector described herein to a subject in need thereof.

In certain embodiments, an rAAV production system useful for producing a vector as described herein is provided.

20 In certain embodiments, a vector comprising an expression cassette is provided, wherein the expression cassette comprises a nucleic acid sequence encoding a functional human CDKL5-co1 (hCDKL5) under control of regulatory sequences which direct the hCDKL5 expression, and wherein the hCDKL5-coding sequence is SEQ ID NO: 22 or a sequence at least about 95% identical to SEQ ID NO: 22. In certain embodiments, a vector 25 comprising an expression cassette is provided, wherein the expression cassette comprises a nucleic acid sequence encoding a functional human CDKL5-2GS (hCDKL5-2GS) under control of regulatory sequences which direct the hCDKL5-2GS expression, and wherein the hCDKL5-2GS-coding sequence is SEQ ID NO: 24 or a sequence at least about 95% identical to SEQ ID NO: 24. In certain embodiments, a vector comprising an expression 30 cassette is provided, wherein the expression cassette comprises a nucleic acid sequence encoding a functional human CDKL5-3GS (hCDKL5-3GS) under control of regulatory sequences which direct the hCDKL5-3GS expression, and wherein the hCDKL5-3GS-

coding sequence is SEQ ID NO: 25 or a sequence at least about 95% identical to SEQ ID NO: 25. In certain embodiments, a vector comprising an expression cassette, wherein the expression cassette comprises a nucleic acid sequence encoding a functional human CDKL5-4GS (hCDKL5-4GS) under control of regulatory sequences which direct the hCDKL5-4GS expression, and wherein the hCDKL5-4GS-coding sequence is SEQ ID NO: 26 or a sequence at least about 95% identical to SEQ ID NO: 26.

5 In certain embodiments, the vector genome further comprises at least two tandem repeats of dorsal root ganglion (drg)-specific miRNA target sequences. In certain embodiments, the rAAV or a composition comprising the rAAV is administrable to a subject 10 in need thereof to ameliorate symptoms of CDD and/or to delay progression of CDD.

In a further aspect, provided herein is a composition comprising a rAAV or a vector as described herein and an aqueous suspension media.

15 In another aspect, a method of treating a subject having CDD, or ameliorating symptoms of CDD, or delaying progression of CDD is provided. The method comprises administrating an effective amount of a rAAV or a vector as described herein to a subject in need thereof. In certain embodiments, the vector or rAAV is administrable to a patient via an intra-cisterna magna injection (ICM).

These and other aspects of the invention are apparent from the following detailed description of the invention.

20

BRIEF DESCRIPTION OF THE DRAWINGS

FIG 1A shows an AAV vector design for an AAV CDKL5 vector genome comprising a 5' AAV inverted terminal repeat (ITR), an expression cassette comprising: a neuronal promoter, an engineered human CDKL5 DNA coding sequence, an enhancer, and 25 poly A, and an AAV 3' ITR. FIG 1B shows an AAV vector design for an AAV CDKL5 vector genome comprising a 5' AAV ITR, and expression cassette comprising: ubiquitin C (UbC) promoter, an engineered human CDKL5 DNA coding sequence, a drg-detargetting miRNA, polyA, and an AAV 3' ITR. FIG 1C shows an AAV vector design for an AAV 30 CDKL5 vector genome comprising a 5' AAV ITR, and expression cassette comprising: a chicken beta-actin hybrid promoter (CBh), an engineered human CDKL5 DNA coding sequence, a drg-detargetting miRNA, polyA, and an AAV 3' ITR.

FIGs 2A and 2B show analysis of mouse hippocampus, as assessed with anti-CDKL5 antibodies (S957D, University of Dundee, UK). Mice were treated with 5×10^{10} GC AAV-hSyn-CDKL5-1co.WPRE via neonatal intracerebroventricular injection. FIG 2A shows CDKL5 expression by Western blotting, charting CDKL5/tubulin levels in wild-type mice 5 with a PBS injection, KO mice with PBS injection, and treated KO mouse. FIG 2B shows CDKL5 activity as determined using the pS222EB2 (Baltussen et al, 2018) levels in wild-type mice with a PBS injection, KO mice with PBS injection, and treated KO mice.

FIG 3 provides a graph of mouse maturation and survival following injection of AAV-hSyn-hCDKL5-1co.WPRE in a CDD mouse model. All injected mice survived 10 treatment, gained weight. They mice did not display any overt signs of adverse outcomes.

FIG 4 provides results from a behavioral assessment in a CDD mice receiving AAV-hSyn-CDKL5-1co.WPRE. This figure charts results from the Elevated Zero Maze, which assesses the balance between risk taking, curiosity and anxiety. The first bar represents wt mice receiving PBS. Wt mice are curious but cautious and spend a limited time out in the 15 open. The middle bar shows Cdkl5-ko mice receiving PBS only, which showed decreased anxiety and spend more time out in the open, they entered the open zone more frequently. After AAV-CDKL5 treatment, behavior of Cdkl5-ko mice reverts to wt behavior (for time in open zone and for entries from closed into open zone).

FIGs 5A and 5B provide results from a behavioral assessment in a CDD mice 20 receiving AAV-hSyn-CDKL5-1co.WPRE. FIG 5A shows exploratory activity of mice in an Open Field Arena, plotted as beam breaks/bin versus time (min). The dotted line shows wt mice, which are curious but within 10 min have explored the arena and calm down. The line with long dashes shows that Cdkl5-ko spend a longer time exploring but eventually calm down. The line with dashes and dots show that after AAV-CDKL5 treatment, activity of 25 Cdkl5-ko mice is reduced, and their overall activity levels resemble wt mice. FIG 5B shows cumulative activity data of total beam breaks taken by mice, which confirms results in FIG 5A.

FIG 6 shows a graph of measured latency to fall (sec) in a motor activity and agility 30 assessment (rotarod) in mice over three consecutive days. The wild-type mice are observed to increase performance while learning over time. The Cdkl5-ko mice show improved performance, compared to wt mice, likely due to the previously observed initial

hyperactivity. After AAV-hSyn-CDKL5-1co.WPRE.treatment, behavior of Cdkl5-ko mice does not revert to wt behavior.

FIGs 7A and 7B show results of hippocampal learning and memory (Y-maze). FIG 7A shows the percentage of spontaneous alterations for the test group and the two control groups. FIG 7B shows the distance moved (m) for the test group and the two control groups. The wt mice show a strong tendency to explore the maze arm that they have not visited recently (spontaneous alteration behavior).

FIGs 8A to 8D illustrate CDKL5 expression levels or activity levels for AAV.CDLK5 vector constructs for the expression isoform 1, isoform 2, isoform 3 or 10 isoform 4. FIG 8A shows quantified expression levels of CDKL5 isoforms 1, 2, 3, and 4 in knock-out mice injected with AAV vectors (5×10^{10} GC, neonatal ICV), as compared to a wild-type mouse injected with vehicle and a knock-out mouse injected with vehicle. FIG 8B shows CDKL5 activity as determined from quantified signal of pS222EB2 from western blot analysis of tissue from treated a wild-type mouse (injected with vehicle (PBS)), a knock-out 15 mouse (injected with vehicle, or the AAV.CDKL5-1co). FIG 8C shows CDKL5 activity as determined from quantified signal of pS222EB2 from western blot analysis of tissue from treated a wild-type mouse (injected with vehicle (PBS)), a knock-out mouse (injected with vehicle, or the AAV.CDKL5-isoform 1, 2, 3 or 4 (from FIG 8A)). FIG 8D shows quantified CDKL5 expression levels of isoform 1 in KO mice (from FIG 8B).

FIGs 9A to 9F show therapeutic efficacy of the AAV.CDKL5 gene therapy in a mouse study comparing different vector doses (5×10^{10} GC, 2.5×10^{10} GC, 1×10^{10} GC, and 6×10^9 GC) in knock out and wild type mice. FIG 9A show body weight gain (g) over a period of 10 weeks for mice treated with AAV.CDKL5 at a dose of 5×10^{10} GC or PBS. FIG 9B show body weight gain (g) over a period of 10 weeks for mice treated with AAV.CDKL5 at a dose of 2.5×10^{10} GC or PBS. FIG 9C shows a dose-dependent results of the hindlimb clasping test for AAV.CDKL5-treated group at a dose of 5×10^{10} GC compared to untreated Cdkl5-ko mice. FIG 9D shows a dose-dependent results of the hindlimb clasping test for AAV.CDKL5-treated group at a dose of 2.5×10^{10} GC compared to untreated Cdkl5-ko mice. FIG 9E shows a dose-dependent results of the hindlimb clasping test for AAV.CDKL5-treated group at a dose of 1×10^{10} GC compared to untreated Cdkl5-ko mice. FIG 9F shows a dose-dependent results of the hindlimb clasping test for AAV.CDKL5-treated group at a dose of 6×10^9 GC compared to untreated Cdkl5-ko mice. WT mice

showed no clasping, while KO mice showed pronounced clasping. After treatment, KO mice displayed a significantly reduced amount of clasping. Injected WT mice were not affected.

FIGs 10A to 10E show therapeutic efficacy of the AAV.CDL5 gene therapy in the CDD ko mouse study. FIGs 10A shows results in nest building (nest quality/score) for the 5 AAV.CDKL5-treated group at a dose of 5×10^{10} GC, compared to untreated Cdkl5-ko mice. FIG 10B shows results from a marble burying tasks with a trend to normalization in the AAV.CDLK5-treated group at a dose of 5×10^{10} GC, compared to WT and Cdkl5-ko mice. FIG 10C shows results in nest building (nest quality/score) for the AAV.CDKL5-treated group at a dose of 2.5×10^{10} GC, compared to untreated Cdkl5-ko mice. FIG 10D shows 10 results from a marble burying tasks with a trend to normalization in the AAV.CDLK5-treated group at a dose of 2.5×10^{10} GC, compared to WT and Cdkl5-ko mice. FIG 10E shows results in nest building (nest quality/score) for the AAV.CDKL5-treated group at a dose of 1×10^{10} GC compared to untreated Cdkl5-ko mice.

FIGs 11A to 11F shows correction of hyperactivity in the ko mice receiving the 15 AAV.CDKL5 treatment as assessed in the open field activity test. FIG 11A shows ambulatory activity/bin vs. time (5-minute intervals to 30 min) in AAV.CDKL5-treated ko mice at a dose of 5×10^{10} GC. FIG 11B shows total activity in AAV.CDKL5-treated ko mice at a dose of 5×10^{10} GC. FIG 11C shows ambulatory activity/bin vs. time (5-minute intervals to 30 min) in AAV.CDKL5-treated ko mice at a dose of 2.5×10^{10} GC. FIG 11D shows total 20 activity in AAV.CDKL5-treated ko mice at a dose of 2.5×10^{10} GC. FIG 11E shows ambulatory activity/bin vs. time (5-minute intervals to 30 min) in AAV.CDKL5-treated ko mice at a dose of 6×10^9 GC. FIG 11F shows total activity in AAV.CDKL5-treated ko mice at a dose of 6×10^9 GC. Normalization of increase risk-taking was observed in the elevated zero maze and normalization of hippocampal learning deficits are seen in the Y-maze in the 25 AAV.CDKL5-treated ko mice.

FIG 12 illustrates that the expression of CDKL5 isoforms 2-4 provide significant correction of the hindlimb clasping phenotype when assessed at dose of 5×10^{10} GC in ko mouse.

FIGs 13A to 13D illustrate a strong trend for correction in KO mice treated with 30 AAV.CDKL5-isoform 1. FIG 13A show activity in elevated in KO mice treated with AAV.CDKL5-isoform 1 at a dose of 5×10^{10} GC. FIG 13B show activity in elevated in KO mice treated with AAV.CDKL5-isoform 1 at a dose of 2.5×10^{10} GC. FIG 13C show activity

in Y-Maze in KO mice treated with AAV.CDKL5-isoform 1 at a dose of 5×10^{10} GC. FIG 13D show activity in Y-Maze in KO mice treated with AAV.CDKL5-isoform 1 at a dose of 2.5×10^{10} GC.

FIGs 14A to 14C show gender specific results in hindlimb clasping following 5 treating of knock-out mice with the AAV.CDKL5-isoform 1. FIG 14A shows hindlimb clasping following treating of male knock-out mice with the AAV.CDKL5-isoform 1. FIG 14B shows hindlimb clasping following treating of female knock-out mice with the AAV.CDKL5-isoform 1. Both hemizygous males and heterozygous females displayed 10 hindlimb clasping in Cdkl5-ko mice, which was significantly reduced after treatment. None of the WT groups showed clasping. FIG 14C shows an ambulatory activity at high dose (5×10^{10} GC, neonatal ICV) with significant improvement in female heterozygous mice.

FIGs 15A and 15B show gender specific results in open field – ambulatory activity 15 in an open field assessment. FIG 15A shows results in ambulatory activity in an open field assessment in males (KO) mouse treated with AAV.CDKL5-isoform 1, plotted as X, Y axis beam breaks over time. FIG 15B shows results in ambulatory activity in an open field assessment in female (ht) mouse treated with AAV.CDKL5-isoform 1, plotted as X, Y axis beam breaks over time. Full correction to wild-type is observed for multiple timepoints in both males (KO) and females (ht) mice treated with AAV.CDKL5-isoform 1.

FIGs 16A and 16B show gender differences in ko mice treated with the 20 AAV.CDKL5-isoform 1 vector. FIG 16A shows results in open field – ambulatory activity in an elevated zero maze assessment in males (KO) mouse treated with AAV.CDKL5-isoform 1, plotted as time spent (s) in an open zone. FIG 16B shows results in open field – ambulatory activity in an elevated zero maze assessment in females (ht) mouse treated with AAV.CDKL5-isoform 1, plotted as time spent (s) in an open zone. Risk-prone behavior 25 is corrected, with a size effect being more pronounced in males.

FIG 17 provides vector distribution in various tissue samples from the NHP study (representative for the 1×10^{14} GC dose). The graph provides rAAV.CDKL5 in gc/diploid genome for various non-neuronal tissue, spinal track, and brain tissue. Strong transduction of dorsal root ganglia (DRG) is observed. Moderate to low transduction of brain tissues is 30 observed, with some leakage into non-neuronal tissues.

FIG 18 provides results quantified hCDKL5 expression (measured by RT-qPCR) in the NHP study shown in cerebellum, frontal cortex, occipital cortex, parietal cortex, and temporal cortex.

FIGs 19A and 19B show results of a dose escalation studies measuring behavioral changes following CDKL5 gene therapy administration to WT mice. FIG 19A shows no significant hindlimb clasp severity score changes in WT mice injected at 7.5×10^{10} GC and 1×10^{11} GC of AAV, as compared with control mice treated with PBS. FIG 19B shows no significant ambulatory activity changes in WT mice injected at 7.5×10^{10} GC and 1×10^{11} GC of AAV, as compared with control mice treated with PBS.

10

DETAILED DESCRIPTION OF THE INVENTION

Compositions and methods for treating CDD are provided herein. An effective amount of a recombinant adeno-associated virus (rAAV) having an AAV capsid (e.g., AAVhu68 or AAV-PHP.B) and packaged therein a vector genome encoding a functional human cyclin dependent kinase like 5 (hCDKL5) is delivered to a subject in need.

I. Human CDKL5

Cyclin dependent kinase like 5 (CDKL5, also known as CFAP247, serine/threonine kinase 9, STK9; Uniprot# 076039) gene is natively located on the short (p) arm of the X chromosome at position 22.13. The N-terminus of the CDLK5 protein acts as a kinase, which is an enzyme that changes the activity of other proteins. Several direct substrates for CDKL5 have been identified (Baltussen et al, 2018; Munoz et al, 2018). The CDKL5 C-terminus is of unknown function.

As used herein, a functional hCDKL5 protein refers to an isoform, a natural variant, a variant, a polymorph, or a truncation of a CKDL5 protein which is not associated with CDD and/or delivery or expression of which may ameliorate symptoms or delay progression of CDD in an animal model or a patient. See, OMIM # 300203, each of the webpages is incorporated herein by reference in its entirety. In certain embodiments, the functional hCDKL5 has an amino acid sequence of SEQ ID NO: 2 (isoform 1) or an amino acid sequence at least about 90 % (e.g., at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9%) identical thereto. In certain embodiment, the functional hCDKL5 protein has an amino acid sequence of SEQ ID NO: 19 (isoform 2) or an amino acid

sequence at least about 90 % (e.g., at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9%) identical thereto. In certain embodiment, the functional hCDKL5 protein has an amino acid sequence of SEQ ID NO: 20 (isoform 3) or an amino acid sequence at least about 90 % (e.g., at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9%) identical thereto. In certain embodiment, the functional hCDKL5 protein has an amino acid sequence of SEQ ID NO: 21 (isoform 4) or an amino acid sequence at least about 90 % (e.g., at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9%) identical thereto. In certain embodiment, the functional hCDKL5 is a truncated hCDKL5 which comprises a methyl-CpG binding domain (MBD) having the sequence and a NCoR/SMRT Interaction Domain (NID). See, WO2018172795A1, which is incorporated herein by reference in its entirety.

In certain embodiments, a functional hCDKL5 protein ameliorates symptoms or delays progression of CDD in an animal model. One exemplified animal model is a CDKL5-ko mouse. Other suitable models are described herein.

The CDD symptoms or progression may be evaluated using various assays/methods, including but not limited to, a survival plot (e.g., Kaplan-Meier survival plot), monitoring body weights, and observing behavior changes (for example, by hind limb clasping, Open Field Assay (motor function), Elevated Zone Maze (anxiety/risk vs. exploration), Y Maze (learning and memory/hippocampus), Marble Burying Assay (inborn behavior and locomotion), Nesting (inborn social behavior), and rotarod assay (motor function, coordination)). In certain embodiment, administration or expression of a functional hCDKL5 protein in an animal model leads to amelioration of CDD symptoms or delay in CDD progression shown by an assay result which is at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 100%, or more than 100% of that obtained in a corresponding wildtype animal. In certain embodiment, administration or expression of a functional hCDKL5 protein in a CDD animal model leads to amelioration of CDD symptoms or delay in CDD progression shown by an improved assay result which is at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 100%, or more than 100% of that obtained from a corresponding non-treated CDD animal.

Provided herein is a nucleic acid sequence encoding a functional hCDKL5 protein, termed as hCDKL5 coding sequence or CDKL5 coding sequence. In certain embodiments, the hCDKL5-coding sequence is SEQ ID NO: 3 or a sequence at least about 95% identical to

SEQ ID NO: 3. In certain embodiments, the hCDKL5 coding sequence is selected from SEQ ID NO: 2 (referred to as CDKL5 or CDKL5co or CDKL5-1 or CDKL5-1co) or NCBI Reference Sequences NM_001037343.1 (referred to as CDKL5 or CDKL5e1; SEQ ID NO: 16) encoding amino acid sequence NP_001032420.1 (SEQ ID NO: 19), NM_001323289.2
5 (SEQ ID NO: 17) encoding amino acid sequence NP_001310218.1 (SEQ ID NO: 20), and NM_003159.2 (SEQ ID NO: 18) encoding amino acid sequence NP_003150.1 (SEQ ID NO: 21), or a nucleic acid sequence at least about 70% (e.g., at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9%) identical thereto. Each of the NCBI Reference Sequences is incorporated herein by reference in its entirety. In certain
10 embodiments, the hCDKL5 coding sequence is a modified or engineered (hCDKL5 or hCDKL5co or CDKL5-1 or CDKL5-1co). The modified or engineered shares less than about 70% (e.g., about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9%) identity to the NCBI Reference Sequences.

In certain embodiments, the hCDKL5 coding sequence is SEQ ID NO: 22 or a
15 nucleic acid sequence at least about 70% (e.g., at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 99.9%) identical thereto. In certain embodiments, the hCDKL5 coding sequence is SEQ ID NO: 24 or a nucleic acid sequence at least about
20 70% (e.g., at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 99.9%) identical thereto. In certain embodiments, the hCDKL5 coding sequence is SEQ ID NO: 25 or a nucleic acid sequence at least about 70% (e.g., at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 99.9%) identical thereto. In certain embodiments, the hCDKL5 coding sequence is SEQ ID NO: 26 or a nucleic acid sequence at least about 70% (e.g., at least about 75%, at least about 80%, at least about 85%,
25 at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 99.9%) identical thereto.
In certain embodiments, the hCDKL5 coding sequence is SEQ ID NO: 27 or a nucleic acid sequence at least about 70% (e.g., at least about 75%, at least about 80%, at least about 85%,
30 at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 99.9%) identical thereto.

In certain embodiments, the hCDKL5-coding sequence is an engineered sequence of SEQ ID NO: 37 or a sequence at least about 95% identical to SEQ ID NO: 37. In certain embodiments, the hCDKL5-coding sequence is an engineered sequence of SEQ ID NO: 38 or a sequence at least about 95% identical to SEQ ID NO: 38. In certain embodiments, the
5 hCDKL5-coding sequence is an engineered sequence of SEQ ID NO: 39 or a sequence at least about 95% identical to SEQ ID NO: 39. In certain embodiments, the hCDKL5-coding sequence is an engineered sequence of SEQ ID NO: 40 or a sequence at least about 95% identical to SEQ ID NO: 40. In certain embodiments, the hCDKL5-coding sequence is an engineered sequence of SEQ ID NO: 41 or a sequence at least about 95% identical to SEQ
10 ID NO: 41. In certain embodiments, the hCDKL5-coding sequence is an engineered sequence of SEQ ID NO: 42 or a sequence at least about 95% identical to SEQ ID NO: 42. In certain embodiments, the hCDKL5-coding sequence is an engineered sequence of SEQ ID NO: 43 or a sequence at least about 95% identical to SEQ ID NO: 43. In certain
15 embodiments, the hCDKL5-coding sequence is an engineered sequence of SEQ ID NO: 44 or a sequence at least about 95% identical to SEQ ID NO: 44. In certain embodiments, the hCDKL5-coding sequence is an engineered sequence of SEQ ID NO: 45 or a sequence at least about 95% identical to SEQ ID NO: 45. In certain embodiments, the hCDKL5-coding sequence is an engineered sequence of SEQ ID NO: 46 or a sequence at least about 95% identical to SEQ ID NO: 46. In certain embodiments, the hCDKL5-coding sequence is an
20 engineered sequence of SEQ ID NO: 47 or a sequence at least about 95% identical to SEQ ID NO: 47. In certain embodiments, the hCDKL5-coding sequence is an engineered sequence of SEQ ID NO: 48 or a sequence at least about 95% identical to SEQ ID NO: 48.

A “nucleic acid”, as described herein, can be RNA, DNA, or a modification thereof, and can be single or double stranded, and can be selected, for example, from a group
25 including: nucleic acid encoding a protein of interest, oligonucleotides, nucleic acid analogues, for example peptide-nucleic acid (PNA), pseudocomplementary PNA (pc-PNA), locked nucleic acid (LNA) etc. Such nucleic acid sequences include, for example, but are not limited to, nucleic acid sequence encoding proteins, for example that act as transcriptional repressors, antisense molecules, ribozymes, small inhibitory nucleic acid sequences, for
30 example but are not limited to RNAi, shRNAi, siRNA, micro RNAi (mRNAi), antisense oligonucleotides etc.

The term “percent (%) identity”, “sequence identity”, “percent sequence identity”, or “percent identical” in the context of nucleic acid sequences refers to the residues in the two sequences which are the same when aligned for correspondence. The length of sequence identity comparison may be over the full-length of the genome, the full-length of a gene coding sequence, or a fragment of at least about 500 to 5000 nucleotides, is desired.

5 However, identity among smaller fragments, e.g., of at least about nine nucleotides, usually at least about 20 to 24 nucleotides, at least about 28 to 32 nucleotides, at least about 36 or more nucleotides, may also be desired.

Percent identity may be readily determined for amino acid sequences over the full-length of a protein, polypeptide, about 32 amino acids, about 330 amino acids, or a peptide fragment thereof or the corresponding nucleic acid sequence coding sequences. A suitable amino acid fragment may be at least about 8 amino acids in length, and may be up to about 700 amino acids. Generally, when referring to “identity”, “homology”, or “similarity” between two different sequences, “identity”, “homology” or “similarity” is determined in reference to “aligned” sequences. “Aligned” sequences or “alignments” refer to multiple nucleic acid sequences or protein (amino acids) sequences, often containing corrections for missing or additional bases or amino acids as compared to a reference sequence.

Alignments are performed using any of a variety of publicly or commercially available Multiple Sequence Alignment Programs. Sequence alignment programs are available for amino acid sequences, e.g., the “Clustal X”, “Clustal Omega” “MAP”, “PIMA”, “MSA”, “BLOCKMAKER”, “MEME”, and “Match-Box” programs. Generally, any of these programs are used at default settings, although one of skill in the art can alter these settings as needed. Alternatively, one of skill in the art can utilize another algorithm or computer program which provides at least the level of identity or alignment as that provided by the referenced algorithms and programs. See, e.g., J. D. Thomson et al, Nucl. Acids. Res., “A comprehensive comparison of multiple sequence alignments”, 27(13):2682-2690 (1999).

Multiple sequence alignment programs are also available for nucleic acid sequences. Examples of such programs include, “Clustal W”, “Clustal Omega”, “CAP Sequence 30 Assembly”, “BLAST”, “MAP”, and “MEME”, which are accessible through Web Servers on the internet. Other sources for such programs are known to those of skill in the art. Alternatively, Vector NTI utilities are also used. There are also a number of algorithms

known in the art that can be used to measure nucleotide sequence identity, including those contained in the programs described above. As another example, polynucleotide sequences can be compared using FastaTM, a program in GCG Version 6.1. FastaTM provides alignments and percent sequence identity of the regions of the best overlap between the 5 query and search sequences. For instance, percent sequence identity between nucleic acid sequences can be determined using FastaTM with its default parameters (a word size of 6 and the NOPAM factor for the scoring matrix) as provided in GCG Version 6.1, herein incorporated by reference.

10 II. Expression Cassette

Provided herein is a nucleic acid sequence comprising the hCDKL5 coding sequence under control of regulatory sequences which direct the hCDKL5 expression in a target cell, also termed as an expression cassette. As used herein, an “expression cassette” refers to a nucleic acid molecule which comprises a coding sequence (e.g., a CDKL5 coding sequence), 15 and regulatory sequences operably linked thereto. In certain embodiments, a vector genome contains two or more expression cassettes. The term “transgene” refers to a DNA sequence from an exogenous source which is inserted into a target cell; typically, the transgene encodes a product (e.g., CDKL5). Typically, such an expression cassette to be packed into a viral vector contains the coding sequence for the gene product described herein flanked by 20 packaging signals of the viral genome and other expression control sequences such as those described herein. The regulatory sequences necessary are operably linked to the hCDKL5 coding sequence in a manner which permits its transcription, translation and/or expression in target cell. As used herein, “operably linked” sequences include sequences which modulate transcription, translation, and/or expression that are contiguous with the hCDKL5 coding 25 sequence and regulatory sequences that act in trans or at a distance to control the hCDKL5 coding sequence. The expression cassette may contain regulatory sequences upstream (5' to) of the gene sequence, e.g., one or more of a promoter, an enhancer, an intron, etc., and one or more of an enhancer, or regulatory sequences downstream (3' to) a gene sequence, e.g., 3' untranslated region (3' UTR) comprising a polyadenylation site, among other elements. Such 30 regulatory sequences typically include, e.g., one or more of a promoter, an enhancer, an intron, a Kozak sequence, a polyadenylation sequence, and a TATA signal. In certain embodiment, the promoter is a tissue-specific promoter, e.g., a CNS-specific or neuron-

specific promoter. In certain embodiments, the promote is a human synapsin promoter (SEQ ID NO: 23). In certain embodiments, an additional or alternative neuron-specific promoter sequence may be selected from neuron-specific enolase (NSE) promoter (Andersen et al., (1993) Cell. Mol. Neurobiol., 13:503 15), neurofilament light chain gene promoter (Piccioli et al., (1991) Proc. Natl. Acad. Sci. USA, 88:5611 5), neuron-specific vgf gene promoter (Piccioli et al., (1995) Neuron, 15:373 84), and/or others.

5 In certain embodiments, the human synapsin promoter has a sequence (e.g., nt 213 to nt 678 of SEQ ID NOs: 1, 3, 5, 7, 9 or SEQ ID NO: 23, also termed as hSyn or Syn herein.

In other embodiments, the promoter is a constitutive promoter, e.g., a chicken beta actin promoter with a cytomegalovirus enhancer (CB7) promoter, a human elongation initiation factor 1 alpha promoter (EF1a) promoter, a human ubiquitin C (UbC) promoter. In certain embodiments, the regulatory sequences direct hCDKL5 expression in central nervous system (CNS) cells.

10 In certain embodiment, the target cell may be a central nervous system cell. In certain embodiments, the target cell is one or more of an excitatory neuron, an inhibitory neuron, a glial cell, a cortex cell, a frontal cortex cell, a cerebral cortex cell, a spinal cord cell. In certain embodiments, the target cell is a peripheral nervous system (PNS) cell, for example a retina cell. Other cells other than those from nervous system may also be chosen as a target cell, such as a monocyte, a B lymphocyte, a T lymphocyte, a NK cell, a lymph node cell, a tonsil cell, a bone marrow mesenchymal cell, a stem cell, a bone marrow stem cell, a heart cell, an epithelium cell, a esophagus cell, a stomach cell, a fetal cut cell, a colon cell, a rectum cell, a liver cell, a kidney cell, a lung cell, a salivary gland cell, a thyroid cell, an adrenal cell, a breast cell, a pancreas cell, an islet of Langerhans cell, a gallbladder cell, a prostate cell, a urinary bladder cell, a skin cell, a uterus cell, a cervix cell, a testis cell, or any other cell which expresses a functional CDKL5 protein in a subject without CDD.

15 In certain embodiments, an additional or alternative promoter sequence may be included as part of the expression control sequences (regulatory sequences), e.g., located between the selected 5' ITR sequence and the coding sequence. Constitutive promoters, regulatable promoters [see, e.g., WO 2011/126808 and WO 2013/04943], tissue specific promoters, or a promoter responsive to physiologic cues may be utilized in the vectors described herein. The promoter(s) can be selected from different sources, e.g., human cytomegalovirus (CMV) immediate-early enhancer/promoter, the SV40 early

enhancer/promoter, the JC polyomavirus promoter, myelin basic protein (MBP) or glial fibrillary acidic protein (GFAP) promoters, herpes simplex virus (HSV-1) latency associated promoter (LAP), rouse sarcoma virus (RSV) long terminal repeat (LTR) promoter, neuron-specific promoter (NSE), platelet derived growth factor (PDGF) promoter, hSYN, melanin-concentrating hormone (MCH) promoter, CBA, matrix metalloprotein promoter (MPP), and the chicken beta-actin promoter.

In addition to a promoter, a vector may contain one or more other appropriate transcription initiation sequences, transcription termination sequences, enhancer sequences, efficient RNA processing signals such as splicing and polyadenylation (polyA) signals; sequences that stabilize cytoplasmic mRNA for example WPRE; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance secretion of the encoded product. An example of a suitable enhancer is the CMV enhancer. Other suitable enhancers include those that are appropriate for desired target tissue indications. In one embodiment, the regulatory sequences comprise one or more expression enhancers. In one embodiment, the regulatory sequences contain two or more expression enhancers. These enhancers may be the same or may differ from one another. For example, an enhancer may include a CMV immediate early enhancer. This enhancer may be present in two copies which are located adjacent to one another. Alternatively, the dual copies of the enhancer may be separated by one or more sequences. In still another embodiment, the expression cassette further contains an intron, e.g., the chicken beta-actin intron. In certain embodiments, the intron is a chimeric intron (CI) – a hybrid intron consisting of a human beta-globin splice donor and immunoglobulin G (IgG) splice acceptor elements. Other suitable introns include those known in the art, e.g., such as are described in WO 2011/126808. Examples of suitable polyA sequences include, e.g., Rabbit globin poly A, SV40, SV50, bovine growth hormone (bGH), human growth hormone, and synthetic polyAs. Optionally, one or more sequences may be selected to stabilize mRNA. An example of such a sequence is a modified WPRE sequence, which may be engineered upstream of the polyA sequence and downstream of the coding sequence (see, e.g., MA Zanta-Boussif, et al, Gene Therapy (2009) 16: 605-619). In certain embodiments, no WPRE sequence is present.

In certain embodiments, an expression cassette refers to nucleic acid molecule with sequence of nt 213-4439 of SEQ ID NO: 1, encoding for amino acid sequence of hCDKL5

(isoform 1; SEQ ID NO: 2). In certain embodiments, an expression cassette refers to nucleic acid molecule with sequence of nt 213-4562 of SEQ ID NO: 5 encoding for amino acid sequence of hCDKL5 (isoform 2 or 2GS SEQ ID NO: 6). In certain embodiments, an expression cassette refers to nucleic acid molecule with sequence of nt 213-4388 of SEQ ID NO: 7 encoding for amino acid sequence of hCDKL5 (isoform 3 or 3GS; SEQ ID NO: 8). In certain embodiments, an expression cassette refers to nucleic acid molecule with sequence of nt 213-4511 of SEQ ID NO: 9 encoding for amino acid sequence of hCDKL5 (isoform 4 or 4GS; SEQ ID NO: 10). In certain embodiments, an expression cassette comprises an engineered nucleic acid sequence selected from SEQ ID NOs: 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, or 48 and encoding for an amino acid sequence of hCDKL5 (isoform 1; SEQ ID NO: 2). In certain embodiments, an expression cassette refers to nucleic acid molecule with sequence of nt 213-4555 of SEQ ID NO: 3, encoding for amino acid sequence of hCDKL5 (isoform 1; SEQ ID NO: 4) and comprising of miRNA183 (SEQ ID NO: 11). In certain embodiments, these expression cassettes further comprise one, two, three, four or more drg-detargetting sequences (i.e., miRNA). See, e.g., PCT/US19/67872, filed December 20, 2019 and now published as WO 2020/132455,

III. rAAV

Provided herein is a recombinant adeno-associated virus (rAAV) useful for treating CDD. The rAAV comprises (a) an AAV capsid; and (b) a vector genome packaged in the AAV capsid of (a). Suitably, the AAV capsid selected targets the cells to be treated. In certain embodiments, the capsid is from Clade F. However, in certain embodiments, another AAV capsid source may be selected. The vector genome comprises inverted terminal repeats (ITR) and a nucleic acid sequence encoding a functional human cyclin dependent kinase like 5 (hCDKL5) under control of regulatory sequences which direct the hCDKL5 expression. In certain embodiments CDKL5 may be referring to CDKL5 or hCDKL5, CDKL5-2GS or hCDKL5-2GS, CDKL5-3GS or hCDKL5-3GS, and CDKL5-4GS or hCDKL5-4GS. In certain embodiments, the hCDKL5-coding sequence is at least about 95% identical to SEQ ID NO: 22 (encoding amino acid sequence of CDKL5-1 or hCDKL5-1; SEQ ID NO: 2). In certain embodiments, the hCDKL5-coding sequence is at least about 95% identical to SEQ ID NO: 24 (encoding amino acid sequence of CDKL5-2GS or hCDKL5-2GS; SEQ ID NO: 6). In certain embodiments, the hCDKL5-coding sequence is at

least about 95% identical to SEQ ID NO: 25 (encoding amino acid sequence of CDKL5-3GS or hCDKL5-3GS; SEQ ID NO: 8). In certain embodiments, the hCDKL5-coding sequence is at least about 95% identical to SEQ ID NO: 26 (encoding amino acid sequence of CDKL5-4GS or hCDKL5-4GS; SEQ ID NO: 10). In certain embodiments, the hCDKL5-coding sequence is less than 80% identical to any one of hCDKL5 transcript variants 1 to 3 (NM_001037343.1 with SEQ ID NO: 16 and encoding amino acid sequence NP_001032420.1 with SEQ ID NO: 19; NM_001323289.2 with SEQ ID NO: 17 and encoding amino acid sequence NP_001310218.1 with SEQ ID NO: 20; NM_003159.2 with SEQ ID NO: 18 encoding amino acid sequence NP_003150.1 with SEQ ID NO: 21). In certain embodiments, the hCDKL5-coding sequence is SEQ ID NOs: 37, 38, 39, 40, 41, 42, 43, 44, 45, 46 and 47 or at least about 95% identical thereto (encoding amino acid sequence of CDKL5-1 or hCDKL5-1; SEQ ID NO: 2). In certain embodiments, the functional hCDKL5 has an amino acid sequence of SEQ ID NO: 2 (CDKL5-1 or hCDKL5-1). In certain embodiments, the functional hCDKL5 has an amino acid sequence of SEQ ID NO: 6 (CDKL5-2GS or hCDKL5-2GS). In certain embodiments, the functional hCDKL5 has an amino acid sequence of SEQ ID NO: 8 (CDKL5-3GS or hCDKL5-3GS). In certain embodiments, the functional hCDKL5 has an amino acid sequence of SEQ ID NO: 10 (CDKL5-4GS or hCDKL5-4GS). In certain embodiments, the regulatory sequences direct hCDKL5 expression in central nervous system cells. In certain embodiments, the regulatory sequences comprise a human Synaspin promoter (hSyn) or a CB7 promoter. In certain embodiments, the regulatory elements comprise one or more of a Kozak sequence, a polyadenylation sequence, an intron, an enhancer, and a TATA signal. In certain embodiments, the vector genome further comprises at least two tandem repeats of dorsal root ganglion (drg)-specific miRNA target sequences, wherein the at least two tandem repeats comprise at least a first miRNA target sequence and at least a second miRNA target sequence which may be the same or different. In certain embodiments, the vector genome is nt 1 to nt 4634 of SEQ ID NO: 1, or nt 1 to nt 4750 of SEQ ID NO: 3, or nt 1 to nt 4757 of SEQ ID NO: 5, or nt 1 to nt 4583 of SEQ ID NO: 7, or nt 1 to nt 4706 of SEQ ID NO: 9 or a nucleic acid sequence at least about 70% (e.g., at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9%) identical thereto.

In certain embodiments, a vector genome refers to nucleic acid molecule comprising SEQ ID NO: 1, encoding for amino acid sequence of hCDKL5 (isoform 1; SEQ ID NO: 2).

In certain embodiments, a vector genome refers to nucleic acid molecule comprising SEQ ID NO: 5 encoding for amino acid sequence of hCDKL5 (isoform 2 or 2GS SEQ ID NO: 6). In certain embodiments, a vector genome refers to nucleic acid molecule comprising SEQ ID NO: 7 encoding for amino acid sequence of hCDKL5 (isoform 3 or 3GS; SEQ ID NO: 8). In 5 certain embodiments, a vector genome refers to nucleic acid molecule comprising SEQ ID NO: 9 encoding for amino acid sequence of hCDKL5 (isoform 4 or 4GS; SEQ ID NO: 10). In certain embodiments, a vector genome refers to nucleic acid molecule comprising SEQ ID NO: 3, encoding for amino acid sequence of hCDKL5 (isoform 1; SEQ ID NO: 4) and comprising of miRNA 183 (SEQ ID NO: 11).

10 In certain embodiments, in addition to the hCDKL5 coding sequence, another non-AAV coding sequence may be included, e.g., a peptide, polypeptide, protein, functional RNA molecule (e.g., miRNA, miRNA inhibitor) or other gene product, of interest. Useful gene products may include miRNAs. miRNAs and other small interfering nucleic acids regulate gene expression via target RNA transcript cleavage/degradation or translational 15 repression of the target messenger RNA (mRNA). miRNAs are natively expressed, typically as final 19-25 non-translated RNA products. miRNAs exhibit their activity through sequence-specific interactions with the 3' untranslated regions (UTR) of target mRNAs. These endogenously expressed miRNAs form hairpin precursors which are subsequently processed into a miRNA duplex, and further into a “mature” single stranded miRNA 20 molecule. This mature miRNA guides a multiprotein complex, miRISC, which identifies target site, e.g., in the 3' UTR regions, of target mRNAs based upon their complementarity to the mature miRNA.

As used herein, an “miRNA target sequence” is a sequence located on the DNA positive strand (5' to 3') and is at least partially complementary to a miRNA sequence, 25 including the miRNA seed sequence. The miRNA target sequence is exogenous to the untranslated region of the encoded transgene product and is designed to be specifically targeted by miRNA in cells in which repression of transgene expression is desired. The term “miR183 cluster target sequence” refers to a target sequence that responds to one or members of the miR183 cluster (alternatively termed family), including miRs-183, -96 and - 30 -182 (as described by Dambal, S. et al. Nucleic Acids Res 43:7173-7188, 2015, which is incorporated herein by reference).

Typically, the miRNA target sequence is at least 7 nucleotides to about 28 nucleotides in length, at least 8 nucleotides to about 28 nucleotides in length, 7 nucleotides to 28 nucleotides, 8 nucleotides to 18 nucleotides, 12 nucleotides to 28 nucleotides in length, about 20 to about 26 nucleotides, about 22 nucleotides, about 24 nucleotides, or about 26 5 nucleotides, and contains at least one consecutive region (e.g., 7 or 8 nucleotides) which is complementary to the miRNA seed sequence. In certain embodiments, the target sequence comprises a sequence with exact complementarity (100%) or partial complementarity to the miRNA seed sequence with some mismatches. In certain embodiments, the target sequence comprises at least 7 to 8 nucleotides which are 100% complementary to the miRNA seed 10 sequence. In certain embodiments, the target sequence consists of a sequence which is 100% complementary to the miRNA seed sequence. In certain embodiments, the target sequence contains multiple copies (e.g., two or three copies) of the sequence which is 100% complementary to the seed sequence. In certain embodiments, the region of 100% complementarity comprises at least 30% of the length of the target sequence. In certain 15 embodiments, the remainder of the target sequence has at least about 80 % to about 99% complementarity to the miRNA. In certain embodiments, in an expression cassette containing a DNA positive strand, the miRNA target sequence is the reverse complement of the miRNA.

As used herein, an “miRNA target sequence” is a sequence located on the DNA 20 positive strand (5’ to 3’) and is at least partially complementary to a miRNA sequence, including the miRNA seed sequence. The miRNA target sequence is exogenous to the untranslated region of the encoded transgene product and is designed to be specifically targeted by miRNA in cells in which repression of transgene expression is desired. The term “miR183 cluster target sequence” refers to a target sequence that responds to one or 25 members of the miR183 cluster (alternatively termed family), including miRs-183, -96 and -182 (as described by Dambal, S. et al. Nucleic Acids Res 43:7173-7188, 2015, which is incorporated herein by reference). Without wishing to be bound by theory, the messenger RNA (mRNA) for the transgene (encoding the gene product) is present in a cell type to which the expression cassette containing the miRNA is delivered, such that specific binding 30 of the miRNA to the 3’ UTR miRNA target sequences results in mRNA silencing and cleavage, thereby reducing or eliminating transgene expression only in the cells that express the miRNA.

Typically, the miRNA target sequence is at least 7 nucleotides to about 28 nucleotides in length, at least 8 nucleotides to about 28 nucleotides in length, 7 nucleotides to 28 nucleotides, 8 nucleotides to 18 nucleotides, 12 nucleotides to 28 nucleotides in length, about 20 to about 26 nucleotides, about 22 nucleotides, about 24 nucleotides, or about 26 5 nucleotides, and contains at least one consecutive region (e.g., 7 or 8 nucleotides) which is complementary to the miRNA seed sequence. In certain embodiments, the target sequence comprises a sequence with exact complementarity (100%) or partial complementarity to the miRNA seed sequence with some mismatches. In certain embodiments, the target sequence comprises at least 7 to 8 nucleotides which are 100% complementary to the miRNA seed 10 sequence. In certain embodiments, the target sequence consists of a sequence which is 100% complementary to the miRNA seed sequence. In certain embodiments, the target sequence contains multiple copies (e.g., two or three copies) of the sequence which is 100% complementary to the seed sequence. In certain embodiments, the region of 100% complementarity comprises at least 30% of the length of the target sequence. In certain 15 embodiments, the remainder of the target sequence has at least about 80 % to about 99% complementarity to the miRNA. In certain embodiments, in an expression cassette containing a DNA positive strand, the miRNA target sequence is the reverse complement of the miRNA.

In certain embodiments, the miRNA target sequence for the at least first and/or at 20 least second miRNA target sequence for the expression cassette mRNA or DNA positive strand is selected from (i) AGTGAATTCTACCAGTGCCATA (miR183, SEQ ID NO: 11); (ii) AGCAAAAATGTGCTAGTGCCAAA (miR-96, SEQ ID NO: 12), (iii) AGTGTGAGTTCTACCATTGCCAAA (miR182, SEQ ID NO: 13). In other embodiments, AGGGATTCTGGGAAACTGGAC (SEQ ID NO: 14) is selected.

25 In certain embodiments, the vector genome or expression cassette contains at least one miRNA target sequence that is a miR-183 target sequence. In certain embodiments, the vector genome or expression cassette contains an miR-183 target sequence that includes AGTGAATTCTACCAGTGCCATA (SEQ ID NO:11), where the sequence complementary to the miR-183 seed sequence is GTGCCAT. In certain embodiments, the vector genome or 30 expression cassette contains more than one copy (e.g., two or three copies) of a sequence that is 100% complementary to the miR-183 seed sequence. In certain embodiments, a miR-183 target sequence is about 7 nucleotides to about 28 nucleotides in length and includes at least

one region that is at least 100% complementary to the miR-183 seed sequence. In certain embodiments, a miR-183 target sequence contains a sequence with partial complementarity to SEQ ID NO: 11 and, thus, when aligned to SEQ ID NO: 11, there are one or more mismatches. In certain embodiments, a miR-183 target sequence comprises a sequence having at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mismatches when aligned to SEQ ID NO: 11, where the mismatches may be non-contiguous. In certain embodiments, a miR-183 target sequence includes a region of 100% complementarity which also comprises at least 30% of the length of the miR-183 target sequence. In certain embodiments, the region of 100% complementarity includes a sequence with 100% complementarity to the miR-183 seed sequence. In certain embodiments, the remainder of a miR-183 target sequence has at least about 80% to about 99% complementarity to miR-183. In certain embodiments, the expression cassette or vector genome includes a miR-183 target sequence that comprises a truncated SEQ ID NO: 11, i.e., a sequence that lacks at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides at either or both the 5' or 3' ends of SEQ ID NO: 11. In certain embodiments, the expression cassette or vector genome comprises a transgene and one miR-183 target sequence. In yet other embodiments, the expression cassette or vector genome comprises at least two, three or four miR-183 target sequences. (i) AGTGAATTCTACCAGTGCCATA (miR183, SEQ ID NO: 11); (ii) AGCAAAAATGTGCTAGTGCCAAA (miR-96, SEQ ID NO: 12).

In certain embodiments, the vector genome or expression cassette contains at least one miRNA target sequence that is a miR-182 target sequence. In certain embodiments, the vector genome or expression cassette contains an miR-182 target sequence that includes AGTGTGAGTTCTACCATTGCCAAA (SEQ ID NO: 13). In certain embodiments, the vector genome or expression cassette contains more than one copy (e.g., two or three copies) of a sequence that is 100% complementary to the miR-182 seed sequence. In certain embodiments, a miR-182 target sequence is about 7 nucleotides to about 28 nucleotides in length and includes at least one region that is at least 100% complementary to the miR-182 seed sequence. In certain embodiments, a miR-182 target sequence contains a sequence with partial complementarity to SEQ ID NO: 13 and, thus, when aligned to SEQ ID NO: 13, there are one or more mismatches. In certain embodiments, a miR-183 target sequence comprises a sequence having at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mismatches when aligned to SEQ ID NO: 13, where the mismatches may be non-contiguous. In certain embodiments, a miR-182

target sequence includes a region of 100% complementarity which also comprises at least 30% of the length of the miR-182 target sequence. In certain embodiments, the region of 100% complementarity includes a sequence with 100% complementarity to the miR-182 seed sequence. In certain embodiments, the remainder of a miR-182 target sequence has at 5 least about 80% to about 99% complementarity to miR-182. In certain embodiments, the expression cassette or vector genome includes a miR-182 target sequence that comprises a truncated SEQ ID NO: 13, i.e., a sequence that lacks at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides at either or both the 5' or 3' ends of SEQ ID NO: 13. In certain embodiments, the expression cassette or vector genome comprises a transgene and one miR-182 target 10 sequence. In yet other embodiments, the expression cassette or vector genome comprises at least two, three or four miR-182 target sequences.

The term “tandem repeats” is used herein to refer to the presence of two or more consecutive miRNA target sequences. These miRNA target sequences may be continuous, i.e., located directly after one another such that the 3' end of one is directly upstream of the 15 5' end of the next with no intervening sequences, or vice versa. In another embodiment, two or more of the miRNA target sequences are separated by a short spacer sequence.

As used herein, as “spacer” is any selected nucleic acid sequence, e.g., of 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 nucleotides in length which is located between two or more consecutive 20 miRNA target sequences. In certain embodiments, the spacer is 1 to 8 nucleotides in length, 2 to 7 nucleotides in length, 3 to 6 nucleotides in length, four nucleotides in length, 4 to 9 nucleotides, 3 to 7 nucleotides, or values which are longer. Suitably, a spacer is a non-coding sequence. In certain embodiments, the spacer may be of four (4) nucleotides. In certain 25 embodiments, the spacer is GGAT. In certain embodiments, the spacer is six (6) nucleotides. In certain embodiments, the spacer is CACGTG or GCATGC.

In certain embodiments, the tandem repeats contain two, three, four or more of the same miRNA target sequence. In certain embodiments, the tandem repeats contain at least 30 two different miRNA target sequences, at least three different miRNA target sequences, or at least four different miRNA target sequences, etc. In certain embodiments, the tandem repeats may contain two or three of the same miRNA target sequence and a fourth miRNA target sequence which is different.

In certain embodiments, there may be at least two different sets of tandem repeats in the expression cassette. For example, a 3' UTR may contain a tandem repeat immediately

downstream of the transgene, UTR sequences, and two or more tandem repeats closer to the 3' end of the UTR. In another example, the 5' UTR may contain one, two or more miRNA target sequences. In another example the 3' may contain tandem repeats and the 5' UTR may contain at least one miRNA target sequence.

5 In certain embodiments, the expression cassette contains two, three, four or more tandem repeats which start within about 0 to 20 nucleotides of the stop codon for the transgene. In other embodiments, the expression cassette contains the miRNA tandem repeats at least 100 to about 4000 nucleotides from the stop codon for the transgene.

In certain embodiments, the spacers between the miRNA target sequences are the
10 same. As used herein, CDKL5 or hCDKL5 refers to isoform 1, unless otherwise specified. Isoforms 2-4 may be specified as: CDKL5-2GS or hCDKL5-2GS, CDKL5-3GS or hCDKL5-3GS, and CDKL5-4GS or hCDKL5-4GS. Expression cassettes and vector genomes with these isoforms may be constructed as described for isoform 1.

See, PCT/US19/67872, filed December 20, 2019, now WO 2020/132455, which is
15 incorporated by reference herein and US Provisional Patent Applications No. 63/023,593, filed May 12, 2020; US Provisional Patent Applications No. 63/038,488, filed June 12, 2020; US Provisional Patent Applications No. 63/043,562, filed June 24, 2020; and US Provisional Patent Applications No. 63/079,299, filed Sept 16, 2020, US Provisional Patent Application No. 63/152,042, filed February 22, 2011, which are hereby incorporated by reference.

20 In certain embodiments, the Clade F AAV capsid is selected from an AA Vhu68 capsid, an AAV9 capsid, an AA Vhu31 capsid, an AA Vhu32 capsid, or an engineered variant of one of these capsids (e.g., AAV-PHP.B). Nucleic acid sequences encoding AA Vhu68 capsid protein, are utilized in the examples below for production of an AAV.hCDKL5 recombinant AAV (rAAV) carrying the vector genome. Additional details relating to
25 AA Vhu68 or AAV-PHP.B are provided in WO 2018/160582, and US 2015/0079038, each of which is incorporated herein by reference in its entirety. The Clade F vectors described herein are well suited for delivery of the vector genome comprising the hCDKL5 coding sequence to cells within the central nervous system, including brain, hippocampus, motor cortex, cerebellum, and motor neurons. These vectors may be used for targeting other cells
30 within the central nervous system (CNS) and certain other tissues and cells outside the CNS.

In certain embodiments, the AAV capsid for the compositions and methods described herein is chosen based on the target cell. In certain embodiment, the AAV capsid

transduces a CNS cell and/or a PNS cell. In certain embodiments, the AAV capsid is selected from a cy02 capsid, a rh43 capsid, an AAV8 capsid, a rh01 capsid, an AAV9 capsid, an rh8 capsid, a rh10 capsid, a bb01 capsid, a hu37 capsid, a rh02 capsid, a rh20 capsid, a rh39 capsid, a rh64 capsid, an AAV6 capsid, an AAV1 capsid, a hu44 capsid, a hu48 capsid, a 5 cy05 capsid a hu11 capsid, a hu32 capsid, a pi2 capsid, or a variation thereof. In certain embodiments, the AAV capsid is a Clade F capsid, such as AAV9 capsid, AAVhu68 capsid, hu31 capsid, hu32 capsid, or a variation thereof. See, e.g., WO 2005/033321 published April 14, 2015, WO 2018/160582, and US 2015/0079038, each of which is incorporated herein by reference in its entirety. In certain embodiments, the AAV capsid is a non-clade F capsid, for 10 example a Clade A, B, C, D, or E capsid. In certain embodiment, the non-Clade F capsid is an AAV1 or a variation thereof. In certain embodiment, the AAV capsid transduces a target cell other than the nervous system cells. In certain embodiments, the AAV capsid is a Clade A capsid (e.g., AAV1, AAV6, AAVrh91), a Clade B capsid (e.g., AAV 2), a Clade C capsid (e.g., hu53), a Clade D capsid (e.g., AAV7), or a Clade E capsid (e.g., rh10). In certain 15 embodiments, the AAV capsid is a Clade A capsid, such as AAVrh91 capsid (nucleic acid sequence of SEQ ID NOs: 33 and 35). See, PCT/US20/030266, filed April 29, 2020, now published WO2020/223231, which is incorporated by reference herein and US Provisional US Patent Application No. 63/065,616, filed April 29, 2019, which is hereby incorporated by reference. See also, US Provisional Application No. 63/065,616, filed August 14, 2020, and 20 US Provisional Patent Application No. 63/109,734, filed November 4, 2020, which are incorporated herein by reference. Still, other AAV capsid may be chosen.

As used herein, the term “clade” as it relates to groups of AAV refers to a group of AAV which are phylogenetically related to one another as determined using a Neighbor-Joining algorithm by a bootstrap value of at least 75% (of at least 1000 replicates) and a 25 Poisson correction distance measurement of no more than 0.05, based on alignment of the AAV vp1 amino acid sequence. The Neighbor-Joining algorithm has been described in the literature. See, e.g., M. Nei and S. Kumar, Molecular Evolution and Phylogenetics (Oxford University Press, New York (2000). Computer programs are available that can be used to implement this algorithm. For example, the MEGA v2.1 program implements the modified 30 Nei-Gojobori method. Using these techniques and computer programs, and the sequence of an AAV vp1 capsid protein, one of skill in the art can readily determine whether a selected AAV is contained in one of the clades identified herein, in another clade, or is outside these

clades. See, e.g., G Gao, et al, J Virol, 2004 Jun; 78(10): 6381-6388, which identifies Clades A, B, C, D, E and F, and provides nucleic acid sequences of novel AAV, GenBank Accession Numbers AY530553 to AY530629. See, also, WO 2005/033321.

A rAAV is composed of an AAV capsid and a vector genome. An AAV capsid is an assembly of a heterogeneous population of vp1, a heterogeneous population of vp2, and a heterogeneous population of vp3 proteins. As used herein when used to refer to vp capsid proteins, the term “heterogeneous” or any grammatical variation thereof, refers to a population consisting of elements that are not the same, for example, having vp1, vp2 or vp3 monomers (proteins) with different modified amino acid sequences.

As used herein when used to refer to vp capsid proteins, the term “heterogeneous” or any grammatical variation thereof, refers to a population consisting of elements that are not the same, for example, having vp1, vp2 or vp3 monomers (proteins) with different modified amino acid sequences. The term “heterogeneous population” as used in connection with vp1, vp2 and vp3 proteins (alternatively termed isoforms), refers to differences in the amino acid sequence of the vp1, vp2 and vp3 proteins within a capsid. The AAV capsid contains subpopulations within the vp1 proteins, within the vp2 proteins and within the vp3 proteins which have modifications from the predicted amino acid residues. These subpopulations include, at a minimum, certain deamidated asparagine (N or Asn) residues. For example, certain subpopulations comprise at least one, two, three or four highly deamidated asparagines (N) positions in asparagine - glycine pairs and optionally further comprising other deamidated amino acids, wherein the deamidation results in an amino acid change and other optional modifications.

In certain embodiments, AAV capsids are provided which have a heterogeneous population of AAV capsid isoforms (i.e., VP1, VP2, VP3) which contain multiple highly deamidated “NG” positions. In certain embodiments, the highly deamidated positions are in the locations identified below, with reference to the predicted full-length VP1 amino acid sequence. In other embodiments, the capsid gene is modified such that the referenced “NG” is ablated and a mutant “NG” is engineered into another position.

As used herein, the terms “target cell” and “target tissue” can refer to any cell or tissue which is intended to be transduced by the subject AAV vector. The term may refer to any one or more of muscle, liver, lung, airway epithelium, central nervous system, neurons, eye (ocular cells), or heart.

As used herein, the term “vector genome” refers to a nucleic acid molecule which is packaged in a viral capsid, for example, an AAV capsid, and is capable of being delivered to a host cell or a cell in a patient. In certain embodiments, the vector genome is an expression cassette having inverted terminal repeat (ITR) sequences necessary for packaging the vector genome into the AAV capsid at the extreme 5' and 3' end and containing therebetween a 5 *CDKL5* gene as described herein operably linked to sequences which direct expression thereof. In certain embodiments, a vector genome may comprise at a minimum from 5' to 3', an AAV 5' ITR, coding sequence(s), and an AAV 3' ITR. In certain embodiments, the ITRs are from AAV2, a different source AAV than the capsid, or other than full-length ITRs may 10 be selected. In certain embodiments, the ITRs are from the same AAV source as the AAV which provides the rep function during production or a transcomplementing AAV. Further, other ITRs may be used. The vector genome is sometimes referred to herein as the “minigene”.

As used herein, the term “host cell” may refer to the packaging cell line in which the 15 rAAV is produced from the plasmid. In the alternative, the term “host cell” may refer to the target cell in which expression of the transgene is desired.

As indicated above, a rAAV is provided which has an AAV capsid which targets the desired cells and a vector genome which comprises, at a minimum, AAV ITRs required to package the vector genome into the capsid, a hCDKL5 coding sequence and regulatory 20 sequences which direct expression therefor. In certain embodiments, the vector genome is a single-stranded AAV vector genome. In certain embodiments, a rAAV vector may be utilized in the invention which contains self-complementary (sc) AAV vector genome.

The AAV sequences of the vector typically comprise the cis-acting 5' and 3' inverted 25 terminal repeat (ITR) sequences (See, e.g., B. J. Carter, in “Handbook of Parvoviruses”, ed., P. Tijsser, CRC Press, pp. 155 168 (1990)). The ITR sequences are about 145 base pairs (bp) in length. Preferably, substantially the entire sequences encoding the ITRs are used in the molecule, although some degree of minor modification of these sequences is permissible. The ability to modify these ITR sequences is within the skill of the art. (See, e.g., texts such 30 as Sambrook et al, “Molecular Cloning. A Laboratory Manual”, 2d ed., Cold Spring Harbor Laboratory, New York (1989); and K. Fisher et al., J. Virol., 70:520 532 (1996)). An example of such a molecule employed in the present invention is a “cis-acting” plasmid containing the transgene, in which the selected transgene sequence and associated regulatory

elements are flanked by the 5' and 3' AAV ITR sequences. In one embodiment, the ITRs are from an AAV different than that supplying a capsid. In one embodiment, the ITR sequences are from AAV2. A shortened version of the 5' ITR, termed ΔITR, has been described in which the D-sequence and terminal resolution site (trs) are deleted. In other embodiments, 5 the full-length AAV 5' and 3' ITRs are used. In certain embodiments, the vector genome includes a shortened AAV2 ITR of 130 base pairs, wherein the external A elements is deleted. The shortened ITR is reverted back to the wild-type length of 145 base pairs during vector DNA amplification using the internal A element as a template. However, ITRs from other AAV sources may be selected. Where the source of the ITRs is from AAV2 and the 10 AAV capsid is from another AAV source, the resulting vector may be termed pseudotyped. However, other configurations of these elements may be suitable.

In certain embodiments, vector genomes are constructed which comprise a 5' AAV ITR - promoter – optional enhancer – optional intron – hCDKL5 coding sequence – polyA – 3' ITR. In certain embodiments, vector genomes are constructed which comprise a 5' AAV 15 ITR - promoter – optional enhancer – optional intron – hCDKL5 coding sequence – optionally repeating miR detargetting sequences - polyA – 3' ITR. In certain embodiments, vector genomes are constructed which comprise a 5' AAV ITR - promoter – optional intron – hCDKL5 coding sequence – optional enhancer - polyA – 3' ITR. In certain embodiments, vector genomes are constructed which comprise a 5' AAV ITR - promoter – optional 20 enhancer – optional intron – hCDKL5 coding sequence – optional enhancer - optionally repeating miR detargetting sequences - polyA – 3' ITR. In certain embodiments, the ITRs are from AAV2. In certain embodiments, more than one promoter is present. In certain embodiments, the enhancer is present in the vector genome. In certain embodiments, more than one enhancer is present. In certain embodiments, an intron is present in the vector 25 genome. In certain embodiments, the enhancer and intron are present. In certain embodiments, the polyA is an SV40 poly A (i.e., a polyadenylation (PolyA) signal derived from Simian Virus 40 (SV40) late genes). In certain embodiments, the polyA is a rabbit beta-globin (RBG) poly A. In certain embodiments, the vector genome comprises, at a minimum: a 5' AAV ITR – hSyn promoter – hCDKL5 coding sequence – poly A – 3' ITR. 30 In certain embodiments, the vector genome comprises, at a minimum, a 5' AAV ITR – CB7 promoter – hCDKL5 coding sequence – RBG poly A – 3' ITR. In certain embodiments, the drg detargetting sequences are one, two, three, four or more miR183 sequences as described

herein and are included in the expression cassette. In certain embodiments the hCDKL5 coding sequence is for CDKL5. In certain embodiments the hCDKL5 coding sequence is for CDKL5-2GS. In certain embodiments the hCDKL5 coding sequence is for CDKL5-3GS. In certain embodiments the hCDKL5 coding sequence is for CDKL5-4GS. Optionally, one or
5 more of these vector genomes includes a WPRE element.

As used herein, a vector genome or a rAAV comprising the vector genome is illustrated herein as AAV.promoter (optional).Kozak (optional).intron (optional).CDKL5 coding sequence (e.g., hCDKL5, hCDKL5co, CDKL5, CDKL5co). miRNA (optional).polyA(optional).Stuffer (optional). In certain embodiments, a rAAV is illustrated
10 herein as AAV capsid.promoter (optional).Kozak (optional).intron (optional).CDKL5 coding sequence. miRNA (optional).polyA (optionl).Stuffer (optional). Optionally, one or more of these vector genomes includes a WPRE element.

In certain embodiments the vector genome comprises, at a minimum a 5' AAV ITR – Ubiquitin C promoter – hCDKL5 coding sequence – one, two, three, four or more miR183 sequences – RBG poly A – 3' ITR. In certain embodiments the vector genome comprises, at
15 a minimum a 5' AAV ITR – Chicken-beta actin hybrid promoter – hCDKL5 coding sequence – one, two, three, four or more miR183 sequences – RBG poly A – 3' ITR. Optionally, one or more of these vector genomes includes a WPRE element.

Additionally, provided herein, is an rAAV production system useful for producing a
20 rAAV as described herein. The production system comprises a cell culture comprising (a) a nucleic acid sequence encoding an AAV capsid protein; (b) the vector genome; and (c) sufficient AAV rep functions and helper functions to permit packaging of the vector genome into the AAV capsid. In certain embodiments, the vector genome is SEQ ID NOs: 1, 3, 5, 7,
25 9, 29 or 31. In certain embodiments, the cell culture is a human embryonic kidney 293 cell culture. In certain embodiments, the AAV rep is from a different AAV. In certain
embodiments, wherein the AAV rep is from AAV2. In certain embodiments, the AAV rep coding sequence and cap genes are on the same nucleic acid molecule, wherein there is
optionally a spacer between the rep sequence and cap gene. In certain embodiments, the
spacer is atgacttaaacccagg (SEQ ID NO: 15).

30 For use in producing an AAV viral vector (e.g., a recombinant (r) AAV), the vector genomes can be carried on any suitable vector, e.g., a plasmid, which is delivered to a packaging host cell. The plasmids useful in this invention may be engineered such that they

are suitable for replication and packaging in vitro in prokaryotic cells, insect cells, mammalian cells, among others. Suitable transfection techniques and packaging host cells are known and/or can be readily designed by one of skill in the art.

Methods for generating and isolating AAVs suitable for use as vectors are known in the art. See generally, e.g., Griege & Samulski, 2005, Adeno-associated virus as a gene therapy vector: Vector development, production and clinical applications, *Adv. Biochem. Engin/Biotechnol.* 99: 119-145; Buning et al., 2008, Recent developments in adeno-associated virus vector technology, *J. Gene Med.* 10:717-733; and the references cited below, each of which is incorporated herein by reference in its entirety. As used herein, a gene therapy vector refers to a rAAV as described herein, which is suitable for use in treating a patient. For packaging a gene into virions, the ITRs are the only AAV components required in cis in the same construct as the nucleic acid molecule containing the gene. The cap and rep genes can be supplied in trans.

In one embodiment, the selected genetic element may be delivered to an AAV packaging cell by any suitable method, including transfection, electroporation, liposome delivery, membrane fusion techniques, high velocity DNA-coated pellets, viral infection and protoplast fusion. Stable AAV packaging cells can also be made. The methods used to make such constructs are known to those with skill in nucleic acid manipulation and include genetic engineering, recombinant engineering, and synthetic techniques. See, e.g., *Molecular Cloning: A Laboratory Manual*, ed. Green and Sambrook, Cold Spring Harbor Press, Cold Spring Harbor, NY (2012).

The term "AAV intermediate" or "AAV vector intermediate" refers to an assembled rAAV capsid which lacks the desired genomic sequences packaged therein. These may also be termed an "empty" capsid. Such a capsid may contain no detectable genomic sequences of an expression cassette, or only partially packaged genomic sequences which are insufficient to achieve expression of the gene product. These empty capsids are non-functional to transfer the gene of interest to a host cell.

The recombinant adeno-associated virus (AAV) described herein may be generated using techniques which are known. See, e.g., WO 2003/042397; WO 2005/033321, WO 2006/110689; US 7588772 B2. Such a method involves culturing a host cell which contains a nucleic acid sequence encoding an AAV capsid protein; a functional rep gene; an expression cassette composed of, at a minimum, AAV inverted terminal repeats (ITRs) and a

transgene; and sufficient helper functions to permit packaging of the expression cassette into the AAV capsid protein. Methods of generating the capsid, coding sequences therefor, and methods for production of rAAV viral vectors have been described. See, e.g., Gao, et al, Proc. Natl. Acad. Sci. U.S.A. 100 (10), 6081-6086 (2003) and US 2013/0045186A1.

5 In one embodiment, a production cell culture useful for producing a recombinant AAVhu68 or AA VRh91 is provided. Such a cell culture contains a nucleic acid which expresses the AAVhu68 capsid protein in the host cell; a nucleic acid molecule suitable for packaging into the AAVhu68 capsid, e.g., a vector genome which contains AAV ITRs and a non-AAV nucleic acid sequence encoding a gene operably linked to regulatory sequences
10 which direct expression of the gene in a host cell; and sufficient AAV rep functions and adenovirus helper functions to permit packaging of the vector genome into the recombinant AAVhu68, or AA VRh91 capsid. In one embodiment, the cell culture is composed of mammalian cells (e.g., human embryonic kidney 293 cells, among others) or insect cells (e.g., Spodoptera frugiperda (Sf9) cells). In certain embodiments, baculovirus provides the
15 helper functions necessary for packaging the vector genome into the recombinant AAVhu68, or AA VRh91 capsid.

20 Optionally the rep functions are provided by an AAV other than hu68 or AAV- PHP.B. In certain embodiments, at least parts of the rep functions are from AAVhu68, or AAVrh91. In another embodiment, the rep protein is a heterologous rep protein other than AAVhu68rep, for example but not limited to, AAV1 rep protein, AAV2 rep protein, AAV3 rep protein, AAV4 rep protein, AAV5 rep protein, AAV6 rep protein, AAV7 rep protein, AAV8 rep protein; or rep 78, rep 68, rep 52, rep 40, rep68/78 and rep40/52; or a fragment thereof; or another source. Any of these AAVhu68 or mutant AAV capsid sequences may be under the control of exogenous regulatory control sequences which direct expression thereof
25 in a host cell.

20 In one embodiment, cells are manufactured in a suitable cell culture (e.g., HEK 293 or Sf9) or suspension. Methods for manufacturing the gene therapy vectors described herein include methods well known in the art such as generation of plasmid DNA used for production of the gene therapy vectors, generation of the vectors, and purification of the
30 vectors. In some embodiments, the gene therapy vector is an AAV vector and the plasmids generated are an AAV cis-plasmid encoding the AAV vector genome and the gene of interest, an AAV trans-plasmid containing AAV rep and cap genes, and an adenovirus helper

plasmid. The vector generation process can include method steps such as initiation of cell culture, passage of cells, seeding of cells, transfection of cells with the plasmid DNA, post-transfection medium exchange to serum free medium, and the harvest of vector-containing cells and culture media. The harvested vector-containing cells and culture media are referred to herein as crude cell harvest. In yet another system, the gene therapy vectors are introduced into insect cells by infection with baculovirus-based vectors. For reviews on these production systems, see generally, e.g., Zhang et al., 2009, Adenovirus-adeno-associated virus hybrid for large-scale recombinant adeno-associated virus production, Human Gene Therapy 20:922-929, the contents of each of which is incorporated herein by reference in its entirety. Methods of making and using these and other AAV production systems are also described in the following US patents, the contents of each of which is incorporated herein by reference in its entirety: US Patent Nos. 5,139,941; 5,741,683; 6,057,152; 6,204,059; 6,268,213; 6,491,907; 6,660,514; 6,951,753; 7,094,604; 7,172,893; 7,201,898; 7,229,823; and 7,439,065.

The crude cell harvest may thereafter be subject method steps such as concentration of the vector harvest, diafiltration of the vector harvest, microfluidization of the vector harvest, nuclease digestion of the vector harvest, filtration of microfluidized intermediate, crude purification by chromatography, crude purification by ultracentrifugation, buffer exchange by tangential flow filtration, and/or formulation and filtration to prepare bulk vector.

A two-step affinity chromatography purification at high salt concentration followed anion exchange resin chromatography are used to purify the vector drug product and to remove empty capsids. These methods are described in more detail in WO 2017/160360 filed December 9, 2016 and its priority documents, US Patent Application Nos. 25 62/322,071, filed April 13, 2016 and 62/226,357, filed December 11, 2015 and entitled “Scalable Purification Method for AAV9”, which is incorporated by reference herein. In certain embodiments, the purification of vector drug product (e.g., AA Vrh91) include those described in more detail in WO2017/100674, filed December 9, 2016, and its priority documents, US Provisional Patent Application Nos. 62/266,351. Filed December 9, 2015, 30 and 62/322,083, filed April 13, 2016 and titled “Scalable Purification Method for AAV1”, which is incorporated herein by reference.

To calculate empty and full particle content, VP3 band volumes for a selected sample (e.g., in examples herein an iodixanol gradient-purified preparation where # of genome copies (GC) = # of particles) are plotted against GC particles loaded. The resulting linear equation ($y = mx + c$) is used to calculate the number of particles in the band volumes of the test article peaks. The number of particles (pt) per 20 μ L loaded is then multiplied by 50 to give particles (pt) /mL. Pt/mL divided by GC/mL gives the ratio of particles to genome copies (pt/GC). Pt/mL–GC/mL gives empty pt/mL. Empty pt/mL divided by pt/mL and $\times 100$ gives the percentage of empty particles.

Generally, methods for assaying for empty capsids and AAV vector particles with packaged genomes have been known in the art. See, e.g., Grimm et al., Gene Therapy (1999) 6:1322-1330; Sommer et al., Molec. Ther. (2003) 7:122-128. To test for denatured capsid, the methods include subjecting the treated AAV stock to SDS-polyacrylamide gel electrophoresis, consisting of any gel capable of separating the three capsid proteins, for example, a gradient gel containing 3-8% Tris-acetate in the buffer, then running the gel until sample material is separated, and blotting the gel onto nylon or nitrocellulose membranes, preferably nylon. Anti-AAV capsid antibodies are then used as the primary antibodies that bind to denatured capsid proteins, preferably an anti-AAV capsid monoclonal antibody, most preferably the B1 anti-AAV-2 monoclonal antibody (Wobus et al., J. Virol. (2000) 74:9281-9293). A secondary antibody is then used, one that binds to the primary antibody and contains a means for detecting binding with the primary antibody, more preferably an anti-IgG antibody containing a detection molecule covalently bound to it, most preferably a sheep anti-mouse IgG antibody covalently linked to horseradish peroxidase. A method for detecting binding is used to semi-quantitatively determine binding between the primary and secondary antibodies, preferably a detection method capable of detecting radioactive isotope emissions, electromagnetic radiation, or colorimetric changes, most preferably a chemiluminescence detection kit. For example, for SDS-PAGE, samples from column fractions can be taken and heated in SDS-PAGE loading buffer containing reducing agent (e.g., DTT), and capsid proteins were resolved on pre-cast gradient polyacrylamide gels (e.g., Novex). Silver staining may be performed using SilverXpress (Invitrogen, CA) according to the manufacturer's instructions or other suitable staining method, i.e., SYPRO ruby or coomassie stains. In one embodiment, the concentration of AAV vector genomes (vg) in column fractions can be measured by quantitative real time PCR (Q-PCR). Samples

are diluted and digested with DNase I (or another suitable nuclease) to remove exogenous DNA. After inactivation of the nuclease, the samples are further diluted and amplified using primers and a TaqMan™ fluorogenic probe specific for the DNA sequence between the primers. The number of cycles required to reach a defined level of fluorescence (threshold cycle, Ct) is measured for each sample on an Applied Biosystems Prism 7700 Sequence Detection System. Plasmid DNA containing identical sequences to that contained in the AAV vector is employed to generate a standard curve in the Q-PCR reaction. The cycle threshold (Ct) values obtained from the samples are used to determine vector genome titer by normalizing it to the Ct value of the plasmid standard curve. End-point assays based on the digital PCR can also be used.

In one aspect, an optimized q-PCR method is used which utilizes a broad spectrum serine protease, e.g., proteinase K (such as is commercially available from Qiagen). More particularly, the optimized qPCR genome titer assay is similar to a standard assay, except that after the DNase I digestion, samples are diluted with proteinase K buffer and treated with proteinase K followed by heat inactivation. Suitably samples are diluted with proteinase K buffer in an amount equal to the sample size. The proteinase K buffer may be concentrated to 2 fold or higher. Typically, proteinase K treatment is about 0.2 mg/mL, but may be varied from 0.1 mg/mL to about 1 mg/mL. The treatment step is generally conducted at about 55 °C for about 15 minutes, but may be performed at a lower temperature (e.g., about 37 °C to about 50 °C) over a longer time period (e.g., about 20 minutes to about 30 minutes), or a higher temperature (e.g., up to about 60 °C) for a shorter time period (e.g., about 5 to 10 minutes). Similarly, heat inactivation is generally at about 95 °C for about 15 minutes, but the temperature may be lowered (e.g., about 70 to about 90 °C) and the time extended (e.g., about 20 minutes to about 30 minutes). Samples are then diluted (e.g., 1000 fold) and subjected to TaqMan analysis as described in the standard assay.

Additionally, or alternatively, droplet digital PCR (ddPCR) may be used. For example, methods for determining single-stranded and self-complementary AAV vector genome titers by ddPCR have been described. See, e.g., M. Lock et al, Hu Gene Therapy Methods, Hum Gene Ther Methods. 2014 Apr;25(2):115-25. doi: 10.1089/hgtb.2013.131. Epub 2014 Feb 14.

In brief, the method for separating rAAVhu68 (or AA VRh91) particles having packaged genomic sequences from genome-deficient AAVhu68 (or AA VRh91) intermediates

involves subjecting a suspension comprising recombinant AAVhu68 (or rh91) viral particles and AAVhu68 (or AA VRh91) capsid intermediates to fast performance liquid chromatography, wherein the AAVhu68 (or AA VRh91) viral particles and AAVhu68 intermediates are bound to a strong anion exchange resin equilibrated at a pH of about 10.2
5 (or about 9.8 for AA VRh91), and subjected to a salt gradient while monitoring eluate for ultraviolet absorbance at about 260 nanometers (nm) and about 280 nm. Although less optimal for rAAVhu68 and AA VRh91, the pH may be in the range of about 10 to 10.4. In this method, the AAV full capsids are collected from a fraction which is eluted when the ratio of A260/A280 reaches an inflection point. In one example, for the Affinity
10 Chromatography step, the diafiltered product may be applied to an affinity resin (Life Technologies) that efficiently captures the AAVhu68 or AA VRh91 serotype. Under these ionic conditions, a significant percentage of residual cellular DNA and proteins flow through the column, while AAV particles are efficiently captured.

The rAAV.hCDKL5 is suspended in a suitable physiologically compatible
15 composition (e.g., a buffered saline). This composition may be frozen for storage, later thawed and optionally diluted with a suitable diluent. Alternatively, the vector may be prepared as a composition which is suitable for delivery to a patient without proceeding through the freezing and thawing steps.

As used herein, the term "NAb titer" a measurement of how much neutralizing
20 antibody (e.g., anti-AAV Nab) is produced which neutralizes the physiologic effect of its targeted epitope (e.g., an AAV). Anti-AAV NAb titers may be measured as described in, e.g., Calcedo, R., et al., Worldwide Epidemiology of Neutralizing Antibodies to Adeno-Associated Viruses. Journal of Infectious Diseases, 2009. 199(3): p. 381-390, which is incorporated by reference herein.

25 The abbreviation "sc" refers to self-complementary. "Self-complementary AAV" refers a construct in which a coding region carried by a recombinant AAV nucleic acid sequence has been designed to form an intra-molecular double-stranded DNA template. Upon infection, rather than waiting for cell mediated synthesis of the second strand, the two complementary halves of scAAV will associate to form one double stranded DNA (dsDNA)
30 unit that is ready for immediate replication and transcription. See, e.g., D M McCarty et al, "Self-complementary recombinant adeno-associated virus (scAAV) vectors promote efficient transduction independently of DNA synthesis", Gene Therapy, (August 2001), Vol

8, Number 16, Pages 1248-1254. Self-complementary AAVs are described in, e.g., U.S. Patent Nos. 6,596,535; 7,125,717; and 7,456,683, each of which is incorporated herein by reference in its entirety.

A "replication-defective virus" or "viral vector" refers to a synthetic or artificial viral particle in which an expression cassette containing a gene of interest is packaged in a viral capsid or envelope, where any viral genomic sequences also packaged within the viral capsid or envelope are replication-deficient; i.e., they cannot generate progeny virions but retain the ability to infect target cells. In one embodiment, the genome of the viral vector does not include genes encoding the enzymes required to replicate (the genome can be engineered to be "gutless" - containing only the gene of interest flanked by the signals required for amplification and packaging of the artificial genome), but these genes may be supplied during production. Therefore, it is deemed safe for use in gene therapy since replication and infection by progeny virions cannot occur except in the presence of the viral enzyme required for replication.

In many instances, rAAV particles are referred to as DNase resistant. However, in addition to this endonuclease (DNase), other endo- and exo- nucleases may also be used in the purification steps described herein, to remove contaminating nucleic acids. Such nucleases may be selected to degrade single stranded DNA and/or double-stranded DNA, and RNA. Such steps may contain a single nuclease, or mixtures of nucleases directed to different targets, and may be endonucleases or exonucleases.

The term "nuclease-resistant" indicates that the AAV capsid has fully assembled around the expression cassette which is designed to deliver a gene to a host cell and protects these packaged genomic sequences from degradation (digestion) during nuclease incubation steps designed to remove contaminating nucleic acids which may be present from the production process.

IV. Other Vector

Provided herein is a vector comprising an expression cassette as described herein. In certain embodiments, the expression cassette comprises a nucleic acid sequence encoding a functional human cyclin dependent kinase like 5 (hCDKL5) under control of regulatory sequences which direct the hCDKL5 expression. In certain embodiments, the hCDKL5-coding sequence encodes a hCDKL5 protein comprising an amino acid sequence of

[MKIPNIGNVMNKFEILGVVGE~~G~~AYGVVLKCRHKETHEIVAIKKFKDSEENEEVKET
 TLRELKMLRTLQENIVELKEAFRRRGKLYLVF~~E~~YVEKNM~~L~~LEEMPGVPPEKV
 KSYIYQLIKAIHWCHKNDIVHRDIKPENLLISHNDVLKLCDFGFARNLSEGNNANYT
 EYVATRWYRSP~~ELL~~LGAPYGKSVD~~M~~WSVG~~C~~ILGELSDGQPLFPG~~E~~SEIDQLFTIQKV
 5 LGPLPSEQMKLFYSNPRFHGLRFPAVNHPQS~~L~~RRLDRSPRS~~A~~KRKPYHVES~~S~~TLSRNQAGKSTALQSHHR
 DRYLTEQCLNHPTFQTQRLLDRSPRS~~A~~KRKPYHVES~~S~~TLSRNQAGKSTALQSHHR
 SNSKDIQNL~~S~~VGLPRADEGLPANESFLNGNLAGASLSPLHTKTYQASSQPGSTS~~K~~D~~L~~
 TN~~NN~~NIPHLLSPKEAKSKTEFDNFIDPKPSEGPGTKYLKSNSRSQQNRHSFMESSQS~~K~~A
 GTLQPNEKQSRHSYIDTIPQSSRSPSYRTKAKSHGALS~~D~~SKSVSNLSEARAQIAEPSTS
 10 RYFPSSC~~L~~D~~L~~NSPTSPT~~P~~TRHSD~~T~~RTLLSPSGRNNRNEGTLDSRRTT~~H~~SKTMEELK
 LPEHMDSSH~~H~~SL~~S~~AP~~H~~ESFSYGLGYTSPFSSQQRPHRHS~~M~~YVTRDKVRAKG~~D~~G~~S~~L
 SIGQGMAARANSLQLLSPQPGEQLPPEMTVARSSVKETSREGTSSFHTRQKSEGGVY
 HDPHSDDGTAPKENRHLYNDPV~~P~~R~~V~~GSFYRVPS~~P~~RD~~N~~S~~H~~ENN~~V~~STRVSSL~~P~~SES
 SSGTNHSKRQPAFDPW~~K~~SPENISHSEQLKEKEKQGFFRSMKKKKKSQ~~T~~VPNSD~~S~~PD
 15 LLTLQKSIHSASTPSSRPKEWRPEKISDLQTQSQLKSLRKL~~L~~HLSSASNHPASSDPRF
 QPLTAQQTKNSFSEIRI~~H~~PLSQASGGSSNIRQEPA~~K~~GR~~P~~ALQLPGQMDPGWHVSSVT
 RSATEG~~P~~SYSEQLGA~~K~~SGP~~N~~GHPYNRTNRSRMPNLNDLKETAL] (SEQ ID NO: 2),
 wherein the hCDKL5-coding sequence is a nucleic acid sequence of SEQ ID NO: 22 or
 which has at least about 95% identical to SEQ ID NO: 22 and encodes the protein of SEQ ID
 20 NO:2. In certain embodiments, the hCDKL5-coding sequence encodes a hCDKL5-2GS
 protein comprising an amino acid sequence of
 [MKIPNIGNVMNKFEILGVVGE~~G~~AYGVVLKCRHKETHEIVAIKKFKDSEENEEVKET
 TLRELKMLRTLQENIVELKEAFRRRGKLYLVF~~E~~YVEKNM~~L~~LEEMPGVPPEKV
 KSYIYQLIKAIHWCHKNDIVHRDIKPENLLISHNDVLKLCDFGFARNLSEGNNANYT
 EYVATRWYRSP~~ELL~~LGAPYGKSVD~~M~~WSVG~~C~~ILGELSDGQPLFPG~~E~~SEIDQLFTIQKV
 25 LGPLPSEQMKLFYSNPRFHGLRFPAVNHPQS~~L~~RRLDRSPRS~~A~~KRKPYHVES~~S~~TLSRNQAGKSTALQSHHR
 DRYLTEQCLNHPTFQTQRLLDRSPRS~~A~~KRKPYHVES~~S~~TLSRNQAGKSTALQSHHR
 SNSKDIQNL~~S~~VGLPRADEGLPANESFLNGNLAGASLSPLHTKTYQASSQPGSTS~~K~~D~~L~~
 TN~~NN~~NIPHLLSPKEAKSKTEFDNFIDPKPSEGPGTKYLKSNSRSQQNRHSFMESSQS~~K~~A
 GTLQPNEKQSRHSYIDTIPQSSRSPSYRTKAKSHGALS~~D~~SKSVSNLSEARAQIAEPSTS
 30 RYFPSSC~~L~~D~~L~~NSPTSPT~~P~~TRHSD~~T~~RTLLSPSGRNNRNEGTLDSRRTT~~H~~SKTMEELK
 LPEHMDSSH~~H~~SL~~S~~AP~~H~~ESFSYGLGYTSPFSSQQRPHRHS~~M~~YVTRDKVRAKG~~D~~G~~S~~L

SIGQGMAARANSLQLLSPQPGEQLPPEMTVARSSVKETSREGTSSFHTRQKSEGGVY
HDPHSDDGTAPKENRHLYNNDPVPRRVGSFYRVPSRPDNSFHENNSTRVSSLSES
SSGTNHSKRQPAFDWKSPENISHSEQLEKEKEKQGFFRSMKKKKKSQTTDSTNGE
NPSIKKSLFPLFNSKNHLKHSSLKKLPVVTPPMVPNSDSDPLLTQKSIHSASTPSSR
5 PKEWRPEKISDLQTQSQPLKSLRKLLHLSSASNHPASSDPRFQPLTAQQTKNSFSEIRI
HPLSQASGGSSNIRQEPAKGRPALQLPGQMDPGWHVSSVTRSATEGPSYSEQLGAK
SGPNGHPYNRTNRSRMPNLNDLKETAL] (SEQ ID NO: 6), wherein the hCDKL5-coding
sequence comprises a nucleic acid sequence of SEQ ID NO: 24 or a sequence which is at
least about 95% identical to SEQ ID NO: 24 and encodes the protein of SEQ ID NO: 6. In
10 certain embodiments, the hCDKL5-coding sequence encodes a hCDKL5-3GS protein
comprising an amino acid sequence of
[MKIPNIGNVMNKFEILGVVGEGAYGVVLKCRHKETHEIVAIKKFKDSEENEEVKET
TLRELKMLRTLKQENIVELKEAFRRRGKLYLVFEYVEKNMELLEEMPNVGVPPEKV
KSYIYQLIKAIHWCHKNDIVHRDIKPENLLISHNDVLKLCDFGFARNLSEGNANYT
15 EYVATRWYRSPPELLLGAPYGKSVDWMSVGCILGELSDGQPLFPGESEIDQLFTIQKV
LGPLPSEQMKLFYSNPRFHGLRFPAVNHPQSLERRYLGILNSVLLDMKNLLKLDPA
DRYLTEQCLNHPTFQTQRLLDRSPSRNQAGKSTALQSHRSNSKDIQNLSVGLPRAD
EGLPANESFLNGNLAGASLSPLHTKYQASSQPGSTSVDLNNNIPHLLSPKEAKSKT
EFDFNIDPKPSEGPGTKYLKSNSRSQQNRHSFMESSQSKAGTLQPNEKQSRHSYIDTI
20 PQSSRSPSYRTKAKSHGALSDSKSVNLSEARAQIAEPSTSRYFPSSCLDLNSPTSPTP
TRHSDTRTLLSPSGRNNRNEGTLDSRRTTRHSKTMEELKLPEHMDSSHSHSLSAPH
ESFSYGLGYTSPFSSQQRPHRHSMYVTRDKVRAKGLDGSLSIGQGMAARANSLQLL
SPQPGEQLPPEMTVARSSVKETSREGTSSFHTRQKSEGGVYHDPHSDDGTAPKENRH
LYNDPVPRRVGSFYRVPSRPDNSFHENNSTRVSSLPESSSGTNHSKRQPAFDPW
25 KSPENISHSEQLEKEKEKQGFFRSMKKKKKSQTVPNSDSDPLLTQKSIHSASTPSSR
PKEWRPEKISDLQTQSQPLKSLRKLLHLSSASNHPASSDPRFQPLTAQQTKNSFSEIRI
HPLSQASGGSSNIRQEPAKGRPALQLPGQMDPGWHVSSVTRSATEGPSYSEQLGAK
SGPNGHPYNRTNRSRMPNLNDLKETAL] (SEQ ID NO: 8), wherein the hCDKL5-
coding sequence comprises a nucleic acid sequence of SEQ ID NO: 25 or which is at least
30 about 95% identical to SEQ ID NO: 25 and encodes the protein of SEQ ID NO: 8. In certain
embodiments, the hCDKL5-coding sequence encodes a hCDKL5-4GS protein comprising an
amino acid sequence of

[MKIPNIGNVMNKFEILGVVGEAGYGVVLKCRHKETHEIVAIKKFKDSEENEEVKET
TLRELKMLRTLQENIVELKEAFRRRGKLYLVFYEYVEKNMLELLEEMPGVPPEKV
KSYIYQLIKAIHWCHKNDIVHRDIKPENLLISHNDVLKLCDFGFARNLSEGNANYT
EYVATRWYRSPPLLGAHYGKSVDWVGCGILGELSDGQPLFPGESIDQLFTIQKV
5 LGPLPSEQMKLFYSNPRFHGLRFPVNHPQSLSERRYLGILNSVLLDMKNLLKLDPA
DRYLTEQCLNHPTFQTQRLLDRSPSRNQAGKSTALQSHRSNSKDIQNLSVGLPRAD
EGLPANESFLNGNLAGASLSPHTKTYQASSQPGSTSVDLTTNNNIPHLLSPKEAKSKT
EFDFNIDPKPSEGPGTKYLKNSRSQQNRHSFMESSQSKAGTLQPNEKQSRHSYIDTI
PQSSRSPSYRTKAOSHGALSDSKSVNLSEARAQIAEPSTSRYFPSSCLDLNSPTSPTP
10 TRHSDTRTLLSPSGRNNRNEGTLDSRRTTRHSKTMEELKLPEHMDSSHSHSLSAPH
ESFSYGLGYTSPFSSQQRPHRHSMYVTRDKVRAKGLDGSLSIGQGMAARANSLQLL
SPQPGEQLPPEMTVARSSVKETSREGTSSFHTRQKSEGGVYHDPSDDGTAPKENRH
LYNDPVPRRVGSFYRVPSPRDNSFHENNSTRVSSLPESSSGTNHSKRQPAFDPW
KSPENISHSEQLKEKEKQGFFRSMKKKKKSQTTDSTNGENPSIKKSLFPLFNSKNHL
15 KHSSLKKLPVVTPPMVPNSDSDPDLTLQKSIHSASTPSSRPKEWRPEKISDLQTQSQP
LKSLRKLLHLSSASNHPASSDPRFQPLTAQQTKNSFSEIRIHPLSQASGGSSNIRQEPA
PKGRPALQLPGQMDPGWHVSSVTRSATEGPSYSEQLGAKSGPNGHPYNRTNRSRMP
NLNDLKETAL] (SEQ ID NO: 10), wherein the hCDKL5-coding sequence comprises a
nucleic acid sequence of SEQ ID NO: 26 or which is at least about 95% identical to SEQ ID
20 NO: 26 and encodes the protein of SEQ ID NO: 10.

In certain embodiments, the vector is a viral vector selected from a recombinant parvovirus, a recombinant lentivirus, a recombinant retrovirus, or a recombinant adenovirus; or a non-viral vector selected from naked DNA, naked RNA, an inorganic particle, a lipid particle, a polymer-based vector, or a chitosan-based formulation. The selected vector may be delivered by any suitable method, including transfection, electroporation, liposome delivery, membrane fusion techniques, high velocity DNA-coated pellets, viral infection and protoplast fusion. The methods used to make such constructs are known to those with skill in nucleic acid manipulation and include genetic engineering, recombinant engineering, and synthetic techniques. See, e.g., Sambrook et al, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, NY.

A "replication-defective virus" or "viral vector" refers to a synthetic or artificial viral particle in which an expression cassette containing a gene of interest is packaged in a viral

capsid or envelope, where any viral genomic sequences also packaged within the viral capsid or envelope are replication-deficient; i.e., they cannot generate progeny virions but retain the ability to infect target cells. In one embodiment, the genome of the viral vector does not include genes encoding the enzymes required to replicate (the genome can be engineered to be "gutless" - containing only the transgene of interest flanked by the signals required for amplification and packaging of the artificial genome), but these genes may be supplied during production. Therefore, it is deemed safe for use in gene therapy since replication and infection by progeny virions cannot occur except in the presence of the viral enzyme required for replication. Such replication-defective viruses may be adeno-associated viruses (AAV), adenoviruses, lentiviruses (integrating or non-integrating), or another suitable virus source.

V. Compositions

Provided herein is a composition comprising an rAAV or a vector as described herein and an aqueous suspension media. In certain embodiments, the suspension is formulated for intravenous delivery, intrathecal administration, or intracerebroventricular administration.

Provided herein are compositions containing at least one rAAV stock and an optional carrier, excipient and/or preservative. As used herein, a "stock" of rAAV refers to a population of rAAV. Despite heterogeneity in their capsid proteins due to deamidation, rAAV in a stock are expected to share an identical vector genome. A stock can include rAAV having capsids with, for example, heterogeneous deamidation patterns characteristic of the selected AAV capsid proteins and a selected production system. The stock may be produced from a single production system or pooled from multiple runs of the production system. A variety of production systems, including but not limited to those described herein, may be selected.

As used herein, "carrier" includes any and all solvents, dispersion media, vehicles, coatings, diluents, antibacterial and antifungal agents, isotonic and absorption delaying agents, buffers, carrier solutions, suspensions, colloids, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Supplementary active ingredients can also be incorporated into the compositions. The phrase "pharmaceutically-acceptable" refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a host. Delivery

vehicles such as liposomes, nanocapsules, microparticles, microspheres, lipid particles, vesicles, and the like, may be used for the introduction of the compositions of the present invention into suitable host cells. In particular, the rAAV vector delivered vector genomes may be formulated for delivery either encapsulated in a lipid particle, a liposome, a vesicle, a 5 nanosphere, or a nanoparticle or the like.

In one embodiment, a composition includes a final formulation suitable for delivery to a subject, e.g., is an aqueous liquid suspension buffered to a physiologically compatible pH and salt concentration. Optionally, one or more surfactants are present in the formulation. In another embodiment, the composition may be transported as a concentrate 10 which is diluted for administration to a subject. In other embodiments, the composition may be lyophilized and reconstituted at the time of administration.

A suitable surfactant, or combination of surfactants, may be selected from among non-ionic surfactants that are nontoxic. In one embodiment, a difunctional block copolymer surfactant terminating in primary hydroxyl groups is selected, e.g., such as Pluronic® F68 15 [BASF], also known as Poloxamer 188, which has a neutral pH, has an average molecular weight of 8400. Other surfactants and other Poloxamers may be selected, i.e., nonionic triblock copolymers composed of a central hydrophobic chain of polyoxypropylene (poly(propylene oxide)) flanked by two hydrophilic chains of polyoxyethylene (poly(ethylene oxide)), SOLUTOL HS 15 (Macrogol-15 Hydroxystearate), LABRASOL 20 (Polyoxy caprylic glyceride), polyoxy 10 oleyl ether, TWEEN (polyoxyethylene sorbitan fatty acid esters), ethanol and polyethylene glycol. In one embodiment, the formulation contains a poloxamer. These copolymers are commonly named with the letter "P" (for poloxamer) followed by three digits: the first two digits x 100 give the approximate molecular mass of the polyoxypropylene core, and the last digit x 10 gives the percentage 25 polyoxyethylene content. In one embodiment Poloxamer 188 is selected. In one embodiment, the surfactant may be present in an amount up to about 0.0005 % to about 0.001% (based on weight ratio, w/w %) of the suspension. In another embodiment, the surfactant may be present in an amount up to about 0.0005 % to about 0.001% (based on volume ratio, v/v %) of the suspension. In yet another embodiment, the surfactant may be 30 present in an amount up to about 0.0005 % to about 0.001% of the suspension, wherein n % indicates n gram per 100 mL of the suspension.

In another embodiment, the composition includes a carrier, diluent, excipient and/or adjuvant. Suitable carriers may be readily selected by one of skill in the art in view of the indication for which the transfer virus is directed. For example, one suitable carrier includes saline, which may be formulated with a variety of buffering solutions (e.g., phosphate buffered saline). Other exemplary carriers include sterile saline, lactose, sucrose, calcium phosphate, gelatin, dextran, agar, pectin, peanut oil, sesame oil, and water. The buffer/carrier should include a component that prevents the rAAV, from sticking to the infusion tubing but does not interfere with the rAAV binding activity in vivo. A suitable surfactant, or combination of surfactants, may be selected from among non-ionic surfactants that are nontoxic. In one embodiment, a difunctional block copolymer surfactant terminating in primary hydroxyl groups is selected, e.g., such as Poloxamer 188 (also known under the commercial names Pluronic® F68 [BASF], Lutrol® F68, Synperonic® F68, Kolliphor® P188) which has a neutral pH, has an average molecular weight of 8400. Other surfactants and other Poloxamers may be selected, i.e., nonionic triblock copolymers composed of a central hydrophobic chain of polyoxypropylene (poly(propylene oxide)) flanked by two hydrophilic chains of polyoxyethylene (poly(ethylene oxide)), SOLUTOL HS 15 (Macrogol-15 Hydroxystearate), LABRASOL (Polyoxy caprylic glyceride), polyoxy -oleyl ether, TWEEN (polyoxyethylene sorbitan fatty acid esters), ethanol and polyethylene glycol. In one embodiment, the formulation contains a poloxamer. These copolymers are commonly named with the letter "P" (for poloxamer) followed by three digits: the first two digits x 100 give the approximate molecular mass of the polyoxypropylene core, and the last digit x 10 gives the percentage polyoxyethylene content. In one embodiment Poloxamer 188 is selected. The surfactant may be present in an amount up to about 0.0005 % to about 0.001% of the suspension.

In certain embodiments, the composition containing the rAAV.hCDKL5 is delivered at a pH in the range of 6.8 to 8, or 7.2 to 7.8, or 7.5 to 8. For intrathecal delivery, a pH above 7.5 may be desired, e.g., 7.5 to 8, or 7.8.

In certain embodiments, the formulation may contain a buffered saline aqueous solution not comprising sodium bicarbonate. Such a formulation may contain a buffered saline aqueous solution comprising one or more of sodium phosphate, sodium chloride, potassium chloride, calcium chloride, magnesium chloride and mixtures thereof, in water, such as a Harvard's buffer. The aqueous solution may further contain Kolliphor® P188, a

poloxamer which is commercially available from BASF which was formerly sold under the trade name Lutrol® F68. The aqueous solution may have a pH of 7.2.

In another embodiment, the formulation may contain a buffered saline aqueous solution comprising 1 mM Sodium Phosphate (Na_3PO_4), 150 mM sodium chloride (NaCl), 5 3mM potassium chloride (KCl), 1.4 mM calcium chloride (CaCl_2), 0.8 mM magnesium chloride (MgCl_2), and 0.001% poloxamer (e.g., Kolliphor®) 188, pH 7.2. See, e.g., harvardapparatus.com/harvard-apparatus-perfusion-fluid.html. In certain embodiments, Harvard's buffer is preferred due to better pH stability observed with Harvard's buffer.

In certain embodiments, the formulation buffer is artificial CSF with Pluronic F68. In 10 other embodiments, the formulation may contain one or more permeation enhancers. Examples of suitable permeation enhancers may include, e.g., mannitol, sodium glycocholate, sodium taurocholate, sodium deoxycholate, sodium salicylate, sodium caprylate, sodium caprate, sodium lauryl sulfate, polyoxyethylene-9-laurel ether, or EDTA.

15 Optionally, the compositions of the invention may contain, in addition to the rAAV and carrier(s), other conventional pharmaceutical ingredients, such as preservatives, or chemical stabilizers. Suitable exemplary preservatives include chlorobutanol, potassium sorbate, sorbic acid, sulfur dioxide, propyl gallate, the parabens, ethyl vanillin, glycerin, phenol, and parachlorophenol. Suitable chemical stabilizers include gelatin and albumin.

The compositions according to the present invention may comprise a 20 pharmaceutically acceptable carrier, such as defined above. Suitably, the compositions described herein comprise an effective amount of one or more AAV suspended in a pharmaceutically suitable carrier and/or admixed with suitable excipients designed for delivery to the subject via injection, osmotic pump, intrathecal catheter, or for delivery by another device or route. In one example, the composition is formulated for intrathecal delivery. In one example, the composition is formulated for intravenous (iv) delivery.

VI. Uses

Provided herein is a method of treating CDD, comprising administrating an effective amount of an rAAV or a vector as described herein to a subject in need thereof.

30 In certain embodiments, an "effective amount" herein is the amount which achieves amelioration of CDD symptoms and/or delayed CDD progression.

The vectors are administered in sufficient amounts to transfect the cells and to provide sufficient levels of gene transfer and expression to provide a therapeutic benefit without undue adverse effects, or with medically acceptable physiological effects, which can be determined by those skilled in the medical arts. Conventional and pharmaceutically acceptable routes of administration include, but are not limited to, direct delivery to a desired organ (e.g., brain, CSF, the liver (optionally via the hepatic artery), lung, heart, eye, kidney,), oral, inhalation, intranasal, intrathecal, intratracheal, intraarterial, intraocular, intravenous, intramuscular, subcutaneous, intradermal, intraparenchymal, intracerebroventricular, intrathecal, ICM, lumbar puncture and other parenteral routes of administration. Routes of administration may be combined, if desired.

Dosages of the viral vector (for example, rAAV) depend primarily on factors such as the condition being treated, the age, weight and health of the patient, and can thus vary among patients. For example, a therapeutically effective human dosage of the viral vector is generally in the range of from about 25 to about 1000 microliters to about 100 mL of solution containing concentrations of from about 1×10^9 to 1×10^{16} vector genome copies. In certain embodiments, a volume of about 1 mL to about 15 mL, or about 2.5 mL to about 10 mL, or about 5 mL suspension is delivered. In certain embodiments, a volume of about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, or about 15 mL suspension is delivered. In certain embodiments, a dose of about 8.9×10^{12} to 2.7×10^{14} GC total is administered in this volume. In certain embodiments, a dose of about 1.1×10^{10} GC/g brain mass to about 3.3×10^{11} GC/g brain mass is administered in this volume. In certain embodiments, a dose of about 3.0×10^9 , about 4.0×10^9 , about 5.0×10^9 , about 6.0×10^9 , about 7.0×10^9 , about 8.0×10^9 , about 9.0×10^9 , about 1.0×10^{10} , about 1.1×10^{10} , about 1.5×10^{10} , about 2.0×10^{10} , about 2.5×10^{10} , about 3.0×10^{10} , about 3.3×10^{10} , about 3.5×10^{10} , about 4.0×10^{10} , about 4.5×10^{10} , about 5.0×10^{10} , about 5.5×10^{10} , about 6.0×10^{10} , about 6.5×10^{10} , about 7.0×10^{10} , about 7.5×10^{10} , about 8.0×10^{10} , about 8.5×10^{10} , about 9.0×10^{10} , about 9.5×10^{10} , about 1.0×10^{11} , about 1.1×10^{11} , about 1.5×10^{11} , about 2.0×10^{11} , about 2.5×10^{11} , about 3.0×10^{11} , about 3.3×10^{11} , about 3.5×10^{11} , about 4.0×10^{11} , about 4.5×10^{11} , about 5.0×10^{11} , about 5.5×10^{11} , about 6.0×10^{11} , about 6.5×10^{11} , about 7.0×10^{11} , about 7.5×10^{11} , about 8.0×10^{11} , about 8.5×10^{11} , about 9.0×10^{11} GC per gram brain mass is administered in this volume.

The dosage is adjusted to balance the therapeutic benefit against any side effects and such dosages may vary depending upon the therapeutic application for which the recombinant vector is employed. The levels of expression of the transgene product can be monitored to determine the frequency of dosage resulting in viral vectors, preferably AAV 5 vectors containing the minigene. Optionally, dosage regimens similar to those described for therapeutic purposes may be utilized for immunization using the compositions of the invention.

The replication-defective virus compositions can be formulated in dosage units to contain an amount of replication-defective virus that is in the range of about 1.0×10^9 GC to 10 1.0×10^{16} GC (to treat a subject) including all integers or fractional amounts within the range, and preferably 1.0×10^{12} GC to 1.0×10^{14} GC for a human patient. In one embodiment, the compositions are formulated to contain at least 1×10^9 , 2×10^9 , 3×10^9 , 4×10^9 , 5×10^9 , 6×10^9 , 7×10^9 , 8×10^9 , or 9×10^9 GC per dose including all integers or fractional amounts within the range. In another embodiment, the compositions are formulated to contain at least 15 1×10^{10} , 2×10^{10} , 3×10^{10} , 4×10^{10} , 5×10^{10} , 6×10^{10} , 7×10^{10} , 8×10^{10} , or 9×10^{10} GC per dose including all integers or fractional amounts within the range. In another embodiment, the compositions are formulated to contain at least 1×10^{11} , 2×10^{11} , 3×10^{11} , 4×10^{11} , 5×10^{11} , 6×10^{11} , 7×10^{11} , 8×10^{11} , or 9×10^{11} GC per dose including all integers or fractional amounts within the range. In another embodiment, the compositions are formulated to contain at least 1×10^{12} , 2×10^{12} , 3×10^{12} , 4×10^{12} , 5×10^{12} , 6×10^{12} , 7×10^{12} , 8×10^{12} , or 9×10^{12} GC per dose including all integers or fractional amounts within the range. In another embodiment, the compositions are formulated to contain at least 1×10^{13} , 2×10^{13} , 3×10^{13} , 4×10^{13} , 5×10^{13} , 6×10^{13} , 7×10^{13} , 8×10^{13} , or 9×10^{13} GC per dose including all integers or fractional amounts within the range. In another embodiment, the compositions are formulated to contain at least 1×10^{14} , 2×10^{14} , 3×10^{14} , 4×10^{14} , 5×10^{14} , 6×10^{14} , 7×10^{14} , 8×10^{14} , or 9×10^{14} GC per dose including all integers or fractional amounts within the range. In another embodiment, the compositions are formulated to contain at least 1×10^{15} , 2×10^{15} , 3×10^{15} , 4×10^{15} , 5×10^{15} , 6×10^{15} , 7×10^{15} , 8×10^{15} , or 9×10^{15} GC per dose including all integers or fractional amounts within the range.

30 In one embodiment, for human application the dose can range from 1×10^{10} to about 1×10^{15} GC per kg body weight including all integers or fractional amounts within the range. In one embodiment, the effective amount of the vector is about 1×10^9 , 2×10^9 , 3×10^9 , 4×10^9 ,

5x10⁹, 6x10⁹, 7x10⁹, 8x10⁹, or 9x10⁹ GC per kg body weight including all integers or fractional amounts within the range. In another embodiment, the effective amount of the vector is about 1x10¹⁰, 2x10¹⁰, 3x10¹⁰, 4x10¹⁰, 5x10¹⁰, 6x10¹⁰, 7x10¹⁰, 8x10¹⁰, or 9x10¹⁰ GC per kg body weight including all integers or fractional amounts within the range. In another 5 embodiment, the effective amount of the vector is about 1x10¹¹, 2x10¹¹, 3x10¹¹, 4x10¹¹, 5x10¹¹, 6x10¹¹, 7x10¹¹, 8x10¹¹, or 9x10¹¹ GC per kg body weight including all integers or fractional amounts within the range. In another embodiment, the effective amount of the vector is about 1x10¹², 2x10¹², 3x10¹², 4x10¹², 5x10¹², 6x10¹², 7x10¹², 8x10¹², or 9x10¹² GC per kg body weight including all integers or fractional amounts within the range. In another 10 embodiment, the effective amount of the vector is about 1x10¹³, 2x10¹³, 3x10¹³, 4x10¹³, 5x10¹³, 6x10¹³, 7x10¹³, 8x10¹³, or 9x10¹³ GC per kg body weight including all integers or fractional amounts within the range. In another embodiment, the effective amount of the vector is about 1x10¹⁴, 2x10¹⁴, 3x10¹⁴, 4x10¹⁴, 5x10¹⁴, 6x10¹⁴, 7x10¹⁴, 8x10¹⁴, or 9x10¹⁴ GC per kg body weight including all integers or fractional amounts within the range. In another 15 embodiment, the effective amount of the vector is about 1x10¹⁵, 2x10¹⁵, 3x10¹⁵, 4x10¹⁵, 5x10¹⁵, 6x10¹⁵, 7x10¹⁵, 8x10¹⁵, or 9x10¹⁵ GC per kg body weight including all integers or fractional amounts within the range.

In one embodiment, for human application the dose can range from 1x10¹⁰ to about 1x10¹⁵ GC per gram (g) brain mass including all integers or fractional amounts within the 20 range. In one embodiment, the effective amount of the vector is about 1x10⁹, 2x10⁹, 3x10⁹, 4x10⁹, 5x10⁹, 6x10⁹, 7x10⁹, 8x10⁹, or 9x10⁹ GC per gram (g) brain mass including all integers or fractional amounts within the range. In another embodiment, the effective amount of the vector is about 1x10¹⁰, 2x10¹⁰, 3x10¹⁰, 4x10¹⁰, 5x10¹⁰, 6x10¹⁰, 7x10¹⁰, 8x10¹⁰, or 9x10¹⁰ GC per gram (g) brain mass including all integers or fractional amounts within the range. In another embodiment, the effective amount of the vector is about 1x10¹¹, 2x10¹¹, 3x10¹¹, 4x10¹¹, 5x10¹¹, 6x10¹¹, 7x10¹¹, 8x10¹¹, or 9x10¹¹ GC per gram (g) brain mass including all integers or fractional amounts within the range. In another embodiment, the effective amount of the vector is about 1x10¹², 2x10¹², 3x10¹², 4x10¹², 5x10¹², 6x10¹², 7x10¹², 8x10¹², or 9x10¹² GC per gram (g) brain mass including all integers or fractional amounts within the range. In another 25 embodiment, the effective amount of the vector is about 1x10¹³, 2x10¹³, 3x10¹³, 4x10¹³, 5x10¹³, 6x10¹³, 7x10¹³, 8x10¹³, or 9x10¹³ GC per gram (g) brain mass including all integers or fractional amounts within the range. In another embodiment, the effective amount of the vector is about 1x10¹⁴, 2x10¹⁴, 3x10¹⁴, 4x10¹⁴, 5x10¹⁴, 6x10¹⁴, 7x10¹⁴, 8x10¹⁴, or 9x10¹⁴ GC per gram (g) brain mass including all integers or fractional amounts within the range. In another 30 embodiment, the effective amount of the vector is about 1x10¹⁵, 2x10¹⁵, 3x10¹⁵, 4x10¹⁵, 5x10¹⁵, 6x10¹⁵, 7x10¹⁵, 8x10¹⁵, or 9x10¹⁵ GC per gram (g) brain mass including all integers or fractional amounts within the range.

embodiment, the effective amount of the vector is about 1×10^{14} , 2×10^{14} , 3×10^{14} , 4×10^{14} , 5×10^{14} , 6×10^{14} , 7×10^{14} , 8×10^{14} , or 9×10^{14} GC per gram (g) brain mass including all integers or fractional amounts within the range. In another embodiment, the effective amount of the vector is about 1×10^{15} , 2×10^{15} , 3×10^{15} , 4×10^{15} , 5×10^{15} , 6×10^{15} , 7×10^{15} , 8×10^{15} , or 9×10^{15} GC per gram (g) brain mass including all integers or fractional amounts within the range.

- 5 These above doses may be administered in a variety of volumes of carrier, excipient or buffer formulation, ranging from about 25 to about 1000 microliters, or higher volumes, including all numbers within the range, depending on the size of the area to be treated, the viral titer used, the route of administration, and the desired effect of the method.
- 10 In one embodiment, the volume of carrier, excipient or buffer is at least about 25 μ L. In one embodiment, the volume is about 50 μ L. In another embodiment, the volume is about 75 μ L. In another embodiment, the volume is about 100 μ L. In another embodiment, the volume is about 125 μ L. In another embodiment, the volume is about 150 μ L. In another embodiment, the volume is about 175 μ L. In yet another embodiment, the volume is about 200 μ L. In another embodiment, the volume is about 225 μ L. In yet another embodiment, the volume is about 250 μ L. In yet another embodiment, the volume is about 275 μ L. In yet another embodiment, the volume is about 300 μ L. In yet another embodiment, the volume is about 325 μ L. In another embodiment, the volume is about 350 μ L. In another embodiment, the volume is about 375 μ L. In another embodiment, the volume is about 400 μ L. In another embodiment, the volume is about 450 μ L. In another embodiment, the volume is about 500 μ L. In another embodiment, the volume is about 550 μ L. In another embodiment, the volume is about 600 μ L. In another embodiment, the volume is about 650 μ L. In another embodiment, the volume is about 700 μ L. In another embodiment, the volume is between about 700 and 1000 μ L.
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In certain embodiments, the dose may be in the range of about 1×10^9 GC/g brain mass to about 1×10^{12} GC/g brain mass. In certain embodiments, the dose may be in the range of about 1×10^{10} GC/g brain mass to about 3×10^{11} GC/g brain mass. In certain embodiments, the dose may be in the range of about 1×10^{10} GC/g brain mass to about 2.5×10^{11} GC/g brain mass. In certain embodiments, the dose may be in the range of about 5×10^{10} GC/g brain mass.

In one embodiment, the viral constructs may be delivered in doses of from at least about 1×10^9 GC to about 1×10^{15} , or about 1×10^{11} to 5×10^{13} GC. Suitable volumes

for delivery of these doses and concentrations may be determined by one of skill in the art. For example, volumes of about 1 μ L to 150 mL may be selected, with the higher volumes being selected for adults. Typically, for newborn infants a suitable volume is about 0.5 mL to about 10 mL, for older infants, about 0.5 mL to about 15 mL may be selected. For 5 toddlers, a volume of about 0.5 mL to about 20 mL may be selected. For children, volumes of up to about 30 mL may be selected. For pre-teens and teens, volumes up to about 50 mL may be selected. In still other embodiments, a patient may receive an intrathecal administration in a volume of about 5 mL to about 15 mL are selected, or about 7.5 mL to about 10 mL. Other suitable volumes and dosages may be determined. The dosage may be 10 adjusted to balance the therapeutic benefit against any side effects and such dosages may vary depending upon the therapeutic application for which the recombinant vector is employed.

The above-described recombinant vectors may be delivered to host cells according to published methods. The rAAV, preferably suspended in a physiologically compatible 15 carrier, may be administered to a human or non-human mammalian patient. In certain embodiments, for administration to a human patient, the rAAV is suitably suspended in an aqueous solution containing saline, a surfactant, and a physiologically compatible salt or mixture of salts. Suitably, the formulation is adjusted to a physiologically acceptable pH, e.g., in the range of pH 6 to 9, or pH 6.5 to 7.5, pH 7.0 to 7.7, or pH 7.2 to 7.8. As the pH of 20 the cerebrospinal fluid is about 7.28 to about 7.32, for intrathecal delivery, a pH within this range may be desired; whereas for intravenous delivery, a pH of about 6.8 to about 7.2 may be desired. However, other pHs within the broadest ranges and these subranges may be selected for other route of delivery.

As used herein, the terms "intrathecal delivery" or "intrathecal administration" refer 25 to a route of administration for drugs via an injection into the spinal canal, more specifically into the subarachnoid space so that it reaches the cerebrospinal fluid (CSF). Intrathecal delivery may include lumbar puncture, intraventricular (including intracerebroventricular (ICV)), suboccipital/intracisternal, and/or C1-2 puncture. For example, material may be introduced for diffusion throughout the subarachnoid space by means of lumbar puncture. In 30 another example, injection may be into the cisterna magna. In certain embodiment, a rAAV, vector, or composition as described herein is administrated to a subject in need via the intrathecal administration. In certain embodiments, the intrathecal administration is

performed as described in US Patent Publication No. 2018-0339065 A1, published November 29, 2019, which is incorporated herein by reference in its entirety.

As used herein, the terms “intracisternal delivery” or “intracisternal administration” refer to a route of administration for drugs directly into the cerebrospinal fluid of the cisterna magna cerebellomedularis, more specifically via a suboccipital puncture or by direct injection into the cisterna magna or via permanently positioned tube.

In certain embodiments, treatment of the composition described herein has minimal to mild asymptomatic degeneration of DRG sensory neurons in animals and/or in human patients, well-tolerated with respect to sensory nerve toxicity and subclinical sensory neuron lesions.

VII. Apparatus and Method For Delivery of a Pharmaceutical Composition into Cerebrospinal Fluid

In one aspect, the vectors provided herein may be administered intrathecally via the method and/or the device provided in this section and described in WO 2018/160582, which is incorporated by reference herein. Alternatively, other devices and methods may be selected. In certain embodiments, the method comprises the steps of CT-guided sub-occipital injection via spinal needle into the cisterna magna of a patient. As used herein, the term Computed Tomography (CT) refers to radiography in which a three-dimensional image of a body structure is constructed by computer from a series of plane cross-sectional images made along an axis. In certain embodiments, the apparatus is described in US Patent Publication No. 2018-0339065 A1, published November 29, 2019, which is incorporated herein by reference in its entirety.

25 VIII. CDD

“Patient” or “subject”, as used herein interchangeably, means a male or female mammalian animal, including a human, a veterinary or farm animal, a domestic animal or pet, and animals normally used for clinical research. In one embodiment, the subject of these methods and compositions is a human patient. In one embodiment, the subject of these methods and compositions is a male or female human. In certain embodiment, the subject of these methods and compositions is diagnosed with CDD and/or with symptoms of CDD .

The methods and compositions may be used for treatment of any of the stages of CDD. In certain embodiments, the patient is about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 month(s) old, or about 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 12, 13, 14, 15, 16, 17, 18 year(s) old. In certain embodiments, the patient is a toddler, e.g., 18 months to 3 years of age. In certain embodiments, the patient is from 3 years to 6 years of age, from 3 years to 12 years of age, from 3 years to 18 years of age, from 3 years to 30 years of age. In certain embodiments, patients are older than 18 years of age.

Symptoms in CDD include seizures that usually begin within the first 3 months of life, and can appear as early as the first week after birth. The types of seizures change with age, and may follow a predictable pattern. The most common types are generalized tonic-clonic seizures, which involve a loss of consciousness, muscle rigidity, and convulsions; tonic seizures, which are characterized by abnormal muscle contractions; and epileptic spasms, which involve short episodes of muscle jerks. Seizures occur daily in most people with CDKL5 deficiency disorder, although they can have periods when they are seizure-free. Seizures in CDKL5 deficiency disorder are typically resistant to treatment.

Development is impaired in children with CDKL5 deficiency disorder. Most have severe intellectual disability and little or no speech. The development of gross motor skills, such as sitting, standing, and walking, is delayed or not achieved. About one-third of affected individuals are able to walk independently. Fine motor skills, such as picking up small objects with the fingers, are also impaired; about half of affected individuals have purposeful use of their hands. Most people with this condition have vision problems (cortical visual impairment).

Other common features of CDKL5 deficiency disorder include repetitive hand movements (stereotypies), such as clapping, hand licking, and hand sucking; teeth grinding (bruxism); disrupted sleep; feeding difficulties; and gastrointestinal problems including constipation and backflow of acidic stomach contents into the esophagus (gastroesophageal reflux). Some affected individuals have episodes of irregular breathing. Distinctive facial features in some people with CDKL5 deficiency disorder include a high and broad forehead, large and deep-set eyes, a well-defined space between the nose and upper lip (philtrum), full lips, widely spaced teeth, and a high roof of the mouth (palate). Other physical differences can also occur, such as an unusually small head size (microcephaly), side-to-side curvature of the spine (scoliosis), and tapered fingers.

As described above, the terms “increase” “decrease” “reduce” “ameliorate” “improve” “delay” or any grammatical variation thereof, or any similar terms indication a change, means a variation of about 5 fold, about 2 fold, about 1 fold, about 90%, about 80%, about 70%, about 60%, about 50%, about 40%, about 30%, about 20%, about 10%, about 5 % compared to the corresponding reference (e.g., untreated control or a subject in normal condition without CDD), unless otherwise specified.

In certain embodiments, the patient receives medications controlling some signs and symptoms associated with the CDD, such as seizures, muscle stiffness, or problems with breathing, sleep, the gastrointestinal tract or the heart.

In certain embodiments, a diuretic agent may be used in co-therapy in a subject in need thereof. Diuretic agent used may be acetazolamide (Diamox) or other suitable diuretics. In some embodiments, the diuretic agent is administered at the time of gene therapy administration. In some embodiments, the diuretic agent is administered prior to gene therapy administration. In some, embodiments the diuretic agent is administered where the volume of injection is 3 mL.

In certain embodiments, co-therapies may be utilized, which comprise co-administration of Cdkl5-isoform 1, isoform 2, isoform 3, and/or isoform 4- expressing vectors, or various two- or three-way combinations thereof. Optionally, co-therapy may further comprise administration of another active agent. In certain embodiments, co-therapy may comprise enzyme replacement therapy.

Optionally, an immunosuppressive co-therapy may be used in a subject in need. Immunosuppressants for such co-therapy include, but are not limited to, a glucocorticoid, steroids, antimetabolites, T-cell inhibitors, a macrolide (e.g., a rapamycin or rapalog), and cytostatic agents including an alkylating agent, an anti-metabolite, a cytotoxic antibiotic, an antibody, or an agent active on immunophilin. The immune suppressant may include a nitrogen mustard, nitrosourea, platinum compound, methotrexate, azathioprine, mercaptopurine, fluorouracil, dactinomycin, an anthracycline, mitomycin C, bleomycin, mithramycin, IL-2 receptor- (CD25-) or CD3-directed antibodies, anti-IL-2 antibodies, ciclosporin, tacrolimus, sirolimus, IFN- β , IFN- γ , an opioid, or TNF- α (tumor necrosis factor-alpha) binding agent. In certain embodiments, the immunosuppressive therapy may be started 0, 1, 2, 3, 4, 5, 6, 7, or more days prior to or after the gene therapy administration. Such immunosuppressive therapy may involve administration of one, two or more drugs

(e.g., glucocorticoids, prednelisone, micophenolate mofetil (MMF) and/or sirolimus (i.e., rapamycin)). Such immunosuppressive drugs may be administrated to a subject in need once, twice or for more times at the same dose or an adjusted dose. Such therapy may involve co-administration of two or more drugs, the (e.g., prednelisone, micophenolate mofetil (MMF) and/or sirolimus (i.e., rapamycin)) on the same day. One or more of these drugs may be continued after gene therapy administration, at the same dose or an adjusted dose. Such therapy may be for about 1 week (7 days), about 60 days, or longer, as needed. In certain embodiments, a tacrolimus-free regimen is selected.

The words "comprise", "comprises", and "comprising" are to be interpreted inclusively rather than exclusively. The words "consist", "consisting", and its variants, are to be interpreted exclusively, rather than inclusively. While various embodiments in the specification are presented using "comprising" language, under other circumstances, a related embodiment is also intended to be interpreted and described using "consisting of" or "consisting essentially of" language.

The term "expression" is used herein in its broadest meaning and comprises the production of RNA or of RNA and protein. With respect to RNA, the term "expression" or "translation" relates in particular to the production of peptides or proteins. Expression may be transient or may be stable.

It is to be noted that the term "a" or "an", refers to one or more, for example, "an enhancer", is understood to represent one or more enhancer(s). As such, the terms "a" (or "an"), "one or more," and "at least one" is used interchangeably herein.

Throughout the specification, exponents are referred to using the term "e" followed by a numerical value (n). It will be understood with this refers to " $x 10^n$ ". For example, "3e9" is the same as 3×10^9 and "1e13" is the same as 1×10^{13} .

As described above, the term "about" when used to modify a numerical value means a variation of $\pm 10\%$, unless otherwise specified.

Unless defined otherwise in this specification, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art and by reference to published texts, which provide one skilled in the art with a general guide to many of the terms used in the present application.

IX. EXAMPLES

The following examples are illustrative only and are not intended to limit the present invention.

Several models for CDD have been developed and may selected for use in evaluating therapeutic effect. These following models are null for CDKL5 expression: g., a Cdkl5-ko mouse having a deletion in exon 6 (Δ exon 6), Wang et al, Proceedings of the National Academy of Sciences Dec 2012, 109 (52) 21516-21521; DOI: 10.1073/pnas.1216988110; a Cdkl5-ko mouse having a deletion in exon 4 (Δ exon 4) (see, Amendola et al. (2014) Mapping Pathological Phenotypes in a Mouse Model of CDKL5 Disorder. PLoS ONE 9(5): e91613. doi.org/10.1371/journal.pone.0091613 (2014); a Cdkl5 (R59X knock-in) model (available from Jackson Laboratory; see, Tang et al (2019), Tang S, et al. Altered NMDAR signaling underlies autistic-like features in mouse models of CDKL5 deficiency disorder. Nat Commun. 2019;10(1):2655. Published 2019 Jun 14. doi:10.1038/s41467-019-10689-w, and a Cdkl5 (D471fs) model (Rodney Samaco, Baylor College of Medicine, Houston, TX).

In this study we show that restoring CDKL5 expression in the CNS of three CDD mouse models (Cdkl5-ko (exon 6), Cdkl5(R59X). Cdkl5(D471fs)) significantly improves disease symptoms. We developed an AAV gene therapy vector comprised of the AAVhu68 capsid, and an expression cassette with the human synapsin promoter and a engineered human CDKL5 transgene. When the AAV-CDKL5 vector was administered to Cdkl5 knock-out mice via neonatal injection into the lateral brain ventricle we detected robust expression in up to 50% of neurons throughout the brain. AAV administration and human CDKL5 expression were tolerated well. The human CDKL5 was mostly localized to the cytoplasm; protein expression and kinase activity persisted for over 4 months. We subjected cohorts of treated Cdkl5-ko mice to a battery of neurobehavior tests and found significant improvement from untreated Cdkl5-ko phenotype towards behavior outcomes observed in wildtype mice. We then repeated the same study in 2 different CDD mouse models that carry patient-derived frameshift mutations (Cdkl5(R59X) and CDKL5(D471fs) models) instead of the gene knock-out. We obtained very similar results, thus reiterating the curative benefit of our CDKL5 gene therapy vector.

CDKL5 is expressed as at least four different isoforms in the human brain. We generated similar AAV gene therapy vectors for the three alternatively spliced isoforms which were all found to express and exhibit kinase activity in transduced mouse brains.

To test for expression of our AAV-CDKL5 gene therapy vector in a larger animal we conducted a study with rhesus macaques. Through infusion into the cerebrospinal fluid via the cisterna magna, we have been able to achieve vector distribution throughout the entire brain at 0.1 to 1 vector copies per diploid genome. Much higher vector transduction was
5 observed in dorsal root ganglia (DRG). Accordingly, *in situ* hybridization with probes specific for the human CDKL5 sequence showed abundant expression in DRG neurons but much sparser expression in cortical grey matter neurons. CDKL5 administration and expression was generally tolerated well in all six rhesus macaques for 60 days, however, we noticed mild axonopathy of spinal white matter tracts.

10 In summary, we show promising evidence that CDKL5 gene therapy provides a lasting curative benefit to model mice and is tolerated well in non-human primates. Further optimization of this approach may eventually offer an option for clinical intervention in children affected by CDD.

15 **Example 1. Materials and Methods.**

Plasmids. The amino acid sequences for four CDKL5 (cyclin-dependent kinase-like 5, Uniprot ID O76039) that express in human brain¹ were reverse translated into a DNA sequence. The coding sequence was further engineered, e.g., by considering codon frequencies found in the human genome, cryptic RNA splice sites and alternative reading
20 frames. The engineered sequence was cloned into an AAV expression plasmid under the control of the human synapsin promoter². The coding sequence is preceded by a Kozak sequence, followed by an WPRE enhancer cassette (Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element), the SV40 poly A sequence, and framed by AAV2 inverted terminal repeats (ITR). To suppress expression in dorsal root ganglia (DRG), in
25 some experiments the above plasmid was modified to contain four repeats of the miR183 binding site (agtgaattctaccagtgccata) (SEQ ID NO: 11) directly after the CDKL5 coding sequence and before the WPRE sequence. AAV CDKL5 vector was produced at the University of Pennsylvania Vector Core, using either as capsid PHP.B³ for mouse studies or AAV9hu68⁴ or AAVrh91 for mouse and non-human primate studies.

30 **Mouse Studies.** All studies involving mice were approved by the University of Pennsylvania IACUC. We obtained Cdkl5-ko mice from Jackson Laboratories

(B6.129(FVB)-Cdkl5tm1.1Joez/J, strain # 021967) and crossed heterozygous female ko mice with wt C57Bl6 males to obtain the following genotypes of littermates: male hemizygous Cdkl5-ko (also referenced as KO mice or mice), male wt, female heterozygous Cdkl5-ko and female wt. Mice received AAV Cdkl5 vector (dose range 1 x 10¹¹ GC to 5 x 5 10¹¹ GC) or vehicle control (sterile phospho-buffered saline) at 18-21 days of age by retro-orbital injections in a total volume of 100 µl. Alternatively, mice were injected at day of birth intracerebroventricular with a dose of 1 x 10¹⁰ GC to 5 x 10¹⁰ GC in a total volume of 2 µl. Mice were housed mixed in regard of genotype and injection product, weighed and observed at least twice a week, and aged to 11 weeks for males or 14 week for females when they 10 were subjected to behavior testing. We did not observe treatment-related morbidity.

Western Blotting and Tissue Staining. After euthanasia, one cortical hemisphere was flash frozen and subsequently protein lysates were generated using RIPA buffer. Western blotting was carried out with antibodies against human Cdkl5 (S957D, University of Dundee, UK), EB2 (ab45767, Abcam) or EB2 phospho222 (pab01032-P, Covalab, UK). The 15 other brain half was fixed overnight in formalin, embedded in paraffin and thin sections were processed for immunofluorescence staining with the same CDKL5 antibodies.

Behavior Testing. Mice underwent one test per day. Testing time of day, operator and environment was kept the same (60 dB white noise background and 1000 lumens incandescent indirect lighting). Mice were habituated in their home cages before each test for 20 minutes. For the Open Field Assay, a new cage with minimal amount of bedding was placed into an infrared beam array (MedAssociates, Inc.). A single mouse was added in the middle of the cage and the number of beam breaks over the next 30 minutes was automatically recorded, separated into beam breaks close to the ground (general activity) and 3 inches above ground (rearing activity). For the Elevated Zero Maze (EZM, Stoelting Co.), 25 an elevated circular platform with two opposite enclosed quadrants and two open was used to allow uninterrupted exploration. A single mouse was placed in the middle of an open quadrant and movement was video-recorded for 15 minutes. For the Y-Maze (Stoelting Co.), an enclosed platform containing three identical arms in the shape of a Y was used. A single mouse was placed in the arm closest to the operator and its movements were video-recorded 30 for 5 minutes. For the Marble Burying Assay, a fresh cage was filled with 3 inches of AlphaDri bedding (Shepherd Specialty Papers) and gently compacted down. 12 solid-blue

marbles were spaced equally onto the bedding and a single mouse placed in the middle of the cage. The number of marbles that were at least half covered by bedding was recorded after 30 minutes.

- Data analysis.** Data was graphed and analyzed using GraphPad Prism software.
- 5 Video files were recorded in mp4 format at 20fps and analyzed using EthoVision XT software (version 14, Noldus Information Technology). For the Nest Building Assay, mice were single-housed overnight in a new cage, supplied with a standardized 2x2 inch nestlet squares (cotton based). After 24 hours, the quality of the nest was scored on a scale of 1-5 and any remaining untouched nestlet material was weighed.

10 **Non-human primate experiments.** All studies involving non-human primates were approved by the University of Pennsylvania IACUC and carried out according to USDA regulations. Non-human primates (NHPs) for the species Macaca mulatta (*rhesus macaques*) were obtained from Covance Research Products, Inc. Quarantine and animal husbandry was performed according to Gene Therapy Program SOPs. In the month before AAV vector administration and throughout the study body weight, temperature, respiratory rate and heart rate was periodically monitored, and blood and CSF samples obtained. Whole blood was used for cell counts and differentials, and a clinical blood chemistry panel. CSF samples were used for blood cell counts and differentials, and total protein quantification. For AAV vector delivery into the CSF via puncture of the cisterna magna, anesthetized macaques were 15 placed on a procedure table in the lateral decubitus position with the head flexed forward. Using aseptic technique, a 21-27 gauge, 1-1.5 inch Quincke spinal needle (Becton Dickinson) was advanced into the sub-occipital space until the flow of CSF is observed. The needle will be directed at the wider superior gap of the cisterna magna to avoid blood contamination and potential brainstem injury. Correct placement of needle puncture will be 20 verified via myelography, using a fluoroscope. 1 mL of CSF was collected for baseline analysis, prior to dosing. After CSF collection, a luer access extension catheter was connected to the spinal needle to facilitate dosing of 1 ml Iohexol (Trade Name: Omnipaque 180 mg/mL, General Electric Healthcare) contrast media. After verifying needle placement, a syringe containing the test article (volume equivalent to 1 mL plus the volume of syringe 25 and linker dead space) was connected to the flexible linker and injected over 30 ± 5 seconds. The needle was removed, and direct pressure applied to the puncture site.

AAVhu68.hSyn.Cdkl5-1co and AAVhu68.hSyn.Cdkl5-1co vector has injected at a dose of up to 3×10^{13} GC/NHP. At study days 0, 14, 18, 41 and the last study day, a neurological assessment was given to all macaques for detailed evaluation of neurological function.

Briefly, evaluation included posture and gait assessment, cranial nerve assessment,

5 proprioceptive assessment and spinal/nerve reflexes. At study day 56, macaques were euthanized, and gross postmortem examination and necropsy was performed. 25 major tissues were harvested from each macaque in duplicate for either snap freezing or fixation in formalin. DNA or RNA was purified from snap-frozen tissues and used for vector biodistribution or transgene expression analysis, respectively. For vector biodistribution, 10 genome copies (GC) per total DNA weight were determined using a TaqMan qPCR assay with probes directed again the polyA region of the transgene cassette and an internal standard. To quantify transgene expression, total RNA was used to generate cDNA via first strand synthesis with polyT oligonucleotides, followed by TaqMan qPCR with probes specific for the transgene that did not cross-react with the endogenous rhesus CDKL5 sequence. For histopathological analysis, macaque tissue was embedded into paraffin and these sections were stained with H&E solution or CDKL5 antibodies, respectively. Same spinal cord sections were incubated with Luxol Fast Blue to stain myelin. All stained tissues sections were reviewed by a board-certified veterinary pathologist, and abnormal findings verified by peer review.

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Example 2: Gene Therapy AAV vector

The expression construct between the ITRs is comprised of the human synapsin promoter (SEQ ID NO: 23), the engineered coding sequence for human CDKL5, isoform 1 (SEQ ID NO: 22), the WPRE expression enhancer (SEQ ID NO: 27) and SV40 poly A sequence (SEQ ID NO: 28) (FIG 1A). Similarly, the alternative expression constructs 5 contain engineered coding sequences for human CDKL5, isoform 2 (CDKL5-2GS or hCDKL5-2GS; SEQ ID NO: 24), 3 (CDKL5-3GS or hCDKL5-3GS; SEQ ID NO: 25) or 4 (CDKL5-4GS or hCDKL5-4GS; SEQ ID NO: 26), respectively, instead of isoform 1. All tested plasmids expressed well in mouse brain, showing minor differences in ability to phosphorylate EB2 (measurement of CDKL5 kinase activity). We found that the WPRE 10 enhancer is required to obtain human CDKL5 expression levels in the mouse brain that resemble wildtype mouse Cdkl5 expression (FIG 2). CDKL5 was fully active, as determined by its ability to phosphorylate its endogenous target EB2 protein (FIG. 2). The expression and localization of CDKL5 was confirmed via IHC. We also generated an alternative expression construct by swapping the human synapsin with the human Ubiquitin C promoter 15 (Ubc) (FIG 1B) or with the chicken-beta actin hybrid promoter (FIG 1C). The AAV vectors AAVrh91.UbC.CDKL5-1co.miR183 and AAVrh91.CBh.CDKL5-1co.miR183, in AAVrh91 capsid, were administered at a dose of 3×10^{10} GC via neonatal ICV in Cdkl5-ko mice and necropsy was performed at P14. Western blot analysis of mouse brain tissue confirmed the expression of CDKL5 following transduction with AAV vector genomes comprising Ubc or 20 CBh promoters. We observed expression of CDKL5 transgene in mouse brain following the transduction with AAVrh91.UbC.CDKL5-1co.miR183 and AAVrh91.CBh.CDKL5-1co.miR183. A higher CDKL5 expression per cell was observed in hippocampus of mouse brain after transduction with AAVrh91.UbC.CDKL5-1co.miR183. While, lower expression 25 of CDKL5 per cell was observed in hippocampus of mouse brain after transduction with AAVrh91.CBh.CDKL5-1co.miR183, wherein. A higher expression of CDKL5 per cell was observed in cortex of mouse brain after transduction with AAVrh91.UbC.CDKL5-1co.miR183. A lower expression of CDKL5 per cell was observed in cortex of mouse brain after transduction with AAVrh91.CBh.CDKL5-1co.miR183. Furthermore, we compared 30 CDKL5 expression in Cdkl5-ko mouse brain following administration of AAVrh91.UbC.CDKL5-1co.miR183 (3×10^{10} GC, neonatal ICV) and AAvg68.hSyn.CDKL5-1co.miR183 (2.5×10^{10} GC). Very similar expression patterns were observed in mouse brain following administration of the AAV as specified. A CDKL5

expression was observed in mouse brain after transduction either with AAVrh91.UbC.CDKL5-1co.miR183 (AAVrh91 capsid; Ubiquitin C promoter) or with AAVhu68.hSyn.CDKL5-1co.miR183 (AAVhu68 capsid; Synapsin promoter). Additionally, varying doses of AAVrh91.UbC.CDKL5-1co.miR183 and AAVrh91.CBh.CDKL5-1co.miR183 at 1×10^{10} GC, 3×10^{10} GC, and 6×10^{10} GC are examined in Cdkl5-ko mice for behavior effects.

The AAV.UbC.CDKL5-1co.miR183 vector showed therapeutic utility in mice similar to when CDKL5 expression was driven by hSyn. The AAVrh91 capsid when used with AAV vector genome comprising an engineered nucleic acid sequence achieved a CDKL5 expression similar to that of AAVhu68. CDKL5 protein levels in mouse brain were higher with the Ubc promoter compared with the hSyn promoter.

Example 3: Pre-clinical therapeutic benefit of CDKL5 gene therapy in CDD mouse model

To test of the therapeutic benefit CDKL5 gene therapy on Cdkl5 deficient mice, we treated cohort of juvenile Cdkl5-ko (also referenced as KO mice or mice) and wild type (wt) littermates with 5×10^{11} GC (5e11 gc) of AAV9-PHP.B-hSyn-hCDKL5-1co.WPRE vector by retro-orbital IV injection. All treatment groups continued to grow at the same rate and no treatment-related deaths were observed. At 10 weeks of age, mice were subjected to the battery of behavior testing. We observed a robust and statistically significant normalization for the treated group in the Elevated Zero Maze and Open Field Activity tests. There was a marginal improvement in the same group in the rotarod and Y-Maze test, and no improvement in thermal sensitivity.

Given that Cdkl5 is important for neurodevelopment, we wondered whether earlier administration of gene therapy might improve therapeutic outcome in mice. We next treated cohorts of neonatal Cdkl5-ko or wt littermates with doses ranging 6×10^9 GC to 5×10^{10} GC of AAVhu68-hSyn-hCDKL5-1co.WPRE vector per mouse by intraventricular (ICV) injection. Behavioral testing started at 10 weeks of age or at 11 to 14 weeks, where indicated. Overall observation across all groups was that the treatment was well tolerated, there was no treatment related deaths, and normal weight gain (FIGS 9A and 9B) and overall development was observed. A dose-dependent expression of CDKL5 transgene in Cdkl5-ko mouse brain at a dose of 5×10^{10} GC, a dose of 2.5×10^{10} GC, and a dose of 1×10^{10} GC was

observed. At 10 weeks of age, KO mice show the characteristic hind limb clasping phenotype, which was substantially ameliorated in treated ko mice. Therapeutic efficacy of CDKL5 gene therapy was measured by hind limb clasping test. A dose dependent improvement was observed of the severity score in treated CDKL5-ko mice (FIGs 9C-9F).

5 Likewise, the persistent hyperactivity and rearing phenotype found in ko mice was normalized towards wildtype activity. Hippocampal learning and memory were highly improved in treated ko mice in the Y-Maze test (spontaneous alteration index). Therapeutic efficacy of CDKL5 gene therapy was further measured by open field activity test, which measures for correction of hyperactivity in KO and AAV-treated mice. A dose-dependent resolution of hyperactivity in treated Cdkl5-ko mice was observed (FIGs 11A-11F). KO mice are poor nest-builders when overnight single-housed in a fresh cage and do not tear up all nesting material. Treated KO mice drastically improved their nest building capabilities in a dose-dependent manner (FIGs 10A, 10C, 10E). Additional two other neurobehavior assays (marble burying and Y-Maze) showed a strong trend to improvement of phenotype in treated 10 Cdkl5-ko mice (FIGs 10B and 10D). EEG phenotype was rescued in a preliminary collaborative study and may become useful as translational biomarker. Validation of outcomes of key assays (hindlimb clasping, ambulatory activity) are additionally performed with an independent experimental cohort. Larger cohort size (N=18/group) led to more robust statistical significance. Validation of outcomes of key assays (hindlimb clasping, 15 ambulatory activity) with alternative CDD mouse models (R59X, D471fs) is also performed. In summary, CDKL5 gene therapy delivers a functional CDKL5 protein to mouse brain and has dose-dependent therapeutic effect for several neurobehavior outcomes in CDD male 20 mouse models.

After finishing behavior testing, brains were harvested when the mice were about 3 months old. Western blotting revealed robust expression of human CDKL5 in Cdkl5-ko brains, even 3 months after AAV administration. Lastly, human CDKL5 showed robust kinase activity when blotting for phosphorylated EB2 protein.

We also examined the outcome of CDKL5 gene therapy with an alternative CDD mouse model. Cdkl5(D471fs) carry a patient point mutation that leads to a premature stop 30 codon. As in patients, no CDKL5 protein and highly reduced EB phosphorylation levels were found in the brains of these mice. Thus, Cdkl5(D471fs) share the absence of Cdkl5 protein with the previously used Cdkl5-ko mice, however, the genetic background is slightly

different due the method of generation for the mouse models. Neonatal pups were injected at the same concentration as before (5×10^{10} GC, neoICV) and showed robust hCDKL5 protein expression and EB2 phosphorylation 3 months later. A small pilot cohort was subjected to behavior testing. AAV treatment was well tolerated and no morbidity was observed. Hind
5 limb clasping was significantly corrected in treated mutant mice; likewise, the mutant phenotype in the Elevated Zero Maze test was corrected to wildtype behavior in treated mutant mice (In Open Zone, Open Zone Entries. Mutant mice show poor interaction and burying behavior with glass marbles arrange in a grid in a new cage, whereas wildtype mice typically burry almost all. Treated mutant mice show a strong correction of their behavior to
10 wildtype mice. Significant improvement was observed in Cdkl5-ko mice in treated group at higher dose of 5×10^{10} GC/mouse.

We also examined the outcome of CDKL5 gene therapy with an alternative CDD mouse model. Cdkl5(R59X) carry a patient point mutation that introduces a premature stop codon. As in patients, no CDKL5 protein and highly reduced EB phosphorylation levels
15 were found in the brains of these mice. Thus, Cdkl5(R59X) share the absence of Cdkl5 protein with the previously used Cdkl5-ko mice, however, the genetic background is slightly different due the method of generation for the mouse models. Neonatal pups were injected at the same concentration as before (5×10^{10} GC, neoICV) and showed robust hCDKL5 protein expression and EB2 phosphorylation 3 months later. A small pilot cohort was subjected to behavior testing. AAV treatment was well tolerated and no morbidity was observed. Hind
20 limb clasping was significantly corrected in treated mutant mice.

We also examined the outcome of CDKL5 gene therapy with alternative CDD mouse model in heterozygous female Cdkl5-ko mice. Such model reflects most CDD patient (CDD females). Overall assessment showed that heterozygous female Cdkl5-ko mouse
25 neurobehavior phenotype is much milder, with later onset and thus makes robust assessment of a therapeutic outcome more difficult. However, a representative data for hindlimb clasping and ambulatory activity at high dose (5×10^{10} GC, neonatal ICV) shows significant improvement (FIGs 14B and 14C).

Additionally, we examined CDKL5 Gene therapy dose escalation in WT mice. WT
30 (C57Bl6/J) mice were injected at 7.5×10^{10} GC and 1×10^{11} GC (i.e., 1.5x or 2x of previously highest used dose) via neonatal ICV. There was no overt effect observed on weight, development, and survival. Mice appeared normal and did not show hindlimb

clasping or activity changes. There was no observed effects in pathologist review of CNS tissues (FIGs 19A and 19B).

Example 4: Utility human CDKL5 isoforms 2 - 4

5 It has been shown that there are at least 4 detectable Cdkl5 mRNA splice variants found in human and mouse brain, but it has not been established if isoforms 2-4 exist as stable proteins. Isoform 1 accounts for >85% of brain CDKL5.

We codon engineered coding sequences for human CDKL4 isoforms 2-4 and cloned them into the same AAV vector as before. AAV9-PHP.B vector coding for isoforms 1-4 was 10 tail IV injected in adult Cdkl5-ko mice and brains harvested after 2 weeks for Western Blot analysis. We found all four isoforms robustly expressing and displaying the expected gel migration patterns. The engineered sequence which yielded expression for isoforms 2-4 that closely resembled the amount of expression of isoform 1, were chosen for follow up. EB2 phosphorylation levels generated by isoform 2 were slightly higher, and by isoforms three or 15 four slightly lower compared to isoform 1.

The three alternative CDKL5 isoforms have been injected in neonatal Cdkl5-ko mice to test how a potential therapeutic benefit compares to that of isoform 1. FIG 12 shows significant correction of clasping phenotype with treatment by alternative CDKL5 isoforms (2, 3, and 4). FIGs 8A to 8D provide CDKL5 expression levels or activity for AAV.CDLK5 20 vector constructs for the expression isoform 1, isoform 2, isoform 3 or isoform 4. FIG 8A shows expression levels in knock-out mice injected with AAV vectors (5×10^{10} GC, neonatal ICV) expressing each one of these isoforms, as compared to a wild-type mouse injected with vehicle and a knock-out mouse injected with vehicle. FIG 8B shows CDKL5 activity as determined using the pS222EB2 in a wild-type mouse injected with vehicle 25 (PBS), a knock-out mouse injected with vehicle, or the AAV.CDKL5-1co. FIG 8C shows CDKL5 activity as determined using the pS222EB2 for the groups in FIG 8A. FIG 8D shows CDKL5 expression levels for the groups in FIG 8B.

In summary, all isoforms expressed as proteins and have comparable catalytic 30 activity. Therapeutic outcome with isoforms 2, 3 or 4 in CDD mouse model are comparable to isoforms 1 when tested head-to-head (5×10^{10} GC, neonatal ICV). Overall, CDKL5 gene therapy with CDKL5 isoform 1 is a promising and safe approach in male CDD model mice with a significant therapeutic benefit.

EXAMPLE 5: Pilot NHP Study for Toxicity and Safety testing of hCDKL5 Gene Therapy

We wanted to investigate the expression pattern and safety profile of the AAV-hSyn-CDKL5-1co.WPRE construct packaged in an AA Vhu68 capsid in NHPs. Vector was generated using the vector genome described herein and production methods which have been previously described. See, e.g., WO 2018/160582, which is incorporated herein by reference. A group of six rhesus macaques (age 4-6 years old) was injected via the cisterna magna (ICM). We tested different conditions:

5 A. dose 1×10^{14} GC, injected in 1 ml buffer
10 B. dose 1×10^{14} GC, injected in 3 or 5ml buffer
 C. dose 3×10^{14} GC, injected in 3ml buffer
 D. dose 1×10^{14} GC, injected in 1ml buffer, after 2 days of pre-treatment with the diuretic acetazolamide (Diamox®) to reduce CSF
15 production.

Briefly, for non-human primate (NHP) study, the toxicity and safety testing of hCDKL5 gene therapy was performed using an AA Vhu68-hSyn-Cdkl5-1co-WPRE vector. In a preliminary study, three different doses were assessed, 3×10^{12} GC/animal, 1×10^{13} GC/animal and 3×10^{13} GC/animal. For the pilot study, a dose of 1×10^{14} GC/animal was selected, and two different volumes (3 mL and 5 mL) were assessed for delivery of the AAV vector to the cerebrospinal fluid (CSF) via cisternal magna. Other study arms used 1×10^{14} GC/animal with a diuretic (e.g., a Diamox brand acetazolamide) or 3×10^{14} GC/animal (subject).

25 Additionally, we tested a dose of 2×10^{12} , 1×10^{13} , and 3×10^{13} GC/ subject. Treatment with CDKL5 vector was well tolerated, no signs of changes in clinical blood chemistry was observed. Observations from cage-side neurological exams found no changes from baseline.

Necropsy was carried out 28 days after injection, followed by molecular analysis, histology and pathology review. Overall, no major transduction differences in main organs outside CNS were found (e.g., transduction of liver has likely already reach a maximum). No

major transduction differences in spinal cord and DRGs (remain at very high transduction rates) were observed, however significant changes in brain tissues depending on the injection parameters became evident. The highest transduction increase of transduction was seen in the cortex. Diamox lead to a slight increase in transduction efficiency through the brain.

5 FIG. 17 shows vector biodistribution for each NHP across non-neuronal tissue, spinal tract tissues and brain tissue. In FIG. 18, only the vector biodistribution data for brain is shown. The results show that there is an observed strong transduction of dorsal root ganglion tissue (DRGs), moderate to low transduction of brain tissues, and present transduction leakage into neuronal tissues. The pathology result indicated mild axonopathy in dorsal
10 white matter tracts. Slight differences in hCDKL5 mRNA levels were observed. There is a trend for higher mRNA expression when a 3ml injection volume was used. We also visualized hCDKL5 mRNA distribution by in-situ hybridization (ISH). Dorsal root ganglia (DRG) showed very strong mRNA expression across all six NHP. In the motor cortex small numbers of transduced neurons were observed (<10 %). Occasionally, small clusters of
15 transduced neurons were found. There was no significant difference in hCDKL5 mRNA positive neurons in the motor cortex across NHPs in this study.

A pathology review did not find any gross lesions across tissues and NHP. The NHP that had received the highest dose showed mild signs of inflammatory cell infiltration in the liver. Additionally, mild to moderate spinal cord axonopathy and DRG satellitosis in all
20 NHPs.

Additionally, we examine vector expression and safety testing in NHPs of AAVrh91.UbC.CDKL5-1co.miR183 and AAVrh91.CBh.CDKL5-1co.miR183 vectors. Vectors are administered to *C. macaques* at a dose of 3×01^{10} GC via ICM route. Pathology analysis and neuro-examination is performed to evaluate the effects of CDKL5 expression
25 following AAV vector administration.

In conclusion, AAV-CDKL5 vector (SEQ ID NO: 1) examined in our studies can be used to achieve stable CDKL5 protein expression in neurons. AAV-CDKL5 gene therapy significantly improved the phenotype of a CDD mouse model. Additionally, AAV-CDKL5 vector can be efficiently delivered via the cisterna magna to non-human primates and
30 expresses in throughout the CNS.

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All documents cited in this specification are incorporated herein by reference, as is US Provisional Patent Application No. 63/016,036, filed April 27, 2020, and US Provisional Patent Application No. 63/091,032, filed October 13, 2020, US Provisional Application No. 63/109,608, filed November 4, 2020. The Sequence Listing filed herewith, labelled "20-25 9196PCT_SeqListing_ST25.txt", and the sequences and text therein are incorporated by reference. While the invention has been described with reference to particular embodiments, it will be appreciated that modifications can be made without departing from the spirit of the invention. Such modifications are intended to fall within the scope of the appended claims.

CLAIMS:

1. A recombinant adeno-associated virus (rAAV) useful for treating CDKL5 deficiency disorder (CDD), wherein the rAAV comprises:
 - (a) an AAV capsid; and
 - (b) a vector genome packaged in the AAV capsid of (a), wherein the vector genome comprises inverted terminal repeats (ITR) and a hCDKL5-coding sequence, which is a nucleic acid sequence encoding a functional human CDKL5 (hCDKL5), under control of regulatory sequences which direct the hCDKL5 expression in central nervous system cells, and wherein the hCDKL5-coding sequence is nt 699 to 3581 of SEQ ID NO: 3 (SEQ ID NO: 22) or a sequence at least about 95% identical to nt 699 to 3581 of SEQ ID NO: 3 (SEQ ID NO: 22).
2. The rAAV according to claim 1, wherein the hCDKL5-coding sequence is less than 80% identical to any one of hCDKL5 transcript variants 1 to 3 (NM_001037343.1 (SEQ ID NO: 16), NM_NM_001323289.2 (SEQ ID NO: 17) and NM_003159.2 (SEQ ID NO: 18)).
3. The rAAV according to claim 1 or 2, wherein the functional hCDKL5 has an amino acid sequence of SEQ ID NO: 2.
4. A recombinant adeno-associated virus (rAAV) useful for treating CDKL5 deficiency disorder (CDD), wherein the rAAV comprises:
 - (a) an AAV capsid; and
 - (b) a vector genome packaged in the AAV capsid of (a), wherein the vector genome comprises inverted terminal repeats (ITR) and a hCDKL5-coding sequence, which is a nucleic acid sequence encoding a functional human CDKL5 (hCDKL5), under control of regulatory sequences which direct the hCDKL5 expression, and wherein the functional hCDKL5 is a functional hCDKL5 isoform 2 (hCDKL5-2GS), a functional hCDKL5 isoform 3 (hCDKL5-3GS) or a functional hCDKL5 isoform 4 (hCDKL5-4GS).

5. The rAAV according to claim 4, wherein the vector genome comprises a nucleic acid sequence encoding the functional hCDKL5-2GS having an amino acid sequence of SEQ ID NO: 6.

6. The rAAV according to claim 4 or 5, wherein the hCDKL5-coding sequence is SEQ ID NO: 24 or a sequence at least 95% identical thereto which encodes SEQ ID NO: 6.

7. The rAAV according to claim 4, wherein the vector genome comprises a nucleic acid sequence encoding the functional hCDKL5-3GS having an amino acid sequence of SEQ ID NO: 8.

8. The rAAV according to claim 4 or 7, wherein the hCDKL5-coding sequence is SEQ ID NO: 25 or a sequence at least 95% identical thereto which encodes SEQ ID NO: 8.

9. The rAAV system according to claim 4, wherein the vector genome comprises a nucleic acid sequence encoding the functional hCDKL5-4GS having the amino acid sequence of SEQ ID NO: 10.

10. The rAAV according to claim 4 or 9, wherein the hCDKL5-coding sequence is SEQ ID NO: 26 or a sequence at least 95% identical thereto which encodes SEQ ID NO: 10.

11. The rAAV according to any one of claims 1 to 10, wherein the regulatory sequences comprise a neuron specific promoter.

12. The rAAV according to any one of claims 1 to 11, wherein the regulatory sequences comprise a human Synapsin promoter

13. The rAAV according to any one of claims 1 to 10, wherein the regulatory sequences comprise a constitutive promoter.

14. The rAAV according to any one of claims 1 to 10 or claim 13, wherein the regulatory sequences comprise a CB7 promoter.

15. The rAAV according to any one of claims 1 to 10 or claim 13, wherein the regulatory sequences comprise a UbC promoter.

16. The rAAV according to any one of claims 1 to 6, wherein the regulatory elements further comprise one or more of a Kozak sequence, an intron, an enhancer, a TATA signal and a polyA sequence.

17. The rAAV according to any one of claims 1 to 6 or 16, wherein the regulatory elements further comprise a WPRE element.

18. The rAAV according to any one of claims 1 to 16, wherein the vector genome further comprises at least two tandem repeats of dorsal root ganglion (drg)-specific miRNA target sequences in the 3' untranslated region of the hCDKL5, wherein the at least two tandem repeats comprise at least a first miRNA target sequence and at least a second miRNA target sequence which may be the same or different, and target miR183 or miR182.

19. The rAAV according to any one of claim 1 to 16 or 18, wherein the miRNA target sequence for the at least first and/or at least second miRNA target sequence for the expression cassette mRNA or DNA positive strand is (i) AGTGAATTCTACCAGTGCCATA (miR183, SEQ ID NO: 11); and (ii) AGCAAAAATGTGCTAGTGCCAAA (SEQ ID NO: 12).

20. The rAAV according to claim 18 or 19, wherein two or more of the miRNA target sequences are separated by a spacer and each spacer is independently selected from one or more of (A) GGAT; (B) CACGTG; or (C) GCATGC.

21. The rAAV according to claim 20, wherein the spacer located between the miRNA target sequences may be located 3' to the first miRNA target sequence and/or 5' to the last miRNA target sequence.

22. The rAAV according to claim 20 or 21, wherein the spacers between the miRNA target sequences are the same.

23. The rAAV according to any one of claims 1 to 22, wherein the capsid is an AAVu68 capsid, an AAV9 capsid, or AA Vrh91 capsid.

24. A composition comprising a stock of rAAV according to any of claims 1 to 23 and an aqueous suspension media.

25. The composition according to claim 24, wherein the suspension is formulated for intravenous administration, intrathecal administration, intra-cisterna magna administration or intracerebroventricular administration.

26. A vector comprising an expression cassette, wherein the expression cassette comprises a hCDKL5-coding sequence, which is a nucleic acid sequence encoding a functional human CDKL5 (hCDKL5), under control of regulatory sequences which direct the hCDKL5 expression, and wherein the hCDKL5-coding sequence is SEQ ID NO: 22 or a sequence at least about 95% identical to SEQ ID NO: 22.

27. The vector according to claim 26, wherein the vector is
a viral vector selected from a recombinant parvovirus, a recombinant lentivirus, a recombinant retrovirus, or a recombinant adenovirus; or
a non-viral vector selected from naked DNA, naked RNA, an inorganic particle, a lipid particle, a polymer-based vector, or a chitosan-based formulation.

28. A method of treating CDD, comprising administrating an effective amount of the rAAV according to any one of claims 1 to 23 or the vector according to claims 26 to 27 to a subject in need thereof.

29. An rAAV production system useful for producing the rAAV according to any of claims 1 to 23, wherein the production system comprises a cell culture comprising:

- (a) a nucleic acid sequence encoding a Clade F capsid protein;
- (b) a vector genome; and
- (c) sufficient AAV rep functions and helper functions to permit packaging of the vector genome into the Clade F capsid.

30. The rAAV production system according to claim 29, wherein the vector genome is SEQ ID NO: 1.

31. The rAAV production system according to claim 29, wherein the vector genome is SEQ ID NO: 29.

32. The rAAV production system according to claim 29, wherein the vector genome is SEQ ID NO: 31.

33. The rAAV production system according to any of claims 29 to 32, wherein the cell culture is a human embryonic kidney 293 cell culture.

34. The system according to any one of claims 29 to 33, wherein the AAV rep is from a different AAV.

35. The rAAV production system according to claim 29, wherein the vector genome is SEQ ID NO: 3.

36. The rAAV production system according to claim 29, wherein the vector genome is SEQ ID NO: 5.

37. The rAAV production system according to claim 29, wherein the vector genome is SEQ ID NO: 7.

38. The rAAV production system according to claim 29, wherein the vector genome is SEQ ID NO: 9.

39. A vector comprising an expression cassette, wherein the expression cassette comprises a hCDKL5-2GS-coding sequence, which is a nucleic acid sequence encoding a functional human CDKL5 isoform 2 (hCDKL5-2GS), under control of regulatory sequences which direct the hCDKL5-2GS expression, and wherein the hCDKL5-2GS-coding sequence is SEQ ID NO: 24 or a sequence at least about 95% identical to SEQ ID NO: 24.

40. A vector comprising an expression cassette, wherein the expression cassette comprises a hCDKL5-3GS-coding sequence, which is a nucleic acid sequence encoding a functional human CDKL5 isoform 3 (hCDKL5-3GS), under control of regulatory sequences which direct the hCDKL5-3GS expression, and wherein the hCDKL5-3GS-coding sequence is SEQ ID NO: 25 or a sequence at least about 95% identical to SEQ ID NO: 25.

41. A vector comprising an expression cassette, wherein the expression cassette comprises a hCDKL5-4GS-coding sequence, which is a nucleic acid sequence encoding a functional human CDKL5 isoform 4 (hCDKL5-4GS), under control of regulatory sequences which direct the hCDKL5-4GS expression, and wherein the hCDKL5-4GS-coding sequence is SEQ ID NO: 26 or a sequence at least about 95% identical to SEQ ID NO: 26.

FIG 1A



FIG 1B

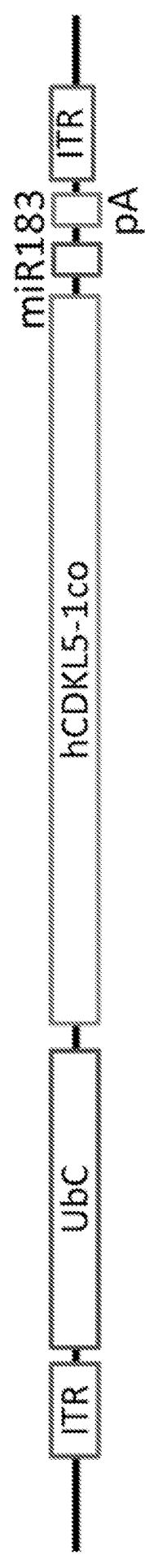


FIG 1C



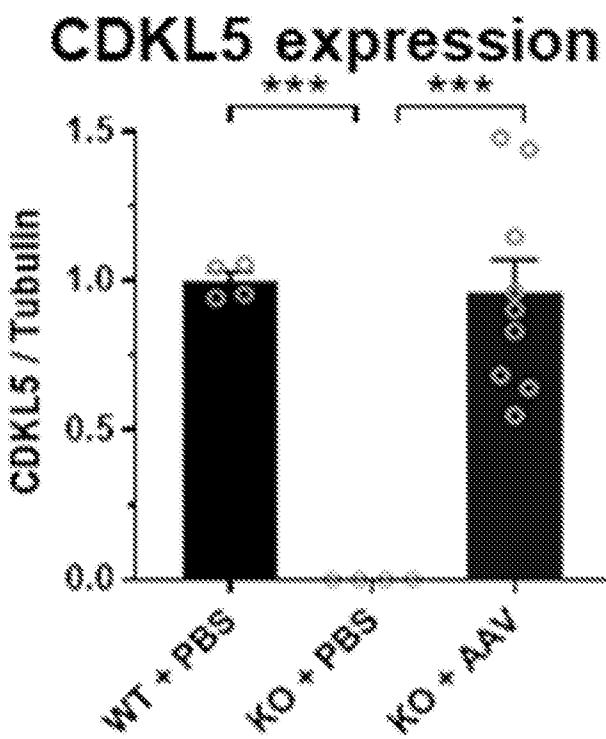
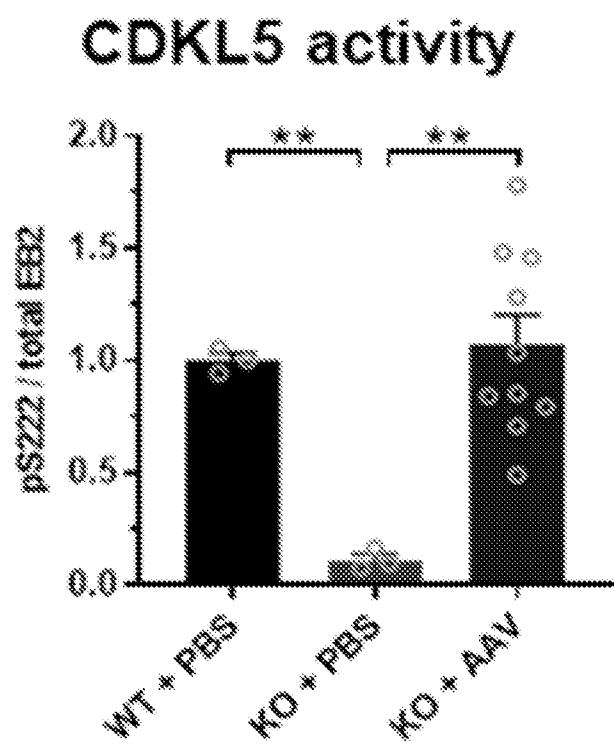
FIG 2A**FIG 2B**

FIG 3

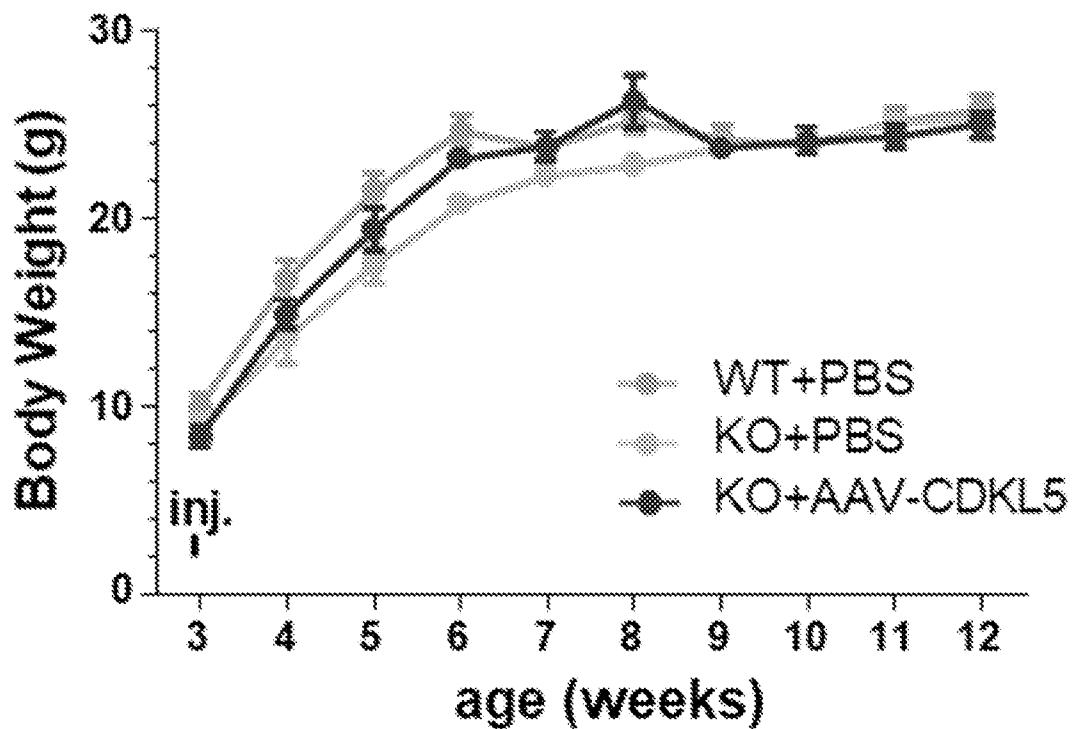


FIG 4

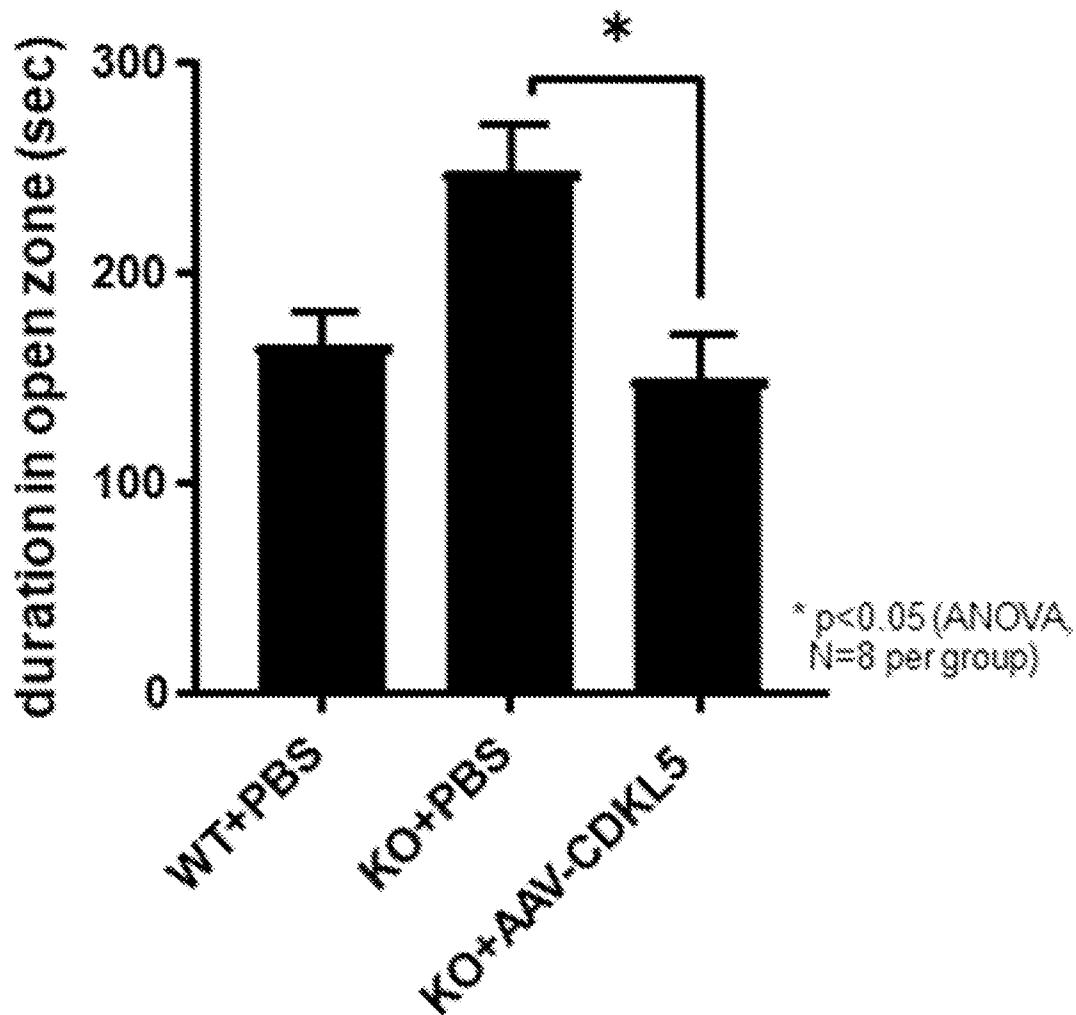


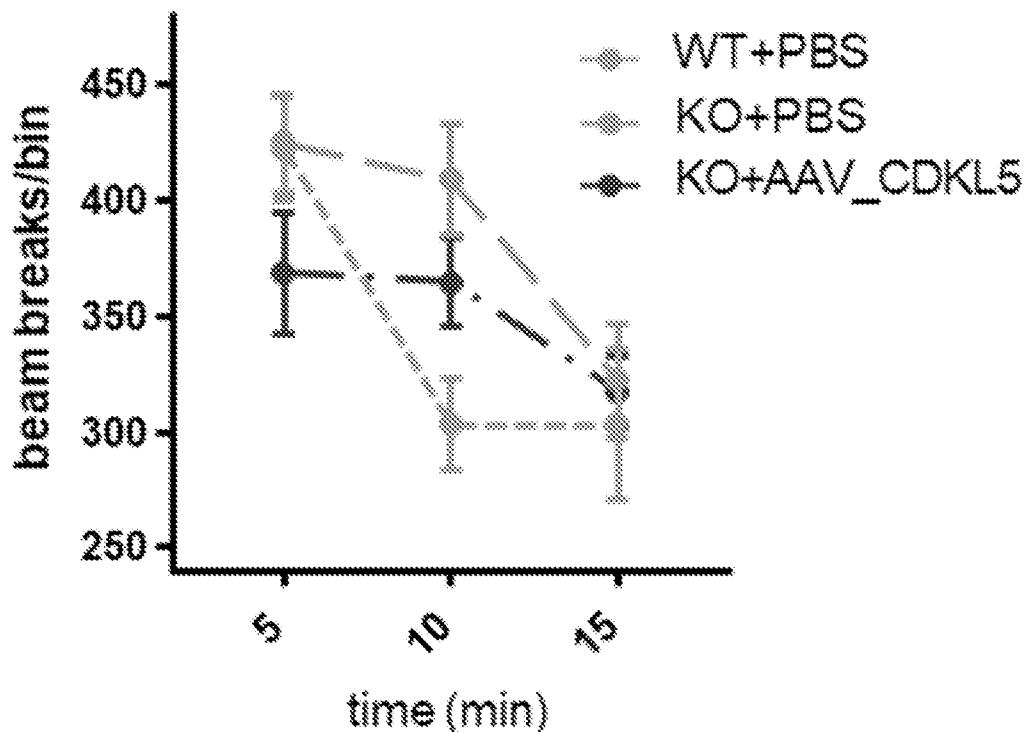
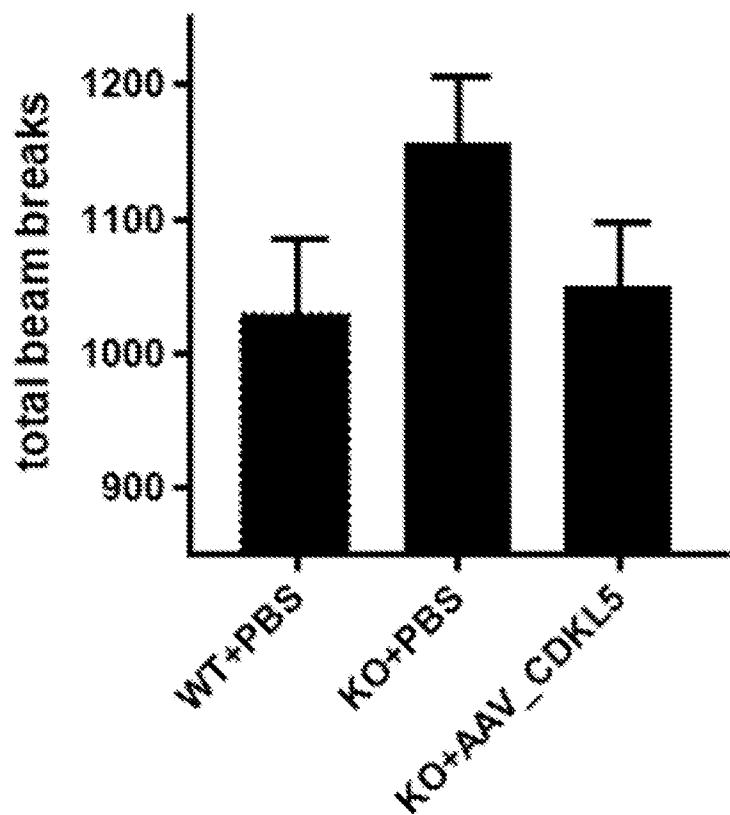
FIG 5A**FIG 5B**

FIG 6

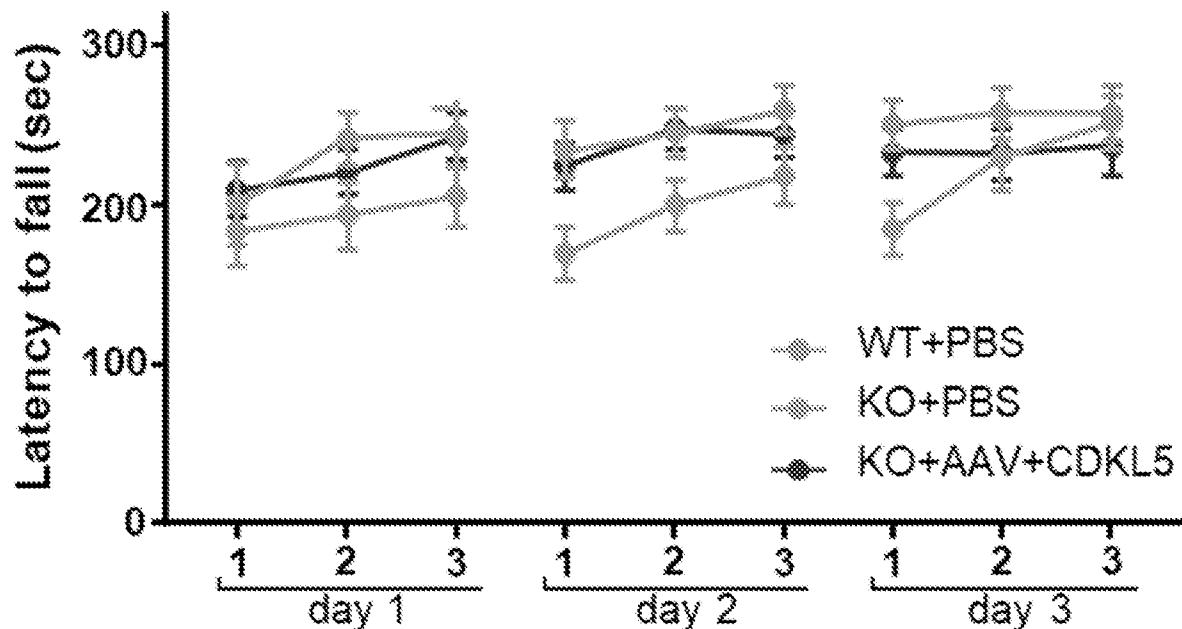


FIG 7A

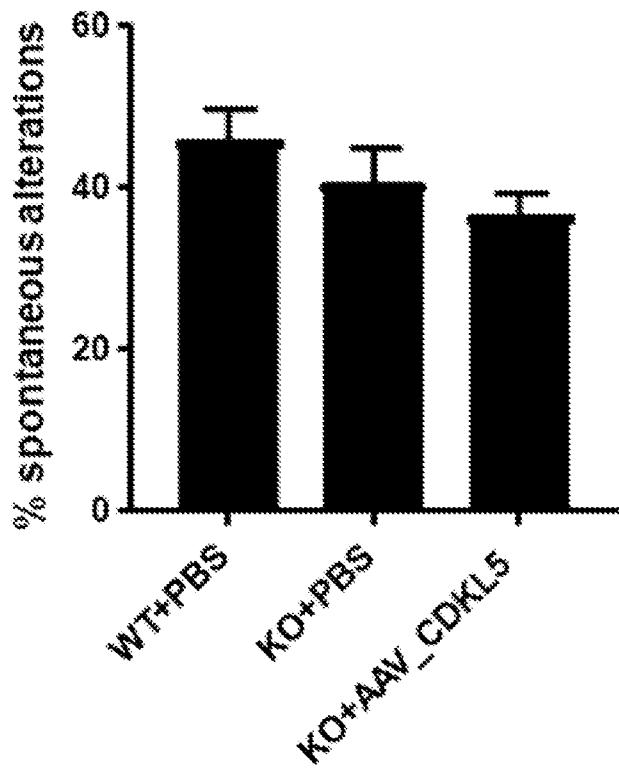


FIG 7B

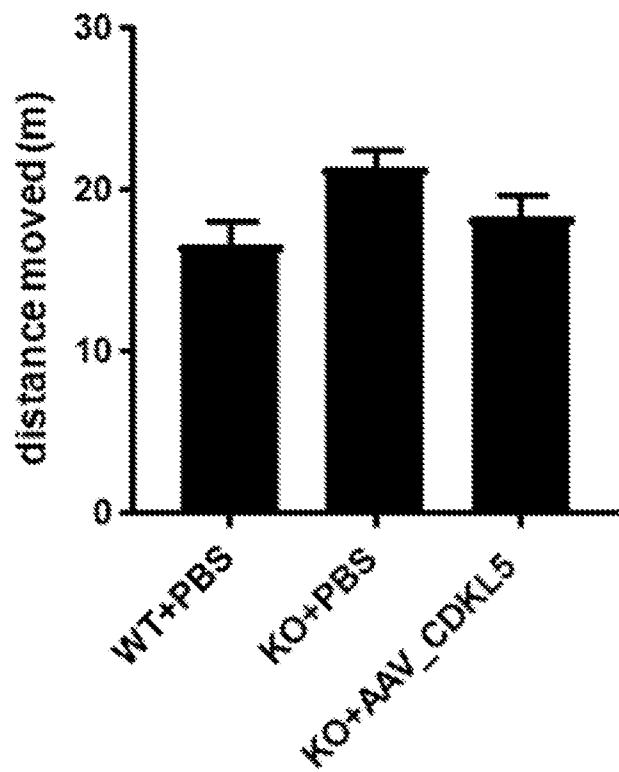


FIG 8A

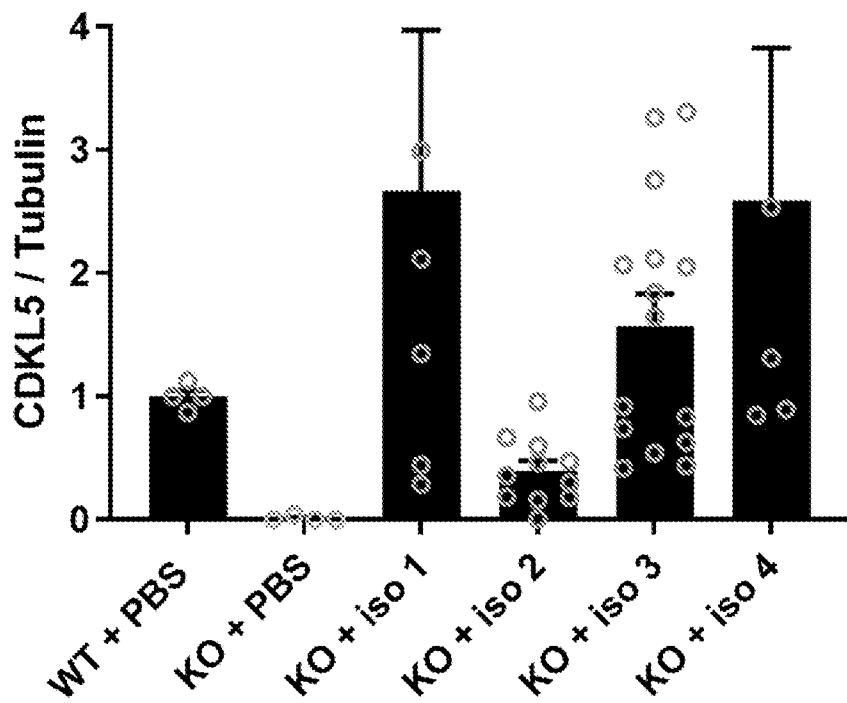


FIG 8B

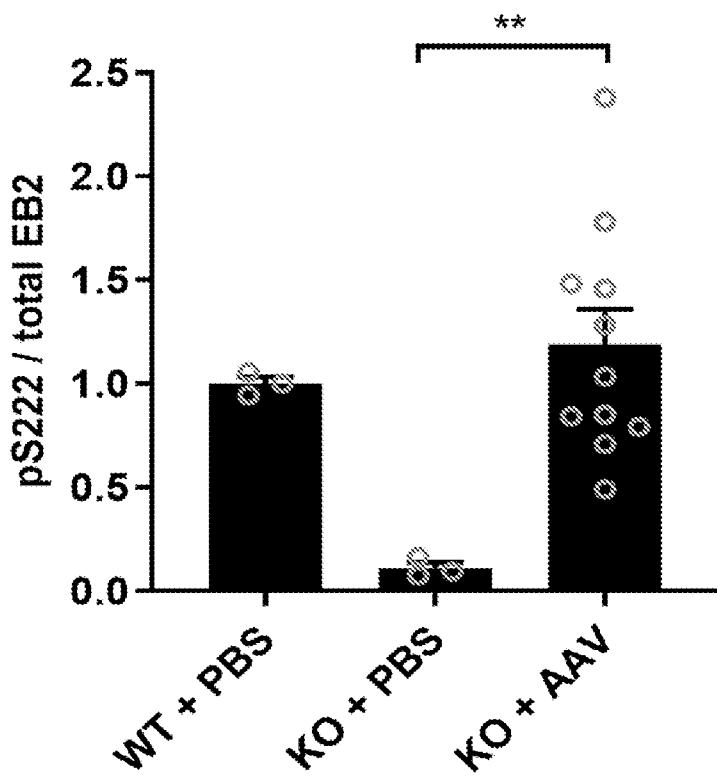
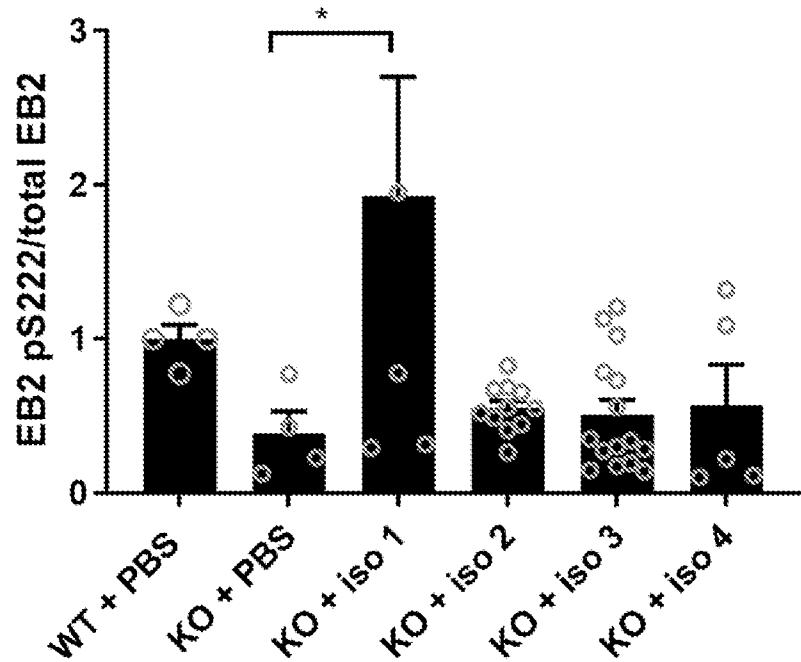


FIG 8C



One-way ANOVA with Sidak's multiple comparison's test

*p<0.5

FIG 8D

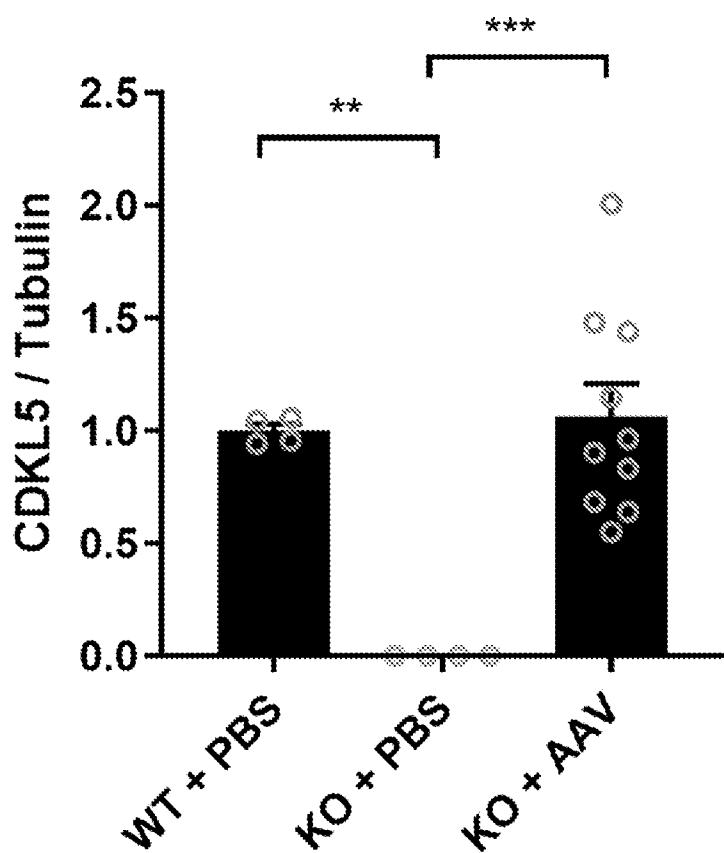


FIG 9A

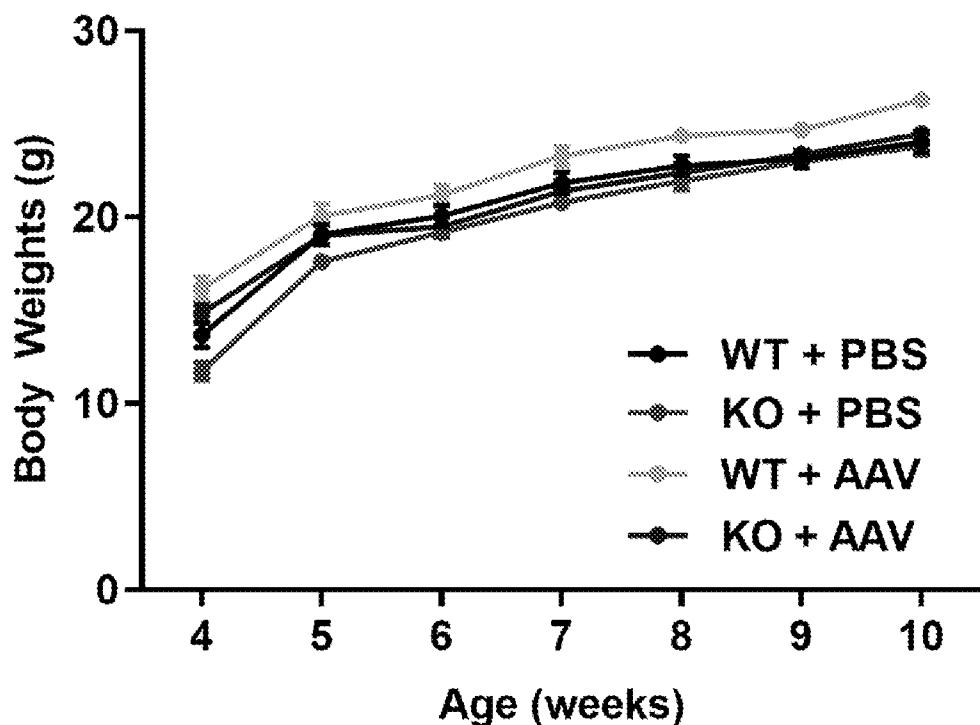


FIG 9B

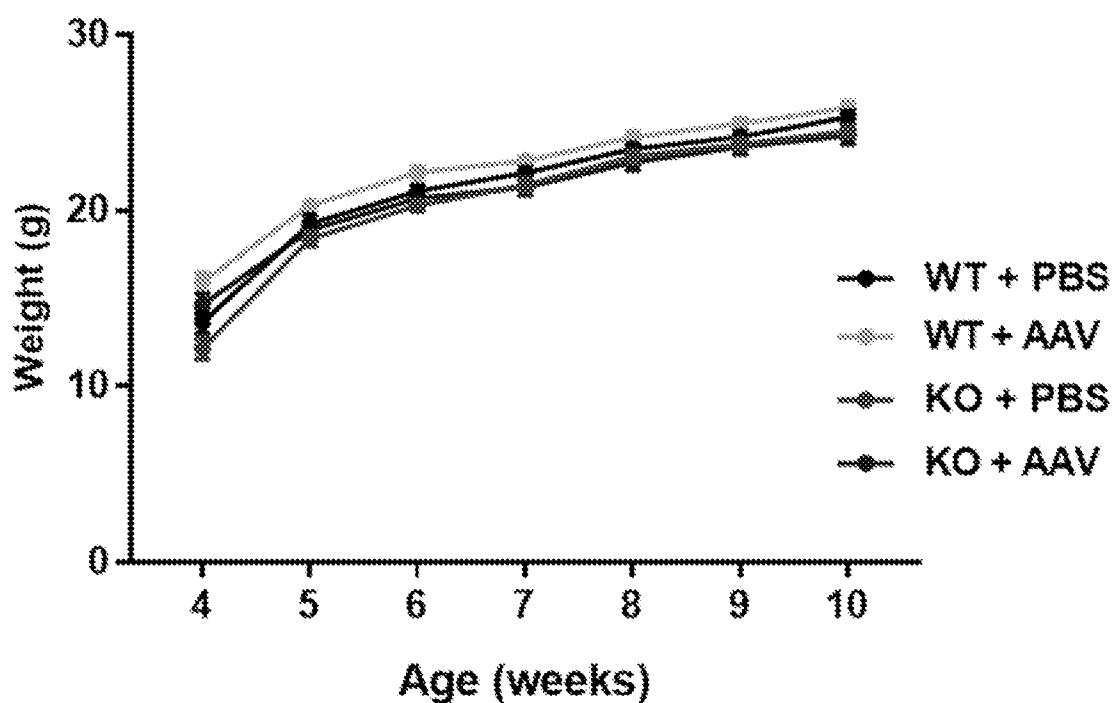


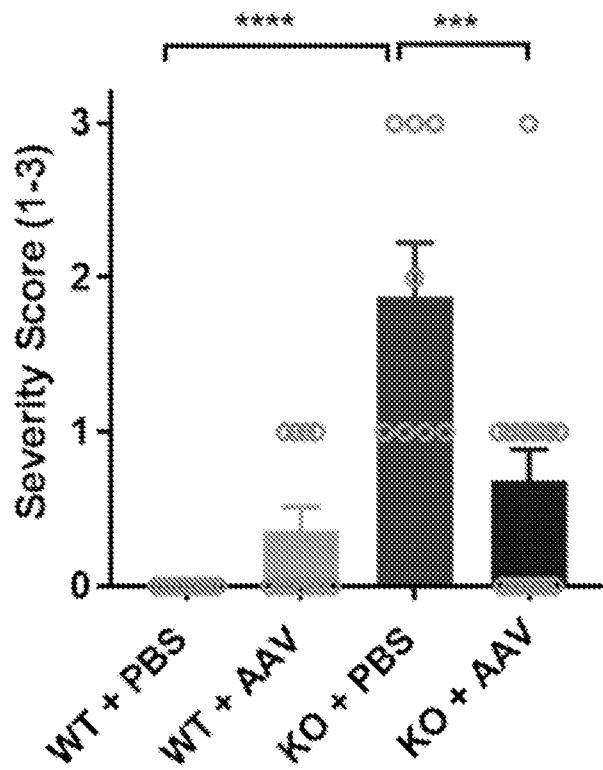
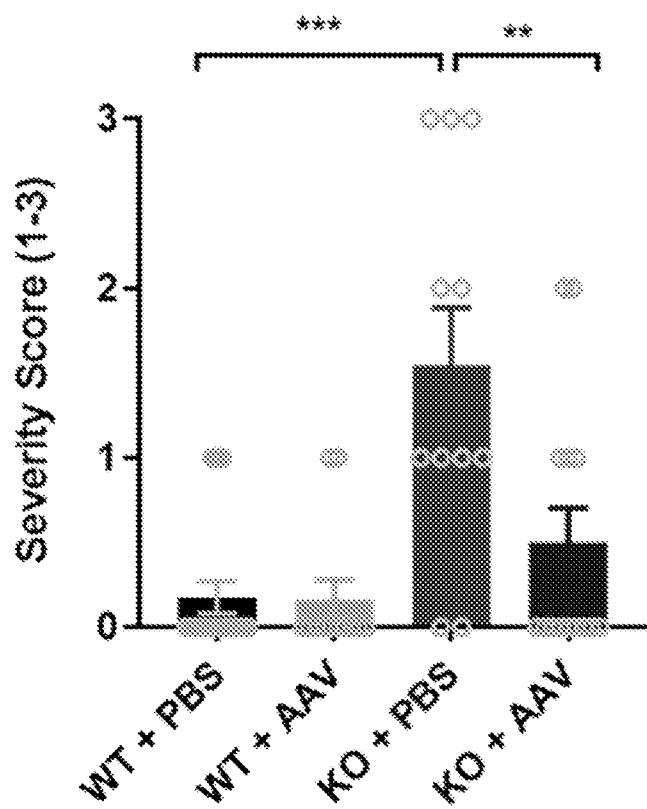
FIG 9C**FIG 9D**

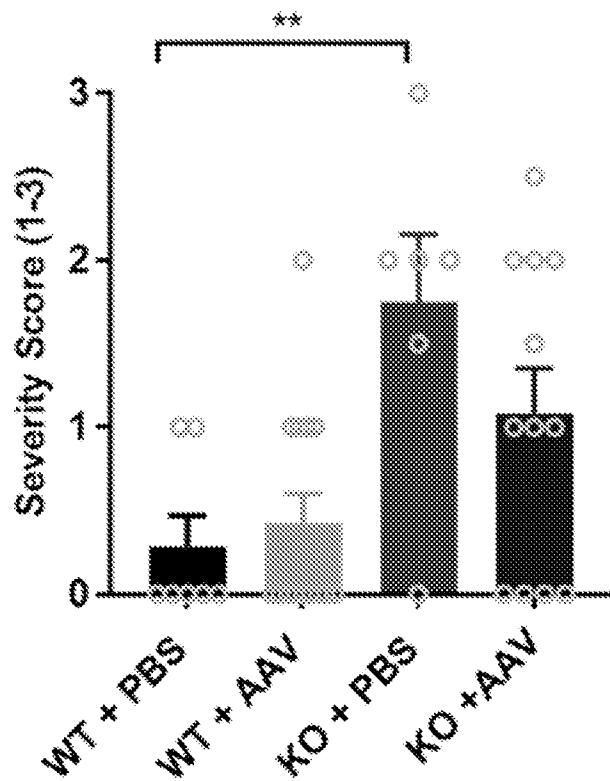
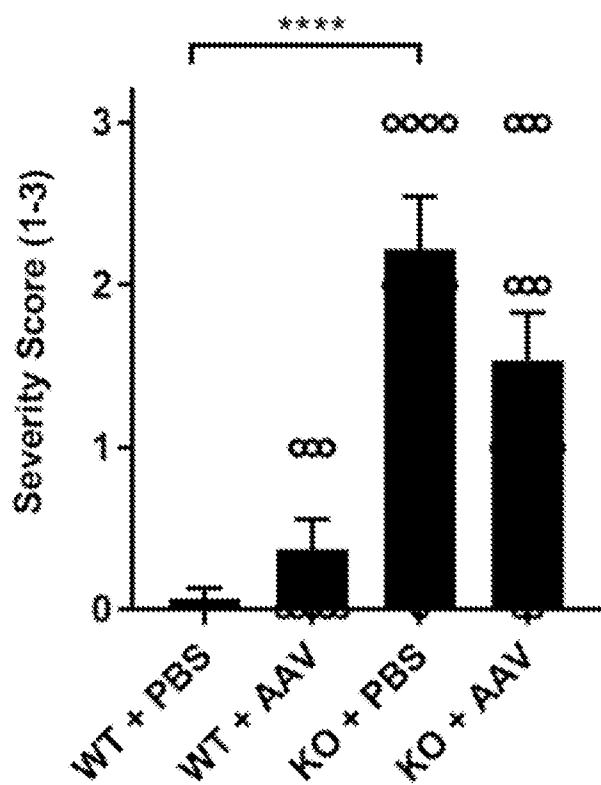
FIG 9E**FIG 9F**

FIG 10A

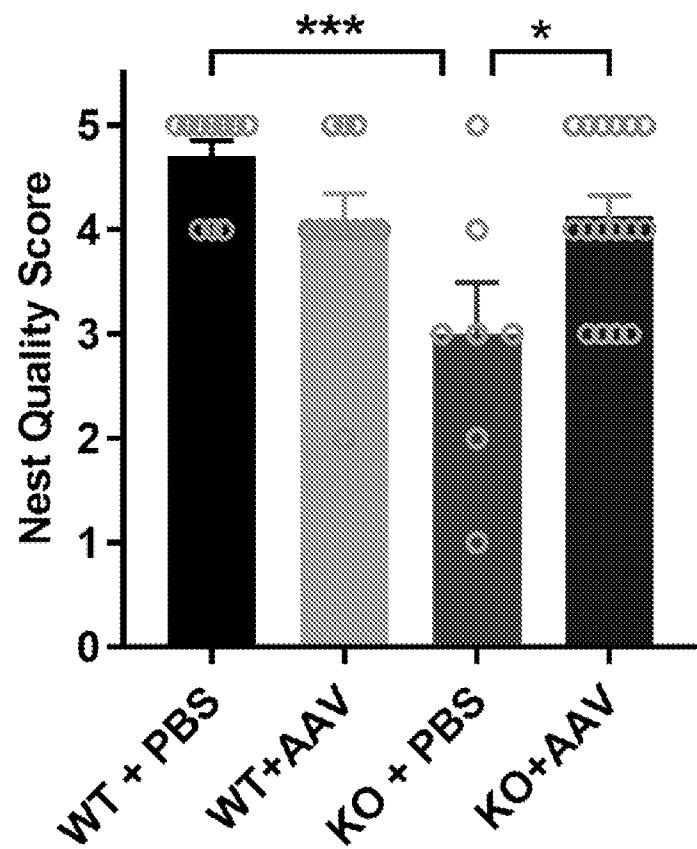


FIG 10B

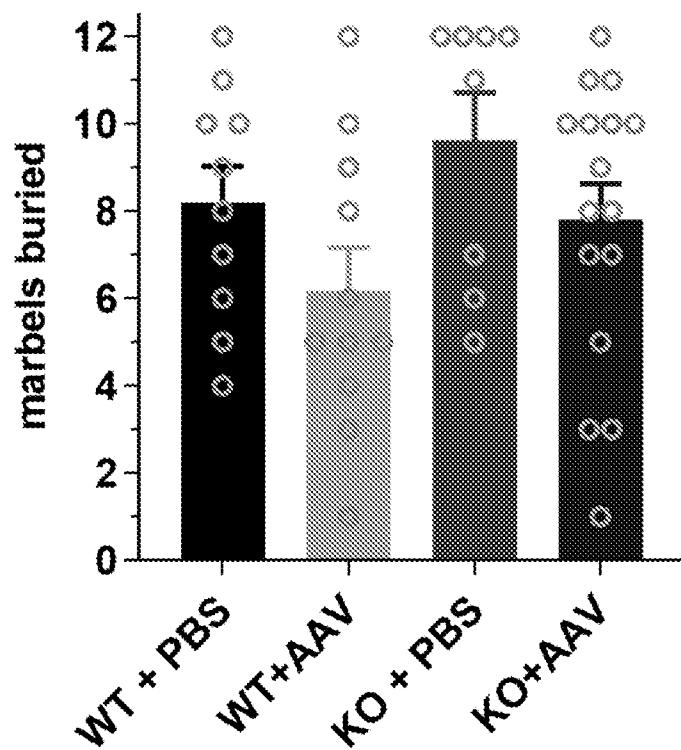


FIG 10C

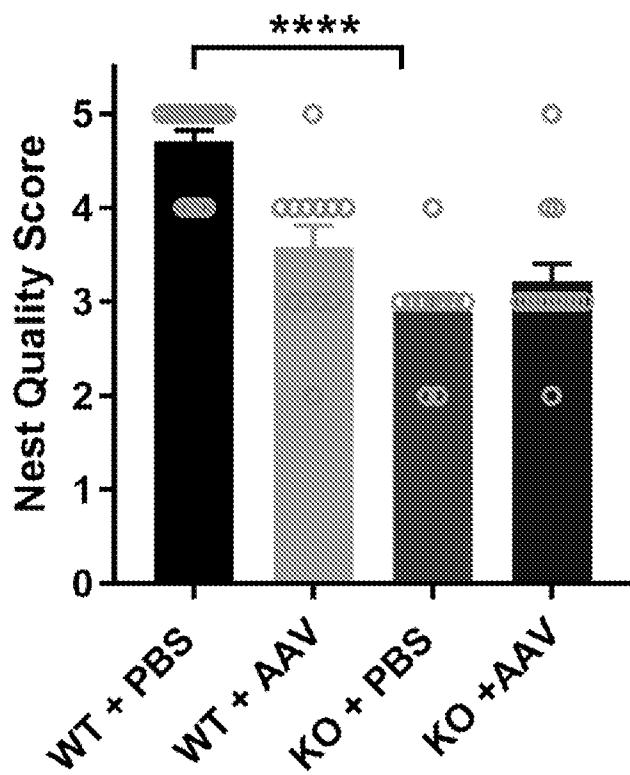


FIG 10D

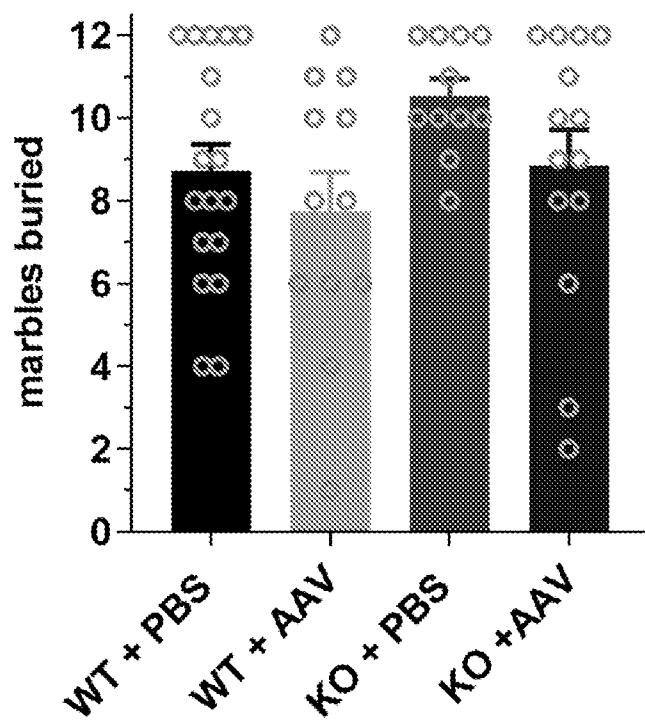


FIG 10E

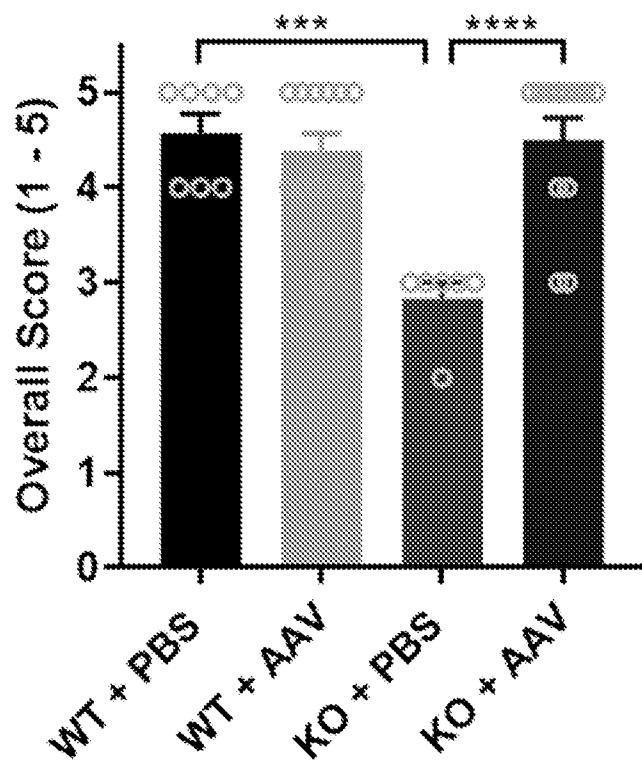


FIG 11A

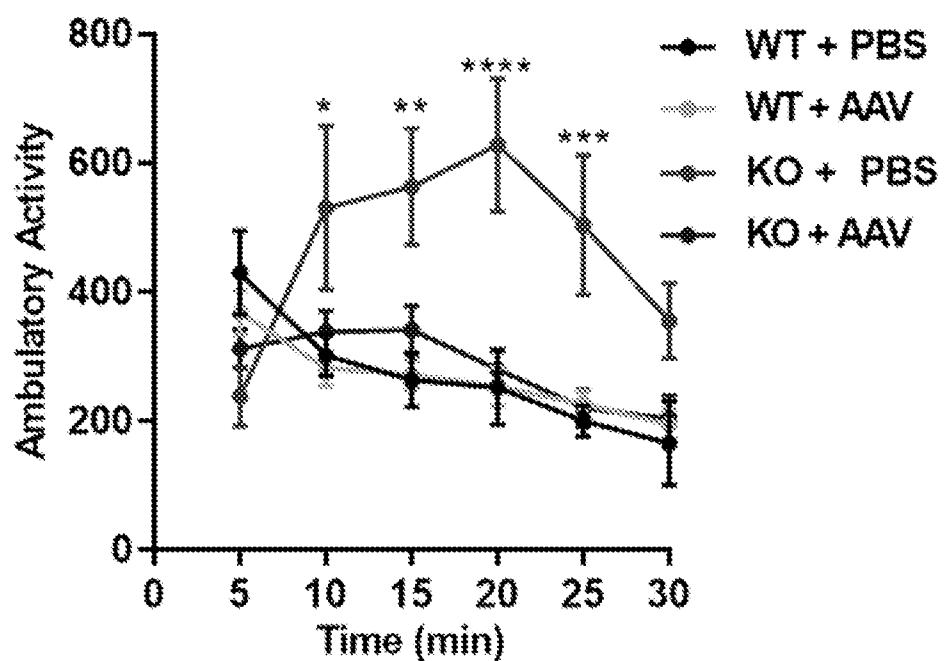


FIG 11B

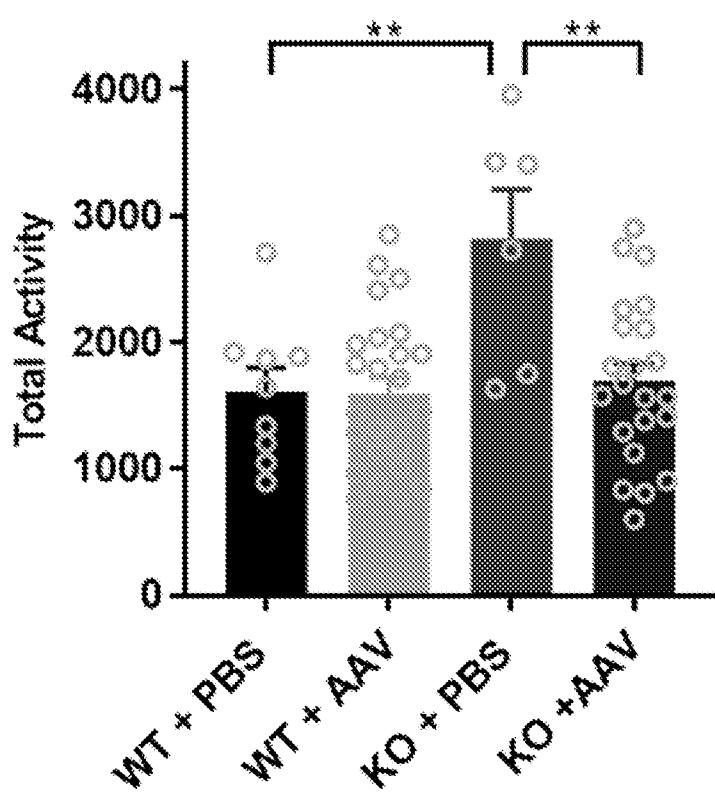


FIG 11C

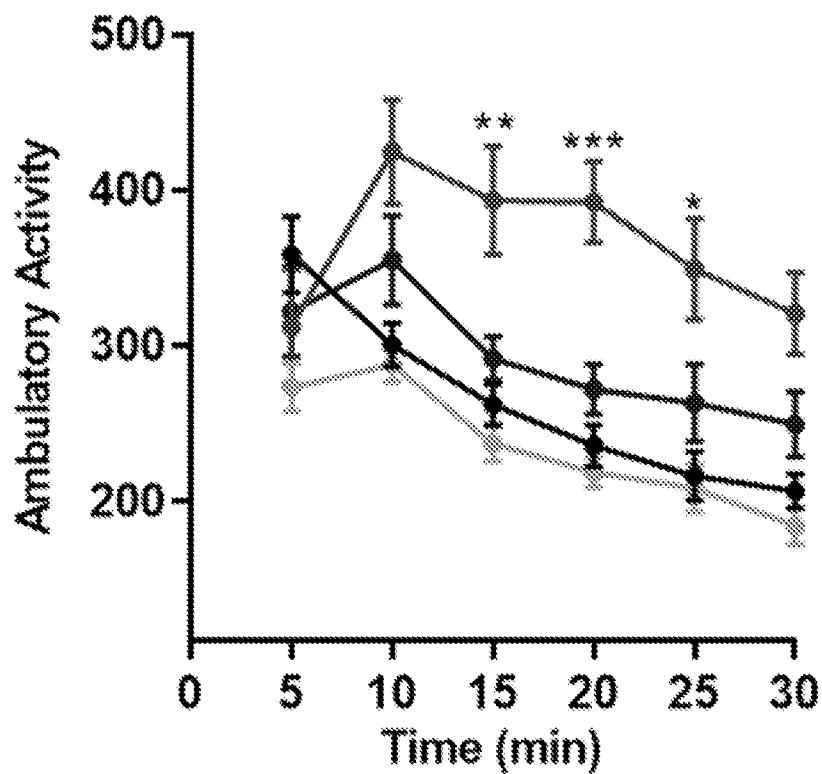


FIG 11D

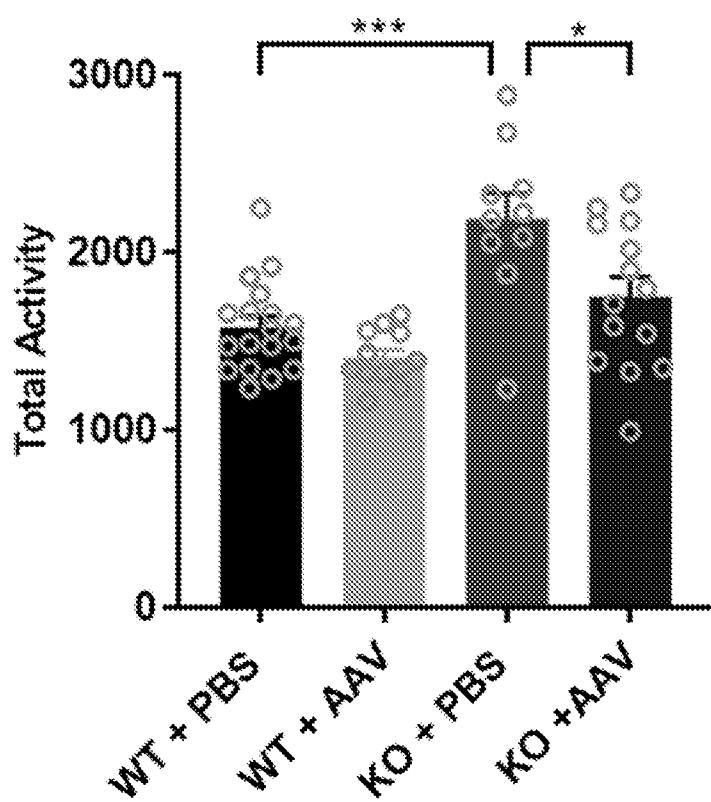


FIG 11E

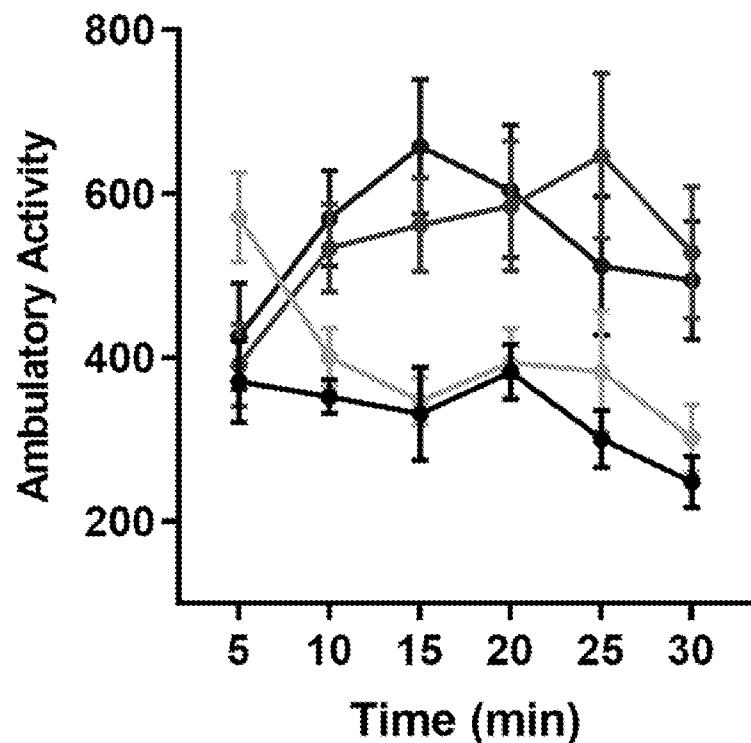


FIG 11F

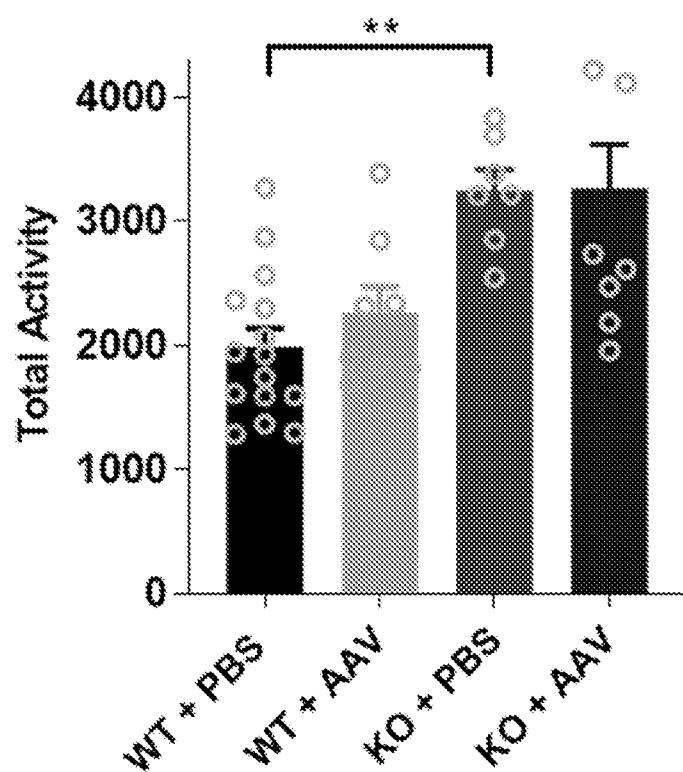


FIG 12

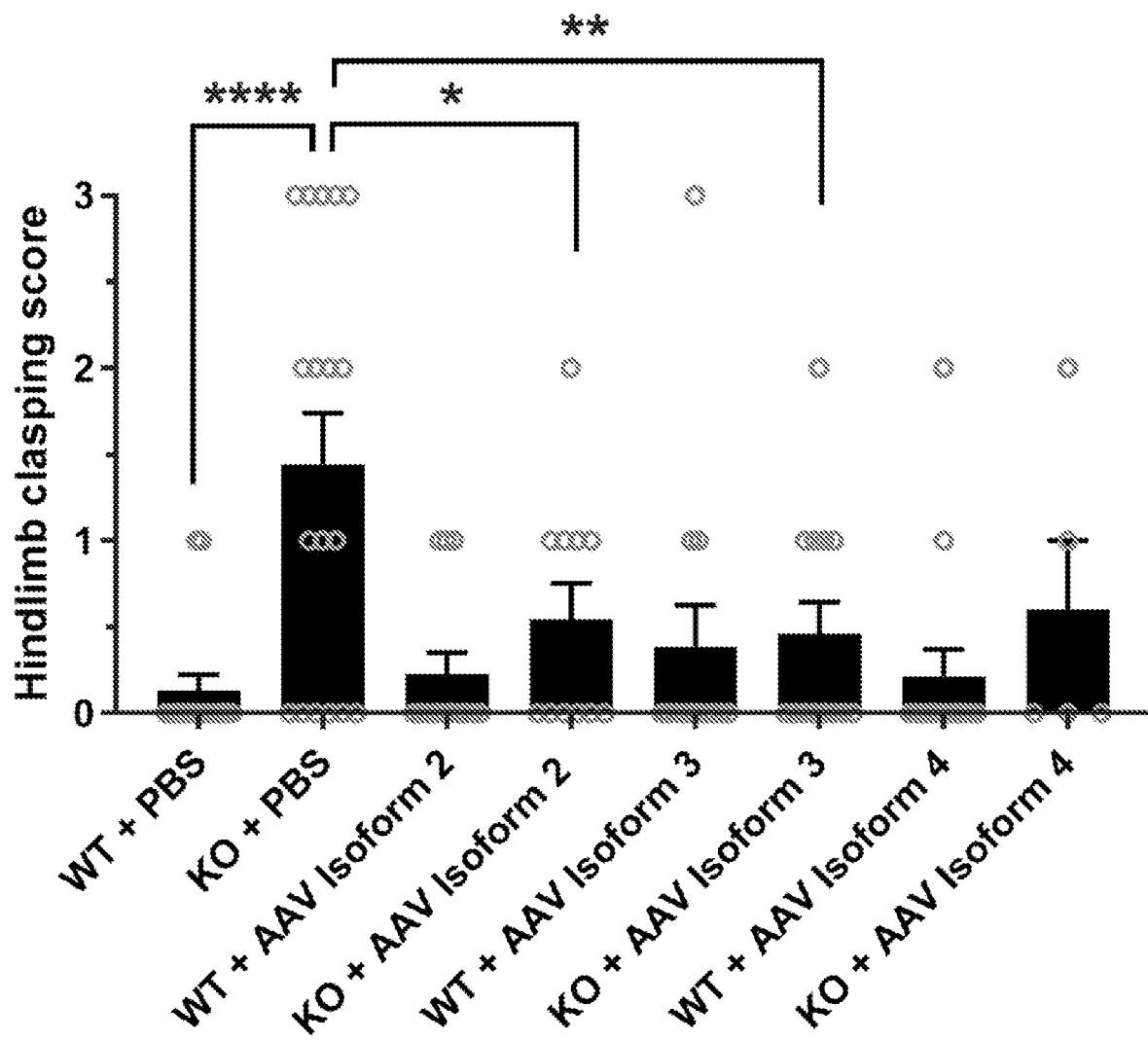


FIG 13A

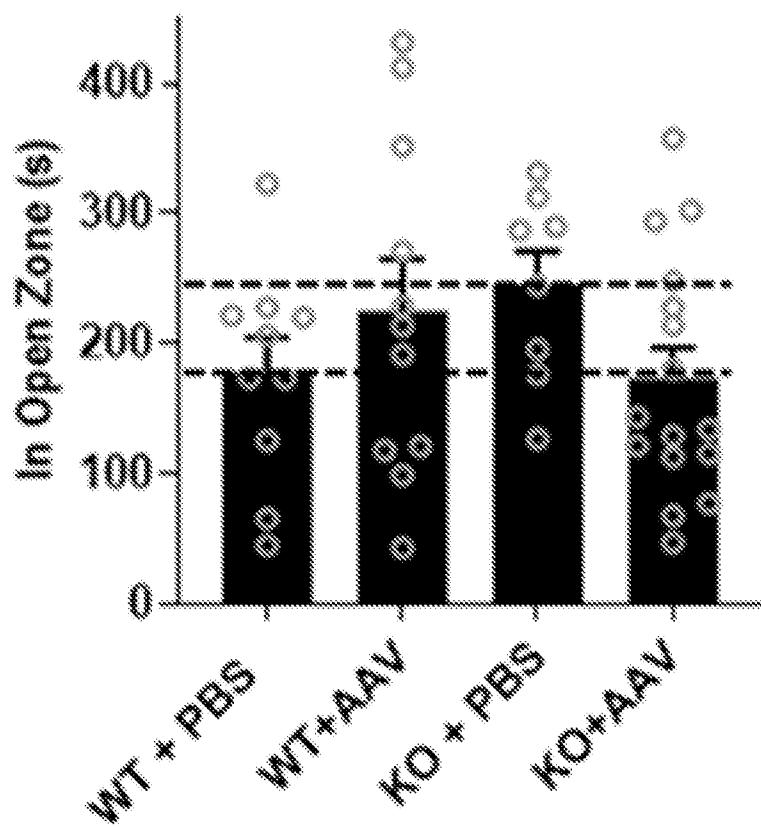


FIG 13B

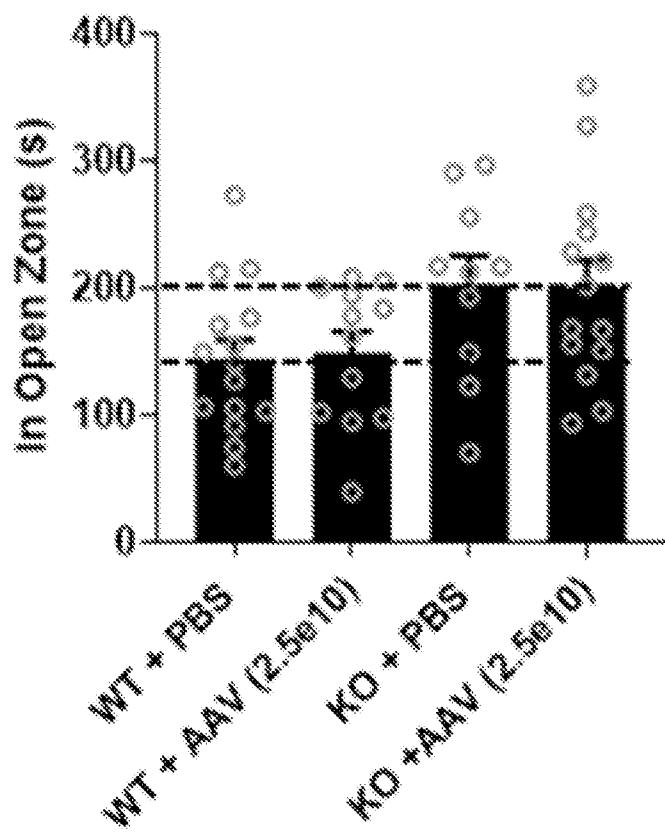


FIG 13C

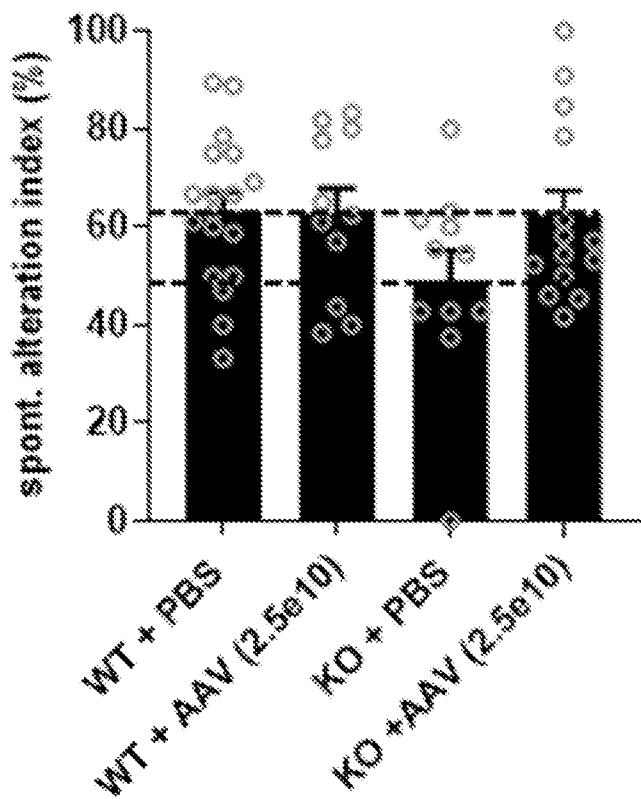


FIG 13D

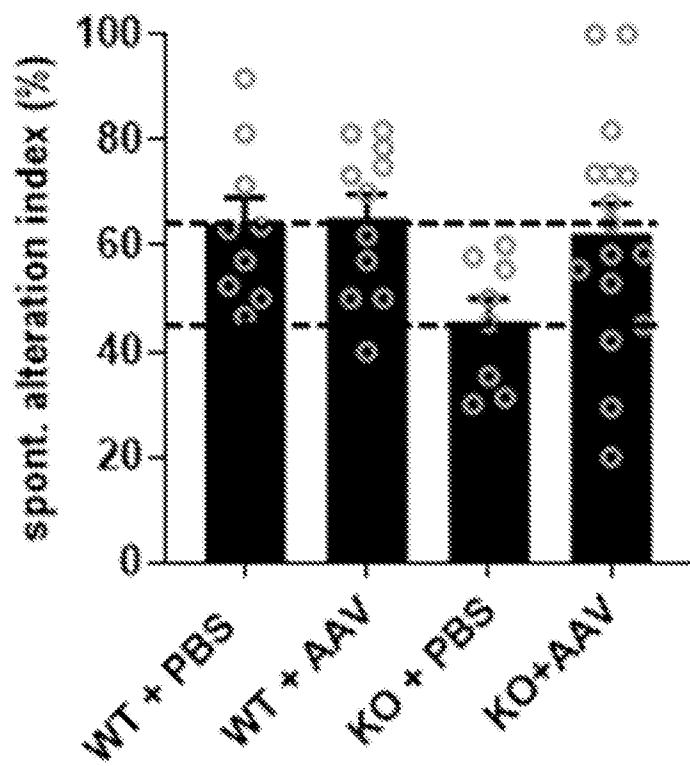
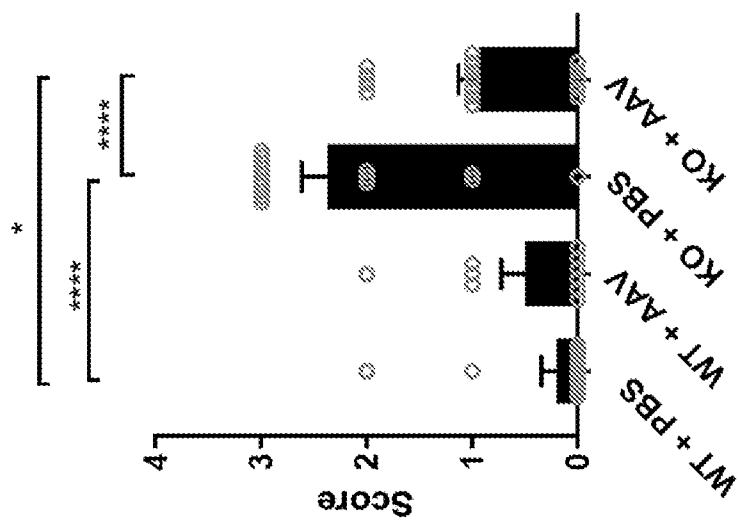


FIG 14A

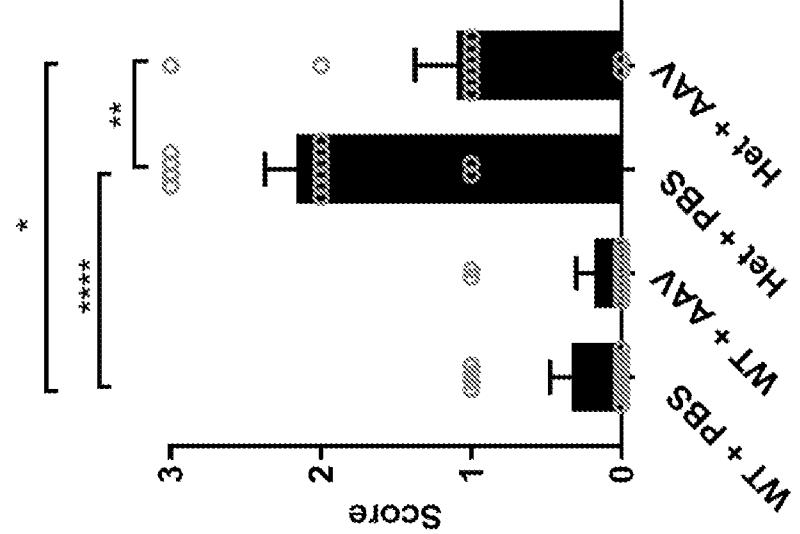
Male Hindlimb clasping



One-way ANOVA with Sidak's multiple comparison's test
*p<0.05, ***p<0.0001

FIG 14B

Female Hindlimb clasping



One-way ANOVA with Sidak's multiple comparison's test
*p<0.05, **p<0.01, ***p<0.0001

FIG 14C

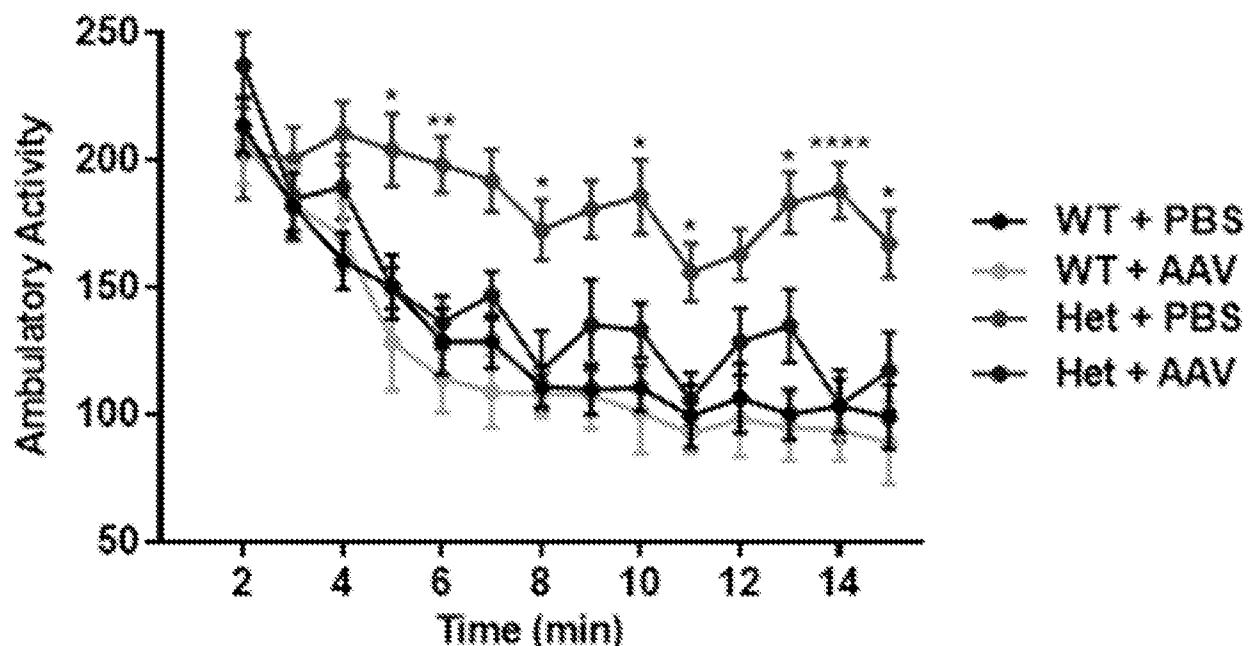


FIG 15A

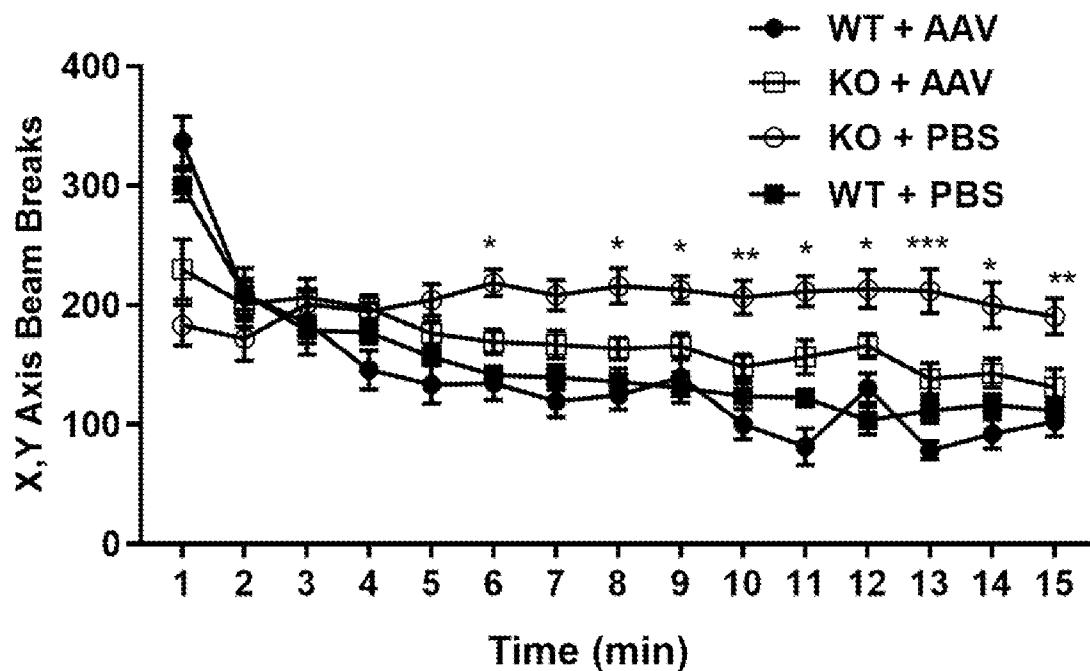
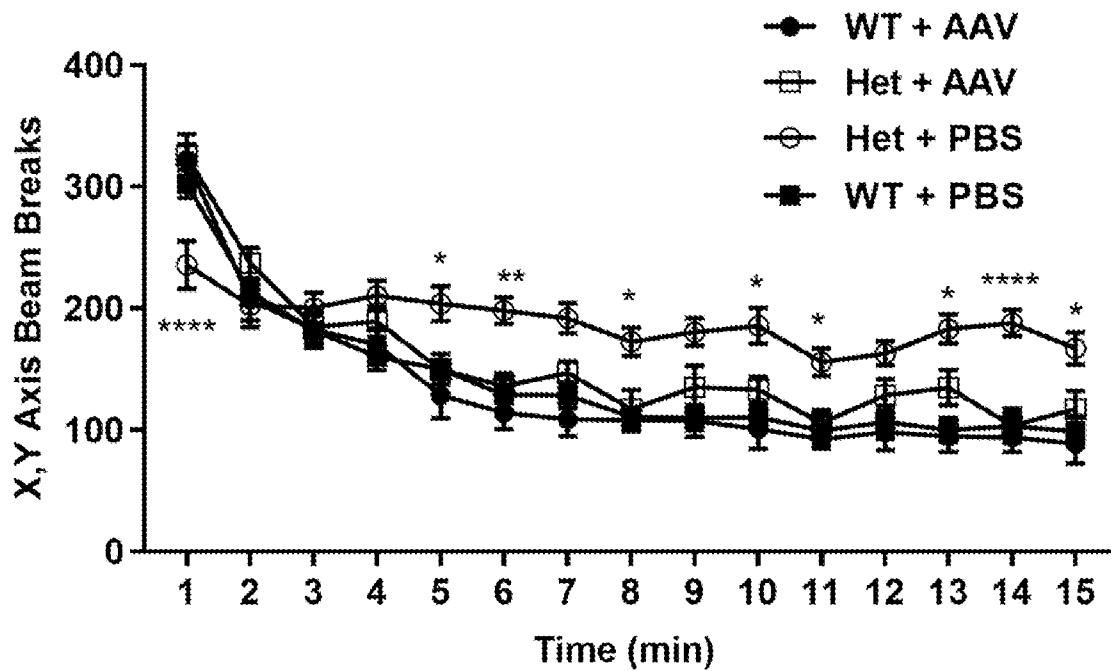


FIG 15B



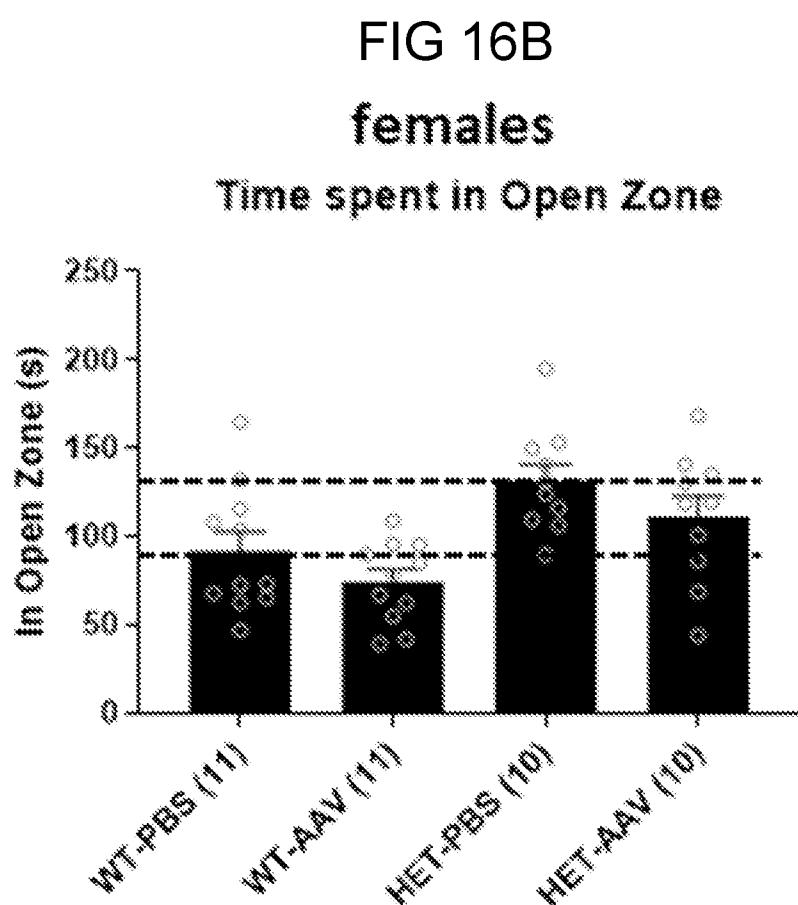
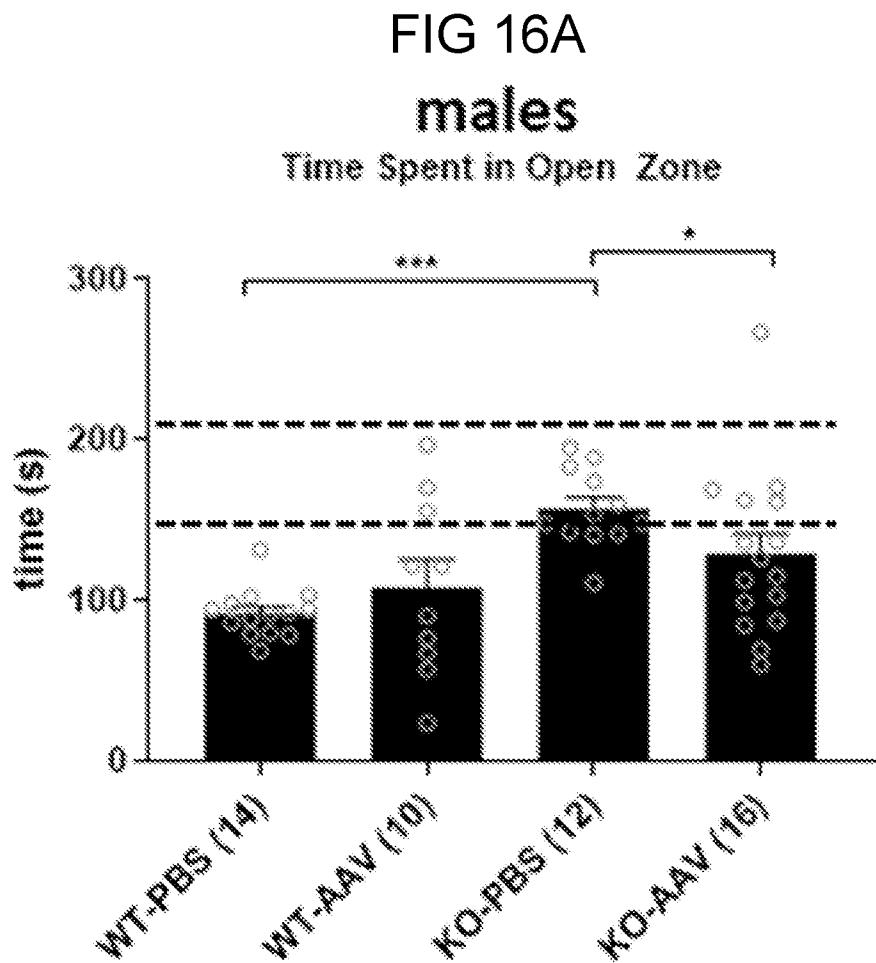


FIG 17

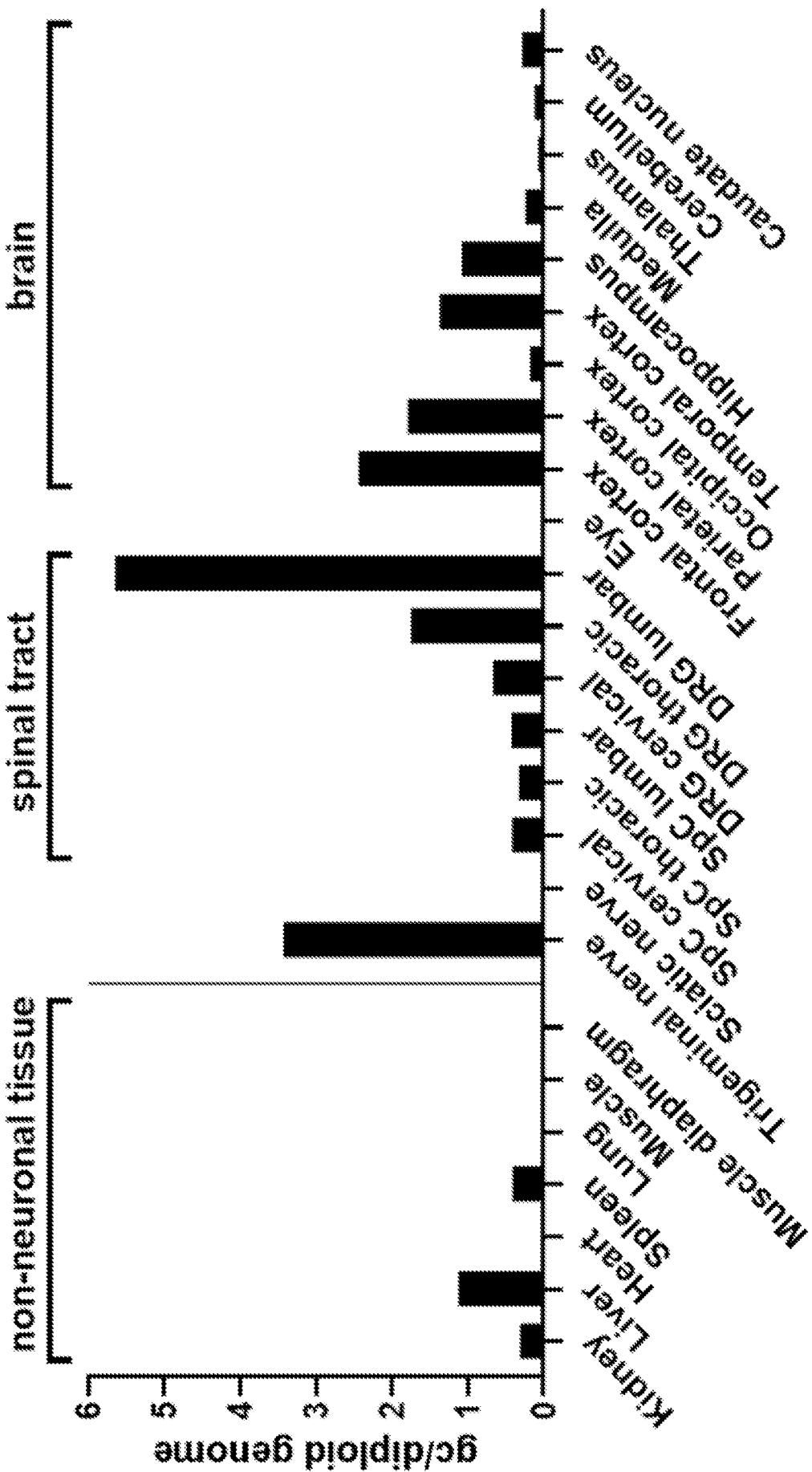


FIG 18

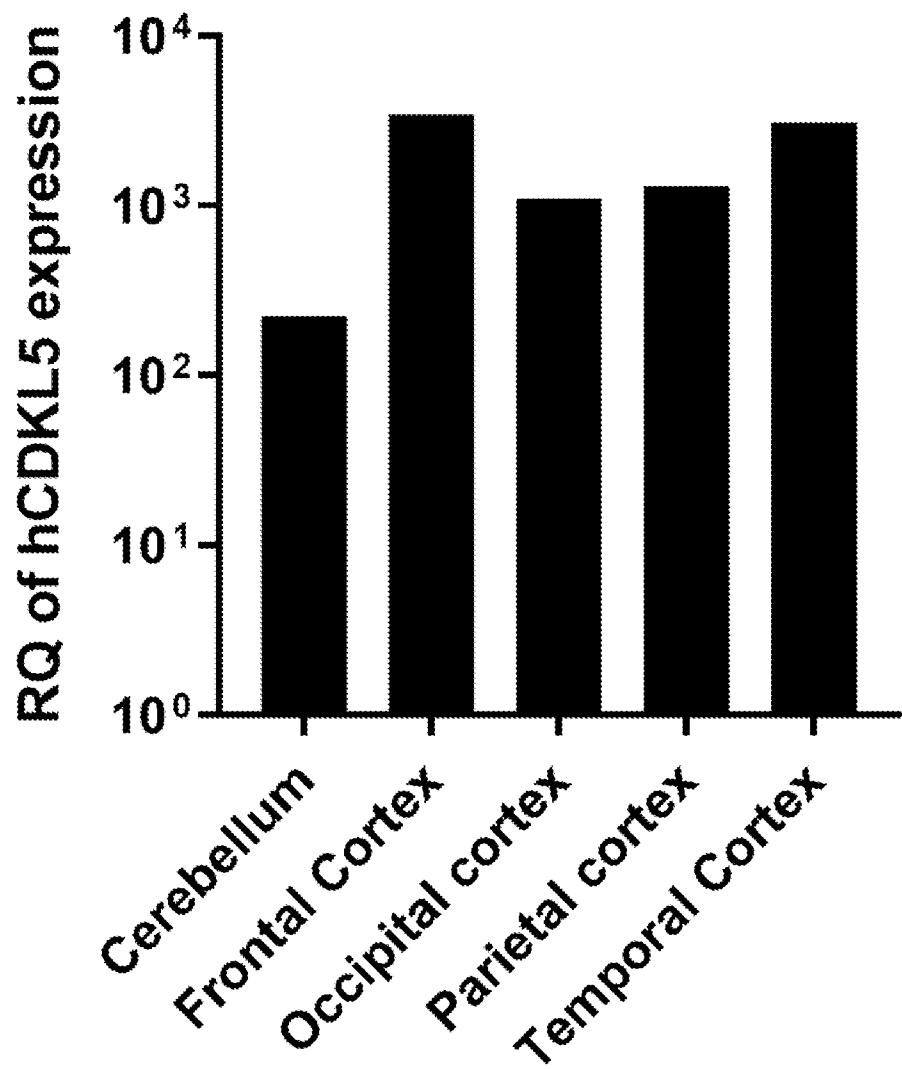


FIG 19A

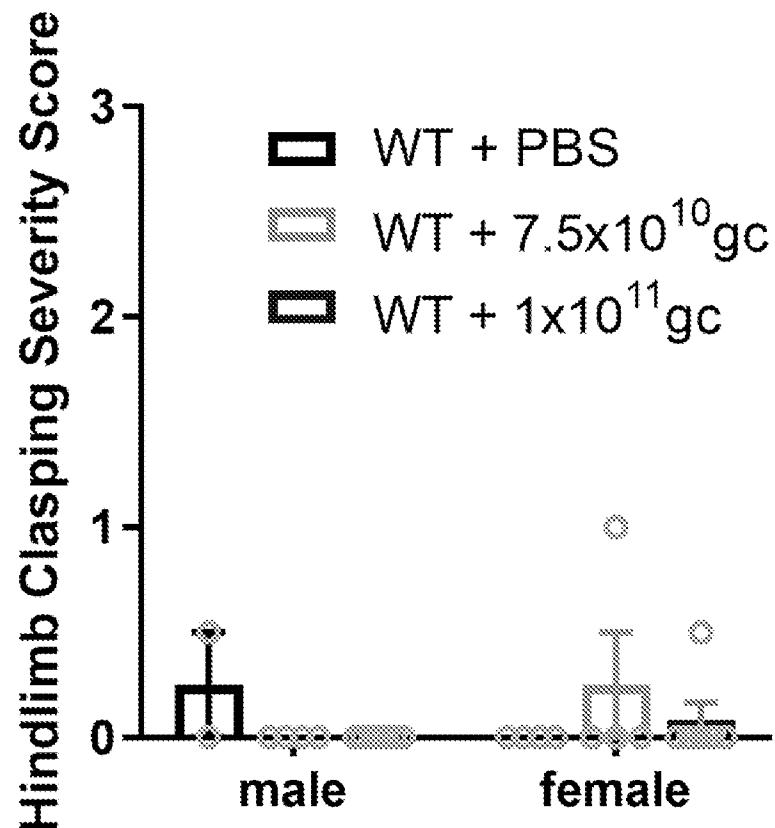
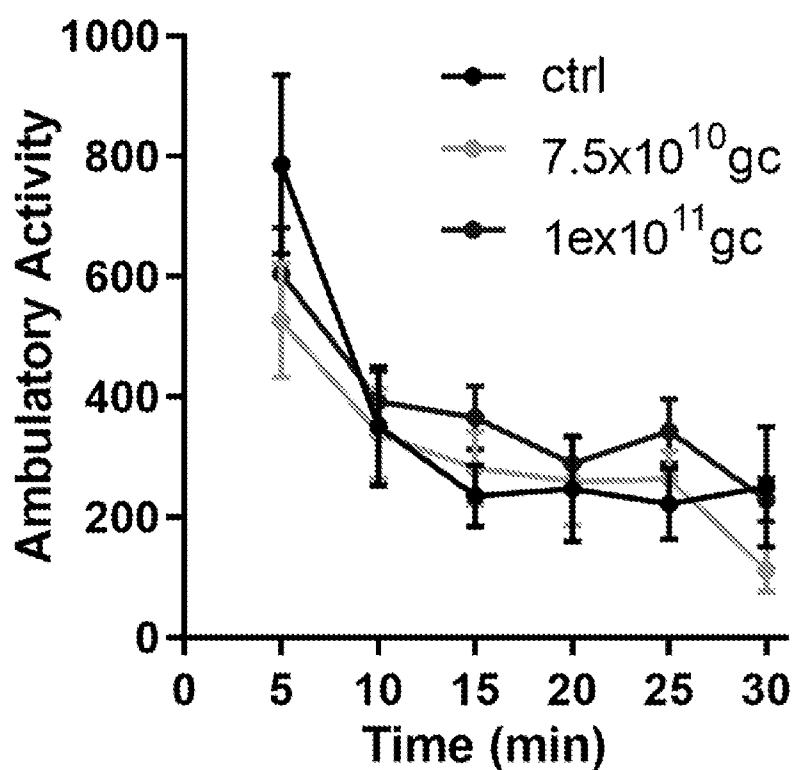


FIG 19A



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 21/29185

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12N 5/00; C12N 9/12 (2021.01)

CPC - C12N 5/00; C12N 9/12

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)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|--|-----------------------|
| A → | GAO et al. Gene replacement ameliorates deficits in mouse and human models of cyclin-dependent kinase-like 5 disorder. Brain. 03 March 2020, Vol. 143, No. 3, pg 811-832; especially pg 812, col 1, para 1; pg 813, col 2, para 2; pg 819, col 1, para 3; pg 819, col 2, para 1; Figure 1E | 1-3, 26-27 |
| A | WO 2017/191274 A2 (CUREVAC AG) 09 November 2017 (09.11.2017); especially claim 1; SEQ ID NO: 15012, residues 1-2713 | 1-3, 26-27 |
| A | US 2014/0010861 A1 (MODERNA THERAPEUTICS) 9 January 2014 (09.01.2014); especially claim 2; SEQ ID NO: 78072, residues 1-2713 | 1-3, 26-27 |

 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
- "D" document cited by the applicant in the international application
- "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

13 September 2021 (13.09.2021)

Date of mailing of the international search report

OCT 04 2021

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-8300Authorized officer
Kari Rodriguez
Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 21/29185

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:
 - in the form of an Annex C/ST.25 text file.
 - on paper or in the form of an image file.
 - b. furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. furnished subsequent to the international filing date for the purposes of international search only:
 - in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
 - on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).
2. In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 21/29185

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.: 11-25, 28-38 because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Continued on Supplemental Page

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-3, 26-27, limited to SEQ ID NOs: 22 and 2

Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 21/29185

Continued from Box No. III Observations where unity of invention is lacking

Group I+, claims 1-10, 26-27, 39-41, directed to a recombinant vector comprising a hCDLK5-coding sequence. The vector will be searched to the extent that the hCDLK5-coding sequence encompasses SEQ ID NO: 22, and the corresponding hCDLK5 amino acid sequence SEQ ID NO: 2. It is believed that claims 1-3, 26-27 encompass this first named invention, and thus these claims will be searched without fee to the extent that the hCDLK5 encompasses SEQ ID NOs: 22 and 2. Additional hCDLK5 sequence(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected hCDLK5 sequence(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be where the functional hCDKL5 is a functional hCDKL5 isoform 2 (hCDKL5-2GS), where the hCDLK5-2GS-coding sequence and amino acid sequence are SEQ ID NOs: 24 and 6, respectively, (claims 4-6, 39).

The inventions listed as Group I+ do not relate to a single special technical feature under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special technical features

The inventions of Group I+ each include the special technical feature of a unique hCDLK5-coding nucleic acid sequence and encoded amino acid sequence, and is considered a distinct technical feature.

No technical features are shared between the hCDLK5-coding nucleic acid and amino acid sequences of Group I+ and accordingly, these groups lack unity a priori.

Additionally, even if the inventions listed as Group I+ were considered to share the technical features of including: a recombinant adeno-associated virus (rAAV) useful for treating CDKLS deficiency disorder (CDD), wherein the rAAV comprises: (a) an AAV capsid; and (b) a vector genome packaged in the AAV capsid of (a), wherein the vector genome comprises inverted terminal repeats (ITR) and a hCDKL5 -coding sequence, which is a nucleic acid sequence encoding a functional human CDKLS (hCDKL5), under control of regulatory sequences which direct the hCDKL5 expression in central nervous system cells, these shared technical features are previously disclosed by the publication entitled "Gene replacement ameliorates deficits in mouse and human models of cyclin-dependent kinase-like 5 disorder" (Brain. 03 March 2020, Vol. 143, No. 3, pg 811-832) by Gao et al. (hereinafter 'Gao').

Gao discloses (instant claim 1) a recombinant adeno-associated virus (rAAV) useful for treating CDKLS deficiency disorder (CDD) (pg 812, col 1, para 1 - "Cyclin-dependent kinase-like 5 (CDKL5) disorder, or CDKL5 deficiency disorder, is a severe, neurodevelopmental disease predominantly affecting females, associated with a full spectrum of co-morbidities."); pg 813, col 2, para 2 - "In this study, we aimed to develop an effective disease modifying gene replacement therapy, utilizing AAV-CDKL5 vectors in in vivo and in vitro models of CDKL5 disorder."), wherein the rAAV comprises:

(a) an AAV capsid (pg 813, col 2, para 2; pg 819, col 2, para 1 - "Furthermore, we produced high-titre rAAV vectors pseudotyped with AAV9, the variant capsid AAV-PHP.B for in vivo and the hybrid capsid AAV-DJ for in vitro applications."); and
(b) a vector genome packaged in the AAV capsid of (a) (pg 813, col 2, para 2; pg 819, col 1, para 4 - "We then cloned the codon-optimized coding region of hCDKL5_1 and hCDKL5_2 downstream of the CBH promoter into the ssAAV2 vector genome..."), wherein the vector genome comprises inverted terminal repeats (ITR) and a hCDKL5-coding sequence, which is a nucleic acid sequence encoding a functional human CDKLS (hCDKL5) (Figure 1E - "Schematics depicting transgene cassettes cloned into the ssAAV vector genome. Expression of haemagglutinin (HA)-tagged hCDKL5_1, Myc-tagged hCDKL5_2 and eGFP were driven by a CBH promoter. CBH = a hybrid form of CBA promoter; ITR = inverted terminal repeat; pA = bovine growth hormone (BGH) polyA."), under control of regulatory sequences which direct the hCDKL5 expression in central nervous system cells (Figure 1E; pg 819, col 1, para 3 - "As human neuronal and glial cells revealed similar cellular expression patterns of hCDKL5 isoforms (Fig. 1B and D), a hybrid form of the chicken b-actin (CBA) promoter, the CBH promoter, was used to drive transgene expression, which provides robust, long-term and ubiquitous expression in all CNS cell types in vivo (Gray et al., 2011b).").

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Group I+ inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

NOTE, continuation of Item 4 above: claims 11-25, 28-38 are held unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).