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Engineered extracellular vesicles comprising fusion proteins

Abstract

Described herein are compositions and techniques related to generation and therapeutic application of artificial synapses. Artificial synapses are engineered extracellular vesicles, including exosomes, which incorporate sticky binders on their surface to anchor signaling domains against biological targets, such as receptors. These engineered additives can be organized in genetic vector constructs, expressed in mammalian cells, wherein the sticky binders attach to extracellular vesicles such as exosomes, thereby presenting their joined signaling domains which are rapidly taken up by recipient cells. Artificial synapses adopt the hallmark biophysical and biochemical features of extracellular vesicles, allowing for rapid deployment and scale-up. Importantly, this strategy can allow for kinetically favorable signal generation and signal propagation. This includes, for example, increasing density of agonist presentation to support receptor clustering—an onerous barrier for traditional receptor targeting strategies.

Inventors: **Pentecost; Mickey (West Hollywood, CA), Bartkowski; Wojciech (La Crescenta, CA)**

Applicant: **DIADEM BIOTHERAPEUTICS, INC.** (Torrance, CA)

Family ID: **1000008748815**

Assignee: **Diadem Biotherapeutics, Inc. (Torrance, CA)**

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Primary Examiner: Landsman; Robert S

Attorney, Agent or Firm: WILSON SONSINI GOODRICH & ROSATI

Background/Summary

CROSS-REFERENCE TO RELATED APPLICATIONS (1) This application is a division of U.S. application Ser. No. 17/377,550, filed Jul. 16, 2021, now U.S. Pat. No. 11,578,116, which is a continuation of International Application No. PCT/US2021/016949, filed Feb. 5, 2021, which designated the U.S., and also claims the benefit of priority under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 62/970,374, filed Feb. 5, 2020, the contents of each of which are incorporated herein by reference in their entirety.

SEQUENCE LISTING
(1) The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on Nov. 9, 2022, is named 085172_000001USD1_ST.26.xml and is 724,326 bytes in size.
FIELD OF THE INVENTION
(2) This invention relates to the generation of artificial synapses or extracellular vesicles, including features of extracellular vesicles engineered to deliver signaling, for therapeutic use, including treatment of immune diseases and cancer.
BACKGROUND
(3) Extracellular vesicles (EVs) play a critical role in intercellular communication by transferring microRNAs, lipids, and proteins to neighboring cells. The delivery of encapsulated molecules within EVs is a highly promising strategy as a therapeutic platform in many contexts, exploiting the unique biophysical and biochemical characteristics of extracellular vesicles (EVs). However, there remains a great need in the art for a flexible and dynamic platform, where specific biological signals can be reliably targeted without off-target effects and that provide a robust cellular response to achieve a therapeutic effect, such as modulating inflammation.
SUMMARY
(4) The compositions and methods provided herein are based, in part, on the discovery that extracellular vesicles can be used to express engineered fusion polypeptides that can modulate biological signal generation. These engineered vesicles, also termed artificial synapses, adopt the hallmark biophysical and biochemical features of extracellular vesicles, but are further engineered with vesicle targeting domains (e.g., sticky binders) and signaling domains, optionally joined by a linker with specific functions. The fusion polypeptides provided herein are designed and produced as nucleic acid constructs (e.g., vectors) and expressed in cells, such as mammalian cells. In particular, the vesicle targeting domain of each fusion polypeptide anchors the polypeptide to the extracellular vesicle lipid membrane, thereby presenting the signaling domain(s) of the

polypeptide. The signaling domains on or within the vesicle membrane can make contact with recipient cells via target polypeptides (e.g., receptors on the extracellular surface of the recipient cell). Importantly, this strategy can allow for kinetically favorable signal generation and signal propagation. This includes, for example, increasing density of agonist presentation to support receptor clustering of a target receptor located on a target cell—an onerous barrier for traditional receptor targeting strategies.

(5) This strategy was applied to alter immune checkpoint signaling, by engineering artificial synapses through genetic constructs with lipid binding glycosylphosphatidylinositol (GPI) sticky binders joined with programmed death-ligand 1 (PD-L1) signaling domain, e.g., human programmed death-ligand 1 (hPD-L1), expressed in cells and capable of attachment to exosomes. Isolation, purification, and analysis of artificial synapses revealed a high density of signaling domains of the hPD-L1-GPI fusion polypeptide. The hPD-L1 artificial synapse exosomes further demonstrated enhanced agonist signaling than soluble PD-L1 ligand alone, supporting receptor clustering on a target cell. When applied to a model of experimental autoimmune uveoretinitis (EAU), a statistically significant reduction in EAU symptoms was observed.

(6) Thus, in one aspect, provided herein is an engineered extracellular vesicle or artificial vesicle comprising: at least one fusion polypeptide comprising: at least one protein of interest (POI) domain; and at least one vesicle targeting domain. In some embodiments of any of the aspects, the engineered extracellular vesicle is an exosome. In some embodiments, of any of the aspects, the fusion protein further comprises at least one linker. In some embodiments of any of the aspects, the POI domain can substantially bind to a target polypeptide.

(7) In another aspect, provided herein is an engineered extracellular vesicle comprising: at least one fusion polypeptide comprising: (i) at least one protein of interest (POI) domain or a fragment thereof; and (ii) at least one vesicle targeting domain, wherein the POI domain is in an extracellular position relative to a lipid membrane of the extracellular vesicle.

(8) In another aspect, provided herein is an engineered extracellular vesicle comprising: (a) a first fusion polypeptide comprising: (i) at least one protein of interest (POI) domain or a fragment thereof; and (ii) at least one vesicle targeting domain, wherein the at least one POI domain is in an extracellular position relative to a lipid membrane of the extracellular vesicle, (b) a second fusion polypeptide comprising: (i) at least one protein of interest (POI) domain or a fragment thereof; and (ii) at least one vesicle targeting domain, wherein the POI domain is in an extracellular position relative to a lipid membrane of the extracellular vesicle, and wherein the at least one vesicle targeting domain is within a lipid membrane of the extracellular vesicle.

(9) In another aspect, provided herein is an extracellular vesicle composition comprising: a plurality of artificial synapses, wherein each artificial synapse comprises (i) an extracellular vesicle; (ii) one or more sticky binders; and (iii) one or more signaling domains.

(10) In another aspect, provided herein is a composition comprising a plurality of the engineered extracellular vesicles provided herein.

(11) In another aspect, provided herein is a composition comprising two or more of the engineered extracellular vesicles provided herein.

(12) In another aspect, provided herein is a composition comprising three or more of the engineered extracellular vesicles provided herein.

(13) In another aspect, provided herein is a method of producing the engineered extracellular vesicle or the compositions provided herein, comprising: (a) providing a population of cells expressing a vector construct encoding one or more sticky binder and one or more signaling domains; and (b) isolating a plurality of artificial synapses from the population of cells.

(14) In another aspect, provided herein is a method of producing the engineered extracellular vesicle or the compositions provided herein, comprising: (a) providing a population of cells expressing a vector construct encoding one or more sticky binder and one or more signaling domains; and (b) isolating a plurality of artificial synapses from the population of cells; and (c)

purifying the plurality of artificial synapses from the population of cells.
(15) In another aspect, provided herein is a method of modulating inflammation in a subject, the method comprising: administering a composition comprising a plurality of engineered extracellular vesicles to a subject in need thereof, wherein the engineered extracellular vesicles comprise at least one fusion polypeptide comprising: (i) at least one protein of interest (POI) domain or a fragment thereof; and (ii) at least one vesicle targeting domain.
(16) In another aspect, provided herein is a use of a composition or engineered extracellular vesicle provided herein for the treatment of an inflammatory disease or condition.
(17) In another aspect, provided herein is a use of a composition or engineered extracellular vesicle provided herein for the treatment of an autoimmune disease or condition.
(18) In another aspect, provided herein is a use of a composition or engineered extracellular vesicle provided herein for the treatment of cancer.
(19) In one embodiment of any of the aspects, the engineered extracellular vesicle is an exosome.
(20) In another embodiment of any of the aspects, the protein of interest (POI) domain or a fragment thereof is a N-terminal domain of the fusion polypeptide. In another embodiment of any of the aspects, the POI domain is selected from the group consisting of: Table 1. In another embodiment of any of the aspects, the POI domain is PD-L1 or a fragment thereof. In another embodiment of any of the aspects, the POI domain is PD-L2 or a fragment thereof. In another embodiment of any of the aspects, the POI domain is FGL1 or a fragment thereof. In another embodiment of any of the aspects, the POI domain is 4-1BBL or a fragment thereof. In another embodiment of any of the aspects, the POI domain is CTLA-4 or a fragment thereof. In another embodiment of any of the aspects, the protein of interest (POI) domain is HVEM or a fragment thereof.
(21) In another embodiment of any of the aspects, the vesicle targeting domain is a C-terminal domain of the fusion polypeptide. In another embodiment of any of the aspects, the vesicle targeting domain is in a luminal position relative to the lipid membrane of the extracellular vesicle. In another embodiment of any of the aspects, the vesicle targeting domain in an exterior position relative to the lipid membrane of the extracellular vesicle. In another embodiment of any of the aspects, the vesicle targeting domain is selected from the group consisting of: Table 3. In another embodiment of any of the aspects, the vesicle targeting domain is selected from the group consisting of: a Glycosylphosphatidylinositol (GPI) anchor, a fatty acylation site, and a prenylation site. In another embodiment of any of the aspects, the vesicle targeting domain is C1C2. In another embodiment of any of the aspects, the vesicle targeting domain is a GPI anchor.
(22) In another embodiment of any of the aspects, the fusion polypeptide comprises at least two POI domains and/or at least two exosome targeting domains.
(23) In another embodiment of any of the aspects, the POI domain substantially binds to one or more of a target polypeptide. In another embodiment of any of the aspects, the target polypeptide is selected from the group consisting of: Table 2.
(24) In another embodiment of any of the aspects, the fusion polypeptide further comprises a peptide linker. In another embodiment of any of the aspects, the fusion polypeptide further comprises a fragment crystallizable region (Fc) domain. In another embodiment of any of the aspects, the linker is in an exterior position relative to the lipid membrane of the extracellular vesicle. In another embodiment of any of the aspects, the linker is a transmembrane linker. In another embodiment of any of the aspects, the linker is in a luminal position relative to the lipid membrane of the extracellular vesicle.
(25) In another embodiment of any of the aspects, the engineered extracellular vesicle does not comprise an endogenous POI polypeptide.
(26) In another embodiment of any of the aspects, the composition further comprises a pharmaceutically acceptable carrier.
(27) In another embodiment of any of the aspects, the one or more sticky binders or the vesicle

targeting domain is selected from the group consisting of: a GPI anchor, a fatty acylation site, and a prenylation site.
(28) In another embodiment of any of the aspects, the signaling domain or the protein of interest comprises one or more of: PD-L1, PD-L2, CTLA-4 (CD152), 4-1BBL (CD137L), HVEM (CD270), FGL1, OX-2 (CD200), Galectin-9, PVR (CD155), Nectin-2 (CD112) isoform alpha, Nectin-2 (CD112) isoform beta, Nectin-2 (CD112) isoform delta, IL-10, TSG-6, B7-H3 (CD276), B7-H4 (VTCN1), B7-H5 (VISTA), B7-H7 (HHLA2), BTNL1, VSIG8, VSIG3 (IGSF11), VSIG4, TIM-3 (HAVCR2), TIM-4 (TIMD4), CEACAM1, BTN3A1, BTN3A2, BTN2A1, BTNL8, BTN2A2, BTN1A1, TIGIT, CD27L (CD70), CD30L (CD153), GITRL, CD40L (CD154), LIGHT (CD258), TL1, CD80, CD86, LFA-3 (CD58), SLAM (CD150), CD40, CD28, CD28H, CD2, LFA-3 (CD58), CD48, CD226, DR3, DcR3, FasL, TIM-1 (CD365), PD-1, or active fragment thereof.
(29) In another embodiment of any of the aspects, the isolating is via size exclusion chromatography. In another embodiment of any of the aspects, the purifying is via multimodal chromatography. In another embodiment of any of the aspects, the method further comprises performing an assay for POI binding to a target polypeptide.
(30) In another embodiment of any of the aspects, the vector construct further encodes a promoter. In another embodiment of any of the aspects, the promoter is a tissue-specific promoter or an inducible promotor.
(31) In one embodiment of any of the aspects, the method further comprises selecting a subject that has or is suspected of having an autoimmune disease or an inflammatory disease or condition. In another embodiment of any of the aspects, the inflammatory disease and/or condition is acute. In another embodiment of any of the aspects, the inflammatory related disease and/or condition is chronic.
(32) In another embodiment of any of the aspects, administering the composition provided herein comprises injection, topical administration, or inhalation.

Description

A BRIEF DESCRIPTION OF THE DRAWINGS
(1) FIG. **1** shows construct representation of fusion polypeptides for labeling an exosome surface with Type I membrane proteins.
(2) FIG. **2**A shows nucleic acid and translated protein sequences of full-length Phosphatidylserine binding: Lactadherin (MFGE8) C1C2. Underlined nucleic acid sequence highlights the sequence translated to the C1C2 protein. Bold and underlined text highlights the C1C2 domain used to anchor signaling domains of interest (i.e., PD-L1 extracellular domain) onto the surface of the Inventors' artificial synapses. FIG. **2**B shows nucleic acid and translated protein sequences of full length CD55 (DAF) Glycosylphosphatidylinositol (GPI) anchor. Bold and underlined text highlights the GPI anchor domain used to anchor signaling domains of interest (i.e., PD-L1 extracellular domain) onto the surface of the Inventors' artificial synapses engineered from exosomes. FIGS. **2**A-**2**B disclose SEQ ID NOS: 199-200, and 196-197, respectively, in order of appearance.
(3) FIG. **3** demonstrates the nucleic acid and translated protein sequence for the Fc linker used in genetically engineered constructs is shown in bold and underlined. FIG. **3** discloses SEQ ID NOS: 219-220, respectively, in order of appearance.
(4) FIG. **4**A demonstrates nucleic acid and translated protein sequence of human PD-L1 (CD274). Bold and underlined sequence highlights the PD-L1 extracellular domain used in the Inventors' artificial synapses engineered from exosomes. FIG. **4**B demonstrates nucleic acid and protein sequence of human PD-L2. Bold and underlined sequence highlights the PD-L2 extracellular domain used in the Inventors' artificial synapses engineered from exosomes. FIG. **4**C shows mRNA

and protein sequence of human CTLA-4 (CD152). Bold and underlined sequence highlights the CTLA-4 extracellular domain used in the Inventors' artificial synapses. FIGS. **4**A-**4**C disclose SEQ ID NOS: 1-2, 5-6, and 9-10, respectively, in order of appearance.

(5) FIG. **5**A shows an exemplary embodiment of pcDNA5-FRT cloning vector with a gene sequence coding for a fusion polypeptide inserted into a multiple cloning site. FIG. **5**B shows an exemplary embodiment of the Gateway® destination vector pEF5-FRT-V5-DEST with a gene sequence coding for a fusion polypeptide inserted into a multiple cloning site. The vectors were used for constitutive high-level expression of fusion polypeptide described herein in mammalian cells. FIG. **5**C shows the nucleic acid and protein sequence for the hCTLA4-Fc-GPI fusion polypeptide wherein the text for the signaling domain is bolded, Fc linker is underlined, and sticky binder is italicized. FIG. **5**D shows the nucleic acid and protein sequence for the hPDL1-GPI-P2A-hHVEM-GPI fusion polypeptide wherein the text for the signaling domain hPDL1 and hHVEM are bolded, P2A sequence is underlined, and sticky binder GPI is italicized. With P2A included, a self-cleaving peptide sequence, artificial synapses with this feature will have both hPDL1-GPI and hHVEM-GPI loaded onto the surface. FIG. **5**E shows the nucleic acid and protein sequence for the hPDL1-GPI-P2A-hFGL1-GPI fusion polypeptide wherein the text for the signaling domain hPDL1 and hFGL1 are bolded, P2A sequence is underlined, and sticky binder GPI is italicized. With P2A included, a self-cleaving peptide sequence, artificial synapses with this feature will have both hPDL1-GPI and FGL1-GPI loaded onto the surface. FIG. **5**F shows the nucleic acid and protein sequence for the hPDL1-GPI fusion polypeptide wherein the text for the signaling domain hPDL1 is bolded and sticky binder GPI is italicized. FIG. **5**G shows the nucleic acid and protein sequence for the hPDL1-Fc-GPI fusion polypeptide wherein the text for the signaling domain hPDL1 is bolded, Fc is underlined, and sticky binder GPI is italicized. FIG. **5**H shows the nucleic acid and protein sequence for the hPDL2-Fc-GPI fusion polypeptide wherein the text for the signaling domain hPDL2 is bolded, Fc is underlined, and sticky binder GPI is italicized. FIG. **5**I shows the nucleic acid and protein sequence for the hPDL1-C1C2 fusion polypeptide wherein the text for the signaling domain hPDL1 is bolded and sticky binder C1C2 is italicized. FIG. **5**J shows the nucleic acid and protein sequence for the hPDL2-C1C2 fusion polypeptide wherein the text for the signaling domain hPDL2 is bolded and sticky binder C1C2 is italicized. FIG. **5**K shows the nucleic acid and protein sequence for the 4F2-h41BBL fusion polypeptide wherein the text for the signaling domain h41BBL is bolded and sticky binder 4F2 is italicized. FIG. **5**L shows the nucleic acid and protein sequence for the hPDL1-4Fc-GPI fusion polypeptide wherein the text for the signaling domain hPDL1 is bolded, 4Fc is underlined, and sticky binder GPI is italicized. FIG. **5**M shows the nucleic acid and protein sequence for the Myr-NanoLuc® Luciferase fusion polypeptide wherein the text for the signaling domain NanoLuc® Luciferase is bolded, and sticky binder Myr is italicized. FIG. **5**N shows the nucleic acid and protein sequence for the Myr-mScarlet fusion polypeptide wherein the text for the signaling domain mScarlet is bolded, and sticky binder Myr is italicized. FIG. **5**O shows the nucleic acid and protein sequence for the secreted isoform of hPDL1 (SecPDL1) fusion polypeptide hSecPDL1-GPI wherein the text for the signaling domain hSecPDL1 is bolded and sticky binder GPI is italicized. FIG. **5**P shows the nucleic acid and protein sequence for the Tfr2-h41BBL fusion polypeptide wherein the text for the signaling domain h41BBL is bolded and sticky binder Tfr2 is italicized. FIG. **5**Q shows the nucleic acid and protein sequence for the CD9tm3-h41BBL fusion polypeptide wherein the text for the signaling domain h41BBL is bolded and sticky binder CD9tm3 is italicized. FIG. **5**R shows the nucleic acid and protein sequence for the Myr/Palm-4F2-h41BBL fusion polypeptide wherein the text for the signaling domain h41BBL is bolded, sticky binder Myr/Palm is underlined, and sticky binder 4F2 is italicized. FIG. **5**S shows the nucleic acid and protein sequence for the Myr/Palm-Link-h41BBL fusion polypeptide wherein the text for the signaling domain h41BBL is bolded, sticky binder Myr/Palm is italicized and underlined, and sticky binder Link (in this embodiment a GSSG linker (SEQ ID NO: 319)) is in regular text (not underlined and not italicized). FIG. **5**T shows the nucleic

acid and protein sequence for the hPDL1-Link-GPI fusion polypeptide wherein the text for the signaling domain hPDL1 is bolded, Link is underlined (in this embodiment a GSSG linker (SEQ ID NO: 319)), and sticky binder GPI is italicized. FIG. **5**U shows the nucleic acid and protein sequence for the secreted isoform of hPDL1 (SecPDL1) fusion polypeptide hSecPDL1-CD9tm2 wherein the text for the signaling domain hSecPDL1 is bolded and sticky binder CD9tm2 is italicized. FIG. **5**V shows the nucleic acid and protein sequence for the secreted isoform of hPDL1 (SecPDL1) fusion polypeptide hSecPDL1-CD9tm2-KRAS wherein the text for the signaling domain hSecPDL1 is bolded, sticky binder CD9tm2 is italicized, and sticky binder KRAS is italicized and underlined. FIG. **5**W shows the nucleic acid and protein sequence for the secreted isoform of hPDL1 (SecPDL1) fusion polypeptide hSecPDL1-CD9tm4 wherein the text for the signaling domain hSecPDL1 is bolded and sticky binder CD9tm4 is italicized. FIG. **5**X shows the nucleic acid and protein sequence for the secreted isoform of hPDL1 (SecPDL1) fusion polypeptide hSecPDL1-CD81 wherein the text for the signaling domain hSecPDL1 is bolded and sticky binder CD81 is italicized. FIG. **5**Y shows the nucleic acid and protein sequence for the hCD200-Fc-GPI fusion polypeptide wherein the text for the signaling domain hCD200 is bolded, Fc is underlined, and sticky binder GPI is italicized, a spacer sequence domain (regular text, not underlined and not italicized) separates hCD200 sequence from the Fc domain, a spacer sequence domain (regular text, not underlined and not italicized) separates Fc sequence from the GPI. FIG. **5**Z shows the nucleic acid and protein sequence for the hFGL1-GPI fusion polypeptide wherein the text for the signaling domain hFGL1 is bolded, and sticky binder GPI is italicized. FIG. **5**AA shows the nucleic acid and protein sequence for the hGal9-Fc-GPI fusion polypeptide wherein the text for the signaling domain hGal9 is bolded, Fc is underlined, and sticky binder GPI is italicized. FIG. **5**BB shows the nucleic acid and protein sequence for the hCD200-GPI fusion polypeptide wherein the text for the signaling domain hCD200 is bolded, and sticky binder GPI is italicized. FIG. **5**CC shows the nucleic acid and protein sequence for the hGal9-GPI fusion polypeptide wherein the text for the signaling domain hGal9 is bolded, and sticky binder GPI is italicized. FIG. **5**DD shows the nucleic acid and protein sequence for the hHVEM-GPI fusion polypeptide wherein the text for the signaling domain hHVEM is bolded, and sticky binder GPI is italicized. FIG. **5**EE shows the nucleic acid and protein sequence for the hPDL2-GPI fusion polypeptide wherein the text for the signaling domain hPDL2 is bolded, and sticky binder GPI is italicized. FIG. **5**FF shows the nucleic acid and protein sequence for the hTSG6-GPI fusion polypeptide wherein the text for the signaling domain hTSG6 is bolded, and sticky binder GPI is italicized. FIG. **5**GG shows the nucleic acid and protein sequence for the hHVEM-Fc-GPI fusion polypeptide wherein the text for the signaling domain hHVEM is bolded, Fc is underlined, and sticky binder GPI is italicized. FIG. **5**HH shows the nucleic acid and protein sequence for the mCTLA4-Fc-GPI fusion polypeptide wherein the text for the signaling domain mCTLA4 is bolded, Fc is underlined, and sticky binder GPI is italicized. FIG. **5**II shows the nucleic acid and protein sequence for the mPDL1-C1C2 fusion polypeptide wherein the text for the signaling domain mPDL1 is bolded and sticky binder C1C2 is italicized. FIG. **5**JJ shows the nucleic acid and protein sequence for the mPDL1-Fc-GPI fusion polypeptide wherein the text for the signaling domain mPDL1 is bolded, Fc is underlined, and sticky binder GPI is italicized. FIG. **5**KK shows the nucleic acid and protein sequence for the mPDL1-GPI fusion polypeptide wherein the text for the signaling domain mPDL1 is bolded and sticky binder GPI is italicized. FIG. **5**LL shows the nucleic acid and protein sequence for the mPDL2-C1C2 fusion polypeptide wherein the text for the signaling domain mPDL2 is bolded and sticky binder C1C2 is italicized. FIG. **5**MM shows the nucleic acid and protein sequence for the mPDL2-Fc-GPI fusion polypeptide wherein the text for the signaling domain mPDL2 is bolded, Fc is underlined, and sticky binder GPI is italicized. FIG. **5**NN shows the nucleic acid and protein sequence for the mPDL1-mFc-GPI fusion polypeptide wherein the text for the signaling domain mPDL2 is bolded, mFc is underlined, and sticky binder GPI is italicized. FIG. **5**OO shows the nucleic acid and protein sequence for the mPDL2-GPI fusion polypeptide wherein the text for the signaling domain mPDL2

is bolded and sticky binder GPI is italicized. FIG. **5**PP shows the nucleic acid and protein sequence for the mPDL1-GPI-P2A-mHVEM-GPI fusion polypeptide wherein the text for the signaling domain mPDL1 and mHVEM are bolded, P2A sequence is underlined, and sticky binder GPI is italicized. With P2A included, a self-cleaving peptide sequence, artificial synapses with this feature will have both mPDL1-GPI and mHVEM-GPI loaded onto the surface. FIG. **5**QQ shows the nucleic acid and protein sequence for the hPDL1-ADAM10 fusion polypeptide wherein the text for the signaling domain mPDL1 is bolded and sticky binder ADAM10 is italicized. FIG. **5**RR shows the nucleic acid and protein sequence for the hPDL1-4Fc-CD9tm2 fusion polypeptide wherein the text for the signaling domain hPDL1 is bolded, 4Fc is underlined, and sticky binder CD9tm2 is italicized. FIG. **5**SS shows the nucleic acid and protein sequence for the fusion polypeptide hPDL1-4Fc-CD9tm2-KRAS wherein the text for the signaling domain hPDL1 is bolded, sticky binder 4Fc is underlined, sticky binder CD9tm2 is italicized, and sticky binder KRAS is italicized and underlined. FIG. **5**TT shows the nucleic acid and protein sequence for the hPDL1-Fc-CD9tm2 fusion polypeptide wherein the text for the signaling domain hPDL1 is bolded, Fc is underlined, and sticky binder CD9tm2 is italicized. FIG. **5**UU shows the nucleic acid and protein sequence for the fusion polypeptide hPDL1-Fc-CD9tm2-KRAS wherein the text for the signaling domain hPDL1 is bolded, sticky binder Fc is underlined, sticky binder CD9tm2 is italicized, and sticky binder KRAS is italicized and underlined. FIG. **5**VV shows the nucleic acid and protein sequence for the mPDL1-mFc-CD9tm2 fusion polypeptide wherein the text for the signaling domain mouse PDL1 (mPDL1) is bolded, mouse mFc (mFc) is underlined, and sticky binder CD9tm2 is italicized. FIG. **5**WW shows the nucleic acid and protein sequence for the fusion polypeptide mPDL1-mFc-CD9tm2-KRAS wherein the text for the signaling domain mPDL1 is bolded, sticky binder mFc is underlined, sticky binder CD9tm2 is italicized, and sticky binder KRAS is italicized and underlined. Wherein mPDL1 and mFc are mouse PDL1 and mouse Fc, respectively. FIGS. **5**C-**5**WW disclose SEQ ID NOS: 223-224, 283-284, 239-240, 225-226, 229-230, 233-234, 227-228, 231-232, 235-238, 243-244, 241-242, 245-282, and 285-316, respectively, in order of appearance.

(6) FIG. **6** shows hPD-L1-Fc-GPI artificial synapse purification via a multimodal resin marketed for exosome purification. Large MW artificial synapses elute in the first fraction as shown by the high hPD-L1 concentration and artificial synapse quantity (2.26E9 synapses/ml) in elution 1. Clean in place (CIP) fractions show bound and eliminated proteins from the Inventors' artificial synapse elution.

(7) FIG. **7** shows hPDL1-Fc-GPI exosome purification via size exclusion chromatography using a resin marketed for exosome purification. Artificial synapses engineered from exosomes eluted from via a multimodal resin may be further purified via size exclusion chromatography using a resin marketed for exosome purification as shown here. Using a size exclusion chromatography, artificial synapses elute in fractions 7-9. Total protein (determined by Qubit™) and hPD-L1 ng/ml (determined by ELISA) of each fraction is shown in the graph. Bars show exosome number per ml (i.e., 1E10 exosomes/ml etc.). Fractions 7-9 contain >99% purified artificial synapses. Fractions 7-9 are pooled and may be concentrated using a filtration device, for example a 10K MWCO Amicon® centrifugal filter. Final purified product may be filtered through a low protein binding filter, for example a 0.2 μm or 0.45 μm PES filter.

(8) FIG. **8** shows hPD-L1 Expression on exosomes, quantity and hPD-L1 concentration was determined in size exclusion chromatography fractions 7-9. Knowing the molecular weight of engineered hPD-L1, the Inventors can determine the number of hPD-L1 molecules per exosome to be approximately between 12 and 40 hPD-L1/exosome. This value is consistent between different purification runs and constructs.

(9) FIG. **9** shows the purification of hPD-L2-Fc-GPI artificial synapses engineered from exosomes via multimodal resin marketed for exosome purification. This graph shows Abs 280 of fractions and quantity of hPDL2 in indicated fractions. Exosomes eluted in Elution 1. Clean in place (CIP) fractions show bound and eliminated proteins from the Inventors' artificial synapse elution.

(10) FIG. **10** shows purification of hPD-L2-Fc-GPI labeled exosomes via size exclusion column as shown here using size exclusion resin marketed for exosome purification. Fractions containing large molecular weight exosomes (Fractions 7-9) showed high hPD-L2 concentration indicating that the purified exosomes contain hPD-L2-Fc-GPI. Total protein (determined by Qubit™) and hPD-L1 ng/ml (determined by ELISA) of each fraction is shown in the graph. Lower molecular weight unbound hPD-L2-Fc-GPI eluted at later fractions.
(11) FIG. **11** shows hCTLA4-Fc-GPI exosome purification via size exclusion column as shown here using size exclusion resin marketed for exosome purification. Using size exclusion chromatography, exosomes elute in fractions 7-9. Total protein (determined by Qubit™) and hCTLA4 ng/ml (determined by ELISA) of each fraction is shown in the graph. Fractions 7-9 are pooled and contain >99% purified exosomes. Pooled exosome fractions may then be concentrated using a filtration device, for example a 10K MWCO Amicon® centrifugal filter. Final purified product may be filtered through a low protein binding filter, for example a 0.2 μm or 0.45 μm PES filter. Knowing the molecular weight of engineered hCTLA-4, the Inventors can determine the number of hCTLA-4 molecules per exosome to be approximately 233 hCTLA-4/exosome.
(12) FIG. **12**A shows PD-1 Signaling Bioassay Method. The Inventors established a method to validate that PD-L1 and PD-L2 artificial synapses engineered from exosomes can bind to cells expressing PD-1 ligand. To perform this validation method, the Inventors modified the PathHunter® PD-1 Signaling Bioassay from DISCOVERX® Briefly, the PathHunter® PD-1 Signaling Bioassay relies on the well-established PathHunter® Enzyme Fragment Complementation (EFC) technology to interrogate receptor activity. EFC consists of a split-galactosidase (β-gal) enzyme: the Enzyme Donor (ED) and Enzyme Acceptor (EA) fragments which independently have no β-gal activity. However, when forced to complement they form an active β-gal enzyme that will hydrolyze substrate to produce a chemiluminescent signal. The PathHunter® PD-1 Signaling Bioassay consists of human cells engineered to stably express an ED-tagged PD-1 receptor, while EA is fused to the phosphotyrosine-binding SH2 domain of the intracellular signaling protein, SHP1. Ligand or antibody-induced activation of the receptor results in phosphorylation of the receptor's cytosolic tail. Ligand engagement, through addition of ligand-presenting artificial synapses engineered from exosomes, results in phosphorylation of PD-1, leading to the recruitment of SHP1-EA. This forces complementation of the EFC components to create an active β-gal enzyme. This active enzyme hydrolyzes substrate to create chemiluminescence as a measure of receptor activity. Addition of an antagonist (e.g., antibody to PD-L1) blocks PD-1 signaling, and will prevent complementation, resulting in a loss of signal. FIG. **12**B shows that the Inventors obtained approximately 10,000× higher increase in Relative Light Units (RLU) in Jurkat signaling cells treated with PD-L1 or PD-L2 labeled artificial synapses when compared to soluble PD-L1-Fc or PD-L2-Fc ligand, respectively. Meaning, it took 10,000× less μg/ml of PD-L1 or PD-L2 on artificial synapses than solubilized PD-L1-Fc or PD-L2 ligand to achieve the same RLU signaling. Shown is a dose-response curve for the PD-L1 and PD-L2 artificial synapses engineered from exosomes vs soluble PD-L1 and PD-L2 signaling bioassay.
(13) FIG. **13**A-**13**C shows experimental EAU outline Test Agent A—unmodified exosomes, Test Agent B—mPDL1-Fc-GPI artificial synapses engineered from exosomes 40 μg/ml, Test Agent C—mPDL1-Fc-GPI artificial synapses engineered from exosomes 400 μg/ml, IRBP-interphotoreceptor retinoid-binding protein (IRBP) peptide, BID-Bis in die (2× daily) p.o.—Per os (orally) (FIG. **13**B) EAU symptoms appear at day 6. 1st intravitreal injection and 2nd intravenous injections are performed on Day 6. There is a statistically significant initial reduction in EAU in mouse PD-L1 (mPD-L1) artificial synapses engineered from exosomes treated rats via either the intravitreal and intravenous delivery modes. 2nd intravitreal and 3rd intravenous injections are performed on Day 12. There appears to be a more rapid rate of resolution in the 1× intravitreal and intravenous groups. (FIG. **13**C) Weight of rats was monitored throughout the study. 3rd intravitreal and 4th intravenous injections are performed on Day 16. There does not appear to be any significant change

in EAU in any of the test groups. The aforementioned results provide proof of principle of successfully treating an autoimmune condition (i.e., EAU) with human cell derived artificial synapses with PD-L1.

(14) FIG. **14** shows 2 types of ligands displayed on the exosome surface (Type I and Type II membrane proteins). Type I membrane proteins wherein the N-Terminus is on the luminal (interior) side of the exosome membrane and the C-Terminus is on the exterior of the exosome.

(15) Type II membrane proteins wherein the N-Terminus is on the exterior while the C-Terminus is on the interior.

(16) FIG. **15** shows a schematic representation of several embodiments of Type I membrane protein constructs, which include but are not limited to: PD-L1, PD-L2, FGL1, OX40L. FIG. **15** discloses "GSSG link" as SEQ ID NO: 319.

(17) FIG. **16** shows a schematic representation of several embodiments of the surface of an extracellular vesicle engineered with a Type I membrane protein of interest (POI) with a variable membrane anchor. Vesicle targeting sequences such as select sequences from 4F2 (CD98), ADAM10, CD298, TFR2, transmembrane portions of CD9, MARCKS, KRAS, and GPI from CD55. Proteins engineered to include a targeting sequence domain may include one or more linkers between the sticky binder and signaling domain (e.g., an Fc linker or a bond sequence wherein the bond sequence may be dimerization or multimerization sequence).

(18) FIG. **17** shows a schematic representation of the surface of an exosome engineered with an extracellular portion of the Type II membrane protein of interest (POI) with transmembrane/exosome targeting domains.

(19) FIG. **18** shows a schematic representation of an exosome engineered with an extracellular portion of the Type II membrane protein 4-1BB.

(20) FIG. **19** demonstrates a construct design for labeling an exosome surface with Type II membrane proteins.

(21) FIG. **20** shows a schematic representation of a construct design for labeling an exosome surface with multiple POI domains operably linked by a cleavable (e.g., P2A) linker.

(22) FIG. **21** shows a flow chart of purification and analytical processes provided herein.

(23) FIG. **22** shows a PD-L1 labeled exosome constructs.

(24) FIG. **23** shows several embodiments of the surface of an exosome engineered with PD-L1. The PD-L1 can be the membrane-bound PD-L1 isotype or secreted PD-L1 (SecPD-L1).

(25) FIG. **24** demonstrates size exclusion chromatography for purifying human PD-L1-GPI (no Fc) exosomes. Left panel: Protein, RNA and DNA measurements in SEC fractions are shown. Invitrogen Qubit™ fluorometric assays were used to measure biomolecules from unmodified concentrated cell media SEC fractions or hPD-L1-Exo-Tag concentrated cell media SEC fractions. PD-L1 was measured using an R&D systems PD-L1 ELISA kit. Right panel shows dot-blot immunoblot analysis of SEC fractions. A 96-well dot blot apparatus was used to immobilize 50 µl of each SEC fraction onto PVDF. Right bottom figures: Exosome size and concentration was measured in fraction 7 by tunable resistive pulse sensing (TRPS).

(26) FIG. **25** demonstrates that GPI anchors hPD-L1 on exosomes.

(27) FIG. **26** demonstrates that a multimodal resin marketed for exosome purification purifies and disaggregates exosomes.

(28) FIG. **27** shows the exosome decoration with hPD-L1-Fc-GPI.

(29) FIG. **28**A shows the exosome decoration with hPD-L1-Fc-GPI. Fraction 7 contained the purified hPD-L1-Fc-GPI vesicles. FIG. **28**B shows size exclusion chromatography (SEC) purification results of various embodiments of human PD-L1 displayed on the surface of extracellular vesicles.

(30) FIG. **29** shows that mouse PD-L1-Fc-GPI exosomes have higher valency than mPD-L1-GPI.

(31) FIG. **30**A-**30**C demonstrates comparison proteomics of transprotein expression and shows that surface labeling on the engineered extracellular vesicles provided herein do not affect the relative

expression of native and associated exosome proteins. FIG. **30**A shows hPD-L1-Fc-GPI. FIG. **30**B shows hPD-L2-FcGPI. FIG. **30**C shows hCTLA4-Fc-GPI.
(32) FIG. **31** shows production of mPD-L1-Fc-GPI in STR Bioreactor.
(33) FIG. **32** shows purification of mPD-L1-Fc-GPI (STR) via SEC. Graph shows mPD-L1 ng/ml vs Total Protein μg/ml.
(34) FIG. **33** shows purification mPDL1-Fc-GPI (STR bioreactor).
(35) FIG. **34** shows a schematic representation of the 4-1BBL labeled exosomes. Top: Vector map showing the N-terminal cystolic domain, a transmembrane (TM) domain, and the POI domain at the C-terminus. Bottom: An embodiment of an engineered EV with a type-II membrane display of the fusion protein.
(36) FIG. **35** shows embodiments of a 4-1BBL display exosome.
(37) FIG. **36**A-**36**B show the protein engineering and purification of 4F2-4-1BBL labeled exosomes. FIG. **36**B confirms that h4-1BBL is displayed on the engineered exosomes.
(38) FIG. **37** shows internal fusion protein loading of exosomes.
(39) FIG. **38** shows internal loading of exosomes with mScarlet (RFP).
(40) FIG. **39**A shows internal loading of exosomes with NanoLuc® luciferase. FIG. **39**B shows tetraspanin characterization of exosomes internally loaded with NanoLuc® luciferase.
(41) FIG. **40**A shows the mechanism of PD-L1 engineered extracellular vesicles induce membrane clustering and receptor agonism on a target cell. An exemplary model of proposed mechanism of extracellular vesicles with a Type I membrane protein signaling domain (PD-L1) promoting receptor clustering on a target cell, wherein receptor clustering promotes increased potency of signal transduction of the target receptor. Antagonist antibodies function well at blocking receptors. Antibodies are poor agonist modalities due to their general inability to cluster receptors. Ligands on a membrane surface are potent agonists, however the cost and cold chain logistics of cell therapies makes commercialization difficult and expensive. Extracellular vesicles engineered with Type I membrane protein are able to induce receptor clustering of target receptors and initiate and propagate a potent signal response on a target cell.
(42) FIG. **40**B shows the mechanism of 4-1BBL engineered extracellular vesicles induce membrane clustering and receptor agonism on a target cell. An exemplary model of proposed mechanism of extracellular vesicles with a Type II membrane protein signaling domain (4-1BBL) promoting receptor clustering on a target cell, wherein receptor clustering promotes increased potency of signal transduction of the target receptor. Soluble ligands are often poor agonist modalities due to their general inability to cluster receptors. Ligands displayed on a membrane surface are potent agonists, however the cost and cold chain logistics of cell therapies makes commercialization difficult and expensive. Extracellular vesicles engineered with Type II membrane protein are able to induce receptor clustering of target receptors and initiate and propagate a potent signal response on a target cell.
DETAILED DESCRIPTION
(43) The compositions and methods provided herein are based, in part, on the discovery that engineered extracellular vesicles (e.g., exosomes) expressing an engineered fusion protein (e.g., PD-L1) reduces inflammation in an animal model of experimental autoimmune uveoretinitis (EAU), an autoimmune disorder. The compositions and methods provided herein are further based, in part, on the discovery that engineered extracellular vesicles produce enhanced signaling compared to an equal quantity of recombinant ligand. Since some cellular receptors, (e.g., PD-1) require clustering or super-clustering to promote a signaling response, it stands to reason that extracellular vesicles engineered to express ligands on their surface wherein the ligands may engage target receptors on target cells and promote clustering of said target receptors thereby promoting a signal response on said target cell.
(44) In one aspect, provided herein is an engineered extracellular vesicle comprising: at least one fusion polypeptide comprising: at least one protein of interest (POI) domain; and at least one

vesicle targeting domain. In some embodiments of any of the aspects, the engineered extracellular vesicle is an exosome. In some embodiments, of any of the aspects, the fusion protein further comprises at least one linker. In some embodiments of any of the aspects, the POI domain can substantially bind to a target polypeptide. In some embodiments of any of the aspects provided herein, the engineered extracellular vesicle is an artificial synapse.

(45) Generally, the extracellular vesicles (e.g., exosomes) provided herein are produced by contacting a population of cells with a nucleic acid construct encoding the fusion proteins provided herein and isolating a plurality of extracellular vesicles. The extracellular vesicles can then be purified by methods provided herein and are formulated for therapeutic use, including but not limited to, for the treatment of autoimmune diseases, cancer, or modulating inflammation in a subject.

(46) The compositions and methods provided herein are specifically designed to exploit the membrane trafficking mechanisms of extracellular vesicles and rely on the hallmark biophysical and biochemical properties of extracellular vesicles, such as exosomes. The vesicles/artificial synapses provided herein are specifically engineered to induce/agonize and propagate biological signaling via a target polypeptide (e.g., by activating a receptor or enzyme or agonizing said receptor or enzyme). Alternatively, the engineered extracellular vesicles provided herein can act as cellular decoys or to reduce or antagonize biological signaling, e.g., by blocking an endogenous ligand from binding to a target cellular receptor and preventing activation of the receptor.

(47) Engineering of the extracellular vesicles provided herein extends these capabilities significantly by incorporating sticky binders attaching to extracellular vesicles such as exosomes, further coupled with signaling domains of choice. For example, attachment of sticky binders to exosomes, along with their linked signaling domains, allows for receptor clustering for biological signal induction/agonism and propagation not otherwise possible. In this aspect, the aforementioned design achieves the aim of an engineered extracellular vesicle by inducing the desired biological signaling in a target recipient cell.

(48) Various aspects and embodiments of the compositions and methods are provided herein in detail below.

Engineered Extracellular Vesicle (EV) Compositions

(49) The compositions provided herein comprises at least one extracellular vesicle (also termed artificial synapse or abbrv: EV), wherein the extracellular vesicle comprises at least one fusion polypeptide or a plurality of fusion polypeptides comprising: at least one vesicle targeting domain (e.g., sticky binders); and at least one protein of interest domain or a fragment thereof (also termed signaling domains).

(50) Extracellular vesicles (EVs) are lipid particles that are released from various cell types that function to transfer “cargo” such as nucleic acids and proteins to other cells. EVs are not able to replicate but serve as cell messengers. EV-mediated signals can be transmitted by all the different biomolecule categories-protein, lipids, nucleic acids and sugars—and the unique package of this information provides both protection and the option of simultaneous delivery of multiple different messengers even to sites remote to the vesicular origin. See, e.g., Yáñez-Mó M, Siljander P R, Andreu Z, et al. Biological properties of extracellular vesicles and their physiological functions. J Extracell Vesicles. 2015; 4:27066. Published 2015 May 14. doi: 10.3402/jev.v4.27066, which is incorporated herein by reference in its entirety. Furthermore, there is an increasing amount of evidence that shows that EVs can modulate a milieu of cellular signaling processes. See, e.g., Yadid et al. *Science Translation Medicine* (2020); Cerqueira de Abreu et al. *Nature Reviews Cardiology* (2020); Zhang W. et al. *Protein J*. (2019); Zha Q B et al. *Tumor Biology*. February 2017; Tan et al. (2016) Recent advances of exosomes in immune modulation and autoimmune diseases, *Autoimmunity,* 49:6, 357-365; Kalluri R, LeBleu V S. et al. The biology, function, and biomedical applications of exosomes. Science. 2020 Feb. 7; 367(6478); which is incorporated herein by reference in its entirety.

(51) There are various types of extracellular vesicles that are named for their site of origin in a cell, size, and structural and/or functional properties. In some embodiments of any of the aspects provided herein, the extracellular vesicle is an exosome, ectosome, macrovesicle, microparticle, apoptotic body, vesicular organelle, oncosome, exosphere, exomeres, or cell derived nanovesicle (CDN) ((e.g., by genesis via grating or shearing cells), liposomes or the like known by one of ordinary skill in the art. In various embodiments, the extracellular vesicle comprises a phospholipid bilayer with an exterior phospholipid layer and an interior phospholipid layer, wherein the exterior phospholipid layer has an external surface and an internal surface, wherein the interior phospholipid layer has an internal surface and an external surface, and the internal surface of the exterior phospholipid layer faces the internal surface of the interior phospholipid layer, and the phospholipid bilayer encloses an internal space, wherein the external surface of the interior phospholipid layer faces the internal space and wherein the external surface of the exterior phospholipid layer faces an extracellular environment, and the external surface of the inner phospholipid layer is the internal surface of the extracellular vesicle.

(52) In various embodiments, the extracellular vesicles range in size from 30 nanometers (nm) to 300 nm. In various embodiments, the plurality of EVs range in size from about 30 nm to about 150 nm. In various embodiments, the plurality of EVs or artificial synapses includes one or more artificial synapses that are about 10 nm to about 250 nm in diameter, including those about 10 nm to about 15 nm, about 15 nm to about 20 nm, about 20 nm to about 25 nm, about 25 nm to about 30 nm, about 30 nm to about 35 nm, about 35 nm to about 40 nm, about 40 nm to about 50 nm, about 50 nm to about 60 nm3 about 60 nm to about 70 nm, about 70 nm to about 80 nm, about 80 nm to about 90 nm, about 90 nm to about 95 nm, about 95 nm to about 100 nm, about 100 nm to about 105 nm, about 105 nm to about 110 nm, about 110 nm to about 115 nm, about 115 nm to about 120 nm, about 120 nm to about 125 nm, about 125 nm to about 130 nm, about 130 nm to about 135 nm, about 135 nm to about 140 nm, about 140 nm to about 145 nm, about 145 nm to about 150 nm, about 150 to about 200 nm, about 200 nm to about 250 nm, about 250 nm or more.

(53) In some embodiments of any of the aspects provided herein, the EV is an exosome. Exosomes are membrane-bound EVs that are produced in the endosomal compartment of most eukaryotic cells. As used herein, the term “exosome” refers to a species of extracellular vesicle between about 20 nm to about 400 μm in diameter, e.g, about 30 nm-200 nm in diameter by inward invagination of a portion of a membrane of an endosome (for example an early or late endosome), wherein the endosome is within a cell comprising a plasma membrane, and the exosome is released from the cell upon fusion of another portion of the endosome membrane with the plasma membrane. An exosome may refer to a species of extracellular vesicle between 20 nm-400 μM in diameter, more preferably 30 nm-200 nm in diameter, that originates by budding of a portion of a plasma membrane from a cell wherein the budded portion of the plasma membrane is released to the extracellular environment.

(54) The EVs (e.g., exosomes or cell derived vesicles) provided herein may comprise cargo, for example, peptides, proteins, nucleic acids, lipids, metabolites, carbohydrates, biomolecules, small molecules, large molecules, vesicles, organelles, or fragments thereof. Exosome cargo may be located within the internal space of the exosome. EV cargo may be membrane bound spanning one or both layers of the exosome phospholipid bilayer (for example a transmembrane protein). EV cargo may be in contact with the exterior or interior surface of the exosome, for example through a covalent bond or a non-covalent bond. The phospholipid bilayer of the EV or exosome provided herein may comprise one or more transmembrane proteins, wherein a portion of the one or more transmembrane membrane proteins is located within the internal space of the exosome. The phospholipid bilayer of the EV or exosome provided herein may comprise one or more transmembrane proteins, wherein a portion of the one or more transmembrane membrane proteins traverses the EV phospholipid bilayer. The phospholipid bilayer of the EV may comprise one or more transmembrane proteins, wherein the one or more transmembrane membrane proteins

comprises a domain on the exterior of the exosome.
(55) In some embodiments of any of the aspects, the extracellular vesicles or exosomes provided herein endogenously express CD81+, CD82+, CD37+, CD63+, CD9+, CD151+, CD105+, or any combination thereof. In various embodiments, the plurality of artificial synapses includes one or more artificial synapses expressing a biomarker. In certain embodiments, the biomarkers are tetraspanins. In other embodiments, the tetraspanins are one or more selected from the group including CD63, CD81, CD82, CD53, CD151, and CD37. In other embodiments, the artificial synapses express one or more lipid raft associated proteins (e.g., glycosylphosphatidylinositol-anchored proteins and flotillin), cholesterol, sphingolipids such as sphingomyelin, and/or hexosylceramides.
(56) In other embodiments, the biological protein is related to exosome formation and packaging of cytosolic proteins, e.g., Hsp70, Hsp90, 14-3-3 epsilon, PKM2, GW182 and AGO2. In certain embodiments, the artificial synapses express CD63, HSP70, CD105 or combinations thereof. In other embodiments, the artificial synapses do not express CD9 or CD81, or express neither. For example, plurality of artificial synapses can include one or more artificial synapses that are CD63+, HSP+, CD105+, CD9−, and CD81−.
(57) The EVs provided herein are specifically engineered to express fusion polypeptides that elicit biological signaling via a target cell. In some embodiments, the fusion polypeptide is overexpressed to elicit a biological response on a target cell or target polypeptide. The engineered EV comprises at least one fusion polypeptide and can comprise a plurality of the same or different fusion polypeptides provided herein. The fusion polypeptides provided herein comprise a protein of interest domain, also termed the signaling domain.
(58) The fusion polypeptides provided herein can comprise one or more of a protein of interest domain, such that expression of said fusion polypeptide is permitted and that the number of POI domains does not impede protein expression or folding. Furthermore, the EVs provided herein can express more than one fusion protein (e.g., encoded by multiple different nucleic acid constructs). One of skill in the art can appreciate that an engineered EV can include one or more combinations of different signaling domains and/or vesicle targeting domains, or that one can use a plurality of engineered EVs, each including one or more vesicle targeting domains and one or more signaling domains.
(59) In some embodiments, the EVs provided herein comprise one or more, two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more fusion proteins. The fusion proteins can be encoded by the same vector or separate vectors. In some embodiments of any of the aspects, the engineered extracellular vesicle comprises at least two POI domains and/or at least two vesicle targeting domains.
(60) In some embodiments, the fusion polypeptide comprises one or more, two or more, three or more, four or more, five or more, or six or more POI domains on the same polypeptide or nucleic acid construct encoding said polypeptide. For example, the fusion polypeptides provided herein can express a fusion polypeptide encoding one or more, two or more, three or more, four or more, five or more, or six or more signaling domains. In another example, the fusion polypeptides provided herein can express a fusion polypeptide encoding an immune checkpoint protein or a protein involved in immune or cell synapse or any combination or fragment thereof.
(61) In some embodiments, the EV comprises one or more, two or more, three or more, four or more, five or more, or six or more fusion polypeptides on the same EV. For example, EVs comprising one or more, two or more, three or more, four or more, five or more, or six or more fusion polypeptides wherein the fusion polypeptides encode a signaling domain. In another example, EVs comprising one or more, two or more, three or more, four or more, five or more, or six or more fusion polypeptides wherein the fusion polypeptides encode for one or more immune checkpoint proteins or proteins involved in immune or cell synapse, or any combination or fragment thereof.

(62) In various embodiments, the signaling domain is a protein or peptide of interest, or a fragment thereof. In various embodiments, the protein of interest (signaling domain) is an immune checkpoint protein. The terms “immune checkpoint protein” or “protein involved in immune or cell synapse” can include but are not limited to adenosine A2A receptor (A2AR), Galectin 9, fibrinogen-like protein 1 (FGL-1), platelet endothelial adhesion factor-1 (PECAM-1), tumor necrosis factor gene 6 protein (TSG-6), Stabilin-1 (STAB-1) also known as Clever-1, Neuropilin 1 (NRP1), Neuropilin 2 (NRP2), semaphorin-3A (SEMA3A), semaphorin-3F (SEMA3F), repulsive guidance molecule B (RGMB) also known as DRG11, T-cell immunoglobulin and mucin domain 3 (TIM-3), T cell immunoreceptor with Ig and ITIM domains (TIGIT), human leukocyte antigen (HLA) class I, HLA class II, high mobility group protein B1 (HMGB1), phosphatidylserine, carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM-1), T-cell receptor (TCR), Src homology 2 domain-containing protein tyrosine phosphatase 1 (SHP-1), SHP-2, F-Box protein 38 (FBXO38), signaling lymphocytic activation molecule (SLAM)-associated protein (SAP) also known as SH2D1A, B7RP1, indoleamine 2,3-dioxygenase (IDO), NADH oxidase 2 (NOX2), tumor necrosis factor receptor (TNFR) superfamily member 18 (TNFRSF18) (also known as activation inducible TNFR family receptor (AITR), glucocorticoid-induced TNFR related (GITR) protein, and CD357), B7-H4 also known as V-set domain containing T-cell activator inhibitor (VTCN1), B7-H5 (also known as V-domain Ig suppressor of T-cell activation (VISTA), platelet receptor Gi24, and stress induced secreted protein 1 (SISP1), B7-H6 (also known as NCR3LG1), B7-H7 (also known as human endogenous retrovirus-H (HERV-H) long terminal repeat-associating protein 2 (HHLA2), apelin receptor (APLNR), interferon gamma (IFN y) receptor, programmed cell death-1 (PD-1), Protein Wnt-5a (WNT5A), serine/threonine-protein kinase PAK4, interleukin 6 (IL-6), interleukin-10 (IL-10), NKG2 family of C-type lectin receptors (for example NKG2A, B, C, D, E, F and H), ligands of NKG2 family, killer cell immunoglobulin-like receptors, CD-2, cluster of differentiation 4 (CD4), CD8, CD27, CD27 ligand (CD27L, also known as CD70), CD28, CD28H (also known as transmembrane and immunoglobulin domain containing 2 (TMIGD2) and Ig containing and proline-rich receptor-1 (IGPR1)), CD39, CD40, CD44, integrin associated protein (CD47), carcinoembryonic antigen related cell adhesion molecule 1 (CEACAM1 also known as CD66a), CD73, B7-1 (also known as CD80), B7-2 (also known as CD86), CD94, CD96, immunoglobulin superfamily member 2 (IGSF2) also known as CD101, nectin cell adhesion molecule 2 (NECTIN2) (also known as herpesvirus entry mediator B (HVEB), poliovirus receptor related 2 (PRR2, PVRL2 and PVRR2) and CD112), poliovirus receptor related immunoglobulin domain containing protein (PVIRG) also known as CD112R, CD122 (also known as IL5RB and P70-75), OX40 (also known as tumor necrosis factor receptor superfamily member 4 (TNFRSF4) and CD134), OX40 ligand (OX40L), 4-1BB (also known as CD137), CD134 (also known as 4-1BB ligand (4-1BBL) and as tumor necrosis factor ligand superfamily member 9 (TNFSF9) and CD137L), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) also known as CD152, CD154 (also known as CD40L), poliovirus receptor (PVR) also known as CD155, killer-cell immunoglobulin-like receptors (KIRs) (for example but not limited to CD158 family, CD158a, CD158g, CD158h, KIR2DL1, KIR2DS1, KIRDS3, and KIR2DS5), CD160, signal-regulatory protein alpha (SIRPα) also known as CD172a, OX-2 also known as CD200, CD200R, lymphocyte-activation gene 3 (LAG-3) also known as CD223, CD226, OX40L also known as CD252, herpes virus entry mediator (HVEM) also known as tumor necrosis factor receptor superfamily member 14 (TNFRSF14) and CD270, B- and T-lymphocyte attenuator (BTLA) also known as CD272, programmed cell death ligand-2 (PD-L2) (also known as B7-DC, PDCDILG2, and CD273), programmed cell death-ligand 1 (PD-L1) (also known as B7-H1 and CD274), B7-H2 (also known as inducible T-cell co-stimulator ligand (ICOSLG), B7RP1, and CD275), B7-H3 also known as CD276, inducible T-cell co-stimulator (ICOS) also known as CD278, programed cell death protein 1 (PD-1) also known as CD279, leukocyte-associated Ig-like receptor-1 (LAIR-1) also known as CD305, collagen family of proteins (for example but not limited to collagen I, collagen II, collagen

III alpha 1, collagen IV, collagen XXIII alpha 1, collagen XXV alpha 1), sialic acid-binding immunoglobulin-type lectin 7 (SIGLEC7) also known as CD328, sialic acid-binding immunoglobulin-type lectin 7 (SIGLEC9) also known as CD329, natural cytotoxicity triggering receptor 3 (NKp30) also known as CD337, or any isoform, fragment, variation thereof, or a ligand to the aforementioned proteins thereof, or the like known by one of ordinary skill in the art. All variants are encompassed by the present invention.

(63) In some embodiments of any of the aspects provided herein, the protein of interest domain (POI domain) comprises a polypeptide or a fragment thereof or a nucleic acid encoding said polypeptide or fragment thereof selected from the group consisting of: Table 1 (below). Non-limiting examples of nucleic acid sequences that encode the POI domains provided herein are also provided in

(64) TABLE-US-00001 TABLE 1 Type I Proteins of Interest Amino Acid Sequence Protein of Transcript Sequence (SEQ ID NO:) Interest Amino Acid Sequence (SEQ ID NO:) Human >NM_014143.4 *Homo sapiens* CD274 molecule (CD274), Programmed transcript variant 1, mRNA death-ligand 1

AGTTCTGCGCAGCTTCCCGAGGCTCCGCACCAGCCGCGCTTCTGTCCGCCTGCAGGG

(PD-L1)

CATTCCAGAAAGATGAGGATATTTGCTGTCTTTATATTCATGACCTACTGGCATTTG
CTGAACGCATTTACTGTCACGGTTCCCAAGGACCTATATGTGGTAGAGTATGGTAGC
AATATGACAATTGAATGCAAATTCCCAGTAGAAAAACAATTAGACCTGGCTGCACTA
ATTGTCTATTGGGAAATGGAGGATAAGAACATTATTCAATTTGTGCATGGAGAGGAA
GACCTGAAGGTTCAGCATAGTAGCTACAGACAGAGGGCCCGGCTGTTGAAGGACCAG
CTCTCCCTGGGAAATGCTGCACTTCAGATCACAGATGTGAAATTGCAGGATGCAGGG
GTGTACCGCTGCATGATCAGCTATGGTGGTGCCGACTACAAGCGAATTACTGTGAAA
GTCAATGCCCCATACAACAAAATCAACCAAAGAATTTTGGTTGTGGATCCAGTCACC
TCTGAACATGAACTGACATGTCAGGCTGAGGGCTACCCCAAGGCCGAAGTCATCTGG
ACAAGCAGTGACCATCAAGTCCTGAGTGGTAAGACCACCACCACCAATTCCAAGAGA
GAGGAGAAGCTTTTCAATGTGACCAGCACACTGAGAATCAACACAACAACTAATGAG
ATTTTCTACTGCACTTTTAGGAGATTAGATCCTGAGGAAAACCATACAGCTGAATTG
GTCATCCCAGAACTACCTCTGGCACATCCTCCAAATGAAAGGACTCACTTGGTAATT
CTGGGAGCCATCTTATTATGCCTTGGTGTAGCACTGACATTCATCTTCCGTTTAAGA
AAAGGGAGAATGATGGATGTGAAAAAATGTGGCATCCAAGATACAAACTCAAAGAAG
CAAAGTGATACACATTTGGAGGAGACGTAATCCAGCATTGGAACTTCTGATCTTCAA
GCAGGGATTCTCAACCTGTGGTTTAGGGGTTCATCGGGGCTGAGCGTGACAAGAGGA
AGGAATGGGCCCGTGGGATGCAGGCAATGTGGGACTTAAAAGGCCCAAGCACTGAAA
ATGGAACCTGGCGAAAGCAGAGGAGGAGAATGAAGAAAGATGGAGTCAAACAGGGAG
CCTGGAGGGAGACCTTGATACTTTCAAATGCCTGAGGGGCTCATCGACGCCTGTGAC
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HLEET (SEQ ID NO: 2) Mouse PD-L1 >NM_021893.3 *Mus musculus* CD274 antigen (Cd274), mRNA
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AAAAA (SEQ ID NO: 3) >NP_068693.l programmed cell death 1 ligand 1 precursor [*Mus musculus*]
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>NP_079515.2 programmed cell death 1 ligand 2 precursor [*Homo sapiens*]
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Mouse PD-L2 >NM_021396.2 *Mus musculus* programmed cell death 1 ligand 2 (Pdcd1lg2), mRNA
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(SEQ ID NO: 9) >NP_005205.2 cytotoxic T-lymphocyte protein 4 isoform CTLA4-TM precursor [*Homo sapiens*]

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SGLFFYSFLLTAVSLSKMLKKRSPLTTGVYVKMPPTEPECEKQFQPYFIPIN (SEQ ID NO: 10) Mouse CTLA- >NM_009843.4 *Mus musculus* cytotoxic T-lymphocyte- 4 (CD152) associated protein 4 (Ctla4), transcript variant 1, mRNA

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NO: 11) >NP_033973.2 cytotoxic T-lymphocyte protein 4 isoform 1 precursor
[*Mus musculus*]
MACLGLRRYKAQLQLPSRTWPFVALLTLLFIPVFSEAIQVTQPSVVLASSHGVASFP
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NO: 12) Human 4- >NM_003811.4 *Homo sapiens* TNF superfamily member 9
1BBL (TNFSF9), mRNA (CD137L)
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>NP_003802.1 tumor necrosis factor ligand superfamily member 9 [*Homo sapiens*]
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>NM_009404.3 *Mus musculus* tumor necrosis factor (ligand) (CD137L) superfamily, member 9 (Tnfsf9), mRNA
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MDQHTLDVEDTADARHPAGTSCPSDAALLRDTGLLADAALLSDTVRPTNAALPTDAA
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DWELSYPNTTSFGLFLVKPDNPWE (SEQ ID NO: 16) Human >NM_003820.4 *Homo sapiens* TNF receptor superfamily member HVEM 14 (TNFRSF14), transcript variant 1, DNA (CD270)
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NO: 17) >NP_003811.2 tumor necrosis factor receptor superfamily member 14
isoform 1 precursor [*Homo sapiens*]
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PNGTLEECQHQTKCSWLVTKAGAGTSSSHWVWWFLSGSLVIVIVCSTVGLIICVKRR
KPRGDVVKVIVSVQRKRQEAEGEATVIEALQAPPDVTTVAVEETIPSFTGRSPNH (SEQ
ID NO: 18) Mouse HVEM >NM_178931.2 *Mus musculus* tumor necrosis factor
receptor (CD270) superfamily, member 14 (herpesvirus entry mediator) (Tnfrsf14),
mRNA
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>NP_849262.1 tumor necrosis factor receptor superfamily member 14 precursor
[*Mus musculus*]
MEPLPGWGSAPWSQAPTDNTFRLVPCVFLLNLLQRISAQPSCRQEEFLVGDECCPMC
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Human FGL1 >NM_004467.4 *Homo sapiens* fibrinogen like 1 (FGL1), transcript
variant 1, mRNA

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>NP_004458.3 fibrinogen-like protein 1 precursor [*Homo sapiens*]
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>NM_145594.2 *Mus musculus* fibrinogen-like protein 1 (Fgl1), mRNA
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>NP_663569.2 fibrinogen-like protein 1 precursor [*Mus musculus*]
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DMSDGGGWTVIQRRSDGSENFNRGWNDYENGFGNFVQNNGEYWLGNKNINLLTIQGD
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ASHQRMKFSTWDRDNDNYQGNCAEEEQSGWWFNRCHSANLNGVYYRGSYRAETDNGV
VWYTWHGWWYSLKSVVMKIRPSDFIPNII (SEQ ID NO: 24) Human OX-2
>NM_005944.7 *Homo sapiens* CD200 molecule (CD200), (CD200) transcript variant 1, mRNA
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TTAAAAGTGTTGACTCCAAA (SEQ ID NO: 25) >NP_005935.4 OX-2 membrane glycoprotein isoform a precursor [*Homo sapiens*]
MERLVIRMPFSHLSTYSLVWVMAAVVLCTAQVQVVTQDEREQLYTPASLKCSLQNAQ
EALIVTWQKKKAVSPENMVTFSENHGVVIQPAYKDKINITQLGLQNSTITFWNITLE

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Mouse OX-2 >NM_010818.3 *Mus musculus* CD200 antigen (Cd200), (CD200) transcript variant 1, mRNA
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LDKGFWFSVPLLLSIVSLVILLILISILLYWKRHRNQERGESSQGMQRMK (SEQ ID NO: 28) Human >NM_009587.3 *Homo sapiens* galectin 9 (LGALS9), transcript Galectin-9 variant 1, mRNA
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TGTTGGCACATTCA (SEQ ID NO: 29) >NP_033665.1 galectin-9 isoform long [*Homo sapiens*]
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31) >NP_034838.2 ga1ectin-9 isoform 1 [*Mus musculus*]
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NTRLHLGYIFLIVFVLAVVIIIAALYTIRRCRHGRALQSNPSERENVQYSSVNGDCR
LNMEPNSTR (SEQ ID NO: 36) Human >NM_002856.3 *Homo sapiens* nectin cell adhesion molecule 2 Nectin-2 (NECTIN2), transcript variant alpha, mRNA (CD112)
GTGACGTCAGCGGGTTCGAACCGCCGGAGCTGAGCGAGAGGCCGGGGGTGCCGAGCC
isoform alpha
GGGCGGGGAGAGCTGGGCCGGGAGAGCAGAACAGGGAGGCTAGAGCGCAGCGGGAAC
CGGCCCGGAGCCGGAGCCGGAGCCCCACAGGCACCTACTAAACCGCCCAGCCGATCG
GCCCCCACAGAGTGGCCCGCGGGCCTCCGGCCGGGCCCAGTCCCCTCCCGGGCCCTC
CATGGCCCGGGCCGCTGCCCTCCTGCCGTCGAGATCGCCGCCGACGCCGCTGCTGTG
GCCGCTGCTGCTGCTGCTGCTCCTGGAAACCGGAGCCCAGGATGTGCGAGTTCAAGT
GCTACCCGAGGTGCGAGGCCAGCTCGGGGGCACCGTGGAGCTGCCGTGCCACCTGCT
GCCACCTGTTCCTGGACTGTACATCTCCCTGGTGACCTGGCAGCGCCCAGATGCACC
TGCGAACCACCAGAATGTGGCCGCCTTCCACCCTAAGATGGGTCCCAGCTTCCCCAG
CCCGAAGCCTGGCAGCGAGCGGCTGTCCTTCGTCTCTGCCAAGCAGAGCACTGGGCA
AGACACAGAGGCAGAGCTCCAGGACGCCACGCTGGCCCTCCACGGGCTCACGGTGGA
GGACGAGGGCAACTACACTTGCGAGTTTGCCACCTTCCCCAAGGGGTCCGTCCGAGG
GATGACCTGGCTCAGAGTCATAGCCAAGCCCAAGAACCAAGCTGAGGCCCAGAAGGT
CACGTTCAGCCAGGACCCTACGACAGTGGCCCTCTGCATCTCCAAAGAGGGCCGCCC
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GTCAGGGACCCTGGCCGGAACTGTCACTGTCACCAGCCGCTTCACCTTGGTGCCCTC
GGGCCGAGCAGATGGTGTCACGGTCACCTGCAAAGTGGAGCATGAGAGCTTCGAGGA
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TGAGGAGGAGGAGGAGGAGGAAGAGAAGGCAGAGAAAGGCCTCATGTTGCCTCCACC
CCCAGCACTCGAGGATGACATGGAGTCCCAGCTGGACGGCTCCCTCATCTCACGGCG
GGCAGTTTATGTGTGACCTGGACACAGACAGAGACAGAGCCAGGCCCGGCCCTCCCG
CCCCCGACCTGACCACGCCGGCCTAGGGTTCCAGACTGGTTGGACTTGTTCGTCTGG
ACGACACTGGAGTGGAACACTGCCTCCCACTTTCTTGGGACTTGGAGGGAGGTGGAA
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AGTGGGGATCCAGAGAGGACCCCCGCCCCCAGAGACTTGGTTTTGGCTCCAGCCTTC
CCCTGGCCCCGTGACACTCAGGAGTTAATAAATGCCTTGGAGGAAAACA (SEQ ID
NO: 37) >NP_002847.1 nectin-2 isoform alpha precursor [*Homo sapiens*]
MARAAALLPSRSPPTPLLWPLLLLLLLETGAQDVRVQVLPEVRGQLGGTVELPCHLL
PPVPGLYISLVTWQRPDAPANHQNVAAFHPKMGPSFPSPKPGSERLSFVSAKQSTGQ
DTEAELQDATLALHGLTVEDEGNYTCEFATFPKGSVRGMTWLRVIAKPKNQAEAQKV
TFSQDPTTVALCISKEGRPPARISWLSSLDWEAKETQVSGTLAGTVTVTSRFTLVPS
GRADGVTVTCKVEHESFEEPALIPVTLSVRYPPEVSISGYDDNWYLGRTDATLSCDV
RSNPEPTGYDWSTTSGTFPTSAVAQGSQLVIHAVDSLFNTTFVCTVTNAVGMGRAEQ
VIFVRETPRASPRDVGPLVWGAVGGTLLVLLLLAGGSLAFILLRVRRRRKSPGGAGG

GASGDGGFYDPKAQVLGNGDPVFWTPVVPGPMEPDGKDEEEEEEEEKAEKGLMLPPP
PALEDDMESQLDGSLISRRAVYV (SEQ ID NO: 38) Mouse Nectin-
>NM_001159724.1 *Mus musculus* nectin cell adhesion 2 (CD112) molecule 2
(Nectin2), transcript variant 2, mRNA isoform alpha
GAGCCCTAGGATCGGCTTGGCGAAGAGGGGCGGGGCCTGTGACGTCATGAGTCCGGC
CCGCTGGAGCTAAGCGAGGGGCCGGGGGGCGCGGATCCTGAGAGCCAGGCGAGGGAA
AGCTGGGCCGAACGAACTGATCCGGGGAGCCGTGAGCGGCGGAAGCCGGCCTGGAGC
CGGACACTTCAGACCCCTGACTGCCCTCCCAGCCGATCGGTACACGAAGAGTGGTCC
CTAGGCACCCCCTGCCCGGGCCCAGTCCCTCCCCGGGCCCCCCATGGCCCGGGCCGC
AGTCCTCCCGCCGTCCAGATTGTCACCGACGCTGCCGTTGTTGCCGCTGCTACTGCT
CCTGCTTCAGGAAACAGGAGCCCAAGATGTGCGGGTACGAGTGCTTCCCGAGGTCCG
GGGCCGCTTGGGAGGCACCGTGGAGTTACCGTGCCACCTGCTCCCACCCACGACGGA
GCGCGTCTCTCAGGTGACCTGGCAGCGCCTGGATGGCACAGTTGTGGCTGCTTTCCA
CCCATCCTTCGGAGTGGATTTCCCCAACTCTCAGTTCAGCAAGGACCGTCTGTCCTT
TGTCAGAGCGAGACCAGAAACAAACGCAGACCTGCGGGATGCCACACTGGCCTTCCG
GGGACTGAGGGTAGAGGACGAGGGCAATTACACCTGCGAGTTTGCCACGTTTCCCAA
CGGTACCCGCAGGGGGGTGACCTGGCTCAGAGTCATAGCCCAGCCTGAGAACCACGC
TGAAGCCCAGGAGGTCACAATTGGCCCCCAGTCGGTGGCTGTAGCCCGCTGTGTCTC
CACTGGGGGCCGCCCCCCTGCCCGAATCACCTGGATCTCATCTCTGGGTGGAGAGGC
CAAAGATACTCAGGAGCCAGGGATACAGGCTGGCACCGTCACTATCATCAGCCGATA
CTCCTTGGTGCCCGTGGGCCGAGCGGATGGCGTCAAGGTCACGTGTAGAGTGGAACA
CGAGAGCTTCGAAGAGCCGATCCTGCTGCCAGTGACCCTCTCTGTGCGCTACCCTCC
AGAAGTATCCATCTCCGGCTATGATGACAACTGGTACCTTGGCCGCAGTGAGGCCAT
ACTGACCTGTGATGTACGAAGCAACCCAGAGCCCACAGACTATGACTGGAGCACGAC
CTCGGGCGTCTTCCCAGCCTCTGCAGTGGCCCAGGGCTCTCAGCTGCTTGTCCACTC
TGTGGATCGAATGGTCAACACTACCTTCATCTGTACAGCCACCAACGCTGTGGGGAC
AGGCCGTGCTGAGCAGGTCATCCTGGTGCGAGACACCCCCCAGGCCTCCCGAGATGT
GGGTCCGCTGGTGTGGGGGGCCGTGGGGGGAACATTGCTGGTGCTACTCCTGGCTGG
GGGGTTCCTGGCCTTGATCCTGCTGAGGGGGAGGAGGAGGCGGAAGAGCCCTGGAGG
AGGAGGAAATGATGGCGACAGAGGATCCTACGATCCAAAGACTCAGGTGTTTGGGAA
CGGGGGTCCTGTCTTCTGGAGGTCAGCATCCCCTGAGCCCATGAGGCCAGATGGCAG
GGAGGAAGATGAGGAGGAGGAGGAAGAAATGAAGGCAGAGGAAGGTCTCATGCTACC
TCCACACGAGTCACCTAAGGACGACATGGAGTCCCATCTGGATGGCTCCCTCATCTC
TCGGCGGGCAGTTTACGTGTGACCCTACGATATAGACACTGGACACATGGAAACACC
AAGTTCCACCCTCACTGCCAACCACACCAATGCCAGCCAGCAACGATGGCTAGGGAC
CGGTTGGACTGGTTCTTCTGGGGCACACTGGAGTTGGAAGGGCACCGCCCCTGCTTT
CAGGATAGAGGACAAGTGGAACCACACAGACTCCTATCTTTAGGGCCTCATGGAGTA
GGGGACCCCAGGAGCGCCATGGTGCACACTCAGGACTCCTCAGAGCTTGCTTTCGGC
CCCAGCCTAGCCCTGGCCCCGAAACACTCAGGAGCTAATAAATGCCTTGTCGGAAAA
AAAAAAAAAAAAAA (SEQ ID NO: 39) >NP_001153196.1 nectin-2 isoform 2
precursor [Mus *musculus*]
MARAAVLPPSRLSPTLPLLPLLLLLLQETGAQDVRVRVLPEVRGRLGGTVELPCHLL
PPTTERVSQVTWQRLDGTVVAAFHPSFGVDFPNSQFSKDRLSFVRARPETNADLRDA
TLAFRGLRVEDEGNYTCEFATFPNGTRRGVTWLRVIAQPENHAEAQEVTIGPQSVAV
ARCVSTGGRPPARITWISSLGGEAKDTQEPGIQAGTVTIISRYSLVPVGRADGVKVT
CRVEHESFEEPILLPVTLSVRYPPEVSISGYDDNWYLGRSEAILTCDVRSNPEPTDY
DWSTTSGVFPASAVAQGSQLLVHSVDRMVNTTFICTATNAVGTGRAEQVILVRDTPQ
ASRDVGPLVWGAVGGTLLVLLLAGGFLALILLRGRRRRKSPGGGGNDGDRGSYDPKT
QVFGNGGPVFWRSASPEPMRPDGREEDEEEEEEMKAEEGLMLPPHESPKDDMESHLD
GSLISRRAVYV (SEQ ID NO: 40) Human >NM_001042724.2 *Homo sapiens*

nectin cell adhesion Nectin-2 molecule 2 (NECTIN2), transcript variant delta, mRNA (CD112)
GTGACGTCAGCGGGTTCGAACCGCCGGAGCTGAGCGAGAGGCCGGGGGTGCCGAGCC
isoform delta
GGGCGGGGAGAGCTGGGCCGGGAGAGCAGAACAGGGAGGCTAGAGCGCAGCGGGAAC
CGGCCCGGAGCCGGAGCCGGAGCCCCACAGGCACCTACTAAACCGCCCAGCCGATCG
GCCCCCACAGAGTGGCCCGCGGGCCTCCGGCCGGGCCCAGTCCCCTCCCGGGCCCTC
CATGGCCCGGGCCGCTGCCCTCCTGCCGTCGAGATCGCCGCCGACGCCGCTGCTGTG
GCCGCTGCTGCTGCTGCTGCTCCTGGAAACCGGAGCCCAGGATGTGCGAGTTCAAGT
GCTACCCGAGGTGCGAGGCCAGCTCGGGGGCACCGTGGAGCTGCCGTGCCACCTGCT
GCCACCTGTTCCTGGACTGTACATCTCCCTGGTGACCTGGCAGCGCCCAGATGCACC
TGCGAACCACCAGAATGTGGCCGCCTTCCACCCTAAGATGGGTCCCAGCTTCCCCAG
CCCGAAGCCTGGCAGCGAGCGGCTGTCCTTCGTCTCTGCCAAGCAGAGCACTGGGCA
AGACACAGAGGCAGAGCTCCAGGACGCCACGCTGGCCCTCCACGGGCTCACGGTGGA
GGACGAGGGCAACTACACTTGCGAGTTTGCCACCTTCCCCAAGGGGTCCGTCCGAGG
GATGACCTGGCTCAGAGTCATAGCCAAGCCCAAGAACCAAGCTGAGGCCCAGAAGGT
CACGTTCAGCCAGGACCCTACGACAGTGGCCCTCTGCATCTCCAAAGAGGGCCGCCC
ACCTGCCCGGATCTCCTGGCTCTCATCCCTGGACTGGGAAGCCAAAGAGACTCAGGT
GTCAGGGACCCTGGCCGGAACTGTCACTGTCACCAGCCGCTTCACCTTGGTGCCCTC
GGGCCGAGCAGATGGTGTCACGGTCACCTGCAAAGTGGAGCATGAGAGCTTCGAGGA
ACCAGCCCTGATACCTGTGACCCTCTCTGTACGCTACCCTCCTGAAGTGTCCATCTC
CGGCTATGATGACAACTGGTACCTCGGCCGTACTGATGCCACCCTGAGCTGTGACGT
CCGCAGCAACCCAGAGCCCACGGGCTATGACTGGAGCACGACCTCAGGCACCTTCCC
GACCTCCGCAGTGGCCCAGGGCTCCCAGCTGGTCATCCACGCAGTGGACAGTCTGTT
CAATACCACCTTCGTCTGCACAGTCACCAATGCCGTGGGCATGGGCCGCGCTGAGCA
GGTCATCTTTGTCCGAGAGACCCCCAACACAGCAGGCGCAGGGGCCACAGGCGGCAT
CATCGGGGGCATCATCGCCGCCATCATTGCTACTGCTGTGGCTGCCACGGGCATCCT
TATCTGCCGGCAGCAGCGGAAGGAGCAGACGCTGCAGGGGGCAGAGGAGGACGAAGA
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CCCCTACTTTGATGCTGGCGCCTCATGCACTGAGCAGGAAATGCCTCGATACCATGA
GCTGCCCACCTTGGAAGAACGGTCAGGACCCTTGCACCCTGGAGCCACAAGCCTGGG
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AGAGGATGAGGAGGGGGAGGAGGAGGAAGAGTATCTGGACAAGATCAACCCCATCTA
TGATGCTCTGTCCTATAGCAGCCCCTCTGATTCCTACCAGGGCAAAGGCTTTGTCAT
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TGATCCCTTAGCTTTCTTGCCAAGGATCTAGTGCCCCCTGACCTCTGGCCAGGCCAC
TGTCAGTTAACACATATGCATTCCATTTGTGATGTCTACCTTGGTGGCTCCACTATG
ACCCCTAACCCATGAGCCCAGAGAAATTCACCGTGATAATGGAATCCTGGCAACCTT
ATCTCATGAGGCAGGAGGTGGGGAAGGTGCTTCTGCACAACCTCTGATCCCAAGGAC
TCCTCTCCCAGACTGTGACCTTAGACCATACCTCTCACCCCCCAATGCCTCGACTCC
CCCAAAATCACAAAGAAGACCCTAGACCTATAATTTGTCTTCAGGTAGTAAATTCCC
AATAGGTCTGCTGGAGTGGGCGCTGAGGGCTCCCTGCTGCTCAGACCTGAGCCCTCC
AGGCAGCAGGGTCCCACTTACCCCCTCCCCACCCTGTTCCCCAAAGGTGGGAAAGAG
GGGATTCCCCAGCCCAAGGCAGGGTTTTCCCAGCACCCTCCTGTAAGCAGAAGTCTC
AGGGTCCAGACCCTTCCCTGAGCCCCCACCCCCACCCCAATTCCTGCCTACCAAGCA
AGCAGCCCCAGCCTAGGGTCAGACAGGGTGAGCCTCATACAGACTGTGCCTTGATGG
CCCCAGCCTTGGGAGAAGAATTTACTGTTAACCTGGAAGACTACTGAATCATTTTAC
CCTTGCCCAGTGGAATAGGACCTAAACATCCCCCTTCCGGGGAAAGTGGGTCATCTG
AATTGGGGGTAGCAATTGATACTGTTTTGTAAACTACATTTCCTACAAAATATGAAT

TTATACTTTGA (SEQ ID NO: 41) >NP_001036189.1 nectin-2 isoform delta precursor [*Homo sapiens*]
MARAAALLPSRSPPTPLLWPLLLLLLLETGAQDVRVQVLPEVRGQLGGTVELPCHLL
PPVPGLYISLVTWQRPDAPANHQNVAAFHPKMGPSFPSPKPGSERLSFVSAKQSTGQ
DTEAELQDATLALHGLTVEDEGNYTCEFATFPKGSVRGMTWLRVIAKPKNQAEAQKV
TFSQDPTTVALCISKEGRPPARISWLSSLDWEAKETQVSGTLAGTVTVTSRFTLVPS
GRADGVTVTCKVEHESFEEPALIPVTLSVRYPPEVSISGYDDNWYLGRTDATLSCDV
RSNPEPTGYDWSTTSGTFPTSAVAQGSQLVIHAVDSLFNTTFVCTVTNAVGMGRAEQ
VIFVRETPNTAGAGATGGIIGGIIAAIIATAVAATGILICRQQRKEQTLQGAEEDED
LEGPPSYKPPTPKAKLEAQEMPSQLFTLGASEHSPLKTPYFDAGASCTEQEMPRYHE
LPTLEERSGPLHPGATSLGSPIPVPPGPPAVEDVSLDLEDEEGEEEEEYLDKINPIY
DALSYSSPSDSYQGKGFVMSRAMYV (SEQ ID NO: 42) Mouse Nectin-2 >NM_008990.3 *Mus musculus* nectin cell adhesion molecule 2 2 (CD112) (Nectin2), transcript variant 1, mRNA isoform beta
GAGCCCTAGGATCGGCTTGGCGAAGAGGGGCGGGGCCTGTGACGTCATGAGTCCGGC
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AGCTGGGCCGAACGAACTGATCCGGGGAGCCGTGAGCGGCGGAAGCCGGCCTGGAGC
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CTAGGCACCCCCTGCCCGGGCCCAGTCCCTCCCCGGGCCCCCCATGGCCCGGGCCGC
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CACTGGGGGCCGCCCCCCTGCCCGAATCACCTGGATCTCATCTCTGGGTGGAGAGGC
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CTCCTTGGTGCCCGTGGGCCGAGCGGATGGCGTCAAGGTCACGTGTAGAGTGGAACA
CGAGAGCTTCGAAGAGCCGATCCTGCTGCCAGTGACCCTCTCTGTGCGCTACCCTCC
AGAAGTATCCATCTCCGGCTATGATGACAACTGGTACCTTGGCCGCAGTGAGGCCAT
ACTGACCTGTGATGTACGAAGCAACCCAGAGCCCACAGACTATGACTGGAGCACGAC
CTCGGGCGTCTTCCCAGCCTCTGCAGTGGCCCAGGGCTCTCAGCTGCTTGTCCACTC
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GGCCACTGGTGGCATCATTGGAGGTATTATCGCTGCCATCATCGCCACCGCAGTGGC
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CAAGCTGGAGGAACCAGAGATGCCCTCTCAACTCTTCACCTTGGGGGCCTCAGAGCA
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GAGCAAAGACTTTTTTGTGTCACGGGCCATGTATGTGTGAGGGAGGCACAGGGGCTC
TGACGTCTCACCTTTCACCCTTGACCCATGAGCTTTCCACCAGTAATCTAGGACACT
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TTAGTGACTCCACCATGACGTCCAATCTATGATGTCTGAGGCAGGCAAACCTGCACA

ACTGGAAACCTGGAGATTTTTATCTCCCTTGGCAGGGAGCTCACCATATCCTTCTGC
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ATGCCACTCAGTATCTCAAGAACCCTTAAAGTCCCAAAGGAATCGGACCTTGCACTT
GTCCTCAGGCAATAGAGTCCAACAGATATGCAAGAACGGGATCAGGGGCTCCCTGTT
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CCTACTCCTGCAGGCAGGAATCTCAGGTTTCTCACACCCTCTCCTCAGCACCCAGGT
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AAGCATTTCCTACATAATATGAGTTTCTACTTTGATAAAGTCTTGTGTTTTCTGTG (SEQ ID NO: 43) >NP_033016.3 nectin-2 isoform 1 precursor [*Mus musculus*]
MARAAVLPPSRLSPTLPLLPLLLLLLQETGAQDVRVRVLPEVRGRLGGTVELPCHLL
PPTTERVSQVTWQRLDGTVVAAFHPSFGVDFPNSQFSKDRLSFVRARPETNADLRDA
TLAFRGLRVEDEGNYTCEFATFPNGTRRGVTWLRVIAQPENHAEAQEVTIGPQSVAV
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CRVEHESFEEPILLPVTLSVRYPPEVSISGYDDNWYLGRSEAILTCDVRSNPEPTDY
DWSTTSGVFPASAVAQGSQLLVHSVDRMVNTTFICTATNAVGTGRAEQVILVRESPS
TAGAGATGGIIGGIIAAIIATAVAGTGILICRQQRKEQRLQAADEEEELEGPPSYKP
PTPKAKLEEPEMPSQLFTLGASEHSPVKTPYFDAGVSCADQEMPRYHELPTLEERSG
PLLLGATGLGPSLLVPPGPNVVEGVSLSLEDEEEDDEEEDFLDKINPIYDALSYPSP
SDSYQSKDFFVSRAMYV (SEQ ID NO: 44) Human IL-10 >NM_000572.3 *Homo sapiens* interleukin 10 (IL10), transcript variant 1, mRNA
ACACATCAGGGGCTTGCTCTTGCAAAACCAAACCACAAGACAGACTTGCAAAAGAAG
GCATGCACAGCTCAGCACTGCTCTGTTGCCTGGTCCTCCTGACTGGGGTGAGGGCCA
GCCCAGGCCAGGGCACCCAGTCTGAGAACAGCTGCACCCACTTCCCAGGCAACCTGC
CTAACATGCTTCGAGATCTCCGAGATGCCTTCAGCAGAGTGAAGACTTTCTTTCAAA
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GCTTGGGGCTTCCTAACTGCTACAAATACTCTTAGGAAGAGAAACCAGGGAGCCCCT
TTGATGATTAATTCACCTTCCAGTGTCTCGGAGGGATTCCCCTAACCTCATTCCCCA
ACCACTTCATTCTTGAAAGCTGTGGCCAGCTTGTTATTTATAACAACCTAAATTTGG
TTCTAGGCCGGGCGCGGTGGCTCACGCCTGTAATCCCAGCACTTTGGGAGGCTGAGG
CGGGTGGATCACTTGAGGTCAGGAGTTCCTAACCAGCCTGGTCAACATGGTGAAACC
CCGTCTCTACTAAAAATACAAAAATTAGCCGGGCATGGTGGCGCGCACCTGTAATCC
CAGCTACTTGGGAGGCTGAGGCAAGAGAATTGCTTGAACCCAGGAGATGGAAGTTGC
AGTGAGCTGATATCATGCCCCTGTACTCCAGCCTGGGTGACAGAGCAAGACTCTGTC

TCAAAAAATAAAAATAAAAATAAATTTGGTTCTAATAGAACTCAGTTTTAACTAGAA
TTTATTCAATTCCTCTGGGAATGTTACATTGTTTGTCTGTCTTCATAGCAGATTTTA
ATTTTGAATAAATAAATGTATCTTATTCACATCA (SEQ ID NO: 45) >NP_000563.1 inter1eukin-10 isoform 1 precursor [*Homo sapiens*]
MHSSALLCCLVLLTGVRASPGQGTQSENSCTHFPGNLPNMLRDLRDAFSRVKTFFQM
KDQLDNLLLKESLLEDFKGYLGCQALSEMIQFYLEEVMPQAENQDPDIKAHVNSLGE
NLKTLRLRLRRCHRFLPCENKSKAVEQVKNAFNKLQEKGIYKAMSEFDIFINYIEAY
MTMKIRN (SEQ ID NO: 46) Mouse IL-10 >NM_010548.2 *Mus musculus* interleukin 10 (Il10), mRNA
ACATTTAGAGACTTGCTCTTGCACTACCAAAGCCACAAGGCAGCCTTGCAGAAAAGA
GAGCTCCATCATGCCTGGCTCAGCACTGCTATGCTGCCTGCTCTTACTGACTGGCAT
GAGGATCAGCAGGGGCCAGTACAGCCGGGAAGACAATAACTGCACCCACTTCCCAGT
CGGCCAGAGCCACATGCTCCTAGAGCTGCGGACTGCCTTCAGCCAGGTGAAGACTTT
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GAGCTGCAGGGCCCTTTGCTATGGTGTCCTTTCAATTGCTCTCATCCCTGAGTTCAG
AGCTCCTAAGAGAGTTGTGAAGAAACTCATGGGTCTTGGGAAGAGAAACCAGGGAGA
TCCTTTGATGATCATTCCTGCAGCAGCTCAGAGGGTTCCCCTACTGTCATCCCCCAG
CCGCTTCATCCCTGAAAACTGTGGCCAGTTTGTTATTTATAACCACCTAAAATTAGT
TCTAATAGAACTCATTTTTAACTAGAAGTAATGCAATTCCTCTGGGAATGGTGTATT
GTTTGTCTGCCTTTGTAGCAGACTCTAATTTTGAATAAATGGATCTTATTCG (SEQ ID NO: 47) >NP_034678.1 inter1eukin-10 precursor [*Mus musculus*]
MPGSALLCCLLLLTGMRISRGQYSREDNNCTHFPVGQSHMLLELRTAFSQVKTFFQT
KDQLDNILLTDSLMQDFKGYLGCQALSEMIQFYLVEVMPQAEKHGPEIKEHLNSLGE
KLKTLRMRLRRCHRFLPCENKSKAVEQVKSDFNKLQDQGVYKAMNEFDIFINCIEAY
MMIKMKS (SEQ ID NO: 48) Human TSG-6 >NM_007115.3 *Homo sapiens* TNF alpha induced protein 6 (TNFAIP6), mRNA
AGTCACATTTCAGCCACTGCTCTGAGAATTTGTGAGCAGCCCCTAACAGGCTGTTAC
TTCACTACAACTGACGATATGATCATCTTAATTTACTTATTTCTCTTGCTATGGGAA
GACACTCAAGGATGGGGATTCAAGGATGGAATTTTTCATAACTCCATATGGCTTGAA
CGAGCAGCCGGTGTGTACCACAGAGAAGCACGGTCTGGCAAATACAAGCTCACCTAC
GCAGAAGCTAAGGCGGTGTGTGAATTTGAAGGCGGCCATCTCGCAACTTACAAGCAG
CTAGAGGCAGCCAGAAAAATTGGATTTCATGTCTGTGCTGCTGGATGGATGGCTAAG
GGCAGAGTTGGATACCCCATTGTGAAGCCAGGGCCCAACTGTGGATTTGGAAAAACT
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AAAAAAAAAAAAAA (SEQ ID NO: 49) >NP_009046.2 tumor necrosis factor-inducible gene 6 protein precursor [*Homo sapiens*]
MIILIYLFLLLWEDTQGWGFKDGIFHNSIWLERAAGVYHREARSGKYKLTYAEAKAV
CEFEGGHLATYKQLEAARKIGFHVCAAGWMAKGRVGYPIVKPGPNCGFGKTGIIDYG
IRLNRSERWDAYCYNPHAKECGGVFTDPKQIFKSPGFPNEYEDNQICYWHIRLKYGQ
RIHLSFLDFDLEDDPGCLADYVEIYDSYDDVHGFVGRYCGDELPDDIISTGNVMTLK
FLSDASVTAGGFQIKYVAMDPVSKSSQGKNTSTTSTGNKNFLAGRFSHL (SEQ ID NO: 50) Mouse TSG-6 >NM_009398.2 *Mus musculus* tumor necrosis factor alpha induced protein 6 (Tnfaip6), mRNA
CCGCTGCTCTGAGAATTTCGTGTGGGCAGCCCCGACATTGTAACCGGCTCTGCAACC
GAAGAGATGGTCGTCCTCCTTTGCTTATGCGTCTTGCTGTGGGAAGAGGCTCACGGA
TGGGGATTCAAGAACGGGATCTTTCATAACTCCATATGGCTTGAACAAGCAGCGGGC
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GCCGTATGTGAATTTGAAGGTGGTCGTCTCGCAACCTACAAGCAGCTAGAGGCAGCC
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CCGCCTACCAAGTGCTGGGATTAAAGGCGTCCACCACCACCGCCCGGCTTCAATATT
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TATTTATTATTTTTCCAAATGTGAAAGCCATATGTAATTATGTGGAAAATTGACAAA
TAAATACAGAGAACTTCAAATGAGTTTTTTTTTTAAATCTCATAATTGTACTACACA
GAAATAACTAATGTTAAAGTTTTTAAATGTTTGTCTTTCATTCATTTTTCTACTTGT
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TGTAACCTGATATTTTTAACCTTGACATTGTATAGCTCAAGCACTTCCCAAGATCTC
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TGGAT (SEQ ID NO: 51) >NP_033424.1 tumor necrosis factor-inducible gene 6 protein precursor [*Mus musculus*]
MVVLLCLCVLLWEEAHGWGFKNGIFHNSIWLEQAAGVYHREARAGRYKLTYAEAKAV
CEFEGGRLATYKQLEAARKIGFHVCAAGWMAKGRVGYPIVKPGPNCGFGKTGIIDYG
IRLNRSERWDAYCYNPHAKECGGVFTDPKRIFKSPGFPNEYDDNQVCYWHIRLKYGQ
RIHLSFLDFDLEHDPGCLADYVEIYDSYDDVHGFVGRYCGDELPEDIISTGNVMTLK
FLSDASVTAGGFQIKYVTVDPASKSSQAKNTSTTGNKKFLPGRFSHL (SEQ ID NO: 52)
Human B7-H3 >NM_001024736.2 *Homo sapiens* CD276 molecule (CD276), (CD276) transcript variant 1, mRNA
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CAGCCGCCTCACAGGAAGATGCTGCGTCGGCGGGGCAGCCCTGGCATGGGTGTGCAT
GTGGGTGCAGCCCTGGGAGCACTGTGGTTCTGCCTCACAGGAGCCCTGGAGGTCCAG
GTCCCTGAAGACCCAGTGGTGGCACTGGTGGGCACCGATGCCACCCTGTGCTGCTCC
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GCCAACCGCACGGCCCTCTTCCCGGACCTGCTGGCACAGGGCAACGCATCCCTGAGG
CTGCAGCGCGTGCGTGTGGCGGACGAGGGCAGCTTCACCTGCTTCGTGAGCATCCGG
GATTTCGGCAGCGCTGCCGTCAGCCTGCAGGTGGCCGCTCCCTACTCGAAGCCCAGC
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CCCCTGACTGGCAACGTGACCACGTCGCAGATGGCCAACGAGCAGGGCTTGTTTGAT
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AGCCCCACAGGAGCCGTGGAGGTCCAGGTCCCTGAGGACCCGGTGGTGGCCCTAGTG
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CAGCTCAACCTCATCTGGCAGCTGACAGACACCAAACAGCTGGTGCACAGTTTCACC
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CTAACTACACTGCACCCTGCGGTTTGCAGGGGGCTCCTGCCTGGCTCCCTGCTCCAC
ACCTCCTCTGTGGCTCAAGGCTTCCTGGATACCTCACCCCCATCCCACCCATAATTC
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GTAAACTGGGGGTATATTTTGGGGAAAATAAATGTCTTTGTAAAAA (SEQ ID NO:
53) >NP_001019907.1 CD276 antigen isoform a precursor [*Homo sapiens*]
MLRRRGSPGMGVHVGAALGALWFCLTGALEVQVPEDPVVALVGTDATLCCSFSPEPG
FSLAQLNLIWQLTDTKQLVHSFAEGQDQGSAYANRTALFPDLLAQGNASLRLQRVRV
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QGVPLTGNVTTSQMANEQGLFDVHSVLRVVLGANGTYSCLVRNPVLQQDAHGSVTIT
GQPMTFPPEALWVTVGLSVCLIALLVALAFVCWRKIKQSCEEENAGAEDQDGEGEGS
KTALQPLKHSDSKEDDGQEIA (SEQ ID NO: 54) Mouse B7-H3 >NM_133983.4
Mus musculus CD276 antigen (Cd276), mRNA (CD276)
CGGCGCGGCGCGCCAAAGTGACCTGGTACAGCCTGGACCCCAAGCTCATCGGCTTTG
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TTCAA (SEQ ID NO: 55) >NP_598744.1 CD276 antigen precursor [*Mus musculus*]
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EDQDGDGEGSKTALRPLKPSENKEDDGQEIA (SEQ ID NO: 56) Human B7-H4 >NM_024626.4 *Homo sapiens* V-set domain containing T cell (VTCN1) activation inhibitor 1 (VTCN1), transcript variant 1, mRNA
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>NP_078902.2 V-set domain-containing T-cell activation inhibitor 1 isoform 1 precursor [*Homo sapiens*]
MASLGQILFWSIISIIIILAGAIALIIGFGISGRHSITVTTVASAGNIGEDGILSCT
FEPDIKLSDIVIQWLKEGVLGLVHEFKEGKDELSEQDEMFRGRTAVFADQVIVGNAS
LRLKNVQLTDAGTYKCYIITSKGKGNANLEYKTGAFSMPEVNVDYNASSETLRCEAP
RWFPQPTVVWASQVDQGANFSEVSNTSFELNSENVTMKVVSVLYNVTINNTYSCMIE
NDIAKATGDIKVTESEIKRRSHLQLLNSKASLCVSSFFAISWALLPLSPYLMLK (SEQ ID NO: 58) Mouse B7-H4 >NM_178594.3 *Mus musculus* V-set domain containing T cell (VTCN1) activation inhibitor 1 (Vtcn1), mRNA

GTGAGTCACAACACCCAGGAGGGCAGCAGCAGGCAGGCAGCTCCACTCACCAAAATC
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(SEQ ID NO: 59) >NP_848709.2 V-set domain containing T-cell activation inhibitor 1 precursor [*Mus musculus*]

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LRLKNVQLTDAGTYTCYIRTSKGKGNANLEYKTGAFSMPEINVDYNASSESLRCEAP
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NDIAKATGDIKVTDSEVKRRSQLQLLNSGPSPCVFSSAFVAGWALLSLSCCLMLR (SEQ
ID NO: 60) Human B7-H5 >NM_022153.2 *Homo sapiens* V-set immunoregulatory receptor (VISTA) (VSIR), mRNA
AGTCGCGGGAGGCTTCCCCGCGCCGGCCGCGTCCCGCCCGCTCCCCGGCACCAGAAG
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>NP_071436.1 V-type immunoglobulin domain-containing suppressor of T-cell activation precursor [*Homo sapiens*]
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ELVRMDSNIQGIENPGFEASPPAQGIPEAKVRHPLSYVAQRQPSESGRHLLSEPSTP
LSPPGPGDVFFPSLDPVPDSPNFEVI (SEQ ID NO: 62) Mouse B7-H5
>NM_028732.4 *Mus musculus* V-set immunoregulatory receptor (VISTA) (Vsir), transcript variant 1, mRNA
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(SEQ ID NO: 63) >NP_083008.1 V-type immunoglobulin domain-containing suppressor of T-cell activation isoform 1 precursor [*Mus musculus*]

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PPGPGDVFFPSLDPVPDSPNSEAI (SEQ ID NO: 64) Human B7-H7 >NM_007072.4 *Homo sapiens* HERV-H LTR-associating 2 (HHLA2) (HHLA2), transcript variant 1, mRNA

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APDNGEENVPLSGKV (SEQ ID NO: 66 Mouse BTNL1 >NM_001111094.1 *Mus musculus* butyrophilin-like 1 (Btnl1), mRNA

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CTGA (SEQ ID NO: 67) >NP_001104564.1 butyrophilin-like protein 1 precursor [*Mus musculus*]
MMKGSPSVPPAGCLLPLLLLLFTGVSGEVSWFSVKGPAEPITVLLGTEATLPCQLSP
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KIAIFLDQEDNDLSFYNMTDETHIFSFAQVPFLGSPYPYFTRNSMGLSATAQP (SEQ ID NO: 68) Human VSIG8 >NM_001013661.1 *Homo sapiens* V-set and immunoglobulin domain containing 8 (VSIG8), mRNA

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ID NO: 69) >NP_001013683.1 V-set and immunoglobulin domain- containing
protein 8 precursor [*Homo sapiens*]
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DCAEGPVQCKNGLLV (SEQ ID NO: 70) Mouse VSIG8 >NM_177723.4 *Mus
musculus* V-set and immunoglobulin domain containing 8 (Vsig8), transcript
variant 1, mRNA
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CAGTGTTAGCACAATAAAGAAACTTAAAGACTGAAAAAAAAAAAAAA (SEQ ID NO: 71) >NP_808391.2 V-set and immunoglobulin domain-containing protein 8 precursor [*Mus musculus*]
MGVRGALHLLLVCLSPALLSAVRINGDGQEVMYLAEGDNVRLGCPYLLDPEDLGTNS
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CADCAQVEQRSCKDGLLV (SEQ ID NO: 72) Human VSIG3 >NM_001015887.3 *Homo sapiens* immunoglobulin superfamily (ISF11) member 11 (IGSF11), transcript variant 2, mRNA
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>NP_001015887.1 immunoglobulin superfamily member 11 isoform b precursor [*Homo sapiens*]
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QSFSFHSGNANIPSIYANGTHLVPGQHKTLVVTANRGSSPQVMSRSNGSVSRKPRPP
HTHSYTISHATLERIGAVPVMVPAQSRAGSLV (SEQ ID NO: 74) Mouse VSIG3
>NM_170599.2 *Mus musculus* immunoglobulin superfamily, (IGSF11) member 11 (Igsf11), mRNA
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SYTVSQMTLERIGAVPVMVPAQSRAGSLV (SEQ ID NO: 76) Human VSIG4 >NM_007268.3 *Homo sapiens* V-set and immunoglobulin domain containing 4 (VSIG4), transcript variant 1, mRNA
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>NP_009199.1 V-set and immunoglobulin domain-containing protein 4 isoform 1 precursor [*Homo sapiens*]
MGILLGLLLLGHLTVDTYGRPILEVPESVTGPWKGDVNLPCTYDPLQGYTQVLVKWL
VQRGSDPVTIFLRDSSGDHIQQAKYQGRLHVSHKVPGDVSLQLSTLEMDDRSHYTCE
VTWQTPDGNQVVRDKITELRVQKLSVSKPTVTTGSGYGFTVPQGMRISLQCQARGSP
PISYIWYKQQTNNQEPIKVATLSTLLFKPAVIADSGSYFCTAKGQVGSEQHSDIVKF
VVKDSSKLLKTKTEAPTTMTYPLKATSTVKQSWDWTTDMDGYLGETSAGPGKSLPVF
AIILIISLCCMVVFTMAYIMLCRKTSQQEHVYEAARAHAREANDSGETMRVAIFASG
CSSDEPTSQNLGNNYSDEPCIGQEYQIIAQINGNYARLLDTVPLDYEFLATEGKSVC (SEQ ID NO: 78) Mouse VSIG4 >NM_177789.5 *Mus musculus* V-set and immunoglobulin domain containing 4 (Vsig4), mRNA
AGCTACCAGCACTTCCAGGTTCTTCAGCAGCAAGAGGATGGAAGGATGAATAGAAGT
AGCTTCAAATAGGATGGAGATCTCATCAGGCTTGCTGTTCCTGGGCCACCTAATAGT
GCTCACCTATGGCCACCCCACCCTAAAAACACCTGAGAGTGTGACAGGGACCTGGAA
AGGAGATGTGAAGATTCAGTGCATCTATGATCCCCTGAGAGGCTACAGGCAAGTTTT
GGTGAAATGGCTGGTAAGACACGGCTCTGACTCCGTCACCATCTTCCTACGTGACTC
CACTGGAGACCATATCCAGCAGGCAAAGTACAGAGGCCGCCTGAAAGTGAGCCACAA
AGTTCCAGGAGATGTGTCCCTCCAAATAAATACCCTGCAGATGGATGACAGGAATCA
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GATCATTGAGCTCCGTGTTCGGAAATATAATCCACCTAGAATCAATACTGAAGCACC
TACAACCCTGCACTCCTCTTTGGAAGCAACAACTATAATGAGTTCAACCTCTGACTT
GACCACTAATGGGACTGGAAAACTTGAGGAGACCATTGCTGGTTCAGGGAGGAACCT
GCCAATCTTTGCCATAATCTTCATCATCTCCCTTTGCTGCATAGTAGCTGTCACCAT
ACCTTATATCTTGTTCCGCTGCAGGACATTCCAACAAGAGTATGTCTATGGAGTGAG
CAGGGTGTTTGCCAGGAAGACAAGCAACTCTGAAGAAACCACAAGGGTGACTACCAT
CGCAACTGATGAACCAGATTCCCAGGCTCTGATTAGTGACTACTCTGATGATCCTTG
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AATGCCCCTACTAAGTCAAGGTCTACTGGCGTAATTACCTGTTACTTATTTACTACT
TGCCTTCAACATAGCTTTCTCCCTGGCTTCCTTTCTTCTTAGACAACCTAAAGTATC
TATCTAGTCTGCCAATTCTGGGGCCATTGAGAAATCCTGGGTTTGGCTAAGAATATA
CTACATGCACCTCAAGAAATCTAGCTTCTGGGCTTCACCCAGAACAATTTTCTTCCT
AGGGCCTTCACAACTCTTCTCCAAACAGCAGAGAAATTCCATAGCAGTAGAGGTTCT
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GTCTAGGATTACTCCTCTTTCTCTAGGGCCAGATGACTTTTAATTGATATTACTATT
GCTACATTATGAATCTAATGCACATGTATTCTTTTGTTGTTAATAAATGTTTAATCA
TGACATCAA(SEQ ID NO: 79) >NP_808457.1 V-set and immunoglobulin domain-containing protein 4 precursor [*Mus musculus*]
MEISSGLLFLGHLIVLTYGHPTLKTPESVTGTWKGDVKIQCIYDPLRGYRQVLVKWL
VRHGSDSVTIFLRDSTGDHIQQAKYRGRLKVSHKVPGDVSLQINTLQMDDRNHYTCE
VTWQTPDGNQVIRDKIIELRVRKYNPPRINTEAPTTLHSSLEATTIMSSTSDLTTNG
TGKLEETIAGSGRNLPIFAIIFIISLCCIVAVTIPYILFRCRTFQQEYVYGVSRVFA
RKTSNSEETTRVTTIATDEPDSQALISDYSDDPCLSQEYQITIRSTMSIPAC (SEQ ID NO: 80) Human Tim-3 >NM_032782.5 *Homo sapiens* hepatitis A virus cellular (HAVCR2) receptor 2 (HAVCR2), mRNA
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CTGCTTCTACACCCCAGCCGCCCCAGGGAACCTCGTGCCCGTCTGCTGGGGCAAAGG
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CCTGACCATAGAGAATGTGACTCTAGCAGACAGTGGGATCTACTGCTGCCGGATCCA
AATCCCAGGCATAATGAATGATGAAAAATTTAACCTGAAGTTGGTCATCAAACCAGC
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GCTTACCACCAGGGGACATGGCCCAGCAGAGACACAGACACTGGGGAGCCTCCCTGA
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CCAACCACCTTATTTTTGAGCTTGGTGTTTTGTCTTTTTCAGAAACTATGAGCTGTG
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CGGCCTCCCAAAGCACTGGGATTACAGGCGTGAGCCACCACATCCAGCCAGTGATCC
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AAGCTATATTTTTCACAGAAGAAGAAGCAGTGACGGGGACACAAATTCTGTTGCCTG
GTGGAAAGAAGGCAAAGGCCTTCAGCAATCTATATTACCAGCGCTGGATCCTTTGAC
AGAGAGTGGTCCCTAAACTTAAATTTCAAGACGGTATAGGCTTGATCTGTCTTGCTT
ATTGTTGCCCCCTGCGCCTAGCACAATTCTGACACACAATTGGAACTTACTAAAAAT

TTTTTTTTACTGTT (SEQ ID NO: 81) >NP_116171.3 hepatitis A virus cellular receptor 2 precursor [*Homo sapiens*]
MFSHLPFDCVLLLLLLLLTRSSEVEYRAEVGQNAYLPCFYTPAAPGNLVPVCWGKGA
CPVFECGNVVLRTDERDVNYWTSRYWLNGDFRKGDVSLTIENVTLADSGIYCCRIQI
PGIMNDEKFNLKLVIKPAKVTPAPTRQRDFTAAFPRMLTTRGHGPAETQTLGSLPDI
NLTQISTLANELRDSRLANDLRDSGATIRIGIYIGAGICAGLALALIFGALIFKWYS
HSKEKIQNLSLISLANLPPSGLANAVAEGIRSEENIYTIEENVYEVEEPNEYYCYVS
SRQQPSQPLGCRFAMP (SEQ ID NO: 82) Mouse Tim-3 >NM_134250.2 *Mus musculus* hepatitis A virus cellular (HAVCR2) receptor 2 (Havcr2), mRNA
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AAAATCCATTAGAGATAACAGCTCTCATGCAGAAGGGAAAACTAATCTCAAATGTTT
TAAAGTAATAAAACTGTACTGGCAAAGTACTTTGAGCATATTTAAA (SEQ ID NO:
83) >NP_599011.2 hepatitis A virus cellular receptor 2 homolog precursor [*Mus musculus*]
MFSGLTLNCVLLLLQLLLARSLENAYVFEVGKNAYLPCSYTLSTPGALVPMCWGKGF
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FPGLMNDKKLELKLDIKAAKVTPAQTAHGDSTTASPRTLTTERNGSETQTLVTLHNN
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SLITLANLPPGGLANAGAVRIRSEENIYTIEENVYEVENSNEYYCYVNSQQPS (SEQ ID
NO: 84) Human Tim-4 >NM_138379.3 *Homo sapiens* T cell immunoglobulin and mucin (TIMD4) domain containing 4 (TIMD4), transcript variant 1, mRNA
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GGTACTTTAGAGACCACAA (SEQ ID NO: 85) >NP_612388.2 T-cell immunoglobulin and mucin domain- containing protein 4 isoform 1 precursor [*Homo sapiens*]
MSKEPLILWLMIEFWWLYLTPVTSETVVTEVLGHRVTLPCLYSSWSHNSNSMCWGKD
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AVPEQNKTTKTGQMDGIPMSMKNEMPISQLLMIIAPSLGFVLFALFVAFLLRGKLME
TYCSQKHTRLDYIGDSKNVLNDVQHGREDEDGLFTL (SEQ ID NO: 86) Mouse Tim-4 >NM_178759.4 *Mus musculus* T cell immunoglobulin and mucin (TIMD4)

domain containing 4 (Timd4), mRNA
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AAAAAATGG (SEQ ID NO: 87) >NP_848874.3 T-cell immunoglobulin and mucin domain- containing protein 4 precursor [*Mus musculus*]
MSKGLLLLWLVTELWWLYLTPAASEDTIIGFLGQPVTLPCHYLSWSQSRNSMC
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NTEDSDSVLNDMSHGRDDEDGIFTL (SEQ ID NO: 88) Human >NM_001712.5 *Homo sapiens* CEA cell adhesion molecule CEACAM1 1 (CEACAM1), transcript variant 1, mRNA

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TCAGGGTTTTCTAACCCTGACACGGACTGTGCATACTTTCCCTCATCCATGCT
GTGCTGTGTTATTTAATTTTTCCTGGCTAAGATCATGTCTGAATTATGTATGA
AAATTATTCTATGTTTTTATAATAAAAATAATATATCAGACATCGA (SEQ ID NO: 89)
>NP_001703.2 carcinoembryonic antigen-related cell adhesion molecule 1 isoform 1 precursor [*Homo sapiens*]
MGHLSAPLHRVRVPWQGLLLTASLLTFWNPPTTAQLTTESMPFNVAEGKEVLL
LVHNLPQQLFGYSWYKGERVDGNRQIVGYAIGTQQATPGPANSGRETIYPNAS
LLIQNVTQNDTGFYTLQVIKSDLVNEEATGQFHVYPELPKPSISSNNSNPVED
KDAVAFTCEPETQDTTYLWWINNQSLPVSPRLQLSNGNRTLTLLSVTRNDTGP
YECEIQNPVSANRSDPVTLNVTYGPDTPTISPSDTYYRPGANLSLSCYAASNP
PAQYSWLINGTFQQSTQELFIPNITVNNSGSYTCHANNSVTGCNRTTVKTIIV
TELSPVVAKPQIKASKTTVTGDKDSVNLTCSTNDTGISIRWFFKNQSLPSSER
MKLSQGNTTLSINPVKREDAGTYWCEVFNPISKNQSDPIMLNVNYNALPQENG
LSPGAIAGIVIGVVALVALIAVALACFLHFGKTGRASDQRDLTEHKPSVSNHT
QDHSNDPPNKMNEVTYSTLNFEAQQPTQPTSASPSLTATEIIYSEVKKQ (SEQ ID NO: 90) Mouse >NM_001039185.1 *Mus musculus* carcinoembryonic CEACAM1 antigen-related cell adhesion molecule 1 (Ceacam1), transcript variant 1, mRNA
AAAGCTCCTTTAAGAAAAGCAGGGCAGATATCAGGGCAGCCTGGCTTAGCAGT
AGTGTTGGAGAAGAAGCTAGCAGGCAGGCAGCAGAGACATGGAGCTGGCCTCA
GCACATCTCCACAAAGGGCAGGTTCCCTGGGGAGGACTACTGCTCACAGCCTC
ACTTTTAGCCTCCTGGAGCCCTGCCACCACTGCTGAAGTCACCATTGAGGCTG
TGCCGCCCCAGGTTGCTGAAGACAACAATGTTCTTCTACTTGTTCACAATCTG
CCCCTGGCGCTTGGAGCCTTTGCCTGGTACAAGGGAAACACTACGGCTATAGA
CAAAGAAATTGCACGATTTGTACCAAATAGTAATATGAATTTCACGGGGCAAG
CATACAGCGGCAGAGAGATAATATACAGCAATGGATCCCTGCTCTTCCAAATG
ATCACCATGAAGGATATGGGAGTCTACACACTAGATATGACAGATGAAAACTA
TCGTCGTACTCAGGCGACTGTGCGATTTCATGTACACCCCATATTATTAAAGC
CCAACATCACAAGCAACAACTCCAATCCCGTGGAGGGTGACGACTCCGTATCA
TTAACCTGTGACTCTTACACTGACCCTGATAATATAAACTACCTGTGGAGCAG
AAATGGTGAAAGCCTTTCAGAAGGTGACAGGCTGAAGCTGTCTGAGGGCAACA
GGACTCTCACTTTACTCAATGTCACGAGGAATGACACAGGACCCTATGTGTGT
GAAACCCGGAATCCAGTGAGTGTCAACCGAAGTGACCCATTCAGCCTGAACAT
TATCTATGGTCCGGACACCCCGATTATATCCCCCTCAGATATTTATTTGCATC
CAGGGTCAAACCTCAACCTCTCCTGCCATGCAGCCTCTAACCCACCTGCACAG
TACTTTTGGCTTATCAATGAGAAGCCCCATGCATCCTCCCAAGAGCTCTTTAT
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CTGTCACTGGCCTCAGTAGGACCACAGTCAAGAACATTACAGTCCTTGAGCCA

GTGACTCAGCCCTTCCTCCAAGTCACCAACACCACAGTCAAAGAACTAGACTC
TGTGACCCTGACCTGCTTGTCGAATGACATTGGAGCCAACATCCAGTGGCTCT
TCAATAGCCAGAGTCTTCAGCTCACAGAGAGAATGACACTCTCCCAGAACAAC
AGCATCCTCAGAATAGACCCTATTAAGAGGGAAGATGCCGGCGAGTATCAGTG
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TAATATTTGACCCAACACAAGGAGGCCTCTCAGATGGCGCCATTGCTGGCATC
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ACACATGCGCGCGCGCACACACACACACACACACACACACACACACACACTTACACA
CACACTCATGCATTCACTCTATTGACTCCTTCAGTGTCTATAGAAGAAAAGGT
GGATCCTGGAGCCTACAGAAAACTCAACCCTTCTAGGCTTTCAAATTTGGCTG
AGAGTGAGGTATCAAAATTTCTCACCCTTTCACTTTCCTGACCCAGATTGTTG
AAAATTGACCTATTCAGAGCACCTTCATTCCCCTCCCAACTCCAAGTCCTGCC
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ACGCCAAACAAAACAAAACAAAACAAAACAAAACAAAACAAAACAAAACAAAA
CAAACCAGAAGCAGGAAATGGCCAGTCCCATATCTTTAAAGGCTGATTGGAAG
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TGTCATGCTTACCCATCTGGGACATTCGAGTCTCTGAACCTTGTGCCCTCACG
CCTGAGCCCTTCTCTGAGCCTTTCTCCAGAAAATCCACTCACAGCAACTAGAG
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GACAGCATCTCTAACATCCACCAGGAAGGTGCCCAGAAAAGCAGAGCTGGTGA
CTTTGGACTGACAGACATCTGGAGTGTGAAAAAGCAGCACAGAGCTAACCTTC
GGAGAGTGTTGAAATTATTTGAAAAGAAGCCATATTTGGAGGTATTGGAGTTT
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TCAGTATGCGAGCTGATCACTTTGCCTTAGGGCCACTAGATTTCTGTCTCCCT
TAGCCCCTCAAGCCCTTTTGATCATGAGTTCCAAACCAAAAATAAATAAATGA
ACAGTGAGGCAGTCCCTTGCAGTACCACTGTCATGGGTCAGGCTAAGCCTCCT
GCTTTTCTGAATTAGTCAAGAAAAGCCTTGGTTTCCCTTTTTCCATCTCTTTA
TCTTGTCTTTCAGATACTGGCCAGAGCCTGGACACTCTTCCTCTGAGATCTCC
AGCTTCTCTGCCTTCTTGTGTTTCTTTTAAACTCTAACAAAAACTGTTCTCAC
CTTCAAAAAATAAAATAATAACAAGCTTTCCACATCCCCACCAAAGAGGGACC
CAGCTAGGTTTCTGGAAACCCAGCACCAGCCTCCAGCTGCCCTTCTGCAGTGT
TTCTGCCTCTGTTTCCCTTTCGTTTTGACTTTTTTCCTTCTTTTGAGACAGAG
TTCCAGCATGGAGCCTGTGCAGGTTTCAATCCCACAGTAACACCTTCTGCAGC
ACCCCACCTGCTCAGACTGCAGCCCTGGCCACCAGGCCTGGCTACCTGGACAT
TCTGTCTGCCCTGCACTCTCAGGAAACCTTGGCCTCTGCTACTGTCTGTTTGG
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GGGCTGTTAGCAGGGTCCTCTCACCTGGCCCTGGCCTTTCTGTAGCCTTGAAT
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AGCAAAGGTTGTATTGGATATCAAGTGTTTGGTCTCACGCCAAGCCAACAGGC
TTTGGAGAGAATTAATTAGTTCTCCTACTCAGGGATTTCTTTCAGTCCTAACA
CAGCCTGTGTATATTTTGCTTCACCCACGCAATGCTGGATTATTTAATTTTGC
CCGGCTTAAGACAAATCTGAGTTACTTGTAAATTTGCTCTATGTTCATAATAA

AAATGTATTATATATCACTGATAGCA (SEQ ID NO: 91) >NP_001034274.1 carcinoembryonic antigen-related cell adhesion molecule 1 isoform 1 precursor [*Mus musculus*]
MELASAHLHKGQVPWGGLLLTASLLASWSPATTAEVTIEAVPPQVAEDNNVLL
LVHNLPLALGAFAWYKGNTTAIDKEIARFVPNSNMNFTGQAYSGREIIYSNGS
LLFQMITMKDMGVYTLDMTDENYRRTQATVRFHVHPILLKPNITSNNSNPVEG
DDSVSLTCDSYTDPDNINYLWSRNGESLSEGDRLKLSEGNRTLTLLNVTRNDT
GPYVCETRNPVSVNRSDPFSLNIIYGPDTPIISPSDIYLHPGSNLNLSCHAAS
NPPAQYFWLINEKPHASSQELFIPNITTNNSGTYTCFVNNSVTGLSRTTVKNI
TVLEPVTQPFLQVTNTTVKELDSVTLTCLSNDIGANIQWLENSQSLQLTERMT
LSQNNSILRIDPIKREDAGEYQCEISNPVSVRRSNSIKLDIIFDPTQGGLSDG
AIAGIVIGVVAGVALIAGLAYFLYSRKSGGGSDQRDLTEHKPSASNHNLAPSD
NSPNKVDDVAYTVLNFNSQQPNRPTSAPSSPRATETVYSEVKKK (SEQ ID NO: 92) Human >NM_007048.6 *Homo sapiens* butyrophilin subfamily 3 BTN3A1 member A1 (BTN3A1), transcript variant 1, mRNA
ATTCCTCACGATGACCCGACAGTCTCTGCTTTCTTTTTCCTTTCTTCCAGAAG
GAGATTTAACCATAGTAGAAAGAATGGAGAACTATTAACTGCCTTTCTTCTGT
GGGCTGTGATTTTCAGAGGGGAATGCTAAGAGGTGATTTTCAATGTTGGGACT
CAAAGGTGAAGACACTGAAGGACAGAATTTTTGGCAGAGGAAAGATCTTCTTC
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ACAGGAAAGAGTGGCATATAGGGGTGTGCAGTAAGAATGTGCAGAGAAAAGGC
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CCCCTAAGAAAGTGGGGGTCTTCCTGGACTATGAGACTGGAGATATCTCATTC
TACAATGCTGTGGATGGATCGCATATTCATACTTTCCTGGACGTCTCCTTCTC
TGAGGCTCTATATCCTGTTTTCAGAATTTTGACCTTGGAGCCCACGGCCCTGA

CTATTTGTCCAGCGTGAAAAGAAGAAGAGAGTTCCTCCAATTCTGACCGAGTG
CTGATCATTCCCTAGAGACACCAGTAACCCCGGGCTTAGCTAACGAAAGTGGG
GAGCCTCAGGCTGAAGTAACTTTTCTCTGCTTCTCCCTGCCCAGCTCAGAGCT
GAGGGCCTCCCCCTCCACAGCAACCAATCACAACCATAAAGCTACAAGCACGC
ACTGAAGCACTTTACTGATACTCATTCAATTATTCATATGACAGTTGTTTGAG
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CATGCAGCCAGAGCCAGCCTTCCTTCAATCAAGGTTTCCAGGCAGAGCAAATA
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GAGTGCAGTGGTGCGATCTCAGCTCCCTGCAACCTCCACCTCCTGGGTTCAAA
CAATTCTCCTGCCTCAGCCTCCCGAGTACTGGGAATATAGGTGCACGCCACCA
CACCCAACAAATTTTTGTACTTTTAGTACAGATGAGGGTTCACTATGTTGGCC
AGGATGGTCTCGATCTCTTGACCTCATGATCCACCCACCTCGGTCTCCCAAAG
TGCTGGGATTACAGGCTTGAGCCACCGGGTGACCGGCTTACAGGGATATTTTT
AATCCCGTTATGGACTCTGTCTCCAGGAGAGGGGTCTATCCACCCCTGCTCAT
TGGTGGATGTTAAACCAATATTCCTTTCAACTGCTGCCTGCTAGGGAAAAACT
ACTCCTCATTATCATCATTATTATTGCTCTCCACTGTATCCCCTCTACCTGGC
ATGTGCTTGTCAAGTTCTAGTTGTTCAATAAATTTGTTAATAATGCTGA (SEQ ID NO: 93) >NP_008979.3 butyrophilin subfamily 3 member Al isoform a precursor [*Homo sapiens*]
MKMASFLAFLLLNFRVCLLLLQLLMPHSAQFSVLGPSGPILAMVGEDADLPCH
LFPTMSAETMELKWVSSSLRQVVNVYADGKEVEDRQSAPYRGRTSILRDGITA
GKAALRIHNVTASDSGKYLCYFQDGDFYEKALVELKVAALGSDLHVDVKGYKD
GGIHLECRSTGWYPQPQIQWSNNKGENIPTVEAPVVADGVGLYAVAASVIMRG
SSGEGVSCTIRSSLLGLEKTASISIADPFFRSAQRWIAALAGTLPVLLLLLGG
AGYFLWQQQEEKKTQFRKKKREQELREMAWSTMKQEQSTRVKLLEELRWRSIQ
YASRGERHSAYNEWKKALFKPADVILDPKTANPILLVSEDQRSVQRAKEPQDL
PDNPERFNWHYCVLGCESFISGRHYWEVEVGDRKEWHIGVCSKNVQRKGWVKM
TPENGFWTMGLTDGNKYRTLTEPRTNLKLPKPPKKVGVFLDYETGDISFYNAV
DGSHIHTFLDVSFSEALYPVFRILTLEPTALTICPA (SEQ ID NO: 94) Human >NM_007047.5 *Homo sapiens* butyrophilin subfamily 3 BTN3A2 member A2 (BTN3A2), transcript variant 1, mRNA
GACTCTTACTGTTTCTCATGGTGAGAAGACAATATTTGCTTTCTCTTTTTCCT
TTCTTCCGGATGAGAGGCTAAGCCATAATAGAAAGAATGGAGAATTATTGATT
GACCGTCTTTATTCTGTGGGCTCTGATTCTCCAATGGGAATACCAAGGGATGG
TTTTCCATACTGGAACCCAAAGGTAAAGACACTCAAGGACAGACATTTTTGGC
AGAGCATAGATGAAAATGGCAAGTTCCCTGGCTTTCCTTCTGCTCAACTTTCA
TGTCTCCCTCCTCTTGGTCCAGCTGCTCACTCCTTGCTCAGCTCAGTTTTCTG
TGCTTGGACCCTCTGGGCCCATCCTGGCCATGGTGGGTGAAGACGCTGATCTG

CCCTGTCACCTGTTCCCGACCATGAGTGCAGAGACCATGGAGCTGAAGTGGGT
AAGTTCCAGCCTAAGGCAGGTGGTGAACGTGTATGCAGATGGAAAGGAAGTGG
AAGACAGGCAGAGTGCACCGTATCGAGGGAGAACTTCGATTCTGCGGGATGGC
ATCACTGCAGGGAAGGCTGCTCTCCGAATACACAACGTCACAGCCTCTGACAG
TGGAAAGTACTTGTGTTATTTCCAAGATGGTGACTTCTATGAAAAAGCCCTGG
TGGAGCTGAAGGTTGCAGCACTGGGTTCTAATCTTCACGTCGAAGTGAAGGGT
TATGAGGATGGAGGGATCCATCTGGAGTGCAGGTCCACCGGCTGGTACCCCCA
ACCCCAAATACAGTGGAGCAACGCCAAGGGAGAGAACATCCCAGCTGTGGAAG
CACCTGTGGTTGCAGATGGAGTGGGCCTATATGAAGTAGCAGCATCTGTGATC
ATGAGAGGCGGCTCCGGGGAGGGTGTATCCTGCATCATCAGAAATTCCCTCCT
CGGCCTGGAAAAGACAGCCAGCATTTCCATCGCAGACCCCTTCTTCAGGAGCG
CCCAGCCCTGGATCGCAGCCCTGGCAGGGACCCTGCCTATCTTGCTGCTGCTT
CTCGCCGGAGCCAGTTACTTCTTGTGGAGACAACAGAAGGAAATAACTGCTCT
GTCCAGTGAGATAGAAAGTGAGCAAGAGATGAAAGAAATGGGATATGCTGCAA
CAGAGCGGGAAATAAGCCTAAGAGAGAGCCTCCAGGAGGAACTCAAGAGGAAA
AAAATCCAGTACTTGACTCGTGGAGAGGAGTCTTCGTCCGATACCAATAAGTC
AGCCTGATGCTCTAATGGAAAAATGGCCCTCTTCAAGCCTGGTGAGGAAATGC
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GCAGAAAAGGGGAACTCATTTAGCTCACGAGTGGTCGAGTGAAGATTGAAAAT
TAACCTCTGAGGGCCAGCACAGCAGCTCATGCCTGTAATCCTAGCACTTTGGA
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GCCGCATCAGGGTATTGGGTTAGGCAGATACTGACCTTACTTTCATTTCCCCT
CTGGTCACTAGACCCCTGGGGCTTTCACCAATGACATTGATGAGAGAATCACA
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CCCATGACCTACCAGACAACCCTGAGAGATTTGAATGGCGTTACTGTGTGCTT
GGCTGTGAAAGCTTCATGTCAGAGAGACACTACTGGGAGGTGGAAGTGGGGGA
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TTTGGGTCAAAATGACACCGGAGAACGGATACTGGACTATGGGCCTGACTGAT
GGGAATAAGTATCGGGCTCTCACTGAGCCCAGAACCAACCTGAAACTTCCTGA
GCCTCCTAGGAAAGTGGGGGTCATCCTGGACTATGAGACTGGACATATCTCGT
TCTACAATGCCACGGATGGATCTCATATCTACACATTTCTGCACGCCTCTTCC
TCTGAGCCTCTGTATCCTGTATTCAGAATTTTGACCTTGGAGCCCACTGCCCT
GACCGTTTGCCCAATACCAAAAGTAGAGAGTTCCCCCGATCCCGACCTAGTGC
CTGATCATTCCCTGGAGATACCACTGACCCCAGGCTTAGCTAATGAAAGTGGG
GAGCCTCAGGCTGAAGTAACATCTCTGCTTCTCCCTGCCCAGCCTGGAGCTAA
GGGTCTCACCCTCCACAACAGCCAGTCAGAACCATAAAGCTACAGGCACACAC
TGAAGCACTTTACTGATATTCATTCAATTATTCCATAGGACAGTTGTTTGAGT
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AAGTGTATTGACTTTACAAAGCAGACAGGAATAGTGAACAACAGAGCTGGGAT
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TAGTCTCATTTGGCAAGTATGGAAGCAGAGGCAGGGCAACATTAAGTAGCTTA
CATAACTCACACGGTAATTTGTGCAGTTGGGAGATGTTCAGCTTCAGTCCCTG
GCCAATTGCCCGTTCTTTTCCAGCCTGATTTTTCCTGCATGGGAAGAGCCCAC
ATGTAGCCCTGAGGTTCCCTTCCCAGGACAGCTCCAGGATCGAGATCACTGTG
AGTGGTTGTGGAGTTAAGACCCCTATGGACTCCTTCCCAGCTGATTATCAGAG
CCTTAGACCCAGCACTCCTTGGATTGGCTCTGCAGAGTGTCTTGGTTGAGAGA
ATAACGTTGCAGTTCCCACAGGGCATGTGACTTTGAAAGAGACTAGAGGCCAC
ACTCAGTTAATAATGGGGCACAGATGTGTTCCCACCCAACAAATGTGATAAGT
GATCGTGCAGCCAGAGCCAGCCTTCCTTCAGTCAAGGTTTCCAGGCAGAGCAA
ATACCCTAGAGATTCTCTGTAATATTGGTAATTTGGATGAAGGAAGCTAGAAG
AATTACAGGGATGTTTTTAATCCCACTATGGACTCAGTCTCCTGGAAAAGGAT
CTGTCCACTCCTGGTCATTGGTGGATGTTAAACCCATATTCCTTTCAACTGCT
GCCTGCTAGGGAAAACTGCTCCTCATTATCATCACTATTATTGCTCACCACTG
TATCCCCTCTACTGGGCAAGTGCTTGTCAAGTTCTAGTTGTTCAATAAATTTG
TTAATAATGCTGA (SEQ ID NO: 95) >NP_008978.2 butyrophilin subfamily 3 member A2 isoform a precursor [*Homo sapiens*]
MKMASSLAFLLLNFHVSLLLVQLLTPCSAQFSVLGPSGPILAMVGEDADLPCH
LFPTMSAETMELKWVSSSLRQVVNVYADGKEVEDRQSAPYRGRTSILRDGITA
GKAALRIHNVTASDSGKYLCYFQDGDFYEKALVELKVAALGSNLHVEVKGYED
GGIHLECRSTGWYPQPQIQWSNAKGENIPAVEAPVVADGVGLYEVAASVIMRG
GSGEGVSCIIRNSLLGLEKTASISIADPFFRSAQPWIAALAGTLPILLLLLAG
ASYFLWRQQKEITALSSEIESEQEMKEMGYAATEREISLRESLQEELKRKKIQ
YLTRGEESSSDTNKSA (SEQ ID NO: 96) Human >NM_007049.5 *Homo sapiens* butyrophilin subfamily 2 BTN2A1 member A1 (BTN2A1), transcript variant 1, mRNA AGATTTCGTTTCCTGCATCTCCAAACATGGCGACCTAGGAGAAGGGGAAGAAC
AATTTTTTCTCCTCTTTTGGGAAGGTTTGTGTCTAGTAGTGCCTGTGCCCCTG
GGCAGATTGGAGAGAAGAGGGACGACTGGAGAATCGTCGAGAACCAGCGGAGA
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CCTCCTCCTCCTCAGCCTGTGTGCACTGGTCTCAGCCCAGTTTATTGTCGTGG
GGCCCACTGATCCCATCTTGGCCACGGTTGGAGAAAACACTACGTTACGCTGC
CATCTGTCACCCGAGAAAAATGCTGAGGACATGGAGGTGCGGTGGTTCCGGTC
TCAGTTCTCCCCCGCAGTGTTTGTGTATAAAGGTGGCAGAGAGAGAACAGAGG
AGCAGATGGAGGAGTACCGAGGAAGAACCACCTTTGTGAGCAAAGACATCAGC
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CTACCGCTGTTACTTCCAAGAAGGCAGGTCCTACGATGAGGCCATCCTGCACC
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GACGGGGGCATCCGGCTGGAGTGCATATCTAGAGGGTGGTACCCAAAGCCCCT
CACAGTGTGGAGGGACCCCTACGGTGGGGTTGCGCCTGCCCTGAAAGAGGTCT
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GACAGTCAGCCTTGTGTCCTAGGCCGGGAGAGCTTCGCTTCAGGGAAACATTA

CTGGGAGGTGGAGGTGGAAAACGTGATTGAGTGGACTGTGGGGGTCTGTAGAG
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ACCTTGGAGATGCATAAAGGGCAATACCGGGCCGTGTCCTCCCCTGATAGGAT
TCTCCCTTTGAAGGAGTCCCTTTGCCGGGTGGGCGTCTTCCTGGACTATGAAG
CTGGAGATGTCTCCTTCTACAACATGAGGGACAGATCGCACATCTACACATGT
CCCCGTTCAGCCTTTTCCGTGCCTGTGAGGCCCTTCTTCAGGTTGGGGTGTGA
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AGAAAGTGTGAGAAGTTGATGGGCAGCAAACCTGCTGTTTAACATCAGGGTGA
CCACATTAAGCCCAGTATTCCAGTTGGCACCAGAAGATATGGACTTGGAATGA
GGCCTACAGGGTTCACCAGGATGTAAGAGGAGAGAGGAATCCACAGGACCACC
AGAGAGGAGAGGGAACCAGATATGCAGATCAGAGATAGAGGAAGTGGAACCAG
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GCGCACATCCACAGGCCTGGACCTGGGATGAAGATGAATGAAGAACATGGATG
CACGTGGATGTAGTTTGGCTCAGGTGTCCCTGCAGTTGGCAAGGAGTCAGTAC
TCAGTCCCTGAGTGTGGCTGAAATTTGAGGTCCTGGCTGAGCCAAGGAGTAAT
GGACCAGATCTACCTCAGTATTCAAGTTCAGTGGGGACACCAGTGGCTTCAAA
CTTCCTGGTTTCATGATATCTTGAGACGCCTTACAAATGATGGAGGATTCCAA
AGAGTTTTTGTTTATTTGGGTTAATATTTGTTGGTATTTATGGCATTTGAGAT
TGAAACTAAGAAATGTTTTAATTTATTACCTTTACAACATTTATTTACATTAC
ATACATACATTTACAACATTTATTAATTTATATTAAAATAGCATGAATAAGCC
AATTATAGGTTAATATAAGTAGAATGTTTGTGAAAAATAAGTATGGTATCCAA
AGCAAAATAAATTTTATTGTGAAGTGTG (SEQ ID NO: 97) >NP_008980.1 butyrophilin subfamily 2 member A1 isoform 1 precursor [*Homo sapiens*]
MESAAALHFSRPASLLLLLLSLCALVSAQFIVVGPTDPILATVGENTTLRCHL
SPEKNAEDMEVRWFRSQFSPAVFVYKGGRERTEEQMEEYRGRTTFVSKDISRG
SVALVIHNITAQENGTYRCYFQEGRSYDEAILHLVVAGLGSKPLISMRGHEDG
GIRLECISRGWYPKPLTVWRDPYGGVAPALKEVSMPDADGLFMVTTAVIIRDK
SVRNMSCSINNTLLGQKKESVIFIPESFMPSVSPCAVALPIIVVILMIPIAVC
IYWINKLQKEKKILSGEKEFERETREIALKELEKERVQKEEELQVKEKLQEEL
RWRRTFLHAVDVVLDPDTAHPDLFLSEDRRSVRRCPFRHLGESVPDNPERFDS
QPCVLGRESFASGKHYWEVEVENVIEWTVGVCRDSVERKGEVLLIPQNGFWTL
EMHKGQYRAVSSPDRILPLKESLCRVGVFLDYEAGDVSFYNMRDRSHIYTCPR
SAFSVPVRPFFRLGCEDSPIFICPALTGANGVTVPEEGLTLHRVGTHQSL (SEQ ID NO: 98) Human >NM_001040462.3 *Homo sapiens* butyrophilin like 8 BTNL8 (BTNL8), transcript variant 2, mRNA
AGAACAGCGCAGTTTGCCCTCCGCTCACGCAGAGCCTCTCCGTGGCTTCCGCA
CCTTGAGCATTAGGCCAGTTCTCCTCTTCTCTCTAATCCATCCGTCACCTCTC
CTGTCATCCGTTTCCATGCCGTGAGGTCCATTCACAGAACACATCCATGGCTC
TCATGCTCAGTTTGGTTCTGAGTCTCCTCAAGCTGGGATCAGGGCAGTGGCAG
GTGTTTGGGCCAGACAAGCCTGTCCAGGCCTTGGTGGGGGAGGACGCAGCATT
CTCCTGTTTCCTGTCTCCTAAGACCAATGCAGAGGCCATGGAAGTGCGGTTCT
TCAGGGGCCAGTTCTCTAGCGTGGTCCACCTCTACAGGGACGGGAAGGACCAG
CCATTTATGCAGATGCCACAGTATCAAGGCAGGACAAAACTGGTGAAGGATTC

TATTGCGGAGGGGCGCATCTCTCTGAGGCTGGAAAACATTACTGTGTTGGATG
CTGGCCTCTATGGGTGCAGGATTAGTTCCCAGTCTTACTACCAGAAGGCCATC
TGGGAGCTACAGGTGTCAGCACTGGGCTCAGTTCCTCTCATTTCCATCACGGG
ATATGTTGATAGAGACATCCAGCTACTCTGTCAGTCCTCGGGCTGGTTCCCCC
GGCCCACAGCGAAGTGGAAAGGTCCACAAGGACAGGATTTGTCCACAGACTCC
AGGACAAACAGAGACATGCATGGCCTGTTTGATGTGGAGATCTCTCTGACCGT
CCAAGAGAACGCCGGGAGCATATCCTGTTCCATGCGGCATGCTCATCTGAGCC
GAGAGGTGGAATCCAGGGTACAGATAGGAGATACCTTTTTCGAGCCTATATCG
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AACTGGACTGGAGAAGAAAGCACGGACAGGCAGAATTGAGAGACGCCCGGAAA
CACGCAGTGGAGGTGACTCTGGATCCAGAGACGGCTCACCCGAAGCTCTGCGT
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CTGAGAAGAGATTTACAAGGAAGAGTGTGGTGGCTTCTCAGAGTTTCCAAGCA
GGGAAACATTACTGGGAGGTGGACGGAGGACACAATAAAAGGTGGCGCGTGGG
AGTGTGCCGGGATGATGTGGACAGGAGGAAGGAGTACGTGACTTTGTCTCCCG
ATCATGGGTACTGGGTCCTCAGACTGAATGGAGAACATTTGTATTTCACATTA
AATCCCCGTTTTATCAGCGTCTTCCCCAGGACCCCACCTACAAAAATAGGGGT
CTTCCTGGACTATGAGTGTGGGACCATCTCCTTCTTCAACATAAATGACCAGT
CCCTTATTTATACCCTGACATGTCGGTTTGAAGGCTTATTGAGGCCCTACATT
GAGTATCCGTCCTATAATGAGCAAAATGGAACTCCCATAGTCATCTGCCCAGT
CACCCAGGAATCAGAGAAAGAGGCCTCTTGGCAAAGGGCCTCTGCAATCCCAG
AGACAAGCAACAGTGAGTCCTCCTCACAGGCAACCACGCCCTTCCTCCCCAGG
GGTGAAATGTAGGATGAATCACATCCCACATTCTTCTTTAGGGATATTAAGGT
CTCTCTCCCAGATCCAAAGTCCCGCAGCAGCCGGCCAAGGTGGCTTCCAGATG
AAGGGGGACTGGCCTGTCCACATGGGAGTCAGGTGTCATGGCTGCCCTGAGCT
GGGAGGGAAGAAGGCTGACATTACATTTAGTTTGCTCTCACTCCATCTGGCTA
AGTGATCTTGAAATACCACCTCTCAGGTGAAGAACCGTCAGGAATTCCCATCT
CACAGGCTGTGGTGTAGATTAAGTAGACAAGGAATGTGAATAATGCTTAGATC
TTATTGATGACAGAGTGTATCCTAATGGTTTGTTCATTATATTACACTTTCAG TAA
(SEQ ID NO: 99) >NP_001035552.1 butyrophilin-like protein 8 isoform 2 precursor [*Homo sapiens*]
MALMLSLVLSLLKLGSGQWQVFGPDKPVQALVGEDAAFSCFLSPKTNAEAMEV
RFFRGQFSSVVHLYRDGKDQPFMQMPQYQGRTKLVKDSIAEGRISLRLENITV
LDAGLYGCRISSQSYYQKAIWELQVSALGSVPLISITGYVDRDIQLLCQSSGW
FPRPTAKWKGPQGQDLSTDSRTNRDMHGLFDVEISLTVQENAGSISCSMRHAH
LSREVESRVQIGDTFFEPISWHLATKVLGILCCGLFFGIVGLKIFFSKFQWKI
QAELDWRRKHGQAELRDARKHAVEVTLDPETAHPKLCVSDLKTVTHRKAPQEV
PHSEKRFTRKSVVASQSFQAGKHYWEVDGGHNKRWRVGVCRDDVDRRKEYVTL
SPDHGYWVLRLNGEHLYFTLNPRFISVFPRTPPTKIGVFLDYECGTISFFNIN
DQSLIYTLTCRFEGLLRPYIEYPSYNEQNGTPIVICPVTQESEKEASWQRASA
IPETSNSESSSQATTPFLPRGEM (SEQ ID NO: 100) Human >NM_006995.5 *Homo sapiens* butyrophilin subfamily 2 BTN2A2 member A2 (BTN2A2), transcript variant 1, mRNA
GGGACTTTTTGGACACCCAGAGAACAGGTCCCAGATACCGAGTCCGCAACTCC
AAACATCGCGATTAATAGGAGGCCTCTGGTCTCTGCCTGCCCTGGGTGCTCAT
GGAACCAGCTGCTGCTCTGCACTTCTCCCTGCCAGCCTCCCTCCTCCTCCTCC
TGCTCCTCCTCCTTCTCAGCCTGTGTGCACTGGTCTCAGCCCAGTTTACTGTC
GTGGGGCCAGCTAATCCCATCCTGGCCATGGTGGGAGAAAACACTACATTACG
CTGCCATCTGTCACCCGAGAAAAATGCTGAGGACATGGAGGTGCGGTGGTTCC

GGTCTCAGTTCTCCCCCGCAGTGTTTGTGTATAAGGGTGGGAGAGAGAGAACA
GAGGAGCAGATGGAGGAGTACCGGGGAAGAATCACCTTTGTGAGCAAAGACAT
CAACAGGGGCAGCGTGGCCCTGGTCATACATAACGTCACAGCCCAGGAGAATG
GGATCTACCGCTGTTACTTCCAAGAAGGCAGGTCCTACGATGAGGCCATCCTA
CGCCTCGTGGTGGCAGGCCTTGGGTCTAAGCCCCTCATTGAAATCAAGGCCCA
AGAGGATGGGAGCATCTGGCTGGAGTGCATATCTGGAGGGTGGTACCCAGAGC
CCCTCACAGTGTGGAGGGACCCCTACGGTGAGGTTGTGCCCGCCCTGAAGGAG
GTTTCCATCGCTGATGCTGACGGCCTCTTCATGGTCACCACAGCTGTGATCAT
CAGAGACAAGTATGTGAGGAATGTGTCCTGCTCTGTCAACAACACCCTGCTCG
GCCAGGAGAAGGAAACTGTCATTTTTATTCCAGAATCCTTTATGCCCAGCGCA
TCTCCCTGGATGGTGGCCCTAGCTGTCATCCTGACCGCATCTCCCTGGATGGT
GTCCATGACTGTCATCCTGGCTGTTTTCATCATCTTCATGGCTGTCAGCATCT
GTTGCATCAAGAAACTTCAAAGGGAAAAAAAGATTCTGTCAGGGGAAAAGAAA
GTTGAACAAGAGGAAAAAGAAATTGCACAGCAACTTCAAGAAGAATTGCGATG
GAGAAGAACATTCTTACATGCTGCTGATGTGGTCCTGGATCCAGACACCGCTC
ATCCCGAGCTCTTCCTGTCAGAGGACCGGAGAAGTGTGAGGCGGGGCCCCTAC
AGGCAGAGAGTGCCTGACAACCCAGAGAGATTCGACAGTCAGCCTTGTGTCCT
GGGATGGGAGAGCTTCGCCTCAGGGAAACATTACTGGGAGGTGGAGGTGGAAA
ACGTGATGGTGTGGACTGTGGGGGTCTGCAGACACAGTGTTGAGAGGAAAGGG
GAGGTCCTGCTGATTCCTCAGAATGGCTTCTGGACCCTGGAGATGTTTGGAAA
CCAATACCGGGCCCTGTCCTCCCCTGAGAGGATTCTCCCTTTGAAGGAGTCCC
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AACATGAGGGACAGATCGCACATCTACACATGTCCCCGTTCAGCCTTTACTGT
GCCTGTGAGGCCCTTCTTCAGGTTAGGGTCTGATGACAGCCCCATCTTCATCT
GCCCTGCACTCACAGGAGCCAGTGGGGTCATGGTGCCTGAAGAGGGCCTGAAA
CTTCACAGAGTGGGGACCCACCAGAGCCTATAGAATCAATTCCTTGGACTCAC
AGCCATGCAGATAAGCCCTGGCCATCTCAGCAGCCACCGCACAACCCCCCTAA
TGAAAGACACGCCCTCCTCCCCTCTGGTCACGTAAGAGAACATCTTCCAGCTG
CCTTTTTCACACCCACTCCAGCCCTCTGCCCCAGTTTTCTCCTCCTCACTAGT
CTGTGGCTTTAGTAGTTCCTTTGCTTGTAATTATGGGATGGGATCCAGGCATA
GGGAACTAGTTGTTTCATAGCTCCCAGTCAAAAAGAAAGTGAGAGAAGCTGTT
GGGCAGCGAACCTACTGTTTAAAATCAGGATAACCACATTAAGCCCAATATGC
CAGTTGGCACCAGATGCTGTGGACTTGGAATGAGGCCAACAGGGTTCACCAGG
ATGAGAGAGGAGAGAGGAATCCACAGGACCACCAGAAGGGAGAGGGAACCAGA
TATGCAGATCAGAGATAGAGGAAGTGGAACCAGAGAGCTGGGAGGGACCAAGG
TTGTAAGGATGGCTAAGTCCCACCATAAGAGCTAAAGGGTCCTGGGAGATGAT
GGCTCATTTCCACCCAACCCCAGGATTTCCACAGCACACACCCACAGGCCTGG
ACCTGGGATGAAGATGAATGAAGAACATGGACTCATGTGGATGTGGTTTGGCT
CAGATGTCCCTGCAATAAACAAGGGGTCAGTACTTAGTCCCTGAGTGTGGTTG
AGGTTTGAGGTCCTGGTCGAGCAGGGCAGTACTGGACCAGGTCTACGTCAGCA
TTCAGGTTCAATGGGGACACCAGTGGCTTCAAACTTCCTGATCTAATTATGTT
TTTAGACACTTAGAAGTTATTGAGGACTTTAAAGAGCTTTTGTTTATTTGGGT
TAATATTTATGACATTTGACATTGAAACAAAAATTTAAAATGTTATCTTTTAA
TTTATGTTAAAATAGCATTAATAAATCAGTTATAGGTTAATGTAGATAGGATG
TTTTGTGAAAAAGCAATCTATTGTGTCCAAATAAAAAAACAAAAAGTGTGACA
CTGGTTAACTTTTTCCAGATCTCATGTCTGGCTTAATAAGAGATATTTGTATT
ATCATATCTGCCTTTGTATTAAACCTATTGGTATATCATAGGTCATGTTAGCT
CAAAAAAACTTTACTGCACACTACTGAGAGAATGAGATGAAAAACGATTAATG
TTTCATTATTATTATTGTGAAAATATTATTAACACTGGGGACTCCTTAAGAGT
ACATCAGAGTTCTCTCTAGGAATCCCAAAACCACATTTTGAAACTAGAATAGT

GGATCCTGGAAGTTAATCCATGTGCTGGTTAATTTTAGATGTCAACCTGACTG
GATTAAGGAATACCTAGACAGCTGGTACAACATTATTTCTGGGTGTGTCTGTG
AGTGTGTTTCCAGAAGAGATTGGCAAGTGAGTCAGTGGGAAATTCTCTCCTTC
TGTTGGCTGGGTGCCCAATACAACAAAAAGGCAGAGGAAAGGCAAATTCTTCT
CTCCTCTGGAGCTGAGACACTCTTCTTCTTCTGCCCTTGGACATCAGAACTCC
TGGCTCTCCGGCCTTTGAACTTCAGGACTTGTACCAGGAGGCCCTGGGTTCTC
AGGCCTTTGGCTTTGGACTGAGAGTTACACAATCAGCTTCCCTGGTTCTGAGG
CTTTCAGACTTAAACTGAGCCATGCTACCAGCATCCCAGGGTCTCCAGCCTAC
AGATGAGCTGTTGTGCGATTTCTTAGCCTCCATAATCACATGAGCCAATCTCC
TTAATAAATGCCTGCTCATAGATCTGTATCTACATCTATATCTGTATGTGCAT
CTATATCTATGCCTATATCTATATCTATATCATATTGATTTTGTCTCTCTGGA
GAACCCTGACTAATAAAATGAGGCATCTAAAA (SEQ ID NO: 101) >NP_008926.2 butyrophilin subfamily 2 member A2 isoform a precursor [*Homo sapiens*]
MEPAAALHFSLPASLLLLLLLLLLSLCALVSAQFTVVGPANPILAMVGENTTL
RCHLSPEKNAEDMEVRWFRSQFSPAVFVYKGGRERTEEQMEEYRGRITFVSKD
INRGSVALVIHNVTAQENGIYRCYFQEGRSYDEAILRLVVAGLGSKPLIEIKA
QEDGSIWLECISGGWYPEPLTVWRDPYGEVVPALKEVSIADADGLFMVTTAVI
IRDKYVRNVSCSVNNTLLGQEKETVIFIPESFMPSASPWMVALAVILTASPWM
VSMTVILAVFIIFMAVSICCIKKLQREKKILSGEKKVEQEEKEIAQQLQEELR
WRRTFLHAADVVLDPDTAHPELFLSEDRRSVRRGPYRQRVPDNPERFDSQPCV
LGWESFASGKHYWEVEVENVMVWTVGVCRHSVERKGEVLLIPQNGFWTLEMFG
NQYRALSSPERILPLKESLCRVGVFLDYEAGDVSFYNMRDRSHIYTCPRSAFT
VPVRPFFRLGSDDSPIFICPALTGASGVMVPEEGLKLHRVGTHQSL (SEQ ID NO: 102) Mouse >NM_175938.3 *Mus musculus* butyrophilin, subfamily 2, BTN2A2 member A2 (Btn2a2), transcript variant 1, mRNA
GAAATTGTGAGACTTGCACGCGGAATGGGTCCTCCGAGGTCTGCTGTCGCGAG
TCCCAGCACTTTGCAAGTAATGGAGAACAGAAAATTCTTTCCTCTCTACTGTA
GCAGTTTGTTCTCTGGTGGCGACTGTGCTCAGCGACAAGTTGGAGAGTAGAGA
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ATTCTAGAAGGTTTTCTGTCCACACGTGACCTAGGTGACTCTGTCCTGAAGAC
CTATGGAGCCTACAACTTCCCTGCGTTCTTGCCCGATAGCCTCCCTTCTCTTC
TTCTTGGTCCTCAGCCTGTTTGTGCTGGTCTCAGCCCAGTTTACTGTCATAGG
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AGGGAAGAGTTGCGCTCATTATCCACAATGTCACAGCCTATGACAATGGCATC
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GCTGTGTGGAGAGACCCCTATGATGAAGTTGTACCTGCCCTGGAGGAGGAGTA
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ACTGCTCTGTGAGGAACATGACCTGCTCTGTCAATAACACTCTGCTCAGCCAG
GAGGTGGAAAGTGTGATTCTCATTCCAGAATCCTTCGTGCCCAGCCTTCCTCT
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CATCTGGAAGCATCTGCCTTGTCAAGAAACACCGCAGGAAGAAATCTATTCTG
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ATACGAGGCTCTTCGAGGCAGAGTGTGCCTGACAACCCTGAGAGATTTGACTG

CCGTCCATGTGTCCTGGGCAGGGAAAGCTTCTCCTCAGGGAAGCATTACTGGG
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GGAGATGTTTGGAAGCCAGTATCGAGCCCTGTCCTCCCCAGAAAAGATCATAC
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GATATTTCTTTCTACAACATGAGAGACAGATCACACATTTACACATGTCCTCC
TGTGACTTTCACTGGGCCCCTGAGACCCTTCTTTAGGCTTGGTTCTGATGACA
GTCCCCTGTTCATCTGTCCAGCATTCACAGGGGCACAGGGAGTTACAATACCT
GAGGGTGGCTTATTCCTATATAAGACAAGACCAATTTCTCAGAGCCTTGTAAG
GAAGCCATAGCTCTCTACACAGTACCATCTGTTGGAGACTAGACCCCATGTCC
TTCAGATCACATGGAGCATCTTCCAGCTGCCACCTTCACACATACTTCAGGCC
CAGTCCTCAGATTACTACATCATTTCTTCTAACTATGGGCCTAGGTAGAGCCA
GTCTTAGGGGACTATTGCTGTAATACAGCTCTCTCCTGAGAAGAAAGTGTGAG
AAGGGCAGAAAACTTGGAGTTTCAACATGCTGCTCTGGTCACAGTGGATATCA
GGCAAGAGCAACAGGGTGGATCAGGATGTAAGAAGTGAGAACTACAGAGGAAG
GAGACAGATAAAGATGAATTGAGGCCGAAGATGGAGGAAATGGACTGAAGAGC
TCTGGGGTAAGCCCTATGTGACAGCTGTGGATAGGTAGGAGCTAATGGTCCAT
TGATATCCAAAGCCAAAGATTTAAATATCACATAGTGTGTCTGGAGTGTATAT
CTGTAGACCTACACATGAGAGGAAACAATCATAGTGATGAACTGGATGTAAGC
TGGCTCAGACGTCCCTACAATAAACACTTCTGAGTTCCATGTCTGTGCTCAGT
AAGAATGGCTTGAGGCTTGCGGTCCATGCTGAGCAGCCAGGTCCACATGAATC
GGATTTACTAGAGTAGGTAGCAGTTCAAGTTCCTTAGGCTCAGGATGTCTTCC
TTTCCCCCAAGCCCTTCCCCCTTCAAGATAGGTCTCACTATGTAGACCAGGCC
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TTCTCTCCCCATCTTTTTGTTATTGCTTTTCAAGACATGGTTTCACTGTGTAT
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GTGCTGGGATTAAAGGTGTGTTACACTACGCCTGGCTTCACTCTATCTCTTCA
GTGTGGGGATTATAGGTTTATACTATCATGCCTAACTAATGTCTGTTGCTGCA
TATGACATTTGAACTTTAGAACAGAAAAACAACTATACATATTAATATATATT
AAACTAATAATAAGC (SEQ ID NO: 103) >NP_787952.2 butyrophilin subfamily 2 member A2 isoform 1 precursor [*Mus musculus*]
MEPTTSLRSCPIASLLFFLVLSLFVLVSAQFTVIGPAEPILAMVGENTTLHCH
LSPERNAEEMEVRWFRWRFFPAVLVYRGHQERPEEQMVAYRGRTTFMRTDISK
GRVALIIHNVTAYDNGIYCCYFQEGRSYDQATMKLMVASLGSEPLIKMKTLED
GSILLECTSEGWYPEPRAVWRDPYDEVVPALEEEYTADREGLFTVTMTIIIRD
CSVRNMTCSVNNTLLSQEVESVILIPESFVPSLPLWMVAVAVTLPVVMLILLT
SGSICLVKKHRRKKSILSAEKEAEYEEKEAARQLQEELRWRRTLLHAADVVLD
PDTAHPELFLSDDQRSVIRGSSRQSVPDNPERFDCRPCVLGRESFSSGKHYWE
VEVENVMVWAIGVCRDSVERKGEALLVPQNGFWTLEMFGSQYRALSSPEKIIP
LKERLHRIAVFLDCEGGDISFYNMRDRSHIYTCPPVTFTGPLRPFFRLGSDDS
PLFICPAFTGAQGVTIPEGGLFLYKTRPISQSLVRKP(SEQ ID NO: 104) Human >NM_001732.3 *Homo sapiens* butyrophilin subfamily 1 BTN1A1 member A1 (BTN1A1), mRNA
AGCTTTCTCACTTGGTAGCAGTGGCCTCTTGTGCCTTTTTCTCCAAGATCACC
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TCATTCTCCTCCAGCTGCCCAAACTGGATTCAGCTCCCTTTGACGTGATTGGA
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CCTGTCTCCGAACGCGAGCGCCGAGCACTTGGAGCTACGCTGGTTCCGAAAGA
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TGCAGTGGAGAACTTCCAAGGGAGAGAAGTTTCCATCTACATCAGAGTCCAGG
AATCCTGATGAAGAAGGTTTGTTCACTGTGGCTGCTTCAGTGATCATCAGAGA
CACTTCTGCGAAAAATGTGTCCTGCTACATCCAGAATCTCCTTCTTGGCCAGG
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ATGAATTCAGCTCTAAAGAGAGACTCCTGGAAGAACTCAAATGGAAAAAGGCT
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TGACTCCTGAGAATGGGTTCTGGGCTGTAGAGTTGTATGGAAATGGGTACTGG
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TGCTGATGGGCCTGAGAGGGTCACAGTCATTGCTAATGCCCAGGACCTTTCTA
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GCACCTTCCAAATCTGTTTCTGTACCAATATTTGGGGGATGGAGGGGTGACTC
AAACTGCTTCTAGTGTTCTCCTAATCCCTTAAGACTAGAACCTATAGGAAACT
ACTTGGAGCAAACTCAAAGGACAGATTAGGGATCGAGATTGGGTCAGGTTAGC
ATGGGGTTGTGGTTGAAATATCTTGGTATCCAGGATAAGGGTATGTGGAAAAA
CAGGCTTTAGGCAAGTGGAAAATTCAAAATGTGCTGTGAAAGGACAATCTCAG
GCTGAAATCCCATAAAGGAACTTGGAGGGAATATTATGATGGAGGGAAGTGAG
GTGAATCCAGGCACATGATGAACACCTGGCTCATCCATAGAGTTTTCACAGCC
TATATCGCAAATTTTCTAAGCCACGTCCTATAGGACAGAGGAGACTGGCCCCA
CTTCTATGGGTCTGAGCTGTGGAAAAGGGAGAGCAGAGAGGAACTGAGATGAG
CAGGGATGAAGGGTCAGGCAGAAAGCGTGATAGAGGAGAGAATTTTTGACAAA
ACTCAAAAGTTGTTTGCACAGCTGTTCTTTGTACCCTGTTCCTTTCTCTGCGC
CCTCCTGTTTCTCCCTTGCCTGGAAGTCATTCCACCCTCAATTTGTTGATCCA
CAAGTTTCCAGTTGTCCTCTTCTTTTTGTTATAGCATCTCTCTATTTCAAAGA
CATTCCTAGAAGTCATCCTTCAGTGATATCACCACTTGCTCAGTCACCATCTC
AACCTTATGTCACCTCAGCCCTCATCTCAATGCCCAAACCCCTTACACACACC
TTCAGTTAGCTTCAACTGCCTCCGTTTCCACACTGTGCACCTTTCACTTTCCC
TACCCAGCTTTCCTACATGCTGCCTCTCCTCAGGGTCCCCTGAATGCTGCATC
ATTGTGTTCAGTGCAGCTGGACTGATTGCACCTGTGTATTTGCCCCTGAGCAC
TTTCCTTTACACATGTGGCTTGTCTTGCCAATAGACTCCAGGCTTATACCTTC
CATTTCCATCGTATTCTCCAGTTTCCAGGATAGACGTTGCTCATCGTCTTTAC

CTAATAAATAAGTTTGTCTGATTGCTGAAA (SEQ ID NO: 105) >NP_001723.2 butyrophilin subfamily 1 member A1 precursor [*Homo sapiens*]
MAVFPSSGLPRCLLTLILLQLPKLDSAPFDVIGPPEPILAVVGEDAELPCRLS
PNASAEHLELRWFRKKVSPAVLVHRDGREQEAEQMPEYRGRATLVQDGIAKGR
VALRIRGVRVSDDGEYTCFFREDGSYEEALVHLKVAALGSDPHISMQVQENGE
ICLECTSVGWYPEPQVQWRTSKGEKFPSTSESRNPDEEGLFTVAASVIIRDTS
AKNVSCYIQNLLLGQEKKVEISIPASSLPRLTPWIVAVAVILMVLGLLTIGSI
FFTWRLYNERPRERRNEFSSKERLLEELKWKKATLHAVDVTLDPDTAHPHLFL
YEDSKSVRLEDSRQKLPEKTERFDSWPCVLGRETFTSGRHYWEVEVGDRTDWA
IGVCRENVMKKGFDPMTPENGFWAVELYGNGYWALTPLRTPLPLAGPPRRVGI
FLDYESGDISFYNMNDGSDIYTFSNVTFSGPLRPFFCLWSSGKKPLTICPIAD
GPERVTVIANAQDLSKEIPLSPMGEDSAPRDADTLHSKLIPTQPSQGAP (SEQ ID NO: 106) Mouse >NM_013483.3 *Mus musculus* butyrophilin, subfamily 1, BTN1A1 member A1 (Btn1a1), mRNA
AACAGCACACAGCCTTCTTCCTTCTGAAGAGCTCTCTCTTTGGCCCCGGGGTG
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AGAGATGGCAGTTCCCACCAACTCCTGCCTCCTGGTCTGTCTGCTCACCCTCA
CTGTCCTACAGCTGCCCACGCTGGATTCGGCAGCTCCCTTCGATGTGACCGCA
CCTCAGGAGCCAGTGTTGGCCCTAGTGGGCTCAGATGCCGAGCTGACCTGTGG
CTTTTCCCCAAACGCGAGCTCAGAATACATGGAGCTGCTGTGGTTTCGACAGA
CGAGGTCGACAGCGGTACTTCTATACCGGGATGGCCAGGAGCAGGAGGGCCAG
CAGATGACGGAGTACCGCGGGAGGGCGACGCTGGCGACAGCCGGGCTTCTAGA
CGGCCGCGCTACTCTGCTGATCCGAGATGTCAGGGTCTCAGACCAGGGGGAGT
ACCGGTGCCTTTTCAAAGACAACGACGACTTCGAGGAGGCCGCCGTATACCTC
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GAAAAAAAA (SEQ ID NO: 107) >NP_038511.1 butyrophilin subfamily 1 member A1 precursor [*Mus musculus*]
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NGPEKVTVIANVQDDIPLSPLGEGCTSGDKDTLHSKLIPFSPSQAAP (SEQ ID NO: 108) Human TIGIT >NM_173799.4 *Homo sapiens* T cell immunoreceptor with Ig and ITIM domains (TIGIT), mRNA
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TATTAACATTA (SEQ ID NO: 109) >NP_776160.2 T-cell immunoreceptor with Ig and ITIM domains precursor [*Homo sapiens*]

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>NP_001139797.1 T-cell immunoreceptor with Ig and ITIM domains precursor [*Mus musculus*]
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EDDYADPQEYFNVLSYRSLESFIAVSKTG (SEQ ID NO: 112) Human
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transcript variant 1, mRNA (CD153)
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TCAACCACTGAAGATGGAGGGTGAGGCACGTCATTTAAAAAAAGTGAAATGTA GC
(SEQ ID NO: 119) >NP_033429.1 tumor necrosis factor ligand superfamily

member 8 [*Mus musculus*]
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Homo sapiens TNF superfamily member 18 GITRL (TNFSF18), mRNA
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KNNTYWGIILLANPQFIS (SEQ ID NO: 122) Mouse GITRL >NM_183391.3 *Mus musculus* tumor necrosis factor (ligand) superfamily, member 18 (Tnfsf18), mRNA
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MEEMPLRESSPQRAERCKKSWLLCIVALLLMLLCSLGTLIYTSLKPTAIESCM
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MAEDLGLSFGETASVEMLPEHGSCRPKARSSSARWALTCCLVLLPFLAGLTTY
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YLGAMFSLQEGDKLMVNVSDISLVDYTKEDKTFFGAFLL (SEQ ID NO: 134) Mouse TL1 >NM_177371.4 *Mus musculus* tumor necrosis factor (ligand) superfamily, member 15 (Tnfsf15), mRNA
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GGGAAGAAACAAAGTTGAACCGTTTCTTTAATAA (SEQ ID NO: 137)
>NP__005182.1 T-lymphocyte activation antigen CD80 precursor [*Homo sapiens*]
MGHTRRQGTSPSKCPYLNFFQLLVLAGLSHFCSGVIHVTKEVKEVATLSCGHN
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RPSDEGTYECVVLKYEKDAFKREHLAEVTLSVKADFPTPSISDFEIPTSNIRR
IICSTSGGFPEPHLSWLENGEELNAINTTVSQDPETELYAVSSKLDFNMTTNH
SFMCLIKYGHLRVNQTFNWNTTKQEHFPDNLLPSWAITLISVNGIFVICCLTY
CFAPRCRERRRNERLRRESVRPV (SEQ ID NO: 138) Mouse CD80 >NM_009855.2
Mus musculus CD80 antigen (Cd80), transcript variant 2, mRNA
GAGTTTTATACCTCAATAGACTCTTACTAGTTTCTCTTTTTCAGGTTGTGAAA
CTCAACCTTCAAAGACACTCTGTTCCATTTCTGTGGACTAATAGGATCATCTT
TAGCATCTGCCGGGTGGATGCCATCCAGGCTTCTTTTTCTACATCTCTGTTTC
TCGATTTTTGTGAGCCTAGGAGGTGCCTAAGCTCCATTGGCTCTAGATTCCTG
GCTTTCCCCATCATGTTCTCCAAAGCATCTGAAGCTATGGCTTGCAATTGTCA
GTTGATGCAGGATACACCACTCCTCAAGTTTCCATGTCCAAGGCTCATTCTTC
TCTTTGTGCTGCTGATTCGTCTTTCACAAGTGTCTTCAGATGTTGATGAACAA
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TGCTGTCTGTCATTGCTGGGAAACTAAAAGTGTGGCCCGAGTATAAGAACCGG
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GCTGAGCCCACTGTGGGTGGTGCTAGCCCTGGGCAGGGGCAGGTGACCCTGGG
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ACTTGATATGTCATGTTTGGTTGGTGTCTGTGGGAGGCCTGCCCTTTTCTGAA
GAGAAGTGGTGGGAGAGTGGATGGGGTGGGGGCAGAGGAAAAGTGGGGGAGAG
GGCCTGGGAGGAGAGGAGGGAGGGGGACGGGGTGGGGGTGGGGAAAACTATGG
TTGGGATGTAAAAACGATAATAATATAAATATTAAATAAAAAGAGAGTATTGA GCAAA

(SEQ ID NO: 139) >NP_033985.3 T-lymphocyte activation antigen CD80 precursor [*Mus musculus*]

MACNCQLMQDTPLLKFPCPRLILLFVLLIRLSQVSSDVDEQLSKSVKDKVLLP
CRYNSPHEDESEDRIYWQKHDKVVLSVIAGKLKVWPEYKNRTLYDNTTYSLII
LGLVLSDRGTYSCVVQKKERGTYEVKHLALVKLSIKADFSTPNITESGNPSAD
TKRITCFASGGFPKPRFSWLENGRELPGINTTISQDPESELYTISSQLDFNTT
RNHTIKCLIKYGDAHVSEDFTWEKPPEDPPDSKNTLVLFGAGFGAVITVVVIV
VIIKCFCKHRSCFRRNEASRETNNSLTFGPEEALAEQTVFL (SEQ ID NO: 140) Human CD86 >NM_175862.5 *Homo sapiens* CD86 molecule (CD86), transcript variant 1, mRNA

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ACATTCTCTTTGTGATGGCCTTOCTGOTCTCTGGTGCTGCTCCTCTGAAGATT
CAAGCTTATTTCAATGAGACTGCAGACCTGCCATGCCAATTTGCAAACTCTCA
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TCTTCAGATCAAGGACAAGGGCTTGTATCAATGTATCATCCATCACAAAAAGC
CCACAGGAATGATTCGCATCCACCAGATGAATTCTGAACTGTCAGTGCTTGCT
AACTTCAGTCAACCTGAAATAGTACCAATTTCTAATATAACAGAAAATGTGTA
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GTGTTTTGCTAAGAACCAAGAATTCAACTATCGAGTATGATGGTGTTATGCAG
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CCTCCCCCAGACCACATTCCTTGGATTACAGCTGTACTTCCAACAGTTATTAT
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CTCCTAGCACCTGGCTAGTTTCTAACATGTTTTGTGCAGCACAGTTTTTAATA
AATGCTTGTTACATTCA (SEQ ID NO: 141) >NP_787058.5 T-lymphocyte activation antigen CD86 isoform 1 precursor [*Homo sapiens*]
MDPQCTMGLSNILFVMAFLLSGAAPLKIQAYFNETADLPCQFANSQNQSLSEL
VVFWQDQENLVLNEVYLGKEKFDSVHSKYMGRTSFDSDSWTLRLHNLQIKDKG
LYQCIIHHKKPTGMIRIHQMNSELSVLANFSQPEIVPISNITENVYINLTCSS
IHGYPEPKKMSVLLRTKNSTIEYDGVMQKSQDNVTELYDVSISLSVSFPDVTS
NMTIFCILETDKTRLLSSPFSIELEDPQPPPDHIPWITAVLPTVIICVMVFCL
ILWKWKKKRPRNSYKCGTNTMEREESEQTKKREKIHIPERSDEAQRVFKSSK
TSSCDKSDTCF (SEQ ID NO: 142) Mouse CD86 >NM_019388.3 *Mus musculus* CD86 antigen (Cd86), mRNA
ATTGCTGAGGAAGAAAGAGGAGCAAGCAGACGCGTAAGAGTGGCTCCTGTAGG
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MDPRCTMGLAILIFVTVLLISDAVSVETQAYFNGTAYLPCPFTKAQNISLSEL
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TVVCVLETESMKISSKPLNFTQEFPSPQTYWKEITASVTVALLLVMLLIIVCH
KKPNQPSRPSNTASKLERDSNADRETINLKELEPQIASAKPNAE (SEQ ID NO: 144) Human LFA-3 >NM_001779.3 *Homo sapiens* CD58 molecule (CD58), (CD58) transcript variant 1, mRNA
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GCCTGCTGCACTGCTTTGGTTTCATCAGCTGTTTTTCCCAACAAATATATGGT
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ATAAATGTAAATATGTACACAAGTGA (SEQ ID NO: 145) >NP_001770.1 lymphocyte function-associated antigen 3 isoform 1 [*Homo sapiens*]
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RHRYALIPIPLAVITTCIVLYMNGILKCDRKPDRTNSN (SEQ ID NO: 146) Human SLAM >NM_003037.5 *Homo sapiens* signaling lymphocytic (CD150) activation molecule family member 1 (SLAMF1), transcript variant 1, mRNA
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>NP_003028.1 signaling lymphocytic activation molecule isoform b precursor [*Homo sapiens*]

MDPKGLLSLTFVLFLSLAFGASYGTGGRMMNCPKILRQLGSKVLLPLTYERIN
KSMNKSIHIVVTMAKSLENSVENKIVSLDPSEAGPPRYLGDRYKFYLENLTLG
IRESRKEDEGWYLMTLEKNVSVQRFCLQLRLYEQVSTPEIKVLNKTQENGTCT
LILGCTVEKGDHVAYSWSEKAGTHPLNPANSSHLLSLTLGPQHADNIYICTVS
NPISNNSQTFSPWPGCRTDPSETKPWAVYAGLLGGVIMILIMVVILQLRRRGK
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QETNSITVYASVTLPES (SEQ ID NO: 148) Mouse SLAM >NM_013730.4 *Mus musculus* signaling lymphocytic (CD150) activation molecule family member 1 (Slamf1), transcript variant 1, mRNA
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GCTACGGAACAGGTGGAGGTGTGATGGATTGCCCAGTGATTCTCCAGAAGCTG
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GAACAAAAGTGTCCGCATCCTCGTCACCATGGCGACGTCCCCAGGAAGCAAAT
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LEKTQCHPCDSGEFSAQWNREIRCHQHRHCEPNQGLRVKKEGTAESDTVCTCK
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KCYPWTRFKVPDASPAGHSCRDGHPHHHFRGVSLYQKGGQETKG (SEQ ID NO: 154) Human CD28 >NM_006139.4 *Homo sapiens* CD28 molecule (CD28), transcript variant 1, mRNA
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(SEQ ID NO: 155) >NP_006130.1 T-cell-specific surface glycoprotein CD28 isoform 1 precursor [*Homo sapiens*]

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GCAATAAATATTTATTCATTGACT (SEQ ID NO: 157) >NP_031668.3 T-cell-specific surface glycoprotein CD28 precursor [*Mus musculus*]
MTLRLLFLALNFFSVQVTENKILVKQSPLLVVDSNEVSLSCRYSYNLLAKEFR
ASLYKGVNSDVEVCVGNGNFTYQPQFRSNAEFNCDGDFDNETVTFRLWNLHVN
HTDIYFCKIEFMYPPPYLDNERSNGTIIHIKEKHLCHTQSSPKLFWALVVVAG
VLFCYGLLVTVALCVIWTNSRRNRLLQSDYMNMTPRRPGLTRKPYQPYAPARD FAAYRP
(SEQ ID NO: 158) Human >NM_144615.2 *Homo sapiens* transmembrane and CD28H immunoglobulin domain containing 2 (TMIGD2), transcript variant 1, mRNA
GGAAGTCTGTCAACTGGGAGGGGGAGAGGGGGGTGATGGGCCAGGAATGGGGT
CCCCGGGCATGGTGCTGGGCCTCCTGGTGCAGATCTGGGCCCTGCAAGAAGCC
TCAAGCCTGAGCGTGCAGCAGGGGCCCAACTTGCTGCAGGTGAGGCAGGGCAG
TCAGGCGACCCTGGTCTGCCAGGTGGACCAGGCCACAGCCTGGGAACGGCTCC
GTGTTAAGTGGACAAAGGATGGGGCCATCCTGTGTCAACCGTACATCACCAAC
GGCAGCCTCAGCCTGGGGGTCTGCGGGCCCCAGGGACGGCTCTCCTGGCAGGC
ACCCAGCCATCTCACCCTGCAGCTGGACCCTGTGAGCCTCAACCACAGCGGGG
CGTACGTGTGCTGGGCGGCCGTAGAGATTCCTGAGTTGGAGGAGGCTGAGGGC
AACATAACAAGGCTCTTTGTGGACCCAGATGACCCCACACAGAACAGAAACCG
GATCGCAAGCTTCCCAGGATTCCTCTTCGTGCTGCTGGGGGTGGGAAGCATGG
GTGTGGCTGCGATCGTGTGGGGTGCCTGGTTCTGGGGCCGCCGCAGCTGCCAG
CAAAGGGACTCAGGTAACAGCCCAGGAAATGCATTCTACAGCAACGTCCTATA
CCGGCCCCGGGGGGCCCCAAAGAAGAGTGAGGACTGCTCTGGAGAGGGGAAGG
ACCAGAGGGGCCAGAGCATTTATTCAACCTCCTTCCCGCAACCGGCCCCCCGC
CAGCCGCACCTGGCGTCAAGACCCTGCCCCAGCCCGAGACCCTGCCCCAGCCC

CAGGCCCGGCCACCCCGTCTCTATGGTCAGGGTCTCTCCTAGACCAAGCCCCA
CCCAGCAGCCGAGGCCAAAAGGGTTCCCCAAAGTGGGAGAGGAGTGAGAGATC
CCAGGAGACCTCAACAGGACCCCACCCATAGGTACACACAAAAAAGGGGGGAT
CGAGGCCAGACACGGTGGCTCACGCCTGTAATCCCAGCAGTTTGGGAAGCCGA
GGCGGGTGGAACACTTGAGGTCAGGGGTTTGAGACCAGCCTGGCTTGAACCTG
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GACAGAGTGAGACTCCGTCTCAAAAAAAACAAAAAGCAGGAGGATTGGGAGCC
TGTCAGCCCCATCCTGAGACCCCGTCCTCATTTCTGTAATGATGGATCTCGCT
CCCACTTTCCCCCAAGAACCTAATAAAGGCTTGTGAAGAAAAAGCAAAAAAAA
AAAAAAAAAA (SEQ ID NO: 159) >NP_653216.2 transmembrane and immunoglobulin domain- containing protein 2 isoform 1 precursor [*Homo sapiens*]
MGSPGMVLGLLVQIWALQEASSLSVQQGPNLLQVRQGSQATLVCQVDQATAWE
RLRVKWTKDGAILCQPYITNGSLSLGVCGPQGRLSWQAPSHLTLQLDPVSLNH
SGAYVCWAAVEIPELEEAEGNITRLFVDPDDPTQNRNRIASFPGFLFVLLGVG
SMGVAAIVWGAWFWGRRSCQQRDSGNSPGNAFYSNVLYRPRGAPKKSEDCSGE
GKDQRGQSIYSTSFPQPAPRQPHLASRPCPSPRPCPSPRPGHPVSMVRVSPRP
SPTQQPRPKGFPKVGEE (SEQ ID NO: 160) Human CD2 >NM_001328609.2 *Homo sapiens* CD2 molecule (CD2), transcript variant 1, mRNA
AGTCTCACTTCAGTTCCTTTTGCATGAAGAGCTCAGAATCAAAAGAGGAAACC
AACCCCTAAGATGAGCTTTCCATGTAAATTTGTAGCCAGCTTCCTTCTGATTT
TCAATGTTTCTTCCAAAGGTGCAGTCTCCAAAGAGATTACGAATGCCTTGGAA
ACCTGGGGTGCCTTGGGTCAGGACATCAACTTGGACATTCCTAGTTTTCAAAT
GAGTGATGATATTGACGATATAAAATGGGAAAAAACTTCAGACAAGAAAAAGA
TTGCACAATTCAGAAAAGAGAAAGAGACTTTCAAGGAAAAAGATACATATAAG
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ACAAGTGGACCACCAGCCTGAGTGCAAAATTCAAGTGCACAGCAGGGAACAAA
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TGGCCAGAGTAATGGGCTCTCTGCCTGGACCCCTCCCAGCCATCCCACTTCTC
TTCCTTTTGCAGAGAAAGGTCTGGACATCTATCTCATCATTGGCATATGTGGA
GGAGGCAGCCTCTTGATGGTCTTTGTGGCACTGCTCGTTTTCTATATCACCAA
AAGGAAAAAACAGAGGAGTCGGAGAAATGATGAGGAGCTGGAGACAAGAGCCC
ACAGAGTAGCTACTGAAGAAAGGGGCCGGAAGCCCCACCAAATTCCAGCTTCA
ACCCCTCAGAATCCAGCAACTTCCCAACATCCTCCTCCACCACCTGGTCATCG
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AGCCTCAGAAGAGGCCTCCTGCTCCGTCGGGCACACAAGTTCACCAGCAGAAA
GGCCCGCCCCTCCCCAGACCTCGAGTTCAGCCAAAACCTCCCCATGGGGCAGC
AGAAAACTCATTGTCCCCTTCCTCTAATTAAAAAAGATAGAAACTGTCTTTTT
CAATAAAAAGCACTGTGGATTTCTGCCCTCCTGATGTGCATATCCGTACTTCC
ATGAGGTGTTTTCTGTGTGCAGAACATTGTCACCTCCTGAGGCTGTGGGCCAC
AGCCACCTCTGCATCTTCGAACTCAGCCATGTGGTCAACATCTGGAGTTTTTG
GTCTCCTCAGAGAGCTCCATCACACCAGTAAGGAGAAGCAATATAAGTGTGAT
TGCAAGAATGGTAGAGGACCGAGCACAGAAATCTTAGAGATTTCTTGTCCCCT
CTCAGGTCATGTGTAGATGCGATAAATCAAGTGATTGGTGTGCCTGGGTCTCA
CTACAAGCAGCCTATCTGCTTAAGAGACTCTGGAGTTTCTTATGTGCCCTGGT
GGACACTTGCCCACCATCCTGTGAGTAAAAGTGAAATAAAAGCTTTGACTAGA (SEQ ID NO: 161) >NP_001315538.1 T-cell surface antigen CD2 isoform 1 precursor

[*Homo sapiens*]
MSFPCKFVASFLLIFNVSSKGAVSKEITNALETWGALGQDINLDIPSFQMSDD
IDDIKWEKTSDKKKIAQFRKEKETFKEKDTYKLFKNGTLKIKHLKTDDQDIYK
VSIYDTKGKNVLEKIFDLKIQERVSKPKISWTCINTTLTCEVMNGTDPELNLY
QDGKHLKLSQRVITHKWTTSLSAKFKCTAGNKVSKESSVEPVSCPGGSILGQS
NGLSAWTPPSHPTSLPFAEKGLDIYLIIGICGGGSLLMVFVALLVFYITKRKK
QRSRRNDEELETRAHRVATEERGRKPHQIPASTPQNPATSQHPPPPPGHRSQA
PSHRPPPPGHRVQHQPQKRPPAPSGTQVHQQKGPPLPRPRVQPKPPHGAAENS LSPSSN
(SEQ ID NO: 162) Mouse CD2 >NM_013486.2 *Mus musculus* CD2 antigen (Cd2), mRNA
GCCTCACCACAGTCCTGACAGAAAGAACTCAGAGTCACCCCTGGGAAAAGAAC
TCTAAAGATGAAATGTAAATTCCTGGGTAGCTTCTTTCTGCTCTTCAGCCTTT
CCGGCAAAGGGGCGGACTGCAGAGACAATGAGACCATCTGGGGTGTCTTGGGT
CATGGCATCACCCTGAACATCCCCAACTTTCAAATGACTGATGATATTGATGA
GGTGCGATGGGTAAGGAGGGGCACCCTGGTCGCAGAGTTTAAAAGGAAGAAGC
CACCTTTTTTGATATCAGAAACGTATGAGGTCTTAGCAAACGGATCCCTGAAG
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TGTGCGGTCTTGCAAGGGACAGATTTTGAACTGAAGCTGTATCAAGGGGAAAC
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GAAGTTGTTAACTGTCCAGAGAAAGGTCTGTCCTTCTATGTCACAGTGGGGGT
CGGTGCAGGAGGACTCCTCTTGGTGCTCTTGGTGGCGCTTTTTATTTTCTGTA
TCTGCAAGAGGAGAAAACGGAACAGGAGGAGAAAAGATGAAGAGCTGGAAATA
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AGCCGCAGCAGCGCAGAATTCAGTGGCGCTCCAAGCTCCTCCTCCACCTGGCC
ATCACCTCCAGACACCTGGCCATCGTCCCTTGCCTCCAGGCCACCGTACCCGT
GAGCACCAGCAGAAGAAGAGACCTCCTCCATCAGGCACACAGATTCACCAGCA
GAAAGGCCCTCCTTTACCCAGACCCCGAGTTCAGCCAAAACCTCCCTGTGGGA
GTGGAGATGGTGTTTCACTGCCGCCCCCTAATTAAGAAGGCAGAGTTCGTCAT
TTCCAATAAAAAGCTGTGTGGATTTATCTTC (SEQ ID NO: 163) >NP_038514.1 T-cell surface antigen CD2 precursor [*Mus musculus*]
MKCKFLGSFFLLFSLSGKGADCRDNETIWGVLGHGITLNIPNFQMTDDIDEVR
WVRRGTLVAEFKRKKPPFLISETYEVLANGSLKIKKPMMRNDSGTYNVMVYGT
NGMTRLEKDLDVRILERVSKPMIHWECPNTTLTCAVLQGTDFELKLYQGETLL
NSLPQKNMSYQWTNLNAPFKCEAINPVSKESKMEVVNCPEKGLSFYVTVGVGA
GGLLLVLLVALFIFCICKRRKRNRRRKDEELEIKASRTSTVERGPKPHSTPAA
AAQNSVALQAPPPPGHHLQTPGHRPLPPGHRTREHQQKKRPPPSGTQIHQQKG
PPLPRPRVQPKPPCGSGDGVSLPPPN (SEQ ID NO: 164) Human LFA-3 >NM_001779.3 *Homo sapiens* CD58 molecule (CD58), (CD58) transcript variant 1, mRNA
GAACTTAGGGCTGCTTGTGGCTGGGCACTCGCGCAGAGGCCGGCCCGACGAGC
CATGGTTGCTGGGAGCGACGCGGGGCGGGCCCTGGGGGTCCTCAGCGTGGTCT
GCCTGCTGCACTGCTTTGGTTTCATCAGCTGTTTTTCCCAACAAATATATGGT
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TGCATGATACCAGAGCATTACAACAGCCATCGAGGACTTATAATGTACTCATG
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AACTGTTGGAAATGTAACTTGAAGCAGCTGCTTTAAGAAGAAATACCCACTAA
CAAAGAACAAGCATTAGTTTTGGCTGTCATCAACTTATTATATGACTAGGTGC
TTGCTTTTTTTGTCAGTAAATTGTTTTTACTGATGATGTAGATACTTTTGTAA
ATAAATGTAAATATGTACACAAGTGA (SEQ ID NO: 165) >NP_001770.1 lymphocyte function-associated antigen 3 isoform 1 [*Homo sapiens*]
MVAGSDAGRALGVLSVVCLLHCFGFISCFSQQIYGVVYGNVTFHVPSNVPLKE
VLWKKQKDKVAELENSEFRAFSSFKNRVYLDTVSGSLTIYNLTSSDEDEYEME
SPNITDTMKFFLYVLESLPSPTLTCALTNGSIEVQCMIPEHYNSHRGLIMYSW
DCPMEQCKRNSTSIYFKMENDLPQKIQCTLSNPLFNTTSSIILTTCIPSSGHS
RHRYALIPIPLAVITTCIVLYMNGILKCDRKPDRTNSN (SEQ ID NO: 166) Human CD48 >NM_001778.4 *Homo sapiens* CD48 molecule (CD48), transcript variant 1, mRNA CTTTTTCTAGCCAGGCTCTCAACTGTCTCCTGCGTTGCTGGGAAGTTCTGGAA
GGAAGCATGTGCTCCAGAGGTTGGGATTCGTGTCTGGCTCTGGAATTGCTACT
GCTGCCTCTGTCACTCCTGGTGACCAGCATTCAAGGTCACTTGGTACATATGA
CCGTGGTCTCCGGCAGCAACGTGACTCTGAACATCTCTGAGAGCCTGCCTGAG
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AGCACCTACATCATGAGGGTGTTGAAAAAGACTGGGAATGAGCAAGAATGGAA
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AGATAGAAGACATGGATGACAACTGTTATCTGAAACTGTCATGTGTGATACCT
GGCGAGTCTGTAAACTACACCTGGTATGGGGACAAAAGGCCCTTCCCAAAGGA
GCTCCAGAACAGTGTGCTTGAAACCACCCTTATGCCACATAATTACTCCAGGT
GTTATACTTGCCAAGTCAGCAATTCTGTGAGCAGCAAGAATGGCACGGTCTGC
CTCAGTCCACCCTGTACCCTGGCCCGGTCCTTTGGAGTAGAATGGATTGCAAG
TTGGCTAGTGGTCACGGTGCCCACCATTCTTGGCCTGTTACTTACCTGAGATG
AGCTCTTTTAACTCAAGCGAAACTTCAAGGCCAGAAGATCTTGCCTGTTGGTG
ATCATGCTCCTCACCAGGACAGAGACTGTATAGGCTGACCAGAAGCATGCTGC
TGAATTATCAACGAGGATTTTCAAGTTAACTTTTAAATACTGGTTATTATTTA
ATTTTATATCCCTTTGTTGTTTTCTAGTACACAGAGATATAGAGATACACATG
CTTTTTTCCCACCCAAAATTGTGACAACATTATGTGAATGTTTTATTATTTTT
TAAAATAAACATTTGATATAATTGTCAATTAACTGAA (SEQ ID NO: 167) >NP_001769.2 CD48 antigen isoform 1 precursor [*Homo sapiens*]
MCSRGWDSCLALELLLLPLSLLVTSIQGHLVHMTVVSGSNVTLNISESLPENY
KQLTWFYTFDQKIVEWDSRKSKYFESKFKGRVRLDPQSGALYISKVQKEDNST
YIMRVLKKTGNEQEWKIKLQVLDPVPKPVIKIEKIEDMDDNCYLKLSCVIPGE
SVNYTWYGDKRPFPKELQNSVLETTLMPHNYSRCYTCQVSNSVSSKNGTVCLS
PPCTLARSFGVEWIASWLVVTVPTILGLLLT (SEQ ID NO: 168) Mouse CD48 >NM_007649.5 *Mus musculus* CD48 antigen (Cd48), transcript variant 1, mRNA
ATACGACTTCCGGTTTTGGGTTTTGCTTCCTGATTGAAGGGCAGGCGCCCTGA
CTTCTCTTACAGTTGTCTCCAGTGTTCTGGGGAAGCTTCTCTAAGTATTATGT

GCTTCATAAAACAGGGATGGTGTCTGGTCCTGGAACTGCTACTGCTGCCCTTG
GGAACTGGATTTCAAGGTCATTCAATACCAGATATAAATGCCACCACCGGCAG
CAATGTAACCCTGAAAATCCATAAGGACCCACTTGGACCATATAAACGTATCA
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CTCATCATTCACAGGATCCTGTTAACCTGACAAGAACTCTTCTCACCCAAGAA
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GCGAAGCAACTCTGGCTCTATTGGACAAAGGAAAATGTGTTACTGAACGTCTG
CGAGAGTTTGCATGCATGCTCTATGAAACAAGCACAGGACCTTGTACAGTGCT
CCACCACTGACCTGTGTGCCCAGTCCTTTACAAAGATTTCAAATCAACCTTTT
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TTTCATATCTCTTTTTAAAATAAATTCTTTTAAAAAACT (SEQ ID NO: 169)
>NP_031675.1 0048 antigen isoform 1 precursor [*Mus musculus*]
MCFIKQGWCLVLELLLLPLGTGFQGHSIPDINATTGSNVTLKIHKDPLGPYKR
ITWLHTKNQKILEYNYNSTKTIFESEFKGRVYLEENNGALHISNVRKEDKGTY
YMRVLRETENELKITLEVFDPVPKPSIEINKTEASTDSCHLRLSCEVKDQHVD
YTWYESSGPFPKKSPGYVLDLIVTPQNKSTFYTCQVSNPVSSKNDTVYFTLPC
DLARSSGVCWTATWLVVTTLIIHRILLT (SEQ ID NO: 170) Human CD226
>NM_006566.4 *Homo sapiens* CD226 molecule (CD226), transcript variant 1, mRNA GCAGATGGGAAGAAGCGTTAGAGCGAGCAGCACTCACATCTCAAGAACCAGCC
TTTCAAACAGTTTCCAGAGATGGATTATCCTACTTTACTTTTGGCTCTTCTTC
ATGTATACAGAGCTCTATGTGAAGAGGTGCTTTGGCATACATCAGTTCCCTTT
GCCGAGAACATGTCTCTAGAATGTGTGTATCCATCAATGGGCATCTTAACACA
GGTGGAGTGGTTCAAGATCGGGACCCAGCAGGATTCCATAGCCATTTTCAGCC
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TCAAATAGCCACATTGTTTCGGAACCTGGAAAGAATGTCACACTCACTTGTCA
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TGCTTTTTCTGATATTAACATAACTTTCAGTTTTTTAAAAAATTAACATTAGC
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CACAAGGAGATAACATGTCAAGTCTATGCGATTGGAAATAAACTAAAGATGAA
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GTTTCACTCATCATATGTGTTCAATATTAATTCATTTTAAGGTTTAGTTGCAC
AAAAGGTAAATGAGAATTAGAAGACTCCATGGGTAAGAGGAGCCACAGAAGTA
AAGCATTGTCAAGGGTTCTATGTCTATATATTTAGATATTAGGCTTCTGAGAA
AAAAACACAATAGGAAGGAAGATGAACACAACAGAGGGCAGAAGGTCTATACG
TCCTGAGGCCTTTTATGCAACGTTTGTTTGTGGAATGTTTTTTAAGAATGTGT
GAGAGTCATTTTAATGTGAAATAAAGACCTACGTCTACA (SEQ ID NO: 171)
>NP_006557.2 CO226 antigen isoform a precursor [*Homo sapiens*]
MDYPTLLLALLHVYRALCEEVLWHTSVPFAENMSLECVYPSMGILTQVEWFKI
GTQQDSIAIFSPTHGMVIRKPYAERVYFLNSTMASNNMTLFFRNASEDDVGYY
SCSLYTYPQGTWQKVIQVVQSDSFEAAVPSNSHIVSEPGKNVTLTCQPQMTWP
VQAVRWEKIQPRQIDLLTYCNLVHGRNFTSKFPRQIVSNCSHGRWSVIVIPDV
TVSDSGLYRCYLQASAGENETFVMRLTVAEGKTDNQYTLFVAGGTVLLLLFVI
SITTIIVIFLNRRRRRERRDLFTESWDTQKAPNNYRSPISTSQPTNQSMDDTR
EDIYVNYPTFSRRPKTRV (SEQ ID NO: 172) Mouse CD226 >NM_178687.2 *Mus musculus* CD226 antigen (Cd226), transcript variant 1, mRNA
ACACAGAAGACTTCTTGACTTCAGGAGACACTGCTGTATGAAACAGTGCTTGC
TATCAGTGGCTGCTGGAAGAGGCTGTGGTGGAAAGAAAACCTCAACTGCAGGC
CAGAGTTGGTTCCCCAAAAGAGGCAAACTCCCAGTGCTAGCCAGAGGCTAGGA
AGCTCTAAGCAACCCACTTATCTGCAAGGAGAGTTACGCCCAAAGAGCATCAA
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TTTTAAGTAGTAGAACACAGTAGTGTAGGAATCTGAGAGCAGAGAGTTTGGGA
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TGCTTCAGAGTTTGATCAGGGCCATCTCTCCCAACTCTGCTGGAACTGAGAGA
ATGCACCTGATGTTGTCTCCATTTTAGATAGAGAAAAAAAAAACCCGAATATT
TATAAAACTAAATAAAACTATAGTTACCTCAAAACTATGGGGATCACTATAAC
ATAGAATAGAATAGAATAGAATAGAATAGAATAGAATAGAATAGAATAG (SEQ ID NO: 173) >NP_848802.2 CD226 antigen isoform a precursor [*Mus musculus*]
MAYVTWLLAILHVHKALCEETLWDTTVRLSETMTLECVYPLTHNLTQVEWTKN
TGTKTVSIAVYNPNHNMHIESNYLHRVHFLNSTVGFRNMSLSFYNASEADIGI
YSCLFHAFPNGPWEKKIKVVWSDSFEIAAPSDSYLSAEPGQDVTLTCQLPRTW
PVQQVIWEKVQPHQVDILASCNLSQETRYTSKYLRQTRSNCSQGSMKSILIIP
NAMAADSGLYRCRSEAITGKNKSFVIRLIITDGGTNKHFILPIVGGLVSLLLV
ILIIIIFILYNRKRRRQVRIPLKEPRDKQSKVATNCRSPTSPIQSTDDEKEDI
YVNYPTFSRRPKPRL (SEQ ID NO: 174) Human DR3 >NM_003790.3 *Homo sapiens* TNF receptor superfamily member 25 (TNFRSF25), transcript variant 2, mRNA
GAAGGCGGAACCACGACGGGCAGAGAGCACGGAGCCGGGAAGCCCCTGGGCGC
CCGTCGGAGGGCTATGGAGCAGCGGCCGCGGGGCTGCGCGGCGGTGGCGGCGG
CGCTCCTCCTGGTGCTGCTGGGGGCCCGGGCCCAGGGCGGCACTCGTAGCCCC
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AGGCTGCCCAGCGGGGCACTACCTGAAGGCCCCTTGCACGGAGCCCTGCGGCA
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CGCCCACTTGCCACCTAGGCGCTCTGGTGGCCCTTGCAGAAGCCCTAAGTACG
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GTGGGTC (SEQ ID NO: 175) >NP_003781.1 tumor necrosis factor receptor superfamily member 25 isoform 2 precursor [*Homo sapiens*]
MEQRPRGCAAVAAALLLVLLGARAQGGTRSPRCDCAGDFHKKIGLFCCRGCPA
GHYLKAPCTEPCGNSTCLVCPQDTFLAWENHHNSECARCQACDEQASQVALEN
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PLLLGATLTYTYRHCWPHKPLVTADEAGMEALTPPPATHLSPLDSAHTLLAPP
DSSEKICTVQLVGNSWTPGYPETQEALCPQVTWSWDQLPSRALGPAAAPTLSP
ESPAGSPAMMLQPGPQLYDVMDAVPARRWKEFVRTLGLREAEIEAVEVEIGRF
RDQQYEMLKRWRQQQPAGLGAVYAALERMGLDGCVEDLRSRLQRGP (SEQ ID NO: 176) Mouse DR3 >NM_033042.4 *Mus musculus* tumor necrosis factor receptor superfamily, member 25 (Tnfrsf25), transcript variant 2, mRNA
CTGCGTGGAGGGGAAATGGGCCAGAGGCTGCTGGCAGGGGGCCTCCTCTGCTG
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CGTAAGAAAAGCAGCTGCTTG (SEQ ID NO: 177) >NP_149031.2 tumor necrosis factor receptor superfamily member 25 isoform 2 precursor [*Mus musculus*]
MEELPRRERSPPGAATPGSTARVLQPLFLPLLLLLLLLGGQGQGGMSGRCDC
ASESQKRYGPFCCRGCPKGHYMKAPCAEPCGNSTCLPCPSDTFLTRDNHFKTD
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EFVRTLGLREAEIEAVEVEICRFRDQQYEMLKRWRQQQPAGLGAIYAALERMG
LEGCAEDLRSRLQRGP (SEQ ID NO: 178) Human DcR3 >NM_003823.4 *Homo sapiens* TNF receptor superfamily member 6b (TNFRSF6B), mRNA
GGACTTGGGCGGCCCCTCCGCAGGCGGACCGGGGGCAAAGGAGGTGGCATGTC
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TTATTTTTATAAAGCTTTTTCATAAAA (SEQ ID NO: 179) >NP_003814.1 tumor necrosis factor receptor superfamily member 6B precursor [*Homo sapiens*]
MRALEGPGLSLLCLVLALPALLPVPAVRGVAETPTYPWRDAETGERLVCAQCP
PGTFVQRPCRRDSPTTCGPCPPRHYTQFWNYLERCRYCNVLCGEREEEARACH
ATHNRACRCRTGFFAHAGFCLEHASCPPGAGVIAPGTPSQNTQCQPCPPGTFS
ASSSSSEQCQPHRNCTALGLALNVPGSSSHDTLCTSCTGFPLSTRVPGAEECE
RAVIDFVAFQDISIKRLQRLLQALEAPEGWGPTPRAGRAALQLKLRRRLTELL
GAQDGALLVRLLQALRVARMPGLERSVRERFLPVH (SEQ ID NO: 180) Human FasL >NM_000639.3 *Homo sapiens* Fas ligand (FASLG), transcript variant 1, mRNA
AGCAGTCAGCAACAGGGTCCCGTCCTTGACACCTCAGCCTCTACAGGACTGAG
AAGAAGTAAAACCGTTTGCTGGGGCTGGCCTGACTCACCAGCTGCCATGCAGC
AGCCCTTCAATTACCCATATCCCCAGATCTACTGGGTGGACAGCAGTGCCAGC
TCTCCCTGGGCCCCTCCAGGCACAGTTCTTCCCTGTCCAACCTCTGTGCCCAG
AAGGCCTGGTCAAAGGAGGCCACCACCACCACCGCCACCGCCACCACTACCAC
CTCCGCCGCCGCCGCCACCACTGCCTCCACTACCGCTGCCACCCCTGAAGAAG
AGAGGGAACCACAGCACAGGCCTGTGTCTCCTTGTGATGTTTTTCATGGTTCT

GGTTGCCTTGGTAGGATTGGGCCTGGGGATGTTTCAGCTCTTCCACCTACAGA
AGGAGCTGGCAGAACTCCGAGAGTCTACCAGCCAGATGCACACAGCATCATCT
TTGGAGAAGCAAATAGGCCACCCCAGTCCACCCCCTGAAAAAAAGGAGCTGAG
GAAAGTGGCCCATTTAACAGGCAAGTCCAACTCAAGGTCCATGCCTCTGGAAT
GGGAAGACACCTATGGAATTGTCCTGCTTTCTGGAGTGAAGTATAAGAAGGGT
GGCCTTGTGATCAATGAAACTGGGCTGTACTTTGTATATTCCAAAGTATACTT
CCGGGGTCAATCTTGCAACAACCTGCCCCTGAGCCACAAGGTCTACATGAGGA
ACTCTAAGTATCCCCAGGATCTGGTGATGATGGAGGGGAAGATGATGAGCTAC
TGCACTACTGGGCAGATGTGGGCCCGCAGCAGCTACCTGGGGGCAGTGTTCAA
TCTTACCAGTGCTGATCATTTATATGTCAACGTATCTGAGCTCTCTCTGGTCA
ATTTTGAGGAATCTCAGACGTTTTTCGGCTTATATAAGCTCTAAGAGAAGCAC
TTTGGGATTCTTTCCATTATGATTCTTTGTTACAGGCACCGAGAATGTTGTAT
TCAGTGAGGGTCTTCTTACATGCATTTGAGGTCAAGTAAGAAGACATGAACCA
AGTGGACCTTGAGACCACAGGGTTCAAAATGTCTGTAGCTCCTCAACTCACCT
AATGTTTATGAGCCAGACAAATGGAGGAATATGACGGAAGAACATAGAACTCT
GGGCTGCCATGTGAAGAGGGAGAAGCATGAAAAAGCAGCTACCAGGTGTTCTA
CACTCATCTTAGTGCCTGAGAGTATTTAGGCAGATTGAAAAGGACACCTTTTA
ACTCACCTCTCAAGGTGGGCCTTGCTACCTCAAGGGGGACTGTCTTTCAGATA
CATGGTTGTGACCTGAGGATTTAAGGGATGGAAAAGGAAGACTAGAGGCTTGC
ATAATAAGCTAAAGAGGCTGAAAGAGGCCAATGCCCCACTGGCAGCATCTTCA
CTTCTAAATGCATATCCTGAGCCATCGGTGAAACTAACAGATAAGCAAGAGAG
ATGTTTTGGGGACTCATTTCATTCCTAACACAGCATGTGTATTTCCAGTGCAA
TTGTAGGGGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTATGACTAAAGAGA
GAATGTAGATATTGTGAAGTACATATTAGGAAAATATGGGTTGCATTTGGTCA
AGATTTTGAATGCTTCCTGACAATCAACTCTAATAGTGCTTAAAAATCATTGA
TTGTCAGCTACTAATGATGTTTTCCTATAATATAATAAATATTTATGTAGATG
TGCATTTTTGTGAAATGAAAACATGTAATAAAAAGTATATGTTAGGATACAAA TAA
(SEQ ID NO: 181) >NP_000630.1 tumor necrosis factor ligand superfamily member 6 isoform 1 [*Homo sapiens*]
MQQPFNYPYPQIYWVDSSASSPWAPPGTVLPCPTSVPRRPGQRRPPPPPPPPP
LPPPPPPPLPPLPLPPLKKRGNHSTGLCLLVMFFMVLVALVGLGLGMFQLFH
LQKELAELRESTSQMHTASSLEKQIGHPSPPPEKKELRKVAHLTGKSNSRSMP
LEWEDTYGIVLLSGVKYKKGGLVINETGLYFVYSKVYFRGQSCNNLPLSHKVY
MRNSKYPQDLVMMEGKMMSYCTTGQMWARSSYLGAVFNLTSADHLYVNVSELS
LVNFEESQTFFGLYKL (SEQ ID NO: 182) Mouse FasL >NM_010177.4 *Mus musculus* Fas ligand (TNF superfamily, member 6) (Fasl), transcript variant 1, mRNA TGAGGCTTCTCAGCTTCAGATGCAAGTGAGTGGGTGTCTCACAGAGAAGCAAA
GAGAAGAGAACAGGAGAAAGGTGTTTCCCTTGACTGCGGAAACTTTATAAAGA
AAACTTAGCTTCTCTGGAGCAGTCAGCGTCAGAGTTCTGTCCTTGACACCTGA
GTCTCCTCCACAAGGCTGTGAGAAGGAAACCCTTTCCTGGGGCTGGGTGCCAT
GCAGCAGCCCATGAATTACCCATGTCCCCAGATCTTCTGGGTAGACAGCAGTG
CCACTTCATCTTGGGCTCCTCCAGGGTCAGTTTTTCCCTGTCCATCTTGTGGG
CCTAGAGGGCCGGACCAAAGGAGACCGCCACCTCCACCACCACCTGTGTCACC
ACTACCACCGCCATCACAACCACTCCCACTGCCGCCACTGACCCCTCTAAAGA
AGAAGGACCACAACACAAATCTGTGGCTACCGGTGGTATTTTTCATGGTTCTG
GTGGCTCTGGTTGGAATGGGATTAGGAATGTATCAGCTCTTCCACCTGCAGAA
GGAACTGGCAGAACTCCGTGAGTTCACCAACCAAAGCCTTAAAGTATCATCTT
TTGAAAAGCAAATAGCCAACCCCAGTACACCCTCTGAAAAAAAAGAGCCGAGG
AGTGTGGCCCATTTAACAGGGAACCCCCACTCAAGGTCCATCCCTCTGGAATG
GGAAGACACATATGGAACCGCTCTGATCTCTGGAGTGAAGTATAAGAAAGGTG

GCCTTGTGATCAACGAAACTGGGTTGTACTTCGTGTATTCCAAAGTATACTTC
CGGGGTCAGTCTTGCAACAACCAGCCCCTAAACCACAAGGTCTATATGAGGAA
CTCTAAGTATCCTGAGGATCTGGTGCTAATGGAGGAGAAGAGGTTGAACTACT
GCACTACTGGACAGATATGGGCCCACAGCAGCTACCTGGGGGCAGTATTCAAT
CTTACCAGTGCTGACCATTTATATGTCAACATATCTCAACTCTCTCTGATCAA
TTTTGAGGAATCTAAGACCTTTTTCGGCTTGTATAAGCTTTAAAAGAAAAAGC
ATTTTAAAATGATCTACTATTCTTTATCATGGGCACCAGGAATATTGTCTTGA
ATGAGAGTCTTCTTAAGACCTATTGAGATTAATTAAGACTACATGAGCCACAA
AGACCTCATGACCGCAAGGTCCAACAGGTCAGCTATCCTTCATTTTCTCGAGG
TCCATGGAGTGGTCCTTAATGCCTGCATCATGAGCCAGATGGAAGGAGGTCTG
TGACTGAGGGACATAAAGCTTTGGGCTGCTGTGTGACAATGCAGAGGCACAGA
GAAAGAACTGTCTGATGTTAAATGGCCAAGAGAATTTTAACCATTGAAGAAGA
CACCTTTACACTCACTTCCAGGGTGGGTCTACTTACTACCTCACAGAGGCCGT
TTTTGAGACATAGTTGTGGTATGAATATACAAGGGTGAGAAAGGAGGCTCATT
TGACTGATAAGCTAGAGACTGAAAAAAAGACAGTGTCTCATTGGCACCATCTT
TACTGTTACCTAATGTTTTCTGAGCCGACCTTTGATCCTAACGGAGAAGTAAG
AGGGATGTTTGAGGCACAAATCATTCTCTACATAGCATGCATACCTCCAGTGC
AATGATGTCTGTGTGTTTGTATGTATGAGAGCAAACAGATTCTAAGGAGTCAT
ATAAATAAAATATGTACATTATGGAGTACATATTAGAAACCTGTTACATTTGA
TGCTAGATATCTGAATGTTTCTTGGCAATAAACTCTAATAGTCTTCAAAATCT
TTTATTATCAGCTACTGATGCTGTTTTTCTTTAATACAACTAGTATTTATGCT
CTGAACATCCTAATGAGGAAAAGACAAATAAAATTATGTTATAGAATACAGAA
ATGCCTTAAGGACATAGACTTTGGAAA (SEQ ID NO: 183) >NP_034307.1 tumor necrosis factor ligand superfamily member 6 isoform 1 [*Mus musculus*]
MQQPMNYPCPQIFWVDSSATSSWAPPGSVFPCPSCGPRGPDQRRPPPPPPPVS
PLPPPSQPLPLPPLTPLKKKDHNTNLWLPVVFFMVLVALVGMGLGMYQLFHLQ
KELAELREFTNQSLKVSSFEKQIANPSTPSEKKEPRSVAHLTGNPHSRSIPLE
WEDTYGTALISGVKYKKGGLVINETGLYFVYSKVYFRGQSCNNQPLNHKVYMR
NSKYPEDLVLMEEKRLNYCTTGQIWAHSSYLGAVFNLTSADHLYVNISQLSLI
NFEESKTFFGLYKL (SEQ ID NO: 184) Human TIM-1 >NM_012206.3 *Homo sapiens* hepatitis A virus cellular (CD365) receptor 1 (HAVCR1), transcript variant 1, mRNA
GACCAGGAGTCAGTTTGGCGGTTATGTGTGGGGAAGAAGCTGGGAAGTCAGGG
GCTGTTTCTGTGGACAGCTTTCCCTGTCCTTTGGAAGGCACAGAGCTCTCAGC
TGCAGGGAACTAACAGAGCTCTGAAGCCGTTATATGTGGTCTTCTCTCATTTC
CAGCAGAGCAGGCTCATATGAATCAACCAACTGGGTGAAAAGATAAGTTGCAA
TCTGAGATTTAAGACTTGATCAGATACCATCTGGTGGAGGGTACCAACCAGCC
TGTCTGCTCATTTTCCTTCAGGCTGATCCCATAATGCATCCTCAAGTGGTCAT
CTTAAGCCTCATCCTACATCTGGCAGATTCTGTAGCTGGTTCTGTAAAGGTTG
GTGGAGAGGCAGGTCCATCTGTCACACTACCCTGCCACTACAGTGGAGCTGTC
ACATCCATGTGCTGGAATAGAGGCTCATGTTCTCTATTCACATGCCAAAATGG
CATTGTCTGGACCAATGGAACCCACGTCACCTATCGGAAGGACACACGCTATA
AGCTATTGGGGGACCTTTCAAGAAGGGATGTCTCTTTGACCATAGAAAATACA
GCTGTGTCTGACAGTGGCGTATATTGTTGCCGTGTTGAGCACCGTGGGTGGTT
CAATGACATGAAAATCACCGTATCATTGGAGATTGTGCCACCCAAGGTCACGA
CTACTCCAATTGTCACAACTGTTCCAACCGTCACGACTGTTCGAACGAGCACC
ACTGTTCCAACGACAACGACTGTTCCAATGACGACTGTTCCAACGACAACTGT
TCCAACAACAATGAGCATTCCAACGACAACGACTGTTCTGACGACAATGACTG
TTTCAACGACAACGAGCGTTCCAACGACAACGAGCATTCCAACAACAACAAGT
GTTCCAGTGACAACAACTGTCTCTACCTTTGTTCCTCCAATGCCTTTGCCCAG

GCAGAACCATGAACCAGTAGCCACTTCACCATCTTCACCTCAGCCAGCAGAAA
CCCACCCTACGACACTGCAGGGAGCAATAAGGAGAGAACCCACCAGCTCACCA
TTGTACTCTTACACAACAGATGGGAATGACACCGTGACAGAGTCTTCAGATGG
CCTTTGGAATAACAATCAAACTCAACTGTTCCTAGAACATAGTCTACTGACGG
CCAATACCACTAAAGGAATCTATGCTGGAGTCTGTATTTCTGTCTTGGTGCTT
CTTGCTCTTTTGGGTGTCATCATTGCCAAAAAGTATTTCTTCAAAAAGGAGGT
TCAACAACTAAGTGTTTCATTTAGCAGCCTTCAAATTAAAGCTTTGCAAAATG
CAGTTGAAAAGGAAGTCCAAGCAGAAGACAATATCTACATTGAGAATAGTCTT
TATGCCACGGACTAAGACCCAGTGGTGCTCTTTGAGAGTTTACGCCCATGAGT
GCAGAAGACTGAACAGACATCAGCACATCAGACGTCTTTTAGACCCCAAGACA
ATTTTTCTGTTTCAGTTTCATCTGGCATTCCAACATGTCAGTGATACTGGGTA
GAGTAACTCTCTCACTCCAAACTGTGTATAGTCAACCTCATCATTAATGTAGT
CCTAATTTTTTATGCTAAAACTGGCTCAATCCTTCTGATCATTGCAGTTTTCT
CTCAAATATGAACACTTTATAATTGTATGTTCTTTTTAGACCCCATAAATCCT
GTATACATCAAAGAGAA (SEQ ID NO: 185) >NP_036338.2 hepatitis A virus cellular receptor 1 isoform a precursor [*Homo sapiens*]
MHPQVVILSLILHLADSVAGSVKVGGEAGPSVTLPCHYSGAVTSMCWNRGSCS
LFTCQNGIVWTNGTHVTYRKDTRYKLLGDLSRRDVSLTIENTAVSDSGVYCCR
VEHRGWFNDMKITVSLEIVPPKVTTTPIVTTVPTVTTVRTSTTVPTTTTVPMT
TVPTTTVPTTMSIPTTTTVLTTMTVSTTTSVPTTTSIPTTTSVPVTTTVSTFV
PPMPLPRQNHEPVATSPSSPQPAETHPTTLQGAIRREPTSSPLYSYTTDGNDT
VTESSDGLWNNNQTQLFLEHSLLTANTTKGIYAGVCISVLVLLALLGVIIAKK
YFFKKEVQQLSVSFSSLQIKALQNAVEKEVQAEDNIYIENSLYATD (SEQ ID NO: 186) Mouse TIM-1 >NM_134248.2 *Mus musculus* hepatitis A virus cellular receptor 1 (Havcr1), transcript variant 1, mRNA
GTCAGTACCATGAATCAGATTCAAGTCTTCATTTCAGGCCTCATACTGCTTCT
CCCAGGCGCTGTGGATTCTTATGTGGAAGTAAAGGGGGTGGTGGGTCACCCTG
TCACACTTCCATGTACTTACTCAACATATCGTGGAATCACAACGACATGTTGG
GGCCGAGGGCAATGCCCATCTTCTGCTTGTCAAAATACACTTATTTGGACCAA
TGGACATCGTGTCACCTATCAGAAGAGCAGTCGGTACAACTTAAAGGGGCATA
TTTCAGAAGGAGATGTGTCCTTGACGATAGAGAACTCTGTTGAGAGTGACAGT
GOTCTGTATTGTTGTCGAGTGGAGATTOCTGOATGGTTTAATGATCAGAAAGT
GACCTTTTCATTGCAAGTTAAACCAGAGATTCCCACACGTCCTCCAAGAAGAC
CCACAACTACAAGGCCCACAGCTACAGGAAGACCCACGACTATTTCAACAAGA
TCCACACATGTACCAACATCAACCAGAGTCTCTACCTCCACTCCTCCAACATC
TACACACACATGGACTCACAAACCAGAACCCACTACATTTTGTCCCCATGAGA
CAACAGCTGAGGTGACAGGAATCCCATCCCATACTCCTACAGACTGGAATGGC
ACTGTGACATCCTCAGGAGATACCTGGAGTAATCACACTGAAGCAATCCCTCC
AGGGAAGCCGCAGAAAAACCCTACTAAGGGCTTCTATGTTGGCATCTGCATCG
CAGCCCTGCTGCTACTGCTCCTTGTGAGCACCGTGGCTATCACCAGGTACATA
CTTATGAAAAGGAAGTCAGCATCTCTAAGCGTGGTTGCCTTCCGTGTCTCTAA
GATTGAAGCTTTGCAGAACGCAGCGGTTGTGCATTCCCGAGCTGAAGACAACA
TCTACATTGTTGAAGATAGACCTTGAGGGGCAGAATGAGTACCAGTGGCCCTC
TGAGGGACCTTCTGCCTGAGATTTATAGAGACTGTCACTGATGTCATAGAGTC
ACACCCATTACAGCGCCAAGGCGATTTTCTGTGTTGGTTCTTCCAGCTGCAGC
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AATTTTGGTATCTGCACCACCTCCGTGTCTCTGCTCACTACAGAGATTCTCTC
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AATACTATTCTATTCTTGGTTACTAAAACCATTACTAAGAGAGGGATAGGAAT
TAAAAGTTGGTGTGAGGGGCCTCCTGAATTTAGAAGCACTTGATTCTGTTTTA

TCTACTTTCTTGAAATGTTACTTCTACCCTTCCCAATGGGTAAAATCATGGGA
GCATGGTGCCCTCATAGATAAATAGAAGAGAGTCTATTGCTGCCAATATAGAT
GGTTATGCTTTCTCATAGCTCTGAAAATATGACACATTTATTATGAGGTTGAT
CTTAGGATAAGGATAGGTGTTTTATGTCAGGAGAGGTTATCATGGTGAATATG
GACCAGCAGACAGCAGTGGAGGAAAATAATGAACCAAGGGATTGAGTTCATTA
GTGCTAATTCTACTCCACTCCTGTCTTTATGCTCCTAAACTTACTGACTGAGC
TCTGAATTAGGTGCTAGGAGGAGACAATGCAGACATGAAAGGGGAAGGAGCGC
CTTCAGGACACAGGCTCTCTGCTGAGAGAAGTCCTATTTGCAGGTGTGATAGA
GGTTGGGACAATCTCTGAGTTGTAAATTTCTAATTGTCTTCAGGCCATATTTA
TAGTTAAATTCATTTCCGAAAGACATAGCATCTTCCCCAATGGGTCAGTTTGT
CAAAATCAATAAAATATTTTGTTTTGCTAAGAATTAAAAAAAAAAAAAAAAA A
(SEQ ID NO: 187) >NP_599009.2 hepatitis A virus cellular receptor 1 homolog isoform a precursor [*Mus musculus*]
MNQIQVFISGLILLLPGAVDSYVEVKGVVGHPVTLPCTYSTYRGITTTCWGRG
QCPSSACQNTLIWTNGHRVTYQKSSRYNLKGHISEGDVSLTIENSVESDSGLY
CCRVEIPGWFNDQKVTFSLQVKPEIPTRPPRRPTTTRPTATGRPTTISTRSTH
VPTSTRVSTSTPPTSTHTWTHKPEPTTFCPHETTAEVTGIPSHTPTDWNGTVT
SSGDTWSNHTEAIPPGKPQKNPTKGFYVGICIAALLLLLLVSTVAITRYILMK
RKSASLSVVAFRVSKIEALQNAAVVHSRAEDNIYIVEDRP (SEQ ID NO: 188) Human PD-1 >NM_005018.3 *Homo sapiens* programmed cell death 1 (PDCD1), mRNA
GCTCACCTCCGCCTGAGCAGTGGAGAAGGCGGCACTCTGGTGGGGCTGCTCCA
GGCATGCAGATCCCACAGGCGCCCTGGCCAGTCGTCTGGGCGGTGCTACAACT
GGGCTGGCGGCCAGGATGGTTCTTAGACTCCCCAGACAGGCCCTGGAACCCCC
CCACCTTCTCCCCAGCCCTGCTCGTGGTGACCGAAGGGGACAACGCCACCTTC
ACCTGCAGCTTCTCCAACACATCGGAGAGCTTCGTGCTAAACTGGTACCGCAT
GAGCCCCAGCAACCAGACGGACAAGCTGGCCGCCTTCCCCGAGGACCGCAGCC
AGCCCGGCCAGGACTGCCGCTTCCGTGTCACACAACTGCCCAACGGGCGTGAC
TTCCACATGAGCGTGGTCAGGGCCCGGCGCAATGACAGCGGCACCTACCTCTG
TGGGGCCATCTCCCTGGCCCCCAAGGCGCAGATCAAAGAGAGCCTGCGGGCAG
AGCTCAGGGTGACAGAGAGAAGGGCAGAAGTGCCCACAGCCCACCCCAGCCCC
TCACCCAGGCCAGCCGGCCAGTTCCAAACCCTGGTGGTTGGTGTCGTGGGCGG
CCTGCTGGGCAGCCTGGTGCTGCTAGTCTGGGTCCTGGCCGTCATCTGCTCCC
GGGCCGCACGAGGGACAATAGGAGCCAGGCGCACCGGCCAGCCCCTGAAGGAG
GACCCCTCAGCCGTGCCTGTGTTCTCTGTGGACTATGGGGAGCTGGATTTCCA
GTGGCGAGAGAAGACCCCGGAGCCCCCCGTGCCCTGTGTCCCTGAGCAGACGG
AGTATGCCACCATTGTCTTTCCTAGCGGAATGGGCACCTCATCCCCCGCCCGC
AGGGGCTCAGCTGACGGCCCTCGGAGTGCCCAGCCACTGAGGCCTGAGGATGG
ACACTGCTCTTGGCCCCTCTGACCGGCTTCCTTGGCCACCAGTGTTCTGCAGA
CCCTCCACCATGAGCCCGGGTCAGCGCATTTCCTCAGGAGAAGCAGGCAGGGT
GCAGGCCATTGCAGGCCGTCCAGGGGCTGAGCTGCCTGGGGGCGACCGGGGCT
CCAGCCTGCACCTGCACCAGGCACAGCCCCACCACAGGACTCATGTCTCAATG
CCCACAGTGAGCCCAGGCAGCAGGTGTCACCGTCCCCTACAGGGAGGGCCAGA
TGCAGTCACTGCTTCAGGTCCTGCCAGCACAGAGCTGCCTGCGTCCAGCTCCC
TGAATCTCTGCTGCTGCTGCTGCTGCTGCTGCTGCCTGCGGCCCGGGGCT
GAAGGCGCCGTGGCCCTGCCTGACGCCCCGGAGCCTCCTGCCTGAACTTGGGG
GCTGGTTGGAGATGGCCTTGGAGCAGCCAAGGTGCCCCTGGCAGTGGCATCCC
GAAACGCCCTGGACGCAGGGCCCAAGACTGGGCACAGGAGTGGGAGGTACATG
GGGCTGGGGACTCCCCAGGAGTTATCTGCTCCCTGCAGGCCTAGAGAAGTTTC
AGGGAAGGTCAGAAGAGCTCCTGGCTGTGGTGGGCAGGGCAGGAAACCCCTCC
ACCTTTACACATGCCCAGGCAGCACCTCAGGCCCTTTGTGGGGCAGGGAAGCT

GAGGCAGTAAGCGGGCAGGCAGAGCTGGAGGCCTTTCAGGCCCAGCCAGCACT
CTGGCCTCCTGCCGCCGCATTCCACCCCAGCCCCTCACACCACTCGGGAGAGG
GACATCCTACGGTCCCAAGGTCAGGAGGGCAGGGCTGGGGTTGACTCAGGCCC
CTCCCAGCTGTGGCCACCTGGGTGTTGGGAGGGCAGAAGTGCAGGCACCTAGG
GCCCCCCATGTGCCCACCCTGGGAGCTCTCCTTGGAACCCATTCCTGAAATTA
TTTAAAGGGGTTGGCCGGGCTCCCACCAGGGCCTGGGTGGGAAGGTACAGGCG
TTCCCCCGGGGCCTAGTACCCCCGCCGTGGCCTATCCACTCCTCACATCCACA
CACTGCACCCCCACTCCTGGGGCAGGGCCACCAGCATCCAGGCGGCCAGCAGG
CACCTGAGTGGCTGGGACAAGGGATCCCCCTTCCCTGTGGTTCTATTATATTA
TAATTATAATTAAATATGAGAGCATGCTAA (SEQ ID NO: 189) >NP_005009.2 programmed cell death protein 1 precursor [*Homo sapiens*]
MQIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPPTFSPALLVVTEGDNATFT
CSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDF
HMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPS
PRPAGQFQTLVVGVVGGLLGSLVLLVWVLAVICSRAARGTIGARRTGQPLKED
PSAVPVFSVDYGELDFQWREKTPEPPVPCVPEQTEYATIVFPSGMGTSSPARR
GSADGPRSAQPLRPEDGHCSWPL (SEQ ID NO: 190) Mouse PD-1 >NM_008798.3 *Mus musculus* programmed cell death 1 (Pdcd1), mRNA
TGAGCAGCGGGGAGGAGGAAGAGGAGACTGCTACTGAAGGCGACACTGCCAGG
GGCTCTGGGCATGTGGGTCCGGCAGGTACCCTGGTCATTCACTTGGGCTGTGC
TGCAGTTGAGCTGGCAATCAGGGTGGCTTCTAGAGGTCCCCAATGGGCCCTGG
AGGTCCCTCACCTTCTACCCAGCCTGGCTCACAGTGTCAGAGGGAGCAAATGC
CACCTTCACCTGCAGCTTGTCCAACTGGTCGGAGGATCTTATGCTGAACTGGA
ACCGCCTGAGTCCCAGCAACCAGACTGAAAAACAGGCCGCCTTCTGTAATGGT
TTGAGCCAACCCGTCCAGGATGCCCGCTTCCAGATCATACAGCTGCCCAACAG
GCATGACTTCCACATGAACATCCTTGACACACGGCGCAATGACAGTGGCATCT
ACCTCTGTGGGGCCATCTCCCTGCACCCCAAGGCAAAAATCGAGGAGAGCCCT
GGAGCAGAGCTCGTGGTAACAGAGAGAATCCTGGAGACCTCAACAAGATATCC
CAGCCCCTCGCCCAAACCAGAAGGCCGGTTTCAAGGCATGGTCATTGGTATCA
TGAGTGCCCTAGTGGGTATCCCTGTATTGCTGCTGCTGGCCTGGGCCCTAGCT
GTCTTCTGCTCAACAAGTATGTCAGAGGCCAGAGGAGCTGGAAGCAAGGACGA
CACTCTGAAGGAGGAGCCTTCAGCAGCACCTGTCCCTAGTGTGGCCTATGAGG
AGCTGGACTTCCAGGGACGAGAGAAGACACCAGAGCTCCCTACCGCCTGTGTG
CACACAGAATATGCCACCATTGTCTTCACTGAAGGGCTGGGTGCCTCGGCCAT
GGGACGTAGGGGCTCAGCTGATGGCCTGCAGGGTCCTCGGCCTCCAAGACATG
AGGATGGACATTGTTCTTGGCCTCTTTGACCAGATTCTTCAGCCATTAGCATG
CTGCAGACCCTCCACAGAGAGCACCGGTCCGTCCCTCAGTCAAGAGGAGCATG
CAGGCTACAGTTCAGCCAAGGCTCCCAGGGTCTGAGCTAGCTGGAGTGACAGC
CCAGCGCCTGCACCAATTCCAGCACATGCACTGTTGAGTGAGAGCTCACTTCA
GGTTTACCACAAGCTGGGAGCAGCAGGCTTCCCGGTTTCCTATTGTCACAAGG
TGCAGAGCTGGGGCCTAAGCCTATGTCTCCTGAATCCTACTGTTGGGCACTTC
TAGGGACTTGAGACACTATAGCCAATGGCCTCTGTGGGTTCTGTGCCTGGAAA
TGGAGAGATCTGAGTACAGCCTGCTTTGAATGGCCCTGTGAGGCAACCCCAAA
GCAAGGGGGTCCAGGTATACTATGGGCCCAGCACCTAAAGCCACCCTTGGGAG
ATGATACTCAGGTGGGAAATTCGTAGACTGGGGGACTGAACCAATCCCAAGAT
CTGGAAAAGTTTTGATGAAGACTTGAAAAGCTCCTAGCTTCGGGGGTCTGGGA
AGCATGAGCACTTACCAGGCAAAAGCTCCGTGAGCGTATCTGCTGTCCTTCTG
CATGCCCAGGTACCTCAGTTTTTTTCAACAGCAAGGAAACTAGGGCAATAAAG
GGAACCAGCAGAGCTAGAGCCACCCACACATCCAGGGGGGCACTTGACTCTCC
CTACTCCTCCTAGGAACCAAAAGGACAAAGTCCATGTTGACAGCAGGGAAGGA

AAGGGGGATATAACCTTGACGCAAACCAACACTGGGGTGTTAGAATCTCCTCA
TTCACTCTGTCCTGGAGTTGGGTTCTGGCTCTCCTTCACACCTAGGACTCTGA
AATGAGCAAGCACTTCAGACAGTCAGGGTAGCAAGAGTCTAGCTGTCTGGTGG
GCACCCAAAATGACCAGGGCTTAAGTCCCTTTCCTTTGGTTTAAGCCCGTTAT
AATTAAATGGTACCAAAAGCTTTAA (SEQ ID NO: 191) >NP_032824.1 programmed cell death protein 1 precursor [*Mus musculus*]
MWVRQVPWSFTWAVLQLSWQSGWLLEVPNGPWRSLTFYPAWLTVSEGANATFT
CSLSNWSEDLMLNWNRLSPSNQTEKQAAFCNGLSQPVQDARFQIIQLPNRHDF
HMNILDTRRNDSGIYLCGAISLHPKAKIEESPGAELVVTERILETSTRYPSPS
PKPEGRFQGMVIGIMSALVGIPVLLLLAWALAVFCSTSMSEARGAGSKDDTLK
EEPSAAPVPSVAYEELDFQGREKTPELPTACVHTEYATIVFTEGLGASAMGRR
GSADGLQGPRPPRHEDGHCSWPL (SEQ ID NO: 317) mScarlet >KY021423.1 Synthetic construct mScarlet gene, partial cds, mRNA
ATGGTGAGCAAGGGCGAGGCAGTGATCAAGGAGTTCATGCGGTTCAAGGTGCA
CATGGAGGGCTCCATGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGG
GCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGC
CCCCTGCCCTTCTCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAG
GGCCTTCACCAAGCACCCCGCCGACATCCCCGACTACTATAAGCAGTCCTTCC
CCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGCCGTG
ACCGTGACCCAGGACACCTCCCTGGAGGACGGCACCCTGATCTACAAGGTGAA
GCTCCGCGGCACCAACTTCCCTCCTGACGGCCCCGTAATGCAGAAGAAGACAA
TGGGCTGGGAAGCGTCCACCGAGCGGTTGTACCCCGAGGACGGCGTGCTGAAG
GGCGACATTAAGATGGCCCTGCGCCTGAAGGACGGCGGCCGCTACCTGGCGGA
CTTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGATGCCCGGCGCCTACA
ACGTCGACCGCAAGTTGGACATCACCTCCCACAACGAGGACTACACCGTGGTG
GAACAGTACGAACGCTCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCT
GTACAAG (SEQ ID NO: 192) >APD76535.1 mScarlet, partial [synthetic construct] MVSKGEAVIKEFMRFKVHMEGSMNGHEFEIEGEGEGRPYEGTQTAKLKVTKGG
PLPFSWDILSPQFMYGSRAFTKHPADIPDYYKQSFPEGFKWERVMNFEDGGAV
TVTQDTSLEDGTLIYKVKLRGTNFPPDGPVMQKKTMGWEASTERLYPEDGVLK
GDIKMALRLKDGGRYLADFKTTYKAKKPVQMPGAYNVDRKLDITSHNEDYTVV
EQYERSEGRHSTGGMDELYK (SEQ ID NO: 193) Nanoluciferase >JQ513379. 1 NanoLuc reporter vector pNL1.1.CMV[Nluc/CMV], complete sequence, mRNA
GGCCTAACTGGCCTCAATATTGGCCATTAGCCATATTATTCATTGGTTATATA
GCATAAATCAATATTGGCTATTGGCCATTGCATACGTTGTATCTATATCATAA
TATGTACATTTATATTGGCTCATGTCCAATATGACCGCCATGTTGGCATTGAT
TATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCA
TATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACC
GCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA
CGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACT
GCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTCCGCCCCCTATTGA
CGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTAC
GGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATG
GTGATGCGGTTTTGGCAGTACACCAATGGGCGTGGATAGCGGTTTGACTCACG
GGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACC
AAAATCAACGGGACTTTCCAAAATGTCGTAATAACCCCGCCCCGTTGACGCAA
ATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGT
GAACCGTCAGATCACTAGAAGCTTTATTGCGGTAGTTTATCACAGTTAAATTG
CTAACGCAGTCAGTGGGCCTCGGCGGCCAAGCTTGGCAATCCGGTACTGTTGG
TAAAGCCACC**ATGGTCTTCACACTCGAAGATTTCGTTGGGGACTGGCGACAGA**

CAGCCGGCTACAACCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTG
TTTCAGAATCTCGGGGTGTCCGTAACTCCGATCCAAAGGATTGTCCTGAGCGG
TGAAAATGGGCTGAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGA
GCGGCGACCAAATGGGCCAGATCGAAAAAATTTTTAAGGTGGTGTACCCTGTG
GATGATCATCACTTTAAGGTGATCCTGCACTATGGCACACTGGTAATCGACGG
GGTTACGCCGAACATGATCGACTATTTCGGACGGCCGTATGAAGGCATCGCCG
TGTTCGACGGCAAAAAGATCACTGTAACAGGGACCCTGTGGAACGGCAACAAA
ATTATCGACGAGCGCCTGATCAACCCCGACGGCTCCCTGCTGTTCCGAGTAAC
CATCAACGGAGTGACCGGCTGGCGGCTGTGCGAACGCATTCTGGCGTAATTCT
AGAGTCGGGGCGGCCGGCCGCTTCGAGCAGACATGATAAGATACATTGATGAG
TTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAAT
TTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTA
ACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAG
GTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTAAAATCGATAAGGATCC
GTCGACCGATGCCCTTGAGAGCCTTCAACCCAGTCAGCTCCTTCCGGTGGGCG
CGGGGCATGACTATCGTCGCCGCACTTATGACTGTCTTCTTTATCATGCAACT
CGTAGGACAGGTGCCGGCAGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTG
CGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAAT
ACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAG
GCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCAT
AGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTG
GCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCC
TCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTT
CTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAG
TTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTC
AGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTA
AGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGC
GAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCT
ACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTC
GGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGG
TGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAG
AAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCA
CGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCT
TTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTT
GGTCTGACAGCGGCCGCAAATGCTAAACCACTGCAGTGGTTACCAGTGCTTGA
TCAGTGAGGCACCGATCTCAGCGATCTGCCTATTTCGTTCGTCCATAGTGGCC
TGACTCCCCGTCGTGTAGATCACTACGATTCGTGAGGGCTTACCATCAGGCCC
CAGCGCAGCAATGATGCCGCGAGAGCCGCGTTCACCGGCCCCCGATTTGTCAG
CAATGAACCAGCCAGCAGGGAGGGCCGAGCGAAGAAGTGGTCCTGCTACTTTG
TCCGCCTCCATCCAGTCTATGAGCTGCTGTCGTGATGCTAGAGTAAGAAGTTC
GCCAGTGAGTAGTTTCCGAAGAGTTGTGGCCATTGCTACTGGCATCGTGGTAT
CACGCTCGTCGTTCGGTATGGCTTCGTTCAACTCTGGTTCCCAGCGGTCAAGC
CGGGTCACATGATCACCCATATTATGAAGAAATGCAGTCAGCTCCTTAGGGCC
TCCGATCGTTGTCAGAAGTAAGTTGGCCGCGGTGTTGTCGCTCATGGTAATGG
CAGCACTACACAATTCTCTTACCGTCATGCCATCCGTAAGATGCTTTTCCGTG
ACCGGCGAGTACTCAACCAAGTCGTTTTGTGAGTAGTGTATACGGCGACCAAG
CTGCTCTTGCCCGGCGTCTATACGGGACAACACCGCGCCACATAGCAGTACTT
TGAAAGTGCTCATCATCGGGAATCGTTCTTCGGGGCGGAAAGACTCAAGGATC
TTGCCGCTATTGAGATCCAGTTCGATATAGCCCACTCTTGCACCCAGTTGATC
TTCAGCATCTTTTACTTTCACCAGCGTTTCGGGGTGTGCAAAAACAGGCAAGC

AAAATGCCGCAAAGAAGGGAATGAGTGCGACACGAAAATGTTGGATGCTCATA
CTCGTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTACTAGTACGTCTC
TCAAGGATAAGTAAGTAATATTAAGGTACGGGAGGTATTGGACAGGCCGCAAT
AAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGAT
AGTACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACAAACTAGCAA
AATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCT (SEQ ID NO: 194) >AFJ15599.1 NanoLuc luciferase [NanoLuc reporter vector pNL1.1.CMV[Nluc/CMV]]
MVFTLEDFVGDWRQTAGYNLDQVLEQGGVSSLFQNLGVSVTPIQRIVLSGENG
LKIDIHVIIPYEGLSGDQMGQIEKIFKVVYPVDDHHFKVILHYGTLVIDGVTP
NMIDYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLINPDGSLLFRVTING
VTGWRLCERILA (SEQ ID NO: 195)

(65) The polypeptides provided in Table 1 above are involved in a range of biological processes, including but not limited to, suppressing the adaptive arm of the immune system (e.g., PD-L1); cellular adhesion (e.g., nectin), immune activation (e.g., HVEM), and the like. The POI domains can also be used to track, purify, or identify the engineered EVs from native EVs (e.g., mScarlet and nanoluciferase). The genes, transcripts, polypeptides, variants, and fragments thereof can be used in any combination from Table 1 to be expressed by an engineered EV provided herein. In some embodiments, the POI domain is the human polypeptide. In some embodiments, the POI domain is a homologue of the human polypeptide (e.g., mouse).

(66) In some embodiments of any of the aspects, the engineered cell or EV provided herein comprises an exogenous nucleic acid encoding one or more exogenous polypeptide(s) selected from the group consisting of: the polypeptides listed in Table 1.

(67) In some embodiments of any of the aspects, the POI domain is PD-L1 or a fragment thereof. In some embodiments of any of the aspects, the POI domain is PD-L2 or a fragment thereof. In some embodiments of any of the aspects, the POI domain is FGL1 or a fragment thereof. In some embodiments of any of the aspects, the POI domain is 4-1BBL or a fragment thereof. In some embodiments of any of the aspects, the POI domain is CTLA or a fragment thereof.

(68) In some embodiments of any of the aspects, the POI domain substantially binds to one or more of a target polypeptide. In some embodiments of any of the aspects, the target polypeptide is a cellular receptor. In some embodiments of any of the aspects, the target polypeptide is an immunosuppressive polypeptide. In some embodiments of any of the aspects, the target polypeptide is an immunostimulatory polypeptide. The engineered exosomes provided herein can be designed to activate, block, or modulate a given target polypeptide with the appropriate POI domain that binds to or modulates the function or expression of the target polypeptide. Non-limiting examples of target polypeptides include those listed in Table 2 (below).

(69) TABLE-US-00002 TABLE 2 Exemplary Target Polypeptides PD-1 VISTA LAG-3 CD44 CD80 BTLA CD112 IL10RA CD86 CD160 CD200R IL10RB CD28 HVEM CD200 Tim-3 ICOS CD2 Galectin 9 TNFRSF25 CD28H SLAM CD150 TIM-3 TNFRSF6B PD-Ll CD58 CD226 CD113 CTLA-4 TIM-1 CD155 CD27 4-1BB (CD137) TIM-4 CD112 CD30 GITR CD40 DR3 LFA-3 (CD58) CD27L CD30L GITRL CD40L CD48 CD244 DcR3 CD28H LFA-3 (CD58) CD98 TNF Receptor TNF receptor Superfamily associated factor members (TRAF) family members Butyrophilin family PD-L2 Nectin TIM family members members B7/CD28 family SLAM family Nectin-like Collagen members members binding receptors family proteins LAIR-1 (CD305)

(70) The EVs provided herein further comprise at least one fusion protein comprising a vesicle targeting domain. In various embodiments, the vesicle targeting domain provided herein is capable of binding or anchoring the fusion polypeptide provided herein to an extracellular vesicle, e.g., via targeting of the phospholipid bilayer membrane. In various embodiments, the vesicle targeting domain is a GPI domain (i.e., GPI linker, GPI anchor), fatty acylation site, or prenylation site. One of skill in the art can appreciate that the aforementioned refer to peptide or protein sites, wherein

covalent lipid attachment supports embedding of the lipid in a cell membrane (i.e., phospholipid bilayer).

(71) Biochemical forces that anchor EV targeting domains to the EV phospholipid bilayer may include, but are not limited to, electrostatic forces, affinity for EVs through protein-protein interactions with natively resident proteins (e.g., CD81, CD63, CD9, ALIX, TSG101. CD98, CD298, MARCKS, PTGFRN, Lactadherin (MFGe8)), association or affinity for negatively or positively curved phospholipids, association or affinity for negatively or positively charged domains of resident membrane associated proteins, etc., or the like.

(72) Additional non-limiting examples of membrane targeting domains that can be used and their properties are further described in detail, e.g., Alberts B, Johnson A, Lewis J, et al., Molecular Biology of the Cell, 4th edition, New York: Garland Science, 2002. Membrane Proteins; Marilyn D.Resh, Fatty acylation of proteins: new insights into membrane targeting of myristoylated and palmitoylated proteins. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. Volume 1451, Issue 1, 12 Aug. 1999, Pages 1-16, doi: 10.1016/S0167-4889 (99) 00075-0; Ann Apolloni, et al., H-ras but Not K-ras Traffics to the Plasma Membrane through the Exocytic Pathway, Molecular and Cellular Biology April 2000, 20 (7) 2475-2487, doi: 10.1128/MCB.20.7.2475-2487.2000; Rosie Dawaliby et. al., Phosphatidylethanolamine Is a Key Regulator of Membrane Fluidity in Eukaryotic Cells, Membrane Biology, VOLUME 291, ISSUE 7, doi: 10.1074/jbc.M115.706523; R. J. Deschenes, Protein Palmitoylation, Encyclopedia of Biological Chemistry (Second Edition), Academic Press, 2013, Pages 645-647, ISBN 9780123786319, doi: 10.1016/B978-O-12-378630-2.00022-0; Charuta C. Palsuledesai and Mark D. Distefano, Protein Prenylation: Enzymes, Therapeutics, and Biotechnology Applications, ACS Chemical Biology 2015 10 (1), 51-62, doi: 10.1021/cb500791f; Hung M E, Leonard J N. Stabilization of exosome-targeting peptides via engineered glycosylation, J Biol Chem, 2015 Mar. 27; 290 (13): 8166-72, doi: 10.1074/jbc.M114.621383; Udenwobele Daniel Ikenna, et. al., Myristoylation: An Important Protein Modification in the Immune Response, Frontiers in Immunology, Vol: 8, 2017, doi: 10.3389/fimmu.2017.00751; Kinoshita Taroh 2020Biosynthesis and biology of mammalian GPI-anchored proteins Open Biol. 10190290, doi: 10.1098/rsob.190290, the contents of which are incorporated herein by reference in their entireties.

(73) In some embodiments, the fusion polypeptide comprises one or more, two or more, three or more, four or more, five or more, or six or more vesicle targeting domains on the same polypeptide or nucleic acid construct encoding said polypeptide. For example, the fusion polypeptides provided herein can comprise PD-L1 and Glycosylphosphatidylinositol (GPI).

(74) In some embodiments, the vesicle targeting domain is a prenylated protein. Prenylated proteins are proteins that have at least one prenylation site. Prenylation occurs when a 15-carbon or 20-carbon, farnesyl or geranylgeranyl isoprenoid, respectively, is covalently bound via a thioether bond to a cysteine at or near the carboxy terminus of a protein. In general, a prenylation site comprises an amino acid sequence CAAX, wherein C represents cysteine, A represents an aliphatic amino acid (glycine, alanine, valine, leucine, or isoleucine), and X represents alanine, methionine, serine, leucine, or glutamine.

(75) In some embodiments, the vesicle targeting domain is a fatty acylated protein. Fatty acylated proteins are proteins that have been modified post-translationally by covalent attachment of one or more fatty acids, generally with a saturated fatty acid that comprises 14-carbon (e.g., myristic acid) via myristoylation or 16-carbons (e.g., palmitic acid) via palmitoylation. For example, proteins destined to become myristoylated begin with the amino acids Met-Gly-X-X-X followed by a serine or threonine at position 6 and lysine or arginine at position 7 and/or 8 wherein X can be any amino acid. The methionine is removed and a myristate is linked to the glycine via an amide bond. Palmitoylation herein means a posttranslational covalent attachment of fatty acids (e.g., palmitic acid) to cysteine (S-palmitoylation), serine and/or threonine (O-palmitoylation), and to the amino group of lysine (N-palmitoylation) of proteins.

(76) Palmitoylated proteins may be acylated by attachment of a thioester linkage to a sulfhydryl group of cysteine, or via a palmitate linked to the amino group of an N-terminal cysteine. Palmitoylation sites may be present near the N- or C-terminus of a protein.
(77) In some embodiments, the vesicle targeting domain is a glycosylphosphatidylinositol (GPI) anchor. A glycosylphosphatidylinositol (GPI) anchor (“GPI anchor”) or “GPI sticky binder” are used interchangeably and refer to a means of stably anchoring a protein to an outer leaflet (e.g., exterior layer of a phospholipid bilayer) of a cell membrane. A GPI anchor comprises a glycan, a phosphoethanolamine linker, a phospholipid tail, and may be modified by various glycan sidechains. The glycan core comprises phosphoinositol, glucosamine, and mannose residues wherein said mannose residues may be modified for example with phosphoethanolamine or carbohydrates. The phosphoethanolamine is amide-bonded to the carboxyl terminus of a protein during the process of GPI attachment. In some embodiments, the vesicle targeting domain may have affinity to EV resident proteins, e.g., CD81, CD63, CD9, ALIX, TSG101, CD98, CD298, MARCKS, PTGFRN, Lactadherin (MFGe8).
(78) Sticky binders can include a sequence for one or more myristoylation and/or palmitoylation (Myr/Palm) sites fused to a transmembrane domain from 4F2 (CD98). For example, the myristoylation sequence from the MARCKS protein may be modified to encode for one or more myristoylation and palmitoylation sites, wherein the modified MARCKS protein sequence is fused to a protein sequence of the transmembrane domain from 4F2 via a covalent peptide bond. A Myr/Palm followed by the 4F2 transmembrane domain can improve loading of the fusion proteins provided herein when compared with 4F2 transmembrane domain alone or Myr/Palm alone.
(79) Non-limiting examples of vesicle targeting domains that enhance fusion polypeptide structure and function on the extracellular vesicles are provided in Table 3 (below).
(80) TABLE-US-00003 TABLE 3 Exosome Targeting Domain Exosome Targeting Domain/Sticky Nucleic Acid Sequence (SEQ ID NO:) Binder Amino Acid Sequence (SEQ ID NO:) Human CD55 >NM_000574.5 *Homo sapiens* CD55 molecule (Cromer blood (DAF) group) (CD55), transcript variant 1, mRNA Glycosylphosphatidylinositol
CTGCTTACTGCAACTCGCTCCGGCCGCTGGGCGTAGCTGCGACTCGGCGGAGTCCCG
(GPI)
GCGGCGCGTCCTTGTTCTAACCCGGCGCGCCATGACCGTCGCGCGGCCGAGCGTGCC
CGCGGCGCTGCCCCTCCTCGGGGAGCTGCCCCGGCTGCTGCTGCTGGTGCTGTTGTG
CCTGCCGGCCGTGTGGGGTGACTGTGGCCTTCCCCCAGATGTACCTAATGCCCAGCC
AGCTTTGGAAGGCCGTACAAGTTTTCCCGAGGATACTGTAATAACGTACAAATGTGA
AGAAAGCTTTGTGAAAATTCCTGGCGAGAAGGACTCAGTGATCTGCCTTAAGGGCAG
TCAATGGTCAGATATTGAAGAGTTCTGCAATCGTAGCTGCGAGGTGCCAACAAGGCT
AAATTCTGCATCCCTCAAACAGCCTTATATCACTCAGAATTATTTTCCAGTCGGTAC
TGTTGTGGAATATGAGTGCCGTCCAGGTTACAGAAGAGAACCTTCTCTATCACCAAA
ACTAACTTGCCTTCAGAATTTAAAATGGTCCACAGCAGTCGAATTTTGTAAAAAGAA
ATCATGCCCTAATCCGGGAGAAATACGAAATGGTCAGATTGATGTACCAGGTGGCAT
ATTATTTGGTGCAACCATCTCCTTCTCATGTAACACAGGGTACAAATTATTTGGCTC
GACTTCTAGTTTTTGTCTTATTTCAGGCAGCTCTGTCCAGTGGAGTGACCCGTTGCC
AGAGTGCAGAGAAATTTATTGTCCAGCACCACCACAAATTGACAATGGAATAATTCA
AGGGGAACGTGACCATTATGGATATAGACAGTCTGTAACGTATGCATGTAATAAAGG
ATTCACCATGATTGGAGAGCACTCTATTTATTGTACTGTGAATAATGATGAAGGAGA
GTGGAGTGGCCCACCACCTGAATGCAGAGGAAAATCTCTAACTTCCAAGGTCCCACC
AACAGTTCAGAAACCTACCACAGTAAATGTTCCAACTACAGAAGTCTCACCAACTTC
TCAGAAAACCACCACAAAAACCACCACACCAAATGCTCAAGCAACACGGAGTACACC
TGTTTCCAGGACAACCAAGCATTTTCATGAAACAACC**CCAAATAAAGGAAGTGGAAC**
CACTTCAGGTACTACCCGTCTTCTATCTGGGCAGACGTGTTTCACGTTGACAGGTTT

GCTTGGGACGCTAGTAACCATGGGCTTGCTGACTTAGCCAAAGAAGAGTTAAGAAGA
AAATACACACAAGTATACAGACTGTTCCTAGTTTCTTAGACTTATCTGCATATTGGA
TAAAATAAATGCAATTGTGCTCTTCATTTAGGATGCTTTCATTGTCTTTAAGATGTG
TTAGGAATGTCAACAGAGCAAGGAGAAAAAAGGCAGTCCTGGAATCACATTCTTAGC
ACACCTACACCTCTTGAAAATAGAACAACTTGCAGAATTGAGAGTGATTCCTTTCCT
AAAAGTGTAAGAAAGCATAGAGATTTGTTCGTATTTAGAATGGGATCACGAGGAAAA
GAGAAGGAAAGTGATTTTTTTCCACAAGATCTGTAATGTTATTTCCACTTATAAAGG
AAATAAAAAATGAAAAACATTATTTGGATATCAAAAGCAAATAAAAACCCAATTCAG
TCTCTTCTAAGCAAAATTGCTAAAGAGAGATGAACCACATTATAAAGTAATCTTTGG
CTGTAAGGCATTTTCATCTTTCCTTCGGGTTGGCAAAATATTTTAAAGGTAAAACAT
GCTGGTGAACCAGGGGTGTTGATGGTGATAAGGGAGGAATATAGAATGAAAGACTGA
ATCTTCCTTTGTTGCACAAATAGAGTTTGGAAAAAGCCTGTGAAAGGTGTCTTCTTT
GACTTAATGTCTTTAAAAGTATCCAGAGATACTACAATATTAACATAAGAAAAGATT
ATATATTATTTCTGAATCGAGATGTCCATAGTCAAATTTGTAAATCTTATTCTTTTG
TAATATTTATTTATATTTATTTATGACAGTGAACATTCTGATTTTACATGTAAAACA
AGAAAAGTTGAAGAAGATATGTGAAGAAAAATGTATTTTTCCTAAATAGAAATAAAT
GATCCCATTTTTTGGTATCATGTAGTATGTGAAATTTATTCTTAAACGTGACTACTT
TATTTCTAAATAAGAAATTCCCTACCTGCTTCCTACAAGCAGTTCAGAATGCCATGC
CTTGGTTGTCCTAGTGTGAATAATTTTCAGCTACTTTAAAATTATATTGTACTTTCT
CAAGCATGTCATATCCTTTCCTATTAGAGTATCTATATTACTTGTTACTGATTTACC
TGAAGGCAATCTGATTAATTTCTAGGTTTTTACCATATTCTTGTCATCTTGCCAATT
ACATTTTAAGTGTTAGACTAGACTAAGATGTACTAGTTGTATAGAATATAACTAGA
TTTATTATGGCAATGTTTATTTTGTCATTTTGCTTCATCTGTTTTGTTGTTGAAGTA
CTTTAAATTTCATACGTTCATGGCATTTCACTGTAAAGACTTTAATGTGTATTTCTT
AAAATAAAACTTTTTTTCCTCCTTAA (SEQ ID NO: 196) >NP_000565.1
complement decay-accelerating factor isoform 1 preproprotein [*Homo sapiens*]
MTVARPSVPAALPLLGELPRLLLLVLLCLPAVWGDCGLPPDVPNAQPALEGRTSFPE
DTVITYKCEESFVKIPGEKDSVICLKGSQWSDIEEFCNRSCEVPTRLNSASLKQPYI
TQNYFPVGTVVEYECRPGYRREPSLSPKLTCLQNLKWSTAVEFCKKKSCPNPGEIRN
GQIDVPGGILFGATISFSCNTGYKLFGSTSSFCLISGSSVQWSDPLPECREIYCPAP
PQIDNGIIQGERDHYGYRQSVTYACNKGFTMIGEHSIYCTVNNDEGEWSGPPPECRG
KSLTSKVPPTVQKPTTVNVPTTEVSPTSQKTTTKTTTPNAQATRSTPVSRTTKHFHE
TT**PNKGSGTTSGTTRLLSGHTCFTLTGLLGTLVTMGLLT** (SEQ ID NO: 197)
Human CD59 >NM_203330.2 *Homo sapiens* CD59 molecule (CD59 blood Glycosylphosphatidylinositol group) (CD59), transcript variant 1, mRNA (GPI)
GGGGCCGGGGGGCGGAGCCTTGCGGGCTGGAGCGAAAGAATGCGGGGGCTGA
GCGCAGAAGCGGCTCGAGGCTGGAAGAGGATCTTGGGCGCCGCCAGTCTCTC
TCTGTTGCCCAAGCTGGAGTGCAGTGGCACAGTCTTGGCTCACTGCAACCTC
CACCTCCTGGGTGCAAGCGATTCTCGTGTCTCAGCCTCTCAAGTAGCTGGGA
TTACAGTCTTTAGCACCAGTTGGTGTAGGAGTTGAGACCTACTTCACAGTAG
TTCTGTGGACAATCACAATGGGAATCCAAGGAGGGTCTGTCCTGTTCGGGCT
GCTGCTCGTCCTGGCTGTCTTCTGCCATTCAGGTCATAGCCTGCAGTGCTAC
AACTGTCCTAACCCAACTGCTGACTGCAAAACAGCCGTCAATTGTTCATCTG
ATTTTGATGCGTGTCTCATTACCAAAGCTGGGTTACAAGTGTATAACAAGTG
TTGGAAGTTTGAGCATTGCAATTTCAACGACGTCACAACCCGCTTGAGGGAA
AATGAGCTAACGTACTACTGCTGCAAGAAG**GACCTGTGTAACTTTAACGAAC**
AGCTTGAAAATGGTGGGACATCCTTATCAGAGAAAACAGTTCTTCTGCTGGT
GACTCCATTTCTGGCAGCAGCCTGGAGCCTTCATCCCTAAGTCAACACCAGG
AGAGCTTCTCCCAAACTCCCCGTTCCTGCGTAGTCCGCTTTCTCTTGCTGCC
ACATTCTAAAGGCTTGATATTTTCCAAATGGATCCTGTTGGGAAAGAATAAA

ATTAGCTTGAGCAACCTGGCTAAGATAGAGGGGCTCTGGGAGACTTTGAAGA
CCAGTCCTGTTTGCAGGGAAGCCCCACTTGAAGGAAGAAGTCTAAGAGTGAA
GTAGGTGTGACTTGAACTAGATTGCATGCTTCCTCCTTTGCTCTTGGGAAGA
CCAGCTTTGCAGTGACAGCTTGAGTGGGTTCTCTGCAGCCCTCAGATTATTT
TTCCTCTGGCTCCTTGGATGTAGTCAGTTAGCATCATTAGTACATCTTTGGA
GGGTGGGGCAGGAGTATATGAGCATCCTCTCTCACATGGAACGCTTTCATAA
ACTTCAGGGATCCCGTGTTGCCATGGAGGCATGCCAAATGTTCCATATGTGG
GTGTCAGTCAGGGACAACAAGATCCTTAATGCAGAGCTAGAGGACTTCTGGC
AGGGAAGTGGGGAAGTGTTCCAGATAGCAGGGCATGAAAACTTAGAGAGGTA
CAAGTGGCTGAAAATCGAGTTTTTCCTCTGTCTTTAAATTTTATATGGGCTT
TGTTATCTTCCACTGGAAAAGTGTAATAGCATACATCAATGGTGTGTTAAAG
CTATTTCCTTGCCTTTTTTTTATTGGAATGGTAGGATATCTTGGCTTTGCCA
CACACAGTTACAGAGTGAACACTCTACTACATGTGACTGGCAGTATTAAGTG
TGCTTATTTTAAATGTTACTGGTAGAAAGGCAGTTCAGGTATGTGTGTATAT
AGTATGAATGCAGTGGGGACACCCTTTGTGGTTACAGTTTGAGACTTCCAAA
GGTCATCCTTAATAACAACAGATCTGCAGGGGTATGTTTTACCATCTGCATC
CAGCCTCCTGCTAACTCCTAGCTGACTCAGCATAGATTGTATAAAATACCTT
TGTAACGGCTCTTAGCACACTCACAGATGTTTGAGGCTTTCAGAAGCTCTTC
TAAAAAATGATACACACCTTTCACAAGGGCAAACTTTTTCCTTTTCCCTGTG
TATTCTAGTGAATGAATCTCAAGATTCAGTAGACCTAATGACATTTGTATTT
TATGATCTTGGCTGTATTTAATGGCATAGGCTGACTTTTGCAGATGGAGGAA
TTTCTTGATTAATGTTGAAAAAAAACCCTTGATTATACTCTGTTGGACAAAC
CGAGTGCAATGAATGATGCTTTTCTGAAAATGAAATATAACAAGTGGGTGAA
TGTGGTTATGGCCGAAAAGGATATGCAGTATGCTTAATGGTAGCAACTGAAA
GAAGACATCCTGAGCAGTGCCAGCTTTCTTCTGTTGATGCCGTTCCCTGAAC
ATAGGAAAATAGAAACTTGCTTATCAAAACTTAGCATTACCTTGGTGCTCTG
TGTTCTCTGTTAGCTCAGTGTCTTTCCTTACATCAATAGGTTTTTTTTTTTT
TTTTTGGCCTGAGGAAGTACTGACCATGCCCACAGCCACCGGCTGAGCAAAG
AAGCTCATTTCATGTGAGTTCTAAGGAATGAGAAACAATTTTGATGAATTTA
AGCAGAAAATGAATTTCTGGGAACTTTTTTGGGGGCGGGGGGGTGGGGAATT
CAGCCACACTCCAGAAAGCCAGGAGTCGACAGTTTTGGAAGCCTCTCTCAGG
ATTGAGATTCTAGGATGAGATTGGCTTACTGCTATCTTGTGTCATGTACCCA
CTTTTTGGCCAGACTACACTGGGAAGAAGGTAGTCCTCTAAAGCAAAATCTG
AGTGCCACTAAATGGGGAGATGGGGCTGTTAAGCTGTCCAAATCAACAAGGG
TCATATAAATGGCCTTAAACTTTGGGGTTGCTTTCTGCAAAAAGTTGCTGTG
ACTCATGCCATAGACAAGGTTGAGTGCCTGGACCCAAAGGCAATACTGTAAT
GTAAAGACATTTATAGTACTAGGCAAACAGCACCCCAGGTACTCCAGGCCCT
CCTGGCTGGAGAGGGCTGTGGCAATAGAAAATTAGTGCCAACTGCAGTGAGT
CAGCCTAGGTTAAATAGAGAGTGTAAGAGTGCTGGACAGGAACCTCCACCCT
CATGTCACATTTCTTCAATGTGACCCTTCTGGCCCCTCTCCTCCTGACAGCG
GAACAATGACTGCCCCGATAGGTGAGGCTGGAGGAAGAATCAGTCCTGTCCT
TGGCAAGCTCTTCACTATGACAGTAAAGGCTCTCTGCCTGCTGCCAAGGCCT
GTGACTTTCTAACCTGGCCTCACGCTGGGTAAGCTTAAGGTAGAGGTGCAGG
ATTAGCAAGCCCACCTGGCTACCAGGCCGACAGCTACATCCTCCAACTGACC
CTGATCAACGAAGAGGGATTCATGTGTCTGTCTCAGTTGGTTCCAAATGAAA
CCAGGGAGCAGGGGAGTTAGGAATCGAACACCAGTCATGCCTACTGGCTCTC
TGCTCGAGAGCCAATACCCTGTGCCCTCCACTCATCTGGATTTACAGGAACT
GTCATAGTGTTCAGTATTGGGTGGTGATAAGCCCATTGGATTGTCCCCTTGG
GGGGATGAGCTAGGGGTGCAAGGAACACCTGATGAGTAGATAAGTGGAGCTC
ATGGTATTTCCTGAAAGATGCTAATCTATTTGCCAAACTTGGTCTTGAATGT

ACTGGGGGCTTCAAGGTATGGGTATATTTTTCTTGTGTCCTTGCAGTTAGCC
CCCATGTCTTATGTGTGTCCTGAAAAAATAAGAGCCTGCCCAAGACTTTGGG
CCTCTTGACAGAATTAACCACTTTTATACATCTGAGTTCTCTTGGTAAGTTC
TTTAGCAGTGTTCAAAGTCTACTAGCTCGCATTAGTTTCTGTTGCTGCCAAC
AGATCTGAACTAATGCTAACAGATCCCCCTGAGGGATTCTTGATGGGCTGAG
CAGCTGGCTGGAGCTAGTACTGACTGACATTCATTGTGATGAGGGCAGCTTT
CTGGTACAGGATTCTAAGCTCTATGTTTTATATACATTTTCATCTGTACTTG
CACCTCACTTTACACAAGAGGAAACTATGCAAAGTTAGCTGGATCGCTCAAG
GTCACTTAGGTAAGTTGGCAAGTCCATGCTTCCCACTCAGCTCCTCAGGTCA
GCAAGTCTACTTCTCTGCCTATTTTGTATACTCTCTTTAATATGTGCCTAGC
TTTGGAAAGTCTAGAATGGGTCCCTGGTGCCTTTTTACTTTGAAGAAATCAG
TTTCTGCCTCTTTTTGGAAAAGAAAACAAAGTGCAATTGTTTTTTACTGGAA
AGTTACCCAATAGCATGAGGTGAACAGGACGTAGTTAGGCCTTCCTGTAAAC
AGAAAATCATATCAAAACACTATCTTCCCATCTGTTTCTCAATGCCTGCTAC
TTCTTGTAGATATTTCATTTCAGGAGAGCAGCAGTTAAACCCGTGGATTTTG
TAGTTAGGAACCTGGGTTCAAACCCTCTTCCACTAATTGGCTATGTCTCTGG
ACAAGTTTTTTTTTTTTTTTTTTTTTAAACCCTTTCTGAACTTTCACTTTCT
ATGTCTACCTCAAAGAATTGTTGTGAGGCTTGAGATAATGCATTTGTAAAGG
GTCTGCCAGATAGGAAGATGCTAGTTATGGATTTACAAGGTTGTTAAGGCTG
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(SEQ ID NO: 199) >NP_005919.2 lactadherin isoform a preproprotein [*Homo sapiens*]

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>NP_002385.3 4F2 cell-surface antigen heavy chain isoform c [*Homo sapiens*]
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transferrin receptor protein 2 isoform 1 [*Homo sapiens*]
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IQQPQRQRPRESYQMGHMRR (SEQ ID NO: 206) Transmembrane >NM_001769.4 *Homo sapiens* CD9 molecule (CD9), domain 2 or transcript variant 1, mRNA transmembrane
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3 from GCCCCCCAGTCCCGCACCCGTTCGGCCCAGGCTAAGTTAGCCCTCACCATGC Human CD9
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CCAGC**TTCTACACAGGAGTCTATATTCTGATCGGAGCCGGCGCCCTCATGAT**
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GGAATGCAGTGGCGCCATCTCAGCTCACTGCAACCTCCATCTCCCAGGTTCA
AGCGATTCTCGTGCCTCGGCCTCCTGAGTAGCTGGGATTACAGGCGTGTGCC
ACTACACTCAACTAATTTTTGTATTTTTAGGAGAGACGGGGTTTCACCCTGT
TGGCCAGGCTGGTCTCGAACTCCTGACCTCAAGTGATTCACCCACCTTGGCC
TCATAAACCTGTTTTGCAGAACTCATTTATTCAGCAAATATTTATTGAGTGC
CTACCAGATGCCAGTCACCACACAAGGCACTGGGTATATGGTATCCCCAAAC
AAGAGACATAATCCCGGTCCTTAGGTAGTGCTAGTGTGGTCTGTAATATCTT
ACTAAGGCCTTTGGTATACGACCCAGAGATAACACGATGCGTATTTTAGTTT
TGCAAAGAAGGGGTTTGGTCTCTGTGCCAGCTCTATAATTGTTTTGCTACGA
TTCCACTGAAACTCTTCGATCAAGCTACTTTATGTAAATCACTTCATTGTTT
TAAAGGAATAAACTTGATTATATTGTTTTTTTATTTGGCATAACTGTGATTC

TTTTAGGACAATTACTGTACACATTAAGGTGTATGTCAGATATTCATATTGA
CCCAAATGTGTAATATTCCAGTTTTCTCTGCATAAGTAATTAAAATATACTT
AAAAATTAATAGTTTTATCTGGGTACAAATAAACAGGTGCCTGAACTAGTTC
ACAGACAAGGAAACTTCTATGTAAAAATCACTATGATTTCTGAATTGCTATG
TGAAACTACAGATCTTTGGAACACTGTTTAGGTAGGGTGTTAAGACTTACAC
AGTACCTCGTTTCTACACAGAGAAAGAAATGGCCATACTTCAGGAACTGCAG
TGCTTATGAGGGGATATTTAGGCCTCTTGAATTTTTGATGTAGATGGGCATT
TTTTTAAGGTAGTGGTTAATTACCTTTATGTGAACTTTGAATGGTTTAACAA
AAGATTTGTTTTTGTAGAGATTTTAAAGGGGGAGAATTCTAGAAATAAATGT
TACCTAATTATTACAGCCTTAAAGACAAAAATCCTTGTTGAAGTTTTTTTAA
AAAAAGCTAAATTACATAGACTTAGGCATTAACATGTTTGTGGAAGAATATA
GCAGACGTATATTGTATCATTTGAGTGAATGTTCCCAAGTAGGCATTCTAGG
CTCTATTTAACTGAGTCACACTGCATAGGAATTTAGAACCTAACTTTTATAG
GTTATCAAAACTGTTGTCACCATTGCACAATTTTGTCCTAATATATACATAG
AAACTTTGTGGGGCATGTTAAGTTACAGTTTGCACAAGTTCATCTCATTTGT
ATTCCATTGATTTTTTTTTTCTTCTAAACATTTTTTCTTCAAACAGTATATA
ACTTTTTTTAGGGGATTTTTTTTTAGACAGCAAAAACTATCTGAAGATTTCC
ATTTGTCAAAAAGTAATGATTTCTTGATAATTGTGTAGTAATGTTTTTTAGA
ACCCAGCAGTTACCTTAAAGCTGAATTTATATTTAGTAACTTCTGTGTTAAT
ACTGGATAGCATGAATTCTGCATTGAGAAACTGAATAGCTGTCATAAAATGA
AACTTTCTTTCTAAAGAAAGATACTCACATGAGTTCTTGAAGAATAGTCATA
ACTAGATTAAGATCTGTGTTTTAGTTTAATAGTTTGAAGTGCCTGTTTGGGA
TAATGATAGGTAATTTAGATGAATTTAGGGGAAAAAAAAAGTTATCTGCAGAT
ATGTTGAGGGCCCATCTCTCCCCCCACACCCCCACAGAGCTAACTGGGTTAC
AGTGTTTTATCCGAAAGTTTCCAATTCCACTGTCTTGTGTTTTCATGTTGAA
AATACTTTTGCATTTTTCCTTTGAGTGCCAATTTCTTACTAGTACTATTTCT
TAATGTAACATGTTTACCTGGAATGTATTTTAACTATTTTTGTATAGTGTAA
ACTGAAACATGCACATTTTGTACATTGTGCTTTCTTTTGTGGGACATATGCA
GTGTGATCCAGTTGTTTTCCATCATTTGGTTGCGCTGACCTAGGAATGTTGG
TCATATCAAACATTAAAAATGACCACTCTTTTAATTGAAATTAACTTTTAAA
TGTTTATAGGAGTATGTGCTGTGAAGTGATCTAAAATTTGTAATATTTTTGT
CATGAACTGTACTACTCCTAATTATTGTAATGTAATAAAAATAGTTACAGTG AC (SEQ ID NO: 211) >NP_004976.2 GTPase KRas isoform b [*Homo sapiens*]
MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCL
LDILDTAGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKRVK
DSEDVPMVLVGNKCDLPSRTVDTKQAQDLARSYGIPFIETSAKTRQGVDDAF
YTLVREIRKHKEKMSKDG**KKKKKKSKTKCVIM** (SEQ ID NO: 212) >Lipid affinity tag nucleotide sequence
AAAAAGAAGAAAAAGAAGAAGAAGACAAAGTGTGTAATTATG (SEQ ID NO: 213) >Lipid affinity tag peptide sequence KKKKKKKKTKCVIM (SEQ ID NO: 214) Myr/Palm tag >NM_002356.7 *Homo sapiens* myristoylated alanine rich protein kinase C substrate (MARCKS), mRNA Human MARCKS modified from
GCACTTGGGCGTTGGACCCCGCATCTTATTAGCAACCAGGGAGATTTCTCCA
TTTTCCTCTTGTCTACAGTGCGGCTACAAATCTGGGATTTTTTTATTACTTC
TTTTTTTTTCGAACTACACTTGGGCTCCTTTTTTTGTGCTCGACTTTTCCAC
CCTTTTTCCCTCCCTCCTGTGCTGCTGCTTTTTGATCTCTTCGACTAAAATT
TTTTTATCCGGAGTGTATTTAATCGGTTCTGTTCTGTCCTCTCCACCACCCC
CACCCCCCTCCCTCCGGTGTGTGTGCCGCTGCCGCTGTTGCCGCCGCCGCTG
CTGCTGCTGCTCGCCCCGTCGTTACACCAACCCGAGGCTCTTTGTTTCCCCT
CTTGGATCTGTTGAGTTTCTTTGTTGAAGAAGCCAGC**ATGGGTGCCCAGTTC**

TCCAAGACCGCAGCGAAGGGAGAAGCCGCCGCGGAGAGGCCTGGGGAGGCGG
CTGTGGCCTCGTCGCCTTCCAAAGCGAACGGACAGGAGAATGGCCACGTGAA
GGTAAACGGCGACGCTTCGCCCGCGGCCGCCGAGTCGGGCGCCAAGGAGGAG
CTGCAGGCCAACGGCAGCGCCCCGGCCGCCGACAAGGAGGAGCCCGCGGCCG
CCGGGAGCGGGGCGGCGTCGCCCTCCGCGGCCGAGAAAGGTGAGCCGGCCGC
CGCCGCTGCCCCCGAGGCCGGGGCCAGCCCGGTAGAGAAGGAGGCCCCCGCG
GAAGGCGAGGCTGCCGAGCCCGGCTCGCCCACGGCCGCGGAGGGAGAGGCCG
CGTCGGCCGCCTCCTCGACTTCTTCGCCCAAGGCCGAGGACGGGGCCACGCC
CTCGCCCAGCAACGAGACCCCGAAAAAAAAAAAAGAAGCGCTTTTCCTTCAAG
AAGTCTTTCAAGCTGAGCGGCTTCTCCTTCAAGAAGAACAAGAAGGAGGCTG
GAGAAGGCGGTGAGGCTGAGGCGCCCGCTGCCGAAGGCGGCAAGGACGAGGC
CGCCGGGGGCGCAGCTGCGGCCGCCGCCGAGGCGGGCGCGGCCTCCGGGGAG
CAGGCAGCGGCGCCGGGCGAGGAGGCGGCAGCGGGCGAGGAGGGGGCGGCGG
GTGGCGACCCGCAGGAGGCCAAGCCCCAGGAGGCCGCTGTCGCGCCAGAGAA
GCCGCCCGCCAGCGACGAGACCAAGGCCGCCGAGGAGCCCAGCAAGGTGGAG
GAGAAAAAGGCCGAGGAGGCCGGGGCCAGCGCCGCCGCCTGCGAGGCCCCCT
CCGCCGCCGGGCCCGGCGCGCCCCCGGAGCAGGAGGCAGCCCCCGCGGAGGA
GCCCGCGGCCGCCGCAGCCTCGTCAGCCTGCGCAGCCCCCTCACAGGAGGCC
CAGCCCGAGTGCAGTCCAGAAGCCCCCCCAGCGGAGGCGGCAGAGTAAAAGA
GCAAGCTTTTGTGAGATAATCGAAGAACTTTTCTCCCCCGTTTGTTTGTTGG
AGTGGTGCCAGGTACTGGTTTTGGAGAACTTGTCTACAACCAGGGATTGATT
TTAAAGATGTCTTTTTTTATTTTACTTTTTTTTTAAGCACCAAATTTTGTTGT
TTTTTTTTTTTCTCCCCTCCCCACAGATCCCATCTCAAATCATTCTGTTAAC
CACCATTCCAACAGGTCGAGGAGAGCTTAAACACCTTCTTCCTCTGCCTTGT
TTCTCTTTTATTTTTTATTTTTTCGCATCAGTATTAATGTTTTTGCATACTT
TGCATCTTTATTCAAAAGTGTAAACTTTCTTTGTCAATCTATGGACATGCCC
ATATATGAAGGAGATGGGTGGGTCAAAAAGGGATATCAAATGAAGTGATGGG
GTCACAATGGGGAAATTGAAGTGGTGCATAACATTGCCAAAATAGTGTGCCA
CTAGAAATGGTGTAAAGGCTGTCTTTTTTTTTTTTTTTAAAAGAAAAGTTATT
ACCATGTATTTTGTGAGGCAGGTTTACAACACTACAAGTCTTGAGTTAAGAA
GGAAAGAGGAAAAAAGAAAAAACACCAATACCCAGATTTAAAAAAAAAAAAA
CGATCATAGTCTTAGGAGTTCATTTAAACCATAGGAACTTTTCACTTATCTC
ATGTTAGCTGTACCAGTCAGTGATTAAGTAGAACTACAAGTTGTATAGGCTT
TATTGTTTATTGCTGGTTTATGACCTTAATAAAGTGTAATTATGTATTACCA
GCAGGGTGTTTTTAACTGTGACTATTGTATAAAAACAAATCTTGATATCCAG
AAGCACATGAAGTTTGCAACTTTCCACCCTGCCCATTTTTGTAAAACTGCAG
TCATCTTGGACCTTTTAAAACACAAATTTTAAACTCAACCAAGCTGTGATAA
GTGGAATGGTTACTGTTTATACTGTGGTATGTTTTTGATTACAGCAGATAAT
GCTTTCTTTTCCAGTCGTCTTTGAGAATAAAGGAAAAAAAATCTTCAGATGC
AATGGTTTTGTGTAGCATCTTGTCTATCATGTTTTGTAAATACTGGAGAAGC
TTTGACCAATTTGACTTAGAGATGGAATGTAACTTTGCTTACAAAAATTGCT
ATTAAACTCCTGCTTAAGGTGTTCTAATTTTCTGTGAGCACACTAAAAGCGA
AAAATAAATGTGAATAAAATGTACAAATTTGTTGTGTTTTTTTATGTTCTAA
TAATACTGAGACTTCTAGGTCTTAGGTTAATTTTTAGGAAGATCTTGCATGC
CATCAGGAGTAAATTTTATTGTGGTTCTTAATCTGAAGTTTTCAAGCTCTGA
AATTCATAATCCGCAGTGTCAGATTACGTAGAGGAAGATCTTACAACATTTC
CATGTCAAATCTGTTACCATTTATTGGCATTTAGTTTTCATTTAAGAATTGA
ACATAATTATTTTTATTGTAGCTATATAGCATGTCAGATTAAATCATTTACA
ACAAAAGGGGTGTGAACCTAAGACTATTTAAATGTCTTATGAGAAAATTTCA
TAAAGCCATTCTCTTGTCATTCAGGTCCAGAAACAAATTTTAAACTGAGTGA

GAGTCTATAGAATCCATACTGCAGATGGGTCATGAAATGTGACCAAATGTGT
TTCAAAAATTGATGGTGTATTACCTGCTATTGTAATTGCTTAGTGCTTGGCT
AATTTCCAAATTATTGCATAATATGTTCTACCTTAAGAAAACAGGTTTATGT
AACAAAGTAATGGTGTTGAATGGATGATGTCAGTTCATGGGCCTTTAGCATA
GTTTTAAGCATCCTTTTTTTTTTTTTTTTTGAAAGTGTGTTAGCATCTTGT
TACTCAAAGGATAAGACAGACAATAATACTTCACTGAATCTTAATAATCTTT
ACTAGTTTACCTCCTCTGCTCTTTGCCACCCGATAACTGGATATCTTTTCCT
TCAAAGGACCCTAAACTGATTGAAATTTAAGATATGTATCAAAAACATTATT
TCATTTAATGCACATCTGTTTTGCTGTTTTTGAGCAGTGTGCAGTTTAGGGT
TCATGATAAATCATTGAACCACATGTGTAACAACTGAATGCCAAATCTTAAA
CTCATTAGAAAAATAACAAATTAGGTTTTGACACGCATTCTTAATTGGAATA
ATGGATCAAAAATAGTGGTTCATGACCTTACCAAACACCCTTGCTACTAATA
AAATCAAATAACACTTAGAAGGGTATGTATTTTTAGTTAGGGTTTCTTGATC
TTGGAGGATGTTTGAAAGTTAAAAATTGAATTTGGTAACCAAAGGACTGATT
TATGGGTCTTTCCTATCTTAACCAACGTTTTCTTAGTTACCTAGATGGCCAA
GTACAGTGCCTGGTATGTAGTAAGACTCAGTAAAAAAGTGGATTTTTAAAAA
TAACTCCCAAAGTGAATAGTCAAAAATCCTGTTAGCAAACTGTTATATATTG
CTAAGTTTGTTCTTTTAACAGCTGGAATTTATTAAGATGCATTATTTTGATT
TTATTCACTGCCTAAAACACTTTGGGTGGTATTGATGGAGTTGGTGGATTTT
CCTCCAAGTGATTAAATGAAATTTGACGTATCTTTTCATCCAAAGTTTTGTA
CATCATGTTTTCTAACGGAAAAAAATGTTAATATGGCTTTTTTTGTATTACTA
AAAATAGCTTTGAGATTAAGGAAAAATAAATAACTCTTGTACAGTTCAGTAT
TGTCTATTAAATCTGTATTGGCAGTATGTATAATGGCATTTGCTGTGGTTAC
AAAATACTTCCTCTGGGTTATAATAATCATTTGATCCAATTCCTATTGCTTG
TAAAATAAAGTTTTACCAGTTGATATAATCAA (SEQ ID NO: 215) >NP_002347.5 myristoylated alanine-rich C-kinase substrate [*Homo sapiens*]
MGAQFSKTAAKGEAAAERPGEAAVASSPSKANGQENGHVKVNGDASPAAAES
GAKEELQANGSAPAADKEEPAAAGSGAASPSAAEKGEPAAAAAPEAGASPVE
KEAPAEGEAAEPGSPTAAEGEAASAASSTSSPKAEDGATPSPSNETPKKKKK
RFSFKKSFKLSGFSFKKNKKEAGEGGEAEAPAAEGGKDEAAGGAAAAAAEAG
AASGEQAAAPGEEAAAGEEGAAGGDPQEAKPQEAAVAPEKPPASDETKAAEE
PSKVEEKKAEEAGASAAACEAPSAAGPGAPPEQEAAPAEEPAAAAASSACAA
PSQEAQPECSPEAPPAEAAE (SEQ ID NO: 216) >Myr/Palm tag modified from Human MARCKS, nucleotide sequence ATGGGTTGCTGTTTCTCCAAGACC (SEQ ID NO: 217) >Myr/Palm tag modified from Human MARCKS, peptide sequence MGCCFSKT (SEQ ID NO: 218)

(81) In some embodiments of any of the aspects provided herein, the fusion polypeptide further comprises a peptide linker. The linker may be flexible, rigid, or cleavable. Further, the linker can be linked directly or via another linker (e.g., a peptide of one, two, three, four, five, six, seven, eight, nine, ten or more amino acids) to the fusion polypeptides described herein. Linkers can be configured according to a specific need, e.g., based on at least one of the following characteristics. In some embodiments of any of the aspects, linkers can be configured to have a sufficient length and flexibility such that it can allow for a cleavage at a target site. In some embodiments of any of the aspects, linkers can be configured to allow multimerization of the fusion polypeptides provided herein. In some embodiments of any of the aspects, linkers can be configured to facilitate expression and purification of the fusion proteins or engineered extracellular vesicles provided herein.

(82) In some embodiments of any of the aspects, a linker can be configured to have any length in a form of a peptide, peptidomimetic, an aptamer, a protein, a nucleic acid (e.g., DNA or RNA), or any combinations thereof. For example, in one embodiment, the linker may be a polypeptide linker

such as Gly-Ser-Ser-Gly (SEQ ID NO: 319) or a variation thereof as known by one of ordinary skill in the art. In another embodiment the linker may be a protein sequence for a self-cleavable peptide. For example, 2A sequences such as P2A, E2A, F2A, and T2A code for self-cleavable peptides by inducing ribosomal slippage on the mRNA at the 2A site which prevents peptide bond formation. The slippage will result in two separate peptides after translation. This allows the expression of two separate proteins from one promoter region. Any combination of the proteins described herein may be expressed with a self-cleavable peptide as known by one of ordinary skill in the art.

(83) In some embodiments of any of the aspects, the polypeptide linker is a non-cleavable linker. In some embodiments of any of the aspects, a linker can be a chemical linker of any length.

(84) In some embodiments of any of the aspects, the linker is an Fc linker. An exemplary nucleic acid sequence encoding an Fc polypeptide is:

(85) TABLE-US-00004 >KY053479.1 Synthetic construct Fc-adiponectin gene, complete cds (SEQ ID NO: 219)

ATGTACAGGATGCAACTCCTGTCTTGCATTGCACTAAGTCTTGCACTTGTC
ACGAACTCGATATCGGCCATGGTTAGATCTGACAAAACTCACACATGCCCA
CCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCC
CCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGC
GTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTAC
GTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAG
TACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGAC
TGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCA
GCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCA
CAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTC
AGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAG
TGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTG
CTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAG
AGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCACGAGGCT
CTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAGCC
AGCGGAAGTGGCGGAGGAGGCGGTCCTGGAGAAGGTGCCTATGTATACCGC
TCAGCATTCAGTGTGGGATTGGAGACTTACGTTACTATCCCCAACATGCCC
ATTCGCTTTACCAAGATCTTCTACAATCAGCAAAACCACTATGATGGCTCC
ACTGGTAAATTCCACTGCAACATTCCTGGGCTGTACTACTTTGCCTACCAC
ATCACAGTCTATATGAAGGATGTGAAGGTCAGCCTCTTCAAGAAGGACAAG
GCTATGCTCTTCACCTATGATCAGTACCAGGAAAATAATGTGGACCAGGCC
TCCGGCTCTGTGCTCCTGCATCTGGAGGTGGGCGACCAAGTCTGGCTCCAG
GTGTATGGGGAAGGAGAGCGTAATGGACTCTATGCTGATAATGACAATGAC
TCCACCTTCACAGGCTTTCTTCTCTACCATGACACCAACTCTAGAAAGCTT
CCTGGAGAAGGTGCCTATGTATACCGCTCAGCATTCAGTGTGGGATTGGAG
ACTTACGTTACTATCCCCAACATGCCCATTCGCTTTACCAAGATCTTCTAC
AATCAGCAAAACCACTATGATGGCTCCACTGGTAAATTCCACTGCAACATT
CCTGGGCTGTACTACTTTGCCTACCACATCACAGTCTATATGAAGGATGTG
AAGGTCAGCCTCTTCAAGAAGGACAAGGCTATGCTCTTCACCTATGATCAG
TACCAGGAAAATAATGTGGACCAGGCCTCCGGCTCTGTGCTCCTGCATCTG
GAGGTGGGCGACCAAGTCTGGCTCCAGGTGTATGGGGAAGGAGAGCGTAAT
GGACTCTATGCTGATAATGACAATGACTCCACCTTCACAGGCTTTCTTCTC
TACCATGACACCAACACTAGTCCTGGAGAAGGTGCCTATGTATACCGCTCA
GCATTCAGTGTGGGATTGGAGACTTACGTTACTATCCCCAACATGCCCATT
CGCTTTACCAAGATCTTCTACAATCAGCAAAACCACTATGATGGCTCCACT
GGTAAATTCCACTGCAACATTCCTGGGCTGTACTACTTTGCCTACCACATC

ACAGTCTATATGAAGGATGTGAAGGTCAGCCTCTTCAAGAAGGACAAGGCT
ATGCTCTTCACCTATGATCAGTACCAGGAAAATAATGTGGACCAGGCCTCC
GGCTCTGTGCTCCTGCATCTGGAGGTGGGCGACCAAGTCTGGCTCCAGGTG
TATGGGGAAGGAGAGCGTAATGGACTCTATGCTGATAATGACAATGACTCC
ACCTTCACAGGCTTTCTTCTCTACCATGACACCAACTAA.

The amino acid sequence of the Fc linker is:

(86) TABLE-US-00005 >Fc Translation (SEQ ID NO: 220)
DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP
EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGK.

(87) In some embodiments of any of the aspects, the linker is a P2A peptide linker. P2A is a self-cleaving peptide sequence allowing for expression of two proteins from one promoter. In some embodiments, the P2A linker is encoded by the nucleic acid sequence: GCTACTAACTTCAGCCTGCTGAAGCAG (SEQ ID NO: 221). The amino acid sequence of P2A is ATNFSLKQAGDVENPGP (SEQ ID NO: 222).

(88) In some embodiments of any of the aspects, the linker provides a multimerization (e.g., dimerization) domain wherein one fusion polypeptide may connect with another fusion polypeptide at each fusion polypeptide's respective multimerization domain. Multimerization of multiple fusion polypeptides will provide multiple fusion polypeptides within close proximity to one another to one or more a target receptor on the target cell, wherein the multiple fusion peptides will enhance receptor clustering on the target cell. Clustering receptors on a target cell will result in enhanced signal transduction. Without receptor clustering a signal may be weaker or not occur all together. For example, Fc domain sequences presented herein dimerize resulting in two fusion polypeptides connected by a covalent bond via the two Fc domains on their respective fusion polypeptide. One preferred embodiment of an Fc domain is from IgG4, herein labeled 4Fc. In other embodiments Fc may be from IgG1, herein labeled Fc. In certain embodiments Fc from other immunoglobulin, (e.g., IgG2, IgG3, etc.) may be used.

(89) Additional non-limiting examples of linkers that can be used and their properties are further described in detail, e.g., in Chen X, Zaro J L, Shen W C. Fusion protein linkers: property, design and functionality. *Adv Drug Deliv Rev.* 2013; 65 (10): 1357-1369. doi: 10.1016/j.addr.2012.09.039; O'Shea E K, Lumb K J, Kim P S. Peptide ‘Velcro’: design of a heterodimeric coiled coil. Curr Biol. 1993 Oct. 1; 3 (10): 658-67. doi: 10.1016/0960-9822 (93) 90063-t. PMID: 15335856; and Müller K M, Arndt K M, Alber T. Protein fusions to coiled-coil domains. Methods Enzymol. 2000; 328:261-82. doi: 10.1016/s0076-6879 (00) 28402-4. PMID: 11075350, the contents of which are incorporated herein by reference in their entireties.

(90) The engineered extracellular vesicle compositions provided herein can comprise variations in the configuration of the POI domain, linkers, and/or vesicle targeting domain. The specific combination and localization of these domains can enhance fusion polypeptide anchoring, function, or therapeutic effect, e.g., modulating inflammation.

(91) Thus, in one aspect, provided herein is an engineered extracellular vesicle comprising: at least one fusion polypeptide comprising: (i) at least one protein of interest (POI) domain or a fragment thereof; and (ii) at least one vesicle targeting domain, wherein the POI domain is in an extracellular position relative to a lipid membrane of the extracellular vesicle.

(92) In some embodiments, the POI domain or a fragment thereof is a N-terminal domain of the fusion polypeptide. In some embodiments, the vesicle targeting domain or a fragment thereof is a C-terminal domain of the fusion polypeptide.

(93) In another aspect, provided herein is an engineered extracellular vesicle comprising: at least one fusion polypeptide comprising: (i) at least one protein of interest (POI) domain or a fragment

thereof; and (ii) at least one vesicle targeting domain, wherein the POI domain is in an extracellular position relative to a lipid membrane of the extracellular vesicle, and wherein the vesicle targeting domain is a transmembrane domain relative to a lipid membrane of the extracellular vesicle.

(94) In some embodiments, the POI domain or a fragment thereof is a C-terminal domain of the fusion polypeptide. In some embodiments, the vesicle targeting domain or a fragment thereof is a N-terminal domain of the fusion polypeptide. In some embodiments, the vesicle targeting domain is in a luminal position relative to the lipid membrane of the extracellular vesicle.

(95) In some embodiments, the linker is in an exterior position relative to the lipid membrane of the extracellular vesicle. In some embodiments, the linker is a transmembrane linker. In some embodiments, the linker is in a luminal position relative to the lipid membrane of the extracellular vesicle.

(96) The engineered extracellular vesicle compositions provided herein can comprise one or more of the following fusion polypeptide sequences in Table 4.

(97) TABLE-US-00006 TABLE 4 Full Length Constructs Nucleic Acid Sequence (SEQ ID NO:) Fusion Polypeptide Amino Acid Sequence (SEQ ID NO:) hCTLA4-Fc-GPI >Artificial sequence; **hCTLA4**-Fc-*GPI*, DNA

ATGGCTTGCCTTGGATTTCAGCGGCACAAGGCTCAGCTGAACCTGGCTACCAGGACC
TGGCCCTGCACTCTCCTGTTTTTTCTTCTCTTCATCCCTGTCTTCTGCAAAGCAATG
CACGTGGCCCAGCCTGCTGTGGTACTGGCCAGCAGCCGAGGCATCGCCAGCTTTGTG
TGTGAGTATGCATCTCCAGGCAAAGCCACTGAGGTCCGGGTGACAGTGCTTCGGCAG
GCTGACAGCCAGGTGACTGAAGTCTGTGCGGCAACCTACATGATGGGGAATGAGTTG
ACCTTCCTAGATGATTCCATCTGCACGGGCACCTCCAGTGGAAATCAAGTGAACCTC
ACTATCCAAGGACTGAGGGCCATGGACACGGGACTCTACATCTGCAAGGTGGAGCTC
ATGTACCCACCGCCATACTACCTGGGCATAGGCAACGGAACCCAGATTTATGTAATT
GATCCAGAACCGTGCCCAGATTCTGACATCGATGACAAAACTCACACATGCCCACCG
TGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCC
AAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTG
AGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCAT
AATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGC
GTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTC
TCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAG
CCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAAC
CAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAG
TGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGAC
TCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAG
CAGGGGAACGTCTTCTCATGCTCCGTGATGCACGAGGCTCTGCACAACCACTACACG
CAGAAGAGCCTCTCCCTGTCTCCGGGTAAAATCGAT*CCAAATAAAGGAAGTGGAACC*
ACTTCAGGTACTACCCGTCTTCTATCTGGGCACACGTGTTTCACGTTGACAGGTTTG
CTTGGGACGCTAGTAACCATGGGCTTGCTGACTTAG (SEQ ID NO: 223) > Artificial sequence; **hCTLA4**-Fc-*GPI*, Amino Acid

MACLGFQRHKAQLNLATRTWPCTLLFFLLFIPVFCKAMHVAQPAVVLASSRGIASFV
CEYASPGKATEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNL
TIQGLRAMDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPDSDIDDKTHTCPP
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKID*PNKGSGT*
TSGTTRLLSGHTCFTLTGLLGTLVTMGLLT (SEQ ID NO: 224) hPDL1-GPI > Artificial sequence; **hPDL1**-*GPI*, DNA

ATGAGGATATTTGCTGTCTTTATATTCATGACCTACTGGCATTTGCTGAACGCATTT

ACTGTCACGGTTCCCAAGGACCTATATGTGGTAGAGTATGGTAGCAATATGACAATT
GAATGCAAATTCCCAGTAGAAAAACAATTAGACCTGGCTGCACTAATTGTCTATTGG
GAAATGGAGGATAAGAACATTATTCAATTTGTGCATGGAGAGGAAGACCTGAAGGTT
CAGCATAGTAGCTACAGACAGAGGGCCCGGCTGTTGAAGGACCAGCTCTCCCTGGGA
AATGCTGCACTTCAGATCACAGATGTGAAATTGCAGGATGCAGGGGTGTACCGCTGC
ATGATCAGCTATGGTGGTGCCGACTACAAGCGAATTACTGTGAAAGTCAATGCCCCA
TACAACAAAATCAACCAAAGAATTTTGGTTGTGGATCCAGTCACCTCTGAACATGAA
CTGACATGTCAGGCTGAGGGCTACCCCAAGGCCGAAGTCATCTGGACAAGCAGTGAC
CATCAAGTCCTGAGTGGTAAGACCACCACCACCAATTCCAAGAGAGAGGAGAAGCTT
TTCAATGTGACCAGCACACTGAGAATCAACACAACAACTAATGAGATTTTCTACTGC
ACTTTTAGGAGATTAGATCCTGAGGAAAACCATACAGCTGAATTGGTCATCCCAGAA
CTACCTCTGGCACATCCTCCAAATGAAAGG*CCAAATAAAGGAAGTGGAACCACTTCA*
GGTACTACCCGTCTTCTATCTGGGCACACGTGTTTCACGTTGACAGGTTTGCTTGGG
ACGCTAGTAACCATGGGCTTGCTGACTTAG (SEQ ID NO: 225) >Amino Acid Sequence; **hPDL1**-GPI, Amino Acid
MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYW
EMEDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRC
MISYGGADYKRITVKVNAPYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSD
HQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPE
LPLAHPPNER*PNKGSGTTSGTTRLLSGHTCFTLTGLLGTLVTMGLLT* (SEQ ID NO: 226) hPDL1-C1C2 >Artificial Sequence; **hPDL1**-*C1C2*, DNA
ATGAGGATATTTGCTGTCTTTATATTCATGACCTACTGGCATTTGCTGAACGCATTT
ACTGTCACGGTTCCCAAGGACCTATATGTGGTAGAGTATGGTAGCAATATGACAATT
GAATGCAAATTCCCAGTAGAAAAACAATTAGACCTGGCTGCACTAATTGTCTATTGG
GAAATGGAGGATAAGAACATTATTCAATTTGTGCATGGAGAGGAAGACCTGAAGGTT
CAGCATAGTAGCTACAGACAGAGGGCCCGGCTGTTGAAGGACCAGCTCTCCCTGGGA
AATGCTGCACTTCAGATCACAGATGTGAAATTGCAGGATGCAGGGGTGTACCGCTGC
ATGATCAGCTATGGTGGTGCCGACTACAAGCGAATTACTGTGAAAGTCAATGCCCCA
TACAACAAAATCAACCAAAGAATTTTGGTTGTGGATCCAGTCACCTCTGAACATGAA
CTGACATGTCAGGCTGAGGGCTACCCCAAGGCCGAAGTCATCTGGACAAGCAGTGAC
CATCAAGTCCTGAGTGGTAAGACCACCACCACCAATTCCAAGAGAGAGGAGAAGCTT
TTCAATGTGACCAGCACACTGAGAATCAACACAACAACTAATGAGATTTTCTACTGC
ACTTTTAGGAGATTAGATCCTGAGGAAAACCATACAGCTGAATTGGTCATCCCAGAA
CTACCTCTGGCACATCCTCCAAATGAAAGGATCGAT*GTCGAGCCACTGGGCATGGAG*
AATGGGAACATTGCCAACTCACAGATCGCCGCCTCATCTGTGCGTGTGACCTTCTTG
GGTTTGCAGCATTGGGTCCCGGAGCTGGCCCGCCTGAACCGCGCAGGCATGGTCAAT
GCCTGGACACCCAGCAGCAATGACGATAACCCCTGGATCCAGGTGAACCTGCTGCGG
AGGATGTGGGTAACAGGTGTGGTGACGCAGGGTGCCAGCCGCTTGGCCAGTCATGAG
TACCTGAAGGCCTTCAAGGTGGCCTACAGCCTTAATGGACACGAATTCGATTTCATC
CATGATGTTAATAAAAAACACAAGGAGTTTGTGGGTAACTGGAACAAAAACGCGGTG
CATGTCAACCTGTTTGAGACCCCTGTGGAGGCTCAGTACGTGAGATTGTACCCCACG
AGCTGCCACACGGCCTGCACTCTGCGCTTTGAGCTACTGGGCTGTGAGCTGAACGGA
TGCGCCAATCCCCTGGGCCTGAAGAATAACAGCATCCCTGACAAGCAGATCACGGCC
TCCAGCAGCTACAAGACCTGGGGCTTGCATCTCTTCAGCTGGAACCCCTCCTATGCA
CGGCTGGACAAGCAGGGCAACTTCAACGCCTGGGTTGCGGGGAGCTACGGTAACGAT
CAGTGGCTGCAGATCTTCCCTGGCAACTGGGACAACCACTCCCACAAGAAGAACTTG
TTTGAGACGCCCATCCTGGCTCGCTATGTGCGCATCCTGCCTGTAGCCTGGCACAAC
CGCATCGCCCTGCGCCTGGAGCTGCTGGGCTGTTAG (SEQ ID NO: 227) >Artificial Sequence, **hPDL1**-*C1C2*, Amino Acid
MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYW

EMEDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRC
MISYGGADYKRITVKVNAPYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSD
HQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPE
LPLAHPPNERID*VEPLGMENGNIANSQIAASSVRVTFLGLQHWVPELARLNRAGMVN*
AWTPSSNDDNPWIQVNLLRRMWVTGVVTQGASRLASHEYLKAFKVAYSLNGHEFDFI
HDVNKKHKEFVGNWNKNAVHVNLFETPVEAQYVRLYPTSCHTACTLRFELLGCELNG
CANPLGLKNNSIPDKQITASSSYKTWGLHLFSWNPSYARLDKQGNFNAWVAGSYGND
QWLQIFPGNWDNHSHKKNLFETPILARYVRILPVAWHNRIALRLELLGC (SEQ ID NO: 228) hPDL1-Fc-GPI >Artificial Sequence; **hPDL1**-Fc-GPI, DNA
ATGAGGATATTTGCTGTCTTTATATTCATGACCTACTGGCATTTGCTGAACGCATTT
ACTGTCACGGTTCCCAAGGACCTATATGTGGTAGAGTATGGTAGCAATATGACAATT
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CAGCATAGTAGCTACAGACAGAGGGCCCGGCTGTTGAAGGACCAGCTCTCCCTGGGA
AATGCTGCACTTCAGATCACAGATGTGAAATTGCAGGATGCAGGGGTGTACCGCTGC
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TACAACAAAATCAACCAAAGAATTTTGGTTGTGGATCCAGTCACCTCTGAACATGAA
CTGACATGTCAGGCTGAGGGCTACCCCAAGGCCGAAGTCATCTGGACAAGCAGTGAC
CATCAAGTCCTGAGTGGTAAGACCACCACCACCAATTCCAAGAGAGAGGAGAAGCTT
TTCAATGTGACCAGCACACTGAGAATCAACACAACAACTAATGAGATTTTCTACTGC
ACTTTTAGGAGATTAGATCCTGAGGAAAACCATACAGCTGAATTGGTCATCCCAGAA
CTACCTCTGGCACATCCTCCAAATGAAAGGATCGATGACAAAACTCACACATGCCCA
CCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAA
CCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGAC
GTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTG
CATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTC
AGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG
GTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGG
CAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAG
AACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTG
GAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTG
GACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGG
CAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCACGAGGCTCTGCACAACCACTAC
ACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAATCGAT*CCAAATAAAGGAAGTGGA*
ACCACTTCAGGTACTACCCGTCTTCTATCTGGGCACACGTGTTTCACGTTGACAGGT
TTGCTTGGGACGCTAGTAACCATGGGCTTGCTGACTTAG (SEQ ID NO: 229) > Artificial Sequence; **hPDL1**-Fc-*GPI*, Amino Acid
MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYW
EMEDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRC
MISYGGADYKRITVKVNAPYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSD
HQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPE
LPLAHPPNERIDDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD
VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
TQKSLSLSPGKID*PNKGSGTTSGTTRLLSGHTCFTLTGLLGTLVTMGLLT* (SEQ ID NO: 230) hPDL2-C1C2 >Artificial Sequence; **hPDL2**-*C1C2*, DNA
ATGATCTTCCTCCTGCTAATGTTGAGCCTGGAATTGCAGCTTCACCAGATAGCAGCT
TTATTCACAGTGACAGTCCCTAAGGAACTGTACATAATAGAGCATGGCAGCAATGTG
ACCCTGGAATGCAACTTTGACACTGGAAGTCATGTGAACCTTGGAGCAATAACAGCC

AGTTTGCAAAAGGTGGAAAATGATACATCCCCACACCGTGAAAGAGCCACTTTGCTG
GAGGAGCAGCTGCCCCTAGGGAAGGCCTCGTTCCACATACCTCAAGTCCAAGTGAGG
GACGAAGGACAGTACCAATGCATAATCATCTATGGGGTCGCCTGGGACTACAAGTAC
CTGACTCTGAAAGTCAAAGCTTCCTACAGGAAAATAAACACTCACATCCTAAAGGTT
CCAGAAACAGATGAGGTAGAGCTCACCTGCCAGGCTACAGGTTATCCTCTGGCAGAA
GTATCCTGGCCAAACGTCAGCGTTCCTGCCAACACCAGCCACTCCAGGACCCCTGAA
GGCCTCTACCAGGTCACCAGTGTTCTGCGCCTAAAGCCACCCCCTGGCAGAAACTTC
AGCTGTGTGTTCTGGAATACTCACGTGAGGGAACTTACTTTGGCCAGCATTGACCTT
CAAAGTCAGATGGAACCCAGGACCCATCCAACTATCGAT*GTCGAGCCACTGGGCATG*
GAGAATGGGAACATTGCCAACTCACAGATCGCCGCCTCATCTGTGCGTGTGACCTTC
TTGGGTTTGCAGCATTGGGTCCCGGAGCTGGCCCGCCTGAACCGCGCAGGCATGGTC
AATGCCTGGACACCCAGCAGCAATGACGATAACCCCTGGATCCAGGTGAACCTGCTG
CGGAGGATGTGGGTAACAGGTGTGGTGACGCAGGGTGCCAGCCGCTTGGCCAGTCAT
GAGTACCTGAAGGCCTTCAAGGTGGCCTACAGCCTTAATGGACACGAATTCGATTTC
ATCCATGATGTTAATAAAAAACACAAGGAGTTTGTGGGTAACTGGAACAAAAACGCG
GTGCATGTCAACCTGTTTGAGACCCCTGTGGAGGCTCAGTACGTGAGATTGTACCCC
ACGAGCTGCCACACGGCCTGCACTCTGCGCTTTGAGCTACTGGGCTGTGAGCTGAAC
GGATGCGCCAATCCCCTGGGCCTGAAGAATAACAGCATCCCTGACAAGCAGATCACG
GCCTCCAGCAGCTACAAGACCTGGGGCTTGCATCTCTTCAGCTGGAACCCCTCCTAT
GCACGGCTGGACAAGCAGGGCAACTTCAACGCCTGGGTTGCGGGGAGCTACGGTAAC
GATCAGTGGCTGCAGATCTTCCCTGGCAACTGGGACAACCACTCCCACAAGAAGAAC
TTGTTTGAGACGCCCATCCTGGCTCGCTATGTGCGCATCCTGCCTGTAGCCTGGCAC
AACCGCATCGCCCTGCGCCTGGAGCTGCTGGGCTGTTAG (SEQ ID NO: 231)

>Artificial Sequence; **hPDL2**-*C1C2*, Amino Acid

MIFLLLMLSLELQLHQIAALFTVTVPKELYIIEHGSNVTLECNFDTGSHVNLGAITA
SLQKVENDTSPHRERATLLEEQLPLGKASFHIPQVQVRDEGQYQCIIIYGVAWDYKY
LTLKVKASYRKINTHILKVPETDEVELTCQATGYPLAEVSWPNVSVPANTSHSRTPE
GLYQVTSVLRLKPPPGRNFSCVFWNTHVRELTLASIDLQSQMEPRTHPTID*VEPLGM*
ENGNIANSQIAASSVRVTFLGLQHWVPELARLNRAGMVNAWTPSSNDDNPWIQVNLL
RRMWVTGVVTQGASRLASHEYLKAFKVAYSLNGHEFDFIHDVNKKHKEFVGNWNKNA
VHVNLFETPVEAQYVRLYPTSCHTACTLRFELLGCELNGCANPLGLKNNSIPDKQIT
ASSSYKTWGLHLFSWNPSYARLDKQGNFNAWVAGSYGNDQWLQIFPGNWDNHSHKKN
LFETPILARYVRILPVAWHNRIALRLELLGC (SEQ ID NO: 232) hPDL2-Fc-GPI

>Artificial Sequence; **hPDL2**-Fc-*GPI*, DNA

ATGATCTTCCTCCTGCTAATGTTGAGCCTGGAATTGCAGCTTCACCAGATAGCAGCT
TTATTCACAGTGACAGTCCCTAAGGAACTGTACATAATAGAGCATGGCAGCAATGTG
ACCCTGGAATGCAACTTTGACACTGGAAGTCATGTGAACCTTGGAGCAATAACAGCC
AGTTTGCAAAAGGTGGAAAATGATACATCCCCACACCGTGAAAGAGCCACTTTGCTG
GAGGAGCAGCTGCCCCTAGGGAAGGCCTCGTTCCACATACCTCAAGTCCAAGTGAGG
GACGAAGGACAGTACCAATGCATAATCATCTATGGGGTCGCCTGGGACTACAAGTAC
CTGACTCTGAAAGTCAAAGCTTCCTACAGGAAAATAAACACTCACATCCTAAAGGTT
CCAGAAACAGATGAGGTAGAGCTCACCTGCCAGGCTACAGGTTATCCTCTGGCAGAA
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GGCCTCTACCAGGTCACCAGTGTTCTGCGCCTAAAGCCACCCCCTGGCAGAAACTTC
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CAAAGTCAGATGGAACCCAGGACCCATCCAACTATCGATGACAAAACTCACACATGC
CCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCA
AAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTG
GACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAG
GTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTG

GTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGC
AAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAA
GGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACC
AAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCC
GTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTG
CTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGG
TGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCACGAGGCTCTGCACAACCAC
TACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAATCGAT*CCAAATAAAGGAAGT*
GGAACCACTTCAGGTACTACCCGTCTTCTATCTGGGCACACGTGTTTCACGTTGACA
GGTTTGCTTGGGACGCTAGTAACCATGGGCTTGCTGACTTAG (SEQ ID NO: 233)
>Artificial Sequence; **hPDL2**-Fc-*GPI*, Amino Acid
MIFLLLMLSLELQLHQIAALFTVTVPKELYIIEHGSNVTLECNFDTGSHVNLGAITA
SLQKVENDTSPHRERATLLEEQLPLGKASFHIPQVQVRDEGQYQCIIIYGVAWDYKY
LTLKVKASYRKINTHILKVPETDEVELTCQATGYPLAEVSWPNVSVPANTSHSRTPE
GLYQVTSVLRLKPPPGRNFSCVFWNTHVRELTLASIDLQSQMEPRTHPTIDDKTHTC
PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKID*PNKGS*
GTTSGTTRLLSGHTCFTLTGLLGTLVTMGLLT (SEQ ID NO: 234) 4F2-h41BBL
>Artificial Sequence; *4F2*-**41BBL**, DNA
ATGAGCCAGGACACCGAGGTGGATATGAAGGAGGTGGAGCTGAATGAGTTAGAGCCC
GAGAAGCAGCCGATGAACGCGGCGTCTGGGGCGGCCATGTCCCTGGCGGGAGCCGAG
AAGAATGGTCTGGTGAAGATCAAGGTGGCGGAAGACGAGGCGGAGGCGGCAGCCGCG
GCTAAGTTCACGGGCCTGTCCAAGGAGGAGCTGCTGAAGGTGGCAGGCAGCCCCGGC
TGGGTACGCACCCGCTGGGCACTGCTGCTGCTCTTCTGGCTCGGCTGGCTCGGCATG
*CTTGCTGGTGCCGTGGTCATAATCGTG***GCCTGCCCCTGGGCCGTGTCCGGGGCTCGC**
GCCTCGCCCGGCTCCGCGGCCAGCCCGAGACTCCGCGAGGGTCCCGAGCTTTCGCCC
GACGATCCCGCCGGCCTCTTGGACCTGCGGCAGGGCATGTTTGCGCAGCTGGTGGCC
CAAAATGTTCTGCTGATCGATGGGCCCCTGAGCTGGTACAGTGACCCAGGCCTGGCA
GGCGTGTCCCTGACGGGGGGCCTGAGCTACAAAGAGGACACGAAGGAGCTGGTGGTG
GCCAAGGCTGGAGTCTACTATGTCTTCTTTCAACTAGAGCTGCGGCGCGTGGTGGCC
GGCGAGGGCTCAGGCTCCGTTTCACTTGCGCTGCACCTGCAGCCACTGCGCTCTGCT
GCTGGGGCCGCCGCCCTGGCTTTGACCGTGGACCTGCCACCCGCCTCCTCCGAGGCT
CGGAACTCGGCCTTCGGTTTCCAGGGCCGCTTGCTGCACCTGAGTGCCGGCCAGCGC
CTGGGCGTCCATCTTCACACTGAGGCCAGGGCACGCCATGCCTGGCAGCTTACCCAG
GGCGCCACAGTCTTGGGACTCTTCCGGGTGACCCCCGAAATCCCAGCCGGACTCCCT
TCACCGAGGTCGGAATAA (SEQ ID NO: 235) >Artificial Sequence; *4F2*-**h41BBL**, Amino Acid
MSQDTEVDMKEVELNELEPEKQPMNAASGAAMSLAGAEKNGLVKIKVAEDEAEAAAA
*AKFTGLSKEELLKVAGSPGWVRTRWALLLLFWLGWLGMLAGAVVIIV***ACPWAVSGAR**
ASPGSAASPRLREGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLA
GVSLTGGLSYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSA
AGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQ
GATVLGLFRVTPEIPAGLPSPRSE (SEQ ID NO: 236) hPDL1-4Fc-GPI >Artificial Sequence; **hPDL1**-4Fc-*GPI*, DNA
ATGAGGATATTTGCTGTCTTTATATTCATGACCTACTGGCATTTGCTGAACGCATTT
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GAAATGGAGGATAAGAACATTATTCAATTTGTGCATGGAGAGGAAGACCTGAAGGTT

CAGCATAGTAGCTACAGACAGAGGGCCCGGCTGTTGAAGGACCAGCTCTCCCTGGGA
AATGCTGCACTTCAGATCACAGATGTGAAATTGCAGGATGCAGGGGTGTACCGCTGC
ATGATCAGCTATGGTGGTGCCGACTACAAGCGAATTACTGTGAAAGTCAATGCCCCA
TACAACAAAATCAACCAAAGAATTTTGGTTGTGGATCCAGTCACCTCTGAACATGAA
CTGACATGTCAGGCTGAGGGCTACCCCAAGGCCGAAGTCATCTGGACAAGCAGTGAC
CATCAAGTCCTGAGTGGTAAGACCACCACCACCAATTCCAAGAGAGAGGAGAAGCTT
TTCAATGTGACCAGCACACTGAGAATCAACACAACAACTAATGAGATTTTCTACTGC
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GTGAGCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGGTG
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AACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTG
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GACTCCGACGGCTCCTTCTTCCTCTACAGCAGGCTAACCGTGGACAAGAGCAGGTGG
CAGGAGGGGAATGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTAC
ACACAGAAGAGCCTCTCCCTGTCTCCGGGTAAA*CCAAATAAAGGAAGTGGAACCACT*
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MISYGGADYKRITVKVNAPYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSD
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LPLAHPPNERESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD
VSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVRVLTVLHQDWLNGKEYKCK
VSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV
EWESNGQPEDNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHY
TQKSLSLSPGK*PNKGSGTTSGTTRLLSGHTCFTLTGLLGTLVTMGLLT* (SEQ ID NO: 238) hPDL1-GPI-P2A- >Artificial Sequence; **hPDL1**-*GPI*-P2A-**hFGL1**-*GPI*, DNA hFGL1-GPI
ATGAGGATATTTGCTGTCTTTATATTCATGACCTACTGGCATTTGCTGAACGCATTT
ACTGTCACGGTTCCCAAGGACCTATATGTGGTAGAGTATGGTAGCAATATGACAATT
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GAAATGGAGGATAAGAACATTATTCAATTTGTGCATGGAGAGGAAGACCTGAAGGTT
CAGCATAGTAGCTACAGACAGAGGGCCCGGCTGTTGAAGGACCAGCTCTCCCTGGGA
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GGTACTACCCGTCTTCTATCTGGGCACACGTGTTTCACGTTGACAGGTTTGCTTGGG
*ACGCTAGTAACCATGGGCTTGCTGACT*GGAAGCGGAGCTACTAACTTCAGCCTGCTG
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(SEQ ID NO: 239) >Artificial Sequence; **hPDL1**-*GPI*-P2A-**hFGL1**-*GPI*, Amino Acid
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EMEDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRC
MISYGGADYKRITVKVNAPYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSD
HQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPE
LPLAHPPNER*PNKGSGTTSGTTRLLSGHTCFTLTGLLGTLVTMGLLT*GSGATNFSLL
KQAGDVEENPGP**MAKVFSFILVTTALTMGREISALEDCAQEQMRLRAQVRLLETRVK**
QQQVKIKQLLQENEVQFLDKGDENTVIDLGSKRQYADCSEIFNDGYKLSGFYKIKPL
QSPAEFSVYCDMSDGGGWTVIQRRSDGSENFNRGWKDYENGFGNFVQKHGEYWLGNK
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GNFHPEVQWWASHQRMKFSTWDRDHDNYEGNCAEEDQSGWWFNRCHSANLNGVYYSG
PYTAKTDNGIVWYTWHGWWYSLKSVVMKIRPNDFIPNVI*PNKGSGTTSGTTRLLSGH*
TCFTLTGLLGTLVTMGLLT (SEQ ID NO: 240) Myr-mScarlet >Artificial Sequence; Myr-**mScarlet**, DNA
*ATGGGTTGCTGTTTCTCCAAGACC*GGCTCGAGCGGC**GTGAGCAAGGGCGAGGCAGTG**
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ACCACCTACAAGGCCAAGAAGCCCGTGCAGATGCCCGGCGCCTACAACGTCGACCGC
AAGTTGGACATCACCTCCCACAACGAGGACTACACCGTGGTGGAACAGTACGAACGC
TCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAG (SEQ ID NO: 241) >Artificial Sequence; *Myr*-**mScarlet**, Amino Acid
*MGCCFSKT*GSSG**VSKGEAVIKEFMRFKVHMEGSMNGHEFEIEGEGEGRPYEGTQTAK**
LKVTKGGPLPFSWDILSPQFMYGSRAFTKHPADIPDYYKQSFPEGFKWERVMNFEDG
GAVTVTQDTSLEDGTLIYKVKLRGTNFPPDGPVMQKKTMGWEASTERLYPEDGVLKG

DIKMALRLKDGGRYLADFKTTYKAKKPVQMPGAYNVDRKLDITSHNEDYTVVEQYER SEGRHSTGGMDELYK (SEQ ID NO: 242) Myr-NanoLuc > Artificial Sequence; *Myr*-**NanoLuc**, DNA Luciferase

*ATGGGTTGCTGTTTCTCCAAGACC*GGCTCGAGCGGC**GTCTTCACACTCGAAGATTTC GTTGGGGACTGGCGACAGACAGCCGGCTACAACCTGGACCAAGTCCTTGAACAGGGA GGTGTGTCCAGTTTGTTTCAGAATCTCGGGGTGTCCGTAACTCCGATCCAAAGGATT GTCCTGAGCGGTGAAAATGGGCTGAAGATCGACATCCATGTCATCATCCCGTATGAA GGTCTGAGCGGCGACCAAATGGGCCAGATCGAAAAAATTTTTAAGGTGGTGTACCCT GTGGATGATCATCACTTTAAGGTGATCCTGCACTATGGCACACTGGTAATCGACGGG GTTACGCCGAACATGATCGACTATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTC GACGGCAAAAAGATCACTGTAACAGGGACCCTGTGGAACGGCAACAAAATTATCGAC GAGCGCCTGATCAACCCCGACGGCTCCCTGCTGTTCCGAGTAACCATCAACGGAGTG ACCGGCTGGCGGCTGTGCGAACGCATTCTGGCGTAA** (SEQ ID NO: 243) >Artificial Sequence; *Myr*-**NanoLuc**, Amino Acid

*MGCCFSKT*GSSGVFTLEDFVGDWRQTAGYNLDQVLEQGGVSSLFQNLGVSVTPIQRI VLSGENGLKIDIHVIIPYEGLSGDQMGQIEKIFKVVYPVDDHHFKVILHYGTLVIDG VTPNMIDYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLINPDGSLLFRVTINGV TGWRLCERILA (SEQ ID NO: 244) hSecPDL1-GPI >Artificial Sequence; **hSecPDL1**-*GPI*, DNA

ATGAGGATATTTGCTGTCTTTATATTCATGACCTACTGGCATTTGCTGAACGCATTT ACTGTCACGGTTCCCAAGGACCTATATGTGGTAGAGTATGGTAGCAATATGACAATT GAATGCAAATTCCCAGTAGAAAAACAATTAGACCTGGCTGCACTAATTGTCTATTGG GAAATGGAGGATAAGAACATTATTCAATTTGTGCATGGAGAGGAAGACCTGAAGGTT CAGCATAGTAGCTACAGACAGAGGGCCCGGCTGTTGAAGGACCAGCTCTCCCTGGGA AATGCTGCACTTCAGATCACAGATGTGAAATTGCAGGATGCAGGGGTGTACCGCTGC ATGATCAGCTATGGTGGTGCCGACTACAAGCGAATTACTGTGAAAGTCAATGCCCCA TACAACAAAATCAACCAAAGAATTTTGGTTGTGGATCCAGTCACCTCTGAACATGAA CTGACATGTCAGGCTGAGGGCTACCCCAAGGCCGAAGTCATCTGGACAAGCAGTGAC CATCAAGTCCTGAGTGGTAAGACCACCACCACCAATTCCAAGAGAGAGGAGAAGCTT TTCAATGTGACCAGCACACTGAGAATCAACACAACAACTAATGAGATTTTCTACTGC ACTTTTAGGAGATTAGATCCTGAGGAAAACCATACAGCTGAATTGGTCATCCCAGGT AATATTCTGAATGTGTCCATTAAAATATGTCTAACACTGTCCCCTAGCACC*CCAAAT AAAGGAAGTGGAACCACTTCAGGTACTACCCGTCTTCTATCTGGGCACACGTGTTTC ACGTTGACAGGTTTGCTTGGGACGCTAGTAACCATGGGCTTGCTGACTTAG*** (SEQ ID NO: 245) >Artificial Sequence; **hSecPDL1**-*GPI*, Amino Acid

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYW EMEDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRC MISYGGADYKRITVKVNAPYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSD HQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPG NILNVSIKICLTLSPST*PNKGSGTTSGTTRLLSGHTCFTLTGLLGTLVTMGLLT*** (SEQ ID NO: 246) Tfr2-h41BBL >Artificial Sequence; *Tfr2*-**h41BBL**, DNA

*ATGGAGCGGCTTTGGGGTCTATTCCAGAGAGCGCAACAACTGTCCCCAAGATCCTCT CAGACCGTCTACCAGCGTGTGGAAGGCCCCCGGAAAGGGCACCTGGAGGAGGAAGAG GAAGACGGGGAGGAGGGGGCGGAGACATTGGCCCACTTCTGCCCCATGGAGCTGAGG GGCCCTGAGCCCCTGGGCTCTAGACCCAGGCAGCCAAACCTCATTCCCTGGGCGGCA GCAGGACGGAGGGCTGCCCCCTACCTGGTCCTGACGGCCCTGCTGATCTTCACTGGG GCCTTCCTACTGGGCTACGTCGCCTTCCGAGGGTCC**GCCTGCCCCTGGGCCGTGTCC GGGGCTCGCGCCTCGCCCGGCTCCGCGGCCAGCCCGAGACTCCGCGAGGGTCCCGAG CTTTCGCCCGACGATCCCGCCGGCCTCTTGGACCTGCGGCAGGGCATGTTTGCGCAG CTGGTGGCCCAAAATGTTCTGCTGATCGATGGGCCCCTGAGCTGGTACAGTGACCCA**

GGCCTGGCAGGCGTGTCCCTGACGGGGGGCCTGAGCTACAAAGAGGACACGAAGGAG
CTGGTGGTGGCCAAGGCTGGAGTCTACTATGTCTTCTTTCAACTAGAGCTGCGGCGC
GTGGTGGCCGGCGAGGGCTCAGGCTCCGTTTCACTTGCGCTGCACCTGCAGCCACTG
CGCTCTGCTGCTGGGGCCGCCGCCCTGGCTTTGACCGTGGACCTGCCACCCGCCTCC
TCCGAGGCTCGGAACTCGGCCTCGGTTTCCAGGGCCGCTTGCTGCACCTGAGTGCCG
GCCAGCGCCTGGGCGTCCATCTTCACACTGAGGCCAGGGCACGCCATGCCTGGCAGC
TTACCCAGGGCGCCACAGTCTTGGGACTCTTCCGGGTGACCCCCGAAATCCCAGCCG
GACTCCCTTCACCGAGGTCGGAATAA (SEQ ID NO: 247) >Artificial Sequence; *Tfr2*-**h41BBL**, Amino Acid
MERLWGLFQRAQQLSPRSSQTVYQRVEGPRKGHLEEEEEDGEEGAETLAHFCPMELR
*GPEPLGSRPRQPNLIPWAAAGRRAAPYLVLTALLIFTGAFLLGYVAFRGS***ACPWAVS**
GARASPGSAASPRLRGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPG
LAGVSLTGGLSYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLR
SAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQL
TQGATVLGLFRVTPEIPAGLPSPRSE (SEQ ID NO: 248) CD9tm3-h41BBL >Artificial Sequence; *CD9tm3*-**h41BBL**, DNA
ATGGGCTGCTGCGGGGCTGTGCAGGAGTCCCAGTGCATGCTGGGACTGTTCTTCGGC
TTCCTCTTGGTGATATTCGCCATTGAAATAGCTGCGGCCATCTGGGGATATTCCCAC
*AAGGATGAG***GCCTGCCCCTGGGCCGTGTCCGGGGCTCGCGCCTCGCCCGGCTCCGCG**
GCCAGCCCGAGACTCCGCGAGGGTCCCGAGCTTTCGCCCGACGATCCCGCCGGCCTC
TTGGACCTGCGGCAGGGCATGTTTGCGCAGCTGGTGGCCCAAAATGTTCTGCTGATC
GATGGGCCCCTGAGCTGGTACAGTGACCCAGGCCTGGCAGGCGTGTCCCTGACGGGG
GGCCTGAGCTACAAAGAGGACACGAAGGAGCTGGTGGTGGCCAAGGCTGGAGTCTAC
TATGTCTTCTTTCAACTAGAGCTGCGGCGCGTGGTGGCCGGCGAGGGCTCAGGCTCC
GTTTCACTTGCGCTGCACCTGCAGCCACTGCGCTCTGCTGCTGGGGCCGCCGCCCTG
GCTTTGACCGTGGACCTGCCACCCGCCTCCTCCGAGGCTCGGAACTCGGCCTTCGGT
TTCCAGGGCCGCTTGCTGCACCTGAGTGCCGGCCAGCGCCTGGGCGTCCATCTTCAC
ACTGAGGCCAGGGCACGCCATGCCTGGCAGCTTACCCAGGGCGCCACAGTCTTGGGA
CTCTTCCGGGTGACCCCCGAAATCCCAGCCGGACTCCCTTCACCGAGGTCGGAATAA
(SEQ ID NO: 249) >Artificial Sequence; *CD9tm3*-**h41BBL**, Amino Acid
*MGCCGAVQESQCMLGLFFGFLLVIFAIEIAAAIWGYSHKDE***ACPWAVSGARASPGSA**
ASPRLREGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTG
GLSYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAAL
ALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLG
LFRVTPEIPAGLPSPRSE (SEQ ID NO: 250) Myr/Palm-4F2- >Artificial Sequence; *Myr/Palm-4F2*-**h41BBL**, DNA h41BBL
*ATGGGTTGCTGTTTCTCCAAGACC*GGCTCGAGOGG*CAGCCAGGACACCGAGGTGGAT*
ATGAAGGAGGTGGAGCTGAATGAGTTAGAGCCCGAGAAGCAGCCGATGAACGCGGCG
TCTGGGGCGGCCATGTCCCTGGCGGGAGCCGAGAAGAATGGTCTGGTGAAGATCAAG
GTGGCGGAAGACGAGGCGGAGGCGGCAGCCGCGGCTAAGTTCACGGGCCTGTCCAAG
GAGGAGCTGCTGAAGGTGGCAGGCAGCCCCGGCTGGGTACGCACCCGCTGGGCACTG
CTGCTGCTCTTCTGGCTCGGCTGGCTCGGCATGCTTGCTGGTGCCGTGGTCATAATC
*GTG***GCCTGCCCCTGGGCCGTGTCCGGGGCTCGCGCCTCGCCCGGCTCCGCGGCCAGC**
CCGAGACTCCGCGAGGGTCCCGAGCTTTCGCCCGACGATCCCGCCGGCCTCTTGGAC
CTGCGGCAGGGCATGTTTGCGCAGCTGGTGGCCCAAAATGTTCTGCTGATCGATGGG
CCCCTGAGCTGGTACAGTGACCCAGGCCTGGCAGGCGTGTCCCTGACGGGGGGCCTG
AGCTACAAAGAGGACACGAAGGAGCTGGTGGTGGCCAAGGCTGGAGTCTACTATGTC
TTCTTTCAACTAGAGCTGCGGCGCGTGGTGGCCGGCGAGGGCTCAGGCTCCGTTTCA
CTTGCGCTGCACCTGCAGCCACTGCGCTCTGCTGCTGGGGCCGCCGCCCTGGCTTTG
ACCGTGGACCTGCCACCCGCCTCCTCCGAGGCTCGGAACTCGGCCTTCGGTTTCCAG

GGCCGCTTGCTGCACCTGAGTGCCGGCCAGCGCCTGGGCGTCCATCTTCACACTGAG
GCCAGGGCACGCCATGCCTGGCAGCTTACCCAGGGCGCCACAGTCTTGGGACTCTTC
CGGGTGACCCCCGAAATCCCAGCCGGACTCCCTTCACCGAGGTCGGAATAA
(SEQ ID NO: 251) >Artificial Sequence; *Myr/Palm-4F2*-**h41BBL**, Amino Acid
*MGCCFSKT*GSSG*SQDTEVDMKEVELNELEPEKQPMNAASGAAMSLAGAEKNGLVKIK*
VAEDEAEAAAAAKFTGLSKEELLKVAGSPGWVRTRWALLLLFWLGWLGMLAGAVVII
*V***ACPWAVSGARASPGSAASPRLREGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDG**
PLSWYSDPGLAGVSLTGGLSYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVS
LALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTE
ARARHAWQLTQGATVLGLFRVTPEIPAGLPSPRSE (SEQ ID NO: 252) Myr/Palm-Link- >Artificial Sequence; Myr/Palm-Link-**41BBL**, DNA 41BBL (41BBL transmembrane domain included)
*ATGGGTTGCTGTTTCTCCAAGACC*GGCTCGAGCGGC**TGGGCCCTGGTCGCGGGGCTG**
CTGCTGCTGCTGCTGCTCGCTGCCGCCTGCGCCGTCTTCCTCGCCTGCCCCTGGGCC
GTGTCCGGGGCTCGCGCCTCGCCCGGCTCCGCGGCCAGCCCGAGACTCCGCGAGGGT
CCCGAGCTTTCGCCCGACGATCCCGCCGGCCTCTTGGACCTGCGGCAGGGCATGTTT
GCGCAGCTGGTGGCCCAAAATGTTCTGCTGATCGATGGGCCCCTGAGCTGGTACAGT
GACCCAGGCCTGGCAGGCGTGTCCCTGACGGGGGGCCTGAGCTACAAAGAGGACACG
AAGGAGCTGGTGGTGGCCAAGGCTGGAGTCTACTATGTCTTCTTTCAACTAGAGCTG
CGGCGCGTGGTGGCCGGCGAGGGCTCAGGCTCCGTTTCACTTGCGCTGCACCTGCAG
CCACTGCGCTCTGCTGCTGGGGCCGCCGCCCTGGCTTTGACCGTGGACCTGCCACCC
GCCTCCTCCGAGGCTCGGAACTCGGCCTTCGGTTTCCAGGGCCGCTTGCTGCACCTG
AGTGCCGGCCAGCGCCTGGGCGTCCATCTTCACACTGAGGCCAGGGCACGCCATGCC
TGGCAGCTTACCCAGGGCGCCACAGTCTTGGGACTCTTCCGGGTGACCCCCGAAATC
CCAGCCGGACTCCCTTCACCGAGGTCGGAATAA (SEQ ID NO: 253) >Artificial Sequence; *Myr/Palm*-Link-**41BBL**, Amino Acid
*MGCCFSKT*GSSG**WALVAGLLLLLLLAAACAVFLACPWAVSGARASPGSAASPRLREG**
PELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDT
KELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPP
ASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFRVTPEI
PAGLPSPRSE (SEQ ID NO: 254) hPDL1-Link-GPI >Artificial Sequence; **hPDL1**-Link-*GPI*, DNA
ATGAGGATATTTGCTGTCTTTATATTCATGACCTACTGGCATTTGCTGAACGCATTT
ACTGTCACGGTTCCCAAGGACCTATATGTGGTAGAGTATGGTAGCAATATGACAATT
GAATGCAAATTCCCAGTAGAAAAACAATTAGACCTGGCTGCACTAATTGTCTATTGG
GAAATGGAGGATAAGAACATTATTCAATTTGTGCATGGAGAGGAAGACCTGAAGGTT
CAGCATAGTAGCTACAGACAGAGGGCCCGGCTGTTGAAGGACCAGCTCTCCCTGGGA
AATGCTGCACTTCAGATCACAGATGTGAAATTGCAGGATGCAGGGGTGTACCGCTGC
ATGATCAGCTATGGTGGTGCCGACTACAAGCGAATTACTGTGAAAGTCAATGCCCCA
TACAACAAAATCAACCAAAGAATTTTGGTTGTGGATCCAGTCACCTCTGAACATGAA
CTGACATGTCAGGCTGAGGGCTACCCCAAGGCCGAAGTCATCTGGACAAGCAGTGAC
CATCAAGTCCTGAGTGGTAAGACCACCACCACCAATTCCAAGAGAGAGGAGAAGCTT
TTCAANGTGACCAGCACACTGAGAATCAACACAACAACTAATGAGATTTTCTACTGC
ACTTTTAGGAGATTAGATCCTGAGGAAAACCATACAGCTGAATTGGGCTCGAGCGGC
CCAAATAAAGGAAGTGGAACCACTTCAGGTACTACCCGTCTTCTATCTGGGCACACG
TGTTTCACGTTGACAGGTTTGCTTGGGACGCTAGTAACCATGGGCTTGCTGACTTAG (SEQ ID NO: 255) >Artificial Sequence; **hPDL1**-Link-*GPI*, Amino Acid
MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYW

EMEDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRC
MISYGGADYKRITVKVNAPYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSD
HQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELGSSG
PNKGSGTTSGTTRLLSGHTCFTLTGLLGTLVTMGLLT (SEQ ID NO: 256) hSecPDL1- >Artificial Sequence; **hSecPDL1**-*CD9tm2*, DNA CD9tm2
ATGAGGATATTTGCTGTCTTTATATTCATGACCTACTGGCATTTGCTGAACGCATTT
ACTGTCACGGTTCCCAAGGACCTATATGTGGTAGAGTATGGTAGCAATATGACAATT
GAATGCAAATTCCCAGTAGAAAAACAATTAGACCTGGCTGCACTAATTGTCTATTGG
GAAATGGAGGATAAGAACATTATTCAATTTGTGCATGGAGAGGAAGACCTGAAGGTT
CAGCATAGTAGCTACAGACAGAGGGCCCGGCTGTTGAAGGACCAGCTCTCCCTGGGA
AATGCTGCACTTCAGATCACAGATGTGAAATTGCAGGATGCAGGGGTGTACCGCTGC
ATGATCAGCTATGGTGGTGCCGACTACAAGCGAATTACTGTGAAAGTCAATGCCCCA
TACAACAAAATCAACCAAAGAATTTTGGTTGTGGATCCAGTCACCTCTGAACATGAA
CTGACATGTCAGGCTGAGGGCTACCCCAAGGCCGAAGTCATCTGGACAAGCAGTGAC
CATCAAGTCCTGAGTGGTAAGACCACCACCACCAATTCCAAGAGAGAGGAGAAGCTT
TTCAATGTGACCAGCACACTGAGAATCAACACAACAACTAATGAGATTTTCTACTGC
ACTTTTAGGAGATTAGATCCTGAGGAAAACCATACAGCTGAATTGGTCATCCCAGGT
AATATTCTGAATGTGTCCATTAAAATATGTCTAACACTGTCCCCTAGCACC*TTCTAC*
ACAGGAGTCTATATTCTGATCGGAGCCGGCGCCCTCATGATGCTGGTGGGCTTCCTG
GGCTGCTGCGGGGCTGTGCAGGAGTCCCAGTGCTAG (SEQ ID NO: 257) >Artificial Sequence; **hSecPDL1**-*CD9tm2*, Amino Acid
MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYW
EMEDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRC
MISYGGADYKRITVKVNAPYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSD
HQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPG
NILNVSIKICLTLSPST*FYTGVYILIGAGALMMLVGFLGCCGAVQESQC* (SEQ ID NO: 258) hSecPDL1- >Artificial Sequence; hSecPDL1-*CD9tm2-*<u>*KRAS*</u>, DNA CD9tm2-
ATGAGGATATTTGCTGTCTTTATATTCATGACCTACTGGCATTTGCTGAACGCATTT
modified KRAS
ACTGTCACGGTTCCCAAGGACCTATATGTGGTAGAGTATGGTAGCAATATGACAATT
GAATGCAAATTCCCAGTAGAAAAACAATTAGACCTGGCTGCACTAATTGTCTATTGG
GAAATGGAGGATAAGAACATTATTCAATTTGTGCATGGAGAGGAAGACCTGAAGGTT
CAGCATAGTAGCTACAGACAGAGGGCCCGGCTGTTGAAGGACCAGCTCTCCCTGGGA
AATGCTGCACTTCAGATCACAGATGTGAAATTGCAGGATGCAGGGGTGTACCGCTGC
ATGATCAGCTATGGTGGTGCCGACTACAAGCGAATTACTGTGAAAGTCAATGCCCCA
TACAACAAAATCAACCAAAGAATTTTGGTTGTGGATCCAGTCACCTCTGAACATGAA
CTGACATGTCAGGCTGAGGGCTACCCCAAGGCCGAAGTCATCTGGACAAGCAGTGAC
CATCAAGTCCTGAGTGGTAAGACCACCACCACCAATTCCAAGAGAGAGGAGAAGCTT
TTCAATGTGACCAGCACACTGAGAATCAACACAACAACTAATGAGATTTTCTACTGC
ACTTTTAGGAGATTAGATCCTGAGGAAAACCATACAGCTGAATTGGTCATCCCAGGT
AATATTCTGAATGTGTCCATTAAAATATGTCTAACACTGTCCCCTAGCACC*TTCTAC*
ACAGGAGTCTATATTCTGATCGGAGCCGGCGCCCTCATGATGCTGGTGGGCTTCCTG
GGCTGCTGCGGGGCTGTGCAGGAGTCCCAGTGC<u>*AAAAAGAAGAAAAAGAAGTCAAAG*</u>
<u>*ACAAAGTGTGTAATTATGTAA*</u> (SEQ ID NO: 259) >Artificial Sequence; **hSecPDL1**-*CD9tm2-*<u>*KRAS*</u>, Amino Acid
MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYW
EMEDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRC
MISYGGADYKRITVKVNAPYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSD
HQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPG
NILNVSIKICLTLSPST*FYTGVYILIGAGALMMLVGFLGCCGAVQESQC*<u>*KKKKKKSK*</u> <u>*TKCVIM*</u>

(SEQ ID NO: 260) hSecPDL1- >Artificial Sequence; **hSecPDL1**-*CD9tm4*, DNA CD9tm4

ATGAGGATATTTGCTGTCTTTATATTCATGACCTACTGGCATTTGCTGAACGCATTT
ACTGTCACGGTTCCCAAGGACCTATATGTGGTAGAGTATGGTAGCAATATGACAATT
GAATGCAAATTCCCAGTAGAAAAACAATTAGACCTGGCTGCACTAATTGTCTATTGG
GAAATGGAGGATAAGAACATTATTCAATTTGTGCATGGAGAGGAAGACCTGAAGGTT
CAGCATAGTAGCTACAGACAGAGGGCCCGGCTGTTGAAGGACCAGCTCTCCCTGGGA
AATGCTGCACTTCAGATCACAGATGTGAAATTGCAGGATGCAGGGGTGTACCGCTGC
ATGATCAGCTATGGTGGTGCCGACTACAAGCGAATTACTGTGAAAGTCAATGCCCCA
TACAACAAAATCAACCAAAGAATTTTGGTTGTGGATCCAGTCACCTCTGAACATGAA
CTGACATGTCAGGCTGAGGGCTACCCCAAGGCCGAAGTCATCTGGACAAGCAGTGAC
CATCAAGTCCTGAGTGGTAAGACCACCACCACCAATTCCAAGAGAGAGGAGAAGCTT
TTCAATGTGACCAGCACACTGAGAATCAACACAACAACTAATGAGATTTTCTACTGC
ACTTTTAGGAGATTAGATCCTGAGGAAAACCATACAGCTGAATTGGTCATCCCAGGT
AATATTCTGAATGTGTCCATTAAAATATGTCTAACACTGTCCCCTAGCACC*ATCGGC*
GCAGTGGGCATCGGCATTGCCGTGGTCATGATATTTGGCATGATCTTCAGTATGATC
TTGTGCTGTGCTATCCGCAGGAACCGCGAGATGGTCTAG (SEQ ID NO: 261)
>Artificial Sequence; **hSecPDL1**-*CD9tm4*, Amino Acid

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYW
EMEDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRC
MISYGGADYKRITVKVNAPYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSD
HQVLSGKTTTTNSKREEKLFNVTSTIAINTTTNEIFYCTFRRLDPEENHTAELVIPG
NILNVSIKICLTLSPST*IGAVGIGIAVVMIFGMIFSMILCCAIRRNREMV* (SEQ ID NO: 262)
hSecPDL1-CD81 >Artificial Sequence; **hSecPDL1**-*CD81*, DNA

ATGAGGATATTTGCTGTCTTTATATTCATGACCTACTGGCATTTGCTGAACGCATTT
ACTGTCACGGTTCCCAAGGACCTATATGTGGTAGAGTATGGTAGCAATATGACAATT
GAATGCAAATTCCCAGTAGAAAAACAATTAGACCTGGCTGCACTAATTGTCTATTGG
GAAATGGAGGATAAGAACATTATTCAATTTGTGCATGGAGAGGAAGACCTGAAGGTT
CAGCATAGTAGCTACAGACAGAGGGCCCGGCTGTTGAAGGACCAGCTCTCCCTGGGA
AATGCTGCACTTCAGATCACAGATGTGAAATTGCAGGATGCAGGGGTGTACCGCTGC
ATGATCAGCTATGGTGGTGCCGACTACAAGCGAATTACTGTGAAAGTCAATGCCCCA
TACAACAAAATCAACCAAAGAATTTTGGTTGTGGATCCAGTCACCTCTGAACATGAA
CTGACATGTCAGGCTGAGGGCTACCCCAAGGCCGAAGTCATCTGGACAAGCAGTGAC
CATCAAGTCCTGAGTGGTAAGACCACCACCACCAATTCCAAGAGAGAGGAGAAGCTT
TTCAATGTGACCAGCACACTGAGAATCAACACAACAACTAATGAGATTTTCTACTGC
ACTTTTAGGAGATTAGATCCTGAGGAAAACCATACAGCTGAATTGGTCATCCCAGGT
AATATTCTGAATGTGTCCATTAAAATATGTCTAACACTGTCCCCTAGCACC*CTGTAC*
CTCATCGGCATTGCTGCCATCGTGGTCGCTGTGATCATGATCTTCGAGATGATCCTG
AGCATGGTGCTGTGCTGTGGCATCCGGAACAGCTCCGTGTACTGA (SEQ ID NO: 263)
>Artificial Sequence; **hSecPDL1**-*CD81*, Amino Acid

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYW
EMEDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRC
MISYGGADYKRITVKVNAPYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSD
HQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPG
NILNVSIKICLTLSPST*LYLIGIAAIVVAVIMIFEMILSMVLCCGIRNSSVY* (SEQ ID NO: 264) hCD200-Fc-GPI >Artificial Sequence; **hCD200**-Fc-*GPI*, DNA

ATGGAGAGGCTGGTGATCAGGATGCCCTTCTCTCATCTGTCTACCTACAGCCTGGTT
TGGGTCATGGCAGCAGTGGTGCTGTGCACAGCACAAGTGCAAGTGGTGACCCAGGAT
GAAAGAGAGCAGCTGTACACACCTGCTTCCTTAAAATGCTCTCTGCAAAATGCCCAG
GAAGCCCTCATTGTGACATGGCAGAAAAAGAAAGCTGTAAGCCCAGAAAACATGGTC

ACCTTCAGCGAGAACCATGGGGTGGTGATCCAGCCTGCCTATAAGGACAAGATAAAC
ATTACCCAGCTGGGACTCCAAAACTCAACCATCACCTTCTGGAATATCACCCTGGAG
GATGAAGGGTGTTACATGTGTCTCTTCAATACCTTTGGTTTTGGGAAGATCTCAGGA
ACGGCCTGCCTCACCGTCTATGTACAGCCCATAGTATCCCTTCACTACAAATTCTCT
GAAGACCACCTAAATATCACTTGCTCTGCCACTGCCCGCCCAGCCCCCATGGTCTTC
TGGAAGGTCCCTCGGTCAGGGATTGAAAATAGTACAGTGACTCTGTCTCACCCAAAT
GGGACCACGTCTGTTACCAGCATCCTCCATATCAAAGACCCTAAGAATCAGGTGGGG
AAGGAGGTGATCTGCCAGGTGCTGCACCTGGGGACTGTGACCGACTTTAAGCAAACC
GTCAACAAAGGCATCGATGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAA
CTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATG
ATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCT
GAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAG
CCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTG
CACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTC
CCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAG
GTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACC
TGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGG
CAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTC
TTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTC
TCATGCTCCGTGATGCACGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCC
CTGTCTCCGGGTAAAATCGAT*CCAAATAAAGGAAGTGGAACCACTTCAGGTACTACC*
CGTCTTCTATCTGGGCACACGTGTTTCACGTTGACAGGTTTGCTTGGGACGCTAGTA
ACCATGGGCTTGCTGACTTAG (SEQ ID NO: 265) >Artificial Sequence; **hCD200**-Fc-*GPI*, Amino Acid
MERLVIRMPFSHLSTYSLVWVMAAVVLCTAQVQVVTQDEREQLYTPASLKCSLQNAQ
EALIVTWQKKKAVSPENMVTFSENHGVVIQPAYKDKINITQLGLQNSTITFWNITLE
DEGCYMCLFNTFGFGKISGTACLTVYVQPIVSLHYKFSEDHLNITCSATARPAPMVF
WKVPRSGIENSTVTLSHPNGTTSVTSILHIKDPKNQVGKEVICQVLHLGTVTDFKQT
VNKGIDDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP
EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG
QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGKID*PNKGSGTTSGTTRLLSGHTCFTLTGLLGTLVTMGLLT* (SEQ ID NO: 266)
hFGL1-GPI >Artificial Sequence; **hFGL1**-*GPI*, DNA
ATGGCAAAGGTGTTCAGTTTCATCCTTGTTACCACCGCTCTGACAATGGGCAGGGAA
ATTTCGGCGCTCGAGGACTGTGCCCAGGAGCAGATGCGGCTCAGAGCCCAGGTGCGC
CTGCTTGAGACCCGGGTCAAACAGCAACAGGTCAAGATCAAGCAGCTTTTGCAGGAG
AATGAAGTCCAGTTCCTTGATAAAGGAGATGAGAATACTGTCATTGATCTTGGAAGC
AAGAGGCAGTATGCAGATTGTTCAGAGATTTTCAATGATGGGTATAAGCTCAGTGGA
TTTTACAAAATCAAACCTCTCCAGAGCCCAGCAGAATTTTCTGTTTATTGTGACATG
TCCGATGGAGGAGGATGGACTGTAATTCAGAGACGATCTGATGGCAGTGAAAACTTT
AACAGAGGATGGAAAGACTATGAAAATGGCTTTGGAAATTTTGTCCAAAAACATGGT
GAATATTGGCTGGGCAATAAAAATCTTCACTTCTTGACCACTCAAGAAGACTACACT
TTAAAAATCGACCTTGCAGATTTTGAAAAAAATAGCCGTTATGCACAATATAAGAAT
TTCAAAGTTGGAGATGAAAAGAATTTCTACGAGTTGAATATTGGGGAATATTCTGGA
ACAGCTGGAGATTCCCTTGCGGGGAATTTTCATCCTGAGGTGCAGTGGTGGGCTAGT
CACCAAAGAATGAAATTCAGCACGTGGGACAGAGATCATGACAACTATGAAGGGAAC
TGCGCAGAAGAAGATCAGTCTGGCTGGTGGTTTAACAGGTGTCACTCTGCAAACCTG
AATGGTGTATACTACAGCGGCCCCTACACGGCTAAAACAGACAATGGGATTGTCTGG
TACACCTGGCATGGGTGGTGGTATTCTCTGAAATCTGTGGTTATGAAAATTAGGCCA

AATGATTTTATTCCAAATGTAATT*CCAAATAAAGGAAGTGGAACCACTTCAGGTACT ACCCGTCTTCTATCTGGGCACACGTGTTTCACGTTGACAGGTTTGCTTGGGACGCTA GTAACCATGGGCTTGCTGACTTAG* (SEQ ID NO: 267) >Artificial Sequence; **hFGL1-***GPI*, Amino Acid

MAKVFSFILVTTALTMGREISALEDCAQEQMRLRAQVRLLETRVKQQQVKIKQLLQE NEVQFLDKGDENTVIDLGSKRQYADCSEIFNDGYKLSGFYKIKPLQSPAEFSVYCDM SDGGGWTVIQRRSDGSENFNRGWKDYENGFGNFVQKHGEYWLGNKNLHFLTTQEDYT LKIDLADFEKNSRYAQYKNFKVGDEKNFYELNIGEYSGTAGDSLAGNFHPEVQWWAS HQRMKFSTWDRDHDNYEGNCAEEDQSGWWFNRCHSANLNGVYYSGPYTAKTDNGIVW YTWHGWWYSLKSVVMKIRPNDFIPNVI*PNKGSGTTSGTTRLLSGHTCFTLTGLLGTL** *VTMGLLT* (SEQ ID NO: 268) hGa19-Fc-GPI >Artificial Sequence; **hGal9**-Fc-*GPI*, DNA

ATGGCCTTCAGCGGTTCCCAGGCTCCCTACCTGAGTCCAGCTGTCCCCTTTTCTGGG ACTATTCAAGGAGGTCTCCAGGACGGACTTCAGATCACTGTCAATGGGACCGTTCTC AGCTCCAGTGGAACCAGGTTTGCTGTGAACTTTCAGACTGGCTTCAGTGGAAATGAC ATTGCCTTCCACTTCAACCCTCGGTTTGAAGATGGAGGGTACGTGGTGTGCAACACG AGGCAGAACGGAAGCTGGGGGCCCGAGGAGAGGAAGACACACATGCCTTTCCAGAAG GGGATGCCCTTTGACCTCTGCTTCCTGGTGCAGAGCTCAGATTTCAAGGTGATGGTG AACGGGATCCTCTTCGTGCAGTACTTCCACCGCGTGCCCTTCCACCGTGTGGACACC ATCTCCGTCAATGGCTCTGTGCAGCTGTCCTACATCAGCTTCCAGAACCCCCGCACA GTCCCTGTTCAGCCTGCCTTCTCCACGGTGCCGTTCTCCCAGCCTGTCTGTTTCCCA CCCAGGCCCAGGGGGCGCAGACAAAAACCTCCCGGCGTGTGGCCTGCCAACCCGGCT CCCATTACCCAGACAGTCATCCACACAGTGCAGAGCGCCCCTGGACAGATGTTCTCT ACTCCCGCCATCCCACCTATGATGTACCCCCACCCCGCCTATCCGATGCCTTTCATC ACCACCATTCTGGGAGGGCTGTACCCATCCAAGTCCATCCTCCTGTCAGGCACTGTC CTGCCCAGTGCTCAGAGGTTCCACATCAACCTGTGCTCTGGGAACCACATCGCCTTC CACCTGAACCCCCGTTTTGATGAGAATGCTGTGGTCCGCAACACCCAGATCGACAAC TCCTGGGGGTCTGAGGAGCGAAGTCTGCCCCGAAAAATGCCCTTCGTCCGTGGCCAG AGCTTCTCAGTGTGGATCTTGTGTGAAGCTCACTGCCTCAAGGTGGCCGTGGATGGT CAGCACCTGTTTGAATACTACCATCGCCTGAGGAACCTGCCCACCATCAACAGACTG GAAGTGGGGGGCGACATCCAGCTGACCCATGTGCAGACAATCGATGACAAAACTCAC ACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTC CCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTG GTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGC GTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTAC AAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAA GCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAG ATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGAC ATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCT CCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAG AGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCACGAGGCTCTGCAC AACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA**ATCGAT***CCAAATAAA GGAAGTGGAACCACTTCAGGTACTACCCGTCTTCTATCTGGGCACACGTGTTTCACG TTGACAGGTTTGCTTGGGACGCTAGTAACCATGGGCTTGCTGACTTAG* (SEQ ID NO: 269) >Artificial Sequence; **hGal9**-Fc-*GPI*, Amino Acid

MAFSGSQAPYLSPAVPFSGTIQGGLQDGLQITVNGTVLSSSGTRFAVNFQTGFSGND IAFHFNPRFEDGGYVVCNTRQNGSWGPEERKTHMPFQKGMPFDLCFLVQSSDFKVMV NGILFVQYFHRVPFHRVDTISVNGSVQLSYISFQNPRTVPVQPAFSTVPFSQPVCFP PRPRGRRQKPPGVWPANPAPITQTVIHTVQSAPGQMFSTPAIPPMMYPHPAYPMPFI

TTILGGLYPSKSILLSGTVLPSAQRFHINLCSGNHIAFHLNPRFDENAVVRNTQIDN
SWGSEERSLPRKMPFVRGQSFSVWILCEAHCLKVAVDGQHLFEYYHRLRNLPTINRL
EVGGDIQLTHVQTID<u>DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV</u>
<u>VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY</u>
<u>KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSD</u>
<u>IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH</u>
<u>NHYTQKSLSLSPGK</u>ID*PNKGSGTTSGTTRLLSGHTCFTLTGLLGTLVTMGLLT* (SEQ ID NO: 270) hCD200-GPI >Artificial Sequence; **hCD200**-*GPI*, DNA
ATGGAGAGGCTGGTGATCAGGATGCCCTTCTCTCATCTGTCTACCTACAGCCTGGTT
TGGGTCATGGCAGCAGTGGTGCTGTGCACAGCACAAGTGCAAGTGGTGACCCAGGAT
GAAAGAGAGCAGCTGTACACACCTGCTTCCTTAAAATGCTCTCTGCAAAATGCCCAG
GAAGCCCTCATTGTGACATGGCAGAAAAAGAAAGCTGTAAGCCCAGAAAACATGGTC
ACCTTCAGCGAGAACCATGGGGTGGTGATCCAGCCTGCCTATAAGGACAAGATAAAC
ATTACCCAGCTGGGACTCCAAAACTCAACCATCACCTTCTGGAATATCACCCTGGAG
GATGAAGGGTGTTACATGTGTCTCTTCAATACCTTTGGTTTTGGGAAGATCTCAGGA
ACGGCCTGCCTCACCGTCTATGTACAGCCCATAGTATCCCTTCACTACAAATTCTCT
GAAGACCACCTAAATATCACTTGCTCTGCCACTGCCCGCCCAGCCCCCATGGTCTTC
TGGAAGGTCCCTCGGTCAGGGATTGAAAATAGTACAGTGACTCTGTCTCACCCAAAT
GGGACCACGTCTGTTACCAGCATCCTCCATATCAAAGACCCTAAGAATCAGGTGGGG
AAGGAGGTGATCTGCCAGGTGCTGCACCTGGGGACTGTGACCGACTTTAAGCAAACC
GTCAACAAAGGC*CCAAATAAAGGAAGTGGAACCACTTCAGGTACTACCCGTCTTCTA*
TCTGGGCACACGTGTTTCACGTTGACAGGTTTGCTTGGGACGCTAGTAACCATGGGC
TTGCTGACTTAG (SEQ ID NO: 271) >Artificial Sequence; **hCD200**-*GPI*, Amino Acid
MERLVIRMPFSHLSTYSLVWVMAAVVLCTAQVQVVTQDEREQLYTPASLKCSLQNAQ
EALIVTWQKKKAVSPENMVTFSENHGVVIQPAYKDKINITQLGLQNSTITFWNITLE
DEGCYMCLFNTFGFGKISGTACLTVYVQPIVSLHYKFSEDHLNITCSATARPAPMVF
WKVPRSGIENSTVTLSHPNGTTSVTSILHIKDPKNQVGKEVICQVLHLGTVTDFKQT
VNKG*PNKGSGTTSGTTRLLSGHTCFTLTGLLGTLVTMGLLT* (SEQ ID NO: 272) hGal9-GPI >Artificial Sequence; **hGa19**-*GPI*, DNA
ATGGCCTTCAGCGGTTCCCAGGCTCCCTACCTGAGTCCAGCTGTCCCCTTTTCTGGG
ACTATTCAAGGAGGTCTCCAGGACGGACTTCAGATCACTGTCAATGGGACCGTTCTC
AGCTCCAGTGGAACCAGGTTTGCTGTGAACTTTCAGACTGGCTTCAGTGGAAATGAC
ATTGCCTTCCACTTCAACCCTCGGTTTGAAGATGGAGGGTACGTGGTGTGCAACACG
AGGCAGAACGGAAGCTGGGGGCCCGAGGAGAGGAAGACACACATGCCTTTCCAGAAG
GGGATGCCCTTTGACCTCTGCTTCCTGGTGCAGAGCTCAGATTTCAAGGTGATGGTG
AACGGGATCCTCTTCGTGCAGTACTTCCACCGCGTGCCCTTCCACCGTGTGGACACC
ATCTCCGTCAATGGCTCTGTGCAGCTGTCCTACATCAGCTTCCAGAACCCCCGCACA
GTCCCTGTTCAGCCTGCCTTCTCCACGGTGCCGTTCTCCCAGCCTGTCTGTTTCCCA
CCCAGGCCCAGGGGGCGCAGACAAAAACCTCCCGGCGTGTGGCCTGCCAACCCGGCT
CCCATTACCCAGACAGTCATCCACACAGTGCAGAGCGCCCCTGGACAGATGTTCTCT
ACTCCCGCCATCCCACCTATGATGTACCCCCACCCCGCCTATCCGATGCCTTTCATC
ACCACCATTCTGGGAGGGCTGTACCCATCCAAGTCCATCCTCCTGTCAGGCACTGTC
CTGCCCAGTGCTCAGAGGTTCCACATCAACCTGTGCTCTGGGAACCACATCGCCTTC
CACCTGAACCCCCGTTTTGATGAGAATGCTGTGGTCCGCAACACCCAGATCGACAAC
TCCTGGGGGTCTGAGGAGCGAAGTCTGCCCCGAAAAATGCCCTTCGTCCGTGGCCAG
AGCTTCTCAGTGTGGATCTTGTGTGAAGCTCACTGCCTCAAGGTGGCCGTGGATGGT
CAGCACCTGTTTGAATACTACCATCGCCTGAGGAACCTGCCCACCATCAACAGACTG
GAAGTGGGGGGCGACATCCAGCTGACCCATGTGCAGACA*CCAAATAAAGGAAGTGGA*
ACCACTTCAGGTACTACCCGTCTTCTATCTGGGCACACGTGTTTCACGTTGACAGGT

TTGCTTGGGACGCTAGTAACCATGGGCTTGCTGACTTAG (SEQ ID NO: 273) >Artificial Sequence; **hGal9**-*GPI*, Amino Acid

MAFSGSQAPYLSPAVPFSGTIQGGLQDGLQITVNGTVLSSSGTRFAVNFQTGFSGND IAFHFNPRFEDGGYVVCNTRQNGSWGPEERKTHMPFQKGMPFDLCFLVQSSDFKVMV NGILFVQYFHRVPFHRVDTISVNGSVQLSYISFQNPRTVPVQPAFSTVPFSQPVCFP PRPRGRRQKPPGVWPANPAPITQTVIHTVQSAPGQMPSTPAIPPMMYPHPAYPMPFI TTILGGLYPSKSILLSGTVLPSAQRFHINLCSGNHIAFHLNPRFDENAVVRNTQIDN SWGSEERSLPRKMPFVRGQSFSVWILCEAHCLKVAVDGQHLFEYYHRLRNLPTINRL EVGGDIQLTHVQT*PNKGSGTTSGTTRLLSGHTCFTLTGLLGTLVTMGLLT*** (SEQ ID NO: 274) hHVEM-GPI >Artificial Sequence; **hHVEM**-*GPI*, DNA

ATGGAGCCTCCTGGAGACTGGGGGCCTCCTCCCTGGAGATCCACCCCCAAAACCGAC GTCTTGAGGCTGGTGCTGTATCTCACCTTCCTGGGAGCCCCCTGCTACGCCCCAGCT CTGCCGTCCTGCAAGGAGGACGAGTACCCAGTGGGCTCCGAGTGCTGCCCCAAGTGC AGTCCAGGTTATCGTGTGAAGGAGGCCTGCGGGGAGCTGACGGGCACAGTGTGTGAA CCCTGCCCTCCAGGCACCTACATTGCCCACCTCAATGGCCTAAGCAAGTGTCTGCAG TGCCAAATGTGTGACCCAGCCATGGGCCTGCGCGCGAGCCGGAACTGCTCCAGGACA GAGAACGCCGTGTGTGGCTGCAGCCCAGGCCACTTCTGCATCGTCCAGGACGGGGAC CACTGCGCCGCGTGCCGCGCTTACGCCACCTCCAGCCCGGGCCAGAGGGTGCAGAAG GGAGGCACCGAGAGTCAGGACACCCTGTGTCAGAACTGCCCCCCGGGGACCTTCTCT CCCAATGGGACCCTGGAGGAATGTCAGCACCAGACCAAGTGCAGCTGGCTGGTGACG AAGGCCGGAGCTGGGACCAGCAGCTCCCACTGGGTACCAAATAAAGGAAGTGGAACC *ACTTCAGGTACTACCCGTCTTCTATCTGGGCACACGTGTTTCACGTTGACAGGTTTG CTTGGGACGCTAGTAACCATGGGCTTGCTGACTTAG* (SEQ ID NO: 275) >Artificial Sequence; **hHVEM**-*GPI*, Amino Acid

MEPPGDWGPPPWRSTPKTDVLRLVLYLTFLGAPCYAPALPSCKEDEYPVGSECCPKC SPGYRVKEACGELTGTVCEPCPPGTYIAHLNGLSKCLQCQMCDPAMGLRASRNCSRT ENAVCGCSPGHFCIVQDGDHCAACRAYATSSPGQRVQKGGTESQDTLCQNCPPGTFS PNGTLEECQHQTKCSWLVTKAGAGTSSSHWV*PNKGSGTTSGTTRLLSGHTCFTLTGL LGTLVTMGLLT*** (SEQ ID NO: 276) hPDL2-GPI >Artificial Sequence; **hPDL2**-*GPI*, DNA

ATGATCTTCCTCCTGCTAATGTTGAGCCTGGAATTGCAGCTTCACCAGATAGCAGCT TTATTCACAGTGACAGTCCCTAAGGAACTGTACATAATAGAGCATGGCAGCAATGTG ACCCTGGAATGCAACTTTGACACTGGAAGTCATGTGAACCTTGGAGCAATAACAGCC AGTTTGCAAAAGGTGGAAAATGATACATCCCCACACCGTGAAAGAGCCACTTTGCTG GAGGAGCAGCTGCCCCTAGGGAAGGCCTCGTTCCACATACCTCAAGTCCAAGTGAGG GACGAAGGACAGTACCAATGCATAATCATCTATGGGGTCGCCTGGGACTACAAGTAC CTGACTCTGAAAGTCAAAGCTTCCTACAGGAAAATAAACACTCACATCCTAAAGGTT CCAGAAACAGATGAGGTAGAGCTCACCTGCCAGGCTACAGGTTATCCTCTGGCAGAA GTATCCTGGCCAAACGTCAGCGTTCCTGCCAACACCAGCCACTCCAGGACCCCTGAA GGCCTCTACCAGGTCACCAGTGTTCTGCGCCTAAAGCCACCCCCTGGCAGAAACTTC AGCTGTGTGTTCTGGAATACTCACGTGAGGGAACTTACTTTGGCCAGCATTGACCTT CAAAGTCAGATGGAACCCAGGACCCATCCAACT*CCAAATAAAGGAAGTGGAACCACT** *TCAGGTACTACCCGTCTTCTATCTGGGCACACGTGTTTCACGTTGACAGGTTTGCTT GGGACGCTAGTAACCATGGGCTTGCTGACTTAG* (SEQ ID NO: 277) >Artificial Sequence; **hPDL2**-*GPI*, Amino Acid

MIFLLLMLSLELQLHQIAALFTVTVPKELYIIEHGSNVTLECNFDTGSHVNLGAITA SLQKVENDTSPHRERATLLEEQLPLGKASFHIPQVQVRDEGQYQCIIIYGVAWDYKY LTLKVKASYRKINTHILKVPETDEVELTCQATGYPLAEVSWPNVSVPANTSHSRTPE GLYQVTSVLRLKPPPGRNFSCVFWNTHVRELTLASIDLQSQMEPRTHPT*PNKGSGTT** *SGTTRLLSGHTCFTLTGLLGTLVTMGLLT*** (SEQ ID NO: 278) hTSG6-GPI >Artificial

Sequence; **hTSG6**-*GPI*, DNA

ATGATCATCTTAATTTACTTATTTCTCTTGCTATGGGAAGACACTCAAGGATGGGGA
TTCAAGGATGGAATTTTTCATAACTCCATATGGCTTGAACGAGCAGCCGGTGTGTAC
CACAGAGAAGCACGGTCTGGCAAATACAAGCTCACCTACGCAGAAGCTAAGGCGGTG
TGTGAATTTGAAGGCGGCCATCTCGCAACTTACAAGCAGCTAGAGGCAGCCAGAAAA
ATTGGATTTCATGTCTGTGCTGCTGGATGGATGGCTAAGGGCAGAGTTGGATACCCC
ATTGTGAAGCCAGGGCCCAACTGTGGATTTGGAAAAACTGGCATTATTGATTATGGA
ATCCGTCTCAATAGGAGTGAAAGATGGGATGCCTATTGCTACAACCCACACGCAAAG
GAGTGTGGTGGCGTCTTTACAGATCCAAAGCAAATTTTTAAATCTCCAGGCTTCCCA
AATGAGTACGAAGATAACCAAATCTGCTACTGGCACATTAGACTCAAGTATGGTCAG
CGTATTCACCTGAGTTTTTTAGATTTTGACCTTGAAGATGACCCAGGTTGCTTGGCT
GATTATGTTGAAATATATGACAGTTACGATGATGTCCATGGCTTTGTGGGAAGATAC
TGTGGAGATGAGCTTCCAGATGACATCATCAGTACAGGAAATGTCATGACCTTGAAG
TTTCTAAGTGATGCTTCAGTGACAGCTGGAGGTTTCCAAATCAAATATGTTGCAATG
GATCCTGTATCCAAATCCAGTCAAGGAAAAAATACAAGTACTACTTCTACTGGAAAT
AAAAACTTTTTAGCTGGAAGATTTAGCCACTTAATCGAT*CCAAATAAAGGAAGTGGA*
ACCACTTCAGGTACTACCCGTCTTCTATCTGGGCACACGTGTTTCACGTTGACAGGT
TTGCTTGGGACGCTAGTAACCATGGGCTTGCTGACTTAG (SEQ ID NO: 279)

>Artificial Sequence; **hTSG6**-*GPI*, Amino Acid

MIILIYLFLLLWEDTQGWGFKDGIFHNSIWLERAAGVYHREARSGKYKLTYAEAKAV
CEFEGGHLATYKQLEAARKIGFHVCAAGWMAKGRVGYPIVKPGPNCGFGKTGIIDYG
IRLNRSERWDAYCYNPHAKECGGVFTDPKQIFKSPGFPNEYEDNQICYWHIRLKYGQ
RIHLSFLDFDLEDDPGCLADYVEIYDSYDDVHGFVGRYCGDELPDDIISTGNVMTLK
FLSDASVTAGGFQIKYVAMDPVSKSSQGKNTSTTSTGNKNFLAGRFSHLID*PNKGSG*
TTSGTTRLLSGHTCFTLTGLLGTLVTMGLLT (SEQ ID NO: 280) hHVEM-Fc-GPI

>Artificial Sequence; **hHVEM**-Fc-*GPI*, DNA

ATGGAGCCTCCTGGAGACTGGGGGCCTCCTCCCTGGAGATCCACCCCCAAAACCGAC
GTCTTGAGGCTGGTGCTGTATCTCACCTTCCTGGGAGCCCCCTGCTACGCCCCAGCT
CTGCCGTCCTGCAAGGAGGACGAGTACCCAGTGGGCTCCGAGTGCTGCCCCAAGTGC
AGTCCAGGTTATCGTGTGAAGGAGGCCTGCGGGGAGCTGACGGGCACAGTGTGTGAA
CCCTGCCCTCCAGGCACCTACATTGCCCACCTCAATGGCCTAAGCAAGTGTCTGCAG
TGCCAAATGTGTGACCCAGCCATGGGCCTGCGCGCGAGCCGGAACTGCTCCAGGACA
GAGAACGCCGTGTGTGGCTGCAGCCCAGGCCACTTCTGCATCGTCCAGGACGGGGAC
CACTGCGCCGCGTGCCGCGCTTACGCCACCTCCAGCCCGGGCCAGAGGGTGCAGAAG
GGAGGCACCGAGAGTCAGGACACCCTGTGTCAGAACTGCCCCCCGGGGACCTTCTCT
CCCAATGGGACCCTGGAGGAATGTCAGCACCAGACCAAGTGCAGCTGGCTGGTGACG
AAGGCCGGAGCTGGGACCAGCAGCTCCCACTGGGTAATCGATGACAAAACTCACACA
TGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCC
CCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTG
GTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTG
GAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGT
GTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAG
TGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCC
AAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATG
ACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATC
GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCC
GTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGC
AGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCACGAGGCTCTGCACAAC
CACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA**ATCGAT***CCAAATAAAGGA*
AGTGGAACCACTTCAGGTACTACCCGTCTTCTATCTGGGCACACGTGTTTCACGTTG

ACAGGTTTGCTTGGGACGCTAGTAACCATGGGCTTGCTGACTTAG (SEQ ID NO: 281) >Artificial Sequence; **hHVEM**-Fc-*GPI*, Amino Acid

MEPPGDWGPPPWRSTPKTDVLRLVLYLTFLGAPCYAPALPSCKEDEYPVGSECCPKC SPGYRVKEACGELTGTVCEPCPPGTYIAHLNGLSKCLQCQMCDPAMGLRASRNCSRT ENAVCGCSPGHFCIVQDGDHCAACRAYATSSPGQRVQKGGTESQDTLCQNCPPGTFS PNGTLEECQHQTKCSWLVTKAGAGTSSSHWVIDDKTHTCPPCPAPELLGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKID*PNKGSGTTSGTTRLLSGHTCFTL TGLLGTLVTMGLLT* (SEQ ID NO: 282) hPDL1-GPI-P2A- >Artificial Sequence; **hPDL1**-*GPI*-P2A-**hHVEM**-*GPI*, DNA hHVEM-GPI

ATGAGGATATTTGCTGTCTTTATATTCATGACCTACTGGCATTTGCTGAACGCATTT ACTGTCACGGTTCCCAAGGACCTATATGTGGTAGAGTATGGTAGCAATATGACAATT GAATGCAAATTCCCAGTAGAAAAACAATTAGACCTGGCTGCACTAATTGTCTATTGG GAAATGGAGGATAAGAACATTATTCAATTTGTGCATGGAGAGGAAGACCTGAAGGTT CAGCATAGTAGCTACAGACAGAGGGCCCGGCTGTTGAAGGACCAGCTCTCCCTGGGA AATGCTGCACTTCAGATCACAGATGTGAAATTGCAGGATGCAGGGGTGTACCGCTGC ATGATCAGCTATGGTGGTGCCGACTACAAGCGAATTACTGTGAAAGTCAATGCCCCA TACAACAAAATCAACCAAAGAATTTTGGTTGTGGATCCAGTCACCTCTGAACATGAA CTGACATGTCAGGCTGAGGGCTACCCCAAGGCCGAAGTCATCTGGACAAGCAGTGAC CATCAAGTCCTGAGTGGTAAGACCACCACCACCAATTCCAAGAGAGAGGAGAAGCTT TTCAATGTGACCAGCACACTGAGAATCAACACAACAACTAATGAGATTTTCTACTGC ACTTTTAGGAGATTAGATCCTGAGGAAAACCATACAGCTGAATTGGTCATCCCAGAA CTACCTCTGGCACATCCTCCAAATGAAAGG*CCAAATAAAGGAAGTGGAACCACTTCA GGTACTACCCGTCTTCTATCTGGGCACACGTGTTTCACGTTGACAGGTTTGCTTGGG ACGCTAGTAACCATGGGCTTGCTGACT***GGAAGCGGAAGCTACTAACTTCAGCCTGCTG AAGCAGGCTGGCGACGTGGAGGAGAACCCTGGACCT**ATGGAGCCTCCTGGAGACTGG GGGCCTCCTCCCTGGAGATCCACCCCCAAAACCGACGTCTTGAGGCTGGTGCTGTAT CTCACCTTCCTGGGAGCCCCCTGCTACGCCCCAGCTCTGCCGTCCTGCAAGGAGGAC GAGTACCCAGTGGGCTCCGAGTGCTGCCCCAAGTGCAGTCCAGGTTATCGTGTGAAG GAGGCCTGCGGGGAGCTGACGGGCACAGTGTGTGAACCCTGCCCTCCAGGCACCTAC ATTGCCCACCTCAATGGCCTAAGCAAGTGTCTGCAGTGCCAAATGTGTGACCCAGCC ATGGGCCTGCGCGCGAGCCGGAACTGCTCCAGGACAGAGAACGCCGTGTGTGGCTGC AGCCCAGGCCACTTCTGCATCGTCCAGGACGGGGACCACTGCGCCGCGTGCCGCGCT TACGCCACCTCCAGCCCGGGCCAGAGGGTGCAGAAGGGAGGCACCGAGAGTCAGGAC ACCCTGTGTCAGAACTGCCCCCCGGGGACCTTCTCTCCCAATGGGACCCTGGAGGAA TGTCAGCACCAGACCAAGTGCAGCTGGCTGGTGACGAAGGCCGGAGCTGGGACCAGC AGCTCCCACTGGGTA***CCAAATAAAGGAAGTGGAACCACTTCAGGTACTACCCGTCTT CTATCTGGGCACACGTGTTTCACGTTGACAGGTTTGCTTGGGACGCTAGTAACCATG GGCTTGCTGACTTAG*** (SEQ ID NO: 283) >Artificial Sequence; **hPDL1**-*GPI*-P2A-**hHVEM**-*GPI*, Amino Acid

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYW EMEDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRC MISYGGADYKRITVKVNAPYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSD HQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPE LPLAHPPNER*PNKGSGTTSGTTRLLSGHTCFTLTGLLGTLVTMGLLTG***SGATNFSLL KQAGDVEENPGP**MEPPGDWGPPPWRSTPKTDVLRLVLYLTFLGAPCYAPALPSCKED EYPVGSECCPKCSPGYRVKEACGELTGTVCEPCPPGTYIAHLNGLSKCLQCQMCDPA MGLRASRNCSRTENAVCGCSPGHFCIVQDGDHCAACRAYATSSPGQRVQKGGTESQD**

TLCQNCPPGTFSPNGTLEECQHQTKCSWINTKAGAGTSSSHWV*PNKGSGTTSGTTRL*
LSGHTCFTLTGLLGTLVTMGLLT (SEQ ID NO: 284) mCTLA4-Fc-GPI >Artificial Sequence; **mCTLA4-**Fc-*GPI*, DNA
ATGGCTTGTCTTGGACTCCGGAGGTACAAAGCTCAACTGCAGCTGCCTTCTAGGACT
TGGCCTTTTGTAGCCCTGCTCACTCTTCTTTTCATCCCAGTCTTCTCTGAAGCCATA
CAGGTGACCCAACCTTCAGTGGTGTTGGCTAGCAGCCATGGTGTCGCCAGCTTTCCA
TGTGAATATTCACCATCACACAACACTGATGAGGTCCGGGTGACTGTGCTGCGGCAG
ACAAATGACCAAATGACTGAGGTCTGTGCCACGACATTCACAGAGAAGAATACAGTG
GGCTTCCTAGATTACCCCTTCTGCAGTGGTACCTTTAATGAAAGCAGAGTGAACCTC
ACCATCCAAGGACTGAGAGCTGTTGACACGGGACTGTACCTCTGCAAGGTGGAACTC
ATGTACCCACCGCCATACTTTGTGGGCATGGGCAACGGGACGCAGATTTATGTCATT
GATCCAGAACCATGCCCGGATTCTGAATCGATGACAAAACTCACACATGCCCACCGT
GCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCA
AGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGA
GCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATA
ATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCG
TCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCT
CCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGC
CCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACC
AGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGT
GGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACT
CCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGC
AGGGGAACGTCTTCTCATGCTCCGTGATGCACGAGGCTCTGCACAACCACTACACGC
AGAAGAGCCTCTCCCTGTCTCCGGGTAAAATCGAT*CCAAATAAAGGAAGTGGAACCA*
CTTCAGGTACTACCCGTCTTCTATCTGGGCACACGTGTTTCACGTTGACAGGTTTGC
TTGGGACGCTAGTAACCATGGGCTTGCTGACTTAG (SEQ ID NO: 285) >Artificial Sequence; **mCTLA4-**Fc-*GPI*, Amino Acid
MACLGLRRYKAQLQLPSRTWPFVALLTLLFIPVFSEAIQVTQPSVVLASSHGVASFP
CEYSPSHNTDEVRVTVLRQTNDQMTEVCATTFTEKNTVGFLDYPFCSGTFNESRVNL
TIQGLRAVDTGLYLCKVELMYPPPYFVGMGNGTQIYVIDPEPCPDSD
IDDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
KID*PNKGSGTTSGTTRLLSGHTCFTLTGLLGTLVTMGLLT* (SEQ ID NO: 286) mPDL1-C1C2 >Artificial Sequence; **mPDL1-***C1C2*, DNA
ATGAGGATATTTGCTGGCATTATATTCACAGCCTGCTGTCACTTGCTACGGGCGTTT
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GAGTGCAGATTCCCTGTAGAACGGGAGCTGGACCTGCTTGCGTTAGTGGTGTACTGG
GAAAAGGAAGATGAGCAAGTGATTCAGTTTGTGGCAGGAGAGGAGGACCTTAAGCCT
CAGCACAGCAACTTCAGGGGGAGAGCCTCGCTGCCAAAGGACCAGCTTTTGAAGGGA
AATGCTGCCCTTCAGATCACAGACGTCAAGCTGCAGGACGCAGGCGTTTACTGCTGC
ATAATCAGCTACGGTGGTGCGGACTACAAGCGAATCACGCTGAAAGTCAATGCCCCA
TACCGCAAAATCAACCAGAGAATTTCCGTGGATCCAGCCACTTCTGAGCATGAACTA
ATATGTCAGGCCGAGGGTTATCCAGAAGCTGAGGTAATCTGGACAAACAGTGACCAC
CAACCCGTGAGTGGGAAGAGAAGTGTCACCACTTCCCGGACAGAGGGGATGCTTCTC
AATGTGACCAGCAGTCTGAGGGTCAACGCCACAGCGAATGATGTTTTCTACTGTACG
TTTTGGAGATCACAGCCAGGGCAAAACCACACAGCGGAGCTGATCATCCCAGAACTG
CCTGCAACACATCCTCCACAGAACAGGACTATCGAT*GTCGAGCCACTGGGCATGGAG*
AATGGGAACATTGCCAACTCACAGATCGCCGCCTCATCTGTGCGTGTGACCTTCTTG

GGTTTGCAGCATTGGGTCCCGGAGCTGGCCCGCCTGAACCGCGCAGGCATGGTCAAT
GCCTGGACACCCAGCAGCAATGACGATAACCCCTGGATCCAGGTGAACCTGCTGCGG
AGGATGTGGGTAACAGGTGTGGTGACGCAGGGTGCCAGCCGCTTGGCCAGTCATGAG
TACCTGAAGGCCTTCAAGGTGGCCTACAGCCTTAATGGACACGAATTCGATTTCATC
CATGATGTTAATAAAAAACACAAGGAGTTTGTGGGTAACTGGAACAAAAACGCGGTG
CATGTCAACCTGTTTGAGACCCCTGTGGAGGCTCAGTACGTGAGATTGTACCCCACG
AGCTGCCACACGGCCTGCACTCTGCGCTTTGAGCTACTGGGCTGTGAGCTGAACGGA
TGCGCCAATCCCCTGGGCCTGAAGAATAACAGCATCCCTGACAAGCAGATCACGGCC
TCCAGCAGCTACAAGACCTGGGGCTTGCATCTCTTCAGCTGGAACCCCTCCTATGCA
CGGCTGGACAAGCAGGGCAACTTCAACGCCTGGGTTGCGGGGAGCTACGGTAACGAT
CAGTGGCTGCAGATCTTCCCTGGCAACTGGGACAACCACTCCCACAAGAAGAACTTG
TTTGAGACGCCCATCCTGGCTCGCTATGTGCGCATCCTGCCTGTAGCCTGGCACAAC
CGCATCGCCCTGCGCCTGGAGCTGCTGGGCTGTTAG (SEQ ID NO: 287) >Artificial Sequence; **mPDL1**-*C1C2*, Amino Acid

MRIFAGIIFTACCHLLRAFTITAPKDLYVVEYGSNVTMECRFPVERELDLLALVVYW
EKEDEQVIQFVAGEEDLKPQHSNFSGRASLPKDQLLKGNAALQITDVKLQDAGVYCC
IISYGGADYKRITLKVNAPYRKINQRISVDPATSEHELICQAEGYPEAEVIWTNSDH
QPVSGKRSVTTSRTEGMLLNVTSSLRVNATANDVFYCTFWRSQPGQNHTAELIIPEL
PATHPPQNRTID*VEPLGMENGNIANSQIAASSVRVTFLGLQHWVPELARLNRAGMVN*
AWTPSSNDDNPWIQVNLLRRMWVTGVVTQGASRLASHEYLKAFKVAYSLNGHEFDFI
HDVNKKHKEFVGNWNKNAVHVNLFETPVEAQYVRLYPTSCHTACTLRFELLGCELNG
CANPLGLKNNSIPDKQITASSSYKTWGLHLFSWNPSYARLDKQGNFNAWVAGSYGND
QWLQIFPGNWDNHSHKKNLFETPILARYVRILPVAWHNRIALRLELLGC (SEQ ID NO: 288) mPDL1-Fc-GPI >Artificial Sequence; **mPDL1**-Fc-*GPI*, DNA

ATGAGGATATTTGCTGGCATTATATTCACAGCCTGCTGTCACTTGCTACGGGCGTTT
ACTATCACGGCTCCAAAGGACTTGTACGTGGTGGAGTATGGCAGCAACGTCACGATG
GAGTGCAGATTCCCTGTAGAACGGGAGCTGGACCTGCTTGCGTTAGTGGTGTACTGG
GAAAAGGAAGATGAGCAAGTGATTCAGTTTGTGGCAGGAGAGGAGGACCTTAAGCCT
CAGCACAGCAACTTCAGGGGGAGAGCCTCGCTGCCAAAGGACCAGCTTTTGAAGGGA
AATGCTGCCCTTCAGATCACAGACGTCAAGCTGCAGGACGCAGGCGTTTACTGCTGC
ATAATCAGCTACGGTGGTGCGGACTACAAGCGAATCACGCTGAAAGTCAATGCCCCA
TACCGCAAAATCAACCAGAGAATTTCCGTGGATCCAGCCACTTCTGAGCATGAACTA
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CAACCCGTGAGTGGGAAGAGAAGTGTCACCACTTCCCGGACAGAGGGGATGCTTCTC
AATGTGACCAGCAGTCTGAGGGTCAACGCCACAGCGAATGATGTTTTCTACTGTACG
TTTTGGAGATCACAGCCAGGGCAAAACCACACAGCGGAGCTGATCATCCCAGAACTG
CCTGCAACACATCCTCCACAGAACAGGACTATCGATGACAAAACTCACACATGCCCA
CCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAA
CCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGAC
GTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTG
CATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTC
AGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG
GTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGG
CAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAG
AACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTG
GAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTG
GACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGG
CAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCACGAGGCTCTGCACAACCACTAC
ACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAATCGAT*CCAAATAAAGGAAGTGGA*
ACCACTTCAGGTACTACCCGTCTTCTATCTGGGCACACGTGTTTCACGTTGACAGGT

TTGCTTGGGACGCTAGTAACCATGGGCTTGCTGACTTAG (SEQ ID NO: 289) >Artificial Sequence; **mPDL1**-Fc-*GPI*, Amino Acid

MRIFAGIIFTACCHLLRAFTITAPKDLYVVEYGSNVTMECRFPVERELDLLALVVYW
EKEDEQVIQFVAGEEDLKPQHSNFRGRASLPKDQLLKGNAALQITDVKLQDAGVYCC
IISYGGADYKRITLKVNAPYRKINQRISVDPATSEHELICQAEGYPEAEVIWTNSDH
QPVSGKRSVTTSRTEGMLLNVTSSLRVNATANDVPYCTFWRSQPGQNHTAELIIPEL
PATHPPQNRTIDDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD
VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
TQKSLSLSPGKID*PNKGSGTTSGTTRLLSGHTCFTLTGLLGTLVTMGLLT* (SEQ ID NO: 290) mPDL1-GPI >Artificial Sequence; **mPDL1**-*GPI*, DNA

ATGAGGATATTTGCTGGCATTATATTCACAGCCTGCTGTCACTTGCTACGGGCGTTT
ACTATCACGGCTCCAAAGGACTTGTACGTGGTGGAGTATGGCAGCAACGTCACGATG
GAGTGCAGATTCCCTGTAGAACGGGAGCTGGACCTGCTTGCGTTAGTGGTGTACTGG
GAAAAGGAAGATGAGCAAGTGATTCAGTTTGTGGCAGGAGAGGAGGACCTTAAGCCT
CAGCACAGCAACTTCAGGGGGAGAGCCTCGCTGCCAAAGGACCAGCTTTTGAAGGGA
AATGCTGCCCTTCAGATCACAGACGTCAAGCTGCAGGACGCAGGCGTTTACTGCTGC
ATAATCAGCTACGGTGGTGCGGACTACAAGCGAATCACGCTGAAAGTCAATGCCCCA
TACCGCAAAATCAACCAGAGAATTTCCGTGGATCCAGCCACTTCTGAGCATGAACTA
ATATGTCAGGCCGAGGGTTATCCAGAAGCTGAGGTAATCTGGACAAACAGTGACCAC
CAACCCGTGAGTGGGAAGAGAAGTGTCACCACTTCCCGGAZAGAGGGGATGCTTCTC
AATGTGACCAGCAGTCTGAGGGTCAACGCCACAGCGAATGATGTTTTCTACTGTACG
TTTTGGAGATCACAGCCAGGGCAAAACCACACAGCGGAGCTGATCATCCCAGAACTG
CCTGCAACACATCCTCCACAGAACAGGACT*CCAAATAAAGGAAGTGGAACCACTTCA*
GGTACTACCCGTCTTCTATCTGGGCACACGTGTTTCACGTTGACAGGTTTGCTTGGG
ACGCTAGTAACCATGGGCTTGCTGACTTAG (SEQ ID NO: 291) >Artificial Sequence; **mPDL1**-*GPI*, Amino Acid

MRIFAGIIFTACCHLLRAFTITAPKDLYVVEYGSNVTMECRFPVERELDLLALVVYW
EKEDEQVIQFVAGEEDLKPQHSNFRGRASLPKDQLLKGNAALQITDVKLQDAGVYCC
IISYGGADYKRITLKVNAPYRKINQRISVDPATSEHELICQAEGYPEAEVIWTNSDH
QPVSGKRSVTTSRTEGMLLNVTSSLRVNATANDVFYCTFWRSQPGQNHTAELIIPEL
PATHPPQNRT*PNKGSGTTSGTTRLLSGHTCFTLTGLLGTLVTMGLLT* (SEQ ID NO: 292) mPDL2-C1C2 >Artificial Sequence; **mPDL2**-*C1C2*, DNA

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AGCCTGGAGTGCGATTTTGACCGCAGAGAATGCACTGAACTGGAAGGGATAAGAGCC
AGTTTGCAGAAGGTAGAAAATGATACGTCTCTGCAAAGTGAAAGAGCCACCCTGCTG
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GATTCCGGGCAGTACCGTTGCCTGGTCATCTGCGGGGCCGCCTGGGACTACAAGTAC
CTGACGGTGAAAGTCAAAGCTTCTTACATGAGGATAGACACTAGGATCCTGGAGGTT
CCAGGTACAGGGGAGGTGCAGCTTACCTGCCAGGCTAGAGGTTATCCCCTAGCAGAA
GTGTCCTGGCAAAATGTCAGTGTTCCTGCCAACACCAGCCACATCAGGACCCCCGAA
GGCCTCTACCAGGTCACCAGTGTTCTGCGCCTCAAGCCTCAGCCTAGCAGAAACTTC
AGCTGCATGTTCTGGAATGCTCACATGAAGGAGCTGACTTCAGCCATCATTGACCCT
CTGAGTCGGATGGAACCCAAAGTCCCCAGAACGATCGAT*GTCGAGCCACTGGGCATG*
GAGAATGGGAACATTGCCAACTCACAGATCGCCGCCTCATCTGTGCGTGTGACCTTC
TTGGGTTTGCAGCATTGGGTCCCGGAGCTGGCCCGCCTGAACCGCGCAGGCATGGTC
AATGCCTGGACACCCAGCAGCAATGACGATAACCCCTGGATCCAGGTGAACCTGCTG
CGGAGGATGTGGGTAACAGGTGTGGTGACGCAGGGTGCCAGCCGCTTGGCCAGTCAT

GAGTACCTGAAGGCCTTCAAGGTGGCCTACAGCCTTAATGGACACGAATTCGATTTC
ATCCATGATGTTAATAAAAAACACAAGGAGTTTGTGGGTAACTGGAACAAAAACGCG
GTGCATGTCAACCTGTTTGAGACCCCTGTGGAGGCTCAGTACGTGAGATTGTACCCC
ACGAGCTGCCACACGGCCTGCACTCTGCGCTTTGAGCTACTGGGCTGTGAGCTGAAC
GGATGCGCCAATCCCCTGGGCCTGAAGAATAACAGCATCCCTGACAAGCAGATCACG
GCCTCCAGCAGCTACAAGACCTGGGGCTTGCATCTCTTCAGCTGGAACCCCTCCTAT
GCACGGCTGGACAAGCAGGGCAACTTCAACGCCTGGGTTGCGGGGAGCTACGGTAAC
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>Artificial Sequence; **mPDL2**-*C1C2*, Amino Acid
MLLLLPILNLSLQLHPVAALFTVTAPKEVYTVDVGSSVSLECDFDRRECTELEGIRA
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LTVKVKASYMRIDTRILEVPGTGEVQLTCQARGYPLAEVSWQNVSVPANTSHIRTPE
GLYQVTSVLRLKPQPSRNFSCMFWNAHMKELTSAIIDPLSRMEPKVPRTID*VEPLGM*
ENGNIANSQIAASSVRVTFLGLQHWVPELARLNRAGMVNAWTPSSNDDNPWIQVNLL
RRMWVTGVVTQGASRLASHEYLKAFKVAYSLNGHEFDFIHDVNKKHKEFVGNWNKNA
VHVNLFETPVEAQYVRLYPTSCHTACTLRFELLGCELNGCANPLGLKNNSIPDKQIT
ASSSYKTWGLHLFSWNPSYARLDKQGNFNAWVAGSYGNDQWLQIFPGNWDNHSHKKN
LFETPILARYVRILPVAWHNRIALRLELLGC (SEQ ID NO: 294) mPDL2-Fc-GPI
>Artificial Sequence; **mPDL2**-Fc-*GPI*, DNA
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TTATTCACCGTGACAGCCCCTAAAGAAGTGTACACCGTAGACGTCGGCAGCAGTGTG
AGCCTGGAGTGCGATTTTGACCGCAGAGAATGCACTGAACTGGAAGGGATAAGAGCC
AGTTTGCAGAAGGTAGAAAATGATACGTCTCTGCAAAGTGAAAGAGCCACCCTGCTG
GAGGAGCAGCTGCCCCTGGGAAAGGCTTTGTTCCACATCCCTAGTGTCCAAGTGAGA
GATTCCGGGCAGTACCGTTGCCTGGTCATCTGCGGGGCCGCCTGGGACTACAAGTAC
CTGACGGTGAAAGTCAAAGCTTCTTACATGAGGATAGACACTAGGATCCTGGAGGTT
CCAGGTACAGGGGAGGTGCAGCTTACCTGCCAGGCTAGAGGTTATCCCCTAGCAGAA
GTGTCCTGGCAAAATGTCAGTGTTCCTGCCAACACCAGCCACATCAGGACCCCCGAA
GGCCTCTACCAGGTCACCAGTGTTCTGCGCCTCAAGCCTCAGCCTAGCAGAAACTTC
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GACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAG
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AAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCC
GTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTG
CTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGG
TGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCACGAGGCTCTGCACAACCAC
TACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAATCGAT*CCAAATAAAGGAAGT*
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>Artificial Sequence; **mPDL2**-Fc-*GPI*, Amino Acid
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LTVKVKASYMRIDTRILEVPGTGEVQLTCQARGYPLAEVSWQNVSVPANTSHIRTPE
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PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKID*PNKGS*
GTTSGTTRLLSGHTCFTLTGLLGTLVTMGLLT (SEQ ID NO: 296) mPDL1-mFc-GPI
>Artificial Sequence; **mPDL1**-mFc-*GPI*, DNA
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GAGTGCAGATTCCCTGTAGAACGGGAGCTGGACCTGCTTGCGTTAGTGGTGTACTGG
GAAAAGGAAGATGAGCAAGTGATTCAGTTTGTGGCAGGAGAGGAGGACCTTAAGCCT
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AATGCTGCCCTTCAGATCACAGACGTCAAGCTGCAGGACGCAGGCGTTTACTGCTGC
ATAATCAGCTACGGTGGTGCGGACTACAAGCGAATCACGCTGAAAGTCAATGCCCCA
TACCGCAAAATCAACCAGAGAATTTCCGTGGATCCAGCCACTTCTGAGCATGAACTA
ATATGTCAGGCCGAGGGTTATCCAGAAGCTGAGGTAATCTGGACAAACAGTGACCAC
CAACCCGTGAGTGGGAAGAGAAGTGTCACCACTTCCCGGACAGAGGGGATGCTTCTC
AATGTGACCAGCAGTCTGAGGGTCAACGCCACAGCGAATGATGTTTTCTACTGTACG
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CCTGCAACACATCCTCCACAGAACAGGACTGGTTGTAAGCCTTGCATATGTACAGTC
CCAGAAGTATCATCTGTCTTCATCTTCCCCCCAAAGCCCAAGGATGTGCTCACCATT
ACTCTGACTCCTAAGGTCACGTGTGTTGTGGTAGACATCAGCAAGGATGATCCCGAG
GTCCAGTTCAGCTGGTTTGTAGATGATGTGGAGGTGCACACAGCTCAGACGCAACCC
CGGGAGGAGCAGTTCAACAGCACTTTCCGCTCAGTCAGTGAACTTCCCATCATGCAC
CAGGACTGGCTCAATGGCAAGGAGTTCAAATGCAGGGTCAACAGTGCAGCTTTCCCT
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TGCTCTGTGTTACATGAGGGCCTGCACAACCACCATACTGAGAAGAGCCTCTCCCAC
TCTCCTGGTAAA*CCAAATAAAGGAAGTGGAACCACTTCAGGTACTACCCGTCTTCTA*
TCTGGGCACACGTGTTTCACGTTGACAGGTTTGCTTGGGACGCTAGTAACCATGGGC
TTGCTGACTTAG (SEQ ID NO: 297) >Artificial Sequence; **mPDL1**-mFc-*GPI*,
Amino Acid
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VQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFP
APIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQ
PAENYKNTQPIMDTDGSYFVYSKLNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSH
SPGK*PNKGSGTTSGTTRLLSGHTCFTLTGLLGTLVTMGLLT* (SEQ ID NO: 298) mPDL2-
GPI >Artificial Sequence; **mPDL2**-*GPI*, DNA
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AGTTTGCAGAAGGTAGAAAATGATACGTCTCTGCAAAGTGAAAGAGCCACCCTGCTG

GAGGAGCAGCTGCCCCTGGGAAAGGCTTTGTTCCACATCCCTAGTGTCCAAGTGAGA
GATTCCGGGCAGTACCGTTGCCTGGTCATCTGCGGGGCCGCCTGGGACTACAAGTAC
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TCAGGTACTACCCGTCTTCTATCTGGGCACACGTGTTTCACGTTGACAGGTTTGCTT
GGGACGCTAGTAACCATGGGCTTGCTGACTTAG (SEQ ID NO: 299) >Artificial Sequence; **mPDL2**-*GPI*, Amino Acid
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GLYQVTSVLRLKPQPSRNFSCMFWNAHMKELTSAIIDPLSRMEPKVPRT*PNKGSGTT*
SGTTRLLSGHTCFTLTGLLGTLVTMGLLT (SEQ ID NO: 300) mPDL1-GPI-P2A- >Artificial Sequence; **mPDL**1-*GPI*-P2A-**mHVEM**-*GPI*, DNA mHVEM-GPI
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GAGTGCAGATTCCCTGTAGAACGGGAGCTGGACCTGCTTGCGTTAGTGGTGTACTGG
GAAAAGGAAGATGAGCAAGTGATTCAGTTTGTGGCAGGAGAGGAGGACCTTAAGCCT
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CAACCCGTGAGTGGGAAGAGAAGTGTCACCACTTCCCGGACAGAGGGGATGCTTCTC
AATGTGACCAGCAGTCTGAGGGTCAACGCCACAGCGAATGATGTTTTCTACTGTACG
TTTTGGAGATCACAGCCAGGGCAAAACCACACAGCGGAGCTGATCATCCCAGAACTG
CCTGCAACACATCCTCCACAGAACAGGACT*CCAAATAAAGGAAGTGGAACCACTTCA*
GGTACTACCCGTCTTCTATCTGGGCACACGTGTTTCACGTTGACAGGTTTGCTTGGG
*ACGCTAGTAACCATGGGCTTGCTGACT*GGAAGCGGAGCTACTAACTTCAGCCTGCTG
AAGCAGGCTGGCGACGTGGAGGAGAACCCTGGACCT**ATGGAACCTCTCCCAGGATGG**
GGGTCGGCACCCTGGAGCCAGGCCCCTACAGACAACACCTTCAGGCTGGTGCCTTGT
GTCTTCCTTTTGAACTTGCTGCAGCGCATCTCTGCCCAGCCCTCATGCAGACAGGAG
GAGTTCCTTGTGGGAGACGAGTGCTGCCCCATGTGCAACCCAGGTTACCATGTGAAG
CAGGTCTGCAGTGAGCATACAGGCACAGTGTGTGCCCCCTGTCCCCCACAGACATAT
ACCGCCCATGCAAATGGCCTGAGCAAGTGTCTGCCCTGCGGAGTCTGTGATCCAGAC
ATGGGCCTGCTGACCTGGCAGGAGTGCTCCAGCTGGAAGGACACTGTGTGCAGATGC
ATCCCAGGCTACTTCTGTGAGAACCAGGATGGGAGCCACTGTTCCACATGCTTGCAG
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AGCACAGACACCACCTGCTCCTCCCAG*CCAAATAAAGGAAGTGGAACCACTTCAGGT*
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CTAGTAACCATGGGCTTGCTGACTTAG (SEQ ID NO: 301) >Artificial Sequence; **mPDL1**-*GPI*-P2A-**mHVEM**-*GPI*, Amino Acid
MRIFAGIIFTACCHLLRAFTITAPKDLYVVEYGSNVTMECRFPVERELDLLALVVYW
EKEDEQVIQFVAGEEDLKPQHSNFRGRASLPKDQLLKGNAALQITDVKLQDAGVYCC
IISYGGADYKRITLKVNAPYRKINQRISVDPATSEHELICQAEGYPEAEVIWTNSDH

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KQAGDVEENPGP**MEPLPGWGSAPWSQAPTDNTFRLVPCVFLLNLLQRISAQPSCRQE**
EFLVGDECCPMCNPGYHVKQVCSEHTGTVCAPCPPQTYTAHANGLSKCLPCGVCDPD
MGLLTWQECSSWKDTVCRCIPGYFCENQDGSHCSTCLQHTTCPPGQRVEKRGTHDQD
TVCADCLTGTFSLGGTQEECLPWTNCSAFQQEVRRGTNSTDTTCSSQ*PNKGSGTTSG*
TTRLLSGHTCFTLTGLLGTLVTMGLLT (SEQ ID NO: 302) hPDL1-ADAM10 >Artificial Sequence; **hPDL1**-*ADAM10*, DNA
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MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKEPVEKQLDLAALIVYW
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GRTITLQPGSPCNDFRGYCDVFMRCRLVDADGPLARLKKAIFSPELYENIAEWIVAH
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hPDL1-4Fc- >Artificial Sequence; **hPDL1**-4Fc-*CD9tm2-KRAS*, DNA CD9tm2-
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modified KRas
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(SEQ ID NO: 307) >Artificial Sequence; **hPDL1**-4Fc-*CD9tm2*-*KRAS*, Amino Acid
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ID NO: 308) hPDL1-Fc- >Artificial Sequence; **hPDL1**-Fc-*CD9tm2*, DNA CD9tm2
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ATTCTGATCGGAGCCGGCGCCCTCATGATGCTGGTGGGCTTCCTGGGCTGCTGCGGG GCTGTGCAGGAGTCCCAGTGCGTAATTATGTAA (SEQ ID NO: 309) >Artificial Sequence; **hPDL1**-Fc-*CD9tm2*, Amino Acid

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYW EMEDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRC MISYGGADYKRITVKVNAPYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSD HQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPE LPLAHPPNERIDDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGKID*FYTGVYILIGAGALMMLVGFLGCCGAVQESQCVIM* (SEQ ID NO: 310) hPDL1-Fc- >Artificial Sequence; **hPDL1**-Fc-*CD9tm2-KRAS*, DNA CD9tm2-

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MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYW EMEDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRC MISYGGADYKRITVKVNAPYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSD HQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPE LPLAHPPNERIDDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV

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(SEQ ID NO: 312) mPDL1-mFc- >Artificial Sequence; **mPDL1**-mFc-*CD9tm2*, DNA CD9tm2
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EKEDEQVIQFVAGEEDLKPQHSNFRGRASLPKDQLLKGNAALQITDVKLQDAGVYCC
IISYGGADYKRITLKVNAPYRKINQRISVDPATSEHELICQAEGYPEAEVIWTNSDH
QPVSGKRSVTTSRTEGMLLNVTSSLRVNATANDVFYCTFWRSQPGQNHTAELIIPEL
PATHPPQNRTGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVDISKDDPE
VQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFP
APIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQ
PAENYKNTQPIMDTDGSYFVYSKLNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSH
SPGK*FYTGVYILIGAGALMMLVGFLGCCGAVQESQCKKKKKKKKTKCVIM* (SEQ ID NO: 316)

(98) In some embodiments of any of the aspects, the fusion polypeptides provided herein comprise two or more POI domains. The specific combinations of POI domains can be used to regulate inflammatory immune responses. Non-limiting examples of additive and synergistic combinations of POIs that can modulate inflammatory signaling pathways are provided in Table 5 (below).

(99) TABLE-US-00007 TABLE 5 Exemplary POI combinations and combined targets for modulating inflammation. COMBINED PUTATIVE ADDITIVE or POIs (LIGANDS) TARGETS SYNERGISTIC MOA PD-L1 or PD-L2 PD-1 Differential use of Shp HVEM BTLA phosphatases. BTLA inhibits both TCR and CD28 phosphorylation (via Shpl) while PD-1 inhibits CD28 phosphorylation (via Shp2). PD-L1 or PD-L2 PD-1 LAG-3 exerts differential FGL1 LAG-3 inhibitory impacts on various types of lymphocytes and shows synergy with PD-1 to inhibit immune responses. PD-L1 or PD-L2 PD-1 PD-1 and Tim-3 have non- CEACAM-1 or GAL9 TIM-3 redundant downstream signaling mechanisms. PD-L1 or PD-L2 PD-1 Differential use of Shp CD155 TIGIT phosphatases. Non-redundantly regulate T cell responses. PD-L1 or PD-L2 PD-1 PD-1 and VISTA non- VSIG3 VISTA redundantly regulate T cell responses. VISTA contains cytosolic SH3 binding domains for adapter proteins. CEACAM-1 or GAL9 TIM-3 TIGIT and TIM-3 have non- CD155 TIGIT redundant downstream signaling mechanisms. PD-L1 or PD-L2 PD-1 PD-1, LAG-3 and TIM-3 have FGL1 LAG-3 non-redundant downstream CEACAM-1 or GAL9 TIM-3 signaling mechanisms.

Methods of Preparing Extracellular Vesicle Compositions

(100) In another aspect, provided herein is a method of preparing an engineered extracellular vesicle provided herein. Generally, the method comprises providing a population of cells expressing a vector construct encoding one or more sticky binder (vesicle targeting domain) and one or more signaling domains (POI domain).
(101) The EVs provided herein can be isolated and purified form any biological source, e.g., cells. The cells that produce the engineered EVs provided herein can be from any viable non-human source or organism. Usually the organism is an animal, vertebrate, or mammal. In some embodiments, the cell described herein is from a human. The cells described herein can be from any tissue isolated from an organism by methods known in the art. The scientific literature provides guidance for one of ordinary skill in the art to isolate, prepare, and culture cells as necessary for use in the compositions and methods described herein. One of skill in the art can appreciate that the cell source of the EVs may alter the cellular protein expression and the native or endogenous cargo within the EV. It is contemplated herein that this can be leveraged for therapeutic effect depending on the disease or disorder being treated.
(102) In some embodiments, the population of cells has been altered by exposure to environmental conditions (e.g., hypoxia), small molecule addition, presence/absence of exogenous factors (e.g., growth factors, cytokines) at the time, or substantially contemporaneous with, isolating the plurality of artificial synapses in a manner altering the regulatory state of the cell. In various embodiments, the cells are HEK 293 cells, MSCs, PER.C, fibrosarcoma HT-1080 or HuH7 cell lines.
(103) The method comprises providing a population of cells and culturing the cells in serum-free or un-concentrated conditioned medium. This includes, for example, artificial synapses secreted into media as conditioned by a population of cells in culture, further including cell lines capable of serial passaging. In certain embodiments, the cells in culture are grown to 10, 20, 30, 40, 50, 60, 70, 80, 90, or 90% or more confluency when artificial synapses (engineered EVs) are isolated.
(104) The methods provided herein further comprise contacting the cells provided herein with a nucleic acid vector encoding the at least one fusion polypeptide provided herein. The vector can be added to the cell culture medium of the cells by methods known in the art and discussed further below.
(105) A vector is a nucleic acid construct designed for delivery to a host cell or for transfer of genetic material between different host cells. As used herein, a vector can be viral or non-viral. The term “vector” encompasses any genetic element that is capable of replication when associated with the proper control elements and that can transfer genetic material to cells. A vector can include, but is not limited to, a cloning vector, an expression vector, a plasmid, phage, transposon, cosmid, artificial chromosome, virus, virion, etc. In some embodiments of any of the aspects, the vector is selected from the group consisting of: a plasmid, a cosmid and a viral vector.
(106) “Expression” refers to the cellular processes involved in producing RNA and proteins and as appropriate, secreting proteins, including where applicable, but not limited to, for example, transcription, transcript processing, translation and protein folding, modification and processing. “Expression products” include RNA transcribed from a gene, and polypeptides obtained by translation of mRNA transcribed from a gene.
(107) In some embodiments, a vector is capable of driving expression of one or more sequences in a mammalian cell; i.e., the vector is a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, 1987. Nature 329:840) and pMT2PC (Kaufman, et al., 1987. *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are typically provided by one or more regulatory elements. For example, commonly used promoters are derived from polyoma, adenovirus 2, cytomegalovirus, simian virus 40, and others disclosed herein and known in the art. For other suitable expression systems for both prokaryotic and eukaryotic cells see, e.g., Chapters 16 and 17 of Sambrook, et al., MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor

Laboratory Press, Cold Spring Harbor, N.Y., 1989.
(108) In some embodiments, the recombinant expression vector is capable of directing expression of the exogenous fusion polypeptide nucleic acid sequence preferentially in a particular cell type (e.g., via tissue-specific regulatory elements).
(109) Tissue-specific and inducible regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert, et al., 1987. *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton, 1988. *Adv. hnmunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore, 1989. *EMBO J.* 8:729-733) and immunoglobulins (Baneiji, et al., 1983. *Cell* 33:729-740; Queen and Baltimore, 1983. *Cell* 33:741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle, 1989. *Proc. Natl. Acad. Sci. USA* 86:5473-5477), pancreas-specific promoters (Edlund, et al., 1985. *Science* 230:912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Pat. No. 4,873,316 and European Application Publication No. 264, 166). Developmentally-regulated promoters are also encompassed, e.g., the murine hox promoters (Kessel and Gruss, 1990. *Science* 249:374-379) and the α-fetoprotein promoter (Campes and Tilghman, 1989. *Genes Dev.* 3:537-546).
(110) In some embodiments, the at least one nucleic acid sequence described herein is delivered to the cell described herein via an integrating vector. Integrating vectors have their delivered genetic material (or a copy of it) permanently incorporated into a host cell chromosome. Non-integrating vectors remain episomal which means the nucleic acid contained therein is never integrated into a host cell chromosome. Examples of integrating vectors include retroviral vectors, lentiviral vectors, hybrid adenoviral vectors, and herpes simplex viral vectors.
(111) In some embodiments, the at least one nucleic acid sequence described herein is delivered to the cell described herein via a non-integrative vector. Non-integrative vectors include non-integrative viral vectors. Non-integrative viral vectors eliminate one of the primary risks posed by integrative retroviruses, as they do not incorporate their genome into the host DNA. One example is the Epstein Barr oriP/Nuclear Antigen-1 (“EBNA1”) vector, which is capable of limited self-replication and known to function in mammalian cells. Containing two elements from Epstein-Barr virus, oriP and EBNA1, binding of the EBNA1 protein to the virus replicon region oriP maintains a relatively long-term episomal presence of plasmids in mammalian cells. This particular feature of the oriP/EBNA1 vector makes it ideal for generation of integration-free host cells. Other non-integrative viral vectors include adenoviral vectors and the adeno-associated viral (AAV) vectors.
(112) Another non-integrative viral vector is RNA Sendai viral vector, which can produce protein without entering the nucleus of an infected cell. The F-deficient Sendai virus vector remains in the cytoplasm of infected cells for a few passages, but is diluted out quickly and completely lost after several passages (e.g., 10 passages). This permits a self-limiting transient expression of a chosen heterologous gene or genes in a target cell. This aspect can be helpful, e.g., for the transient introduction of reprogramming factors, among other uses. As noted above, in some embodiments, the nucleic acid sequence described herein is expressed in the cells from a viral vector.
(113) A “viral vector” includes a nucleic acid vector construct that includes at least one element of viral origin and has the capacity to be packaged into a viral vector particle. The viral vector can contain a nucleic acid encoding a polypeptide described herein in place of non-essential viral genes. The vector and/or particle can be utilized for the purpose of transferring nucleic acids into cells either in vitro or in vivo.
(114) The nucleic acids described herein can be delivered using any transfection reagent or other physical means that facilitates entry of nucleic acids into a cell. Methods of non-viral delivery of nucleic acids include lipofection, nucleofection, microinjection, electroporation, biolistics, virosomes, liposomes, immunoliposomes, polycation or lipid: nucleic acid conjugates, naked DNA, artificial virions, and agent-enhanced uptake of DNA. Lipofection is described in e.g., U.S. Pat. Nos. 5,049,386, 4,946,787; and 4,897,355) and lipofection reagents are sold commercially (e.g.,

Transfectam™ and Lipofectin™). Cationic and neutral lipids that are suitable for efficient receptor-recognition lipofection of polynucleotides include those of Felgner, WO 91/17424; WO 91/16024. Delivery can be to cells (e.g., in vitro or ex vivo administration) or target tissues (e.g., in vivo administration).

(115) The preparation of lipid: nucleic acid complexes, including targeted liposomes such as immunolipid complexes, is well known to one of skill in the art (see, e.g., Crystal, Science 270:404-410 (1995); Blaese et al., Cancer Gene Ther. 2:291-297 (1995); Behr et al., Bioconjugate Chem. 5:382-389 (1994); Remy et al., Bioconjugate Chem. 5:647-654 (1994); Gao et al., Gene Therapy 2:710-722 (1995); Ahmad et al., Cancer Res. 52:4817-4820 (1992); U.S. Pat. Nos. 4,186,183, 4,217,344, 4,235,871, 4,261,975, 4,485,054, 4,501,728, 4,774,085, 4,837,028, and 4,946,787).

(116) An “agent that increases cellular uptake” is a molecule that facilitates transport of a molecule, e.g., nucleic acid, or peptide or polypeptide, or other molecule that does not otherwise efficiently transit the cell membrane across a lipid membrane. For example, a nucleic acid can be conjugated to a lipophilic compound (e.g., cholesterol, tocopherol, etc.), a cell penetrating peptide (CPP) (e.g., penetratin, TAT, Syn1B, etc.), or a polyamine (e.g., spermine). Further examples of agents that increase cellular uptake are disclosed, for example, in Winkler (2013). *Oligonucleotide conjugates for therapeutic applications. Ther. Deliv.* 4 (7); 791-809. The one or more nucleic acid sequences encoding the fusion polypeptides provided herein can be delivered to the cell by any method discussed above or known in the art.

(117) In some embodiments of any of the aspects, the vectors provided herein comprise a nucleic acid modification by methods known in the art. In some embodiments, the cell can be genetically manipulated to express one or more vectors, each encoding one or more vesicle targeting domains and/or one or more signaling domains. In certain embodiments, the population of cells has been genetically manipulated. This includes, for example, knockout (KO) or transgenic (TG) cell lines, wherein an endogenous gene has been removed and/or an exogenous introduced in a stable, persistent manner. In certain embodiments, this further includes transient knockdown of one or more genes and associated coding and non-coding transcripts within the population of cells, via any number of methods known in the art, such as introduction of dsRNA, siRNA, microRNA, etc. This further includes transient expression of one or more genes and associated coding and non-coding transcripts within the population of cells, via any number of methods known in the art, such as introduction of a vector, plasmid, artificial plasmid, replicative and/or non-replicative virus, etc.

(118) In certain embodiments the cell population has been manipulated to knockout the expression of one or more endogenous gene sequences that encode for metalloendopeptidases. In certain embodiments the cell population has been manipulated to knockout the expression of one or more endogenous gene sequences that code for metalloproteinases. In certain embodiments the cell population has been manipulated to knockout the expression of one or more endogenous gene sequences that encode for a disintegrin and metalloproteinase (ADAM). For example, the cell population can be manipulated to knock of the expression of one or more gene sequences that encode for ADAM1, ADAM2, ADAM7, ADAM8, ADAM9, ADAM10, ADAM11, ADAM12, ADAM15, ADAM17, ADAM18, ADAM19, ADAM20, ADAM21, ADAM22, ADAM23, ADAM28, ADAM29, ADAM30, ADAM33, etc.

(119) In certain embodiments the cell population has been manipulated to knockout the expression of one or more endogenous genes that encode for enzymes that hydrolyze the inositol phosphate linkage in proteins anchored by phosphatidylinositol glycans, thereby preventing the release of proteins attached to the plasma membrane via GPI anchors. For example, the cell population can be manipulated to knock of the expression of phosphatidylinositol-glycan-specific phospholipase D (GPLD1).

(120) In certain embodiments, the population of cells has been genetically manipulated. This includes, for example, knock-in of an exogenous genetic sequence, wherein the exogenous genetic

sequence is expressed in a stable, persistent manner. In certain embodiments, the cell population has been manipulated to knock-in recombinase recognition sequences (e.g., FRT), transgenic reporters such as antibiotic resistance genes, fluorescent or enzymatic reporter genes, etc. or the like.

(121) In some embodiments, the method comprises a step of isolating the engineered extracellular vesicles provided herein. Particulates within the medium are removed by a series of specific centrifugation steps and the media is filtered. The general method of isolating extracellular vesicles as provided herein is depicted in FIG. **21** of the working examples. Methods of isolating and purifying the extracellular vesicles and exosomes are known in the art and further described, e.g., in Whitford W, Guterstam P. Exosome manufacturing status. Future Med Chem. 2019 May; 11(10):1225-1236. doi: 10.4155/fmc-2018-0417. PMID: 31280675, Patel D B, Santoro M, Born L J, Fisher J P, Jay S M. Towards rationally designed biomanufacturing of therapeutic extracellular vesicles: impact of the bioproduction microenvironment. Biotechnol Adv. 2018 December; 36 (8):2051-2059 . . . doi: 10.1016/j.biotechadv.2018.09.001. Epub 2018 Sep. 12. PMID: 30218694; PMCID: PMC6250573, Ng K S, Smith J A, McAteer M P, Mead B E, Ware J, Jackson F O, Carter A, Ferreira L, Bure K, Rowley J A, Reeve B, Brindley D A, Karp J M. Bioprocess decision support tool for scalable manufacture of extracellular vesicles. Biotechnol Bioeng. 2019 February; 116 (2):307-319. doi: 10.1002/bit.26809. Epub 2018 Nov. 8. PMID: 30063243; PMCID: PMC6322973, Paganini C, Capasso Palmiero U, Pocsfalvi G, Touzet N, Bongiovanni A, Arosio P. Scalable Production and Isolation of Extracellular Vesicles: Available Sources and Lessons from Current Industrial Bioprocesses. Biotechnol J. 2019 October; 14 (10):e1800528. doi: 10.1002/biot.201800528. Epub 2019 Jul. 8. PMID: 31140717, which are incorporated herein by reference in their entireties.

(122) In some embodiments, isolating the plurality of engineered EVs (artificial synapses) includes precipitation, centrifugation, filtration, immuno-separation, tangential flow, liquid chromatography, and/or flow fractionation. For example, differential ultracentrifugation has become a technique wherein secreted exosomes are isolated from the supernatants of cultured cells. This approach allows for separation of exosomes from non-membranous particles, by exploiting their relatively low buoyant density. Size exclusion allows for their separation from biochemically similar, but biophysically different microvesicles, which possess larger diameters of up to 1,000 nm. Differences in floatation velocity further allows for separation of differentially sized exosomes. In general, exosome sizes will possess a diameter ranging from 30-300 nm, including sizes of 30-150 nm. Further purification may rely on specific properties of the particular exosomes of interest. This includes, for example, use of immunoadsorption with a protein of interest to select specific vesicles with exoplasmic or outward orientations.

(123) Among current methods (differential centrifugation, discontinuous density gradients, immunoaffinity, ultrafiltration and liquid chromatography (e.g., fast protein liquid chromatography (FPLC)), differential ultracentrifugation is the most commonly used for exosome isolation. This technique utilizes increasing centrifugal force from 2000×g to 10,000×g to separate the medium- and larger-sized particles and cell debris from the exosome pellet at 100,000×g. Centrifugation alone allows for significant separation/collection of exosomes from a conditioned medium, although it is insufficient to remove various protein aggregates, genetic materials, particulates from media and cell debris that are common contaminants. Enhanced specificity of exosome purification may deploy sequential centrifugation in combination with ultrafiltration, or equilibrium density gradient centrifugation in a sucrose density gradient, to provide for the greater purity of the exosome preparation (flotation density 1.1-1.2 g/ml) or application of a discrete sugar cushion in preparation.

(124) Ultrafiltration can be used to purify exosomes without compromising their biological activity. Membranes with different pore sizes-such as 100 kDa molecular weight cut-off (MWCO) or 300 kDa MWCO and gel filtration to eliminate smaller particles—have been used to avoid the use of a

nonneutral pH or non-physiological salt concentration. Currently available tangential flow filtration (TFF) systems are scalable (to >10,000 L), allowing one to not only purify, but concentrate the exosome fractions, and such approaches are less time consuming than differential centrifugation. Liquid Chromatography can also be used to purify exosomes to homogeneously sized particles and preserve their biological activity as the preparation is maintained at a physiological pH and salt concentration.

(125) Other chemical methods have exploit differential solubility of exosomes for precipitation techniques, addition to volume-excluding polymers (e.g., polyethylene glycols (PEGs)), possibly combined additional rounds of centrifugation or filtration. For example, a precipitation reagent, ExoQuick®, can be added to conditioned cell media to quickly and rapidly precipitate a population of exosomes, although re-suspension of pellets prepared via this technique may be difficult. Flow field-flow fractionation (FIFFF) is an elution-based technique that is used to separate and characterize macromolecules (e.g., proteins) and nano- to micro-sized particles (e.g., organelles and cells) and which has been successfully applied to fractionate exosomes from culture media.

(126) Beyond these techniques relying on general biochemical and biophysical features, focused techniques may be applied to isolated specific exosomes of interest. This includes relying on antibody immunoaffinity to recognizing certain exosome-associated antigens. Conjugation to magnetic beads, chromatography matrices, plates or microfluidic devices allows isolating of specific exosome populations of interest as may be related to their production from a parent cell of interest or associated cellular regulatory state. Other affinity-capture methods use lectins which bind to specific saccharide residues on the exosome surface.

(127) In several embodiments, isolating a plurality of artificial synapses from the population of cells includes centrifugation of the cells and/or media conditioned by the cells. In several embodiments, ultracentrifugation is used. In several embodiments, isolating a plurality of artificial synapses from the population of cells is via size-exclusion filtration. In other embodiments, isolating a plurality of artificial synapses from the population of cells includes use of discontinuous density gradients, immunoaffinity, ultrafiltration, tangential flow and/or liquid chromatography.

(128) In certain embodiments, differential ultracentrifugation includes using centrifugal force from 1000-2000×g, 2000-3000×g, 3000-4000×g, 4000-5000×g, 5000×g-6000×g, 6000-7000×g, 7000-8000×g, 8000-9000×g, 9000-10,000×g, to 10,000×g or more to separate larger-sized particles from a plurality of artificial synapses derived from the cells.

(129) In other embodiments, isolating a plurality of artificial synapses from the population of cells includes use of filtration or ultrafiltration. In certain embodiments, a size exclusion membrane with different pore sizes is used. For example, a size exclusion membrane can include use of a filter with a pore size of 0.1-0.5 micron (μm), 0.5-1.0 μm, 1-2.5 μm, 2.5-5 μm, 5 or more μm. In certain embodiments, the pore size is about 0.2 μm. In certain embodiments, filtration or ultrafiltration includes size exclusion ranging from 100-500 daltons (Da), 500-1 kDa, 1-2 kDa, 2-5 kDa, 5-10 kDa, 10-25 kDa, 25-50 kDa, 50-100 kDa, 100-250 kDa, 250-500 kDa, 500 or more kDa. In certain embodiments, the size exclusion is for about 2-5 kDa. In certain embodiments, the size exclusion is for about 3 kDa. In other embodiments, filtration or ultrafiltration includes size exclusion includes use of hollow fiber membranes capable of isolating particles ranging from 100-500 daltons (Da), 500-1 kDa, 1-2 kDa, 2-5 kDa, 5-10 kDa, 10-25 kDa, 25-50 kDa, 50-100 kDa, 100-250 kDa, 250-500 kDa, 500 or more kDa. In certain embodiments, the size exclusion is for about 2-5 kDa. In certain embodiments, the size exclusion is for about 3 kDa. In other embodiments, a molecular weight cut-off (MWCO) gel filtration capable of isolating particles ranging from 100-500 daltons (Da), 500-1 kDa, 1-2 kDa, 2-5 kDa, 5-10 kDa, 10-25 kDa, 25-50 kDa, 50-100 kDa, 100-250 kDa, 250-500 kDa, 500 or more kDa. In certain embodiments, the size exclusion is for about 2-5 kDa. In certain embodiments, the size exclusion is for about 3 kDa. In various embodiments, such systems are used in combination with variable fluid flow systems. In certain embodiments, a size exclusion membrane with different pore sizes is used to purify extracellular vesicles from a solution

comprising undesirable proteins or nucleic acids.
(130) In other embodiments, isolating a plurality of artificial synapses from the population of cells includes use of tangential flow filtration (TFF) systems are used purify and/or concentrate the exosome fractions. In other embodiments, isolating a plurality of artificial synapses from the population of cells includes use of liquid chromatography can also be used to purify artificial synapses to homogeneously sized particles. In various embodiments, density gradients as used, such as centrifugation in a sucrose density gradient or application of a discrete sugar cushion in preparation.
(131) In other embodiments, isolating a plurality of artificial synapses from the population of cells includes use of a precipitation reagent. For example, a precipitation reagent, ExoQuick®, can be added to conditioned cell media to quickly and rapidly precipitate a population of artificial synapses. In other embodiments, isolating a plurality of artificial synapses from the population of cells includes use of volume-excluding polymers (e.g., polyethylene glycols (PEGs)) are used. In another embodiment, isolating a plurality of artificial synapses from the population of cells includes use of flow field-flow fractionation (FIFFF), an elution-based technique.
(132) In certain embodiments, isolating a plurality of artificial synapses from the population of cells includes use of one or more capture agents to isolate one or more artificial synapses possessing specific biomarkers or containing particular biological molecules. In one embodiment, one or more capture agents include at least one antibody. For example, antibody immunoaffinity recognizing exosome-associated antigens is used to capture specific artificial synapses. In other embodiments, the at least one antibody are conjugated to a fixed surface, such as magnetic beads, chromatography matrices, plates or microfluidic devices, thereby allowing isolation of the specific exosome populations of interest. In other embodiments, isolating a plurality of artificial synapses from the population of cells includes use of one or more capture agents that is not an antibody. This includes, for example, use of a “bait” molecule presenting an antigenic feature complementary to a corresponding molecule of interest on the exosome surface, such as a receptor or other coupling molecule. In one embodiment, the non-antibody capture agent is a lectin capable of binding to polysaccharide residues on the exosome surface.
(133) In other embodiments, isolating a plurality of artificial synapses from the population of cells includes use of ion exchange chromatography. In other embodiments, isolating a plurality of artificial synapses from the population of cells includes use of anion exchange chromatography. In other embodiments, isolating a plurality of artificial synapses from the population of cells includes use of caion exchange chromatography. In certain embodiments, ion exchange chromatography comprises a chromatography resin with a functional group selected from the group consisting of diethylaminoethyl (DEAE), quaternary aminoethyl (QAE), quaternary ammonium (Q), carboxymethyl (CM), sulfopropyl (SP), or methyl sulfate(S). In certain embodiments, ion exchange chromatography comprises a chromatography resin which may have properties of a weak acid, strong acid, weak base, or strong basic. In certain embodiments, ion exchange chromatography comprises a chromatography selected from the group consisting of DEAE cellulose, DEAE Sephadex, Mono Q, Mini Q, HiTrap Capto, Capto Core 700, HiPrep Q, QAE Sephadex, Q Sepharose, CM Cellulose, SP Sepharose, SOURCE S, EAH-Sepharose, sulfoxyethyl cellulose, CM Sephadex, or CM Sepharose. Isolating a plurality of artificial synapses can be prepared by any of a variety of ion exchange chromatography techniques that are known in the art.
(134) In other embodiments, isolating a plurality of artificial synapses from the population of cells includes use of a nuclease enzyme (e.g., a DNase or RNase). For example, a working concentration of Benzonase® nuclease may be added to an extracellular vesicle sample preparation in the presence of a divalent cation, for example 1-2 mM Mg.sup.2+, 2-5 mM Mg.sup.2+, 10-20 mM Mg.sup.2+, 20-50 mM Mg.sup.2+, 50-100 mM Mg.sup.2+, or more than 100 mM Mg.sup.2+.
(135) Following isolation and purification of the engineered EVs provided herein, EVs can be further evaluated for the desired structural and functional properties by methods known in the art.

For example, the engineered exosomes provided herein can be assayed for functional activity on a target cell using a cell-based bioassays (e.g., those commercially available, PROMEGA® DISCOVERX®), ligand-receptor binding assays, vesicle flow cytometric assays, enzyme-linked immunosorbent assays, tunable resistive pulse sensing (TRPS), nanoparticle tracking analysis (NTA), surface plasmon resonance (SSPR), nucleotide sequencing, lipidomics, proteomics, colorimetric assays, fluorescence assays, luminescence assays, immunoblotting, radioimmunoassays, electron microscopy, or EV automated analysis (e.g., EXOVIEW®). Additional methods of characterizing EVs are found, e.g., in Zhang Y, Bi J, Huang J, Tang Y, Du S, Li P. Exosome: A Review of Its Classification, Isolation Techniques, Storage, Diagnostic and Targeted Therapy Applications. Int J Nanomedicine. 2020 Sep. 22; 15:6917-6934. doi: 10.2147/IJN.S264498. PMID: 33061359; PMCID: PMC7519827, Kluszczyńska K, Czernek L, Cypryk W, Pęczek Ł, Düchler M. Methods for the Determination of the Purity of Exosomes. Curr Pharm Des. 2019; 25(42):4464-4485. doi: 10.2174/1381612825666191206162712. PMID: 31808383, Nolan J P, Duggan E. Analysis of Individual Extracellular Vesicles by Flow Cytometry. Methods Mol Biol. 2018; 1678:79-92. doi: 10.1007/978-1-4939-7346-O_5. PMID: 29071676; Doyle L M, Wang M Z. Overview of Extracellular Vesicles, Their Origin, Composition, Purpose, and Methods for Exosome Isolation and Analysis. Cells. 2019 Jul. 15; 8(7):727. doi: 10.3390/cells8070727. PMID: 31311206; PMCID: PMC6678302, Pugholm L H, Revenfeld A L, Søndergaard E K, Jørgensen M M. Antibody-Based Assays for Phenotyping of Extracellular Vesicles. Biomed Res Int. 2015; 2015:524817. doi: 10.1155/2015/524817. Epub 2015 Dec. 3. PMID: 26770974; PMCID: PMC4681819, Shao H, Im H, Castro C M, Breakefield X, Weissleder R, Lee H. New Technologies for Analysis of Extracellular Vesicles. Chem Rev. 2018 Feb. 28; 118(4):1917-1950. doi: 10.1021/acs.chemrev.7b00534. Epub 2018 Jan. 31. PMID: 29384376; PMCID: PMC6029891, which are incorporated herein by reference in their entireties.

Pharmaceutical Compositions

(136) Provided herein are compositions comprising the engineered extracellular vesicles (artificial synapses) provided herein.

(137) In one aspect, provided herein is a composition comprising: a plurality of the engineered extracellular vesicles provided herein. In some embodiments of any of the aspects, the compositions and engineered EVs provided herein further comprise a pharmaceutically acceptable carrier.

(138) For clinical use of the methods and compositions described herein, administration of the engineered EVs/artificial synapses provided herein can include formulation into pharmaceutical compositions or pharmaceutical formulations for parenteral administration, e.g., intravenous; mucosal, e.g., intranasal; ocular, or other mode of administration. In some embodiments, the engineered EVs described herein can be administered along with any pharmaceutically acceptable carrier compound, material, or composition which results in an effective treatment in the subject. Thus, a pharmaceutical formulation for use in the methods described herein can contain the engineered EVs described herein in combination with one or more pharmaceutically acceptable ingredients. The phrase “pharmaceutically acceptable” refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. The phrase “pharmaceutically acceptable carrier” as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent, media, encapsulating material, manufacturing aid (e.g., lubricant, talc magnesium, calcium or zinc stearate, or steric acid), or solvent encapsulating material, involved in maintaining the stability, solubility, or activity of, an engineered EV as described herein. Each carrier must be “acceptable” in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. The terms “excipient,” “carrier,” “pharmaceutically

acceptable carrier" or the like are used interchangeably herein.
(139) The engineered EVs provided herein can be formulated for administration of the compound to a subject in solid, liquid, or gel form, including those adapted for the following: (1) parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; (2) transdermally; (3) transmucosally; (4) via bronchoalveolar lavage.
(140) In some embodiments, the compositions described herein comprise a particle or polymer-based vehicle. Exemplary particle or polymer-based vehicles include, but are not limited to, nanoparticles, microparticles, polymer microspheres, or polymer-drug conjugates.
(141) In one embodiment of any of the aspects, the compositions described herein further comprise a lipid vehicle. Exemplary lipid vehicles include, but are not limited to, liposomes, phospholipids, micelles, lipid emulsions, and lipid-drug complexes.
(142) Formulations can be adapted for delivery to the airway, e.g., to address respiratory inflammation. Such formulations can be adapted for delivery as an aerosol, e.g., for inhalation. In some embodiments, the compositions described herein are formulated for aerosol administration, nebulizer administration, tracheal lavage administration, or for a pulmonary delivery device.
(143) As used herein, the term "pulmonary delivery device" refers to a device used to deliver a therapeutic dose of a composition of the present invention to the respiratory system including, but not limited to, a nebulizer, metered-dose inhaler, or dry powder inhaler.
(144) Examples of nebulizers include, but are not limited to, soft mist inhalers (for example Respimat® Boehringer Ingelheim) jet nebulizers (use compressed gas or air), ultrasonic nebulizers (produce aerosols using a piezoelectric crystal vibrating at high frequencies), and vibrating mesh nebulizers.
(145) As used herein, the term "jet nebulizer" refers to a device that flows compressed air or gas through a composition of the present invention for aerosolization. The aerosolized composition of the present invention may be inhaled by a patient. Jet nebulizer may include, but is not limited to, jet nebulizers with a corrugated tube, jet nebulizers with a collection bag, breath enhanced jet nebulizers, breath actuated jet nebulizers, and metered-dose inhalers. Examples of jet nebulizers include, but are not limited to, Circulaire (Westmed INC, Tucson, AZ), Pari Inhalierboy (PARI, Midlothian, VA), Pari LC Plus (PARI, Midlothian, VA), NebuTech (Salter Labs, Arvin, CA), AeroEclipse (Monoghan/Trudell Medical International, London, Ontario, Canada), and Maxin MA-2 (MA-2; Clinova Medical AB, Malmö, Sweden). Examples of ultrasonic nebulizers include, but are not limited to, De Vilbiss-Pulmosonic (Somerset, PA), Omron-Microair (Omron, Kyoto, Japan), Omron NE-U17 (Omron, Kyoto, Japan), Rhone Poulenc-Rorer-Fisoneb (Sanofi, Paris, France), and Beurer Nebulizer IH30 (Beurer GmbH, Neu-Ulm, Germany).
(146) As used herein, the term "mesh nebulizer" refers to forcing a liquid, gel, fluid, solution, tincture, or the like through apertures in a mesh or aperture plate to generate aerosol. Mesh nebulizer may include, but is not limited to, active mesh nebulizers and passive mesh nebulizers. Examples of active mesh nebulizers include, but is not limited to, Aeroneb® (Aerogen, Galway, Ireland) and eFlow® (PARI, Midlothian, VA). Examples of passive mesh nebulizers are, but not limited to, I-neb (Philips Respironics, Newark, USA), AKITA (Activaero, Gemunden/Wohra, Germany), and Microair NE-U22® (Omron, Kyoto, Japan).
(147) For use as aerosols, the compositions described herein can be prepared in a solution or suspension and may be packaged in a pressurized aerosol container together with suitable propellants, for example, hydrocarbon propellants like propane, butane, or isobutane with conventional excipients.
(148) The engineered EVs provided herein can also be administered in a non-pressurized form such as in a nebulizer or atomizer that reduces a liquid to a fine spray. Preferably, by such nebulization small liquid droplets of uniform size are produced from a larger body of liquid in a controlled manner. Nebulization can be achieved by any suitable means therefor, including by using many

nebulizers known and marketed today. For example, an AEROMIST™ pneumatic nebulizer available from Inhalation Plastic, Inc. of Niles, Ill.
(149) When the active ingredients are adapted to be administered, either together or individually, via nebulizer(s) they can be in the form of a nebulized aqueous suspension or solution, with or without a suitable pH or tonicity adjustment, either as a unit dose or multi-dose device.
(150) Furthermore, any suitable gas can be used to apply pressure during the nebulization, with preferred gases to date being those which are chemically inert. Exemplary gases including, but are not limited to, nitrogen, argon, or helium can be used to advantage.
(151) In some embodiments, the compositions described herein can also be administered directly to the airways in the form of a dry powder. Thus, the engineered EVs can be administered via an inhaler. Exemplary inhalers include metered dose inhalers and dry powdered inhalers.
(152) A metered dose inhaler or “MDI” is a pressure resistant canister or container filled with a product such as a pharmaceutical composition dissolved in a liquefied propellant or micronized particles suspended in a liquefied propellant. The propellants which can be used include chlorofluorocarbons, hydrocarbons or hydrofluoroalkanes. Commonly used propellants are P134a (tetrafluoroethane) and P227 (heptafluoropropane) each of which may be used alone or in combination. They are optionally used in combination with one or more other propellants and/or one or more surfactants and/or one or more other excipients, for example ethanol, a lubricant, an anti-oxidant and/or a stabilizing agent.
(153) As used herein, the term “dry powder inhaler” refers to a device that delivers a therapeutic dose of a composition of the present invention in a powdered form without propellants to the respiratory system. A dry powder inhaler (i.e., Turbuhaler™ (Astra AB)) is a system operable with a source of pressurized air to produce dry powder particles of a pharmaceutical composition that is compacted into a very small volume. Examples of dry powder inhalers include, but are not limited to, Spinhaler® (Fisons Pharmaceuticals, Rochester, NY), Rotahaler® (GlaxoSmithKline, NC), Turbuhaler® (AstraZeneca, UK), and Diskhaler® (GlaxoSmithKline, NC).
(154) Dry powder aerosols for inhalation therapy are generally produced with mean diameters primarily in the range of <5 μm. As the diameter of particles exceeds 3 μm, there is increasingly less phagocytosis by macrophages. However, increasing the particle size also has been found to minimize the probability of particles (possessing standard mass density) entering the airways and acini due to excessive deposition in the oropharyngeal or nasal regions.
(155) Suitable powder compositions include, by way of illustration, powdered preparations including the engineered EVs described herein. These can be intermixed with lactose, or other inert powders acceptable for intrabronchial administration. The powder compositions can be administered via an aerosol dispenser or encased in a breakable capsule which may be inserted by the patient or clinician into a device that punctures the capsule and blows the powder out in a steady stream suitable for inhalation. The compositions can include propellants, surfactants, and co-solvents and may be filled into conventional aerosol containers that are closed by a suitable metering valve.
(156) Aerosols for the delivery to the respiratory tract are described, for example, by Adjei, A. and Garren, J. Pharm. Res., 1:565-569 (1990); Zanen, P. and Lamm, J.-W. J. Int. J. Pharm., 114:111-115 (1995); Gonda, I. “Aerosols for delivery of therapeutic and diagnostic agents to the respiratory tract,” in Critical Reviews in Therapeutic Drug Carrier Systems, 6:273-313 (1990); Anderson et al., Am. Rev. Respir. Dis., 140:1317-1324 (1989)) and have potential for the systemic delivery of peptides and proteins as well (Patton and Platz, Advanced Drug Delivery Reviews, 8:179-196 (1992)); Timsina et al., Int. J. Pharm., 101:1-13 (1995); and Tansey, I. P., Spray Technol. Market, 4:26-29 (1994); French, D. L., Edwards, D. A. and Niven, R. W., Aerosol Sci., 27:769-783 (1996); Visser, J., Powder Technology 58:1-10 (1989)); Rudt, S. and R. H. Muller, J. Controlled Release, 22:263-272 (1992); Tabata, Y, and Y. Ikada, Biomed. Mater. Res., 22:837-858 (1988); Wall, D. A., Drug Delivery, 2:10 1-20 1995); Patton, J. and Platz, R., Adv. Drug Del. Rev., 8:179-196 (1992);

Bryon, P., Adv. Drug. Del. Rev., 5:107-132 (1990); Patton, J. S., et al., Controlled Release, 28:15 79-85 (1994); Damms, B. and Bains, W., Nature Biotechnology (1996); Niven, R. W., et al., Pharm. Res., 12 (9); 1343-1349 (1995); and Kobayashi, S., et al., Pharm. Res., 13 (1): 80-83 (1996), the contents of each of which are incorporated herein by reference in their entirety.

(157) Microemulsification technology can improve bioavailability of some lipophilic (water insoluble) pharmaceutical agents. Examples include Trimetrine (Dordunoo, S. K., et al., Drug Development and Industrial Pharmacy, 17 (12), 1685-1713, 1991 and REV 5901 (Sheen, P. C., et al., J Pharm Sci 80 (7), 712-714, 1991). Among other things, microemulsification provides enhanced bioavailability by preferentially directing absorption to the lymphatic system instead of the circulatory system, which thereby bypasses the liver, and prevents destruction of the cell-based compositions in the hepatobiliary circulation.

(158) The engineered EVs described herein can be formulated with an amphiphilic carrier. Amphiphilic carriers are saturated and monounsaturated polyethyleneglycolyzed fatty acid glycerides, such as those obtained from fully or partially hydrogenated various vegetable oils. Such oils may advantageously consist of tri-, di-, and mono-fatty acid glycerides and di- and mono-polyethyleneglycol esters of the corresponding fatty acids, with a particularly preferred fatty acid composition including capric acid 4-10, capric acid 3-9, lauric acid 40-50, myristic acid 14-24, palmitic acid 4-14 and stearic acid 5-15%. Another useful class of amphiphilic carriers includes partially esterified sorbitan and/or sorbitol, with saturated or mono-unsaturated fatty acids (SPAN-series) or corresponding ethoxylated analogs (TWEEN-series).

(159) Commercially available amphiphilic carriers are particularly contemplated, including Gelucire-series, Labrafil, Labrasol, or Lauroglycol (all manufactured and distributed by Gattefosse Corporation, Saint Priest, France), PEG-mono-oleate, PEG-di-oleate, PEG-mono-laurate and di-laurate, Lecithin, Polysorbate 80, etc. (produced and distributed by a number of companies in USA and worldwide).

(160) The engineered EV compositions provided herein can be formulated with hydrophilic polymers. Hydrophilic polymers are water-soluble, can be covalently attached to a vesicle-forming lipid, and which are tolerated in vivo without toxic effects (i.e., are biocompatible). Suitable polymers include polyethylene glycol (PEG), polylactic (also termed polylactide), polyglycolic acid (also termed polyglycolide), a polylactic-polyglycolic acid copolymer, and polyvinyl alcohol. Other hydrophilic polymers which may be suitable include polyvinylpyrrolidone, polymethoxazoline, polyethyloxazoline, polyhydroxypropyl methacrylamide, polymethacrylamide, polydimethylacrylamide, and derivatized celluloses such as hydroxymethylcellulose or hydroxyethylcellulose.

(161) In certain embodiments, a pharmaceutical composition as described herein comprises a biocompatible polymer selected from the group consisting of polyamides, polycarbonates, polyalkylenes, polymers of acrylic and methacrylic esters, polyvinyl polymers, polyglycolides, polysiloxanes, polyurethanes and co-polymers thereof, celluloses, polypropylene, polyethylenes, polystyrene, polymers of lactic acid and glycolic acid, polyanhydrides, poly(ortho) esters, poly(butic acid), poly(valeric acid), poly(lactide-co-caprolactone), polysaccharides, proteins, polyhyaluronic acids, polycyanoacrylates, and blends, mixtures, or copolymers thereof.

(162) In certain embodiments, a pharmaceutical composition described herein is formulated as a liposome. Liposomes can be prepared by any of a variety of techniques that are known in the art. See, e.g., U.S. Pat. No. 4,235,871; Published PCT applications WO 96/14057; New RRC, Liposomes: A practical approach, IRL Press, Oxford (1990), pages 33-104; Lasic DD, Liposomes from physics to applications, Elsevier Science Publishers BV, Amsterdam, 1993.

(163) Therapeutic formulations of the engineered EV compositions as described herein can be prepared for storage by with optional pharmaceutically acceptable carriers, excipients or stabilizers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are

nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

(164) Vaccine or other pharmaceutical compositions comprising an engineered EV composition as described herein can contain a pharmaceutically acceptable salt, typically, e.g., sodium chloride, and preferably at about physiological concentrations. The formulations of the vaccine or other pharmaceutical compositions described herein can contain a pharmaceutically acceptable preservative. In some embodiments, the preservative concentration ranges from 0.1 to 2.0%, typically v/v. Suitable preservatives include those known in the pharmaceutical arts. Benzyl alcohol, phenol, m-cresol, methylparaben, and propylparaben are examples of preservatives. The formulations of the vaccine or other pharmaceutical compositions described herein can include a pharmaceutically acceptable surfactant at a concentration of 0.005 to 0.02%.

(165) Therapeutic pharmaceutical compositions described herein can also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other.

(166) In some embodiments in which the engineered EVs are formulated for use in or with a vaccine, the vaccine composition can be formulated with the engineered EVs as an adjuvant. In other embodiments the vaccine composition can be formulated with the engineered EVs and an additional adjuvant, e.g., as known in the art.

(167) As used herein in the context of immunization, immune response and vaccination, the term “adjuvant” refers to any substance than when used in combination with a specific antigen produces a more robust immune response than the antigen alone. When incorporated into a vaccine formulation, an adjuvant acts generally to accelerate, prolong, or enhance the quality of specific immune responses to the vaccine antigen(s). Adjuvants typically promote the accumulation and/or activation of accessory cells or factors to enhance antigen-specific immune responses and thereby enhance the efficacy of vaccines, i.e., antigen-containing or encoding compositions used to induce protective immunity against the antigen.

(168) Adjuvants, in general, include adjuvants that create a depot effect, immune-stimulating adjuvants, and adjuvants that create a depot effect and stimulate the immune system. An adjuvant that creates a depot effect is an adjuvant that causes the antigen to be slowly released in the body, thus prolonging the exposure of immune cells to the antigen. This class of adjuvants includes but is not limited to alum (e.g., aluminum hydroxide, aluminum phosphate); emulsion-based formulations including mineral oil, non-mineral oil, water-in-oil or oil-in-water-in oil emulsion, oil-in-water emulsions such as Seppic ISA series of Montanide adjuvants (e.g., Montanide ISA 720; AirLiquide, Paris, France); MF-59 (a squalene-in-water emulsion stabilized with Span 85 and Tween 80; Chiron Corporation, Emeryville, Calif.); and PROVAX™ (an oil-in-water emulsion containing a stabilizing detergent and a micelle-forming agent; IDEC Pharmaceuticals Corporation, San Diego, Calif.).

(169) An immune-stimulating adjuvant is an adjuvant that causes activation of a cell of the immune system. It may, for instance, cause an immune cell to produce and secrete cytokines and interferons. This class of adjuvants includes but is not limited to saponins purified from the bark of

the *Q. saponaria* tree, such as QS21 (a glycolipid that elutes in the 21st peak with HPLC fractionation; Aquila Biopharmaceuticals, Inc., Worcester, Mass.); poly[di(carboxylatophenoxy)phosphazene (PCPP polymer; Virus Research Institute, USA); derivatives of lipopolysaccharides such as monophosphoryl lipid A (MPL; Ribi ImmunoChem Research, Inc., Hamilton, Mont.), muramyl dipeptide (MDP; Ribi) and threonyl-muramyl dipeptide (t-MDP; Ribi); OM-174 (a glucosamine disaccharide related to lipid A; OM Pharma SA, Meyrin, Switzerland); and *Leishmania* elongation factor (a purified *Leishmania* protein; Corixa Corporation, Seattle, Wash.). This class of adjuvants also includes CpG DNA.

(170) Adjuvants that create a depot effect and stimulate the immune system are those compounds which have both of the above-identified functions. This class of adjuvants includes but is not limited to ISCOMS (immunostimulating complexes which contain mixed saponins, lipids and form virus-sized particles with pores that can hold antigen; CSL, Melbourne, Australia); SB-AS2 (SmithKline Beecham adjuvant system #2 which is an oil-in-water emulsion containing MPL and QS21: SmithKline Beecham Biologicals [SBB], Rixensart, Belgium); SB-AS4 (SmithKline Beecham adjuvant system #4 which contains alum and MPL; SBB, Belgium); non-ionic block copolymers that form micelles such as CRL 1005 (these contain a linear chain of hydrophobic polyoxypropylene flanked by chains of polyoxyethylene; Vaxcel, Inc., Norcross, Ga.); and Syntex Adjuvant Formulation (SAF, an oil-in-water emulsion containing Tween 80 and a nonionic block copolymer; Syntex Chemicals, Inc., Boulder, Colo.).

(171) The active ingredients of the pharmaceutical compositions described herein can also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

(172) In some embodiments, sustained-release preparations can be used. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing a composition described herein in which the matrices are in the form of shaped articles, e.g., films, or microcapsule. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and y ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(−)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated, the composition can remain in the body for a long time (e.g., up to about 1 hour, between 1-12 hours, 12-24 hours, 24 hours to 2 days, 2-3 days, 3-4 days, 4-5 days, 5-6 days, 6-7 days, 1-2 weeks, 3-4 weeks, 4 weeks to 2 months, 2-3 months, 3-4 months, 4-5 months, 5-6 months, or more than 6 months, or a variation thereof), denature, or aggregate as a result of exposure to moisture at 37° C., resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S—S— bond formation through thio-disulfide interchange, stabilization can be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

Administration, Dosing, Efficacy

(173) The engineered EV compositions, pharmaceutical compositions, or vaccine compositions described herein can be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being

treated, the particular subject being treated, the clinical condition of the individual subject, the cause of the disorder, the site of delivery of the vaccine composition, the method of administration, the scheduling of administration, and other factors known to medical practitioners.

(174) Generally, application of artificial synapses as therapy will take into account similar parameters as other therapeutic strategies, including concentration, timing of delivery, and sustained bioavailability at injury/disease site. Extracellular vesicle can be delivered via a number of routes: intravenous, intracoronary, and intramyocardial. Extracellular vesicles (e.g., exosomes), also allow for new delivery routes that were previously infeasible for cell therapy, such as inhalation or injection. These various approaches are described below, including injection, topical application, enteral administration, and pulmonary delivery.

(175) The engineered EV compositions provided herein can be administered to a subject in need thereof by any appropriate route which results in an effective treatment in the subject. As used herein, the terms “administering,” and “introducing” are used interchangeably and refer to the placement of a composition provided herein into a subject by a method or route which results in at least partial localization of such compositions at a desired site, such as a site of inflammation or a tumor, such that a desired effect(s) is produced. The compositions can be administered to a subject by any mode of administration that delivers the composition systemically or to a desired surface or target, and can include, but is not limited to, injection, infusion, instillation, and inhalation administration. To the extent that the composition can be protected from inactivation in the gut, oral administration forms are also contemplated. “Injection” includes, without limitation, intravenous, intramuscular, intra-arterial, intrathecal, intraventricular, intracapsular, intraorbital, retro-orbital, intravitreal, intraocular, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, sub capsular, subarachnoid, intraspinal, intracerebral, intratarsal, and intrasternal, intratumoral injection, and infusion or the like as known in the art.

(176) A therapeutic does of the present invention may be delivered to a patient by means of controlled release, for example but not limited to, implantable pump and implantable cannulas to provide continuous access to the venous or arterial system.

(177) Topical application refers to applying or spreading a composition of the present invention onto surfaces on or in the body, both internally and/or externally, in a therapeutically effective amount for local and/or systemic treatment. Topical application may be epicutaneuos wherein a composition of the present invention may be directly applied onto a localized surface of the skin or mucous membranes. Topical application may include transdermal application wherein a composition of the present invention may be absorbed into the body to obtain systemic delivery and systemic distribution. Topical application formulations may include, but are not limited to, creams, foams, gels, lotions, solutions, ointments, dermal patch, transdermal patches, powder, solid, sponge, tape, vapor, paste, film, liposomes, balm, shampoo, spray, or tincture or the like or a combination thereof. A therapeutic dose of a composition of the present invention may be delivered vaginally (for example a vaginal suppository, vaginal ring, douche, intrauterine device, intravesical infusion, and the like) or urethra or the like or a combination thereof.

(178) Enteral administration refers to a composition of the present invention administered via the gastrointestinal tract in a therapeutically effective amount for local or systemic treatment. Enteral administration may include, but is not limited to, delivery of a composition of the present invention via the mouth, sublingual, esophagus, gastric (for example the stomach), small intestines, large intestines or rectum. Oral delivery of the present invention may include, but is not limited to, the use of a capsule, pastille, pill, tablet, solution, gel, suspension, emulsion, syrup, elixir, tincture, mouthwash, lozenges, chewing gum, lollipop, cream, foam, solution, powder, solid, vapor, liposomes, spray, or tincture osmotic-controlled release oral delivery system, or the like. Gastric delivery may involve the use of a tube or nasal passage that leads directly to the stomach, for example, a percutaneous endoscopic gastrostomy tube. Gastric delivery may involve direct injection made through the abdominal wall. Rectal delivery may involve, but is not limited to, the

use of a suppository, ointment, enema, murphy drip, or the like. A therapeutic does of the present invention may be delivered to a patient by means of controlled release, for example but not limited to, controlled release drug delivery pellet or pill.

(179) Inhalation (i.e., pulmonary delivery, pulmonary administration refers to delivery to the respiratory system through the respiratory route, including but not limited to, intranasal administration, oral administration, and oral inhalative administration (e.g., intratracheal instillation and intratracheal inhalation) of a therapeutically effective amount for local or systemic treatment. Pulmonary delivery of a therapeutically effective amount of a composition of the present invention may be achieved by dispersion, for example by using a syringe. Pulmonary delivery of a composition of the present invention may be achieved by aerosol administration, wherein aerosol administration may deposit a therapeutically effective amount of the present invention by gravitational sedimentation, inertial impaction, or diffusion.

(180) Intravenous delivery technique can occur through a peripheral or central venous catheter. As the simplest delivery mode, this technique avoids the risk of an invasive procedure. However, intravenous may be regarded as a comparatively inefficient and less localized delivery method, as a high percentage of infused cell exosomes may become sequestered in organs such as the lung, liver, or spleen. Such sequestration may result in few or no cellular exosomes reaching broader circulation or have unintended systemic effects following their distribution.

(181) In certain embodiments, administration can include delivery to a tissue or organ site that is the same as the site of diseased and/or dysfunctional tissue. In certain embodiments, administration can include delivery to a tissue or organ site that is different from the site or diseased and/or dysfunctional tissue. In certain embodiments, the delivery is via inhalation or oral administration. In various embodiments, administration of artificial synapses can include combinations of multiple delivery techniques.

(182) In some embodiments, the compositions described herein are administered by aerosol administration, nebulizer administration, or tracheal lavage administration.

(183) The term “effective amount” as used herein refers to the amount of an engineered EV composition needed to alleviate or prevent at least one or more symptom of a disease or disorder (e.g., autoimmune disease or cancer), and relates to a sufficient amount of pharmacological composition to provide the desired effect, e.g., reduce the pathology, or any symptom associated with or caused by a disease. The term “therapeutically effective amount” therefore refers to an amount of an engineered EV composition or vaccine composition described herein using the methods as disclosed herein, that is sufficient to affect a particular disease state when administered to a typical subject. An effective amount as used herein would also include an amount sufficient to delay the development of a symptom of the disease, alter the course of a symptom disease (for example, but not limited to, slow the progression of a symptom of the disease), or reverse a symptom of the disease. Thus, it is not possible to specify the exact “effective amount.” However, for any given case, an appropriate “effective amount” can be determined by one of ordinary skill in the art using only routine experimentation.

(184) Effective amounts, toxicity, and therapeutic efficacy can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dosage can vary depending upon the dosage form employed and the route of administration utilized. The dose ratio between toxic and therapeutic effects is the therapeutic index and can be expressed as the ratio LD50/ED50. Compositions and methods that exhibit large therapeutic indices are preferred. A therapeutically effective dose can be estimated initially from cell culture assays. Also, a dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (i.e., the concentration of the engineered EVs or fusion polypeptides provided herein), which achieves a half-maximal inhibition of symptoms) as determined in cell culture, or in an appropriate animal model. Levels of therapeutic engineered Evs

in plasma can be measured, for example, by high performance liquid chromatography, enzyme linked immunosorbent assay (ELISA), flow cytometry, FACS sorting, western blot, mass spectroscopy, tunable resistive pulse sensing, EXOVIEW®, qRT-PCR, next generation sequencing (NGS), or by any analysis technique known by one of ordinary skill in the art. The effects of any particular dosage can be monitored by a suitable bioassay. The dosage can be determined by a physician and adjusted, as necessary, to suit observed effects of the treatment.

(185) The engineered EV compositions, pharmaceutical compositions, or vaccine compositions described herein can be formulated, in some embodiments, with one or more additional therapeutic agents currently used to prevent or treat the infection, for example. The effective amount of such other agents depends on the amount of an engineered EV in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as used herein before or about from 1 to 99% of the heretofore employed dosages.

(186) The dosage ranges for the pharmaceutical compositions described herein depend upon the potency and encompass amounts large enough to produce the desired effect. The dosage should not be so large as to cause unacceptable adverse side effects. Generally, the dosage will vary with the age, condition, health, and sex of the patient and can be determined by one of skill in the art. The dosage can also be adjusted by the individual physician in the event of any complication. In some embodiments, the dosage ranges from 0.001 mg/kg body weight to 100 mg/kg body weight. In some embodiments, the dose range is from 5 μg/kg body weight to 100 μg/kg body weight. Alternatively, the dose range can be titrated to maintain serum levels between 0.1 μg/mL and 1000 μg/mL. For systemic administration, subjects can be administered a therapeutic amount, such as, e.g., 0.1 mg/kg, 0.5 mg/kg, 1.0 mg/kg, 2.0 mg/kg, 2.5 mg/kg, 5 mg/kg, 7.5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 40 mg/kg, 50 mg/kg, or more. These doses can be administered by one or more separate administrations, or by continuous infusion. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until, for example, the infection is treated, as measured by the methods described above or known in the art. However, other dosage regimens can be useful.

(187) In various embodiments, the quantities of artificial synapses that are administered to achieve these effects range from 1×10.sup.6 to 1×10.sup.7, 1×10.sup.7 to 1×10.sup.8, 1×10.sup.8 to 1×10.sup.9, 1×10.sup.9 to 1×10.sup.10, 1×10.sup.10 to 1×10.sup.11, 1×10.sup.11 to 1×10.sup.12, 1×10.sup.12 to 1×10.sup.13, 1×10.sup.13 to 1×10.sup.14, 1×10.sup.14 to 1×10.sup.15, 1×10.sup.15 or more Evs/artificial synapses. In other embodiments, the numbers of artificial synapses are relative to the number of cells used in a clinically relevant dose for a cell-therapy method. For example, defining an effective dose range, dosing regimen and route of administration, may be guided by studies using fluorescently labeled artificial synapses, and measuring target tissue retention, which can be >10×, >50×, or >100× background, as measured 5, 10, 15, 30, or 30 or more min as a screening criterion. In certain embodiments, >100× background measured at 30 mins is a baseline measurement for a low and high dose that is then assess for safety and bioactivity (e.g., using MRI endpoints: scar size, global and regional function of the target organ being treated). In various embodiments, single doses are compared to two, three, four, four or more sequentially-applied doses. In various embodiments, the repeated or sequentially-applied doses are provided for treatment of an acute disease and/or condition. In various embodiments, the repeated or sequentially-applied doses are provided for treatment of a chronic disease and/or condition. In other embodiments, administration of the plurality of artificial synapses is adjunctive to standard therapy.

(188) In other embodiments, administering a composition includes 1×10.sup.10 or more artificial synapses in a single dose. In various embodiments, exosome quantity may be defined by protein quantity, such as dosages including 1-10, 10-25, 25-50, 50-75, 75-100, or 100 or more mg exosome protein. In other embodiments, a single dose is administered multiple times to the subject. In other

embodiments, administering a composition consists of one or more of: injection, topical administration, enteral, intravenous, intra-arterial, or inhalation.
(189) In various embodiments, exosome quantity may be defined by protein quantity, such as dosages including 1-10, 10-25, 25-50, 50-75, 75-100, or 100 or more mg exosome protein. In various embodiments, administering a composition includes multiple dosages of the artificial synapses. In various embodiments, the repeated or sequentially-applied doses are provided for treatment of an acute disease and/or condition. In various embodiments, the repeated or sequentially-applied doses are provided for treatment of a chronic disease and/or condition.
(190) In other embodiments, administering a composition including a plurality of artificial synapses to the subject is adjunctive to standard therapy.
(191) The duration of a therapy using the methods described herein will continue for as long as medically indicated or until a desired therapeutic effect (e.g., those described herein) is achieved. In certain embodiments, the administration of the vaccine composition described herein is continued for 1 month, 2 months, 4 months, 6 months, 8 months, 10 months, 1 year, 2 years, 3 years, 4 years, 5 years, 10 years, 20 years, or for a period of years up to the lifetime of the subject.
(192) As will be appreciated by one of skill in the art, appropriate dosing regimens for a given composition can comprise a single administration/immunization or multiple ones. Subsequent doses may be given repeatedly at time periods, for example, about two weeks or greater up through the entirety of a subject's life, e.g., to provide a sustained preventative effect. Subsequent doses can be spaced, for example, about two weeks, about three weeks, about four weeks, about one month, about two months, about three months, about four months, about five months, about six months, about seven months, about eight months, about nine months, about ten months, about eleven months, or about one year after a primary immunization.
(193) The precise dose to be employed in the formulation will also depend on the route of administration and should be decided according to the judgment of the practitioner and each patient's circumstances. Ultimately, the practitioner or physician will decide the amount of the engineered EV or composition thereof to administer to particular subjects.
Methods of Modulating Inflammation and Treating Autoimmune Diseases
(194) The artificial synapses/engineered Evs and compositions thereof provided herein can be deployed in a therapeutic strategy against virtually any injury/disease, as providing a platform for altering biological signaling. This includes, for example, inflammation and immune signaling, which plays a role in virtually all injuries and diseases in living organisms.
(195) Thus, described herein is a method of modulating inflammation, including selecting a subject afflicted with an inflammatory related disease and/or condition; and administering to the subject a composition including a plurality of artificial synapses (engineered Evs) to the subject, wherein administration of the composition modulates inflammation.
(196) As used herein, the term “inflammation” or “inflamed” refers to activation or recruitment of the immune system or immune cells (e.g., T cells, B cells, macrophages). A tissue that has inflammation can become reddened, white, swollen, hot, painful, sensitivity, exhibit a loss of function, or have a film or mucus. Methods of identifying inflammation are well known in the art. Inflammation typically occurs following injury, infection by a microorganism, exposure to a substance (e.g., a toxin, chemical, or dust) or autoimmune dysfunction. Onset of inflammation may be rapid (e.g., immediately following injury) or slow (e.g., repeated exposure to an irritant such as a chemical over time) with a duration of minutes, hours, days, months, years, or an individual's life.
(197) Inflammation plays a vital role in alerting the immune system of potential danger and damage within a body. Inflammation is necessary to control and repair injury. For example, acute inflammation is a response to physical trauma, infection, and stress. Acute inflammation helps prevent further injury and triggers healing and recovery. Unfortunately, inflammation can become excessive and inappropriately active, lasting beyond the typical recovery time from an injury or infection. Wherein healthy inflammation helps a body respond to injury, chronic inflammation

perpetuates injury and may lead to negative consequences to one's health. In particular, autoimmune diseases are chronic diseases from a host's immune system attacking itself, often due to aberrant biological signaling in the host. Restoring normal homeostatic signaling via application of artificial synapses, particularly targeting immune checkpoints, represents a highly promising avenue. For example, surface bound immune-checkpoint proteins or fragments thereof may modulate immune cell stimulation and affect suppression of immune cell function when delivered via artificial synapses. Injection, inhalation, ingestion or topical application of artificial synapses with surface bound immune-checkpoint proteins or fragments thereof may be used to treat immune, auto-immune, inflammatory, and auto-inflammatory conditions. Examples include chronic obstructive pulmonary disease (COPD) which is an inflammatory, progressive, life-threatening lung disease, psoriasis, a common chronic noncommunicable inflammatory skin disease, arthritis, a debilitating and painful degeneration of joints, among others well-understood to one of skill in the art.

(198) In other embodiments, the inflammatory related disease and/or condition is acute, for example septicemia. In other embodiments, the inflammatory related disease and/or condition is chronic, for example chronic obstructive pulmonary disease. In other embodiments, the inflammatory condition is an autoimmune disease wherein the autoimmune disease and/or condition is one or more of: polymyositis, dermatomyositis, Graves' disease, Hashimoto's thyroiditis, myasthenia gravis, vasculitis, multiple sclerosis, psoriasis, rheumatoid arthritis, psoriatic arthritis, scleroderma, systemic lupus erythematosus, inflammatory bowel disease, Crohn's disease, hyperthyroidism, autoimmune adrenal insufficiency, Sjogren syndrome, type I diabetes mellitus, autoimmune hemolytic anemia, idiopathic thrombocytopeniarpura, myasthenia gravis, ulcerative colitis, uveitis, polyarteritis nodosa, relapsing polychondritis, Behcet's disease, reactive arthritis, ankylosing spondylitis, Guillain-Barre syndrome, or optic neuropathy. In other embodiments, the disease and/or condition is chronic obstructive pulmonary disease, rheumatoid arthritis, uveoretinitis, psoriasis, and eczema. In other embodiments, the disease and/or condition is irritable bowel disease, multiple sclerosis or lupus.

(199) In other embodiments, the inflammatory related disease and/or condition is an ocular disease. As used herein, the terms “ocular disease”, “eye disorder” and “eye disease” are used interchangeably and refer to a disease or disorder that affects the health and/or vision of either one or both eyes or the general area of the eye(s), eye lid(s), or area surrounding or in near proximity to the eye(s). Eye disease may include, but are not limited to, macular degeneration (e.g., age-related macular degeneration), cataracts, diabetic retinopathy, diabetic macular edema, eye floaters, eye flashes, glaucoma, amblyopia, strabismus, retinitis (e.g., CMV retinitis), color blindness, keratoconus, retinal detachment, eyelid twitching, ocular hypertension, blepharitis, uveitis, Bietti's crystalline dystrophy, blepharospasm, cornea and corneal diseases, dry eye, histoplasmosis, macular hole, macular pucker, conjunctivitis, presbyopia, retinoblastoma, retinitis pigmentosa, retinopathy, Stargardt disease, Usher syndrome, uveal Coloma, and vitreous detachment, or the like.

(200) Described herein is a method for treatment including, selecting a subject in need of treatment, administering a composition including a plurality of artificial synapses to the individual, wherein administration of the composition treats the subject. In certain embodiments, the subject is in need to treatment for a disease and/or condition involving tissue damage or dysfunction.

(201) Described herein is a method of treating an autoimmune disease, inflammation, inflammatory disease or condition, or cancer in a subject, the method comprising: administering to a subject an engineered EV or composition thereof as provided herein to the subject.

(202) Measured or measurable parameters include clinically detectable markers of disease, for example, elevated or depressed levels of a clinical or biological marker, as well as parameters related to a clinically accepted scale of symptoms or markers for a disease or disorder. It will be understood, however, that the total usage of the compositions and formulations as disclosed herein

will be decided by the attending physician within the scope of sound medical judgment. The exact amount required will vary depending on factors such as the type of disease being treated.
(203) Non-limiting examples of clinical tests that can be used to assess autoimmune diseases, inflammatory conditions, or inflammation parameters include blood tests, skin biopsy, MRI, eye examination, ocular pressure tests, etc. Where necessary or desired, animal models of injury or disease can be used to gauge the effectiveness of a particular composition as described herein. For example, an EAU animal model, as demonstrated in the working examples can be used.
(204) In various embodiments, administration of the plurality of artificial synapses alters gene expression in the damaged or dysfunctional tissue, improves viability of the damaged tissue, and/or enhances regeneration or production of new tissue in the individual. In various embodiments, administration of the plurality of artificial synapses alters gene expression in the damaged or dysfunctional tissue, improves viability of the damaged tissue, and/or enhances regeneration or production of new tissue in the individual.
(205) In various embodiments, the damaged or dysfunctional tissue is in need of repair, regeneration, or improved function due to an acute event. Acute events include, but are not limited to, trauma such as laceration, crush or impact injury, shock, loss of blood or oxygen flow, infection, chemical or heat exposure, poison or venom exposure, drug overuse or overexposure, and the like. Other sources of damage also include, but are not limited to, injury, age-related degeneration, cancer, and infection. In several embodiments, the regenerative cells used to prepare the engineered EVs provided herein are from the same tissue type as is in need of repair or regeneration. In several other embodiments, the regenerative cells are from a tissue type other than the tissue in need of repair or regeneration. In some embodiments, the engineered EVs provided herein are derived from the subject being treated. In some embodiments, the engineered EVs are derived from a donor subject.
(206) In other embodiments, the damaged or dysfunctional tissue is in need of repair, regeneration, or improved function due to damage from chronic disease.
Some Selected Definitions
(207) All references cited herein are incorporated by reference in their entirety as though fully set forth. Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Allen et al., *Remington: The Science and Practice of Pharmacy* 22.sup.nd *ed*., Pharmaceutical Press (Sep. 15, 2012); Hornyak et al., *Introduction to Nanoscience and Nanotechnology*, CRC Press (2008); Singleton and Sainsbury, *Dictionary of Microbiology and Molecular Biology* 3.sup.rd *ed., revised ed*., J. Wiley & Sons (New York, NY 2006); Smith, *March's Advanced Organic Chemistry Reactions, Mechanisms and Structure* 7.sup.th *ed*., J. Wiley & Sons (New York, NY 2013); Singleton, *Dictionary of DNA and Genome Technology* 3.sup.rd *ed*., Wiley-Blackwell (Nov. 28, 2012); and Green and Sambrook, *Molecular Cloning: A Laboratory Manual* 4*th ed*., Cold Spring Harbor Laboratory Press (Cold Spring Harbor, NY 2012), provide one skilled in the art with a general guide to many of the terms used in the present application. For references on the preparation and structure of antibodies and fusion polypeptides, see, e.g., Greenfield, *Antibodies A Laboratory Manual* 2.sup.nd *ed*., Cold Spring Harbor Press (Cold Spring Harbor NY, 2013); Köhler and Milstein, *Derivation of specific antibody-producing tissue culture and tumor lines by cell fusion*, Eur. J. Immunol. 1976 Jul. 6 (7): 511-9; Queen and Selick, Humanized immunoglobulins, U.S. Pat. No. 5,585,089 (1996 December); and Riechmann et al., *Reshaping human antibodies for therapy*, Nature 1988 Mar. 24, 332 (6162): 323-7. See also, Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991), Lanzavecchia et al., Eur. J. Immunol. 17, 105 (1987)), Huston et al., Proc. Natl. Acad. Sci. U.S.A., 85, 5879-5883 (1988), Bird et al., Science 242, 423-426 (1988), Brinkman et al. mAbs Vol 9, No. 2, 182-212 (2017), Chothia & Lesk, J. Mol. Biol, 196:901-917 (1987), Chothia et al., Nature 342:877-883 (1989)), Holliger et al. (1993) Proc. Natl. Acad. Sci.

USA 90:6444-6448; Poljak (1994) Structure 2:1121-1123); Kontermann and Dubel eds., Antibody Engineering, Springer-Verlag, N.Y. (2001), p. 790 (ISBN 3-540-41354-5, Zapata et al. (1995) Protein Eng. 8 (10): 1057-1062; Morrison, et al., Proc. Natl. Acad. Sci. USA, 81:6851 (1984), U.S. Pat. Nos. 4,816,567, 5,693,780, which are incorporated herein by reference in their entireties.

(208) One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. Indeed, the present invention is in no way limited to the methods and materials described. For purposes of the present invention, the following terms are defined below.

(209) As used herein, the term “extracellular vesicle” and “vesicle” are used interchangeably and refer to a particle, wherein the particle comprises a phospholipid bilayer that encloses an internal space and an exterior surface and may or may not be derived from a cell. The size of extracellular vesicles can range between 20 nm to 3 μm in diameter but may be smaller than 20 nm or larger than 3 μm. Examples of extracellular vesicles include, but is not limited to, exosomes (for example small exosomes and large exosomes), ectosomes, macrovesicles, microparticles, apoptotic bodies, vesicular organelles, oncosomes (for examples large oncosomes), exospheres, exomeres, cell derived nanovesicles (CDN) (e.g., by genesis via grating or shearing cells), liposomes or the like known by one of ordinary skill in the art. Extracellular vesicles may originate naturally via known or unknown biosynthetic pathways. Extracellular vesicles may be promoted to originate by using mechanical methods such as cell grating or cell shearing wherein a cell is grated or sheared causing portions or parts of the cell membrane to from vesicles. For example, CDNs may be formed by using mechanical methods such as cell grating or cell shearing wherein a cell is grated or sheared causing portions or parts of the cell membrane to from vesicles. Additional non-limiting examples of mechanical methods that can be used to form cell derived nanovesicles are further described in detail, e.g., Goh, W. J., Zou, S., Ong, W. Y. et al. Bioinspired Cell-Derived Nanovesicles versus Exosomes as Drug Delivery Systems: a Cost-Effective Alternative. *Sci Rep* 7, 14322 (2017). doi: 10.1038/s41598-017-14725-x, the contents of which are incorporated herein by reference in their entireties.

(210) Extracellular vesicles comprise cargo, wherein the term “cargo” refers to peptides, proteins, nucleic acids, lipids, metabolites, carbohydrates, biomolecules, small molecules, large molecules, vesicles, organelles, or fragments thereof. In some embodiments, cargo may refer to existing drugs or therapeutics known in the art. Extracellular vesicle cargo may be located within the internal space of the extracellular vesicle. Extracellular vesicle cargo may be membrane bound and span one or both layers of the extracellular vesicle phospholipid bilayer (for example a transmembrane protein). Extracellular vesicle cargo may be in contact with the external or internal surface of the extracellular vesicle, for example through a covalent bond or a non-covalent bond. The phospholipid bilayer of the extracellular vesicle may comprise one or more transmembrane proteins, wherein a portion of the one or more transmembrane membrane proteins is located within the internal space of the extracellular vesicle. The phospholipid bilayer of the extracellular vesicle may comprise one or more transmembrane proteins, wherein the one or more transmembrane membrane proteins comprises a domain on the exterior of the extracellular vesicle. The phospholipid bilayer of the extracellular vesicle may comprise one or more transmembrane proteins, wherein the one or more transmembrane membrane proteins comprises a domain on the interior of the extracellular vesicle. Cargo may refer to a protein on the luminal side (e.g., in the internal space) of the extracellular vesicle wherein said protein encodes a vesicle targeting domain that may be in contact with the interior phospholipid layer of the extracellular vesicle. Cargo may refer to a protein on the luminal side (e.g., in the internal space) of the extracellular vesicle wherein said protein encodes a vesicle targeting domain that may be in contact with the interior phospholipid layer of the extracellular vesicle and wherein said protein may be presented into the internal space of the extracellular vesicle.

(211) As used herein, the terms “sticky binder” and “vesicle targeting domain” and “anchor

protein" are used interchangeably and refer to a protein that is covalently or non-covalently attached to at least one lipid wherein the one or more lipid is embedded within a membrane (e.g., a cell membrane), and the lipid serves to anchor the protein to the membrane. The terms "sticky binder" and "vesicle targeting domain" and "anchor protein" can also mean a protein sequence that encodes for one or more transmembrane domains wherein the one or more transmembrane domains spans at least partly through a phospholipid bilayer, for example the phospholipid bilayer of an extracellular vesicle. The transmembrane domain can be of a Type I or Type II membrane protein. Transmembrane domains can be structurally identified using methods known to those of skill in the art, such as sequence analysis programs that identify hydrophobic and hydrophilic domains (for example TMHMM Server, v. 2.0-DTU, Erik L. L. Sonnhammer, Gunnar von Heijne, and Anders Krogh: *A hidden Markov model for predicting transmembrane helices in protein sequences. In Proc. of Sixth Int. Conf. on Intelligent Systems for Molecular Biology, p* 175-182 Ed J. Glasgow, T. Littlejohn, F. Major, R. Lathrop, D. Sankoff, and C. Sensen Menlo Park, CA: AAAI Press, 1998, which is incorporated herein by reference in its entirety.)

(212) A vesicle targeting domain may include, but is not limited to, one or more prenylation site, fatty acylation site, and/or glycosylphosphatidylinositol (GPI) linked protein. One preferred embodiment of a vesicle targeting domain is the GPI sequence from CD55. Another preferred embodiment of a vesicle targeting domain is the GPI sequence from CD59. Another embodiment of a vesicle targeting domain is the C1C2 domain from MFGE8. Other embodiments of sequences for vesicle targeting domains include transmembrane regions of CD9 (for example transmembrane 2 or 3 of CD9, CD9tm2 or CD9tm3, respectively), K-Ras (for example K-Ras4A and K-Ras4B), transmembrane domain from A Disintegrin and Metalloproteinase Domain-containing protein 10 (ADAM10, also known as CDw156 or CD156c) or other ADAM proteins. Vesicle targeting domains may include one or more sequences from 4F2 (for example 4F2 encoded by the solute carrier family 3 member 2 (SLC3A2) gene which makes up the heavy subunit of CD98). Vesicle targeting domains can include a sequence for one or more myristoylation sites. For example, the protein sequence for a myristoylation site from myristoylated alanine-rich C-kinase substrate (MARCKS) protein. Vesicle targeting domains can include a sequence for one or more palmitoylation sites. For example, the myristoylation sequence from the MARCKS protein may be modified to encode for a palmitoylation site. All variants, isoforms, or fragments or the like known by one of ordinary skill in the art are encompassed by the present invention.

(213) Vesicle targeting domains may include transmembrane sequences from *Homo sapiens* transferrin receptor 2 (TFR2), transcript variant 1 (transferrin receptor protein 2 isoform 1) or versions therefore. In a preferred embodiment, the vesicle targeting domain may be a transmembrane domain from CD298.

(214) As used herein, the terms "proteins" and "peptides" and "polypeptides" are used interchangeably herein to designate a series of amino acid residues connected to the other by peptide bonds between the alpha-amino and carboxy groups of adjacent residues. Although "protein" is often used in reference to relatively large polypeptides, and "peptide" is often used in reference to small polypeptides, usage of these terms in the art overlaps and varies. The term "peptide" as used herein refers to peptides, polypeptides, proteins and fragments of proteins, unless otherwise noted. The terms "protein" and "peptide" are used interchangeably herein when referring to a gene product and fragments thereof. Thus, exemplary peptides or proteins include gene products, naturally occurring proteins, homologs, orthologs, paralogs, fragments and other equivalents, variants, fragments, and analogs of the foregoing.

(215) As used herein, the term "linker" refers to a synthetic protein sequence of amino acids that is used to connect two polypeptide domains via peptide bonds.

(216) As used herein, the term "fusion protein" refers to a single chimeric protein comprising a protein of interest (e.g., checkpoint protein) joined to an exogenous protein or protein fragment (e.g., an anchor protein), wherein the components of the fusion protein are linked to each other by

peptide-bonds, either directly or through a peptide linker. The anchor protein of the fusion protein may enhance incorporation of the fusion protein onto and/or into the membrane of a vesicle, for example the internal and/or external leaflet of the phospholipid bilayer of an exosome membrane. The fusion protein may have at least a part of an amino acid sequence of an immune checkpoint protein or proteins involved in immune synapses. The fusion protein may have at least a part of an amino acid sequence of A2AR, VTCN1, Galectin 9, FGL-1, PECAM-1, TSG-6, STAB-1, NRP1, NRP2, SEMA3A, SEMA3F, RGMB, TIM-3, TIGIT, HLA class I, HLA class II, VISTA, HMGB1, phosphatidylserine, T-cell receptor (TCR), SHP-1, SHP-2, FBXO38, SH2D1A, B7RP1, IDO, NOX2, TNFRSF18, B7-H4, B7-H5, SISP1, B7-H6, B7-H7, APLNR, IFN y, PD-1, WNT5A, IL-6, IL-10, NKG2 family of C-type lectin receptors, ligands of NKG2 family, killer cell immunoglobulin-like receptors, CD2, CD4, CD8, CD27, CD27 ligand (CD70), CD28, CD28H, CD39, CD40, CD44, CD47, CD63, CD66a, CD80, B7-2, CD86, CD73, CD94, CD96, CD101, CD112, CD112R, CD122, CD134, CD137 (4-1BB), CD137 ligand (4-1BBL), CD152, CD154, CD155, CD158, CD158a, CD158g, CD158h, KIR2DL1, KIR2DS1, KIRDS3, KIR2DS5, CD160, CD172a, CD200, CD200R, CD223, CD226, CD252, CD270, CD272, CD273, CD274, CD275, CD276, CD278, CD279 (PD-1), CD279 ligand (PD-L1/PDL-2), CD328, CD329, and/or CD337. The fusion protein may have a polypeptide linker sequence (e.g., an Fc domain and/or a GSSG linker (SEQ ID NO: 319)), followed by an amino acid sequence coding for an anchor protein sequence (e.g., a prenylation site, fatty acylation site, or a GPI sequence) or any isoform, fragment, variation thereof, or a ligand to the aforementioned proteins thereof, or the like known by one of ordinary skill in the art. All variants are encompassed by the present invention.

(217) As used herein, the term “immune synapse” and “cell synapse” are used interchangeably and refer to cell-to-cell interaction wherein said interaction results in activation, suppression, and/or adhesion of either one or more cells. Immune synapse or cell synapse are mediated by proteins that may be cytoplasmic, membrane bound, membrane associated, and/or secreted. Immune or cell synapses may be mediated by one or more “immune checkpoint proteins” which herein refers to any protein that is involved in maintaining immune homeostasis or plays a role in regulating immune activation or suppression. Immune checkpoint proteins may be cytoplasmic, membrane bound, membrane associated, and/or secreted.

(218) As used herein, the term “fragment” or “active fragment” refers to a portion of a nucleic acid or polypeptide provided herein that retains the ability to be expressed by the engineered EVs provided herein. In some embodiments, the active fragment retains the ability to activate a target polypeptide, thereby increasing the activity of said target polypeptide (e.g., suppressing an immune response).

(219) As used herein, the terms “specifically bind” and/or “specifically recognize” or “substantially binds” refers to the affinity of a binding molecule for a target molecule compared to the binding molecule's affinity for non-target molecules. A binding molecule (e.g., a POI domain) that specifically binds a target molecule (e.g., a target polypeptide provided herein) does not substantially recognize or bind non-target molecules. e.g., an antibody “specifically binds” and/or “specifically recognize” another molecule, meaning that this interaction is dependent on the presence of the binding specificity of the molecule structure, e.g., an antigenic epitope. As used herein, “non-specific binding” and “background binding” refers to the interaction that does not depend on the presence of specific structure (e.g., a specific antigenic epitopes). Methods of measuring binding of a polypeptide to a target are known in the art (e.g., differential scanning calorimetry, isothermal titration calorimetry, spectroscopy, crystallography, surface plasmon resonance, co-immunoprecipitation, pulldown assays, crosslinking, yeast two-hybrid system, tandem affinity purification-mass spectroscopy, protein microarrays, bio-layer interferometry, far-Western blots, computational prediction, analytical ultracentrifugation, light scattering, fluorescence spectroscopy, resonance energy transfer, ELISA or ELISPOT assays, or any other assays known in the art).

(220) As used herein, the terms “treat,” “treatment,” “treating,” or “amelioration” refer to therapeutic treatments, wherein the object is to reverse, alleviate, ameliorate, inhibit, slow down or stop the progression or severity of a condition associated with, a disease or disorder. The term “treating” includes reducing or alleviating at least one adverse effect or symptom of a condition, disease or disorder associated with an infection or a cancer. Treatment is generally “effective” if one or more symptoms or clinical markers are reduced. Alternatively, treatment is “effective” if the progression of a disease is reduced or halted. That is, “treatment” includes not just the improvement of symptoms or markers, but also a cessation or at least slowing of progress or worsening of symptoms that would be expected in absence of treatment. Beneficial or desired clinical results include, but are not limited to, alleviation of one or more symptom(s), diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. The term “treatment” of a disease also includes providing relief from the symptoms or side-effects of the disease (including palliative treatment).
(221) As used herein “preventing” or “prevention” refers to any methodology where the disease state does not occur due to the actions of the methodology (such as, for example, administration of a composition or construct as described herein). In one aspect, it is understood that prevention can also mean that the disease is not established to the extent that occurs in untreated controls. Accordingly, prevention of a disease encompasses a reduction in the likelihood that a subject can develop the disease, relative to an untreated subject (e.g., a subject who is not treated with the methods or compositions described herein).
(222) As used herein, the terms “autoimmune condition” and “autoimmune disease” are used interchangeably and refer to any disease characterized by abnormal functioning of the immune system and may include, but is not limited to, achalasia, Addison's disease, adult Still's disease, agammaglobulinemia, alopecia areata, amyloidosis, ankylosing spondylitis, anti-GBM/Anti-TBM nephritis, antiphospholipid syndrome, autoimmune angioedema, autoimmune dysautonomia, autoimmune encephalomyelitis, autoimmune hepatitis, autoimmune inner ear disease (AIED), autoimmune myocarditis, autoimmune oophoritis, autoimmune orchitis, autoimmune pancreatitis, autoimmune retinopathy, autoimmune urticaria, axonal & neuronal neuropathy (AMAN), Baló disease, Behcet's disease, benign mucosal pemphigoid, bullous pemphigoid, Castleman disease, celiac disease, Chagas disease, chronic inflammatory demyelinating polyneuropathy (CIDP), chronic recurrent multifocal osteomyelitis (CRMO), Churg-Strauss syndrome (CSS), eosinophilic granulomatosis (EGPA), cicatricial pemphigoid, Cogan's syndrome, cold agglutinin disease, congenital heart block, coxsackie myocarditis, CREST syndrome, Crohn's disease, dermatitis herpetiformis, dermatomyositis, Devic's disease (neuromyelitis optica), discoid lupus, Dressler's syndrome, endometriosis, eosinophilic esophagitis (EoE), eosinophilic fasciitis, erythema nodosum, essential mixed cryoglobulinemia, Evans syndrome, fibromyalgia, fibrosing alveolitis, giant cell arteritis (temporal arteritis), giant cell myocarditis, glomerulonephritis, Goodpasture's syndrome, granulomatosis with polyangiitis, Graves' disease, Guillain-Barre syndrome, Hashimoto's thyroiditis, hemolytic anemia, Henoch-Schonlein purpura (HSP), Herpes gestationis or pemphigoid gestationis (PG), hidradenitis suppurativa (HS) (acne inversa), hypogammalglobulinemia, IgA nephropathy, IgG4-related sclerosing disease, immune thrombocytopenia purpura (ITP), inclusion body myositis (IBM), interstitial cystitis (IC), juvenile arthritis, type 1 diabetes, juvenile myositis (JM), Kawasaki disease, Lambert-Eaton syndrome, leukocytoclastic vasculitis, lichen planus, lichen sclerosus, ligneous conjunctivitis, linear IgA disease (LAD), lupus, lyme disease chronic, Meniere's disease, microscopic polyangiitis (MPA), mixed connective tissue disease (MCTD), Mooren's ulcer, Mucha-Habermann disease, multifocal motor neuropathy (MMN) or MMNCB, multiple sclerosis, myasthenia gravis, myositis, narcolepsy, neonatal Lupus, neuromyelitis optica, neutropenia, ocular cicatricial pemphigoid, optic neuritis, palindromic rheumatism (PR), PANDA, paraneoplastic cerebellar degeneration (PCD), paroxysmal

nocturnal hemoglobinuria (PNH), Parry Romberg syndrome, pars planitis (peripheral uveitis), Parsonage-Turner syndrome, pemphigus, peripheral neuropathy, perivenous encephalomyelitis, pernicious anemia (PA), POEMS syndrome, polyarteritis nodosa, polyglandular syndromes type I, II, III, polymyalgia rheumatica, polymyositis, postmyocardial infarction syndrome, postpericardiotomy syndrome, primary biliary cirrhosis, primary sclerosing cholangitis, progesterone dermatitis, psoriasis, psoriatic arthritis, pure red cell aplasia (PRCA), pyoderma gangrenosum, Raynaud's phenomenon, reactive arthritis, reflex sympathetic dystrophy, relapsing polychondritis, restless legs syndrome (RLS), retroperitoneal fibrosis, rheumatic fever, rheumatoid arthritis, sarcoidosis, Schmidt syndrome, scleritis, scleroderma, Sjögren's syndrome, sperm & testicular autoimmunity, stiff person syndrome (SPS), subacute bacterial endocarditis (SBE), Susac's syndrome, sympathetic ophthalmia (SO), takayasu's arteritis, temporal arteritis/Giant cell arteritis, thrombocytopenia purpura (TTP), Tolosa-Hunt syndrome (THS), transverse myelitis, type 1 diabetes, ulcerative colitis (UC), undifferentiated connective tissue disease (UCTD), uveitis, vasculitis, vitiligo, Vogt-Koyanagi-Harada disease. An autoimmune condition or autoimmune diseases may be caused by, but not limited to, a natural predisposition, an infection (e.g., bacteria or virus), drugs, vaccination, environmental triggers (e.g., toxins or chemicals such as dust, silica, oil, benzene, tri- or per-chloroethylene etc.), stress, cancer, blood or tissue or organ transplantation, or unknown etiology. Autoimmune disorders may result in but not limited to the destruction of body tissue, abnormal growth of an organ or tissue, changes in organ or tissue function (e.g., changes in blood vessels, connective tissue, function of endocrine glands, joints, muscles, blood cells, skin, etc.).

(223) As used herein, the term “cancer” refers to a hyperproliferation of cells that exhibit a loss of normal cellular control that results in unregulated growth, lack of differentiation, local tissue invasion, and metastasis. The methods and compositions described herein can be used for the treatment of solid tumors (e.g., cancer) or non-solid tumors, such as leukemia, blood cell cancers, and the like. Solid tumors can be found in bones, muscles, the brain, or organs, and can be sarcomas or carcinomas. Where the methods and compositions described herein can overcome barriers of tumor treatment, including, but not limited to barriers to treatment or inhibition of metastases, it is contemplated that aspects of the technology described herein can be used to treat all types of solid and non-solid tumor cancers, including cancers not listed in the instant specification. The compositions and methods described herein, without limitation, include methods of treating cancer, methods of inhibiting metastases, and methods of inducing an anti-tumor immune response.

(224) As used herein, the terms “subject”, “individual”, “host”, and “patient” are used interchangeably and may refer to any animal, mammal, bird, fish, reptile, and amphibian, for example, human, monkey, dog, cat, horse, pig, cattle, ox, donkey, rabbit, sheep, goat, mouse, rat, guinea pig, llama, chicken, goose, duck, turkey, or the like receiving or registered to receive a therapeutic amount of a composition of the present invention for medical care or treatment.

(225) As used herein, the term “injection” refers to any process or method which allows the person skilled in the art to administer any therapeutic to a target site by penetration. Examples of injection are, but not limited to, subcutaneous, subcuticular, subcapsular, subarachnoid, intradermal, intramuscular, intravenous, intra-arterial, intraventricular, intracapsular, intraorbital, intraocular, intrathoracic, intraperitoneal, intravitreal, retro-orbital, intranasal, intracerebral, intrathymic, intraspinal, intrasternal, intra-articular, intracavernous, intracardiac, intraosseous, intrathecal, transtracheal, epidural, or the like as known in the art. A therapeutic does of the present invention may be delivered to a patient by means of controlled release, for example but not limited to, implantable pump and implantable cannulas to provide continuous access to the venous or arterial system.

(226) As used herein, the term “topical application” refers to applying or spreading a composition of the present invention onto surfaces on or in the body, both internally and/or externally, in a

therapeutically effective amount for local and/or systemic treatment. Topical application may be epicutaneuos wherein a composition of the present invention may be directly applied onto a localized surface of the skin or mucous membranes. Topical application may include transdermal application wherein a composition of the present invention may be absorbed into the body to obtain systemic delivery and systemic distribution. For example, a transdermal patch may be applied onto the body to deliver a therapeutic dose of a composition of the invention presented herein. Topical application formulations may include, but are not limited to, creams, foams, gels, lotions, solutions, ointments, dermal patch, transdermal patches, powder, solid, sponge, tape, vapor, paste, film, liposomes, balm, shampoo, spray, or tincture. A therapeutic dose of a composition of the present invention may be delivered vaginally (for example a vaginal suppository, vaginal ring, douche, intrauterine device, intravesical infusion, and the like) or urethra.

(227) As used herein, the term “enteral administration” refers to a composition of the present invention administered via the gastrointestinal tract in a therapeutically effective amount for local or systemic treatment. Enteral administration may include, but is not limited to, delivery of a composition of the present invention via the mouth, sublingual, esophagus, gastric (for example the stomach), small intestines, large intestines or rectum. Oral delivery of the present invention may include, but is not limited to, the use of a capsule, pastille, pill, tablet, solution, gel, suspension, emulsion, syrup, elixir, tincture, mouthwash, lozenges, chewing gum, lollipop, osmotic-controlled release oral delivery system, or the like. Gastric delivery may involve the use of a tube or nasal passage that leads directly to the stomach, for example, a percutaneous endoscopic gastrostomy tube. Gastric delivery may involve direct injection made through the abdominal wall. Rectal delivery may involve, but is not limited to, the use of a suppository, ointment, enema, murphy drip, or the like. A therapeutic does of the present invention may be delivered to a patient by means of controlled release, for example but not limited to, controlled release drug delivery pellet or pill.

(228) As used herein, the terms “pulmonary system” or “respiratory system” are used interchangeably and refer, but are not limited, to the respiratory region, conducting airways, nasal cavity, sinuses, nasopharynx, oropharynx, larynx, trachea, bronchi, bronchioles, respiratory bronchioles, alveolar ducts, alveolar sacs, respiratory epithelium (e.g., alveolar epithelial cells), endothelial cells, or the like.

(229) As used herein, the terms “pulmonary delivery” and “pulmonary administration” are used interchangeably and refer to delivering a composition of the present invention to the respiratory system through the respiratory route, including but not limited to, intranasal administration, oral administration, and oral inhalative administration (e.g., intratracheal instillation and intratracheal inhalation) of a therapeutically effective amount for local or systemic treatment. Pulmonary delivery of a therapeutically effective amount of a composition of the present invention may be achieved by dispersion, for example by using a syringe. Pulmonary delivery of a composition of the present invention may be achieved by aerosol administration, wherein aerosol administration may deposit a therapeutically effective amount of the present invention by gravitational sedimentation, inertial impaction, or diffusion.

(230) Pulmonary delivery of a therapeutically effective amount of a composition of the present invention may be deposited on any mucus layer of the respiratory system, for example, but not limited to, the mucus layer which coats the walls of conducting airways, the smaller airway, and/or alveolar space.

(231) As used herein, an “appropriate control” refers to an untreated, otherwise identical cell or population (e.g., a subject who was not administered the composition described herein, or was administered by only a subset of agents provided herein, as compared to a non-control cell).

(232) As used herein, a “reference level” can refer to one or more parameters or markers as measured for a normal, otherwise unaffected cell population or tissue (e.g., a biological sample obtained from a healthy subject, or a biological sample obtained from the subject at a prior time point, or a biological sample that has not yet been contacted with a pathogen as described herein).

For measuring or monitoring therapeutic efficacy, a level determined prior to treatment or earlier in treatment can also provide a reference level for a given parameter or value.
(233) As used herein, the term “modulates” refers to an effect including increasing or decreasing a given parameter as those terms are defined herein.
(234) The terms “increased,” “increase,” “increases,” or “enhance” or “activate” are all used herein to generally mean an increase of a property, level, or other parameter by a statistically significant amount; for the avoidance of any doubt, the terms “increased”, “increase” or “enhance” or “activate” means an increase of at least 10% as compared to a reference level, for example an increase of at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90% or up to and including a 100% increase or any increase between 10-100% as compared to a reference level, or at least about a 2-fold, or at least about a 3-fold, or at least about a 4-fold, or at least about a 5-fold or at least about a 10-fold increase, at least about a 20-fold increase, at least about a 50-fold increase, at least about a 100-fold increase, at least about a 1000-fold increase or more as compared to a reference level. For example, increasing activity can refer to activating a receptor or a signaling pathway (e.g., antibody production or inflammation).
(235) The terms “decrease”, “reduced”, “reduction”, or “inhibit” are all used herein to mean a decrease or lessening of a property, level, or other parameter by a statistically significant amount. In some embodiments of any of the aspects, “reduce,” “reduction” or “decrease” or “inhibit” typically means a decrease by at least 10% as compared to a reference level (e.g., the absence of a given treatment) and can include, for example, a decrease by at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or more. As used herein, “reduction” or “inhibition” does not encompass a complete inhibition or reduction as compared to a reference level. “Complete inhibition” is a 100% inhibition as compared to a reference level. A decrease can be preferably down to a level accepted as within the range of normal for an individual without a given disorder.
(236) As used herein the term “comprising” or “comprises” is used in reference to compositions, methods, and respective component(s) thereof, that are essential to the method or composition, yet open to the inclusion of unspecified elements, whether essential or not.
(237) As used herein the term “consisting essentially of” refers to those elements required for a given embodiment. The term permits the presence of elements that do not materially affect the basic and novel or functional characteristic(s) of that embodiment.
(238) The term “consisting of” refers to compositions, methods, and respective components thereof as described herein, which are exclusive of any element not recited in that description of the embodiment.
(239) As used in this specification and the appended claims, the singular forms “a,” “an,” and “the” include plural references unless the context clearly dictates otherwise. Thus for example, references to “the method” includes one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure and so forth. Similarly, the word “or” is intended to include “and” unless the context clearly indicates otherwise. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of this disclosure, suitable methods and materials are described below.
(240) The abbreviation, “e.g.” is derived from the Latin exempli gratia, and is used herein to indicate a non-limiting example. Thus, the abbreviation “e.g.” is synonymous with the term “for example.”
(241) The abbreviation, “etc.” is derived from the Latin et cetera, and is used herein to indicate a non-limiting list. Thus, the abbreviation “etc.,” is synonymous with the term “and other similar

things", or "and so forth".
(242) Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein should be understood as modified in all instances by the term "about." The term "about" when used in connection with percentages can mean±1%.
(243) The term "statistically significant" or "significantly" refers to statistical significance and generally means a two-standard deviation (2SD) difference, above or below a reference value. Additional definitions are provided in the text of individual sections below.
(244) It should be understood that this invention is not limited to the particular methodology, protocols, and reagents, etc., described herein and as such may vary. The terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which is defined solely by the claims.
(245) As used herein and in the claims, the singular forms include the plural reference and vice versa unless the context clearly indicates otherwise. The term "or" is inclusive unless modified, for example, by "either." Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein should be understood as modified in all instances by the term "about."
(246) Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs.
(247) It is to be understood that the foregoing description and the following examples are illustrative only and are not to be taken as limitations upon the scope of the invention. Various changes and modifications to the disclosed embodiments, which will be apparent to those of skill in the art, may be made without departing from the spirit and scope of the present invention. Further, all patents, patent applications, and publications identified are expressly incorporated herein by reference for the purpose of describing and disclosing, for example, the methodologies described in such publications that might be used in connection with the present invention. These publications are provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention or for any other reason. All statements as to the date or representation as to the contents of these documents are based on the information available to the applicants and do not constitute any admission as to the correctness of the dates or contents of these documents.
(248) All patents and other publications identified are expressly incorporated herein by reference for the purpose of describing and disclosing, for example, the methodologies described in such publications that could be used in connection with the present invention. These publications are provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention or for any other reason. All statements as to the date or representation as to the contents of these documents is based on the information available to the applicants and does not constitute any admission as to the correctness of the dates or contents of these documents.
(249) Some embodiments of the technology described herein can be defined according to any of the following numbered paragraphs: 1. An engineered extracellular vesicle comprising: at least one fusion polypeptide comprising: (i) at least one protein of interest (POI) domain or a fragment thereof; and (ii) at least one vesicle targeting domain, wherein the POI domain is in an extracellular position relative to a lipid membrane of the extracellular vesicle. 2. The engineered extracellular vesicle of paragraph 1, wherein the extracellular vesicle is an exosome. 3. The engineered extracellular vesicle of paragraph 1 or paragraph 2, wherein the protein of interest (POI) domain or a fragment thereof is a N-terminal domain of the fusion polypeptide. 4. The engineered

extracellular vesicle of any one of paragraphs 1-3, wherein the vesicle targeting domain is a C-terminal domain of the fusion polypeptide. 5. The engineered extracellular vesicle of any one of paragraphs 1-4, wherein the fusion polypeptide comprises at least two POI domains and/or at least two exosome targeting domains. 6. The engineered extracellular vesicle of any one of paragraphs 1-5, wherein the fusion polypeptide further comprises a peptide linker. 7. The engineered extracellular vesicle of any one of paragraphs 1-6, wherein the fusion polypeptide further comprises a fragment crystallizable region (Fc) domain. 8. The engineered extracellular vesicle of any one of paragraphs 1-7, wherein the vesicle targeting domain is in a luminal position relative to the lipid membrane of the extracellular vesicle. 9. The engineered extracellular vesicle of any one of paragraphs 1-7, wherein the vesicle targeting domain in an exterior position relative to the lipid membrane of the extracellular vesicle. 10. The engineered extracellular vesicle of any one of paragraphs 1-9, wherein the POI domain is selected from the group consisting of: Table 1. 11. The engineered extracellular vesicle of any one of paragraphs 1-10, wherein the POI domain is PD-L1 or a fragment thereof. 12. The engineered extracellular vesicle of any one of paragraphs 1-11, wherein the POI domain is PD-L2 or a fragment thereof. 13. The engineered extracellular vesicle of any one of paragraphs 1-12, wherein the POI domain is FGL1 or a fragment thereof. 14. The engineered extracellular vesicle of any one of paragraphs 1-13, wherein the POI domain is 4-1BBL or a fragment thereof. 15. The engineered extracellular vesicle of any one of paragraphs 1-14, wherein the POI domain is CTLA-4 or a fragment thereof. 16. The engineered extracellular vesicle of any one of paragraphs 1-15, wherein the POI domain substantially binds to one or more of a target polypeptide. 17. The engineered extracellular vesicle of paragraph 16, wherein the target polypeptide is selected from the group consisting of: Table 2. 18. The engineered extracellular vesicle of any one of paragraphs 1-17, wherein the vesicle targeting domain is selected from the group consisting of: Table 3. 19. The engineered extracellular vesicle of any one of paragraphs 1-18, wherein the linker is in an exterior position relative to the lipid membrane of the extracellular vesicle. 20. The engineered extracellular vesicle of any one of paragraphs 1-18, wherein the linker is a transmembrane linker. 21. The engineered extracellular vesicle of any one of paragraphs 1-18, wherein the linker is in a luminal position relative to the lipid membrane of the extracellular vesicle. 22. The engineered extracellular vesicle of any one of paragraphs 1-21, wherein the extracellular vesicle does not comprise an endogenous POI polypeptide. 23. A composition comprising a plurality of the engineered extracellular vesicles of any one of paragraphs 1-22. 24. The composition of paragraph 23, further comprising a pharmaceutically acceptable carrier. 25. An engineered extracellular vesicle comprising: (a) a first fusion polypeptide comprising: (i) at least one protein of interest (POI) domain or a fragment thereof; and (ii) at least one vesicle targeting domain, wherein the at least one POI domain is in an extracellular position relative to a lipid membrane of the extracellular vesicle, (b) a second fusion polypeptide comprising: (i) at least one protein of interest (POI) domain or a fragment thereof; and (ii) at least one vesicle targeting domain, wherein the POI domain is in an extracellular position relative to a lipid membrane of the extracellular vesicle, and wherein the at least one vesicle targeting domain is within a lipid membrane of the extracellular vesicle. 26. A composition comprising two or more of the engineered extracellular vesicles selected from any one of paragraphs 1-25. 27. An extracellular vesicle composition comprising: a plurality of artificial synapses, wherein each artificial synapse comprises (i) an extracellular vesicle; (ii) one or more sticky binders; and (iii) one or more signaling domains. The composition of paragraph 27, wherein the extracellular vesicle comprises an exosome. 28. The composition of paragraph 27, wherein the one or more sticky binders is selected from the group consisting of: a GPI anchor, a fatty acylation site, and a prenylation site. 30. The composition of paragraph 27, wherein the signaling domain comprises one or more of: PD-L1, PD-L2, CTLA-4 (CD152), 4-1BBL (CD137L), HVEM (CD270), FGL1, OX-2 (CD200), Galectin-9, PVR (CD155), Nectin-2 (CD112) isoform alpha, Nectin-2 (CD112) isoform beta, Nectin-2 (CD112) isoform delta, IL-10, TSG-6, B7-H3 (CD276), B7-H4 (VTCN1), B7-H5

(VISTA), B7-H7 (HHLA2), BTNL1, VSIG8, VSIG3 (IGSF11), VSIG4, TIM-3 (HAVCR2), TIM-4 (TIMD4), CEACAM1, BTN3A1, BTN3A2, BTN2A1, BTNL8, BTN2A2, BTN1A1, TIGIT, CD27L (CD70), CD30L (CD153), GITRL, CD40L (CD154), LIGHT (CD258), TL1, CD80, CD86, LFA-3 (CD58), SLAM (CD150), CD40, CD28, CD28H, CD2, LFA-3 (CD58), CD48, CD226, DR3, DcR3, FasL, TIM-1 (CD365), PD-1, or active fragment thereof. 29. A method of producing the engineered extracellular vesicle or the composition of any one of paragraphs 1-30, comprising: (a) providing a population of cells expressing a vector construct encoding one or more sticky binder and one or more signaling domains; and (b) isolating a plurality of artificial synapses from the population of cells. 30. A method of producing the engineered extracellular vesicle or the composition of any one of paragraphs 1-30, comprising: (a) providing a population of cells expressing a vector construct encoding one or more sticky binder and one or more signaling domains; and (b) isolating a plurality of artificial synapses from the population of cells; and (c) purifying the plurality of artificial synapses from the population of cells. 33. The method of paragraph 31 or paragraph 32, the isolating is via size exclusion chromatography. 34. The method of paragraph 32, wherein the purifying is via multimodal chromatography. 35. The method of any of paragraphs 31-34, further comprising performing an assay for POI binding to a target polypeptide. 36. The method of paragraph 35, wherein the vector construct further encodes a promoter. 37. The method of paragraph 36, wherein the promoter is a tissue-specific promoter or an inducible promotor. 38. A method of modulating inflammation in a subject, the method comprising: administering a composition comprising a plurality of engineered extracellular vesicles to a subject in need thereof, wherein the engineered extracellular vesicles comprise at least one fusion polypeptide comprising: (i) at least one protein of interest (POI) domain or a fragment thereof; and (ii) at least one vesicle targeting domain. 39. The method of paragraph 38, wherein the extracellular vesicle comprises an exosome. 40. The method of any one of paragraphs 38-39, further comprising selecting a subject that has or is suspected of having an autoimmune disease or an inflammatory disease or condition. 41. The method of any one of paragraphs 38-40, wherein the vesicle targeting domain is selected from the group consisting of: a Glycosylphosphatidylinositol (GPI) anchor, a fatty acylation site, and a prenylation site. 42. The method of any one of paragraphs 38-41, wherein the vesicle targeting domain is a GPI anchor. 43. The method of any one of paragraphs 38-41, wherein the vesicle targeting domain is C1C2. 44. The method of any one of paragraphs 38-43, wherein the protein of interest (POI) domain comprises one or more of: PD-L1, PD-L2, CTLA-4 (CD152), 4-1BBL (CD137L), HVEM (CD270), FGL1, OX-2 (CD200), Galectin-9, PVR (CD155), Nectin-2 (CD112) isoform alpha, Nectin-2 (CD112) isoform beta, Nectin-2 (CD112) isoform delta, IL-10, TSG-6, B7-H3 (CD276), B7-H4 (VTCN1), B7-H5 (VISTA), B7-H7 (HHLA2), BTNL1, VSIG8, VSIG3 (IGSF11), VSIG4, TIM-3 (HAVCR2), TIM-4 (TIMD4), CEACAM1, BTN3A1, BTN3A2, BTN2A1, BTNL8, BTN2A2, BTN1A1, TIGIT, CD27L (CD70), CD30L (CD153), GITRL, CD40L (CD154), LIGHT (CD258), TL1, CD80, CD86, LFA-3 (CD58), SLAM (CD150), CD40, CD28, CD28H, CD2, LFA-3 (CD58), CD48, CD226, DR3, DcR3, FasL, TIM-1 (CD365), PD-1, or active fragment thereof. 45. The method of any one of paragraphs 38-44, wherein the protein of interest (POI) domain is PD-L1 or a fragment thereof. 46. The method of any one of paragraphs 38-44, wherein the protein of interest (POI) domain is PD-L2 or a fragment thereof. 47. The method of any one of paragraphs 38-44, wherein the protein of interest (POI) domain is CTLA-4 or a fragment thereof. 48. The method of any one of paragraphs 38-44, wherein the protein of interest (POI) domain is HVEM or a fragment thereof. 49. The method of paragraph 40, wherein the inflammatory disease and/or condition is acute. 50. The method of paragraph 40, wherein the inflammatory related disease and/or condition is chronic. 51. The method of paragraph 38, wherein administering the composition comprises injection, topical administration, or inhalation. 52. Use of a composition comprising a plurality of engineered extracellular vesicles, the engineered extracellular vesicles each comprising: at least one fusion polypeptide comprising: (i) at least one protein of interest (POI) domain or a fragment thereof; and (ii) at least one vesicle

targeting domain for the treatment of an inflammatory disease or condition. 53. Use of a composition comprising a plurality of engineered extracellular vesicles, the engineered extracellular vesicles each comprising: at least one fusion polypeptide comprising: (i) at least one protein of interest (POI) domain or a fragment thereof; and (ii) at least one vesicle targeting domain for the treatment of an autoimmune disease or condition. 54. Use of a composition comprising a plurality of engineered extracellular vesicles, the engineered extracellular vesicles each comprising: at least one fusion polypeptide comprising: (i) at least one protein of interest (POI) domain or a fragment thereof; and (ii) at least one vesicle targeting domain for the treatment of cancer.

EXAMPLES

(250) The following examples are provided by way of illustration, not limitation.

Example 1

Design of Artificial Synapse

(251) As described, artificial synapses are engineered to induce and propagate biological signaling, including for example, antagonist and agonist signaling. Artificial synapses are designed to include hallmark biophysical and biochemical features of extracellular vesicles, further including vesicle targeting domains and signaling domains. Vesicle targeting domains capable of attaching to extracellular vesicles such as exosomes, signaling domains, optionally including a linker (e.g., Fc linker), can be organized in genetic vector constructs. Designs are shown in FIG. **1**.

(252) Sticky binders are extracellular vesicle targeting sequences. Preliminary extracellular vesicle targeting sequences of interest are from, but not limited to, 4F2 (CD98), ADAM10, CD298, TFR2, transmembrane domains of CD9, MARCKS, KRAS, etc. or the like as appreciate by one of ordinary skill in the art. The Inventors discovered high efficiency when proteins are engineered with a GPI domain. Optionally, linker regions such as an Fc linker between the vesicle targeting domains and signaling domains can be added.

(253) A variety of signaling domains are of interest with proof-of-concept examples including PD-L1, PD-L2 and CTLA-4 (CD152). Artificial synapses including these three signaling domains are shown in FIGS. **2-5**.

(254) Each of these elements are described in the following non-limiting examples.

Example 2

Genetic Constructs

(255) Examples of constructs including these variable elements (e.g., sticky binders GPI or C1C2, or signaling domains including PD-L1, PD-L2 and CTLA-4 (CD152) were engineered into vectors shown in FIGS. **2-5**.

Example 3

Purification of hPD-L1 Tagged Artificial Synapses by a Multimodal Resin Marketed for Exosome Purification

(256) Upon expression of hPD-L1-Fc-GPI in mammalian cells, artificial synapses were further purified using a size exclusion resin marketed for exosome purification. Large MW artificial synapses elute in the first fraction as shown by the high hPD-L1 concentration and exosome quantity (2.26E9 artificial synapses/ml) in elution 1. Clean in place (CIP) fractions show bound and eliminated proteins from the Inventors' exosome elution. Results are shown in FIG. **6**.

Example 4

hPDL1-Fc-GPI Exosome Purification-Size Exclusion Chromatography Column

(257) Artificial synapses engineered from exosomes such as hPDL1-Fc-GPI after elution from size exclusion resin marketed for exosome purification can be further purified via a size exclusion column as shown here. Using a size exclusion chromatography (SEC), artificial synapses elute in fractions 7-9. Total protein (determined by Qubit™) and hPD-L1 ng/ml (determined by ELISA) of each fraction is shown in the graph. Bars show exosome number per ml (i.e., 1E10 artificial synapses/ml etc.). Fractions 7-9 contain >99% purified artificial synapses. Fractions 7-9 are pooled and may be concentrated using a filtration device, for example a 10K MWCO Amicon®

Centrifugal Filter. Final purified product is filtered through a low protein binding 0.2 μm or 0.45 μm filter, for example a PES filter. Results are shown in FIG. **7**.

Example 5

hPD-L1 Expression on Artificial Synapses

(258) Exosome quantity and hPD-L1 concentration was determined in SEC fractions 7-9. Knowing the molecular weight of engineered hPD-L1, the Inventors can determine the number of hPD-L1 molecules per exosome to be approximately between 12 to 40 PD-L1/exosome. This value is consistent between different purification runs and constructs. Results are shown in FIG. **8**.

Example 6

Purification of hPD-L2-Fc-GPI Artificial Synapses Via Multimodal Resin Chromatography Marketed for Exosome Purification

(259) This graph shows Abs 280 of multimodal resin chromatography fractions and quantity of hPDL2 in indicated fractions. Artificial synapses eluted in Elution 1.

(260) Clean in place (CIP) fractions show bound and eliminated proteins from the Inventors' exosome elution. Results are shown in FIG. **9**.

Example 7

PD-L2 Purification Via Size Exclusion Chromatography

(261) Artificial synapses engineered from artificial synapses such as hPDL2-GPI after elution from size exclusion resin size exclusion resin marketed for exosome purification are further purified via size exclusion chromatography as shown. Results are shown in FIG. **10**.

Example 8

hCTLA4-Fc-GPI Exosome Purification Via Size Exclusion Chromatography

(262) Using size exclusion chromatography marketed for exosome purification, artificial synapses elute in fractions 7-9. Total protein (determined by Qubit™) and hPD-L1 ng/ml (determined by ELISA) of each fraction is shown in the graph. Fractions 7-9 are pooled and contain >99% purified artificial synapses. Pooled artificial synapses engineered from artificial synapses fractions may then be concentrated using a filtration device, for example a 10K MWCO Amicon®. Final purified product is filtered through a low protein binding filter, for example a 0.2 μm or 0.45 μm PES filter. Results are shown in FIG. **11**.

Example 9

PD-L1 and PD-L2 In Vitro Assay from DISCOVERX®

(263) To perform this validation method, the Inventors modified the PathHunter® PD-1 Signaling Bioassay from DISCOVERX® Briefly, the PathHunter® PD-1 Signaling Bioassay relies on the well-established PathHunter® Enzyme Fragment Complementation (EFC) technology to interrogate receptor activity. EFC consists of a split β-galactosidase (β-gal) enzyme: the Enzyme Donor (ED) and Enzyme Acceptor (EA) fragments which independently have no β-gal activity. However, when forced to complement they form an active β-gal enzyme that will hydrolyze substrate to produce a chemiluminescent signal. The PathHunter® PD-1 Signaling Bioassay consists of human cells engineered to stably express an ED-tagged PD-1 receptor, while EA is fused to the phosphotyrosine-binding SH2 domain of the intracellular signaling protein, SHP1. Ligand or antibody-induced activation of the receptor results in phosphorylation of the receptor's cytosolic tail. The SH2-domain fused to EA binds the phosphorylated receptor, forcing complementation of ED and EA, resulting in formation of an active β-gal enzyme, which hydrolyzes the substrate to produce a chemiluminescent signal. Full-length PD-1 receptor was engineered with a small β-gal fragment (ED in red) fused to its C-terminus, and the SH2-domain of SHP1 was engineered with the complementing β-gal fragment (EA). These constructs were stably expressed in Jurkat cells (produced by DISCOVERX®), while PD-L1 and PD-L2 was stably expressed on artificial synapses produced by Diadem Biotherapeutics. Artificial synapses were engineered to have surface expressed human PD-L1 or PD-L2. Briefly, the gene sequence coding for the extracellular domain of human PD-L1 or PD-L2 was linked to the exosome via a

glycosylphosphatidylinositol (GPI) linker with an Fc domain between the linker and PD-L1 or PD-L2 (PD-L1-Fc-GPI and PD-L2-Fc-GPI). Additional variations of the Inventors' PD-L1 and PD-L2 artificial synapses include cloning a C1C2 linker (from MFGE8) in place of the GPI linker, and with or without the Fc domain. The Inventors also cloned murine versions of PD-L1 and PD-L2 extracellular domains in place of the human PD-L1 and PD-L2 all variations. Ligand engagement, through addition of ligand-presenting artificial synapses, results in phosphorylation of PD-1, leading to the recruitment of SHP1-EA

(264) The Inventors obtained approximately 1000× higher increase in Relative Light Units (RLU) in Jurkat signaling cells treated with PD-L1 or PD-L2 labeled artificial synapses when compared to soluble PD-L1-Fc or PD-L2-Fc ligand, respectively. Meaning, it took 1000× less μg/ml of PD-L1 or PD-L2 on artificial synapses than solubilized PD-L1-Fc or PD-L2 ligand to achieve the same RLU signaling. Results are shown in FIG. **12**.

Example 10

PD-L1 In Vivo Assay-Experimental Autoimmune Uveoretinitis (EAU) in Lewis Rats Bioassay

(265) Experimental autoimmune uveoretinitis (EAU) is an organ-specific, T lymphocyte-mediated autoimmune disease, which serves as a model for several human ocular inflammations of an apparently autoimmune nature. There is a statistically significant initial reduction in EAU in mPDL1 artificial synapse treated rats via either the intravitreal and intravenous delivery modes. 2nd intravitreal and 3rd intravenous injections are performed on Day 12. There appears to be a more rapid rate of resolution in the 1× intravitreal and intravenous groups. (C) Simplified view of aforementioned results. (D) Weight of rats was monitored throughout the study. 3rd intravitreal and 4th intravenous injections are performed on Day 16. There does not appear to be any significant change in EAU in any of the test groups. The aforementioned results provide proof of principle of successfully immunizing the rats with human cell derived artificial synapses with mouse PDL1 injected into rats. Results are shown in FIG. **13**.

Example 11

Engineered Exosome Multivalent Display

(266) The inventors have developed the following 3 types of protein display on or within exosomes: Type I membrane proteins wherein the N-Terminus is on the luminal (interior) side of the exosome membrane and the C-Terminus is on the exterior of the exosome. Type II membrane proteins wherein the N-Terminus is on the exterior while the C-Terminus is on the interior. Luminal internally loaded proteins which are linked to the exosome by a Myristoylation/Palmitoylation site which attaches proteins to the interior of the exosome membrane.

(267) FIGS. **14**-**21** demonstrate the various embodiments of the engineered extracellular vesicles.

(268) Additional embodiments or ligands displayed on the exosome surface (Type I and Type II membrane proteins) and internal luminal display can include the following: Type I: PD-L1, PD-L2, FGL1, OX40L. Type II: 4-1BBL, GITRL, CD27L, CD30L Luminal: NanoLuc® luciferase; Green fluorescent protein (GFP) (e.g., eGFP, etc.); Red fluorescent protein (RFP) (e.g., mScarlet, mCherry, mRuby, tdTomato, etc.); Cyan fluorescent protein (CFP); Yellow fluorescent protein (YFP); A therapeutic protein; and CRISPR/CAS-9

(269) FIG. **20** shows an exemplary multiple protein display construct. Sequences such as P2A, E2A, F2A, and T2A induce ribosomal slippage which prevent peptide bond formation, meaning that a single mRNA transcript with a 2A sequence will result in two separate peptides after translation. This allows the expression of two separate proteins from one promoter region and thus loading of two proteins on an exosome. Any combination of the proteins of interest domains provided herein can be engineered. Furthermore, a cell line with multiple transgene inserts under separate promoter control. Either method can be used to label Type I, Type II, and luminal display proteins.

Example 12a

Designed and Engineered Human Fusion Polypeptide Constructs

(270) The inventors have designed, engineered, and purified the following human fusion polypeptide constructs for therapeutic use (FIG. **5**A-FIG. **5**WW): pEF5-FRT-hPDL1-C1C2 (FIG. **5**I) pEF5-FRT-hPDL2-C1C2 (FIG. **5**J) pEF5-FRT-hPDL1-GPI-P2A-hFGL1-GPI (FIG. **5**E) pEF5-FRT-hCTLA4-Fc-GPI (FIG. **5**C) pEF5-FRT-hPDL2-Fc-GPI (FIG. **5**H) pEF5-FRT-hPD-L1-GPI-P2A-hHVEM-GPI (FIG. **5**D) pEF5-FRT-hPDL1-GPI (FIG. **5**F) pcDNA5-FRT-hSecPDL1-GPI (FIG. **5**O) pcDNA5-FRT-hPDL1-GPI (FIG. **5**F) pcDNA5-FRT-hPDL1-Link-GPI (FIG. **5**T) pcDNA5-FRT-4F2-h41BBL (FIG. **5**K) pcDNA5-FRT-Tfr2-h41BBL (FIG. **5**P) pEF5-FRT-hPDL1-Fc-GPI (FIG. **5**G) pcDNA5-FRT-CD9tm3-h41BBL (FIG. **5**Q) pcDNA5-FRT-hPDL1-Fc-GPI (FIG. **5**G) pcDNA5-FRT-hPDL1-4Fc-CD9tm2 (FIG. **5**RR) pcDNA5-FRT-hPDL1-Fc-CD9tm2KRAS (FIG. **5**UU) pcDNA5-FRT-hPDL1-4Fc-CD9tm2KRAS (FIG. **5**SS) pcDNA5-FRT-hPDL1-4Fc-GPI (FIG. **5**L) pcDNA5-FRT-hPDL1-ADAM10 (FIG. **5**QQ) pcDNA5-FRT-MyrPalm-4F2-h41BBL (FIG. **5**R) pcDNA5-FRT-MyrPalm-h41BBL (FIG. **5**S) pcDNA5-FRT-hPDL1-Fc-CD9tm2 (FIG. **5**TT) pcDNA5-FRT-hSecPDL1-CD9tm4 (FIG. **5**W) pcDNA5-FRT-hSecPDL1-CD9tm2KRas (FIG. **5**V) pcDNA5-FRT-hSecPDL1-CD9tm2 (FIG. **5**U) pcDNA5-FRT-hSecPDL1-CD81 (FIG. **5**X) pEF5-FRT-hCD200-Fc-GPI (FIG. **5**Y) pEF5-FRT-hCD200-GPI (FIG. **5**BB) pEF5-FRT-hTSG6-GPI (FIG. **5**FF) pEF5-FRT-hPDL2-GPI (FIG. **5**EE) pEF5-FRT-hFGL-1-GPI (FIG. **5**Z) pEF5-FRT-hHVEM-GPI (FIG. **5**DD) pEF5-FRT-hGal9-GPI (FIG. **5**CC) pEF5-FRT-hHVEM-Fc-GPI (FIG. **5**GG); and pEF5-FRT-hGal9-Fc-GPI (FIG. **5**AA)

Example 12b

Designed and Engineered Fusion Polypeptide Constructs

(271) The inventors have designed, engineered, and purified the following mouse fusion polypeptide constructs for therapeutic use (FIG. **5**A-FIG. **5**WW): pcDNA5-FRT-mPDL1-mFc-CD9tm2KRAS (FIG. **5**WW) pcDNA5-FRT-mPDL1-mFc-CD9tm2 (FIG. **5**VV) pcDNA5-FRT-mPDL1-mFc-GPI (FIG. **5**NN) pcDNA5-FRT-mPDL1-GPI (FIG. **5**KK) pEF5-FRT-mPDL2-GPI (FIG. **5**OO) pEF5-FRT-mPDL1-GPI-P2A-mHVEM-GPI (FIG. **5**PP) pEF5-FRT-mPDL1-GPI (FIG. **5**KK) pEF5-FRT-mPDL2-Fc-GPI (FIG. **5** MM) pEF5-FRT-mPDL1-Fc-GPI (FIG. **5**JJ) pEF5-FRT-mCTLA4-Fc-GPI (FIG. **5**HH) pEF5-FRT-mPDL1-C1C2 (FIG. **5**II); and pEF5-FRT-mPDL2-C1C2 (FIG. **5**LL).

Example 12c

Designed and Engineered Luminal Loaded Fusion Polypeptide Constructs

(272) The inventors have designed, engineered, and purified the following fusion polypeptide constructs for internal luminal loading of the fusion polypeptide: pcDNA5-FRT-Myr-NanoLuc (FIG. **5**M) pcDNA5-FRT-Myr-mScarlet (FIG. **5**N)

Example 13

Purification of Exosomes Labeled with Type I Membrane Fusion Polypeptides

(273) The inventors have purified engineered EVs, including hPD-L1-GPI; hPDL1-Fc-GPI; hPDL2-Fc-GPI; hCTLA4-Fc-GPI; mPDL1-GPI; and mPD-L1-Fc-GPI. The process for purification and analytical processing of the engineered EVs are shown in the flow chart provided in FIG. **21**.

(274) Size exclusion chromatography was performed to purify hPD-L1-GPI (no Fc) exosomes (FIG. **24**). Protein, RNA and DNA measurements in SEC fractions. Invitrogen Qubit™ fluorometric assays were used to measure biomolecules from unmodified concentrated cell media SEC fractions or hPD-L1-Exo-Tag concentrated cell media SEC fractions. PD-L1 was measured using an R&D systems PD-L1 ELISA kit. Dot-blot immunoblot analysis of SEC fractions. A 96-well dot blot apparatus was used to immobilize 50 µl of each SEC fraction onto PVDF. Exosome size and concentration was measured in fraction 7 by tunable resistive pulse sensing (TRPS). It was confirmed that GPI anchors the hPD-L1 fusion protein onto the exosomes (FIG. **25**).

(275) Furthermore, a commercially available multimodal exosome purification resin was also used to purify and isolate PD-L1-GPI exosomes and PD-L1-Fc-GPI exosomes. Fraction 7 was further analyzed by dot blots (FIG. **28**A-**28**B). In particular, FIG. **28**B shows SEC purification results of various embodiments of human PD-L1 displayed on the surface of extracellular vesicles. One

embodiment is the hPD-L1-4Fc-GPI (CMV) construct as seen in the top dot blot (stained with rabbit monoclonal anti-PD-L1 antibody). Another embodiment is the hPD-L1-4Fc-GPI (EF1a) as seen in the top dot blot (stained with rabbit monoclonal anti-PD-L1 antibody).

(276) Large MW exosomes elute in the first fraction as shown by the high hPD-L1 concentration and exosome quantity (2.26E9 exosomes/ml) in elution 1. Clean in place (CIP) fractions show bound and eliminated proteins from our exosome elution. Exosome quantity and hPD-L1 concentration was determined in SEC fractions 7-9. Knowing the molecular weight of engineered hPD-L1, we can determine the number of hPD-L1 molecules per exosome to be approximately 12 PD-L1/exosome. This value is consistent between different purification runs and constructs (FIG. **8**).

(277) Human hPD-L2 and hCTLA-4-Fc-GPI SEC fractions were purified. In addition, purification of the mouse PD-L1-FcGPI exosomes was performed (FIG. **29**). The mouse Fc-PD-L1 expressing exosomes have a higher valency than those that do not comprise the Fc linker.

Example 14

Comparative Proteomics Analysis of the Engineered EVs

(278) Fc-GPI enables high density display and has a higher abundance than endogenous PTGFRN or CD81. Therefore, comparison proteomics of transprotein expression and surface labeling on the engineered exosomes, hPD-L1-Fc-GPI; hPD-L2-Fc-GPI; and hCTLA-Fc-GPI, was performed to determine the effects on endogenous protein expression in engineered exosomes. It was confirmed that the fusion polypeptide expression does not affect the relative expression of native and associated exosome proteins. However, the trans protein may crowd out abundant proteins like CD81 (data not shown).

Example 15

Scale-Up Production and Purification of mPD-L1-Fc-GPI Exosomes Using Microcarriers in a Stirred Tank Single-Use Bioreactor (STR)

(279) 1E7 HEK 293 cells were utilized for the production of mPDL1-Fc-GPI exosomes. Cells were passaged on SoloHill® Microcarriers up to Passage 4, at which point cells were expanded in a 2.5 L Stirred Tank Single-Use Bioreactor. Passage 4 cells were cultured for an additional 5 days and media was harvested on Day 5 and used for exosome purification. The general aim and process is provided below

(280) AIM: Utilize SoloHill®'s Xeno-free microcarrier technology to scale up cells for engineering EVs and evaluate Microcarrier-stir tank bioreactor technology for production of therapeutic exosomes in the Xeno-free medium conditions.

(281) Passage 1:

(282) Thaw vial (1.00E+07) of cells and seed Corning® T-150 & CellSTACK®2 tissue culture treated flask at 1.00E+04 cells per cm.sup.2 seed density.

(283) Perform 100% medium exchange from both flasks on day 3.

(284) Harvest Corning® T-150 & CellSTACK®2 flasks on day 4 post seeding and seed spinner microcarrier culture.

(285) Passage 2:

(286) Expand cells in 2×200 mL spinner flasks at 10 cm.sup.2/mL microcarrier density using SoloHill®'s Xeno-free prototype microcarrier.

(287) Seed microcarrier cultures at 1.00E+04 cells per cm.sup.2 seed density and T-25 as flatware control flask.

(288) Perform 80% batch volume medium exchange from spinners and T-25 flasks on day 3.

(289) Harvest both microcarrier and T-25 flasks on day 4 post seeding and seed spinner microcarrier culture.

(290) Passage 3:

(291) Expand cells in 3×300 mL spinner flasks at 10 cm.sup.2/mL microcarrier density using SoloHill®'s Xeno-free prototype microcarrier.

(292) Seed microcarrier cultures at 1.00E+04 cells per cm.sup.2 seed density and T-25 as flatware control flask.
(293) Perform 80% batch volume medium exchange from spinners and T-25 flasks on day 3.
(294) Harvest both microcarrier and T-25 flasks on day 4 post seeding.
(295) Seed microcarrier-stir tank bioreactor for exosome production.
(296) Passage 4:
(297) Expand cells into a 2.5 L microcarrier-stir tank at 10 cm.sup.2/mL surface area to medium ratio.
(298) Seed cultures at 1.00E+04 cells per cm.sup.2 seed density and T-25 as flatware control flask.
(299) Perform 80% batch volume medium exchange on day 2.
(300) On day 3 rinse all cultures with 2×cell culture volumes of DPBS containing Ca and Mg.
(301) Add exosome production medium (DMEM-1% GlutaMAX™) to all cultures at 10 cm.sup.2/mL surface area to medium volume ratio.
(302) On day 5 collect harvest spent medium from all cultures, filter using 0.45 μm Nalgene rapid flow system and freeze at −20° C.
(303) Procedures:
(304) Medium Composition DMEM 1× (Corning ref #10-013-CV) 1% GlutaMAX™ (Thermo ref #35050061) 3% Human platelet lysate (Stemulate from Cook Reagentec PG-NH-500)
Cell Harvest Protocol for Planar Culture
(305) Settle microcarriers and remove maximum volume of spent medium without removing microcarriers.
(306) Wash microcarrier culture with DPBS 2× time at 0.1 mL/cm.sup.2 volume to surface area ratio.
(307) Add 37° C. warmed TrypLE™ 5× enzyme at 0.012 mL/cm.sup.2 and incubate flask at room temperature for ˜15 minutes.
(308) Add complete medium at 0.024 mL/cm.sup.2 to quench TrypLE™ 5× activity.
(309) Perform viable cell count using NC200 cell count instrument.
(310) Nuclei Count Protocol for Microcarrier Culture
(311) Obtain 4-5 mL of microcarrier culture from bioreactor or spinner flask
(312) Settle microcarriers and remove maximum volume of spent medium without removing microcarriers.
(313) Add 1.5 mL NucleoCounter® Reagent A to macrocarrier sample tube and vortex at high speed for a minute.
(314) Add 1.5 mL NucleoCounter® Reagent B to macrocarrier sample tube and vortex at high speed for a minute.
(315) Perform nuclei count using NC200 nuclei count instrument.
(316) Medium Collection from STR Bioreactor
(317) Stop all controls and settle microcarriers in the bioreactor vessel.
(318) Pump out medium through screen bag into collection bottle at 200 mL/minute flowrate using peristaltic pump.
(319) Inside BSC pour medium into 0.45 μm Nalgene™ Rapid-Flow™ filter system and remove free floating cells.
(320) Freeze medium bottles in minus 20° C. freezer.
(321) Medium Collection from Spinner Flasks
(322) Inside BSC pour microcarrier culture into 0.45 μm Nalgene™ Rapid-Flow™ system and remove free floating cells as well as microcarriers.
(323) Freeze medium bottles in minus 20° C. freezer.
(324) TABLE-US-00008 Cell culture set points Tem- Dis- Incubator perature Agitation solved Oxygen CO.sub.2 ° C. rpm (DO) % pH setting % T-Flask 37 n/a n/a n/a 5 ± 1 CellSTACK 2 37 n/a n/a n/a 5 ± 1 Spinner flask 37 35 n/a n/a 5 ± 1 STR bioreactor 37 35 50 7.35 n/a

(325) FIG. **31** shows mPDL1-Fc-GPI production, growth parameters, and analyte concentrations from a 2.6-L culture in a Stirred Tank Single-Use (STR) bioreactor. Day 2: 80% batch volume medium was exchanged (1st increase in glucose and decreased in lactate) Day 3: rinse culture with 2× cell culture volumes of DPBS containing Ca and Mg. (2nd increase in glucose and decreased in lactate). Add exosome production medium (DMEM-1% GlutaMAX™) to culture at 10 cm.sup.2/mL surface area to medium volume ratio.

(326) mPDL1 was purified using the purification process outlined above (FIGS. **32-33**).

Example 16

PD-L1-Fc-GPI and PDL2-Fc-GPI Exosomes Increase PD-1 Signaling

(327) The purified exosomes were tested using the modified DISCOVERX® Assay in FIG. **12**A. Approximately a 1000× increase in Relative Light Units (RLU) was achieved for Jurkat signaling cells treated with PD-L1 or PD-L2 labeled exosomes when compared to soluble PD-L1-Fc or PD-L2-Fc ligands alone, respectively. Therefore, it takes 1000× less μg/ml of PD-L1 or PD-L2 on the engineered exosomes to activate PD-1 over solubilized ligands, PD-L1-Fc or PD-L2, achieve the same RLU signaling. FIG. **12**B show a dose-response curves for the PD-L1 and PD-L2 exosomes vs soluble PD-L1 and PD-L2 signaling bioassay. FIG. **12**B shows dose-response curves for the PD-L1 and PD-L2 exosomes comprising an Fc linker and GPI sticky binder vs. soluble ligands with an Fc domain linker. These results show that the PD-L1 and PD-L2 polypeptides fused with the Fc and GPI domains on EVs have a more potent effect on PD-1 signaling than the soluble ligands alone.

Example 17

In Vivo Assay—Therapeutic Effect of mPD-L1 Exosomes in an Experimental Autoimmune Uveoretinitis (EAU) Model in Lewis Rats

(328) Lewis rats were challenged with retinal antigen interphotoreceptor retinoid-binding protein (IRBP) peptide. This model can be used to study anterior and posterior chamber dependent EAU. Rats were immunized on Day 1 with EAU presenting typically at Day 6. Clinical scores in the rat were determined. The EAU dosing schedule is shown in FIG. **13**A. EAU dosing test article are shown in the following table.

(329) TABLE-US-00009 EAU dosing test articles Unmodified mPD-Ll-Fc-GPI mPD-L1-Fc-GPI mPD-L1 Exosomes (IVT) Exosomes 1X (IVT) Exosomes 10X (IVT) Exosomes (IV) Dose 2 ul 2 ul 2 ul 5 ml/kg Total protein 40 ug/ml 40 ug/ml 400 ug/ml 40 ug/ml concentration Total protein 80 ng/eye 80 ng/eye 800 ng/eye 50 ug/animal administered Exosome 5.7 × 10.sup.10/ml 2.34 × 10.sup.10/ml 2.34 × 10.sup.11/ml 2.34 × 10.sup.10/ml concentration Total exosomes 4.7 × 10.sup.7 4.7 × 10.sup.7 4.7 × 10.sup.8 2.93 × 10.sup.10 administered *IVT-intravitreal, IV-intravenous

(330) The study design is outlined below:

(331) TABLE-US-00010 Group Test Article N Route Concentration Dosage Regimen 1 Cyclosporine 8 p.o. 1 mg/mL 10 mg/kg BID from day 0 to Day 20 2 Negative control (PBS 8 Intravitreal both 1× 2-3 μL Day 6, Day 12, vehicle) eyes and Day 16 3 Unmodified exosomes 8 Intravitreal both 1× (~40 ug/ml) 2-3 μL Day 6 and Day (Control exosomes) eyes 12 4 mPD-L1-Fc-GPI (40 8 Intravitreal both 1× (~40 ug/ml) 2-3 μL Day 6, Day 12, ug/ml) eyes and Day 16 5 mPD-Ll-Fc-GPI (40 4 Intravenous 1× (~40 ug/ml) 5 mL/kg Day 1, Day 6, ug/ml) Injection Day 12, and Day 16 6 No IRBP peptide but 4 Intravitreal both 1× (~40 ug/ml) 2-3 μL Day 6, Day 12, treated with Test Agent eyes and Day 16 B (for tolerability) 7 mPD-L1-Fc-GPI (400 8 Intravitreal both 1× (400 ug/ml) 2-3 μL Day 6, Day 12, ug/ml) eyes and Day 16

(332) Clinical Scores were determined as follows:

(333) TABLE-US-00011 EAU Clinical Scores in Rats Score Clinical Criteria 0 No disease; eye is translucent and reflects light(red reflex) 0.5 Dilated blood vessels in the iris (trace) 1 Engorged blood vessels in the iris; abnormal pupil contraction 2 Hazy anterior chamber;decreased red reflex 3 Moderately opaque anterior chamber,but pupil still visible; dull red reflex 4 Opaque anterior

chamber and obscured pupil; red reflex absent; proptosis
Each higher grade includes the criteria of the preceding one.
(334) It was discovered that there is a statistically significant initial reduction in EAU in mPDL1 exosome treated rats via either the intravitreal and intravenous delivery modes as compared with untreated animals (FIG. **13**A). Rat weight did not change post immunization (FIG. **13**C).
Example 18
Purification of Exosomes Labeled with Type II Membrane Proteins
(335) The inventors designed, engineered, and purified pcDNA5-FRT-4F2-4-1BBL exosomes by the methods provided herein (FIG. **34**). Several embodiments of the 4-1BBL labeled exosomes are shown in FIG. **35**. Cell expression of the 4F2-4-1BBL was confirmed (data not shown). FIGS. **92**A-**92**B shows the purification of 4F2-4-1BBL exosomes.
Example 19
Purification of Luminal Labeled Exosomes (Internal Loading)
(336) In addition to Type I and Type II display fusion proteins on the surface of an EV, exosomes can be loaded with fusion proteins that are localized to the lumen of the phospholipid bilayer of the exosome (FIG. **37**). The Myr/Palm sequence used herein when fused to mScarlet the fusion protein into the luminal interior of extracellular vesicles. Fluorescence at an excitation wavelength 470 nm and emission wavelength of 665-720 nm peaks in SEC fractions 7, 8, and 9. SEC fractions 7, 8, and 9 contain exosomes as demonstrated by the dot blot. Fraction 8 was further analyzed for exosome quantification using an EXOVIEW® system (FIG. **38**). Unmodified exosomes do not show fluorescence. Exosomes show near 80% loading with Myr/Palm-mScarlet. The remaining 20% were out of the detection limit. Thus, nearly 100% internal loading was achieved using the specific Myr/Palm sequence.
(337) NanoLuc® luciferase expressing exosomes were also purified with the Myr/Palm sequence incorporated into the vector encoding the fusion polypeptide. A Qubit™ fluorometer was used to measure total protein and PROMEGA® Nano-Glo® substrate and plate luminometer to measure luminescence (FIG. **39**A). Tetraspanin characterization of exosomes was performed and determined that the NanoLuc® luciferase exosomes were internally loaded and purified in fraction 8 (FIG. **39**B).

Claims

1. An engineered extracellular vesicle comprising a fusion protein, the fusion protein comprising: at least two signaling domains, each of the at least two signaling domains being a same signaling domain and selected from the group consisting of either full length or active fragments of 4-1BBL (CDI37L), CD27L (CD70), CD30L (CD153), mCD30L, GITRL, CD40L (CD154), mCD140L, LIGHT (CD258), TL1, mTL1, FasL, NKG2 family, CD94, CD98, OX40L (CD252), and 4-1BB/TNFRSF4/CD137; at least one linker, and at least one vesicle targeting domain linked to the at least two signaling domains; wherein the at least one linker comprises a fragment crystallizable region (Fc) domain, wherein the at least one vesicle targeting domain comprises a Type II transmembrane domain, and wherein the at least two signaling domains are in an extracellular position relative to a lipid membrane of the extracellular vesicle.
2. The engineered extracellular vesicle of claim 1, wherein the at least one vesicle targeting domain comprises a fatty acylation site or a prenylation site, whereby the at least one vesicle targeting domain is embedded in a phospholipid bilayer of the engineered extracellular vesicle through covalent lipid attachment to the fatty acylation site or the prenylation site.
3. The engineered extracellular vesicle of claim 1, wherein the at least one vesicle targeting domain is selected from the group consisting of sequences encoding peptides or protein domains from ADAM10, TFR2, and MARCKS comprising modified myristoylation and palmitoylation tags; sequences encoding prenylation sites and fatty acylation sites; and sequences encoding lipid

affinity tags derived from KRAS, CD81, CD63, ALIX, TSG101, CD98, CD298, GPI, CD9, and CD105.

4. The engineered extracellular vesicle of claim 1, wherein the at least one vesicle targeting domain comprises at least two exosome targeting domains.

5. The engineered extracellular vesicle of claim 1, further comprising a tetraspanin selected from the group consisting of CD9, CD63, CD81, CD82, CD53, and CD37.

6. The engineered extracellular vesicle of claim 5, wherein the at least two signaling domains comprise three identical signaling domains.

7. The engineered extracellular vesicle of claim 1, comprising a plurality of the fusion protein, and wherein the density of the plurality of fusion protein is configured to support receptor clustering on a target cell.

8. The engineered extracellular vesicle of claim 1, wherein the at least two signaling domains substantially bind to one or more of a target polypeptide.

9. The engineered extracellular vesicle of claim 1, further comprising one or more secondary fusion proteins, each comprising a secondary signaling domain different from the at least two signaling domains of the fusion protein.

10. The engineered extracellular vesicle of claim 9, wherein the secondary signaling domains are each independently selected from the group consisting of either full-length or active fragments of PD-L1 (CD274), PD-L2 (CD273), CTLA-4 (CD152), 4-1BBL (CD137L), HVEM (CD270), FGL1, OX-2 (CD200), Galectin-9, PVR (CD155), Nectin-2 (CD112) isophorm alpha, Nectin-2 (CD112) isophorm delta, mNectin-2 beta, IL-10, TSG-6, B7-H3 (CD276), B7-H4 (VTCN1), B7-H5 (VISTA), B7-H7 (HHLA2), VSIG8, VSIG3 (IGSF11), VSIG4, Tim-3 (HAVCR2), Tim-4, CEACAM1 (CD66a), BTN3A1, BTN3A2, BTN2A1, BTNLR, BTN2A2, mBTN2A2, BTN1A1, TIGIT, CD27L (CD70), CD30L (CD153), mCD30L, GITRL, CD40L (CD154), mCD140L, LIGHT (CD258), TL1, mTL1, B7-1 (CD80), B7-2 (CD86), LFA-3 (CD58), SLAM (CD150), mSLAM, CD40, CD28, mCD28, CD28H/TMIGD2/IGPR1, CD2, CD48, CD226, DR3, DcR3, FasL, Tim-1 (CD365), PD-1 (CD279), mScarlet, Nanoluciferase, A2AR, PECAM-1, STAB-1, Clever-I, NRP1, NRP2, SEMA3A, SEMA3F, RGMB/DRG11, HLA DII, HMGB1, TCR, SHP-1, SHP-2, FBOX38, SH2DIA, B7RP1, IDO, NOX2, TNFRSF18/GITR/CD357, SISP1, B7-H6/NCR3LG1, APLNR, IFNg receptor, WNTSA, PAK4, IL-6, NKG2 family, NKG2 family ligands, Killer cell Ig-like receptors, CD4, CD5, CD27, CD39, CD44, CD47, CD73, CD94, CD96, CD98, 1GSF2/CD101, PVIRG/CD112R, IL5RB/CD122, OX40L (CD252), 4-1BB/TNFRSF4/CD137, KIRs/CD158 family, CD160, SIRP alpha/CD172a, CD200R, LAG-3/CD223, CD244, BTLA/CD272, B7H2/ICOSLG/B7RP1/CD275, ICOS/CD278, LIAR-1/CD305, Collagen family members, SIGLEC7/CD328, SIGLEC9/CD329, NKp30/CD337, TNFR superfamily, Nectin-like binding receptors, Nectin, IL10RA, IL10RB, TNFRSF25, TNFRSF6B, CDI13, CD30, TRAF family members, and TIM family members.

11. The engineered extracellular vesicle of claim 9, wherein the one or more secondary fusion proteins each comprises a secondary vesicle targeting domain linked to its secondary signaling domain.

12. The engineered extracellular vesicle of claim 11, wherein the one or more secondary vesicle targeting domains are each independently selected from the group consisting of sequence prides or protein domains from ADAM10, TFR2, and MARCKS comprising modified myristoylation and palmitoylation tags; sequences encoding prenylation sites and fatty acylation sites; and sequences encoding lipid affinity tags derived from KRAS, CD81, CD63, ALIX, TSG101, CD98, CD298, GPL CD9, and CD105.

13. An engineered extracellular vesicle comprising a fusion protein, the fusion protein comprising: at least two signaling domains, each of the at least two signaling domains being a same signaling domain and selected from the group consisting of either full-length of active fragments of 4-1BBL (CD137L), CD27L (CD70), CD30L (CD153), mCD30L, GITRL, CD40L (CD154), mCD140L,

LIGHT (CD258), TL1, mTL1 FasL, NKG2 family, CD94, CD98, OX40L (CD252), and 4-1BB/TNFRSF4/CD137; at least one linker, and at least one vesicle targeting domain linked to the at least two signaling domains; wherein the at least one linker comprises a fragment crystallizable region (Fc) domain and is between the at lea st two signaling domains and the at least one vesicle targeting domain, wherein the at least one vesicle targeting domain comprises a Type II transmembrane domain, and wherein the at least two signaling domains are in an extracellular position relative to a lipid membrane of the extracellular vesicle.

14. The engineered extracellular vesicle of claim 13, wherein the linker is an Fc from IgG1, Fc from IgG2, Fc from IgG3, or Fc from IgG4, or wherein the linker further comprises Gly-Ser-Ser-Gly (SEQ ID NO: 319), a cleavable 2A sequence, P2A, E2A, F2A, T2A, or (GGGGS (SEQ ID NO: 320)) n.

15. The engineered extracellular vesicle of claim 13, wherein the at least one vesicle targeting domain comprises a sequence encoding a fatty acylation site or a prenylation site, whereby the at least one vesicle targeting domain is embedded in a phospholipid bilayer of the engineered extracellular vesicle through covalent lipid attachment to the fatty acylation site or the prenylation site.

16. The engineered extracellular vesicle of claim 13, wherein the at least one vesicle targeting domain is selected from the group consisting of sequences encoding peptides or protein domains from ADAM10, TFR2, and MARCKS comprising modified myristoylation and palmitoylation tags; sequences encoding prenylation sites and fatty acylation sites; and sequences encoding lipid affinity tags derived from KRAS, CD81, CD63, ALIX, TSG101, CD98, CD298, GPI, CD9, and CD105.

17. The engineered extracellular vesicle of claim 13, further comprising a tetraspanin selected from the group consisting of CD9, CD63, CD81, CD82, CD53, and CD37.

18. The engineered extracellular vesicle of claim 17, wherein the at least two signaling domains comprise three identical signaling domains.

19. The engineered extracellular vesicle of claim 13, comprising a plurality of the fusion protein, and wherein the density of the plurality of fusion protein is configured to support receptor clustering on a target cell.

20. The engineered extracellular vesicle of claim 13, further comprising one or more secondary fusion proteins, each comprising a secondary signaling domain different from the signaling domain of the fusion protein.

21. The engineered extracellular vesicle of claim 20, wherein the secondary signaling domains are each independently selected from the group consisting of either full-length or active fragments of PD-L1, PD-L2, CTLA-4 (CD152), 4-1BBL (CD137L), HVEM (CD270), FOL1, OX-2 (CD200), Galectin-9, PVR (CD155), Nectin-2 (CD112) isophorm alpha, Nectin-2 (CD112) isophorm delta, mNectin-2 beta, IL-10, TSG-6, B7-H3 (CD276), B7-H4 (VTCN1), B7-H5 (VISTA), B7-H7 (HHLA2), VSIG8, VSIG3 (IGSF11), VSIG4, Tim-3 (HAVCR2), Tim-4, CEACAM1 (CD66a), BTN3A1, BTN3A2, BTN2A1, BTNL8, BTN2A2, mBTN2A2, BTN1A1, TIGIT, CD27L (CD70), CD30L (CD153), mCD30L, GITRL, CD40L (CD154), mCD140L, LIGHT (CD258), TL1, mTL1, B7-1 (CD80), B7-2 (CD86), LFA-3 (CD58), SLAM (CD150), mSLAM, CD40, CD28, mCD28, CD28H/TMIGD2/IGPR1, CD2, CD48, CD226, DR3, DcR3, FasL, Tim-1 (CD365), PD-1 (CD279), mScarlet, Nanoluciferase, AZAR, PECAM-I, STAB-1, Clever-1, NRP1, NRP2, SEMA3A, SEMA3F, RGMB/DRG11, HLA I/II, HMGB1, TCR, SHP-1, SHP-2, FBOX38, SH2D1A, B7RP1, IDO, NOX2, TNERSF18/GITR/CD357, SISP1, B7-H6/NCR3LG1, APLNR, IFNg receptor, WNTSA, PAK4, IL-6, NKG2 family, NKG2 family ligands, Killer cell Ig-like receptors, CD4, CD8, CD27, CD39, CD44, CD47, CD73, CD94, CD96, CD98, IGSF2/CD101, NECTIN2/CD112, PVIRO/CD112R, ILSRB/CD122, OX40L (CD252), 4-1BB/TNFRSF4/CD137, CTLA-4/CD152, KIRs/CD158 family, CD160, SIRP alpha/CD172a, CD200R, LAG-3/CD223, CD244, BTLA/CD272, B7H2/ICOSLG/B7RP1/CD275, ICOS/CD278, LIAR-1/CD305, Collagen

family members, SIGLEC7/CD328, SIGLEC9/CD329, NKp30/CD337, TNER superfamily, Nectin-like binding receptors, Nectin, IL10RA, IL10RB, TNERSF25, TNFRSF6B, CD113, CD30, TRAF family members, and TIM family members.

22. The engineered extracellular vesicle of claim 20, wherein the one or more secondary fusion proteins each comprises a secondary vesicle targeting domain linked to its secondary signaling domain.

23. The engineered extracellular vesicle of claim 22, wherein the one or more secondary vesicle targeting domains are each independently selected from the group consisting of sequences encoding peptides or protein domains from ADAM10, TFR2, and MARCKS comprising modified myristoylation and palmitoylation tags; sequences encoding prenylation sites and fatty acylation sites; and sequences encoding lipid affinity s derived from KRAS, CD81, CD63, ALIX, TSG101, CD98, CD298, GPI, CD9, and CD105.