COMPOSITIONS AND METHODS FOR COMPARTMENT-SPECIFIC CARGO DELIVERY

Cross-Reference to Related Applications

This application claims priority to U.S. provisional applications: 62/,767,394 filed November 14, 2018, entitled "COMPOSITIONS AND METHODS FOR COMPARTMENT-SPECIFIC CARGO DELIVERY", the contents of which are incorporated by reference in their entirety for all purposes.

Incorporation by Reference of Sequence Listing

The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled 186152002641SEQLIST.TXT, created November 14, 2019 which is 807 kilobytes in size. The information in the electronic format of the Sequence Listing is incorporated by reference in its entirety.

BACKGROUND

Cell–cell fusion is required in biological processes as diverse as fertilization, development, immune response, and tumorigenesis.

SUMMARY

The present disclosure provides technologies relating to fusosomes and their use to deliver membrane proteins to target cells. In some embodiments, a fusosome comprises a lipid bilayer, a lumen surrounded by the lipid bilayer, a fusogen, and a cargo that includes a membrane protein payload agent, In some embodiments, such cargo may be or comprise a membrane protein itself; in some embodiments, such cargo may be or comprise a nucleic acid that encodes (or is complementary to a nucleic acid that encodes) a membrane protein.

Enumerated Embodiments

Membrane delivery

1. A fusosome comprising:

(a) a lipid bilayer comprising a plurality of lipids derived from a source cell;

(b) a lumen (e.g., comprising cytosol) surrounded by the lipid bilayer;

(c) a fusogen that is exogenous or overexpressed relative to the source cell, e.g., wherein the fusogen is disposed in the lipid bilayer; and

(d) a membrane protein payload agent (e.g., which is exogenous or overexpressed relative to the source cell) that comprises or encodes one or more of:

i) a chimeric antigen receptor;

ii) an integrin membrane protein payload, e.g., chosen from Table 7;

iii) an ion channel protein chosen from Table 8;

iv) a pore forming protein, e.g., chosen from Tables 9 and 10;

v) a Toll-Like Receptor, e.g., chosen from Table 11;

vi) an interleukin receptor payload, e.g., chosen from Table 12;

vii) a cell adhesion protein chosen from Tables 13-14;

viii) a transport protein chosen from Table 17;

ix) a signal sequence that is heterologous relative to the naturally-occurring membrane protein; or

x) a signal sequence listed in Table 6;

wherein the fusosome does not comprise a nucleocapsid protein or a viral matrix protein.

2. A fusosome comprising:

(a) a lipid bilayer comprising a plurality of lipids derived from a source cell;

(b) a lumen (e.g., comprising cytosol) surrounded by the lipid bilayer;

(c) a fusogen that is exogenous or overexpressed relative to the source cell, e.g., wherein the fusogen is disposed in the lipid bilayer; and

(d) a membrane protein payload agent (e.g., which is exogenous or overexpressed relative to the source cell) that comprises or encodes a T cell receptor;

wherein optionally the fusosome does not comprise a nucleocapsid protein or a viral matrix protein.

3. A fusosome comprising:

(a) a lipid bilayer comprising a plurality of lipids derived from a source cell;

(b) a lumen (e.g., comprising cytosol) surrounded by the lipid bilayer;

(c) a fusogen that is exogenous or overexpressed relative to the source cell, e.g., wherein the fusogen is disposed in the lipid bilayer; and

(d) a membrane protein payload agent that is exogenous or overexpressed relative to the source cell;

and

wherein one or more of:

i) the fusosome comprises or is comprised by a cytobiologic;

ii) the fusogen is present at a copy number, e.g., per fusosome, of at least, or no more than, 10, 50, 100, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000, 100,000,000, 500,000,000, or 1,000,000,000 copies, e.g., as measured by an assay of Example 29;

iii) the fusosome comprises a therapeutic agent at a copy number, e.g., per fusosome, of at least, or no more than, 10, 50, 100, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000, 100,000,000, 500,000,000, or 1,000,000,000copies, e.g., as measured by an assay of Example 43;

iv) the fusosome comprises a lipid wherein one or more of CL, Cer, DAG, HexCer, LPA, LPC, LPE, LPG, LPI, LPS, PA, PC, PE, PG, PI, PS, CE, SM and TAG is within 75% of the corresponding lipid level in the source cell;

v) the fusosome comprises a proteomic composition similar to that of the source cell, e.g., using an assay of Example 42;

vi) the fusosome is capable of signal transduction, e.g., transmitting an extracellular signal, e.g., AKT phosphorylation in response to insulin, or glucose (e.g., labeled glucose, e.g., 2-NBDG) uptake in response to insulin, e.g., by at least 10% more than a negative control, e.g., an otherwise similar fusosome in the absence of insulin, e.g., using an assay of Example 63;

vii) the fusosome targets a tissue, e.g., liver, lungs, heart, spleen, pancreas, gastrointestinal tract, kidney, testes, ovaries, brain, reproductive organs, central

nervous system, peripheral nervous system, skeletal muscle, endothelium, inner ear, or eye, when administered to a subject, e.g., a mouse, e.g., wherein at least 0.1%, or 10%, of the fusosomes in a population of administered fusosomes are present in the target tissue after 24 hours, e.g., by an assay of Example 87 or 100; or

viii) the source cell is selected from a neutrophil, a granulocyte, a mesenchymal stem cell, a bone marrow stem cell, an induced pluripotent stem cell, an embryonic stem cell, a myeloblast, a myoblast, a hepatocyte, or a neuron e.g., retinal neuronal cell.

4. A fusosome comprising:

(a) a lipid bilayer comprising a plurality of lipids derived from a source cell;

(b) a lumen (e.g., comprising cytosol) surrounded by the lipid bilayer;

(c) a fusogen that are exogenous or overexpressed relative to the source cell, e.g., wherein the fusogen is disposed in the lipid bilayer; and

(d) a membrane protein payload agent that:

i) comprises DNA that encodes a membrane protein; or

ii) comprises RNA, e.g., mRNA, that encodes a membrane protein that is exogenous or overexpressed relative to the source cell,

wherein the fusosome does not comprise a nucleocapsid protein or a viral matrix protein.

5. A fusosome comprising:

(a) a lipid bilayer comprising a plurality of lipids derived from a source cell;

(b) a lumen (e.g., comprising cytosol) surrounded by the lipid bilayer;

(c) a non-viral, e.g., mammalian, fusogen that is exogenous or overexpressed relative to the source cell, wherein the mammalian fusogen is not Alzheimer's beta-amyloid peptide or fertilin; and

(d) a membrane protein payload agent which is exogenous or overexpressed relative to the source cell,

wherein optionally the fusosome does not comprise a nucleocapsid protein or a viral matrix

protein.

6. A fusosome comprising:

(a) a lipid bilayer comprising a plurality of lipids derived from a source cell;

(b) a lumen (e.g., comprising cytosol) surrounded by the lipid bilayer;

(c) a fusogen that are exogenous or overexpressed relative to the source cell, e.g., wherein the fusogen is disposed in the lipid bilayer; and

(d) a membrane protein payload agent that is exogenous or overexpressed relative to the source cell;

wherein the fusosome comprises an enucleated cell, and

wherein optionally the fusosome does not comprise a nucleocapsid protein or a viral matrix protein.

7. A fusosome comprising:

(a) a lipid bilayer comprising a plurality of lipids derived from a source cell;

(b) a lumen (e.g., comprising cytosol) surrounded by the lipid bilayer;

(c) a fusogen that are exogenous or overexpressed relative to the source cell, e.g., wherein the fusogen is disposed in the lipid bilayer; and

(d) a membrane protein payload agent that is exogenous or overexpressed relative to the source cell;

and wherein one or more of:

i) the fusosome comprises or is comprised by a cytobiologic;

ii) the fusosome comprises an enucleated cell;

iii) the fusosome comprises an inactivated nucleus;

iv) the fusosome fuses at a higher rate with a target cell than with a non-target cell, e.g., by at least at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, e.g., in an assay of Example 54;

v) the fusosome fuses at a higher rate with a target cell than non-target fusosomes,

e.g., by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, e.g., in an assay of Example 54;

vi) the fusosome fuses with target cells at a rate such that the membrane protein payload agent in the fusosome is delivered to at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, of target cells after 24, 48, or 72 hours, e.g., in an assay of Example 54;

vii) the fusogen is present at a copy number, per fusosome, of at least, or no more than, 10, 50, 100, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000, 100,000,000, 500,000,000, or 1,000,000,000 copies, e.g., as measured by an assay of Example 29;

viii) the fusosome comprises the membrane protein payload agent at a copy number, per fusosome, of at least, or no more than, 10, 50, 100, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000, 100,000,000, 500,000,000, or 1,000,000,000 copies, e.g., as measured by an assay of Example 43;

ix) the ratio of the copy number of the fusogen to the copy number of the membrane protein payload agent is between 1,000,000:1 and 100,000:1, 100,000:1 and 10,000:1, 10,000:1 and 1,000:1, 1,000:1 and 100:1, 100:1 and 50:1, 50:1 and 20:1, 20:1 and 10:1, 10:1 and 5:1, 5:1 and 2:1, 2:1 and 1:1, 1:1 and 1:2, 1:2 and 1:5, 1:5 and 1:10, 1:10 and 1:20, 1:20 and 1:50, 1:50 and 1:100, 1:100 and 1:1,000, 1:1,000 and 1:10,000, 1:10,000 and 1:100,000, or 1:100,000 and 1:1,000,000;

x) the fusosome comprises a lipid composition substantially similar to that of the source cell or wherein one or more of CL, Cer, DAG, HexCer, LPA, LPC, LPE, LPG, LPI, LPS, PA, PC, PE, PG, PI, PS, CE, SM and TAG is within 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75% of the corresponding lipid level in the source cell;

xi) the fusosome comprises a proteomic composition similar to that of the source cell, e.g., using an assay of Example 42;

xii) the fusosome comprises a ratio of lipids to proteins that is within 10%, 20%, 30%,

40%, or 50% of the corresponding ratio in the source cell, e.g., as measured using an assay of Example 49;

xiii) the fusosome comprises a ratio of proteins to nucleic acids (e.g., DNA) that is within 10%, 20%, 30%, 40%, or 50% of the corresponding ratio in the source cell, e.g., as measured using an assay of Example 50;

xiv) the fusosome comprises a ratio of lipids to nucleic acids (e.g., DNA) that is within 10%, 20%, 30%, 40%, or 50% of the corresponding ratio in the source cell, e.g., as measured using an assay of Example 51;

xv) the fusosome has a half-life in a subject, e.g., in a mouse, that is within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% of the half life of a reference cell, e.g., the source cell, e.g., by an assay of Example 75;

xvi) the fusosome transports glucose (e.g., labeled glucose, e.g., 2-NBDG) across a membrane, e.g., by at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% more than a negative control, e.g., an otherwise similar fusosome in the absence of glucose, e.g., as measured using an assay of Example 64;

xvii) the fusosome comprises esterase activity in the lumen that is within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of that of the esterase activity in a reference cell, e.g., the source cell or a mouse embryonic fibroblast, e.g., using an assay of Example 66;

xviii) the fusosome comprises a metabolic activity (e.g., citrate synthase activity) level that is within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the metabolic activity (e.g., citrate synthase activity) in a reference cell, e.g., the source cell, e.g., as described in Example 68;

xix) the fusosome comprises a respiration level (e.g., oxygen consumption rate) that is within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the respiration level (e.g., oxygen consumption rate) in a reference cell, e.g., the source cell, e.g., as described in Example 69;

xx) the fusosome comprises an Annexin-V staining level of at most 18,000, 17,000, 16,000, 15,000, 14,000, 13,000, 12,000, 11,000, or 10,000 MFI, e.g., using an assay

of Example 70, or wherein the fusosome comprises an Annexin-V staining level at least 5%, 10%, 20%, 30%, 40%, or 50% lower than the Annexin-V staining level of an otherwise similar fusosome treated with menadione in the assay of Example 70, or wherein the fusosome comprises an Annexin-V staining level at least 5%, 10%, 20%, 30%, 40%, or 50% lower than the Annexin-V staining level of a macrophage treated with menadione in the assay of Example 70,

xxi) the fusosome has a miRNA content level of at least at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or greater than that of the source cell, e.g., by an assay of Example 39;

xxii) the fusosome has a soluble : non-soluble protein ratio that is within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or greater than that of the source cell, e.g., within 1%-2%, 2%-3%, 3%-4%, 4%-5%, 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, or 80%-90% of that of the source cell, e.g., by an assay of Example 47;

xxiii) the fusosome has an LPS level less than 5%, 1%, 0.5%, 0.01%, 0.005%, 0.0001%, 0.00001% or less of the LPS content of the source cell, e.g., as measured by mass spectrometry as described in Example 48;

xxiv) the fusosome and/or compositions or preparations thereof, are capable of signal transduction, e.g., transmitting an extracellular signal, e.g., AKT phosphorylation in response to insulin, or glucose (e.g., labeled glucose, e.g., 2-NBDG) uptake in response to insulin, e.g., by at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% more than a negative control, e.g., an otherwise similar fusosome in the absence of insulin, e.g., using an assay of Example 63;

xxv) the fusosome targets a tissue, e.g., liver, lungs, heart, spleen, pancreas, gastrointestinal tract, kidney, testes, ovaries, brain, reproductive organs, central nervous system, peripheral nervous system, skeletal muscle, endothelium, inner ear, or eye, when administered to a subject, e.g., a mammal, e.g., an experimental mammal (e.g., a mouse), a domesticated animal (e.g., a pet or farm animal), or a human, wherein at least 0.1%, 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the fusosomes in a population

of administered fusosomes are present in the target tissue after 24, 48, or 72 hours, e.g., by an assay of Example 87 or 100;

xxvi) the fusosome has juxtacrine-signaling level of at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% greater than the level of juxtacrine signaling induced by a reference cell, e.g., the source cell or a bone marrow stromal cell (BMSC), e.g., by an assay of Example 71;

xxvii) the fusosome has paracrine-signaling level of at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% greater than the level of paracrine signaling induced by a reference cell, e.g., the source cell or a macrophage, e.g., by an assay of Example 72;

xxviii) the fusosome polymerizes actin at a level within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% compared to the level of polymerized actin in a reference cell, e.g., the source cell or a C2C12 cell, e.g., by the assay of Example 73;

xxix) the fusosome has a membrane potential within about 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% of the membrane potential of a reference cell, e.g., the source cell or a C2C12 cell, e.g., by an assay of Example 74, or wherein the fusosome has a membrane potential of about -20 to -150mV, -20 to -50mV, -50 to -100mV, or -100 to -150mV;

xxx) the fusosome and/or compositions or preparations thereof, are capable of extravasation from blood vessels, e.g., at a rate at least 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% the rate of extravasation of the source cell, e.g., using an assay of Example 57, e.g., wherein the source cell is a neutrophil, lymphocyte, B cell, macrophage, or NK cell;

xxxi) the fusosome and/or compositions or preparations thereof, are capable of crossing a cell membrane, e.g., an endothelial cell membrane or the blood brain barrier;

xxxii) the fusosome and/or compositions or preparations thereof, are capable of secreting a protein, e.g., at a rate at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% greater than areference cell, e.g., a mouse embryonic fibroblast or the source cell, e.g., using an assay of Example 62;

xxxiii) the fusosome meets a pharmaceutical or good manufacturing practices (GMP) standard;

xxxiv) the fusosome was made according to good manufacturing practices (GMP);

xxxv) a pharmaceutical preparation comprising a plurality of fusosomes as described herein has a pathogen level below a predetermined reference value, e.g., is substantially free of pathogens;

xxxvi) a pharmaceutical preparation comprising a plurality of fusosomes as described herein has a contaminant level below a predetermined reference value, e.g., is substantially free of contaminants;

xxxvii) a pharmaceutical preparation comprising a plurality of fusosomes as described herein has low immunogenicity, e.g., as described herein;

xxxviii) the source cell is selected from a neutrophil, a granulocyte, a mesenchymal stem cell, a bone marrow stem cell, an induced pluripotent stem cell, an embryonic stem cell, a myeloblast, a myoblast, a hepatocyte, or a neuron e.g., retinal neuronal cell; or

xxxix) the source cell is other than a 293 cell, HEK cell, human endothelial cell, or a human epithelial cell, monocyte, macrophage, dendritic cell, or stem cell.

8. A fusosome comprising:

(a) a lipid bilayer,

(b) a lumen (e.g., comprising cytosol) surrounded by the lipid bilayer;

(c) a fusogen that is exogenous or overexpressed relative to the source cell, e.g., wherein the fusogen is disposed in the lipid bilayer,

(d) a membrane protein payload agent, e.g., a membrane protein exogenous to the source cell,

wherein the fusosome is derived from a source cell; and

wherein the fusosome has partial or complete nuclear inactivation (e.g. lacks an intact nucleus as found in the source cell, nuclear removal/enucleation, non-functional nucleus, etc.).

9. A fusosome comprising:

(a) a lipid bilayer,

(b) a lumen (e.g., comprising cytosol) surrounded by the lipid bilayer;

(c) a fusogen that is exogenous or overexpressed relative to the target cell, e.g., wherein the fusogen is disposed in the lipid bilayer (e.g., wherein the fusogen is endogenous or exogenous to the source cell), and

(d) a membrane protein payload agent (e.g., which is exogenous or overexpressed relative to the source cell) that:

i) comprises or encodes a chimeric antigen receptor;

ii) comprises or encodes an integrin membrane protein payload, e.g., chosen from Table 7;

iii) comprises or encodes an ion channel protein chosen from Table 8;

iv) comprises or encodes a pore forming protein, e.g., chosen from Tables 9 and 10;

v) comprises or encodes a Toll-Like Receptor, e.g., chosen from Table 11;

vi) comprises or encodes an interleukin receptor payload, e.g., chosen from Table 12;

vii) comprises or encodes a cell adhesion protein chosen from Tables 13-14;

viii) comprises or encodes a transport protein chosen from Table 17;

ix) comprises or encodes a signal sequence that is heterologous relative to the naturally-occurring membrane protein;

x) comprises or encodes a signal sequence listed in Table 6;

wherein the fusosome does not comprise viral capsid or viral envelope proteins.

10. A fusosome comprising:

(a) a lipid bilayer comprising a plurality of lipids derived from a source cell;

(b) a lumen (e.g., comprising cytosol) surrounded by the lipid bilayer;

(c) a fusogen that is exogenous or overexpressed relative to the source cell, e.g., wherein the fusogen is disposed in the lipid bilayer; and

(d) a membrane protein payload agent (e.g., which is exogenous or overexpressed

relative to the source cell) that comprises or encodes one or more of:

i) a lipid-anchored protein;

ii) an extracellular protein that binds a transmembrane protein;

iii) an extracellular protein that lacks a transmembrane domain;

iv) a protein that partially spans a membrane (e.g., a membrane of the target cell or the fusosome) and does not completely span the membrane (e.g., the protein comprises an in-plane membrane helix, or the protein comprises a hydrophobic loop that does not completely span the membrane); or

v) the protein does not comprise a transmembrane domain, wherein the protein interacts with a membrane surface, e.g., through electropstatic or ionic interactions;

wherein the fusosome does not comprise a viral structural protein, e.g., a viral capsid protein or a viral envelope protein.

11. A fusosome comprising:

(a) a lipid bilayer,

(b) a lumen (e.g., comprising cytosol) surrounded by the lipid bilayer,

(c) a fusogen that is exogenous relative to the source cell or an overexpressed fusogen, e.g., wherein the fusogen is disposed in the lipid bilayer,

(d) a membrane protein payload agent, and

(e) a functional nucleus,

wherein the fusosome is derived from a source cell.

12. The fusosome of any of the preceding embodiments, wherein a plurality of the fusosomes, when contacted with a target cell population in the presence of an inhibitor of endocytosis, and when contacted with a reference target cell population not treated with the inhibitor of endocytosis, delivers the cargo to at least 30%, 40%, 50%, 60%, 70%, or 80% of the number of cells in the target cell population compared to the reference target cell population.

13. The fusosome of any of the preceding embodiments, wherein:

(i) when the plurality of fusosomes are contacted with a cell population comprising target cells and non-target cells, the cargo is present in at least 2-fold, 5-fold, 10-fold, 20-fold, 50-fold, or 100-fold more target cells than non-target cells, or

(ii) the fusosomes of the plurality fuse at a higher rate with a target cell than with a non-target cell by at least at least 50%.

14. The fusosome of any of the preceding embodiments, wherein:

i) the fusogen is present at a copy number of at least 1,000 copies, e.g., as measured by an assay of Example 29; or

ii) the ratio of the copy number of the fusogen to the copy number of the membrane protein payload agent is between 1,000,000:1 and 100,000:1, 100,000:1 and 10,000:1, 10,000:1 and 1,000:1, 1,000:1 and 100:1, 100:1 and 50:1, 50:1 and 20:1, 20:1 and 10:1, 10:1 and 5:1, 5:1 and 2:1, 2:1 and 1:1, 1:1 and 1:2, 1:2 and 1:5, 1:5 and 1:10, 1:10 and 1:20, 1:20 and 1:50, 1:50 and 1:100, 1:100 and 1:1,000, 1:1,000 and 1:10,000, 1:10,000 and 1:100,000, or 1:100,000 and 1:1,000,000.

15. The fusosome of any of the preceding embodiments, wherein the fusosome comprises a membrane protein payload agent at a copy number of at least 1,000 copies, e.g., as measured by an assay of Example 43.

16. The fusosome of any of the preceding embodiments, wherein the membrane protein payload agent is a membrane protein, disposed in the fusosome lipid bilayer.

17. The fusosome of any of the preceding embodiments, wherein the membrane protein payload agent is a nucleic acid, disposed in the fusosome lumen, that encodes a membrane protein.

18. The fusosome of embodiment 17, wherein the nucleic acid is or comprises RNA.

19. The fusosome of embodiment 18, wherein the RNA is or comprises a pre-mRNA or an mRNA.

20. The fusosome of embodiment 17, wherein the nucleic acid is or comprises DNA.

21. The fusosome of embodiment 20, wherein the DNA is or comprises a gDNA or a cDNA.

22. The fusosome of any of embodiments 17-21, wherein the nucleic acid further comprises one or more sequences encoding one or more signal sequences, e.g., wherein a target cell translocates a protein comprising a signal sequence to the cell membrane of the target cell.

23. The fusosome of embodiment 22, wherein the one or more signal sequences is or comprise a sequence selected from Table 6.

24. The fusosome of any of embodiments 17-23, wherein the nucleic acid includes one or more regulatory elements that direct expression of sequences encoding the membrane protein by the target cell.

25. The fusosome of any of the preceding embodiments, wherein the membrane protein payload agent is or comprises a sequence of SEQ ID NOs: 8144-16131 of U.S. Patent Publication No. 2016/0289674.

26. The fusosome of any of embodiments 1-24, wherein the membrane protein payload agent is or comprises a fragment, variant, or homolog of a sequence of SEQ ID NOs: 8144-16131 of U.S. Patent Publication No. 2016/0289674.

27. The fusosome of any of embodiments 1-24, wherein the membrane protein payload agent is or comprises a nucleic acid encoding a protein comprising a sequence of SEQ ID NOs: 8144-16131 of U.S. Patent Publication No. 2016/0289674.

28. The fusosome of any of embodiments 1-24, wherein the membrane protein payload agent is or comprises a nucleic acid encoding a protein comprising a fragment, variant, or homolog of a sequence of SEQ ID NOs: 8144-16131 of U.S. Patent Publication No. 2016/0289674.

29. The fusosome of any of embodiments 1-24, wherein the membrane protein payload agent is or comprises a protein selected from Tables 7-17.

30. The fusosome of any of embodiments 1-24, wherein the membrane protein payload agent is or comprises a fragment, variant, or homolog of a protein selected from Tables 7-17.

31. The fusosome of any of embodiments 1-24, wherein the membrane protein payload agent is or comprises a nucleic acid encoding a protein which is or comprises a protein selected from Tables 7-17.

32. The fusosome of any of embodiments 1-24, wherein the membrane protein payload agent is or comprises a nucleic acid encoding a protein comprising a fragment, variant, or homolog of a protein selected from Tables 7-17.

33. The fusosome of any of embodiments 1-24, wherein the membrane protein payload agent is or comprises a chimeric antigen receptor (CAR) comprising an antigen binding domain.

34. The fusosome of embodiment 33, wherein the CAR is or comprises a first generation CAR comprising an antigen binding domain, a transmembrane domain, and signaling domain.

35. The fusosome of embodiment 33, wherein the CAR is or comprises a second generation CAR comprising an antigen binding domain, a transmembrane domain, and two signaling domains.

36. The fusosome of embodiment 33, wherein the CAR is or comprises a third generation CAR comprising an antigen binding domain, a transmembrane domain, and at least three signaling

domains.

37. The fusosome of embodiment 33, wherein the CAR is or comprises a fourth generation CAR comprising an antigen binding domain, a transmembrane domain, three or four signaling domains, and a domain which upon successful signaling of the CAR induces expression of a cytokine gene.

38. The fusosome of any of embodiments 33-37, wherein the antigen binding domain is or comprises an scFv or Fab.

39. The fusosome of any of embodiments 33-38, wherein the antigen binding domain targets an antigen characteristic of a neoplastic cell.

40. The fusosome of embodiment 39, wherein the antigen characteristic of a neoplastic cell is selected from a cell surface receptor, an ion channel-linked receptor, an enzyme-linked receptor, a G protein-coupled receptor, receptor tyrosine kinase, tyrosine kinase associated receptor, receptor-like tyrosine phosphatase, receptor serine/ threonine kinase, receptor guanylyl cyclase, histidine kinase associated receptor, Epidermal Growth Factor Receptors (EGFR) (including ErbB1/EGFR, ErbB2/HER2, ErbB3/HER3, and ErbB4/HER4), Fibroblast Growth Factor Receptors (FGFR) (including FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, FGF7, FGF18, and FGF21) Vascular Endothelial Growth Factor Receptors (VEGFR) (including VEGF-A, VEGF-B, VEGF-C, VEGF-D, and PIGF), RET Receptor and the Eph Receptor Family (including EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, EphA8, EphA9, EphA10, EphB1, EphB2. EphB3, EphB4, and EphB6), CXCR1, CXCR2, CXCR3, CXCR4, CXCR6, CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR8, CFTR, CIC-1, CIC-2, CIC-4, CIC-5, CIC-7, CIC-Ka, CIC-Kb, Bestrophins, TMEM16A, GABA receptor, glycin receptor, ABC transporters, NAV1.1, NAV1.2, NAV1.3, NAV1.4, NAV1.5, NAV1.6, NAV1.7, NAV1.8, NAV1.9, sphingosin-1-phosphate receptor (S1P1R), NMDA channel, transmembrane protein, multispan transmembrane protein, T-cell receptor motifs; T-cell alpha chains; T-cell β chains; T-cell γ chains; T-cell δ chains; CCR7; CD3; CD4; CD5; CD7; CD8; CD11b; CD11c; CD16; CD19; CD20; CD21 ; CD22; CD25; CD28;

CD34; CD35; CD40; CD45RA; CD45RO; CD52; CD56; CD62L; CD68; CD80; CD95; CD117; CD127; CD133; CD137 (4-1 BB); CD163; F4/80; IL-4Ra; Sca-1 ; CTLA-4; GITR; GARP; LAP; granzyme B; LFA-1 ; transferrin receptor; NKp46, perforin, CD4+; Th1; Th2; Th17; Th40; Th22; Th9; Tfh, Canonical Treg. FoxP3+; Tr1; Th3; Treg17; $T_{RE}G$; CDCP1, NT5E, EpCAM, CEA, gpA33, Mucins, TAG-72, Carbonic anhydrase IX, PSMA, Folate binding protein, Gangliosides (e.g., CD2, CD3, GM2), Lewis-γ^2, VEGF, VEGFR 1/2/3, $\alpha V\beta 3$, $\alpha 5\beta 1$, ErbB1/EGFR, ErbB1/HER2, ErB3, c-MET, IGF1R, EphA3, TRAIL-R1, TRAIL-R2, RANKL, FAP, Tenascin, PDL-1, BAFF, HDAC, ABL, FLT3, KIT, MET, RET, IL-1β, ALK, RANKL, mTOR, CTLA-4, IL-6, IL-6R, JAK3, BRAF, PTCH, Smoothened, PIGF, ANPEP, TIMP1, PLAUR, PTPRJ, LTBR, or ANTXR1, Folate receptor alpha (FRa), ERBB2 (Her2/neu), EphA2, IL-13Ra2, epidermal growth factor receptor (EGFR), Mesothelin, TSHR, CD19, CD123, CD22, CD30, CD171, CS-1, CLL-1, CD33, EGFRvIII , GD2, GD3, BCMA, MUC16 (CA125), L1CAM, LeY, MSLN, IL13Rα1, L1-CAM, Tn Ag, prostate specific membrane antigen (PSMA), ROR1, FLT3, FAP, TAG72, CD38, CD44v6, CEA, EPCAM, B7H3, KIT, interleukin-11 receptor a (IL-11Ra), PSCA, PRSS21, VEGFR2, LewisY, CD24, platelet-derived growth factor receptor-beta (PDGFR-beta), SSEA-4, CD20, MUC1, NCAM, Prostase, PAP, ELF2M, Ephrin B2, IGF-1 receptor, CAIX, LMP2, gplOO, bcr-abl, tyrosinase, Fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, Folate receptor beta, TEM1/CD248, TEM7R, CLDN6, GPRC5D, CXORF61, CD97, CD179a, ALK, Polysialic acid, PLACl, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-la, MAGE-A1, legumain, HPV E6, E7, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, prostein, survivin, telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, Androgen receptor, Cyclin B1, MYCN, RhoC, TRP-2, CYPIB I, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2, CD79a, CD79b, CD72, LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, GPC3, FCRL5, IGLL1, a neoantigen, CD133, CD15, CD184, CD24, CD56, CD26, CD29, CD44, HLA-A, HLA-B, HLA-C, (HLA-A,B,C) CD49f, CD151 CD340, CD200, tkrA, trkB, or trkC, or an antigenic fragment or antigenic portion thereof.

41. The fusosome of any of embodiments 33-38, wherein the antigen binding domain targets an antigen characteristic of a T-cell.

42. The fusosome of embodiment 41, wherein the antigen characteristic of a T-cell is selected from a cell surface receptor, a membrane transport protein (e.g., an active or passive transport protein such as, for example, an ion channel protein, a pore-forming protein, etc.), a transmembrane receptor, a membrane enzyme, and/or a cell adhesion protein characteristic of a T-cell. In some embodiments, an antigen characteristic of a T-cell may be a G protein-coupled receptor, receptor tyrosine kinase, tyrosine kinase associated receptor, receptor-like tyrosine phosphatase, receptor serine/ threonine kinase, receptor guanylyl cyclase, histidine kinase associated receptor, AKT1; AKT2; AKT3; ATF2; BCL10; CALM1; CD3D (CD3δ); CD3E (CD3ε); CD3G (CD3γ); CD4; CD8; CD28; CD45; CD80 (B7-1); CD86 (B7-2); CD247 (CD3ζ); CTLA4 (CD152); ELK1; ERK1 (MAPK3); ERK2; FOS; FYN; GRAP2 (GADS); GRB2; HLA-DRA; HLA-DRB1; HLA-DRB3; HLA-DRB4; HLA-DRB5; HRAS; IKBKA (CHUK); IKBKB; IKBKE; IKBKG (NEMO); IL2; ITPR1; ITK; JUN; KRAS2; LAT; LCK; MAP2K1 (MEK1); MAP2K2 (MEK2); MAP2K3 (MKK3); MAP2K4 (MKK4); MAP2K6 (MKK6); MAP2K7 (MKK7); MAP3K1 (MEKK1); MAP3K3; MAP3K4; MAP3K5; MAP3K8; MAP3K14 (NIK); MAPK8 (JNK1); MAPK9 (JNK2); MAPK10 (JNK3); MAPK11 (p38β); MAPK12 (p38γ); MAPK13 (p38δ); MAPK14 (p38α); NCK; NFAT1; NFAT2; NFKB1; NFKB2; NFKBIA; NRAS; PAK1; PAK2; PAK3; PAK4; PIK3C2B; PIK3C3 (VPS34); PIK3CA; PIK3CB; PIK3CD; PIK3R1; PKCA; PKCB; PKCM; PKCQ; PLCY1; PRF1 (Perforin); PTEN; RAC1; RAF1; RELA; SDF1; SHP2; SLP76; SOS; SRC; TBK1; TCRA; TEC; TRAF6; VAV1; VAV2; or ZAP70.

43. The fusosome of any of embodiments 33-38, wherein the antigen binding domain targets an antigen characteristic of an autoimmune or inflammatory disorder.

44. The fusosome of embodiment 43, wherein the autoimmune or inflammatory disorder is selected from chronic graft-vs-host disease (GVHD), lupus, arthritis, immune complex glomerulonephritis, goodpasture, uveitis, hepatitis, systemic sclerosis or scleroderma, type I diabetes, multiple sclerosis, cold agglutinin disease, Pemphigus vulgaris, Grave's disease,

autoimmune hemolytic anemia, Hemophilia A, Primary Sjogren's Syndrome, thrombotic thrombocytopenia purrpura, neuromyelits optica, Evan's syndrome, IgM mediated neuropathy, cyroglobulinemia, dermatomyositis, idiopathic thrombocytopenia, ankylosing spondylitis, bullous pemphigoid, acquired angioedema, chronic urticarial, antiphospholipid demyelinating polyneuropathy, and autoimmune thrombocytopenia or neutropenia or pure red cell aplasias, while exemplary non-limiting examples of alloimmune diseases include allosensitization (see, for example, Blazar et al., 2015, Am. J. Transplant, 15(4):931-41) or xenosensitization from hematopoietic or solid organ transplantation, blood transfusions, pregnancy with fetal allosensitization, neonatal alloimmune thrombocytopenia, hemolytic disease of the newborn, sensitization to foreign antigens such as can occur with replacement of inherited or acquired deficiency disorders treated with enzyme or protein replacement therapy, blood products, and gene therapy.

45. The fusosome of embodiment 43 or embodiment 44, wherein the antigen characteristic of an an autoimmune or inflammatory disorder is selected from a cell surface receptor, an ion channel-linked receptor, an enzyme-linked receptor, a G protein-coupled receptor, receptor tyrosine kinase, tyrosine kinase associated receptor, receptor-like tyrosine phosphatase, receptor serine/ threonine kinase, receptor guanylyl cyclase, or histidine kinase associated receptor. In some embodiments, a CAR antigen binding domain binds to a ligand expressed on B cells, plasma cells, plasmablasts, CD10, CD19, CD20, CD22, CD24, CD27, CD38, CD45R, CD138, CD319, BCMA, CD28, TNF, interferon receptors, GM-CSF, ZAP-70, LFA-1, CD3 gamma, CD5 or CD2.

46. The fusosome of any of embodiments 33-38, wherein the antigen binding domain targets an antigen characteristic of an infectious disease.

47. The fusosome of embodiment 46, wherein the infectious disease is selected from HIV, hepatitis B virus, hepatitis C virus, Human herpes virus, Human herpes virus 8 (HHV-8, Kaposi sarcoma-associated herpes virus (KSHV)), Human T-lymphotrophic virus-1 (HTLV-1), Merkel cell polyomavirus (MCV), Simian virus 40 (SV40), Eptstein-Barr virus, CMV, human papillomavirus.

48. The fuosome of embodiment 46 or embodiment 47, wherein the antigen characteristic of an infectious disease is selected from a cell surface receptor, an ion channel-linked receptor, an enzyme-linked receptor, a G protein-coupled receptor, receptor tyrosine kinase, tyrosine kinase associated receptor, receptor-like tyrosine phosphatase, receptor serine/ threonine kinase, receptor guanylyl cyclase, histidine kinase associated receptor, HIV Env, gpl20, or CD4-induced epitope on HIV-1 Env.

49. The fusosome of any of embodiments 33-48, wherein the transmembrane domain comprises at least a transmembrane region of the alpha, beta or zeta chain of a T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, or functional variant thereof.

50. The fusosome of any of embodiments 33-49, wherein the transmembrane domain comprises at least a transmembrane region(s) of CD8α, CD8β, 4-1BB/CD137, CD28, CD34, CD4, FcεRIγ, CD16, OX40/CD134, CD3ζ, CD3ε, CD3γ, CD3δ, TCRα, TCRβ, TCRζ, CD32, CD64, CD64, CD45, CD5, CD9, CD22, CD37, CD80, CD86, CD40, CD40L/CD154, VEGFR2, FAS, and FGFR2B, or functional variant thereof.

51. The fusosome of any of embodiments 33-50, wherein the CAR comprises at least one signaling domain selected from one or more of B7-1/CD80; B7-2/CD86; B7-H1/PD-L1; B7-H2; B7-H3; B7-H4; B7-H6; B7-H7; BTLA/CD272; CD28; CTLA-4; Gi24/VISTA/B7-H5; ICOS/CD278; PD-1; PD-L2/B7-DC; PDCD6); 4-1BB/TNFSF9/CD137; 4-1BB Ligand/TNFSF9; BAFF/BLyS/TNFSF13B; BAFF R/TNFRSF13C; CD27/TNFRSF7; CD27 Ligand/TNFSF7; CD30/TNFRSF8; CD30 Ligand/TNFSF8; CD40/TNFRSF5; CD40/TNFSF5; CD40 Ligand/TNFSF5; DR3/TNFRSF25; GITR/TNFRSF18; GITR Ligand/TNFSF18; HVEM/TNFRSF14; LIGHT/TNFSF14; Lymphotoxin-alpha/TNF-beta; OX40/TNFRSF4; OX40 Ligand/TNFSF4; RELT/TNFRSF19L; TACI/TNFRSF13B; TL1A/TNFSF15; TNF-alpha; TNF RII/TNFRSF1B); 2B4/CD244/SLAMF4; BLAME/SLAMF8; CD2; CD2F-10/SLAMF9; CD48/SLAMF2; CD58/LFA-3; CD84/SLAMF5; CD229/SLAMF3; CRACC/SLAMF7; NTB-

A/SLAMF6; SLAM/CD150); CD2; CD7; CD53; CD82/Kai-1; CD90/Thy1; CD96; CD160; CD200; CD300a/LMIR1; HLA Class I; HLA-DR; Ikaros; Integrin alpha 4/CD49d; Integrin alpha 4 beta 1; Integrin alpha 4 beta 7/LPAM-1; LAG-3; TCL1A; TCL1B; CRTAM; DAP12; Dectin-1/CLEC7A; DPPIV/CD26; EphB6; TIM-1/KIM-1/HAVCR; TIM-4; TSLP; TSLP R; lymphocyte function associated antigen-1 (LFA-1); NKG2C, a CD3 zeta domain, an immunoreceptor tyrosine-based activation motif (ITAM), CD27, CD28, 4-1BB, CD134/OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, or functional fragment thereof.

52. The fusosome of any of embodiments 33-51, wherein the CAR comprises a CD3 zeta domain or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof.

53. The fusosome of any of embodiments 33-51, wherein the CAR comprises (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; and (ii) a CD28 domain, or a 4-1BB domain, or functional variant thereof.

54. The fusosome of any of embodiments 33-51, wherein the CAR comprises a (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; (ii) a CD28 domain or functional variant thereof; and (iii) a 4-1BB domain, or a CD134 domain, or functional variant thereof.

55. The fusosome of any of embodiments 33-53, wherein the CAR comprises a (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; (ii) a CD28 domain or functional variant thereof; (iii) a 4-1BB domain, or a CD134 domain, or functional variant thereof; and (iv) a cytokine or costimulatory ligand transgene.

56. The fusosome of any of embodiments 33-53, wherein the CAR further comprises one or more spacers.

57. The fusosome of embodiment 56, wherein the spacer is a first spacer between the antigen binding domain and the transmembrane domain.

58. The fusosome of embodiment 56 or embodiment 57, wherein the first spacer includes at least a portion of an immunoglobulin constant region or variant or modified version thereof.

59. The fusosome of any of embodiments 56-58, wherein the spacer is a second spacer between the transmembrane domain and a signaling domain.

60. The fusosome of embodiment 59, wherein the second spacer is an oligopeptide.

61. The fusosome of embodiment 60, wherein the oligopeptide comprises glycine-serine doublets.

62. The fusosome of embodiment 18-32, wherein the membrane protein is selected from a cell surface receptor, an ion channel-linked receptor, an enzyme-linked receptor, a G protein-coupled receptor, receptor tyrosine kinase, tyrosine kinase associated receptor, receptor-like tyrosine phosphatase, receptor serine/ threonine kinase, receptor guanylyl cyclase, histidine kinase associated receptor, Epidermal Growth Factor Receptors (EGFR) (including ErbB1/EGFR, ErbB2/HER2, ErbB3/HER3, and ErbB4/HER4), Fibroblast Growth Factor Receptors (FGFR) (including FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, FGF7, FGF18, and FGF21) Vascular Endothelial Growth Factor Receptors (VEGFR) (including VEGF-A, VEGF-B, VEGF-C, VEGF-D, and PIGF), RET Receptor and the Eph Receptor Family (including EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, EphA8, EphA9, EphA10, EphB1, EphB2. EphB3, EphB4, and EphB6), CXCR1, CXCR2, CXCR3, CXCR4, CXCR6, CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR8, CFTR, CIC-1, CIC-2, CIC-4, CIC-5, CIC-7, CIC-Ka, CIC-Kb, Bestrophins, TMEM16A, GABA receptor, glycin receptor, ABC transporters, NAV1.1, NAV1.2, NAV1.3, NAV1.4, NAV1.5, NAV1.6, NAV1.7, NAV1.8, NAV1.9, sphingosin-1-phosphate receptor (S1P1R), NMDA channel, transmembrane protein, multispan transmembrane protein, T-cell receptor motifs; T-cell alpha chains; T-cell β chains; T-cell γ chains; T-cell δ chains; CCR7; CD3;

CD4; CD5; CD7; CD8; CD11b; CD11c; CD16; CD19; CD20; CD21 ; CD22; CD25; CD28; CD34; CD35; CD40; CD45RA; CD45RO; CD52; CD56; CD62L; CD68; CD80; CD95; CD117; CD127; CD133; CD137 (4-1 BB); CD163; F4/80; IL-4Ra; Sca-1 ; CTLA-4; GITR; GARP; LAP; granzyme B; LFA-1 ; transferrin receptor; NKp46, perforin, CD4+; Th1; Th2; Th17; Th40; Th22; Th9; Tfh, Canonical Treg. FoxP3+; Tr1; Th3; Treg17; $T_{RE}G$; CDCP1, NT5E, EpCAM, CEA, gpA33, Mucins, TAG-72, Carbonic anhydrase IX, PSMA, Folate binding protein, Gangliosides (e.g., CD2, CD3, GM2), Lewis-γ^2, VEGF, VEGFR 1/2/3, $\alpha V\beta 3$, $\alpha 5\beta 1$, ErbB1/EGFR, ErbB1/HER2, ErB3, c-MET, IGF1R, EphA3, TRAIL-R1, TRAIL-R2, RANKL, FAP, Tenascin, PDL-1, BAFF, HDAC, ABL, FLT3, KIT, MET, RET, IL-1β, ALK, RANKL, mTOR, CTLA-4, IL-6, IL-6R, JAK3, BRAF, PTCH, Smoothened, PIGF, ANPEP, TIMP1, PLAUR, PTPRJ, LTBR, or ANTXR1, Folate receptor alpha (FRa), ERBB2 (Her2/neu), EphA2, IL-13Ra2, epidermal growth factor receptor (EGFR), Mesothelin, TSHR, CD19, CD123, CD22, CD30, CD171, CS-1, CLL-1, CD33, EGFRvIII , GD2, GD3, BCMA, MUC16 (CA125), L1CAM, LeY, MSLN, IL13Rα1, L1-CAM, Tn Ag, prostate specific membrane antigen (PSMA), ROR1, FLT3, FAP, TAG72, CD38, CD44v6, CEA, EPCAM, B7H3, KIT, interleukin-11 receptor a (IL-11Ra), PSCA, PRSS21, VEGFR2, LewisY, CD24, platelet-derived growth factor receptor-beta (PDGFR-beta), SSEA-4, CD20, MUC1, NCAM, Prostase, PAP, ELF2M, Ephrin B2, IGF-1 receptor, CAIX, LMP2, gplOO, bcr-abl, tyrosinase, Fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, Folate receptor beta, TEM1/CD248, TEM7R, CLDN6, GPRC5D, CXORF61, CD97, CD179a, ALK, Polysialic acid, PLACl, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-la, MAGE-A1, legumain, HPV E6, E7, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, prostein, survivin, telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, Androgen receptor, Cyclin B1, MYCN, RhoC, TRP-2, CYPIB I, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2, CD79a, CD79b, CD72, LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, GPC3, FCRL5, IGLL1, a neoantigen, CD133, CD15, CD184, CD24, CD56, CD26, CD29, CD44, HLA-A, HLA-B, HLA-C, (HLA-A,B,C) CD49f, CD151 CD340, CD200, tkrA, trkB, or trkC.

63. The fusosome of any of the preceding embodiments, wherein the fusosome enters the target cell by endocytosis, e.g., wherein the level of membrane protein payload agent delivered via an endocytic pathway for a given fusosome is 0.01-0.6, 0.01-0.1, 0.1-0.3, or 0.3-0.6, or at least at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater than a chloroquine treated reference cell, e.g., using an assay of Example 91.

64. The fusosome of any of the preceding embodiments, wherein the fusosome enters the target cell by a non-endocytic pathway, e.g., wherein the level of membrane protein payload agent delivered via a non-endocytic pathway for a given fusosome is 0.1-0.95, 0.1-0.2, 0.2-0.3, 0.3-0.4, 0.4-0.5, 0.5-0.6, 0.6-0.7, 0.7-0.8, 0.8-0.9, 0.9-0.95, or at least at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater than a chloroquine treated reference cell, e.g., using an assay of Example 90.

65. The fusosome of any of the preceding embodiments, wherein:

i) the membrane protein payload agent is a membrane protein, or a nucleic acid (e.g., a DNA, a gDNA, a cDNA, an RNA, a pre-mRNA, an mRNA, etc.) encoding or complementary to one that encodes, a membrane protein, e.g., a chimeric antigen receptor (CAR);

ii) the membrane protein is or comprises a receptor, such as an antigen receptor, which in some embodiments may be a natural receptor or an engineered receptor e.g., a CAR;

iii) the membrane protein is or comprises an integrin;

iv) the membrane protein is or comprises a T cell receptor;

v) the membrane protein is or comprises a membrane transport protein such as an ion channel protein or a pore-forming protein (e.g., a hemolysin or colicin);

vi) the membrane protein is or comprises a toll-like receptor;

vii) the membrane protein is or comprises an interleukin receptor;

viii) the membrane protein is or comprises a membrane enzyme; or

ix) the membrane protein is or comprises a cell adhesion protein (e.g., cadherin protein, selectin protein, mucin protein, etc.).

66. The fusosome of any of the preceding embodiments, wherein:

i) the membrane protein is an integral membrane protein;

ii) the membrane protein is a peripheral membrane protein;

iii) the membrane protein is temporarily associated with a membrane;

iv) the membrane protein is a protein that is associated with, and/or wholly or partially spans (e.g., as a transmembrane protein) a target cell's membrane;

v) the membrane protein is an integral monotopic protein (i.e., associated with only one side of a membrane);

vi) the membrane protein is or becomes associated with (e.g., is partly or wholly present on) an outer surface of a target cell's membrane; or

vii) the membrane protein is or becomes associated with (e.g., is partly or wholly present on) an inner surface of a target cell's membrane.

67. The fusosome of any of the preceding embodiments, wherein:

i) the membrane protein is a therapeutic membrane protein; or

ii) the membrane protein is or comprises a receptor (e.g., a cell surface receptor and/or a transmembrane receptor), a cell surface ligand, a membrane transport protein (e.g., an active or passive transport protein such as, for example, an ion channel protein, a pore-forming protein [e.g., a toxin protein], etc), a membrane enzyme, and/or a cell adhesion protein).

68. The fusosome of any of the preceding embodiments, wherein:

i) the membrane protein comprises a sequence of a naturally-occurring membrane protein;

ii) the membrane protein is or comprises a variant or modified version of a naturally-occurring membrane protein;

iii) the membrane protein is or comprises an engineered membrane protein; or

iv) the membrane protein is or comprises a fusion protein.

69. The fusosome of any of the preceding embodiments, wherein the fusosome delivers a membrane protein payload agent to the membrane of a target cell, e.g., as described herein.

70. The fusosome of embodiment 69, wherein the fusosome is further capable of delivering (e.g., delivers) one or more agents, e.g., proteins, nucleic acids (e.g., a DNA, a gDNA, a cDNA, an RNA, a pre-mRNA, an mRNA, etc.), organelles, or and/or metabolites to the cytosol of the target cell.

71. A fusosome composition or preparation, comprising a plurality of fusosomes, wherein at least one fusosome comprises:

(a) a lipid bilayer comprising a plurality of lipids derived from a source cell;

(b) a lumen (e.g., comprising cytosol) surrounded by the lipid bilayer;

(c) a fusogen that is exogenous or overexpressed relative to the source cell, e.g., wherein the fusogen is disposed in the lipid bilayer;

(d) a membrane protein payload agent, e.g., as described herein.

72. A frozen purified fusosome composition or preparation comprising a plurality of fusosomes comprising a membrane protein payload agent described herein, wherein the preparation is frozen at a temperature that is at or less than, 4, 0, -4, -10, -12, -16, -20, -80, or -160°C.

73. The fusosome composition of embodiment 71 or embodiment 72, where at least one fusosome of the plurality of fusosomes is derived from a source cell.

74. The fusosome composition of any of embodiments 71-73, wherein the fusosome is at a temperature of less than 4, 0, -4, -10, -12, -16, -20, -80, or -160 °C.

75. The fusosome composition of any of embodiments 71-74, wherein the plurality of fusosomes comprises at least about 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13}, 10^{14}, or 10^{15}fusosomes.

76. The fusosome composition of any of embodiments 71-75, wherein the plurality of

fusosomes are the same.

77. The fusosome composition of embodiment 76, wherein the plurality of fusosomes are the same if at least 0.01%-0.05%, 0.05%-0.1%, 0.1%-0.5%, 0.5%- 1%, 1%-2%, 2%-3%, 3%-4%, 4%-5%, 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, or 80%-90% of the fusosomes in the fusosome composition share at least one property selected from:

comprise the same fusogen;

produced using the same type of source cell; or

comprise the same membrane protein payload agent.

78. The fusosome composition of any of embodiments 71-75, wherein the plurality of fusosomes are different.

79. The fusosome composition of any of embodiments 71-77, wherein the plurality of fusosomes is derived from two or more types of source cells.

80. The fusosome composition of any of embodiments 71-79, which has a volume of at least 1 μL, 2 μL. 5 μL, 10 μL, 20 μL, 50 μL, 100 μL, 200 μL, 500 μL, 1 mL, 2 mL, 5 mL, or 10 mL.

81. The fusosome composition of any of embodiments 71-80, wherein the plurality of fusosomes comprises at least 0.01%-0.05%, 0.05%-0.1%, 0.1%-0.5%, 0.5%- 1%, 1%-2%, 2%-3%, 3%-4%, 4%-5%, 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, or 80%-90% fusosomes which do not comprise a functional nucleus.

82. The fusosome composition of any of embodiments 71-81, wherein the plurality of fusosomes comprises at least 0.01%-0.05%, 0.05%-0.1%, 0.1%-0.5%, 0.5%- 1%, 1%-2%, 2%-3%, 3%-4%, 4%-5%, 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, or 80%-90% fusosomes which do not comprise a nucleus.

83. The fusosome composition of any of embodiments 71-82, wherein the plurality of

fusosomes comprises at least 0.01%-0.05%, 0.05%-0.1%, 0.1%-0.5%, 0.5%- 1%, 1%-2%, 2%-3%, 3%-4%, 4%-5%, 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, or 80%-90% fusosomes which are substantially free of nuclear DNA.

84. The fusosome composition of any of embodiments 71-83, wherein the plurality of fusosomes comprises at least 0.01%-0.05%, 0.05%-0.1%, 0.1%-0.5%, 0.5%- 1%, 1%-2%, 2%-3%, 3%-4%, 4%-5%, 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, or 80%-90% fusosomes which do not comprise functional mitochondria.

85. The fusosome composition of any of embodiments 71-84, wherein the plurality of fusosomes comprises at least 0.01%-0.05%, 0.05%-0.1%, 0.1%-0.5%, 0.5%- 1%, 1%-2%, 2%-3%, 3%-4%, 4%-5%, 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, or 80%-90% fusosomes which do not comprise mitochondria.

86. The fusosome composition of any of embodiments 71-85, which comprises less than 0.01%, 0.05%, 0.1%, 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 4%, 5%, or 10% source cells by protein mass or less than 0.01%, 0.05%, 0.1%, 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 4%, 5%, or 10% of source cells having a functional nucleus.

87. The fusosome composition of any of embodiments 71-86, wherein the fusosome composition comprises at least 0.01%-0.05%, 0.05%-0.1%, 0.1%-0.5%, 0.5%- 1%, 1%-2%, 2%-3%, 3%-4%, 4%-5%, 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, or 80%-90% fusosomes wherein:

i) the fusogen is present at a copy number of at least 1,000 copies per fusosome, e.g., as measured by an assay of Example 29; or

ii) the ratio of the copy number of the fusogen to the copy number of the membrane protein payload agent per fusosome is between 1,000,000:1 and 100,000:1, 100,000:1 and 10,000:1, 10,000:1 and 1,000:1, 1,000:1 and 100:1, 100:1 and 50:1, 50:1 and 20:1, 20:1 and 10:1, 10:1 and 5:1, 5:1 and 2:1, 2:1 and 1:1, 1:1 and 1:2, 1:2 and 1:5, 1:5 and 1:10, 1:10 and 1:20, 1:20 and 1:50, 1:50 and 1:100, 1:100 and 1:1,000, 1:1,000 and 1:10,000, 1:10,000 and 1:100,000, or

1:100,000 and 1:1,000,000.

88. The fusosome composition of any of embodiments 71-87, wherein the fusosome composition comprises at least 0.01%-0.05%, 0.05%-0.1%, 0.1%-0.5%, 0.5%- 1%, 1%-2%, 2%-3%, 3%-4%, 4%-5%, 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, or 80%-90% fusosomes wherein the the membrane protein payload agent is present at a copy number of at least 1,000 copies per fusosome, e.g., as measured by an assay of Example 43.

89. The fusosome composition of any of embodiments 71-77, wherein the plurality of fusosomes has a mean diameter of at least about 50 nm, about 80 nm, about 100 nm, about 200 nm, about 500 nm, about 1000 nm, about 1200 nm, about 1400 nm, or about 1500 nm.

90. The fusosome composition of any of embodiments 71-89, wherein the plurality of fusosomes comprises fusosomes having a diameter within the range of about 10 nm to about 100 μm.

91. The fusosome composition of any of embodiments 71-89, wherein the plurality comprises fusosomes having a size within the range of about 20 nm to about 200 nm, about 50 nm to about 200 nm, about 50 nm to about 100 nm, about 50 nm to about 150 nm, or about 100 nm to about 150 nm.

92. The fusosome composition of any of embodiments 71-91, wherein at least 50% of fusosomes in the plurality have a diameter within 10%, 20%, 30%, 40%, or 50% of the mean diameter of the fusosomes in the fusosome composition.

93. The fusosome composition of any of embodiments 71-92, wherein the plurality comprises fusosomes having a volume within the range of about 500 nm^3 to about 0.0006 mm^3, or about 4,000 nm^3 to about 0.005 μm^3, about 65,000 nm^3 to about 0.005 μm^3, about 65,000 nm^3 to about 0.0006 μm^3, about 65,000 nm^3 to about 0.002 μm^3, or about 0.0006 μm^3 to about 0.002 μm^3.

94. The fusosome composition of any of embodiments 71-93, wherein at least 50% of fusosomes in the plurality have a volume within 10%, 20%, 30%, 40%, or 50% of the mean volume of the fusosomes in the fusosome composition.

95. The fusosome composition of any of embodiments 71-94, wherein at least 50% of fusosomes in the plurality have a copy number of the fusogen within 10%, 20%, 30%, 40%, or 50% of the mean fusogen copy number in the fusosomes in the fusosome composition.

96. The fusosome composition of any of embodiments 71-95, wherein at least 50% of fusosomes in the plurality have a copy number of the protein membrane payload within 10%, 20%, 30%, 40%, or 50% of the mean protein membrane payload copy number in the fusosomes in the fusosome composition.

97. The fusosome composition or pharmaceutical composition of any of the preceding embodiments, wherein:

i) a membrane protein payload agent is partially or wholly disposed in a fusosome lumen;

ii) a membrane protein payload agent is associated with (e.g., partially or wholly located within) a fusosome's lipid bilayer; or

i) the relevant membrane protein is associated with and/or partially or wholly displayed on the fusosome's external surface.

98. A method of manufacturing a fusosome composition, comprising:

a) providing a source cell comprising, e.g., expressing, a fusogen;

b) producing a fusosome from the source cell, wherein the fusosome comprises a lipid bilayer, a lumen, a fusogen, and a membrane protein payload agent, thereby making a fusosome; and

c) formulating the fusosome, e.g., as a pharmaceutical composition suitable for administration to a subject, wherein one or more of:

i) the source cell is other than a 293 cell, HEK cell, human endothelial cell, or a human epithelial cell;

ii) the fusogen is other than a viral protein;

iii) the fusosome and/or compositions or preparations thereof has a density of other than between 1.08 g/mL and 1.12 g/mL, e.g.,

iv) the fusosome and/or compositions or preparations thereof has a density of 1.25 g/mL +/- 0.05, e.g., as measured by an assay of Example 33;

v) the fusosome is not captured by the scavenger system in circulation or by Kupffer cells in the sinus of the liver;

vi) the fusosome is not captured by the reticulo-endothelial system (RES) in a subject, e.g., by an assay of Example 76;

vii) when a plurality of fusosomes are administered to a subject, less than 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, of the plurality are not captured by the RES after 24 hours, e.g., by an assay of Example 76;

viii) the fusosome has a diameter of greater than 5 μm, 6 μm, 7 μm, 8 μm, 10 μm, 20 μm, 50 μm, 100 μm, 150 μm, or 200 μm.

ix) the fusosome comprises a cytobiologic;

x) the fusosome comprises an enucleated cell; or

xi) the fusosome comprises an inactivated nucleus.

99. The method of embodiment 98, wherein providing a source cell expressing a fusogen comprises expressing an exogenous fusogen in the source cell or upregulating expression of an endogenous fusogen in the source cell.

100. The method of embodiment 98 or embodiment 99, which comprises inactivating the nucleus of the source cell.

101. The method of any of embodiments 98-100, which comprises introducing a membrane protein payload (e.g., a nucleic acid or protein) into a fusosome, e.g., by electroporation.

102. A method of manufacturing a fusosome drug product composition, comprising:

a) providing, e.g., producing, a plurality of fusosomes according to any of embodiments 1-70, a fusosome composition of any of embodiments 71-97, or a pharmaceutical composition of embodiment 97; and

b) assaying one or more fusosomes from the plurality to determine whether one or more (e.g., 2, 3, or all) of the following standards are met:

i) the fusosome fuses at a higher rate with a target cell than with a non-target cell, e.g., by at least at least 10% e.g., in an assay of Example 54;

ii) the fusosome fuses at a higher rate with a target cell than with other fusosomes, e.g., by at least 50% e.g., in an assay of Example 54;

iii) the fusosome fuses with target cells at a rate such that an agent in the fusosome is delivered to at least 10% of target cells after 24 hours, e.g., in an assay of Example 54;

iv) the fusogen is present at a copy number of at least 1,000 copies, e.g., as measured by an assay of Example 29;

v) the fusosome comprises a membrane protein payload agent at a copy number of at least 1,000 copies, e.g., as measured by an assay of Example 43;

vi) the ratio of the copy number of the fusogen to the copy number of the membrane protein payload agent is between 1,000,000:1 and 100,000:1, 100,000:1 and 10,000:1, 10,000:1 and 1,000:1, 1,000:1 and 100:1, 100:1 and 50:1, 50:1 and 20:1, 20:1 and 10:1, 10:1 and 5:1, 5:1 and 2:1, 2:1 and 1:1, 1:1 and 1:2, 1:2 and 1:5, 1:5 and 1:10, 1:10 and 1:20, 1:20 and 1:50, 1:50 and 1:100, 1:100 and 1:1,000, 1:1,000 and 1:10,000, 1:10,000 and 1:100,000, or 1:100,000 and 1:1,000,000;

vii) the fusosome comprises a lipid composition wherein one or more of CL, Cer, DAG, HexCer, LPA, LPC, LPE, LPG, LPI, LPS, PA, PC, PE, PG, PI, PS, CE, SM and TAG is within 75% of the corresponding lipid level in the source cell;

viii) the fusosome comprises a proteomic composition similar to that of the source cell, e.g., using an assay of Example 42;

ix) the fusosome comprises a ratio of lipids to proteins that is within 10%, 20%, 30%, 40%, or 50% of the corresponding ratio in the source cell, e.g., as measured using an assay of Example 49;

x) the fusosome comprises a ratio of proteins to nucleic acids (e.g., DNA) that is within 10%, 20%, 30%, 40%, or 50% of the corresponding ratio in the source cell, e.g., as measured using an assay of Example 50;

xi) the fusosome comprises a ratio of lipids to nucleic acids (e.g., DNA) that is within 10%, 20%, 30%, 40%, or 50% of the corresponding ratio in the source cell, e.g., as measured using an assay of Example 51;

xii) the fusosome has a half-life in a subject, e.g., in a mouse, that is within 90% of the half-life of a reference cell, e.g., the source cell, e.g., by an assay of Example 75;

xiii) the fusosome transports glucose (e.g., labeled glucose, e.g., 2-NBDG) across a membrane, e.g., by at least 10% more than a negative control, e.g., an otherwise similar fusosome in the absence of glucose, e.g., as measured using an assay of Example 64;

xiv) the fusosome comprises esterase activity in the lumen that is within 90% of that of the esterase activity in a reference cell, e.g., the source cell or a mouse embryonic fibroblast, e.g., using an assay of Example 66;

xv) the fusosome comprises a metabolic activity level that is within 90% of the metabolic activity (e.g., citrate synthase activity) in a reference cell, e.g., the source cell, e.g., as described in Example 68;

xvi) the fusosome comprises a respiration level (e.g., oxygen consumption rate) that is within 90% of the respiration level in a reference cell, e.g., the source cell, e.g., as described in Example 69;

xvii) the fusosome comprises an Annexin-V staining level of at most 18,000, 17,000, 16,000, 15,000, 14,000, 13,000, 12,000, 11,000, or 10,000 MFI, e.g., using an assay of Example 70, or wherein the fusosome comprises an Annexin-V staining level at least 5%, 10%, 20%, 30%, 40%, or 50% lower than the Annexin-V staining level of an otherwise similar fusosome treated with menadione in the assay of Example 70, or wherein the fusosome comprises an Annexin-V staining

level at least 5%, 10%, 20%, 30%, 40%, or 50% lower than the Annexin-V staining level of a macrophage treated with menadione in the assay of Example 70;

xviii) the fusosome has a miRNA content level of at least 1% than that of the source cell, e.g., by an assay of Example 39;

xix) the fusosome has a soluble : non-soluble protein ratio is within 90% of that of the source cell, e.g., by an assay of Example 47;

xx) the fusosome has an LPS level less than 5% of the lipid content of fusosomes, e.g., as measured by an assay of Example 48;

xxi) the fusosome and/or compositions or preparations thereof, are capable of signal transduction, e.g., transmitting an extracellular signal, e.g., AKT phosphorylation in response to insulin, or glucose (e.g., labeled glucose, e.g., 2-NBDG) uptake in response to insulin, e.g., by at least 10% more than a negative control, e.g., an otherwise similar fusosome in the absence of insulin, e.g., using an assay of Example 63;

xxii) the fusosome has juxtacrine-signaling level of at least 5% greater than the level of juxtacrine signaling induced by a reference cell, e.g., the source cell or a bone marrow stromal cell (BMSC), e.g., by an assay of Example 71;

xxiii) the fusosome has paracrine-signaling level of at least 5% greater than the level of paracrine signaling induced by a reference cell, e.g., the source cell or a macrophage, e.g., by an assay of Example 72;

xxiv) the fusosome polymerizes actin at a level within 5% compared to the level of polymerized actin in a reference cell, e.g., the source cell or a C2C12 cell, e.g., by the assay of Example 73;

xxv) the fusosome has a membrane potential within about 5% of the membrane potential of a reference cell, e.g., the source cell or a C2C12 cell, e.g., by an assay of Example 74, or wherein the fusosome has a membrane potential of about -20 to -150mV, -20 to -50mV, -50 to -100mV, or -100 to -150mV;

xxvi) the fusosome and/or compositions or preparations thereof, are capable of secreting a protein, e.g., at a rate at least 5% greater than a reference cell, e.g., a mouse embryonic fibroblast, e.g., using an assay of Example 62; or

xxvii) the fusosome has low immunogenicity, e.g., as described herein; and

c) (optionally) approving the plurality of fusosomes or fusosome composition for release if one or more of the standards is met;

thereby manufacturing a fusosome drug product composition.

103. A method of manufacturing a fusosome composition, comprising:

a) providing a source cell comprising, e.g., expressing, a fusogen;

b) producing a fusosome from the source cell, wherein the fusosome comprises a lipid bilayer, a lumen, a fusogen and a membrane protein payload agent, thereby making a fusosome; and

c) formulating the fusosome, e.g., as a pharmaceutical composition suitable for administration to a subject.

104. A method of manufacturing a fusosome composition, comprising:

a) providing, e.g., producing, a plurality of fusosomes or a fusosome preparation described herein; and

b) assaying a sample of the plurality (e.g., of the preparation) to determine whether one or more (e.g., 2, 3, or more) standards are met.

105. The method of embodiment 104, wherein the standard(s) are chosen from:

i) fusosomes in the sample fuse at a higher rate with a target cell than with a non-target cell, e.g., by at least at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, e.g., in an assay of Example 54;

ii) fusosomes in the sample fuse at a higher rate with a target cell than other fusosomes, e.g., by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, e.g., in an assay of Example 54;

iii) fusosomes in the sample fuse with target cells at a rate such that a membrane protein

payload agent in the fusosome is delivered to at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, of target cells after 24, 48, or 72 hours, e.g., in an assay of Example 54;

iv) the fusogen is present at a copy number, per fusosome (e.g., on average in the sample), of at least, or no more than, 10, 50, 100, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000, 100,000,000, 500,000,000, or 1,000,000,000 copies, e.g., as measured by an assay of Example 29;

v) the membrane protein payload agent is detectable in fusosomes of the sample (e.g., on average in the sample) at a copy number of at least, or no more than, 10, 50, 100, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000 1,000,000, 5,000,000, 10,000,000, 50,000,000, 100,000,000, 500,000,000, or 1,000,000,000 copies, e.g., as measured by an assay of Example 43;

vi) the ratio of the copy number of the fusogen to the copy number of the membrane protein payload agent is between 1,000,000:1 and 100,000:1, 100,000:1 and 10,000:1, 10,000:1 and 1,000:1, 1,000:1 and 100:1, 100:1 and 50:1, 50:1 and 20:1, 20:1 and 10:1, 10:1 and 5:1, 5:1 and 2:1, 2:1 and 1:1, 1:1 and 1:2, 1:2 and 1:5, 1:5 and 1:10, 1:10 and 1:20, 1:20 and 1:50, 1:50 and 1:100, 1:100 and 1:1,000, 1:1,000 and 1:10,000, 1:10,000 and 1:100,000, or 1:100,000 and 1:1,000,000;

vii) fusosomes of the sample are characterized by a lipid composition substantially similar to that of the source cell or wherein one or more of CL, Cer, DAG, HexCer, LPA, LPC, LPE, LPG, LPI, LPS, PA, PC, PE, PG, PI, PS, CE, SM and TAG is within 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, or 75% of the corresponding lipid level in the source cell;

viii) fusosomes of the sample are characterized by a proteomic composition similar to that of the source cell, e.g., using an assay of Example 42;

ix) fusosomes of the sample are characterized by a ratio of lipids to proteins that is within 10%, 20%, 30%, 40%, or 50% of the corresponding ratio in the source cell, e.g., as measured using an assay of Example 49;

x) fusosomes of the sample are characterized by a ratio of proteins to nucleic acids (e.g., DNA) that is within 10%, 20%, 30%, 40%, or 50% of the corresponding ratio in the source cell, e.g., as measured using an assay of Example 50;

xi) fusosomes of the sample are characterized by a ratio of lipids to nucleic acids (e.g.,

DNA) that is within 10%, 20%, 30%, 40%, or 50% of the corresponding ratio in the source cell, e.g., as measured using an assay of Example 51;

xii) fusosomes of the sample are characterized by a half-life in a subject, e.g., in a an experimental animal such as a mouse, that is within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% of the half life of a reference cell, e.g., the source cell, e.g., by an assay of Example 75;

xiii) fusosomes of the sample are characterized in that they transport glucose (e.g., labeled glucose, e.g., 2-NBDG) across a membrane, e.g., by at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% more than a negative control, e.g., fusosomes of an otherwise similar sample in the absence of glucose, e.g., as measured using an assay of Example 64;

xiv) fusosomes of the sample are characterized by esterase activity in the lumen that is within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of that of the esterase activity in a reference cell, e.g., the source cell or a mouse embryonic fibroblast, e.g., using an assay of Example 66;

xv) fusosomes of the sample are characterized by a metabolic activity (e.g., citrate synthase activity) level that is within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the metabolic activity, e.g., citrate synthase activity, in a reference cell, e.g., the source cell, e.g., as described in Example 68;

xvi) fusosomes of the sample are characterized by a respiration level (e.g., oxygen consumption rate) that is within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the respiration level in a reference cell, e.g., the source cell, e.g., as described in Example 69;

xvii) fusosomes of the sample are characterized by an Annexin-V staining level of at most 18,000, 17,000, 16,000, 15,000, 14,000, 13,000, 12,000, 11,000, or 10,000 MFI, e.g., using an assay of Example 70, or wherein the fusosome comprises an Annexin-V staining level at least 5%, 10%, 20%, 30%, 40%, or 50% lower than the Annexin-V staining level of an otherwise similar fusosome treated with menadione in the assay of Example 70, or wherein the fusosome comprises an Annexin-V staining level at least 5%, 10%, 20%, 30%, 40%, or 50% lower than the Annexin-V staining level of a macrophage treated with menadione in the assay of Example 70,

xviii) fusosomes of the sample are characterized by a miRNA content level of at least at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or greater than that of the source cell, e.g., by an assay of Example 39;

xix) the fusosome has a soluble : non-soluble protein ratio is within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or greater than that of the source cell, e.g., within 1%-2%, 2%-3%, 3%-4%, 4%-5%, 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, or 80%-90% of that of the source cell, e.g., by an assay of Example 47;

xx) fusosomes of the sample are characterized by an LPS level less than 5%, 1%, 0.5%, 0.01%, 0.005%, 0.0001%, 0.00001% or less of the LPS content of the source cell or a reference cell, e.g., as measured by an assay of Example 48;

xxi) fusosomes of the sample are capable of signal transduction, e.g., transmitting an extracellular signal, e.g., AKT phosphorylation in response to insulin, or glucose (e.g., labeled glucose, e.g., 2-NBDG) uptake in response to insulin, e.g., by at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% more than a negative control, e.g., an otherwise similar fusosome in the absence of insulin, e.g., using an assay of Example 63;

xxii) fusosomes of the sample are characterized by a juxtacrine-signaling level of at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% greater than the level of juxtacrine signaling induced by a reference cell, e.g., the source cell or a bone marrow stromal cell (BMSC), e.g., by an assay of Example 71;

xxiii) fusosomes of the sample are characterized by a paracrine-signaling level of at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% greater than the level of paracrine signaling induced by a reference cell, e.g., the source cell or a macrophage, e.g., by an assay of Example 72;

xxiv) fusosomes of the sample are characterized in that they polymerize actin at a level within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% compared to the level of polymerized actin in a reference cell, e.g., the source cell or a C2C12 cell, e.g., by the assay of Example 73;

xxv) fusosomes of the sample are characterized by a membrane potential within about 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% of the membrane

potential of a reference cell, e.g., the source cell or a C2C12 cell, e.g., by an assay of Example 74, or wherein the fusosome has a membrane potential of about -20 to -150mV, -20 to -50mV, -50 to -100mV, or -100 to -150mV;

xxvi) fusosomes of the sample are capable of secreting a protein, e.g., at a rate at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% greater than the a reference cell, e.g., a mouse embryonic fibroblast, e.g., using an assay of Example 62; or

xxvii) fusosomes of the sample are characterized by low immunogenicity, e.g., as described herein.

106. The method of embodiment 104 or 105, further comprising:

c) approving the plurality of fusosomes or fusosome composition for release if one or more of the standards is met or (optionally) formulating the plurality of fusosomes or the fusosome preparation as a drug product if the one or more standards is met.

107. A method of manufacturing a fusosome composition, comprising:

a) providing a source cell comprising, e.g., expressing, a fusogen;

b) producing a fusosome from the source cell, wherein the fusosome comprises a lipid bilayer, a lumen, a fusogen, and a membrane protein payload agent, thereby making a fusosome; and

c) formulating the fusosome, e.g., as a pharmaceutical composition suitable for administration to a subject, wherein one or more of:

i) the source cell is other than a 293 cell, HEK cell, human endothelial cell, or a human epithelial cell;

ii) the fusogen is other than a viral protein;

iii) the fusosome and/or compositions or preparations thereof has a density of other than between 1.08 g/mL and 1.12 g/mL, e.g.,

iv) the fusosome and/or compositions or preparations thereof has a density of 1.25 g/mL +/- 0.05, e.g., as measured by an assay of Example 33;

v) the fusosome is not captured by the scavenger system in circulation or by Kupffer cells in the sinus of the liver;

vi) the fusosome is not captured by the reticulo-endothelial system (RES) in a subject, e.g., by an assay of Example 76;

vii) when a plurality of fusosomes are administered to a subject, less than 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, of the plurality are not captured by the RES after 24 hours, e.g., by an assay of Example 76;

viii) the fusosome has a diameter of greater than 5 μm, 6 μm, 7 μm, 8 μm, 10 μm, 20 μm, 50 μm, 100 μm, 150 μm, or 200 μm.

ix) the fusosome comprises a cytobiologic;

x) the fusosome comprises an enucleated cell; or

xi) the fusosome comprises an inactivated nucleus.

108. A method of manufacturing a fusosome composition, comprising:

a) providing a plurality of fusosomes, a fusosome composition, or a pharmaceutical composition as described herein; and

b) assaying one or more fusosomes from the plurality to determine whether one or more (e.g., 2, 3, or all) of the following standards are met:

i) the fusosome fuses at a higher rate with a target cell than with a non-target cell, e.g., by at least at least 10% e.g., in an assay of Example 54;

ii) the fusosome fuses at a higher rate with a target cell than with other fusosomes, e.g., by at least 50% e.g., in an assay of Example 54;

iii) the fusosome fuses with target cells at a rate such that an agent in the fusosome is delivered to at least 10% of target cells after 24 hours, e.g., in an assay of Example 54;

iv) the fusogen is present at a copy number of at least 1,000 copies, e.g., as measured by an assay of Example 29;

v) the fusosome comprises a membrane protein payload agent at a copy number of at least 1,000 copies, e.g., as measured by an assay of Example 43;

vi) the ratio of the copy number of the fusogen to the copy number of the membrane protein payload agent is between 1,000,000:1 and 100,000:1, 100,000:1 and 10,000:1, 10,000:1 and 1,000:1, 1,000:1 and 100:1, 100:1 and 50:1, 50:1 and

20:1, 20:1 and 10:1, 10:1 and 5:1, 5:1 and 2:1, 2:1 and 1:1, 1:1 and 1:2, 1:2 and 1:5, 1:5 and 1:10, 1:10 and 1:20, 1:20 and 1:50, 1:50 and 1:100, 1:100 and 1:1,000, 1:1,000 and 1:10,000, 1:10,000 and 1:100,000, or 1:100,000 and 1:1,000,000;

vii) the fusosome comprises a lipid composition wherein one or more of CL, Cer, DAG, HexCer, LPA, LPC, LPE, LPG, LPI, LPS, PA, PC, PE, PG, PI, PS, CE, SM and TAG is within 75% of the corresponding lipid level in the source cell;

viii) the fusosome comprises a proteomic composition similar to that of the source cell, e.g., using an assay of Example 42;

ix) the fusosome comprises a ratio of lipids to proteins that is within 10%, 20%, 30%, 40%, or 50% of the corresponding ratio in the source cell, e.g., as measured using an assay of Example 49;

x) the fusosome comprises a ratio of proteins to nucleic acids (e.g., DNA) that is within 10%, 20%, 30%, 40%, or 50% of the corresponding ratio in the source cell, e.g., as measured using an assay of Example 50;

xi) the fusosome comprises a ratio of lipids to nucleic acids (e.g., DNA) that is within 10%, 20%, 30%, 40%, or 50% of the corresponding ratio in the source cell, e.g., as measured using an assay of Example 51;

xii) the fusosome has a half-life in a subject, e.g., in a mouse, that is within 90% of the half-life of a reference cell, e.g., the source cell, e.g., by an assay of Example 75;

xiii) the fusosome transports glucose (e.g., labeled glucose, e.g., 2-NBDG) across a membrane, e.g., by at least 10% more than a negative control, e.g., an otherwise similar fusosome in the absence of glucose, e.g., as measured using an assay of Example 64;

xiv) the fusosome comprises esterase activity in the lumen that is within 90% of that of the esterase activity in a reference cell, e.g., the source cell or a mouse embryonic fibroblast, e.g., using an assay of Example 66;

xv) the fusosome comprises a metabolic activity level that is within 90% of the metabolic activity (e.g., citrate synthase activity) in a reference cell, e.g., the source cell, e.g., as described in Example 68;

xvi) the fusosome comprises a respiration level (e.g., oxygen consumption rate) that is within 90% of the respiration level in a reference cell, e.g., the source cell, e.g., as described in Example 69;

xvii) the fusosome comprises an Annexin-V staining level of at most 18,000, 17,000, 16,000, 15,000, 14,000, 13,000, 12,000, 11,000, or 10,000 MFI, e.g., using an assay of Example 70, or wherein the fusosome comprises an Annexin-V staining level at least 5%, 10%, 20%, 30%, 40%, or 50% lower than the Annexin-V staining level of an otherwise similar fusosome treated with menadione in the assay of Example 70, or wherein the fusosome comprises an Annexin-V staining level at least 5%, 10%, 20%, 30%, 40%, or 50% lower than the Annexin-V staining level of a macrophage treated with menadione in the assay of Example 70;

xviii) the fusosome has a miRNA content level of at least 1% than that of the source cell, e.g., by an assay of Example 39;

xix) the fusosome has a soluble : non-soluble protein ratio is within 90% of that of the source cell, e.g., by an assay of Example 47;

xx) the fusosome has an LPS level less than 5% of the lipid content of fusosomes, e.g., as measured by an assay of Example 48;

xxi) the fusosome and/or compositions or preparations thereof, are capable of signal transduction, e.g., transmitting an extracellular signal, e.g., AKT phosphorylation in response to insulin, or glucose (e.g., labeled glucose, e.g., 2-NBDG) uptake in response to insulin, e.g., by at least 10% more than a negative control, e.g., an otherwise similar fusosome in the absence of insulin, e.g., using an assay of Example 63;

xxii) the fusosome has juxtacrine-signaling level of at least 5% greater than the level of juxtacrine signaling induced by a reference cell, e.g., the source cell or a bone marrow stromal cell (BMSC), e.g., by an assay of Example 71;

xxiii) the fusosome has paracrine-signaling level of at least 5% greater than the level of paracrine signaling induced by a reference cell, e.g., the source cell or a macrophage, e.g., by an assay of Example 72;

xxiv) the fusosome polymerizes actin at a level within 5% compared to the level

of polymerized actin in a reference cell, e.g., the source cell or a C2C12 cell, e.g., by the assay of Example 73;

xxv) the fusosome has a membrane potential within about 5% of the membrane potential of a reference cell, e.g., the source cell or a C2C12 cell, e.g., by an assay of Example 74, or wherein the fusosome has a membrane potential of about -20 to -150mV, -20 to -50mV, -50 to -100mV, or -100 to -150mV;

xxvi) the fusosome and/or compositions or preparations thereof, are capable of secreting a protein, e.g., at a rate at least 5% greater than a reference cell, e.g., a mouse embryonic fibroblast, e.g., using an assay of Example 62; or

xxvii) the fusosome has low immunogenicity, e.g., as described herein; and

c) optionally, approving the plurality of fusosomes or fusosome composition for release if one or more of the standards is met;

thereby manufacturing a fusosome drug product composition.

109. A method of delivering a membrane protein payload agentto a subject, comprising administering to the subject a fusosome composition comprising a plurality of fusosomes according to any of embodiments 1-70, a fusosome composition of any of embodiments 71-97, or a pharmaceutical composition of embodiment 97, wherein the fusosome composition is administered in an amount and/or time such that the membrane protein payload agent is delivered.

110. A method of delivering a fusosome composition or preparation comprising a membrane protein payload agent, e.g., as described herein, to a human subject, a target tissue, or a cell, the method comprising administering to the human subject, or contacting the target tissue or the cell with, a fusosome composition comprising a plurality of fusosomes described herein, a fusosome composition described herein, or a pharmaceutical composition described herein, thereby administering the fusosome composition to the subject.

111. A method of delivering a membrane protein payload agent to a subject, a target tissue, or a cell, comprising administering to the subject, or contacting the target tissue or the cell with, a fusosome composition or preparation described herein (e.g., a pharmaceutical composition

described herein), wherein the fusosome composition or preparation is administered in an amount and/or time such that the membrane protein payload agent is delivered.

112. A method of delivering a membrane protein payload agent to a subject, for example comprising:

a) administering to the subject first fusogen, under conditions that allow for disposition of the first fusogen in one or more target cells in the subject, wherein one or more of:

i) administering the first fusogen comprises administering a nucleic acid encoding the first fusogen, under conditions that allow for expression of the first fusogen in the one or more target cells, or

ii) the first fusogen does not comprise a coiled-coil motif, and

b) administering to the human subject a fusosome composition or preparation as described herein, comprising a plurality of fusosomes comprising a second fusogen and a membrane protein payload agent, wherein the second fusogen is compatible with the first fusogen, wherein the plurality of fusosomes further comprise a membrane protein payload agent,

thereby delivering the membrane protein payload agent to the subject.

113. A method of modulating, e.g., enhancing, a biological function in a subject, comprising:

a) administering to the subject first fusogen, under conditions that allow for disposition of the first fusogen in one or more target cells in the subject, wherein one or more of:

i) administering the first fusogen comprises administering a nucleic acid encoding the first fusogen, under conditions that allow for expression of the first fusogen in the one or more target cells, or

ii) the first fusogen does not comprise a coiled-coil motif, and

b) administering to the human subject a fusosome composition or preparation as described herein, comprising a plurality of fusosomes comprising a second fusogen, wherein the second fusogen is compatible with the first fusogen, wherein the plurality of fusosomes further comprise a membrane protein payload agent,

thereby modulating the biological function in the subject.

114. A method of delivering or targeting a membrane protein function to a subject, comprising administering to the subject a fusosome composition or preparation described herein which comprises a membrane protein payload agent, wherein the fusosome composition or preparation is administered in an amount and/or time such that the membrane protein function is delivered or targeted in the subject, optionally wherein the subject has a cancer, an inflammatory disorder, autoimmune disease, a chronic disease, inflammation, damaged organ function, an infectious disease, a degenerative disorder, a genetic disease, or an injury.

115. A method of administering a fusosome composition to a human subject, comprising:

a) administering to the subject a first fusogen, under conditions that allow for disposition of the first fusogen in one or more target cells in the subject, wherein one or more of:

i) administering the first fusogen comprises administering a nucleic acid encoding the first fusogen, under conditions that allow for expression of the first fusogen in the one or more target cells, or

ii) the first fusogen does not comprise a coiled-coil motif, and

b) administering to the human subject a fusosome composition comprising a plurality of fusosomes comprising a second fusogen, wherein the second fusogen is compatible with the first fusogen, wherein the plurality of fusosomes further comprise a membrane protein payload agent;

thereby administering the fusosome composition to the subject.

116. A method of delivering a membrane protein payload agent to a subject, comprising:

a) administering to the subject first fusogen, under conditions that allow for disposition of the first fusogen in one or more target cells in the subject, wherein one or more of:

i) administering the first fusogen comprises administering a nucleic acid encoding the first fusogen, under conditions that allow for expression of the first fusogen in the one or more target cells, or

ii) the first fusogen does not comprise a coiled-coil motif, and

b) administering to the human subject a fusosome composition comprising a plurality of fusosomes comprising a second fusogen and a therapeutic agent, wherein the second

fusogen is compatible with the first fusogen, wherein the plurality of fusosomes further comprise a membrane protein payload agent;

thereby delivering the membrane protein payload agent to the subject.

117. A method of modulating, e.g., enhancing, a biological function in a subject, comprising:

a) administering to the subject first fusogen, under conditions that allow for disposition of the first fusogen in one or more target cells in the subject, wherein one or more of:

i) administering the first fusogen comprises administering a nucleic acid encoding the first fusogen, under conditions that allow for expression of the first fusogen in the one or more target cells, or

ii) the first fusogen does not comprise a coiled-coil motif, and

b) administering to the human subject a fusosome composition comprising a plurality of fusosomes comprising a second fusogen, wherein the second fusogen is compatible with the first fusogen, wherein the plurality of fusosomes further comprise a membrane protein payload agent;

thereby modulating the biological function in the subject.

118. The method of embodiment 117, wherein the biological function is selected from:

a) modulating, e.g., increasing or decreasing, an interaction between two cells;

b) modulating, e.g. increasing or decreasing, an immune response;

c) modulating, e.g. increasing or decreasing, recruitment of cells to a target tissue;

d) decreasing the growth rate of a cancer; or

e) reducing the number of cancerous cells in the subject.

119. The method of any of embodiments 115-118, wherein the membrane protein payload agent is exogenous or overexpressed relative to the source cell.

120. The method of any of embodiments 115-119, wherein the membrane protein payload agent comprises or encodes one or more of:

i) a chimeric antigen receptor;

ii) an integrin membrane protein payload, e.g., chosen from Table 7;
iii) an ion channel protein chosen from Table 8;
iv) a pore forming protein, e.g., chosen from Tables 9 and 10;
v) a Toll-Like Receptor, e.g., chosen from Table 11;
vi) an interleukin receptor payload, e.g., chosen from Table 12;
vii) a cell adhesion protein chosen from Tables 13-14;
viii) a transport protein chosen from Table 17;
ix) a signal sequence that is heterologous relative to the naturally-occurring membrane protein; or
x) a signal sequence listed in Table 6.

121. The method of any of embodiments 115-120, wherein the membrane protein payload agent is other than, does not comprise, does not encode, or is not complementary to a sequence that encodes, a connexin, CFTR, thyrotropin receptor, myelin protein zero, melacortin 4, myelin proteolipid protein, low-density lipoprotein receptor, ABC transporter, CD81, mCAT-1, CXCR4, CD4, CCR5, sialic acid-rich proteins, claudins, CD21, T-cell receptors, B cell receptors, TNFR1, CD63, GLUT4, VEGF, or ICAM.

122. The method of any of embodiments 115-121, wherein the membrane protein payload agent comprises or encodes a chimeric protein which does not bind a cell surface marker or target cell moiety of a target cell and which does not comprise a fluorescent protein.

123. The method of any of embodiments 115-122, wherein the membrane protein payload agent:

a) comprises a therapeutic protein, e.g., a therapeutic protein described herein;

b) comprises a Golgi apparatus protein, a secreted protein, or an endoplasmic reticulum protein, or a combination thereof;

c) does not comprise one or more of: a dimer (e.g., a dimer that is exogenous to the source cell), a heterodimer (e.g., a heterodimer that is exogenous to the source cell), or a dimerization domain (e.g., a dimerization domain in a polypeptide that is exogenous to the source cell);

d) comprises a nucleic acid (e.g., DNA or RNA) encoding a membrane protein.

124. The method of any of embodiments 115-123, wherein the membrane protein payload agent comprises or encodes:

a) a membrane protein that comprises a transmembrane domain;

b) a lipid-anchored protein;

c) a protein that binds a transmembrane protein (e.g., an extracellular protein that binds an extracellular portion of a transmembrane protein or an intracellular protein that binds an intracellular portion of a transmembrane protein);

d) a protein that lacks a transmembrane domain;

e) a protein that partially spans a membrane (e.g., a membrane of the target cell or the fusosome) and does not completely span the membrane (e.g., comprising an in-plane membrane helix or the protein comprises a hydrophobic loop that does not completely span the membrane); or

f) a protein that does not comprise a transmembrane domain, wherein the protein interacts with a membrane surface, e.g., through electrostatic or ionic interactions.

125. The method of any of embodiments 115-124, wherein the first fusogen is not a lipopeptide.

126. The method of any of embodiments 109-125, further comprising a step of:

monitoring one or more of cancer progression, tumor recession, tumor volume, decrease in neoplastic cell number, quantity of fused cells, quantity of fused cells comprising a membrane protein payload agent, quantity of fused cells expressing a nucleic acid protein payload, and quantity of membrane protein disposed in membrane of a fused cell.

127. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the membrane protein payload agent is or comprises:

a) a sequence of SEQ ID NOs: 8144-16131 of U.S. Patent Publication No. 2016/0289674;

b) a fragment, variant, or homolog of a sequence of SEQ ID NOs: 8144-16131 of U.S.

Patent Publication No. 2016/0289674;

c) a nucleic acid encoding a protein comprising a sequence of SEQ ID NOs: 8144-16131 of U.S. Patent Publication No. 2016/0289674; or

d) a nucleic acid encoding a protein comprising a fragment, variant, or homolog of a sequence of SEQ ID NOs: 8144-16131 of U.S. Patent Publication No. 2016/0289674.

128. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the membrane protein payload agent is or comprises:

a) a protein selected from Tables 7-17;

b) a fragment, variant, or homolog of a protein selected from Tables 7-17;

c) a nucleic acid encoding a protein which is or comprises a protein selected from Tables 7-17; or

d) a nucleic acid encoding a protein comprising a fragment, variant, or homolog of a protein selected from Tables 7-17.

129. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the membrane protein payload agent is or comprises a chimeric antigen receptor (CAR) comprising an antigen binding domain.

130. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the membrane protein provided by or as a membrane protein payload agent as described herein is or comprises an immunoglobulin moiety or entity (e.g., an antibody, a Fab, an scFV, an scFab, a sdAb, a duobody, a minibody, a nanobody, a diabody, a zybody, a camelid antibody, a BiTE, a quadroma, or a bsDb).

131. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the membrane protein provided by or as a membrane protein payload agent as described herein is or comprises one or more cell surface ligands (e.g., 1, 2, 3, 4, 5, 10, 20, 50, or more cell surface ligands), and/or wherein the membrane protein provided by or as a membrane protein payload agent as described herein presents one or more cell surface ligands,

e.g., to a target cell.

132. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, comprises (e.g., is capable of delivering to the target cell) a plurality of agents (e.g., at least 2, 3, 4, 5, 10, 20, or 50 agents), wherein at least one agent is or comprises a membrane protein payload; optionally, wherein one or more of the agents is or comprises an inhibitory nucleic acid (e.g., siRNA or miRNA) and/or an mRNA.

133. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, comprises (e.g., is capable of delivering to the target cell) a membrane protein payload agent are capable of reprogramming or transdifferentiating a target cell, e.g., the fusosome (and/or composition thereof) comprises one or more agents that induce reprogramming or transdifferentiation of a target cell.

134. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein:

a) the fusosome delivers to a target cell at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% of a membrane protein payload agent (e.g., a therapeutic agent, e.g., a therapeutic agent that is endogenous or exogenous relative to the source cell) comprised by the fusosome;

b) the fusosomes that fuse with the target cell(s) deliver to the target cell an average of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% of the membrane protein payload agent (e.g., a therapeutic membrane protein payload agent, e.g., an endogenous therapeutic membrane protein payload agent or a therapeutic membrane protein payload agent that is exogenous relative to the source cell) comprised by the fusosomes that fuse with the target cell(s); and/o

c) the fusosome composition delivers to a target tissue at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% of the membrane protein payload agent

(e.g., a membrane protein payload agent agent, e.g., a therapeutic membrane protein payload agent that is exogenous relative ot he source cell) comprised by the fusosome composition.

135. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, delivers or is capable of delivering (e.g., deliver) a membrane protein payload agent (e.g., a therapeutic agent) that is characterized by a half-life in a subject that is longer than the half-life of the fusosome, e.g., by at least 10%, 20%, 50%, 2-fold, 5-fold, or 10-fold.

136. The fusosome, fusosome composition, fusosome preparation, or method of embodiment 306, wherein the fusosome delivers the therapeutic agent to the target cell, optionally wherein the agent is present after the fusosome is no longer present or detectable.

137. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome comprises a therapeutic membrane protein payload agent, e.g., a therapeutic membrane protein payload agent, e.g., a therapeutic membrane protein payload agent that is exogenous or endogenous relative to the source cell, optionally wherein the therapeutic membrane protein payload agent is chosen from one or more of a protein, e.g., a transmembrane protein, a cell surface protein, a secreted protein, a receptor, an antibody; a nucleic acid, e.g., DNA, a chromosome (e.g. a human artificial chromosome), RNA, or mRNA.

Delivery to the nucleus

138. A fusosome comprising:

(a) a lipid bilayer comprising a plurality of lipids derived from a source cell;

(b) a lumen (e.g., comprising cytosol) surrounded by the lipid bilayer;

(c) a fusogen that is exogenous or overexpressed relative to the source cell, e.g., wherein the fusogen is disposed in the lipid bilayer; and

(d) a nuclear payload agent, e.g., a nuclear protein payload agent (e.g., which is exogenous or overexpressed relative to the source cell), wherein:

i) when a target cell is contacted with a plurality of the fusosomes, the nuclear

protein payload agent or a protein encoded therein becomes present in the nucleus of the target cell at a level at least 10%, 20%, 50%, 100%, 2-fold, 5-fold, 10-fold, 20-fold, 50-fold, or 100-fold higher than the level of the nuclear protein payload agent in the cytoplasm of the target cell, e.g., by an assay of any of Examples 117-122;

ii) when a population of target cells is contacted with a plurality of the fusosomes, the nuclear protein payload agent or a protein encoded therein becomes enriched (e.g., present in the nucleus of the target cell at a level at least 50% higher than the level of the nuclear protein payload agent in the cytoplasm of the target cell) in the nucleus of **at least** 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of cells in the population of target cells, e.g., as measured in an assay of any of Examples 117-122;

iii) when a population of target cells is contacted with a plurality of the fusosomes, the nuclear protein payload agent or a protein encoded therein becomes present in a sub-population (the "fused population") of the population of target cells, wherein the nuclear protein payload agent or protein encoded therein becomes enriched in the nucleus (e.g., present in the nucleus of the target cell at a level at least 50% higher than the level of the nuclear protein payload agent in the cytosol of the target cell), in at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of cells in the **fused population**, e.g., as measured in an assay of any of Examples 117-122

iv) when a population of target cells is contacted with a plurality of the fusosomes, the nuclear protein payload agent or a protein encoded therein becomes present in a **sub-population** (the "recipient cell population") of the population of target cells, wherein the nuclear protein payload agent or protein encoded therein becomes enriched in the nucleus (e.g., present in the nucleus of the target cell at a level at least 50% higher than the level of the nuclear protein payload agent in the cytoplasm of the target cell), in at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of

cells in the recipient cell population, e.g., as measured in an assay of any of Examples 117-122;

v) at least 1, 2, 5, 10, 20, 50, 100, 200, 500, or 1,000 copies of the nuclear protein payload agent, or a protein encoded by a nucleic acid nuclear protein payload agent, are present in the nucleus in a target cell, e.g., as measured in an assay of Example 117 or 118;

vi) the nuclear protein payload agent comprises or encodes one or more of a transcriptional activator, transcriptional repressor, epigenetic modifier, histone acetyltransferase, histone deacetylase, histone methyltransferase, DNA methyltransferase, a DNA nickase, a site-specific DNA editing enzyme e.g. a deaminase, DNA transposase, DNA integrase, an RNA editor, an RNA splicing factor, a PIWI protein;

vii) the nuclear protein payload agent comprises or encodes one or more of a basic helix-loop helix motif, leucine zipper motif, helix-turn-helix motif, homeodomain motif, a winged helix motif, a winged helix-turn motif, a HMG-box domain, a Wor3 domain, an OB-fold domain, a zinc finger motif, a TAL effector, or a B3 domain;

viii) the nuclear protein payload agent comprises a **complex** comprising a homodimer, a heterodimer, a homotrimer, a heterotrimer, a homotetramer, or a heterotetramer;

ix) the nuclear protein payload agent comprises or encodes a heterologous nuclear localization signal, and wherein the nuclear protein payload agent does not comprise a site-specific nuclease such as Cre;

x) the nuclear protein payload agent comprises or encodes a nuclear localization signal listed in Table 6-1, optionally wherein the NLS comprises an amino acid sequence set forth in any one of SEQ ID NOS: 128-507 and 605-626; or

xi) the nuclear protein payload agent comprises or encodes a protein that is preferentially enriched in the nucleoplasm, nucleolus, perionucleolar region, a Cajal body, a clastosome, a gems nuclear body, a histone locus

body (HLB), a nuclear speckle, a nuclear stress body, a paraspeckle, a PML body, a polycomb body; or

xii) the nuclear protein payload agent comprises or encodes a protein comprising a first domain that binds an endogenous protein present (at least partly) in the cytoplasm of a target cell and a second domain that promotes nuclear import, e.g., an NLS.

139. The fusosome of embodiment 138, wherein the payload that is in an endocytic vesicle is in the cytoplasm of a target cell.

140. A fusosome comprising:

(a) a lipid bilayer comprising a plurality of lipids derived from a source cell;

(b) a lumen (e.g., comprising cytosol) surrounded by the lipid bilayer;

(c) a fusogen that is exogenous or overexpressed relative to the source cell, e.g., wherein the fusogen is disposed in the lipid bilayer; and

(d) a nuclear payload agent, e.g., a nuclear protein payload agent (e.g., which is exogenous or overexpressed relative to the source cell), wherein:

i) the fusosome comprises or is comprised by a cytobiologic;

ii) the fusogen is present at a copy number of at least 1,000 copies, e.g., as measured by an assay of Example 29;

iii) the fusosome comprises a therapeutic agent at a copy number of at least 1,000 copies, e.g., as measured by an assay of Example 43;

iv) the fusosome comprises a lipid wherein one or more of CL, Cer, DAG, HexCer, LPA, LPC, LPE, LPG, LPI, LPS, PA, PC, PE, PG, PI, PS, CE, SM and TAG is within 75% of the corresponding lipid level in the source cell;

v) the fusosome comprises a proteomic composition similar to that of the source cell, e.g., using an assay of Example 42;

vi) the fusosome is capable of signal transduction, e.g., transmitting an extracellular signal, e.g., AKT phosphorylation in response to insulin, or glucose (e.g., labeled glucose, e.g., 2-NBDG) uptake in response to insulin,

e.g., by at least 10% more than a negative control, e.g., an otherwise similar fusosome in the absence of insulin, e.g., using an assay of Example 63;

vii) the fusosome targets a tissue, e.g., liver, lungs, heart, spleen, pancreas, gastrointestinal tract, kidney, testes, ovaries, brain, reproductive organs, central nervous system, peripheral nervous system, skeletal muscle, endothelium, inner ear, or eye, when administered to a subject, e.g., a mouse, e.g., wherein at least 0.1%, or 10%, of the fusosomes in a population of administered fusosomes are present in the target tissue after 24 hours, e.g., by an assay of Example 87 or 100; or

viii) the source cell is selected from a neutrophil, a granulocyte, a mesenchymal stem cell, a bone marrow stem cell, an induced pluripotent stem cell, an embryonic stem cell, a myeloblast, a myoblast, a hepatocyte, or a neuron e.g., retinal neuronal cell.

141. A fusosome comprising:

(a) a lipid bilayer comprising a plurality of lipids derived from a source cell;

(b) a lumen (e.g., comprising cytosol) surrounded by the lipid bilayer;

(c) a fusogen that is exogenous or overexpressed relative to the source cell, e.g., wherein the fusogen is disposed in the lipid bilayer; and

(d) a nuclear payload agent comprising (i) a nucleic acid, and (ii) a protein that promotes nuclear import of the nucleic acid.

142. The fusosome of any of the preceding embodiments, wherein the nuclear protein payload agent (e.g., which is exogenous or overexpressed relative to the source cell) comprises or encodes one or more of:

i) a transcriptional activator, e.g., a transcriptional activator of Table 17-1;

ii) a transcriptional repressor, e.g., a transcriptional repressor of Table 17-1;

iii) an epigenetic modifier, e.g., an epigenetic modifier of Table 17-1;

iv) a histone acetyltransferase, e.g., a histone acetyltransferase of Table 17-1;

v) a histone deacetylase, e.g., a histone deacetylase of Table 17-1;

vi) a histone methyltransferase, e.g., a histone methyltransferase of Table 17-1;
vii) a DNA methyltransferase, e.g., a DNA methyltransferase of Table 17-1;
viii) a DNA nickase, e.g., a DNA nickase as described herein;
ix) a site-specific DNA editing enzyme, e.g., a deaminase, e.g., of Table 17-1;
x) a DNA transposase, e.g., a DNA transposase as described herein;
xi) a DNA integrase, e.g., a DNA integrase as described herein;
xii) an RNA editor, e.g., an RNA editor of Table 17-1;
xiii) an RNA splicing factor, e.g., an RNA splicing factor of Table 17-1; or
xiv) a PIWI protein, e.g., a PIWI protein as described herein;

143. The fusosome of any of the preceding embodiments, wherein the nuclear protein payload agent comprises or encodes a protein having a NLS which is the same as the NLS in a naturally-occurring counterpart of the protein.

144. The fusosome of any of the preceding embodiments, wherein the nuclear protein payload agent comprises or encodes a protein having a NLS which is not comprised by a naturally-occurring counterpart of the protein.

145. The fusosome of any of the preceding embodiments, wherein the nuclear protein payload agent comprises or encodes: a transcription factor, a nuclease, a recombinase, an epigenetic factor, a post-transcriptional RNA modification factor (e.g., an mRNA splicing factor), a non-coding RNA (e.g. a snRNA or snoRNA) or ribonucleic protein (e.g. a snRNP or snoRNP), a structural protein (e.g. a lamin or karysokeletal protein).

146. The fusosome of any of the preceding embodiments, wherein the nuclear protein payload agent comprises or encodes an antibody molecule, e.g., a Fab, an scFv, an scFab, a sdAb, a duobody, a minibody, a nanobody, a diabody, a zybody, a camelid antibody, a BiTE, a quadroma, or a bsDb.

147. The fusosome of any of the preceding embodiments, wherein the nuclear protein payload

agent comprises or encodes a fusion protein comprising an effector domain and a localization domain.

148. The fusosome of embodiment 147, wherein the localization domain comprises a TAL domain, a Zinc Finger domain, a Cas9 domain, or a meganuclease domain.

149. The fusosome of embodiment 147 or 148, wherein the effector domain comprises a nuclease, recombinase, integrase, base editor (deaminase), transcription factor, or epigenetic modifier.

150. The fusosome of any of the preceding embodiments, wherein the nuclear protein payload agent comprises or encodes a gene editing protein or nucleic acid, e.g., Cas9 or a guide RNA.

151. The fusosome of any of the preceding embodiments, wherein the nuclear payload agent comprises a functional RNA.

152. The fusosome of any of the preceding embodiments, wherein the nuclear payload agent comprises a nuclear protein payload agent, e.g., a protein or an mRNA encoding the protein.

153. The fusosome of any of the preceding embodiments, wherein the nuclear payload agent comprises (i) a nucleic acid, and (ii) a protein that promotes nuclear import of the nucleic acid.

154. The fusosome of embodiment 153, wherein the nucleic acid comprises a binding site, and the protein that promotes nuclear import binds to the binding site.

155. The fusosome of embodiment 154, wherein the binding site comprises a TetR binding site and the protein comprises TetR.

156. The fusosome of any of the preceding embodiments, wherein the nuclear payload agent comprises a synthetic transcription factor, e.g., a fusion protein comprising (i) a DNA binding

domain, e.g., a specific DNA binding domain, e.g., TAL domain, ZF domain, or Cas9 domain and (ii) a transcriptional activator domain or transcriptional repressor domain.

157. The fusosome of any of the preceding embodiments, wherein the nuclear payload agent comprises a synthetic epigenetic modifier, e.g., a fusion protein comprising (i) a DNA binding domain and (ii) an epigenetic modifier domain.

158. The fusosome of any of the preceding embodiments, wherein the nuclear protein payload agent is or comprises a protein selected from Table 17-1.

159. The fusosome of any of the preceding embodiments, wherein the nuclear protein payload agent is or comprises a fragment, variant, or homolog of a protein selected from Tables 17-1.

160. The fusosome of any of the preceding embodiments, wherein the nuclear protein payload agent is or comprises a nucleic acid encoding a protein which is or comprises a protein selected from Tables 17-1.

161. The fusosome of any of the preceding embodiments, wherein the nuclear protein payload agent is or comprises a nucleic acid encoding a protein comprising a fragment, variant, or homolog of a protein selected from Tables 17-1.

Organellar delivery

162. A fusosome comprising:

(a) a lipid bilayer comprising a plurality of lipids derived from a source cell;

(b) a lumen surrounded by the lipid bilayer;

(c) a fusogen that is exogenous or overexpressed relative to the source cell, e.g., wherein the fusogen is disposed in the lipid bilayer; and

(d) an organellar payload agent, e.g., a organellar protein payload agent (e.g., which is exogenous or overexpressed relative to the source cell), wherein, when a target cell is contacted with a plurality of the fusosomes, the organellar protein payload agent or polypeptide encoded

therein becomes enriched in mitochondria of the target cell at a level at least 10%, 20%, 50%, 100%, 2-fold, 5-fold, 10-fold, 20-fold, 50-fold, or 100-fold higher than the level of the organellar protein payload agent in the cytoplasm or the target cell plasma membrane.

163. A fusosome comprising:

(a) a lipid bilayer comprising a plurality of lipids derived from a source cell;

(b) a lumen surrounded by the lipid bilayer;

(c) a fusogen that is exogenous or overexpressed relative to the source cell, e.g., wherein the fusogen is disposed in the lipid bilayer; and

(d) an organellar protein payload agent that comprises or encodes a heterologous signal sequence to the organellar protein payload agent (e.g., a signal sequence of Table 6 or 6-1) that is sufficient to enrich the organellar protein payload agent, or polypeptide encoded therein, in a cytoplasmic organelle of the target cell, optionally wherein the heterologous signal sequence is set forth in any one of SEQ ID NOS: 39-127 and 605-626.

164. A fusosome comprising:

(a) a lipid bilayer comprising a plurality of lipids derived from a source cell;

(b) a lumen surrounded by the lipid bilayer, wherein the lumen does not comprise an organelle;

(c) a fusogen that is exogenous or overexpressed relative to the source cell, e.g., wherein the fusogen is disposed in the lipid bilayer; and

(d) an organellar protein payload agent (e.g., which is exogenous or overexpressed relative to the source cell), wherein, when a target cell is contacted with a plurality of the fusosomes, the organellar protein payload agent or polypeptide encoded therein becomes enriched in one or more of the Golgi apparatus, endoplasmic reticulum, vacuole, acrosome, autophagosome, centriole, glycosome, glyoxysome, hydrogenosome, melanosome, mitosome, cnidocyst, peroxisome, proteasome, vesicle, or stress granule of the target cell relative to the cyosol or plasma membrane of the target cell.

165. A fusosome comprising:

(a) a lipid bilayer comprising a plurality of lipids derived from a source cell;

(b) a lumen surrounded by the lipid bilayer, wherein the lumen does not comprise an organelle;

(c) a fusogen that is exogenous or overexpressed relative to the source cell, e.g., wherein the fusogen is disposed in the lipid bilayer; and

(d) an organellar protein payload agent that comprises a nucleic acid encoding a polypeptide, wherein, when a target cell is contacted with a plurality of the fusosomes, the encoded polypeptide becomes enriched in the lysosome and/or endosome, relative to the cytosol or plasma membrane of the target cell.

166. A fusosome comprising:

(a) a lipid bilayer comprising a plurality of lipids derived from a source cell;

(b) a lumen surrounded by the lipid bilayer;

(c) a fusogen that is exogenous or overexpressed relative to the source cell, e.g., wherein the fusogen is disposed in the lipid bilayer; and

(d) an exogenous or overexpressed organellar protein payload agent, wherein the organellar protein payload agent comprises or encodes a polypeptide chosen from a mitochondrial membrane protein, a outer mitochondrial membrane protein, an inner mitochondrial membrane protein, a mitochondrial inner boundary membrane protein, a mitochondrial cristal membrane protein, a mitochondrial DNA protein, a mitochondrial intermembrane space protein, a mitochondrial matrix protein, a mitochondrial matrix granule protein, a mitochondrial cristae protein, a mitochondrial ribosome protein, a mitochondrial intracristal protein, a mitochondrial peripheral space protein, a peroxisomal membrane protein, a peroxisomal crystalloid core protein, a peroxisomal matrix protein, a peroxin.

167. A fusosome comprising:

(a) a lipid bilayer comprising a plurality of lipids derived from a source cell;

(b) a lumen surrounded by the lipid bilayer;

(c) a fusogen that is exogenous or overexpressed relative to the source cell, e.g.,

wherein the fusogen is disposed in the lipid bilayer; and

(d) an exogenous or overexpressed organellar protein payload agent, wherein the organellar protein payload agent comprises or encodes a polypeptide chosen from:

i) a metabolic protein (e.g., an electron transport chain protein or ATP synthase)

ii) a protease;

iii) a chaperone;

iv) a protein transport protein;

v) a mitochondrial ribosome protein;

vi) a mitochondrial transcription protein; or

vii) a mitochondrial replication protein.

168. A fusosome comprising:

(a) a lipid bilayer comprising a plurality of lipids derived from a source cell;

(b) a lumen surrounded by the lipid bilayer;

(c) a fusogen that is exogenous or overexpressed relative to the source cell, e.g., wherein the fusogen is disposed in the lipid bilayer; and

(d) an organellar protein payload agent (e.g., which is exogenous or overexpressed relative to the source cell), that comprises or encodes a first domain that binds an endogenous protein present (at least partly) in the cytosol or nucleus of a target cell and a second domain that promotes import into a cytoplasmic organelle of the target cell.

169. A fusosome comprising:

(a) a lipid bilayer comprising a plurality of lipids derived from a source cell;

(b) a lumen surrounded by the lipid bilayer;

(c) a fusogen that is exogenous or overexpressed relative to the source cell, e.g., wherein the fusogen is disposed in the lipid bilayer; and

(d) an organellar protein payload agent comprising (i) a nucleic acid, and (ii) a protein that promotes import of the nucleic acid into a cytoplasmic organelle.

170. A fusosome comprising:

(a) a lipid bilayer comprising a plurality of lipids derived from a source cell;

(b) a lumen surrounded by the lipid bilayer;

(c) a fusogen that is exogenous or overexpressed relative to the source cell, e.g., wherein the fusogen is disposed in the lipid bilayer; and

(d) an organellar protein payload agent (e.g., which is exogenous or overexpressed relative to the source cell), wherein the organellar protein payload agent comprises a nucleic acid (e.g., DNA or RNA), e.g., a functional RNA or a nucleic acid encoding a protein.

171. A fusosome comprising:

(a) a lipid bilayer comprising a plurality of lipids derived from a source cell;

(b) a lumen (e.g., comprising cytosol) surrounded by the lipid bilayer;

(c) a fusogen that is exogenous or overexpressed relative to the source cell, e.g., wherein the fusogen is disposed in the lipid bilayer; and

(d) an organellar payload agent, e.g., a organellar protein payload agent (e.g., which is exogenous or overexpressed relative to the source cell), wherein:

ix) the fusosome comprises or is comprised by a cytobiologic;

x) the fusogen is present at a copy number of at least 1,000 copies, e.g., as measured by an assay of Example 29;

xi) the fusosome comprises a therapeutic agent at a copy number of at least 1,000 copies, e.g., as measured by an assay of Example 43;

xii) the fusosome comprises a lipid wherein one or more of CL, Cer, DAG, HexCer, LPA, LPC, LPE, LPG, LPI, LPS, PA, PC, PE, PG, PI, PS, CE, SM and TAG is within 75% of the corresponding lipid level in the source cell;

xiii) the fusosome comprises a proteomic composition similar to that of the source cell, e.g., using an assay of Example 42;

xiv) the fusosome is capable of signal transduction, e.g., transmitting an extracellular signal, e.g., AKT phosphorylation in response to insulin, or glucose (e.g., labeled glucose, e.g., 2-NBDG) uptake in response to insulin, e.g., by at least 10% more than a negative control, e.g., an otherwise similar fusosome in the absence of insulin, e.g., using an assay of Example 63;

xv) the fusosome targets a tissue, e.g., liver, lungs, heart, spleen, pancreas, gastrointestinal tract, kidney, testes, ovaries, brain, reproductive organs, central nervous system, peripheral nervous system, skeletal muscle, endothelium, inner ear, or eye, when administered to a subject, e.g., a mouse, e.g., wherein at least 0.1%, or 10%, of the fusosomes in a population of administered fusosomes are present in the target tissue after 24 hours, e.g., by an assay of Example 87 or 100; or

xvi) the source cell is selected from a neutrophil, a granulocyte, a mesenchymal stem cell, a bone marrow stem cell, an induced pluripotent stem cell, an embryonic stem cell, a myeloblast, a myoblast, a hepatocyte, or a neuron e.g., retinal neuronal cell.

172. A fusosome comprising:

(a) a lipid bilayer comprising a plurality of lipids derived from a source cell;

(b) a lumen (e.g., comprising cytosol) surrounded by the lipid bilayer;

(c) a fusogen that is exogenous or overexpressed relative to the source cell, e.g., wherein the fusogen is disposed in the lipid bilayer; and

(d) an organellar payload agent, e.g., a organellar protein payload agent (e.g., which is exogenous or overexpressed relative to the source cell), wherein:

i) the fusosome comprises or is comprised by a cytobiologic;

ii) the fusosome comprises an enucleated cell;

iii) the fusosome comprises an inactivated nucleus;

iv) the fusosome fuses at a higher rate with a target cell than with a non-target cell, e.g., by at least at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 20-fold, 50-fold, or 100-fold, e.g., in an assay of Example 54;

v) the fusosome fuses at a higher rate with a target cell than with other fusosomes, e.g., by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 20-fold, 50-fold, or 100-fold, e.g., in an assay of Example 54;

vi) the fusosome fuses with target cells at a rate such that an agent in the fusosome is delivered to at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, of target cells after 24, 48, or 72 hours, e.g., in an assay of Example 54;

vii) the fusogen is present at a copy number of at least, or no more than, 10, 50, 100, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000, 100,000,000, 500,000,000, or 1,000,000,000 copies, e.g., as measured by an assay of Example 29;

viii) the fusosome comprises a therapeutic agent at a copy number of at least, or no more than, 10, 50, 100, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000, 100,000,000, 500,000,000, or 1,000,000,000 copies, e.g., as measured by an assay of Example 43 or 156;

ix) the ratio of the copy number of the fusogen to the copy number of the therapeutic agent is between 1,000,000:1 and 100,000:1, 100,000:1 and 10,000:1, 10,000:1 and 1,000:1, 1,000:1 and 100:1, 100:1 and 50:1, 50:1 and 20:1, 20:1 and 10:1, 10:1 and 5:1, 5:1 and 2:1, 2:1 and 1:1, 1:1 and 1:2, 1:2 and 1:5, 1:5 and 1:10, 1:10 and 1:20, 1:20 and 1:50, 1:50 and 1:100, 1:100 and 1:1,000, 1:1,000 and 1:10,000, 1:10,000 and 1:100,000, or 1:100,000 and 1:1,000,000;

x) the fusosome comprises a lipid composition substantially similar to that of the source cell or wherein one or more of CL, Cer, DAG, HexCer, LPA, LPC, LPE, LPG, LPI, LPS, PA, PC, PE, PG, PI, PS, CE, SM and TAG is within 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75% of the corresponding lipid level in the source cell;

xi) the fusosome comprises a proteomic composition similar to that of the source cell, e.g., using an assay of Example 42 or 155;

xii) the fusosome comprises a ratio of lipids to proteins that is within 10%, 20%, 30%, 40%, or 50% of the corresponding ratio in the source cell, e.g., as measured using an assay of Example 49;

xiii) the fusosome comprises a ratio of proteins to nucleic acids (e.g., DNA) that is within 10%, 20%, 30%, 40%, or 50% of the corresponding ratio in the source cell, e.g., as measured using an assay of Example 50;

xiv) the fusosome comprises a ratio of lipids to nucleic acids (e.g., DNA) that is within 10%, 20%, 30%, 40%, or 50% of the corresponding ratio in the source cell, e.g., as measured using an assay of Example 51 or 159;

xv) the fusosome has a half-life in a subject, e.g., in a mouse, that is within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% of the half life of a reference cell, e.g., the source cell, e.g., by an assay of Example 75;

xvi) the fusosome transports glucose (e.g., labeled glucose, e.g., 2-NBDG) across a membrane, e.g., by at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% more

(e.g., about 11.6% more) than a negative control, e.g., an otherwise similar fusosome in the absence of glucose, e.g., as measured using an assay of Example 64;

xvii) the fusosome comprises esterase activity in the lumen that is within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of that of the esterase activity in a reference cell, e.g., the source cell or a mouse embryonic fibroblast, e.g., using an assay of Example 66;

xviii) the fusosome comprises a metabolic activity level that is within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the citrate synthase activity in a reference cell, e.g., the source cell, e.g., as described in Example 68;

xix) the fusosome comprises a respiration level (e.g., oxygen consumption rate) that is within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the respiration level in a reference cell, e.g., the source cell, e.g., as described in Example 69;

xx) the fusosome comprises an Annexin-V staining level of at most 18,000, 17,000, 16,000, 15,000, 14,000, 13,000, 12,000, 11,000, or 10,000 MFI, e.g., using an assay of Example 70, or wherein the fusosome comprises an Annexin-V staining level at least 5%, 10%, 20%, 30%, 40%, or 50% lower than the Annexin-V staining level of an otherwise similar fusosome treated with menadione in the assay of Example 70, or wherein the fusosome comprises an Annexin-V staining level at least 5%, 10%, 20%, 30%, 40%, or 50% lower than the Annexin-V staining level of a macrophage treated with menadione in the assay of Example 70,

xxi) the fusosome has a miRNA content level of at least at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or greater than that of the source cell, e.g., by an assay of Example 39;

xxii) the fusosome has a soluble : non-soluble protein ratio is within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or greater than that of the source cell, e.g., within 1%-2%, 2%-3%, 3%-4%, 4%-5%, 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, or 80%-90% of that of the source cell, e.g., by an assay of Example 47;

xxiii) the fusosome has an LPS level less than 5%, 1%, 0.5%, 0.01%, 0.005%, 0.0001%, 0.00001% or less of the LPS content of the source cell, e.g., as measured by mass spectrometry, e.g., in an assay of Example 48;

xxiv) the fusosome is capable of signal transduction, e.g., transmitting an extracellular signal, e.g., AKT phosphorylation in response to insulin, or glucose (e.g., labeled glucose, e.g., 2-NBDG) uptake in response to insulin, e.g., by at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%,

60%, 70%, 80%, 90%, 100% more than a negative control, e.g., an otherwise similar fusosome in the absence of insulin, e.g., using an assay of Example 63;

xxv) the fusosome targets a tissue, e.g., liver, lungs, heart, spleen, pancreas, gastrointestinal tract, kidney, testes, ovaries, brain, reproductive organs, central nervous system, peripheral nervous system, skeletal muscle, endothelium, inner ear, or eye, when administered to a subject, e.g., a mouse, e.g., wherein at least 0.1%, 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the fusosomes in a population of administered fusosomes are present in the target tissue after 24, 48, or 72 hours, e.g., by an assay of Example 87 or 100;

xxvi) the fusosome has juxtacrine-signaling level of at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% greater than the level of juxtacrine signaling induced by a reference cell, e.g., the source cell or a bone marrow stromal cell (BMSC), e.g., by an assay of Example 71;

xxvii) the fusosome has paracrine-signaling level of at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% greater than the level of paracrine signaling induced by a reference cell, e.g., the source cell or a macrophage, e.g., by an assay of Example 72;

xxviii) the fusosome polymerizes actin at a level within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% compared to the level of polymerized actin in a reference cell, e.g., the source cell or a C2C12 cell, e.g., by the assay of Example 73;

xxix) the fusosome has a membrane potential within about 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% of the membrane potential of a reference cell, e.g., the source cell or a C2C12 cell, e.g., by an assay of Example 74, or wherein the fusosome has a membrane potential of about -20 to -150mV, -20 to -50mV, -50 to -100mV, or -100 to -150mV;

xxx) the fusosome is capable of extravasation from blood vessels, e.g., at a rate at least 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% the rate of extravasation of the source cell or of a cell of the same type as the source cell, e.g., using an assay of Example 57, e.g., wherein the source cell is a neutrophil, lymphocyte, B cell, macrophage, or NK cell;

xxxi) the fusosome is capable of crossing a cell membrane, e.g., an endothelial cell membrane or the blood brain barrier;

xxxii) the fusosome is capable of secreting a protein, e.g., at a rate at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% greater than a reference cell, e.g., a mouse embryonic fibroblast, e.g., using an assay of Example 62;

xxxiii) the fusosome meets a pharmaceutical or good manufacturing practices (GMP) standard;

xxxiv) the fusosome was made according to good manufacturing practices (GMP);

xxxv) the fusosome has a pathogen level below a predetermined reference value, e.g., is substantially free of pathogens;

xxxvi) the fusosome has a contaminant level below a predetermined reference value, e.g., is substantially free of contaminants;

xxxvii) the fusosome has low immunogenicity, e.g., as described herein;

xxxviii) the source cell is selected from a neutrophil, a granulocyte, a mesenchymal stem cell, a bone marrow stem cell, an induced pluripotent stem cell, an embryonic stem cell, a myeloblast, a myoblast, a hepatocyte, or a neuron e.g., retinal neuronal cell; or

xxxix) the source cell is other than a 293 cell, HEK cell, human endothelial cell, or a human epithelial cell, monocyte, macrophage, dendritic cell, or stem cell.

173. The fusosome of any of the preceding embodiments, wherein the cytoplasmic organelle is chosen from a mitochondrion, Golgi apparatus, lysosome, endoplasmic reticulum, vacuole, endosome, acrosome, autophagosome, centriole, glycosome, glyoxysome, hydrogenosome, melanosome, mitosome, cnidocyst, peroxisome, proteasome, vesicle, or stress granule.

174. The fusosome of any of the preceding embodiments, wherein the cytoplasmic organelle is a membrane-bound organelle.

10a. The fusosome of any of the preceding embodiments, wherein the organellar payload agent becomes enriched in one or more of the mitochondrial matrix, mitochondrial intermembrane space, mitochondrial outer membrane, mitochondrial inner membrane, or mitochondrial christae.

175. The fusosome of any of the preceding embodiments, wherein the organellar protein payload agent or polypeptide encoded therein comprises a protein of Table 17-2.

176. The fusosome of any of the preceding embodiments, wherein, when a population of target cells is contacted with a plurality of the fusosomes, the organellar protein payload agent or a polypeptide encoded therein becomes enriched in the cytoplasmic organelle (e.g., present in the cytoplasmic organelle of the target cell at a level at least 50% higher than the level of the organellar protein payload agent in the cytosol of the target cell) of at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of cells in the population of target cells, e.g., as measured in an

assay of Example 123 or 124.

177. The fusosome of any of the preceding embodiments, wherein, when a population of target cells is contacted with a plurality of the fusosomes, the organellar protein payload agent or a polypeptide encoded therein becomes present in a sub-population (the "fused population") of the population of target cells, wherein the organellar payload agent or protein encoded therein becomes enriched in a cytoplasmic organelle (e.g., present in the cytoplasmic of the target cell at a level at least 50% higher than the level of the organellar protein payload agent or polypeptide encoded therein in the **cytosol** of the target cell), in at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of cells in the fused population, e.g., as measured in an assay of Example 123 or 124.

178. The fusosome of any of the preceding embodiments, wherein at least 1, 2, 5, 10, 20, 50, 100, 200, 500, or 1,000 copies of the organellar protein payload agent, or a polypeptide encoded therein, are present in the cytoplasmic organelle in a target cell contacted with a plurality of the fusosomes.

179. The fusosome of any of the preceding embodiments, wherein the organellar protein payload agent comprises or encodes one or more of a basic helix-loop helix motif, leucine zipper motif, helix-turn-helix motif, homeodomain motif, a winged helix motif, a winged helix-turn motif, a HMG-box domain, a Wor3 domain, an OB-fold domain, a zinc finger motif, a TAL effector, or a B3 domain.

180. The fusosome of any of the preceding embodiments, wherein the organellar protein payload agent comprises or encodes a protein complex comprising a homodimer, a heterodimer, a homotrimer, a heterotrimer, a homotetramer, or a heterotetramer.

181. The fusosome of any of the preceding embodiments, wherein the organellar protein payload agent comprises or encodes a protein having a signal sequence which is the same as the signal sequence in a naturally-occurring counterpart of the protein.

182. The fusosome of any of the preceding embodiments, wherein the organellar protein payload agent comprises or encodes a protein having a signal sequence which is not comprised by a naturally-occurring counterpart of the protein.

183. The fusosome of any of the preceding embodiments, wherein the organellar protein payload agent comprises or encodes an antibody molecule, e.g., a Fab, an scFV, an scFab, a sdAb, a duobody, a minibody, a nanobody, a diabody, a zybody, a camelid antibody, a BiTE, a quadroma, or a bsDb.

184. The fusosome of any of the preceding embodiments, wherein the organellar payload agent comprises a functional RNA.

185. The fusosome of any of the preceding embodiments, wherein the organellar payload agent comprises an organellar protein payload agent, e.g., a protein or an mRNA encoding the protein.

186. The fusosome of any of the preceding embodiments, wherein the organellar payload agent does not comprise or encode a reporter protein or a fluorescent protein, e.g., GFP.

187. The fusosome of any of the preceding embodiments, wherein the lumen does not comprise an organelle.

188. The fusosome of any of the preceding embodiments, wherein the organellar protein payload agent, or protein encoded therein, is not a cell membrane protein or secreted protein.

189. The fusosome of any of the preceding embodiments, wherein the amount of viral capsid protein in the fusosome composition is less than 1% of total protein.

190. A fusosome comprising a chondrisome and a fusogen.

191. The fusosome of any of the preceding embodiments, which has partial or complete nuclear inactivation (e.g. nuclear removal).

192. The fusosome of any of the preceding embodiments, wherein the source cell is a cell grown under adherent or suspension conditions.

193. The fusosome of any of the preceding embodiments, wherein the source cell is a primary cell, a cultured cell, an immortalized cell, or a cell line (e.g., myelobast cell line, e.g., C2C12).

194. The fusosome of any of the preceding embodiments, wherein the source cell is an endothelial cell, a fibroblast, a blood cell (e.g., a macrophage, a neutrophil, a granulocyte, a leukocyte), a stem cell (e.g., a mesenchymal stem cell, an umbilical cord stem cell, bone marrow stem cell, a hematopoietic stem cell, an induced pluripotent stem cell e.g., an induced pluripotent stem cell derived from a subject's cells), an embryonic stem cell (e.g., a stem cell from embryonic yolk sac, placenta, umbilical cord, fetal skin, adolescent skin, blood, bone marrow, adipose tissue, erythropoietic tissue, hematopoietic tissue), a myoblast, a parenchymal cell (e.g., hepatocyte), an alveolar cell, a neuron (e.g., a retinal neuronal cell) a precursor cell (e.g., a retinal precursor cell, a myeloblast, myeloid precursor cells, a thymocyte, a meiocyte, a megakaryoblast, a promegakaryoblast, a melanoblast, a lymphoblast, a bone marrow precursor cell, a normoblast, or an angioblast), a progenitor cell (e.g., a cardiac progenitor cell, a satellite cell, a radial gial cell, a bone marrow stromal cell, a pancreatic progenitor cell, an endothelial progenitor cell, a blast cell), or an immortalized cell (e.g., HeLa, HEK293, HFF-1, MRC-5, WI-38, IMR 90, IMR 91, PER.C6, HT-1080, or BJ cell).

195. The fusosome of any of the preceding embodiments, wherein the source cell is allogeneic, e.g., obtained from a different organism of the same species as the target cell.

196. The fusosome of any of the preceding embodiments, wherein the source cell is autologous, e.g., obtained from the same organism as the target cell.

197. The fusosome of any of the preceding embodiments, wherein the source cell is heterologous, e.g., obtained from an organism of a different species from the target cell.

198. The fusosome of any of the preceding embodiments, wherein the source cell is selected from a white blood cell or a stem cell.

199. The fusosome of any of the preceding embodiments, wherein the source cell is selected from a neutrophil, a lymphocyte (e.g., a T cell, a B cell, a natural killer cell), a macrophage, a granulocyte, a mesenchymal stem cell, a bone marrow stem cell, an induced pluripotent stem cell, an embryonic stem cell, or a myeloblast.

200. The fusosome of any of the preceding embodiments, wherein the source cell is other than a 293 cell, HEK cell, human endothelial cell, or a human epithelial cell, monocyte, macrophage, dendritic cell, or stem cell.

201. The fusosome of any of the preceding embodiments, wherein:

i) the source cell is not transformed or immortalized; or

ii) the source cell is transformed, or immortalized using a method other than adenovirus-mediated immortalization, e.g., immortalized by spontaneous mutation, or telomerase expression.

202. The fusosome of any of the preceding embodiments, wherein the fusosome is from a source cell having a modified genome, e.g., having reduced immunogenicity (e.g., by genome editing to remove MHC complexes).

203. The fusosome of any of the preceding embodiments, wherein the source cell is from a cell culture treated with an anti-inflammatory signal.

204. The fusosome of any of the preceding embodiments, wherein the source cell is substantially non-immunogenic, e.g., using an assay described herein.

205. The fusosome of any of the preceding embodiments, wherein the source cell is a recombinant cell.

206. The fusosome of any of the preceding embodiments, wherein the source cell comprises further comprises a second agent that is exogenous to the source cell, e.g., a therapeutic agent, e.g., a protein or a nucleic acid (e.g., an RNA, e.g., an mRNA or miRNA).

207. The fusosome of embodiment 206, wherein the second agent is present at least, or no more than, 10, 20, 50, 100, 200, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000 or 1,000,000 copies comprised by the fusosome, or is present at an average level of at least, or no more than, 10, 20, 50, 100, 200, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000 or 1,000,000 copies per fusosome.

208. The fusosome of any of the preceding embodiments, wherein the fusosome has an altered, e.g., increased or decreased level of one or more endogenous molecules as compared to the source cell, e.g., protein or nucleic acid, e.g., due to treatment of the source cell, e.g., mammalian source cell with a siRNA or gene editing enzyme.

209. The fusosome of embodiment 208, wherein the fusosome comprises at least, or no more than, 10, 20, 50, 100, 200, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000 or 1,000,000 copies of the endogenous molecule, or is present at an average level of at least, or no more than, 10, 20, 50, 100, 200, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000 or 1,000,000 copies of the endogenous molecule per fusosome.

210. The fusosome of embodiment 208 or embodiment 209, wherein the endogenous molecule (e.g., an RNA or protein) is present at a concentration of at least 1, 2, 3, 4, 5, 10, 20, 50, 100, 500, 10^3, 5.0 x 10^3, 10^4, 5.0 x 10^4, 10^5, 5.0 x 10^5, 10^6, 5.0 x 10^6, 1.0 x 10^7, 5.0 x 10^7, or 1.0 x 10^8 greater than its concentration in the source cell.

211. The fusosome of embodiment 208 or embodiment 209, wherein the endogenous molecule (e.g., an RNA or protein) is present at a concentration of at least 1, 2, 3, 4, 5, 10, 20, 50, 100, 500, 10^3, 5.0 x 10^3, 10^4, 5.0 x 10^4, 10^5, 5.0 x 10^5, 10^6, 5.0 x 10^6, 1.0 x 10^7, 5.0 x 10^7, or 1.0 x 10^8 less than its concentration in the source cell.

212. The fusosome of any of the preceding embodiments, wherein the fusosome has a miRNA content level of at least 1% than that of the source cell, e.g., by an assay of Example 39.

213. The fusosome of embodiment 206 or 207, wherein the second agent, e.g., therapeutic agent, is selected from a protein, protein complex (e.g., comprising at least 2, 3, 4, 5, 10, 20, or 50 proteins, e.g., at least at least 2, 3, 4, 5, 10, 20, or 50 different proteins) polypeptide, nucleic acid (e.g., DNA, chromosome, or RNA, e.g., mRNA, siRNA, or miRNA) or small molecule.

214. The fusosome of any of the preceding embodiments, wherein the fusosome comprises a proteomic composition similar to that of the source cell, e.g., using an assay of Example 42.

215. The fusosome of any of the preceding embodiments, wherein the fusosome has a soluble : non-soluble protein ratio within 90% of that of the source cell, e.g., by an assay of Example 47.

216. The fusosome of any of the preceding embodiments, which has a diameter that is less than about 0.01%-0.05%, 0.05%-0.1%, 0.1%-0.5%, 0.5%- 1%, 1%-2%, 2%-3%, 3%-4%, 4%-5%, 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, or 80%-90% the size of the source cell, e.g., as measured by an assay of Example 30.

217. The fusosome of any of the preceding embodiments, wherein the fusosome has a diameter that is less than about 0.01% or 1%, of that of the source cell, e.g., as measured by an assay of Example 30.

218. The fusosome of any of the preceding embodiments, which has a volume that is less than about 0.01%-0.05%, 0.05%-0.1%, 0.1%-0.5%, 0.5%- 1%, 1%-2%, 2%-3%, 3%-4%, 4%-5%, 5%-

10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, or 80%-90%% of the volume of the source cell.

219. The fusosome of any of the preceding embodiments, wherein the fusogen is a protein fusogen.

220. The fusosome of any of the preceding embodiments, wherein the fusogen is a mammalian fusogen or a viral fusogen.

221. The fusosome of any of the preceding embodiments, wherein the fusogen does not promote vesicle formation from a source cell.

222. The fusosome of any of the preceding embodiments, wherein the fusogen comprises an enucleated cell.

223. The fusosome of any of the preceding embodiments, wherein the fusogen:

i) is other than a viral protein;

ii) the fusogen is other than a fusogenic glycoprotein;

iii) the fusogen is a mammalian protein other than fertilin-beta;

iv) the fusogen is other than VSVG, a SNARE protein, or a secretory granule protein; or

v) the fusogen is other than TAT, TAT-HA2, HA-2, gp41, Alzheimer's beta-amyloid peptide, a Sendai virus protein, or amphipathic net-negative peptide (WAE 11).

224. The fusosome of any of the preceding embodiments, wherein:

i) the fusogen is present at a copy number of at least 1,000 copies, e.g., as measured by an assay of Example 29; or

ii) the ratio of the copy number of the fusogen to the copy number of the payload (e.g., membrane payload agent, nuclear payload agent, or organellar payload agent) is between 1,000,000:1 and 100,000:1, 100,000:1 and 10,000:1, 10,000:1 and

1,000:1, 1,000:1 and 100:1, 100:1 and 50:1, 50:1 and 20:1, 20:1 and 10:1, 10:1 and 5:1, 5:1 and 2:1, 2:1 and 1:1, 1:1 and 1:2, 1:2 and 1:5, 1:5 and 1:10, 1:10 and 1:20, 1:20 and 1:50, 1:50 and 1:100, 1:100 and 1:1,000, 1:1,000 and 1:10,000, 1:10,000 and 1:100,000, or 1:100,000 and 1:1,000,000.

225. The fusosome of any of the preceding embodiments, wherein:

i) the fusosome was not made by loading the fusosome with a therapeutic or diagnostic substance;

ii) the source cell was not loaded with a therapeutic or diagnostic substance;

iii) the fusosome does not comprise doxorubicin, dexamethasone, cyclodextrin; polyethylene glycol, a micro RNA e.g., miR125, VEGF receptor, ICAM-1, E-selectin, iron oxide, a fluorescent protein e.g., GFP or RFP, a nanoparticle, or an RNase, or does not comprise a form of any of the foregoing that is exogenous to the source cell; or

iv) the fusosome further comprises a therapeutic agent that is exogenous to the source cell and comprises one or more post-translational modifications, e.g., glycosylation.

226. The fusosome of any of the preceding embodiments, wherein the fusogen is a viral fusogen, e.g., HA, HIV-1 ENV, gp120, or VSV-G, or wherein the fusogen is a mammalian fusogen, e.g., a SNARE, a Syncytin, myomaker, myomixer, myomerger, or FGFRL1.

227. The fusosome or fusosome of any of the preceding embodiments, wherein the fusogen is active at a pH of 4-5, 5-6, 6-7, 7-8, 8-9, or 9-10.

228. The fusosome of any of the preceding embodiments, wherein the fusogen is not active at a pH of 4-5, 5-6, 6-7, 7-8, 8-9, or 9-10.

229. The fusosome of any of the preceding embodiments wherein the fusogen is a lipid fusogen, e.g., oleic acid, glycerol mono-oleate, a glyceride, diacylglycerol, or a modified unsaturated fatty

acid.

230. The fusosome of any of the preceding embodiments wherein the fusogen is a chemical fusogen, e.g., PEG.

231. The fusosome of any of the preceding embodiments wherein the fusogen is a small molecule fusogen, e.g., halothane, an NSAID such as meloxicam, piroxicam, tenoxicam, and chlorpromazine.

232. The fusosome of any of the preceding embodiments, wherein the fusogen is recombinant.

233. The fusosome of any of the preceding embodiments, wherein the fusogen is biochemically incorporated, e.g., the fusogen is provided as a purified protein and contacted with a fusosome lipid bilayer under conditions that allow for association of the fusogen with the lipid bilayer, or biosynthetically incorporated, e.g., expressed in a source cell under conditions that allow the fusogen to associate with the fusosome lipid bilayer.

234. The fusosome of any of the preceding embodiments, wherein the fusosome has one or more of the following properties:

i) the fusosome comprises or is comprised by a cytobiologic;

ii) the fusosome comprises or is comprised by an enucleated cell; or

iii) the fusosome comprises an inactivated nucleus.

234A. The fusosome of any of the preceding embodiments, wherein the fusosome comprises a payload (e.g., a membrane payload agent, nuclear payload agent, or organellar payload agent) at a copy number of at least 1,000 copies, e.g., as measured by an assay of Example 43.

235. The fusosome of any of the preceding embodiments, wherein the fusosome has one or more of the following properties:

i) the fusosome comprises a lipid wherein one or more of CL, Cer, DAG, HexCer,

LPA, LPC, LPE, LPG, LPI, LPS, PA, PC, PE, PG, PI, PS, CE, SM and TAG is within 75% of the corresponding lipid level in the source cell;

ii) the fusosome comprises a ratio of lipids to proteins that is within 10%, 20%, 30%, 40%, or 50% of the corresponding ratio in the source cell, e.g., as measured using an assay of Example 49;

iii) the fusosome comprises a ratio of proteins to nucleic acids (e.g., DNA) that is within 10%, 20%, 30%, 40%, or 50% of the corresponding ratio in the source cell, e.g., as measured using an assay of Example 50; or

iv) the fusosome comprises a ratio of lipids to nucleic acids (e.g., DNA) that is within 10%, 20%, 30%, 40%, or 50% of the corresponding ratio in the source cell, e.g., as measured using an assay of Example 51.

236. The fusosome of any of the preceding embodiments, wherein one or more of:

i) the fusosome fuses at a higher rate with a target cell than with a non-target cell, e.g., by at least at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% (e.g., 10%), e.g., in an assay of Example 54;

ii) the fusosome fuses at a higher rate with a target cell than with other fusosomes, e.g., by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% (e.g., 50%), e.g., in an assay of Example 54;

iii) the fusosome fuses with target cells at a rate such that an agent in the fusosome is delivered to at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% (e.g., 10%) of target cells after 24, 48, or 72 hours, e.g., in an assay of Example 54;

vi) the fusosome transports glucose (e.g., labeled glucose, e.g., 2-NBDG) across a membrane, e.g., by at least 10% more than a negative control, e.g., an otherwise similar fusosome in the absence of glucose, e.g., as measured using an assay of Example 64;

v) the fusosome comprises esterase activity in the lumen that is within 90% of that of the esterase activity in a reference cell, e.g., the source cell or a mouse embryonic fibroblast, e.g., using an assay of Example 66;

vi) the fusosome comprises a metabolic activity level that is within 90% of the

metabolic activity (e.g., citrate synthase activity) in a reference cell, e.g., the source cell, e.g., as described in Example 68;

vii) the fusosome comprises a respiration level (e.g., oxygen consumption rate) that is within 90% of the respiration level in a reference cell, e.g., the source cell, e.g., as described in Example 69;

viii) the fusosome comprises an Annexin-V staining level of at most 18,000, 17,000, 16,000, 15,000, 14,000, 13,000, 12,000, 11,000, or 10,000 MFI, e.g., using an assay of Example 70, or wherein the fusosome comprises an Annexin-V staining level at least 5%, 10%, 20%, 30%, 40%, or 50% lower than the Annexin-V staining level of an otherwise similar fusosome treated with menadione in the assay of Example 70, or wherein the fusosome comprises an Annexin-V staining level at least 5%, 10%, 20%, 30%, 40%, or 50% lower than the Annexin-V staining level of a macrophage treated with menadione in the assay of Example 70;

ix) the fusosome has an LPS level less than 5% of the lipid content of fusosomes, e.g., as measured by an assay of Example 48;

x) the fusosome has juxtacrine-signaling level of at least 5% greater than the level of juxtacrine signaling induced by a reference cell, e.g., the source cell or a bone marrow stromal cell (BMSC), e.g., by an assay of Example 71;

xi) the fusosome has paracrine-signaling level of at least 5% greater than the level of paracrine signaling induced by a reference cell, e.g., the source cell or a macrophage, e.g., by an assay of Example 72;

xii) the fusosome polymerizes actin at a level within 5% compared to the level of polymerized actin in a reference cell, e.g., the source cell or a C2C12 cell, e.g., by the assay of Example 73; or

xiii) the fusosome and/or compositions or preparations thereof, are capable of secreting a protein, e.g., at a rate at least 5% greater than a reference cell, e.g., a mouse embryonic fibroblast, e.g., using an assay of Example 62.

237. The fusosome of any of the preceding embodiments, wherein one or more of:

i) the fusosome has a membrane potential within about 5% of the membrane potential

of a reference cell, e.g., the source cell or a C2C12 cell, e.g., by an assay of Example 74, or wherein the fusosome has a membrane potential of about -20 to -150mV, -20 to -50mV, -50 to -100mV, or -100 to -150mV;

ii) the fusosome and/or compositions or preparations thereof, are capable of extravasation from blood vessels, e.g., at a rate at least 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% the rate of extravasation of a cell of the same type as the source cell, e.g., using an assay of Example 57, e.g., wherein the source cell is a neutrophil, lymphocyte, B cell, macrophage, or NK cell;

iii) the fusosome and/or compositions or preparations thereof, are capable of crossing a cell membrane, e.g., an endothelial cell membrane or the blood brain barrier, e.g., at a rate at least 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% that of a cell of the same type as the source cell;

iv) the fusosome meets a pharmaceutical or good manufacturing practices (GMP) standard;

v) the fusosome was made according to good manufacturing practices (GMP);

vi) the fusosome has a pathogen level below a predetermined reference value, e.g., is substantially free of pathogens;

vii) the fusosome has a contaminant level below a predetermined reference value, e.g., is substantially free of contaminants; or

viii) the fusosome has low immunogenicity, e.g., as described herein.

238. The fusosome of any of the preceding embodiments, wherein the fusosome is or comprises a chondrisome.

239. The fusosome of any of the preceding embodiments, wherein the fusosome has a half-life in a subject, e.g., in a mouse, that is within 90% of the half-life of a reference cell, e.g., the source cell, e.g., by an assay of Example 75.

240. The fusosome preparation of any of the preceding embodiments, wherein:

i) the fusosome and/or compositions or preparations thereof has a density of other than between 1.08 g/mL and 1.12 g/mL;

ii) the fusosome and/or compositions or preparations thereof has a density of >1.12 g/mL, e.g., 1.25 g/mL +/- 0.1, 1.25 g/mL +/- 0.05, e.g., as measured by an assay of Example 33; or

iii) the fusosome and/or compositions or preparations thereof has a density of <1, 1-1.1, 1.05-1.15, 1.1-1.2, 1.15-1.25, 1.2-1.3, 1.25-1.35, or >1.35 g/mL, e.g., by an assay of Example 33.

241. The fusosome of any of the preceding embodiments, wherein one or more of:

i) the fusosome is not captured by the scavenger system in circulation or by Kupffer cells in the sinus of the liver;

ii) the fusosome is not captured by the reticulo-endothelial system (RES) in a subject, e.g., by an assay of Example 76; or

iii) when a plurality of fusosomes are administered to a subject, less than 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, of the plurality are not captured by the RES after 24 hours, e.g., by an assay of Example 76.

242. The fusosome of any of the preceding embodiments, wherein:

i) the fusosome meets a pharmaceutical or good manufacturing practices (GMP) standard;

ii) the fusosome was made according to good manufacturing practices (GMP);

iii) the fusosome has a pathogen level below a predetermined reference value, e.g., is substantially free of pathogens; or

iv) the fusosome has a contaminant level below a predetermined reference value, e.g., is substantially free of contaminants.

243. The fusosome of any of the preceding embodiments, wherein:

i) the fusosome has a diameter of greater than 5 μm, 6 μm, 7 μm, 8 μm, 10 μm, 20 μm, 50 μm, 100 μm, 150 μm, or 200 μm.

ii) the fusosome has a diameter of other than between 40 and 150 nm, e.g., greater than 150 nm, 200 nm, 300 nm, 400 nm, or 500 nm;

iii) the fusosome has a diameter of at least 80 nm, 100 nm, 200 nm, 500 nm, 1000 nm, 1200 nm, 1400 nm, or 1500 nm;

iv) the fusosome has a diameter of less than 80 nm, 100 nm, 200 nm, 500 nm, 1000 nm, 1200 nm, 1400 nm, or 1500 nm; or

v) the fusosome has a diameter of at least about 10 nm, 20 nm, 30 nm, 40 nm, 50 nm, 60 nm, 70 nm, 80 nm, 90 nm, 100 nm, 150 nm, or 200 nm, e.g., as measured by an assay of Example 32.

244. The fusosome of any of the preceding embodiments, wherein the fusosome further comprises in its lumen a polypeptide selected from an enzyme, antibody, or anti-viral polypeptide.

245. The fusosome of any of the preceding embodiments, wherein the fusosome does not comprise CD63 or GLUT4.

246. The fusosome of any of the preceding embodiments, wherein the fusosome:

i) does not comprise a virus, is not infectious, or does not propagate in a host cell;

ii) is not a VLP (virus like particle);

iii) does not comprise a viral structural protein, e.g., a viral capsid protein, e.g., a viral nucleocapsid protein, or wherein the amount of viral capsid protein is less than 10%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.2%, or 0.1% of total protein, e.g., by an assay of Example 53;

iv) does not comprise a viral matrix protein;

v) does not comprise a viral non-structural protein;

vi) comprises less than 10, 50, 100, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000, 100,000,000, 500,000,000, 1,000,000,000 copies of a viral structural protein; or

vii) is not a virosome.

247. The fusosome of any of the preceding embodiments, wherein the fusosome comprises a viral structural protein and/or a viral matrix protein.

248. The fusosome of any of the preceding embodiments, wherein:

i) the ratio of the copy number of the fusogen to the copy number of viral structural protein on the fusosome is at least 1,000,000:1, 100,000:1, 10,000:1, 1,000:1, 100:1, 50:1 1, 20:1, 10:1, 5:1, or 1:1;

ii) the ratio of the copy number of the fusogen to the copy number of viral matrix protein on the fusosome is at least 1,000,000:1, 100,000:1, 10,000:1, 1,000:1, 100:1, 50:1, 20:1, 10:1, 5:1, or 1:1.

249. The fusosome of any of the preceding embodiments, wherein the fusosome is unilamellar or multilamellar.

250. The fusosome of any of the preceding embodiments, wherein:

i) the fusosome does not comprise a water-immiscible droplet;

ii) the fusosome comprises an aqueous lumen and a hydrophilic exterior.

251. The fusosome of any of the preceding embodiments, wherein the fusosome is substantially free of one or more of the following organelles: a mitochondrion, Golgi apparatus, lysosome, endoplasmic reticulum, vacuole, endosome, acrosome, autophagosome, centriole, glycosome, glyoxysome, hydrogenosome, melanosome, mitosome, cnidocyst, peroxisome, proteasome, vesicle, and stress granule.

252. The fusosome of any of the preceding embodiments, wherein the fusosome has a lower number of an organelle as compared to the source cell, where the organelle is selected from: a mitochondrion, Golgi apparatus, lysosome, endoplasmic reticulum, vacuole, endosome, acrosome, autophagosome, centriole, glycosome, glyoxysome, hydrogenosome, melanosome, mitosome, cnidocyst, peroxisome, proteasome, vesicle, and stress granule.

253. The fusosome of any of the preceding embodiments, wherein:

i) the fusosome is not an exosome;

ii) the fusosome is a microvesicle;

iii) the fusosome comprises a non-mammalian fusogen;

iv) the fusosome has been engineered to incorporate a fusogen;

v) the fusosome comprises a fusogen that is exogenous to the source cell;

vi) the fusosome comprises one or more organelles, e.g., a mitochondrion, Golgi apparatus, lysosome, endoplasmic reticulum, vacuole, endosome, acrosome, autophagosome, centriole, glycosome, glyoxysome, hydrogenosome, melanosome, mitosome, cnidocyst, peroxisome, proteasome, vesicle, stress granule, or a combination thereof;

vii) the fusosome comprises a cytoskeleton or a component thereof, e.g., actin, Arp2/3, formin, coronin, dystrophin, keratin, myosin, or tubulin;

viii) the fusosome and/or compositions or preparations thereof does not have a flotation density of 1.08–1.22 g/mL, or has a density of at least 1.18–1.25 g/mL, or 1.05–1.12 g/mL, e.g., in a sucrose gradient centrifugation assay, e.g., as described in Théry et al., "Isolation and characterization of exosomes from cell culture supernatants and biological fluids." Curr Protoc Cell Biol. 2006 Apr; Chapter 3:Unit 3.22;

ix) the fusosome lipid bilayer is enriched for ceramides or sphingomyelins or a combination thereof compared to the source cell, or the fusosome lipid bilayer is not enriched (e.g., is depleted) for glycolipids, free fatty acids, or phosphatidylserine, or a combination thereof, compared to the source cell;

x) the fusosome comprises Phosphatidyl serine (PS) or CD40 ligand or both of PS and CD40 ligand, e.g., when measured in an assay of Example 52;

xi) the fusosome is enriched for PS compared to the source cell, e.g., in a plurality of fusosomes at least 5%, 10% 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% are positive for PS;

xii) the fusosome is substantially free of acetylcholinesterase (AChE), or contains less than 0.001, 0.002, 0.005, 0.01,0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100,

200, 500, or 1000 AChE activity units/μg of protein, e.g., by an assay of Example 67;

xiii) the fusosome is substantially free of a Tetraspanin family protein (e.g., CD63, CD9, or CD81), an ESCRT-related protein (e.g., TSG101, CHMP4A-B, or VPS4B), Alix, TSG101, MHCI, MHCII, GP96, actinin-4, mitofilin, syntenin-1, TSG101, ADAM10, EHD4, syntenin-1, TSG101, EHD1, flotillin-1, heat-shock 70-kDa proteins (HSC70/HSP73, HSP70/HSP72), or any combination thereof, or contains less than 0.05%, 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 5%, or 10% of any individual exosomal marker protein and/or less than 0.05%, 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, or 25% of total exosomal marker proteins of any of said proteins, or is de-enriched for any one or more of these proteins compared to the source cell, or is not enriched for any one or more of these proteins, e.g., by an assay of Example 44;

xiv) the fusosome comprises a level of Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) that is below 500, 250, 100, 50, 20, 10, 5, or 1 ng GAPDH/μg total protein or below the level of GAPDH in the source cell, e.g., less than 1%, 2.5%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, less than the level of GAPDH per total protein in ng/μg in the source cell, e.g., using an assay of Example 45;

xv) the fusosome is enriched for one or more endoplasmic reticulum proteins (e.g., calnexin), one or more proteasome proteins, or one or more mitochondrial proteins, or any combination thereof, e.g., wherein the amount of calnexin is greater than 500, 250, 100, 50, 20, 10, 5, or 1 ng Calnexin / μg total protein, or wherein the fusosome comprises more Calnexin per total protein in ng/μg compared to the source cell by 1%, 2.5%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, e.g., using an assay of Example 46;

xvi) the fusosome comprises an agent that is exogenous to the source cell (e.g., protein, mRNA, or siRNA) e.g., as measured using an assay of Example 39 or 40; or

xvii) the fusosome can be immobilized on a mica surface by atomic force microscopy for at least 30 min.

254. The fusosome of any of the preceding embodiments, wherein:

i) the fusosome is an exosome;

ii) the fusosome is not a microvesicle;

iii) the fusosome does not comprise an organelle;

iv) the fusosome does not comprise a cytoskeleton or a component thereof, e.g., actin, Arp2/3, formin, coronin, dystrophin, keratin, myosin, or tubulin ;

v) the fusosome and/or compositions or preparations thereof has flotation density of 1.08–1.22 g/mL, e.g., in a sucrose gradient centrifugation assay;

vi) the fusosome lipid bilayer is not enriched (e.g., is depleted) for ceramides or sphingomyelins or a combination thereof compared to the source cell, or the fusosome lipid bilayer is enriched for glycolipids, free fatty acids, or phosphatidylserine, or a combination thereof, compared to the source cell;

vii) the fusosome does not comprise, or is depleted for relative to the source cell, Phosphatidyl serine (PS) or CD40 ligand or both of PS and CD40 ligand, e.g., when measured in an assay of Example 52;

viii) the fusosome is not enriched (e.g., is depleted) for PS compared to the source cell, e.g., in a plurality of fusosomes less than 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% are positive for PS;

ix) the fusosome comprises acetylcholinesterase (AChE), e.g. at least 0.001, 0.002, 0.005, 0.01,0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, 200, 500, or 1000 AChE activity units/μg of protein , e.g., by an assay of Example 67;

x) the fusosome comprises a Tetraspanin family protein (e.g., CD63, CD9, or CD81), an ESCRT-related protein (e.g., TSG101, CHMP4A-B, or VPS4B), Alix, TSG101, MHCI, MHCII, GP96, actinin-4, mitofilin, syntenin-1, TSG101, ADAM10, EHD4, syntenin-1, TSG101, EHD1, flotillin-1, heat-shock 70-kDa proteins (HSC70/HSP73, HSP70/HSP72), or any combination thereof, e.g., contains more than 0.05%, 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 5%, or 10% of any

individual exosomal marker protein and/or less than 0.05%, 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, or 25% of total exosomal marker proteins of any of said proteins, or is enriched for any one or more of these proteins compared to the source cell, e.g., by an assay of Example 44;

xi) the fusosome comprises a level of Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) that is above 500, 250, 100, 50, 20, 10, 5, or 1 ng GAPDH/μg total protein or below the level of GAPDH in the source cell, e.g., at least 1%, 2.5%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, greater than the level of GAPDH per total protein in ng/μg in the source cell, e.g., using an assay of Example 45;

xii) the fusosome is not enriched for (e.g., is depleted for) one or more endoplasmic reticulum proteins (e.g., calnexin), one or more proteasome proteins, or one or more mitochondrial proteins, or any combination thereof, e.g., wherein the amount of calnexin is less than 500, 250, 100, 50, 20, 10, 5, or 1 ng Calnexin / μg total protein, or wherein the fusosome comprises less Calnexin per total protein in ng/μg compared to the source cell by 1%, 2.5%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, e.g., using an assay of Example 46; or

xiii) the fusosome cannot be immobilized on a mica surface by atomic force microscopy for at least 30 min.

255. The fusosome of any of the preceding embodiments, wherein:

i) the fusosome does not comprise a VLP;

ii) the fusosome does not comprise a virus;

iii) the fusosome does not comprise a replication-competent virus;

iv) the fusosome does not comprise a viral protein, e.g., a viral structural protein, e.g., a capsid protein or a viral matrix protein;

v) the fusosome does not comprise a capsid protein from an enveloped virus;

vi) the fusosome does not comprise a nucleocapsid protein; or

vii) the fusogen is not a viral fusogen.

256. The fusosome of any of the preceding embodiments, wherein the fusosome comprises cytosol.

257. The fusosome of any of the preceding embodiments, wherein:

i) the fusosome does not form a teratoma when implanted into subject, e.g., by an assay of Example 102;

ii) the fusosome and/or compositions or preparations thereof, are capable of chemotaxis, e.g., at a speed at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% compared to a reference cell, e.g., a macrophage, e.g., using an assay of Example 58;

iii) the fusosome and/or compositions or preparations thereof, are capable of homing, e.g., at the site of an injury, wherein the fusosome is from a human cell, e.g., using an assay of Example 59, e.g., wherein the source cell is a neutrophil; or

iv) the fusosome and/or compositions or preparations thereof, are capable of phagocytosis, e.g., wherein phagocytosis by the fusosome is detectable within .5, 1, 2, 3, 4, 5, or 6 hours in using an assay of Example 60, e.g., wherein the source cell is a macrophage.

258. The fusosome of any of the preceding embodiments, wherein the fusosome has one or more of the following characteristics:

a) comprises one or more endogenous proteins from a source cell, e.g., membrane proteins or cytosolic proteins;

b) comprises at least 10, 20, 50, 100, 200, 500, 1000, 2000, or 5000 different proteins;

c) comprises at least 1, 2, 5, 10, 20, 50, or 100 different glycoproteins;

d) at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% by mass of the proteins in the fusosome are naturally-occurring proteins;

e) comprises at least 10, 20, 50, 100, 200, 500, 1000, 2000, or 5000 different RNAs; or

f) comprises at least 2, 3, 4, 5, 10, or 20 different lipids, e.g., selected from CL, Cer, DAG, HexCer, LPA, LPC, LPE, LPG, LPI, LPS, PA, PC, PE, PG, PI, PS, CE, SM and TAG.

259. The fusosome of any of the preceding embodiments, wherein the fusosome has been manipulated to have, or wherein the fusosome is not a naturally occurring cell and has, or wherein the nucleus is not naturally one, two, three, four, five or more of the following properties:

i) the partial nuclear inactivation results in a reduction of at least 50%, 60%, 70%, 80%, 90% or more in nuclear function, e.g., a reduction in transcription or DNA replication, or both, e.g., wherein transcription is measured by an assay of Example 19 and DNA replication is measured by an assay of Example 20;

ii) the fusosome is not capable of transcription or has transcriptional activity of less than 1%, 2.5%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of that of the transcriptional activity of a reference cell, e.g., the source cell, e.g., using an assay of Example 19;

iii) the fusosome is not capable of nuclear DNA replication or has nuclear DNA replication of less than 1%, 2.5% 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the nuclear DNA replication of a reference cell, e.g., the source cell, e.g., using an assay of Example 20;

iv) the fusosome lacks chromatin or has a chromatin content of less than 1%, 2.5% 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the of the chromatin content of a reference cell, e.g., the source cell, e.g., using an assay of Example 37;

v) the fusosome lacks a nuclear membrane or has less than 50%, 40%, 30%, 20%, 10%, 5%, 4%, 3%, 2%, or 1% the amount of nuclear membrane of a reference cell, e.g., the source cell or a Jurkat cell, e.g., by an assay of Example 36;

vi) the fusosome lacks functional nuclear pore complexes or has reduced nuclear import or export activity, e.g., by at least 50%, 40%, 30%, 20%, 10%, 5%, 4%, 3%, 2%, or 1% by an assay of Example 36, or the fusosome lacks on or more of a nuclear pore protein, e.g., NUP98 or Importin 7;

vii) the fusosome does not comprise histones or has histone levels less than 1%, of the histone level of the source cell (e.g., of H1, H2a, H2b, H3, or H4), e.g., by an assay of Example 37;

viii) the fusosome comprises less than 20, 10, 5, 4, 3, 2, or 1 chromosome;

ix) nuclear function is eliminated;

x) the fusosome is an enucleated mammalian cell;

xi) the nucleus is removed or inactivated, e.g., extruded by mechanical force, by radiation or by chemical ablation; or

xii) the fusosome is from a mammalian cell having DNA that is completely or partially removed, e.g., during interphase or mitosis.

260. The fusosome of any of the preceding embodiments, wherein the fusosome comprises mtDNA or vector DNA.

261. The fusosome of any of the preceding embodiments, which does not comprise DNA or is substantially free of DNA.

262. The fusosome of any of the preceding embodiments, wherein the fusosome does not comprise a functional nucleus.

263. The fusosome of any of the preceding embodiments, wherein the fusosome does not comprise a nucleus.

264. The fusosome of any of the preceding embodiments, wherein the fusosome is substantially free of nuclear DNA.

265. The fusosome of any of the preceding embodiments, wherein:

ii) the fusosome does not comprise Cre or GFP, e.g., EGFP; or

iii) the fusosome further comprises a protein that is exogenous to the source cell and is other than Cre or GFP, e.g., EGFP.

266. The fusosome of any of the preceding embodiments, wherein the fusosome further comprises a nucleic acid (e.g., a DNA, a gDNA, a cDNA, an RNA, a pre-mRNA, an mRNA, an miRNA, or an siRNA) or protein (e.g., an antibody), wherein the nucleic acid or protein is exogenous to the source cell, e.g., in the lumen.

267. The fusosome of any of the preceding embodiments, wherein the fusosome does not comprise mitochondria or is substantially free of mitochondria.

268. The fusosome of any of the preceding embodiments, which retains one, two, three, four, five six or more of any of the characteristics for 5 days or less, e.g., 4 days or less, 3 days or less, 2 days or less, 1 day or less, e.g., about 12-72 hours, after administration into a subject, e.g., a human subject.

269. The fusosome of any of the preceding embodiments, which associates with and/or binds a target cell or a surface feature of a target cell.

270. The fusosome of any of the preceding embodiments, which fuses to the target cell at the surface of the target cell.

271. The fusosome of any of the preceding embodiments, which promotes fusion to a target cell in a lysosome-independent manner.

272. The fusosome of any of the preceding embodiments, wherein the fusosome and/or fusosome contents enters the target cell by endocytosis or via a non-endocytic pathway.

273. The fusosome of any of the preceding embodiments, wherein at least a portion of the fusosome lipid bilayer becomes incorporated in the target cell membrane.

274. The fusosome of embodiment 273, wherein the fusosome enters the target cell by

endocytosis, e.g., wherein the level of payload (e.g., membrane payload agent, nuclear payload agent, or organellar payload agent) delivered via an endocytic pathway for a given fusosome is 0.01-0.6, 0.01-0.1, 0.1-0.3, or 0.3-0.6, or at least at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater than a chloroquine treated reference cell, e.g., using an assay of Example 91.

275. The fusosome of embodiment 273, wherein the fusosome enters the target cell by a non-endocytic pathway, e.g., wherein the level of payload (e.g., membrane payload agent, nuclear payload agent, or organellar payload agent) delivered via a non-endocytic pathway for a given fusosome is 0.1-0.95, 0.1-0.2, 0.2-0.3, 0.3-0.4, 0.4-0.5, 0.5-0.6, 0.6-0.7, 0.7-0.8, 0.8-0.9, 0.9-0.95, or at least at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater than a chloroquine treated reference cell, e.g., using an assay of Example 90.

276. The fusosome of any of the preceding embodiments, wherein the target cell is in an organism.

277. The fusosome of any of the preceding embodiments, wherein the target cell is a primary cell isolated from an organism.

278. The fusosome of any of the preceding embodiments, wherein the target cell is selected from an endothelial cell, a fibroblast, a blood cell (e.g., a macrophage, a neutrophil, a granulocyte, a leukocyte), a stem cell (e.g., a mesenchymal stem cell, an umbilical cord stem cell, bone marrow stem cell, a hematopoietic stem cell, an induced pluripotent stem cell e.g., an induced pluripotent stem cell derived from a subject's cells), an embryonic stem cell (e.g., a stem cell from embryonic yolk sac, placenta, umbilical cord, fetal skin, adolescent skin, blood, bone marrow, adipose tissue, erythropoietic tissue, hematopoietic tissue), a myoblast, a parenchymal cell (e.g., hepatocyte), an alveolar cell, a neuron (e.g., a retinal neuronal cell) a precursor cell (e.g., a retinal precursor cell, a myeloblast, myeloid precursor cells, a thymocyte, a meiocyte, a megakaryoblast, a promegakaryoblast, a melanoblast, a lymphoblast, a bone marrow precursor cell, a normoblast, or an angioblast), a progenitor cell (e.g., a cardiac progenitor cell, a satellite cell, a radial gial cell, a

bone marrow stromal cell, a pancreatic progenitor cell, an endothelial progenitor cell, a blast cell), or an immortalized cell (e.g., HeLa, HEK293, HFF-1, MRC-5, WI-38, IMR 90, IMR 91, PER.C6, HT-1080, or BJ cell).

279. The fusosome of any of the preceding embodiments, wherein the target cell is other than a HeLa cell, or the target cell is not transformed or immortalized.

280. The fusosome of any of the preceding embodiments, wherein the target cell is transformed or immortalized.

281. The fusosome of any of the preceding embodiments, wherein the target cell is selected from a white blood cell or a stem cell.

282. The fusosome of any of the preceding embodiments, wherein the target cell is selected from a neutrophil, a lymphocyte (e.g., a T cell, a B cell, a natural killer cell), a macrophage, a granulocyte, a mesenchymal stem cell, a bone marrow stem cell, an induced pluripotent stem cell, an embryonic stem cell, or a myeloblast.

283. The fusosome of any of the preceding embodiments, wherein the fusosome comprises a targeting domain which localizes the fusosome to a target cell.

284. The fusosome of embodiment 283, wherein the targeting domain interacts with a target cell moiety on the target cell.

285. The fusosome of embodiment 284, wherein the target cell moiety is a cell surface feature.

286. The fusosome of embodiment 284 or embodiment 285, wherein the fusosome does not comprise said target cell moiety.

287. The fusosome of any of the preceding embodiments, wherein the fusosome comprises a

fusogen which interacts with a fusogen binding partner on the target cell, thereby allowing the fusosome to bind or fuse to the target cell.

288. The fusosome of embodiment 287, wherein the fusosome does not comprise said fusogen binding partner.

289. The fusosome of any of embodiments 283-288, wherein the targeting domain is not part of the fusogen.

290. The fusosome of any of embodiments 283-288, wherein the fusogen comprises the targeting domain.

291. The fusosome of any of embodiments 283-289, wherein the fusogen binding partner is or is a portion of a different entity from the target cell moiety.

292. The fusosome of any of embodiments 283-289, wherein the fusogen binding partner is or is a portion of the target cell moiety.

293. The fusosome of any of the preceding embodiments, further comprising an organelle, e.g., a therapeutically effective number of organelles, disposed in the lumen.

294. The fusosome of any of the preceding embodiments, wherein:

i) the source cell is other than a dendritic cell or tumor cell, e.g., the source cell is selected from an endothelial cell, a macrophage, a neutrophil, a granulocyte, a leukocyte, a stem cell (e.g., a mesenchymal stem cell, a bone marrow stem cell, an induced pluripotent stem cell, an embryonic stem cell), a myeloblast, a myoblast, a hepatocyte, or a neuron e.g., retinal neuronal cell;

ii) the fusogen is other than a fusogenic glycoprotein;

iii) the fusogen is a mammalian protein other than fertilin-beta;

iv) the fusosome has low immunogenicity, e.g., as described herein;

v) the fusosome meets a pharmaceutical or good manufacturing practices (GMP) standard;

vi) a pharmaceutical preparation comprising a plurality of the fusosomes was made according to good manufacturing practices (GMP);

vii) a pharmaceutical preparation comprising a plurality of the fusosomes has a pathogen level below a predetermined reference value, e.g., is substantially free of pathogens; or

viii) a pharmaceutical preparation comprising a plurality of the fusosomes has a contaminant level below a predetermined reference value, e.g., is substantially free of contaminants.

295. A fusosome composition or fusosome preparation, comprising a plurality of fusosomes according to any of the preceding embodiments.

296. A fusosome composition comprising a plurality of fusosomes, wherein at least one fusosome comprises:

(a) a lipid bilayer comprising a plurality of lipids derived from a source cell;

(b) a lumen (e.g., comprising cytosol) surrounded by the lipid bilayer;

(c) a fusogen that is exogenous or overexpressed relative to the source cell, e.g., wherein the fusogen is disposed in the lipid bilayer;

(d) a payload (e.g., a membrane payload agent, nuclear payload agent, or organellar payload agent), e.g., membrane protein payload agent, nuclear protein payload agent, or organellar protein payload agent, e.g., as described herein.

297. The fusosome composition of embodiment 295 or embodiment 296, where at least one fusosome of the plurality of fusosomes is derived from a source cell.

298. The fusosome composition of any of embodiments 295-297, wherein the fusosome is at a temperature of less than 4, 0, -4, -10, -12, -16, -20, -80, or -160°C.

299. The fusosome composition of any of embodiments 295-298, wherein the plurality of

fusosomes comprises at least about 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13}, 10^{14}, or 10^{15}fusosomes.

300. The fusosome composition of any of embodiments 295-299, wherein the plurality of fusosomes are the same.

301. The fusosome composition of embodiment 300, wherein the plurality of fusosomes are the same if at least 0.01%-0.05%, 0.05%-0.1%, 0.1%-0.5%, 0.5%- 1%, 1%-2%, 2%-3%, 3%-4%, 4%-5%, 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, or 80%-90% of the fusosomes in the fusosome composition share at least one property selected from:

comprise the same fusogen;

produced using the same type of source cell; or

comprise the same payload (e.g., a membrane payload agent, nuclear payload agent, or organellar payload agent).

302. The fusosome composition of any of embodiments 295-299, wherein the plurality of fusosomes are different.

303. The fusosome composition of any of embodiments 295-302, wherein the plurality of fusosomes is derived from two or more types of source cells.

304. The fusosome composition of any of embodiments 295-303, which has a volume of at least 1 μL, 2 μL. 5 μL, 10 μL, 20 μL, 50 μL, 100 μL, 200 μL, 500 μL, 1 mL, 2 mL, 5 mL, or 10 mL.

305. The fusosome composition of any of embodiments 295-304, wherein the plurality of fusosomes comprises at least 0.01%-0.05%, 0.05%-0.1%, 0.1%-0.5%, 0.5%- 1%, 1%-2%, 2%-3%, 3%-4%, 4%-5%, 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, or 80%-90% fusosomes which do not comprise a functional nucleus.

306. The fusosome composition of any of embodiments 295-305, wherein the plurality of

fusosomes comprises at least 0.01%-0.05%, 0.05%-0.1%, 0.1%-0.5%, 0.5%- 1%, 1%-2%, 2%-3%, 3%-4%, 4%-5%, 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, or 80%-90% fusosomes which do not comprise a nucleus.

307. The fusosome composition of any of embodiments 295-306, wherein the plurality of fusosomes comprises at least 0.01%-0.05%, 0.05%-0.1%, 0.1%-0.5%, 0.5%- 1%, 1%-2%, 2%-3%, 3%-4%, 4%-5%, 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, or 80%-90% fusosomes which are substantially free of nuclear DNA.

308. The fusosome composition of any of embodiments 295-307, wherein the plurality of fusosomes comprises at least 0.01%-0.05%, 0.05%-0.1%, 0.1%-0.5%, 0.5%- 1%, 1%-2%, 2%-3%, 3%-4%, 4%-5%, 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, or 80%-90% fusosomes which do not comprise functional mitochondria.

309. The fusosome composition of any of embodiments 295-308, wherein the plurality of fusosomes comprises at least 0.01%-0.05%, 0.05%-0.1%, 0.1%-0.5%, 0.5%- 1%, 1%-2%, 2%-3%, 3%-4%, 4%-5%, 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, or 80%-90% fusosomes which do not comprise mitochondria.

310. The fusosome composition of any of embodiments 295-309, which comprises less than 0.01%, 0.05%, 0.1%, 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 4%, 5%, or 10% source cells by protein mass or less than 0.01%, 0.05%, 0.1%, 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 4%, 5%, or 10% of source cells having a functional nucleus.

311. The fusosome composition of any of embodiments 295-310, wherein the fusosome composition comprises at least 0.01%-0.05%, 0.05%-0.1%, 0.1%-0.5%, 0.5%- 1%, 1%-2%, 2%-3%, 3%-4%, 4%-5%, 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, or 80%-90% fusosomes wherein:

i) the fusogen is present at a copy number of at least 1,000 copies per fusosome, e.g., as measured by an assay of Example 29; or

ii) the ratio of the copy number of the fusogen to the copy number of the payload (e.g., a membrane payload agent, nuclear payload agent, or organellar payload agent) per fusosome is between 1,000,000:1 and 100,000:1, 100,000:1 and 10,000:1, 10,000:1 and 1,000:1, 1,000:1 and 100:1, 100:1 and 50:1, 50:1 and 20:1, 20:1 and 10:1, 10:1 and 5:1, 5:1 and 2:1, 2:1 and 1:1, 1:1 and 1:2, 1:2 and 1:5, 1:5 and 1:10, 1:10 and 1:20, 1:20 and 1:50, 1:50 and 1:100, 1:100 and 1:1,000, 1:1,000 and 1:10,000, 1:10,000 and 1:100,000, or 1:100,000 and 1:1,000,000.

312. The fusosome composition of any of embodiments 295-311, wherein the fusosome composition comprises at least 0.01%-0.05%, 0.05%-0.1%, 0.1%-0.5%, 0.5%- 1%, 1%-2%, 2%-3%, 3%-4%, 4%-5%, 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, or 80%-90% fusosomes wherein the payload (e.g., a membrane payload agent, nuclear payload agent, or organellar payload agent) is present at a copy number of at least 1,000 copies per fusosome, e.g., as measured by an assay of Example 43.

313. The fusosome composition of any of embodiments 295-312, wherein the plurality of fusosomes has a mean diameter of at least about 50 nm, about 80 nm, about 100 nm, about 200 nm, about 500 nm, about 1000 nm, about 1200 nm, about 1400 nm, or about 1500 nm.

314. The fusosome composition of any of embodiments 295-313, wherein the plurality of fusosomes comprises fusosomes having a diameter within the range of about 10 nm to about 100 μm.

315. The fusosome composition of any of embodiments 295-314, wherein the plurality comprises fusosomes having a size within the range of about 20 nm to about 200 nm, about 50 nm to about 200 nm, about 50 nm to about 100 nm, about 50 nm to about 150 nm, or about 100 nm to about 150 nm.

316. The fusosome composition of any of embodiments 295-315, wherein at least 50% of fusosomes in the plurality have a diameter within 10%, 20%, 30%, 40%, or 50% of the mean diameter of the fusosomes in the fusosome composition.

317. The fusosome composition of any of embodiments 295-316, wherein the plurality comprises fusosomes having a volume within the range of about 500 nm^3 to about 0.0006 mm^3, or about 4,000 nm^3 to about 0.005 μm^3, about 65,000 nm^3 to about 0.005 μm^3, about 65,000 nm^3 to about 0.0006 μm^3, about 65,000 nm^3 to about 0.002 μm^3, or about 0.0006 μm^3 to about 0.002 μm^3.

318. The fusosome composition of any of embodiments 295-317, wherein at least 50% of fusosomes in the plurality have a volume within 10%, 20%, 30%, 40%, or 50% of the mean volume of the fusosomes in the fusosome composition.

319. The fusosome composition of any of embodiments 295-318, wherein at least 50% of fusosomes in the plurality have a copy number of the fusogen within 10%, 20%, 30%, 40%, or 50% of the mean fusogen copy number in the fusosomes in the fusosome composition.

320. The fusosome composition of any of embodiments 295-319, wherein at least 50% of fusosomes in the plurality have a copy number of the payload (e.g., a membrane payload agent, nuclear payload agent, or organellar payload agent) within 10%, 20%, 30%, 40%, or 50% of the mean payload (e.g., a membrane payload agent, nuclear payload agent, or organellar payload agent) copy number in the fusosomes in the fusosome composition.

321. The fusosome composition of any of the preceding embodiments, wherein less than 10%, 5%, 4%, 3%, 2%, or 1% of fusosomes in the composition comprise an organelle in the lumen, e.g., the lumen does not comprise one or more of a mitochondrion, Golgi apparatus, lysosome, endoplasmic reticulum, mitochondria, vacuole, endosome, acrosome, autophagosome, centriole, glycosome, glyoxysome, hydrogenosome, melanosome, mitosome, cnidocyst, peroxisome, proteasome, vesicle, or stress granule.

322. A pharmaceutical composition comprising the fusosome composition or preparation of any of the preceding embodiments and a pharmaceutically acceptable carrier.

323. The fusosome composition or pharmaceutical composition of any of the preceding embodiments, which has been maintained at a predetermined temperature for at least 1, 2, 3, 6, or 12 hours; 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, or 4 weeks; 1, 2, 3, or 6 months; or 1, 2, 3, 4, or 5 years.

324. The fusosome composition or pharmaceutical composition of embodiment 323, wherein the predetermined temperature is selected from about 4, 0, -4, -10, -12, -16, -20, -80, or -160°C.

325. The fusosome composition or pharmaceutical composition of embodiment 323 or embodiment 324, which has an activity of at least 50%, 60%, 70%, 80%, 90%, 95%, or 99% of the activity of the plurality before maintenance at said temperature, e.g., by one or more of:

i) the fusosome fuses at a higher rate with a target cell than with a non-target cell, e.g., by at least at least 10%, e.g., in an assay of Example 54;
ii) the fusosome fuses at a higher rate with a target cell than with other fusosomes, e.g., by at least 50%, e.g., in an assay of Example 54;
iii) the fusosome fuses with target cells at a rate such that an agent in the fusosome is delivered to at least 10% of target cells after 24 hours, e.g., in an assay of Example 54; or
iv) the fusogen is present at a copy number of at least 50%, 60%, 70%, 80%, 90%, 95%, or 99% of the fusogen copy number of the plurality before maintenance at said temperature, e.g., as measured by an assay of Example 29.

326. The fusosome composition or pharmaceutical composition of any of the preceding embodiments, which is stable on storage at a temperature of less than 4°C for at least 1, 2, 3, 6, or 12 hours; 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, or 4 weeks; 1, 2, 3, or 6 months; or 1, 2, 3, 4, or 5 years.

327. The fusosome composition or pharmaceutical composition of any of the preceding embodiments, which is stable on storage at a temperature of less than -20°C for at least 1, 2, 3, 6, or 12 hours; 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, or 4 weeks; 1, 2, 3, or 6 months; or 1, 2, 3, 4, or 5 years.

328. The fusosome composition or pharmaceutical composition of any of the preceding embodiments, which is stable on storage at a temperature of less than -80°C for at least 1, 2, 3, 6,

or 12 hours; 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, or 4 weeks; 1, 2, 3, or 6 months; or 1, 2, 3, 4, or 5 years.

329. The fusosome composition or pharmaceutical composition of any of embodiments 326-328, wherein the composition is considered stable if it has an activity of at least 50%, 60%, 70%, 80%, 90%, 95%, or 99% of the activity of the plurality before storage at said temperature for said time period, e.g., by one or more of:

i) the fusosome fuses at a higher rate with a target cell than with a non-target cell, e.g., by at least at least 10%, e.g., in an assay of Example 54;

ii) the fusosome fuses at a higher rate with a target cell than with other fusosomes, e.g., by at least 50%, e.g., in an assay of Example 54;

iii) the fusosome fuses with target cells at a rate such that an agent in the fusosome is delivered to at least 10% of target cells after 24 hours, e.g., in an assay of Example 54; or

iv) the fusogen is present at a copy number of at least 50%, 60%, 70%, 80%, 90%, 95%, or 99% of the fusogen copy number of the plurality before maintenance at said temperature, e.g., as measured by an assay of Example 29.

330. The pharmaceutical composition of any of embodiments 322-329, having one or more of the following characteristics:

a) the pharmaceutical composition meets a pharmaceutical or good manufacturing practices (GMP) standard;

b) the pharmaceutical composition was made according to good manufacturing practices (GMP);

c) the pharmaceutical composition has a pathogen level below a predetermined reference value, e.g., is substantially free of pathogens;

d) the pharmaceutical composition has a contaminant level below a predetermined reference value, e.g., is substantially free of contaminants; or

e) the pharmaceutical composition has low immunogenicity, e.g., as described herein.

331. A method of manufacturing a fusosome composition, comprising:

a) providing a source cell comprising, e.g., expressing, a fusogen;

b) producing a fusosome from the source cell, wherein the fusosome comprises a lipid bilayer, a lumen, a fusogen, and a payload (e.g., a membrane payload agent, nuclear payload agent, or organellar payload agent), e.g., membrane protein payload agent, nuclear protein payload agent, or organellar protein payload agent, thereby making a fusosome; and

c) formulating the fusosome, e.g., as a pharmaceutical composition suitable for administration to a subject, wherein one or more of:

xii) the source cell is other than a 293 cell, HEK cell, human endothelial cell, or a human epithelial cell;

xiii) the fusogen is other than a viral protein;

xiv) the fusosome and/or compositions or preparations thereof has a density of other than between 1.08 g/mL and 1.12 g/mL, e.g.,

xv) the fusosome and/or compositions or preparations thereof has a density of 1.25 g/mL +/- 0.05, e.g., as measured by an assay of Example 33;

xvi) the fusosome is not captured by the scavenger system in circulation or by Kupffer cells in the sinus of the liver;

xvii) the fusosome is not captured by the reticulo-endothelial system (RES) in a subject, e.g., by an assay of Example 76;

xviii) when a plurality of fusosomes are administered to a subject, less than 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, of the plurality are not captured by the RES after 24 hours, e.g., by an assay of Example 76;

xix) the fusosome has a diameter of greater than 5 μm, 6 μm, 7 μm, 8 μm, 10 μm, 20 μm, 50 μm, 100 μm, 150 μm, or 200 μm.

xx) the fusosome comprises a cytobiologic;

xxi) the fusosome comprises an enucleated cell; or

xxii) the fusosome comprises an inactivated nucleus.

332. The method of embodiment 331, wherein providing a source cell expressing a fusogen

comprises expressing an exogenous fusogen in the source cell or upregulating expression of an endogenous fusogen in the source cell.

333. The method of embodiment 331 or embodiment 332, which comprises inactivating the nucleus of the source cell.

334. A method of manufacturing a fusosome composition, comprising:

i) providing a plurality of fusosomes according to any of embodiments 1-70, a fusosome composition of any of embodiments 71-97, or a pharmaceutical composition of embodiment 97; and

ii) formulating the plurality of fusosomes, fusosome composition, or pharmaceutical composition , e.g., as a fusosome drug product suitable for administration to a subject.

335. The method of any of embodiments 331-334, wherein the fusosome composition comprises at least 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13}, 10^{14}, or 10^{15} fusosomes.

336. The method of any of embodiments 331-335, wherein the fusosome composition comprises a volume of at least 10 mL, 20 mL, 50 mL, 100 mL, 200 mL, 500 mL, 1 L, 2 L, 5 L, 10 L, 20 L, or 50 L.

337. The method of any of embodiments 331-336, which comprises enucleating the source cell, e.g., mammalian cell, e.g., by chemical enucleation, use of mechanical force e.g. use of a filter or centrifuge, least partial disruption of the cytoskeleton.

338. The method of any of embodiments 331-337, which comprises expressing a fusogen or other membrane protein in the source cell.

339. The method of any of embodiments 331-338, which comprises one or more of: vesiculation, hypotonic treatment, extrusion, or centrifugation.

340. The method of any of embodiments 331-339, which comprises genetically expressing an exogenous agent in the cell or loading the exogenous agent into the source cell or fusosome.

341. The method of any of embodiments 331-340, which comprises contacting the source cell with DNA encoding a polypeptide agent, e.g., before inactivating the nucleus, e.g., enucleating the source cell.

342. The method of any of embodiments 331-341, which comprises contacting the source cell with RNA encoding a polypeptide agent, e.g., before or after inactivating the nucleus, e.g., enucleating the source cell.

343. The method of any of embodiments 331-342, which comprises introducing a payload, e.g., a membrane payload agent, nuclear payload agent, or organellar payload agent (e.g., a nucleic acid or protein), into a fusosome, e.g., by electroporation.

344. The method of any of embodiments 331-343 wherein the source cell is an endothelial cell, a fibroblast, a blood cell (e.g., a macrophage, a neutrophil, a granulocyte, a leukocyte), a stem cell (e.g., a mesenchymal stem cell, an umbilical cord stem cell, bone marrow stem cell, a hematopoietic stem cell, an induced pluripotent stem cell e.g., an induced pluripotent stem cell derived from a subject's cells), an embryonic stem cell (e.g., a stem cell from embryonic yolk sac, placenta, umbilical cord, fetal skin, adolescent skin, blood, bone marrow, adipose tissue, erythropoietic tissue, hematopoietic tissue), a myoblast, a parenchymal cell (e.g., hepatocyte), an alveolar cell, a neuron (e.g., a retinal neuronal cell) a precursor cell (e.g., a retinal precursor cell, a myeloblast, myeloid precursor cells, a thymocyte, a meiocyte, a megakaryoblast, a promegakaryoblast, a melanoblast, a lymphoblast, a bone marrow precursor cell, a normoblast, or an angioblast), a progenitor cell (e.g., a cardiac progenitor cell, a satellite cell, a radial gial cell, a bone marrow stromal cell, a pancreatic progenitor cell, an endothelial progenitor cell, a blast cell), or an immortalized cell (e.g., HeLa, HEK293, HFF-1, MRC-5, WI-38, IMR 90, IMR 91, PER.C6, HT-1080, or BJ cell).

345. The method of any of embodiments 331-344, wherein the fusosome is from a mammalian cell having a modified genome, e.g., having reduced immunogenicity (e.g., by genome editing to remove MHC complexes).

346. The method of any of embodiments 331-345, wherein the source cell is from a cell culture treated with an anti-inflammatory signal.

347. The method of any of embodiments 331-346, further comprising contacting the source cell of step a) with an anti-inflammatory signal, e.g., before or after inactivating the nucleus, e.g., enucleating the cell.

348. A method of manufacturing a fusosome drug product composition, comprising:

a) providing, e.g., producing, a plurality of fusosomes according to any of embodiments 1-70, a fusosome composition of any of embodiments 71-97, or a pharmaceutical composition of embodiment 97; and

b) assaying one or more fusosomes from the plurality to determine whether one or more (e.g., 2, 3, or all) of the following standards are met:

i) the fusosome fuses at a higher rate with a target cell than with a non-target cell, e.g., by at least at least 10% e.g., in an assay of Example 54;

ii) the fusosome fuses at a higher rate with a target cell than with other fusosomes, e.g., by at least 50% e.g., in an assay of Example 54;

iii) the fusosome fuses with target cells at a rate such that an agent in the fusosome is delivered to at least 10% of target cells after 24 hours, e.g., in an assay of Example 54;

iv) the fusogen is present at a copy number of at least 1,000 copies, e.g., as measured by an assay of Example 29;

v) the fusosome comprises a payload (e.g., membrane payload agent, nuclear payload agent, or organellar payload agent) at a copy number of at least 1,000 copies, e.g., as measured by an assay of Example 43;

vi) the ratio of the copy number of the fusogen to the copy number of the payload (e.g.,

membrane payload agent, nuclear payload agent, or organellar payload agent) is between 1,000,000:1 and 100,000:1, 100,000:1 and 10,000:1, 10,000:1 and 1,000:1, 1,000:1 and 100:1, 100:1 and 50:1, 50:1 and 20:1, 20:1 and 10:1, 10:1 and 5:1, 5:1 and 2:1, 2:1 and 1:1, 1:1 and 1:2, 1:2 and 1:5, 1:5 and 1:10, 1:10 and 1:20, 1:20 and 1:50, 1:50 and 1:100, 1:100 and 1:1,000, 1:1,000 and 1:10,000, 1:10,000 and 1:100,000, or 1:100,000 and 1:1,000,000;

vii) the fusosome comprises a lipid composition wherein one or more of CL, Cer, DAG, HexCer, LPA, LPC, LPE, LPG, LPI, LPS, PA, PC, PE, PG, PI, PS, CE, SM and TAG is within 75% of the corresponding lipid level in the source cell;

viii) the fusosome comprises a proteomic composition similar to that of the source cell, e.g., using an assay of Example 42;

ix) the fusosome comprises a ratio of lipids to proteins that is within 10%, 20%, 30%, 40%, or 50% of the corresponding ratio in the source cell, e.g., as measured using an assay of Example 49;

x) the fusosome comprises a ratio of proteins to nucleic acids (e.g., DNA) that is within 10%, 20%, 30%, 40%, or 50% of the corresponding ratio in the source cell, e.g., as measured using an assay of Example 50;

xi) the fusosome comprises a ratio of lipids to nucleic acids (e.g., DNA) that is within 10%, 20%, 30%, 40%, or 50% of the corresponding ratio in the source cell, e.g., as measured using an assay of Example 51;

xii) the fusosome has a half-life in a subject, e.g., in a mouse, that is within 90% of the half-life of a reference cell, e.g., the source cell, e.g., by an assay of Example 75;

xiii) the fusosome transports glucose (e.g., labeled glucose, e.g., 2-NBDG) across a membrane, e.g., by at least 10% more than a negative control, e.g., an otherwise similar fusosome in the absence of glucose, e.g., as measured using an assay of Example 64;

xiv) the fusosome comprises esterase activity in the lumen that is within 90% of that of the esterase activity in a reference cell, e.g., the source cell or a mouse embryonic fibroblast, e.g., using an assay of Example 66;

xv) the fusosome comprises a metabolic activity level that is within 90% of the

metabolic activity (e.g., citrate synthase activity) in a reference cell, e.g., the source cell, e.g., as described in Example 68;

xvi) the fusosome comprises a respiration level (e.g., oxygen consumption rate) that is within 90% of the respiration level in a reference cell, e.g., the source cell, e.g., as described in Example 69;

xvii) the fusosome comprises an Annexin-V staining level of at most 18,000, 17,000, 16,000, 15,000, 14,000, 13,000, 12,000, 11,000, or 10,000 MFI, e.g., using an assay of Example 70, or wherein the fusosome comprises an Annexin-V staining level at least 5%, 10%, 20%, 30%, 40%, or 50% lower than the Annexin-V staining level of an otherwise similar fusosome treated with menadione in the assay of Example 70, or wherein the fusosome comprises an Annexin-V staining level at least 5%, 10%, 20%, 30%, 40%, or 50% lower than the Annexin-V staining level of a macrophage treated with menadione in the assay of Example 70;

xviii) the fusosome has a miRNA content level of at least 1% than that of the source cell, e.g., by an assay of Example 39;

xix) the fusosome has a soluble : non-soluble protein ratio is within 90% of that of the source cell, e.g., by an assay of Example 47;

xx) the fusosome has an LPS level less than 5% of the lipid content of fusosomes, e.g., as measured by an assay of Example 48;

xxi) the fusosome and/or compositions or preparations thereof, are capable of signal transduction, e.g., transmitting an extracellular signal, e.g., AKT phosphorylation in response to insulin, or glucose (e.g., labeled glucose, e.g., 2-NBDG) uptake in response to insulin, e.g., by at least 10% more than a negative control, e.g., an otherwise similar fusosome in the absence of insulin, e.g., using an assay of Example 63;

xxii) the fusosome has juxtacrine-signaling level of at least 5% greater than the level of juxtacrine signaling induced by a reference cell, e.g., the source cell or a bone marrow stromal cell (BMSC), e.g., by an assay of Example 71;

xxiii) the fusosome has paracrine-signaling level of at least 5% greater than the level of paracrine signaling induced by a reference cell, e.g., the source cell or a

macrophage, e.g., by an assay of Example 72;

xxiv) the fusosome polymerizes actin at a level within 5% compared to the level of polymerized actin in a reference cell, e.g., the source cell or a C2C12 cell, e.g., by the assay of Example 73;

xxv) the fusosome has a membrane potential within about 5% of the membrane potential of a reference cell, e.g., the source cell or a C2C12 cell, e.g., by an assay of Example 74, or wherein the fusosome has a membrane potential of about -20 to -150mV, -20 to -50mV, -50 to -100mV, or -100 to -150mV;

xxvi) the fusosome and/or compositions or preparations thereof, are capable of secreting a protein, e.g., at a rate at least 5% greater than a reference cell, e.g., a mouse embryonic fibroblast, e.g., using an assay of Example 62; or

xxvii) the fusosome has low immunogenicity, e.g., as described herein; and

c) (optionally) approving the plurality of fusosomes or fusosome composition for release if one or more of the standards is met;

thereby manufacturing a fusosome drug product composition.

349. A method of manufacturing a fusosome drug product composition, comprising:

a) providing, e.g., producing, providing a plurality of fusosomes according to any of embodiments 1-70, a fusosome composition of any of embodiments 71-97, or a pharmaceutical composition of embodiment 97; and

b) assaying one or more fusosomes from the plurality to determine the presence or level of one or more of the following factors:

i) an immunogenic molecule, e.g., an immunogenic protein, e.g., as described herein;

ii) a pathogen, e.g., a bacterium or virus; or

iii) a contaminant;

c) (optionally) approving the plurality of fusosomes or fusosome composition for release if one or more of the factors is below a reference value;

thereby manufacturing a fusosome drug product composition.

350. The method of embodiment 349, wherein if a detectable level, e.g., a value above a reference value, is determined, a sample containing the plurality of fusosomes or fusosome composition is discarded.

351. A method of manufacturing a fusosome composition, comprising:

a) providing a plurality of fusosomes described herein or a fusosome composition described herein; and

b) formulating the fusosomes, e.g., as a pharmaceutical composition suitable for administration to a subject.

352. A method of manufacturing a fusosome composition, comprising:

a) providing, e.g., producing, a plurality of fusosomes or a fusosome preparation described herein; and

b) assaying a sample of the plurality (e.g., of the preparation) to determine whether one or more (e.g., 2, 3, or more) standards are met.

353. A method of manufacturing a fusosome composition, comprising:

a) providing, e.g., producing, a plurality of fusosomes described herein or a fusosome composition or preparation described herein; and

b) assaying a sample of the plurality or preparation to determine the presence or level of one or more of the following factors:

i) an immunogenic molecule, e.g., an immunogenic protein, e.g., as described herein;

ii) a pathogen, e.g., a bacterium or virus; or

iii) a contaminant (e.g., a nuclear structure or component such as nuclear DNA); and

c) (optionally) approving the plurality of fusosomes or fusosome preparation for release if one or more of the factors is deviates significantly (e.g., by more than a specified amount) from a reference value or (optionally) formulating the plurality of fusosomes or the fusosome preparation as a drug product if the one or more factors does not significantly deviate (e.g., does not deviate

by more than the specified about) from the reference value.

354. A method of manufacturing a fusosome composition, comprising:

i) providing a plurality of fusosomes, a fusosome composition, or a pharmaceutical composition as described herein; and

ii) formulating the plurality of fusosomes, fusosome composition, or pharmaceutical composition , e.g., as a fusosome drug product suitable for administration to a subject.

355. A method of manufacturing a fusosome composition, comprising:

a) providing a plurality of fusosomes, a fusosome composition, or a pharmaceutical composition as described herein; and

b) assaying one or more fusosomes from the plurality to determine the presence or level of one or more of the following factors:

i) an immunogenic molecule, e.g., an immunogenic protein, e.g., as described herein;

ii) a pathogen, e.g., a bacterium or virus; or

iii) a contaminant;

c) (optionally) approving the plurality of fusosomes or fusosome composition for release if one or more of the factors is below a reference value;

thereby manufacturing a fusosome drug product composition.

356. The method of any of embodiments 102-356, wherein one or more of the following is present:

i) the source cell is other than a 293 cell, HEK cell, human endothelial cell, or a human epithelial cell;

ii) the fusogen is other than a viral protein;

iii) a preparation comprising a plurality of the fusosomes has a density of other than between 1.08 g/mL and 1.12 g/mL;

iv) a preparation comprising a plurality of the fusosomes has a density of 1.25 g/mL +/- 0.05, e.g., as measured by an assay of Example 33;

v) the fusosome is not substantially captured by the scavenger system in circulation or by Kupffer cells in the sinus of the liver;

vi) the fusosome is not substantially captured by the reticulo-endothelial system (RES) in a subject, e.g., by an assay of Example 76;

vii) when a plurality of fusosomes are administered to a subject, less than 1%, 2%, 3%, 4%, 5% , 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the plurality are captured by the RES after 24, 48, or 72 hours, e.g., by an assay of Example 76;

viii) the fusosome has a diameter of greater than 5 μm, 6 μm, 7 μm, 8 μm, 10 μm, 20 μm, 50 μm, 100 μm, 150 μm, or 200 μm.

ix) the fusosome comprises a cytobiologic;

x) the fusosome comprises an enucleated cell; or

xi) the fusosome comprises an inactivated nucleus.

357. A method of administering a fusosome composition to a subject, e.g., a human subject, comprising administering to the subject a fusosome composition comprising a plurality of fusosomes according to any of embodiments 1-70, a fusosome composition of any of embodiments 71-97, or a pharmaceutical composition of embodiment 97, thereby administering the fusosome composition to the subject.

358. A method of delivering a payload (e.g., a membrane payload agent, nuclear payload agent, or organellar payload agent) to a subject, comprising administering to the subject a fusosome composition comprising a plurality of fusosomes according to any of embodiments 1-70, a fusosome composition of any of embodiments 71-97, or a pharmaceutical composition of embodiment 97, wherein the fusosome composition is administered in an amount and/or time such that the payload is delivered.

359. A method of modulating, e.g., enhancing, a biological function in a subject, comprising administering to the subject, or contacting the target tissue or the cell with, a plurality of fusosomes according to any of embodiments 1-70, a fusosome composition of any of embodiments 71-97, or a pharmaceutical composition of embodiment 97, thereby modulating the biological function in

the subject.

360. The method of embodiment 359, wherein the biological function is selected from:

a) modulating, e.g., increasing or decreasing, an interaction between two cells;

b) modulating, e.g. increasing or decreasing, an immune response;

c) modulating, e.g. increasing or decreasing, recruitment of cells to a target tissue;

d) decreasing the growth rate of a cancer; or

e) .reducing the number of cancerous cells in the subject.

361. A method of delivering a function to a subject, comprising administering to the subject a plurality of fusosomes according to any of embodiments 1-70, a fusosome composition of any of embodiments 71-97, or a pharmaceutical composition of embodiment 97, wherein the fusosome composition is administered in an amount and/or time such that the function in the subject is delivered.

362. A method of targeting a function to a subject, comprising administering to the subject a plurality of fusosomes according to any of embodiments 1-70, a fusosome composition of any of embodiments 71-97, or a pharmaceutical composition of embodiment 97, wherein the fusosome composition is administered in an amount and/or time such that the function in the subject is targeted.

363. A method of delivering or targeting a function to a subject, comprising administering to the subject a fusosome composition comprising a plurality of fusosomes, a fusosome composition, or a pharmaceutical composition as described herein, wherein the fusosome composition is administered in an amount and/or time such that the function in the subject is delivered or targeted.

364. A method of treating a disease or disorder in a patient comprising administering to the subject a plurality of fusosomes according to any of embodiments 1-70, a fusosome composition of any of embodiments 71-97, or a pharmaceutical composition of embodiment 97, wherein the fusosome composition is administered in an amount and/or time such that the disease or disorder

is treated.

365. The method of embodiment 364, wherein the disease or disorder is selected from cancer, autoimmune disorder, or infectious disease.

366. The method of any of embodiments 357-365, wherein the plurality of fusosomes has a local effect.

367. The method of any of embodiments 357-365, wherein the plurality of fusosomes has a distal effect.

368. The method of any of embodiments 357-365, wherein the plurality of fusosomes has a systemic effect.

369. The method of any of embodiments 357-368, wherein the subject has a cancer.

370. The method of embodiment 369, wherein the subject has a cancer and the fusosome comprises a neoantigen.

371. The method of any of embodiments 357-370, wherein the fusosome composition is administered to the subject at least 1, 2, 3, 4, or 5 times.

372. The method of any of embodiments 357-370, wherein the fusosome composition is administered to the subject systemically (e.g., orally, parenterally, subcutaneously, intravenously, intramuscularly, intraperitoneally) or locally.

373. The method of any of embodiments 357-372, wherein the fusosome composition is administered to the subject such that the fusosome composition reaches a target tissue selected from liver, lungs, heart, spleen, pancreas, gastrointestinal tract, kidney, testes, ovaries, brain, reproductive organs, central nervous system, peripheral nervous system, skeletal muscle,

endothelium, inner ear, or eye.

374. The method of any of embodiments 357-373, wherein the fusosome composition is co-administered with an immunosuppressive agent, e.g., a glucocorticoid, cytostatic, antibody, or immunophilin modulator.

375. The method of any of embodiments 357-374, wherein the fusosome composition is co-administered with an immunostimulatory agent, e.g., an adjuvant, interleukin, cytokine, or chemokine.

376. The method of any of the preceding embodiments, wherein the method comprises delivering an agent to the cytosol of a target cell, optionally wherein the cytosol-delivered agent is a protein (or a nucleic acid encoding, or complementary to one encoding, the protein, e.g., an e.g., a DNA, a gDNA, a cDNA, an RNA, a pre-mRNA, an mRNA, etc. encoding the protein).

377. A method of administering a fusosome composition to a human subject, comprising:

a) administering to the subject a first fusogen, under conditions that allow for disposition of the first fusogen in one or more target cells in the subject, wherein one or more of:

i) administering the first fusogen comprises administering a nucleic acid encoding the first fusogen, under conditions that allow for expression of the first fusogen in the one or more target cells, or

ii) the first fusogen does not comprise a coiled-coil motif, and

b) administering to the human subject a fusosome composition comprising a plurality of fusosomes comprising a second fusogen, wherein the second fusogen is compatible with the first fusogen, wherein the plurality of fusosomes further comprise a payload (e.g., membrane payload agent, nuclear payload agent, or organellar payload agent);

thereby administering the fusosome composition to the subject.

378. A method of delivering a payload (e.g., membrane payload agent, nuclear payload agent, or organellar payload agent) to a subject, comprising:

a) administering to the subject first fusogen, under conditions that allow for disposition of the first fusogen in one or more target cells in the subject, wherein one or more of:

i) administering the first fusogen comprises administering a nucleic acid encoding the first fusogen, under conditions that allow for expression of the first fusogen in the one or more target cells, or

ii) the first fusogen does not comprise a coiled-coil motif, and

b) administering to the human subject a fusosome composition comprising a plurality of fusosomes comprising a second fusogen and a therapeutic agent, wherein the second fusogen is compatible with the first fusogen, wherein the plurality of fusosomes further comprise a payload (e.g., membrane payload agent, nuclear payload agent, or organellar payload agent);

thereby delivering the payload (e.g., membrane payload agent, nuclear payload agent, or organellar payload agent) to the subject.

379. A method of modulating, e.g., enhancing, a biological function in a subject, comprising:

a) administering to the subject first fusogen, under conditions that allow for disposition of the first fusogen in one or more target cells in the subject, wherein one or more of:

i) administering the first fusogen comprises administering a nucleic acid encoding the first fusogen, under conditions that allow for expression of the first fusogen in the one or more target cells, or

ii) the first fusogen does not comprise a coiled-coil motif, and

b) administering to the human subject a fusosome composition comprising a plurality of fusosomes comprising a second fusogen, wherein the second fusogen is compatible with the first fusogen, wherein the plurality of fusosomes further comprise a payload (e.g., membrane payload agent, nuclear payload agent, or organellar payload agent);

thereby modulating the biological function in the subject.

380. The method of any of embodiments 377-379, wherein the payload (e.g., membrane payload agent, nuclear payload agent, or organellar payload agent) is exogenous or overexpressed relative to the source cell.

381. The method of any of embodiments 377-379, wherein the payload (e.g., membrane payload agent, nuclear payload agent, or organellar payload agent) comprises or encodes one or more of:

i) a transcriptional activator, e.g., a transcriptional activator of Table 17-1;
ii) a transcriptional repressor, e.g., a transcriptional repressor of Table 171;
iii) an epigenetic modifier, e.g., an epigenetic modifier of Table 17-1;
iv) a histone acetyltransferase, e.g., a histone acetyltransferase of Table 17-1;
v) a histone deacetylase, e.g., a histone deacetylase of Table 17-1;
vi) a histone methyltransferase, e.g., a histone methyltransferase of Table 17-1;
vii) a DNA methyltransferase, e.g., a DNA methyltransferase of Table 17-1;
viii) a DNA nickase, e.g., a DNA nickase as described herein;
ix) a site-specific DNA editing enzyme, e.g., a deaminase, e.g., of Table 17-1;
x) a DNA transposase, e.g., a DNA transposase as described herein;
xi) a DNA integrase, e.g., a DNA integrase as described herein;
xii) an RNA editor, e.g., an RNA editor of Table 17-1;
xiii) an RNA splicing factor, e.g., an RNA splicing factor of Table 17-1; or
xiv) a PIWI protein, e.g., a PIWI protein as described herein.

382. The method of any of the preceding embodiments, wherein the plurality of fusosomes has a local effect or has a distal effect.

383. The method of any of the preceding embodiments, comprising providing a source cell expressing a fusogen comprises expressing an exogenous fusogen in the source cell or upregulating expression of an endogenous fusogen in the source cell.

384. The method of any of the preceding embodiments, comprising inactivating the nucleus of the source cell.

385. The method of any of the preceding embodiments, comprising enucleating a mammalian cell, e.g., by chemical enucleation, use of mechanical force e.g., use of a filter or centrifuge, at

least partial disruption of the cytoskeleton, or a combination thereof.

386. The method of any of the preceding embodiments, comprising expressing a fusogen or other membrane protein in the source cell.

387. The method of any of the preceding embodiments, comprising one or more of: vesiculation, hypotonic treatment, extrusion, or centrifugation.

388. The method of any of the preceding embodiments, comprising genetically expressing an exogenous agent in the source cell or loading the exogenous agent into the source cell or fusosome.

389. The method of any of the preceding embodiments, comprising contacting the cell with DNA encoding a polypeptide agent, e.g., before inactivating the nucleus, e.g., enucleating the cell.

390. The method of any of the preceding embodiments, comprising contacting the cell with RNA encoding a polypeptide agent, e.g., before or after inactivating the nucleus, e.g., enucleating the cell.

391. The method of any of the preceding embodiments, comprising introducing a therapeutic agent (e.g., a nucleic acid or protein) into a fusosome, e.g., by electroporation.

392. The method of any of the preceding embodiments, wherein the fusosome is from a mammalian cell having a modified genome, e.g., to reduce immunogenicity (e.g., by genome editing, e.g., to remove an MHC protein); optionally wherein the method further coprises contacting the source cell of step a) with an immunosuppressive agent, e.g., before or after inactivating the nucleus, e.g., enucleating the cell.

393. The method of any of the preceding embodiments, wherein if a detectable level, e.g., a value above a reference value, is determined, a sample containing the plurality of fusosomes or fusosome composition or preparation is discarded.

394. The method of any of the preceding embodiments, further comprising a step of:

a) monitoring one or more of cancer progression, tumor recession, tumor volume, decrease in neoplastic cell number, quantity of fused cells, quantity of fused cells comprising a payload (e.g., a membrane payload agent, nuclear payload agent, or organellar payload agent), quantity of fused cells expressing a nucleic acid protein payload, and quantity of membrane protein disposed in membrane of a fused cell; and/or

b) monitoring adverse events in the organism; optionally wherein the adverse event includes one or more of cytokine release syndrome, fever, tachycardia, chills, anorexia, nausea, vomiting, myalgia, headaches, capillary leak syndrome, hypotension, pulmonary edema, coagulopathy, renal dysfunction, kidney injury, macrophage-activation syndrome, hemophagocytic lymphohistiocytosis, organ failure, cerebral edema, bystander inflammation from T cell activation, neurologic symptoms, encephalopathy, confusion, hallucination, delirium, obtundation, aphasia, seizures, B-cell aplasia, tumor lysis syndrome, and graft versus host disease.

395. The method of any of embodiments 377-379, wherein the first fusogen is not a lipopeptide.

396. The method of any of embodiments 357-395, further comprising a step of:

monitoring one or more of quantity of fused cells, quantity of fused cells comprising a payload (e.g., membrane payload agent, nuclear payload agent, or organellar payload agent), quantity of fused cells expressing a payload (e.g., membrane payload agent, nuclear payload agent, or organellar payload agent), and quantity of membrane protein disposed in the nucleus of a fused cell.

397. The method of any of embodiments 357-396, further comprising a step of monitoring adverse events in the organism.

398. The method of embodiment 397, wherein the adverse event includes one or more of cytokine release syndrome, fever, tachycardia, chills, anorexia, nausea, vomiting, myalgia, headaches, capillary leak syndrome, hypotension, pulmonary edema, coagulopathy, renal

dysfunction, kidney injury, macrophage-activation syndrome, hemophagocytic lymphohistiocytosis, organ failure, cerebral edema, bystander inflammation from T cell activation, neurologic symptoms, encephalopathy, confusion, hallucination, delirium, obtundation, aphasia, seizures, B-cell aplasia, tumor lysis syndrome, and graft versus host disease.

399. The method of any of embodiments 357-398, wherein the organism is a human.

400. The method of embodiment 399, wherein the human has a disease, disorder, or condition.

401. The method of embodiment 400, wherein presence of the payload (e.g., membrane payload agent, nuclear payload agent, or organellar payload agent, e.g., membrane protein payload agent, nuclear protein payload agent, or organellar protein payload agent) in the cell membrane lipid bilayer of the target cell improves one or more symptoms of the disease, disorder, or condition.

402. The fusosome, fusosome composition, fusosome preparation, or method any of the preceding embodiments, wherein the CAR is or comprises:

a) a first generation CAR comprising an antigen binding domain, a transmembrane domain, and signaling domain (e.g., one, two or three signaling domains);

b) a third generation CAR comprising an antigen binding domain, a transmembrane domain, and at least three signaling domains;

c) a fourth generation CAR comprising an antigen binding domain, a transmembrane domain, three or four signaling domains, and a domain which upon successful signaling of the CAR induces expression of a cytokine gene;

optionally wherein the antigen binding domain is or comprises an scFv or Fab.

403. The fusosome, fusosome composition, fusosome preparation, or method of embodiment 401 or 402, wherein a CAR antigen binding domain binds to a ligand expressed on B cells, plasma cells, plasmablasts, CD10, CD19, CD20, CD22, CD24, CD27, CD38, CD45R, CD138, CD319, BCMA, CD28, TNF, interferon receptors, GM-CSF, ZAP-70, LFA-1, CD3 gamma, CD5 or CD2.

404. The fusosome, fusosome composition, fusosome preparation, or method of any of embodiments 401-403, wherein the CAR transmembrane domain comprises at least a transmembrane region of the alpha, beta or zeta chain of a T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, or functional variant thereof.

405. The fusosome, fusosome composition, fusosome preparation, or method of any of embodiments 401-404, wherein the transmembrane domain comprises at least a transmembrane region(s) of CD8α, CD8β, 4-1BB/CD137, CD28, CD34, CD4, FcεRIγ, CD16, OX40/CD134, CD3ζ, CD3ε, CD3γ, CD3δ, TCRα, TCRβ, TCRζ, CD32, CD64, CD64, CD45, CD5, CD9, CD22, CD37, CD80, CD86, CD40, CD40L/CD154, VEGFR2, FAS, and FGFR2B, or functional variant thereof.

406. The fusosome, fusosome composition, fusosome preparation, or method of any of embodiments 401-405, wherein the CAR comprises at least one signaling domain selected from one or more of B7-1/CD80; B7-2/CD86; B7-H1/PD-L1; B7-H2; B7-H3; B7-H4; B7-H6; B7-H7; BTLA/CD272; CD28; CTLA-4; Gi24/VISTA/B7-H5; ICOS/CD278; PD-1; PD-L2/B7-DC; PDCD6); 4-1BB/TNFSF9/CD137; 4-1BB Ligand/TNFSF9; BAFF/BLyS/TNFSF13B; BAFF R/TNFRSF13C; CD27/TNFRSF7; CD27 Ligand/TNFSF7; CD30/TNFRSF8; CD30 Ligand/TNFSF8; CD40/TNFRSF5; CD40/TNFSF5; CD40 Ligand/TNFSF5; DR3/TNFRSF25; GITR/TNFRSF18; GITR Ligand/TNFSF18; HVEM/TNFRSF14; LIGHT/TNFSF14; Lymphotoxin-alpha/TNF-beta; OX40/TNFRSF4; OX40 Ligand/TNFSF4; RELT/TNFRSF19L; TACI/TNFRSF13B; TL1A/TNFSF15; TNF-alpha; TNF RII/TNFRSF1B); 2B4/CD244/SLAMF4; BLAME/SLAMF8; CD2; CD2F-10/SLAMF9; CD48/SLAMF2; CD58/LFA-3; CD84/SLAMF5; CD229/SLAMF3; CRACC/SLAMF7; NTB-A/SLAMF6; SLAM/CD150); CD2; CD7; CD53; CD82/Kai-1; CD90/Thy1; CD96; CD160; CD200; CD300a/LMIR1; HLA Class I; HLA-DR; Ikaros; Integrin alpha 4/CD49d; Integrin alpha 4 beta 1; Integrin alpha 4 beta 7/LPAM-1; LAG-3; TCL1A; TCL1B; CRTAM; DAP12; Dectin-1/CLEC7A; DPPIV/CD26; EphB6; TIM-1/KIM-1/HAVCR; TIM-4; TSLP; TSLP R; lymphocyte function associated antigen-1 (LFA-1); NKG2C, a CD3 zeta domain, an immunoreceptor tyrosine-based activation motif (ITAM), CD27, CD28, 4-

1BB, CD134/OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, or functional fragment thereof.

407. The fusosome, fusosome composition, fusosome preparation, or method of any of embodiments 401-406, wherein the CAR comprises a CD3 zeta domain or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof. In some embodiments, the CAR comprises (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; (ii) a CD28 domain, or a 4-1BB domain, or functional variant thereof, and/or (iii) a 4-1BB domain, or a CD134 domain, or functional variant thereof.

408. The fusosome, fusosome composition, fusosome preparation, or method of any of embodiments 401-407, wherein the CAR further comprises one or more spacers, e.g., wherein the spacer is a first spacer between the antigen binding domain and the transmembrane domain, optionally wherein the first spacer includes at least a portion of an immunoglobulin constant region or variant or modified version thereof, optionally wherein the spacer is a second spacer between the transmembrane domain and a signaling domain, optionally wherein the second spacer is an oligopeptide, e.g., wherein the oligopeptide comprises glycine-serine doublets.

409. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the antigen binding domain targets an antigen characteristic of a neoplastic cell.

410. The fusosome, fusosome composition, fusosome preparation, or method of embodiment 409, wherein the antigen characteristic of a neoplastic cell is selected from a cell surface receptor, an ion channel-linked receptor, an enzyme-linked receptor, a G protein-coupled receptor, receptor tyrosine kinase, tyrosine kinase associated receptor, receptor-like tyrosine phosphatase, receptor serine/ threonine kinase, receptor guanylyl cyclase, histidine kinase associated receptor, Epidermal Growth Factor Receptors (EGFR) (including ErbB1/EGFR, ErbB2/HER2, ErbB3/HER3, and ErbB4/HER4), Fibroblast Growth Factor Receptors (FGFR) (including FGF1, FGF2, FGF3,

FGF4, FGF5, FGF6, FGF7, FGF18, and FGF21) Vascular Endothelial Growth Factor Receptors (VEGFR) (including VEGF-A, VEGF-B, VEGF-C, VEGF-D, and PIGF), RET Receptor and the Eph Receptor Family (including EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, EphA8, EphA9, EphA10, EphB1, EphB2. EphB3, EphB4, and EphB6), CXCR1, CXCR2, CXCR3, CXCR4, CXCR6, CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR8, CFTR, CIC-1, CIC-2, CIC-4, CIC-5, CIC-7, CIC-Ka, CIC-Kb, Bestrophins, TMEM16A, GABA receptor, glycin receptor, ABC transporters, NAV1.1, NAV1.2, NAV1.3, NAV1.4, NAV1.5, NAV1.6, NAV1.7, NAV1.8, NAV1.9, sphingosin-1-phosphate receptor (S1P1R), NMDA channel, transmembrane protein, multispan transmembrane protein, T-cell receptor motifs; T-cell alpha chains; T-cell β chains; T-cell γ chains; T-cell δ chains; CCR7; CD3; CD4; CD5; CD7; CD8; CD11b; CD11c; CD16; CD19; CD20; CD21 ; CD22; CD25; CD28; CD34; CD35; CD40; CD45RA; CD45RO; CD52; CD56; CD62L; CD68; CD80; CD95; CD117; CD127; CD133; CD137 (4-1 BB); CD163; F4/80; IL-4Ra; Sca-1 ; CTLA-4; GITR; GARP; LAP; granzyme B; LFA-1 ; transferrin receptor; NKp46, perforin, CD4+; Th1; Th2; Th17; Th40; Th22; Th9; Tfh, Canonical Treg. FoxP3+; Tr1; Th3; Treg17; TREG; CDCP1, NT5E, EpCAM, CEA, gpA33, Mucins, TAG-72, Carbonic anhydrase IX, PSMA, Folate binding protein, Gangliosides (e.g., CD2, CD3, GM2), Lewis-γ2, VEGF, VEGFR 1/2/3, αVβ3, α5β1, ErbB1/EGFR, ErbB1/HER2, ErB3, c-MET, IGF1R, EphA3, TRAIL-R1, TRAIL-R2, RANKL, FAP, Tenascin, PDL-1, BAFF, HDAC, ABL, FLT3, KIT, MET, RET, IL-1β, ALK, RANKL, mTOR, CTLA-4, IL-6, IL-6R, JAK3, BRAF, PTCH, Smoothened, PIGF, ANPEP, TIMP1, PLAUR, PTPRJ, LTBR, or ANTXR1, Folate receptor alpha (FRa), ERBB2 (Her2/neu), EphA2, IL-13Ra2, epidermal growth factor receptor (EGFR), Mesothelin, TSHR, CD19, CD123, CD22, CD30, CD171, CS-1, CLL-1, CD33, EGFRvIII , GD2, GD3, BCMA, MUC16 (CA125), L1CAM, LeY, MSLN, IL13R□1, L1-CAM, Tn Ag, prostate specific membrane antigen (PSMA), ROR1, FLT3, FAP, TAG72, CD38, CD44v6, CEA, EPCAM, B7H3, KIT, interleukin-11 receptor a (IL-11Ra), PSCA, PRSS21, VEGFR2, LewisY, CD24, platelet-derived growth factor receptor-beta (PDGFR-beta), SSEA-4, CD20, MUC1, NCAM, Prostase, PAP, ELF2M, Ephrin B2, IGF-1 receptor, CAIX, LMP2, gplOO, bcr-abl, tyrosinase, Fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, Folate receptor beta, TEM1/CD248, TEM7R, CLDN6, GPRC5D, CXORF61, CD97, CD179a, ALK, Polysialic acid, PLACl, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-la, MAGE-A1, legumain,

HPV E6, E7, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, prostein, survivin, telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, Androgen receptor, Cyclin B1, MYCN, RhoC, TRP-2, CYPIB I, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2, CD79a, CD79b, CD72, LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, GPC3, FCRL5, IGLL1, a neoantigen, CD133, CD15, CD184, CD24, CD56, CD26, CD29, CD44, HLA-A, HLA-B, HLA-C, (HLA-A,B,C) CD49f, CD151 CD340, CD200, tkrA, trkB, or trkC, or an antigenic fragment or antigenic portion thereof.

411. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the antigen binding domain targets an antigen characteristic of a T-cell.

412. The fusosome, fusosome composition, fusosome preparation, or method of embodiment 411, wherein the antigen characteristic of a T-cell is selected from a cell surface receptor, a membrane transport protein (e.g., an active or passive transport protein such as, for example, an ion channel protein, a pore-forming protein, etc.), a transmembrane receptor, a membrane enzyme, and/or a cell adhesion protein characteristic of a T-cell.

413. The fusosome, fusosome composition, fusosome preparation, or method of embodiment 411 or 412, wherein the antigen characteristic of a T-cell may be a G protein-coupled receptor, receptor tyrosine kinase, tyrosine kinase associated receptor, receptor-like tyrosine phosphatase, receptor serine/ threonine kinase, receptor guanylyl cyclase, histidine kinase associated receptor, AKT1; AKT2; AKT3; ATF2; BCL10; CALM1; CD3D (CD3δ); CD3E (CD3ε); CD3G (CD3γ); CD4; CD8; CD28; CD45; CD80 (B7-1); CD86 (B7-2); CD247 (CD3ζ); CTLA4 (CD152); ELK1; ERK1 (MAPK3); ERK2; FOS; FYN; GRAP2 (GADS); GRB2; HLA-DRA; HLA-DRB1; HLA-DRB3; HLA-DRB4; HLA-DRB5; HRAS; IKBKA (CHUK); IKBKB; IKBKE; IKBKG (NEMO); IL2; ITPR1; ITK; JUN; KRAS2; LAT; LCK; MAP2K1 (MEK1); MAP2K2 (MEK2); MAP2K3

(MKK3); MAP2K4 (MKK4); MAP2K6 (MKK6); MAP2K7 (MKK7); MAP3K1 (MEKK1); MAP3K3; MAP3K4; MAP3K5; MAP3K8; MAP3K14 (NIK); MAPK8 (JNK1); MAPK9 (JNK2); MAPK10 (JNK3); MAPK11 (p38β); MAPK12 (p38γ); MAPK13 (p38δ); MAPK14 (p38α); NCK; NFAT1; NFAT2; NFKB1; NFKB2; NFKBIA; NRAS; PAK1; PAK2; PAK3; PAK4; PIK3C2B; PIK3C3 (VPS34); PIK3CA; PIK3CB; PIK3CD; PIK3R1; PKCA; PKCB; PKCM; PKCQ; PLCY1; PRF1 (Perforin); PTEN; RAC1; RAF1; RELA; SDF1; SHP2; SLP76; SOS; SRC; TBK1; TCRA; TEC; TRAF6; VAV1; VAV2; or ZAP70.

414. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the antigen binding domain targets an antigen characteristic of an autoimmune or inflammatory disorder.

415. The fusosome, fusosome composition, fusosome preparation, or method of embodiment 414, wherein the autoimmune or inflammatory disorder is selected from chronic graft-vs-host disease (GVHD), lupus, arthritis, immune complex glomerulonephritis, goodpasture, uveitis, hepatitis, systemic sclerosis or scleroderma, type I diabetes, multiple sclerosis, cold agglutinin disease, Pemphigus vulgaris, Grave's disease, autoimmune hemolytic anemia, Hemophilia A, Primary Sjogren's Syndrome, thrombotic thrombocytopenia purrpura, neuromyelits optica, Evan's syndrome, IgM mediated neuropathy, cyroglobulinemia, dermatomyositis, idiopathic thrombocytopenia, ankylosing spondylitis, bullous pemphigoid, acquired angioedema, chronic urticarial, antiphospholipid demyelinating polyneuropathy, and autoimmune thrombocytopenia or neutropenia or pure red cell aplasias, while exemplary non-limiting examples of alloimmune diseases include allosensitization (see, for example, Blazar et al., 2015, Am. J. Transplant, 15(4):931-41) or xenosensitization from hematopoietic or solid organ transplantation, blood transfusions, pregnancy with fetal allosensitization, neonatal alloimmune thrombocytopenia, hemolytic disease of the newborn, sensitization to foreign antigens such as can occur with replacement of inherited or acquired deficiency disorders treated with enzyme or protein replacement therapy, blood products, and gene therapy, optionally wherein the antigen characteristic of an an autoimmune or inflammatory disorder is selected from a cell surface receptor, an ion channel-linked receptor, an enzyme-linked receptor, a G protein-coupled receptor,

receptor tyrosine kinase, tyrosine kinase associated receptor, receptor-like tyrosine phosphatase, receptor serine/ threonine kinase, receptor guanylyl cyclase, or histidine kinase associated receptor.

416. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the antigen binding domain targets an antigen characteristic of an infectious disease.

417. The fusosome, fusosome composition, fusosome preparation, or method of embodiment 416, wherein the infectious disease is selected from HIV, hepatitis B virus, hepatitis C virus, Human herpes virus, Human herpes virus 8 (HHV-8, Kaposi sarcoma-associated herpes virus (KSHV)), Human T-lymphotrophic virus-1 (HTLV-1), Merkel cell polyomavirus (MCV), Simian virus 40 (SV40), Eptstein-Barr virus, CMV, human papillomavirus, optionally wherein the antigen characteristic of an infectious disease is selected from a cell surface receptor, an ion channel-linked receptor, an enzyme-linked receptor, a G protein-coupled receptor, receptor tyrosine kinase, tyrosine kinase associated receptor, receptor-like tyrosine phosphatase, receptor serine/ threonine kinase, receptor guanylyl cyclase, histidine kinase associated receptor, HIV Env, gpl20, or CD4-induced epitope on HIV-1 Env.

418. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the target cell comprises an aggregated or misfolded membrane protein.

419. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome and/or compositions or preparations thereof, are capable of reducing levels (e.g., reduces levels) of the aggregated or misfolded protein in the target cell, or a method herein comprises reducing levels of the aggregated or misfolded protein in the target cell.

420. The fusosome, fusosome composition, fusosome preparation, or method of any of the

preceding embodiments, wherein the fusosome and/or compositions or preparations thereof, are capable of delivering (e.g., deliver) a membrane protein to the cell membrane of a target cell.

421. The fusosome, fusosome composition, fusosome preparation, or method of embodiment 420, wherein delivering the protein comprises delivering a nucleic acid (e.g., a DNA, a gDNA, a cDNA, an RNA, a pre-mRNA, an mRNA, etc.) encoding the protein to the target cell such that the target cell produces the protein and localizes it to the membrane.

422. The fusosome, fusosome composition, fusosome preparation, or method of embodiment 420 or 421, wherein the fusosome comprises, or the method further comprises delivering, the protein, and fusion of the fusosome with the target cell transfers the protein to the cell membrane of the target cell.

423. The fusosome, fusosome composition, fusosome preparation, or method of any of embodiments 420-422, wherein the protein comprises a cell surface ligand or an antibody that binds a cell surface receptor.

424. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome further comprises, or the method further comprises delivering, a second agent that comprises or encodes a second cell surface ligand or antibody that binds a cell surface receptor, and optionally further comprising or encoding one or more additional cell surface ligands or antibodies that bind a cell surface receptor (e.g., 1, 2, 3, 4, 5, 10, 20, 50, or more).

425. The fusosome, fusosome composition, fusosome preparation, or method of embodiment 424, wherein the first agent and the second agent form a complex, wherein optionally the complex further comprises one or more additional cell surface ligands.

426. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the agent comprises or encodes a cell surface receptor, e.g., a

cell surface that is exogenous or overexpressed relative to the source cell.

427. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosomes further comprise, or the method further comprises delivering, a second agent that comprises or encodes a second cell surface receptor, and optionally further comprises or encodes one or more additional cell surface receptors (e.g., 1, 2, 3, 4, 5, 10, 20, 50, or more cell surface receptors).

428. The fusosome, fusosome composition, fusosome preparation, or method of embodiment 427, wherein the second agent, e.g., therapeutic agent, is selected from a protein, protein complex (e.g., comprising at least 2, 3, 4, 5, 10, 20, or 50 proteins, e.g., at least at least 2, 3, 4, 5, 10, 20, or 50 different proteins) polypeptide, nucleic acid (e.g., DNA, chromosome, or RNA, e.g., mRNA, siRNA, or miRNA) or small molecule.

429. The fusosome, fusosome composition, fusosome preparation, or method of embodiment 427 or 428, wherein the first agent and the second agent form a complex, wherein optionally the complex further comprises one or more additional cell surface receptors, optionally wherein the the agent comprises or encodes an antigen or an antigen presenting protein.

430. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, delivers or is capable of delivering (e.g., deliver) a secreted agent, e.g., a secreted protein to a target site (e.g., an extracellular region), e.g., by delivering a nucleic acid (e.g., a DNA, a gDNA, a cDNA, an RNA, a pre-mRNA, an mRNA, etc.) encoding the protein to the target cell under conditions that allow the target cell to produce and secrete the protein.

431. The fusosome, fusosome composition, fusosome preparation, or method of embodiment 430, wherein the secreted protein is endogenous or exogenous relative to the source cell or relative to the target cell.

432. The fusosome, fusosome composition, fusosome preparation, or method of embodiment 430 or 431, wherein the secreted protein comprises a protein therapeutic, e.g., an antibody molecule, a cytokine, an enzyme, an autocrine signalling molecule, a paracrine signalling molecule, or a secretory granule.

433. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, delivers or is capable of delivering (e.g., deliver) a membrane protein or a secreted protein that is or comprises an antigen.

434. The fusosome, fusosome composition, fusosome preparation, or method of embodiment 433, wherein the fusosome, and/or composition or preparation thereof, delivers or is capable of delivering (e.g., deliver) a membrane protein or a secreted protein that is or comprises an antigen antigen presenting protein, optionally together (e.g., as a complex) with an antigen.

435. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, donates or is capable of donating (e.g., donate) one or more cell surface receptors to a target cell (e.g., an immune cell).

436. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the target cell is or comprises a tumor cell.

437. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, delivers or is capable of delivering (e.g., deliver) a membrane or secreted protein that is or comprises an immunostimulatory ligand, an antigen presenting protein, a tumor suppressor protein, a pro-apoptotic protein, or a receptor or binding partner for any of the foregoing.

438. The fusosome, fusosome composition, fusosome preparation, or method of any of the

preceding embodiments, wherein the fusosome comprises an agent (e.g., a membrane payload agent, nuclear payload agent, or organellar payload agent, and optionally at least one second agent) that is immunomodulatory, e.g., immunostimulatory.

439. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, causes or is capable of causing (e.g., cause) the target cell to present an antigen.

440. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, delivers or is capable of delivering (e.g., deliver) a nucleic acid to a target cell, e.g., to transiently modify gene expression in the target cell or to modify, for example by integration into, the genome of the target cell, for example to cause expression of a membrane protein (or secreted protein) as described herein.

441. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, delivers or is capable of delivering (e.g., deliver) a protein (e.g., a membrane protein such as a transporter protein or a secreted protein such as an immunosuppressive protein) to a target cell so that a protein deficiency of the target cell is rescued, at least transiently.

442. The fusosome, fusosome composition, fusosome preparation, or method of embodiment 441, the membrane protein may include one or more covalently-associated non-peptide moieties such as, for example, one or more carbohydrate moieties, lipid moieties, polyethylene glycol moieties, small molecules, etc, and combinations thereof.

443. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, causes or is capable of causing (e.g., cause) a target cell to secrete a protein, e.g., a therapeutic protein.

444. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome having a cell surface ligand is from a source cell chosen from a neutrophil (e.g., and the target cell is a tumor-infiltrating lymphocyte), dendritic cell (e.g., and the target cell is a naïve T cell), or neutrophil (e.g., and the target is a tumor cell or virus-infected cell).

445. The fusosome, fusosome composition, fusosome preparation, or method of embodiment 444, wherein the fusosome comprises a membrane complex, e.g., a complex comprising at least 2, 3, 4, or 5 proteins, e.g., a homodimer, heterodimer, homotrimer, heterotrimer, homotetramer, or heterotetramer.

446. The fusosome, fusosome composition, fusosome preparation, or method of embodiment 444 or 445, wherein the fusosome comprises an antibody, e.g., a toxic antibody, e.g., the fusosome and/or compositions or preparations thereof, are capable of delivering (e.g., deliver) the antibody to the target site, e.g., by homing to a target site.

447. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the source cell is an NK cell or a neutrophil.

448. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the membrane protein is selected from a cell surface receptor, an ion channel-linked receptor, an enzyme-linked receptor, a G protein-coupled receptor, receptor tyrosine kinase, tyrosine kinase associated receptor, receptor-like tyrosine phosphatase, receptor serine/ threonine kinase, receptor guanylyl cyclase, histidine kinase associated receptor, Epidermal Growth Factor Receptors (EGFR) (including ErbB1/EGFR, ErbB2/HER2, ErbB3/HER3, and ErbB4/HER4), Fibroblast Growth Factor Receptors (FGFR) (including FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, FGF7, FGF18, and FGF21) Vascular Endothelial Growth Factor Receptors (VEGFR) (including VEGF-A, VEGF-B, VEGF-C, VEGF-D, and PIGF), RET Receptor and the Eph Receptor Family (including EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, EphA8, EphA9, EphA10, EphB1, EphB2. EphB3, EphB4, and EphB6), CXCR1, CXCR2, CXCR3,

CXCR4, CXCR6, CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR8, CFTR, CIC-1, CIC-2, CIC-4, CIC-5, CIC-7, CIC-Ka, CIC-Kb, Bestrophins, TMEM16A, GABA receptor, glycin receptor, ABC transporters, NAV1.1, NAV1.2, NAV1.3, NAV1.4, NAV1.5, NAV1.6, NAV1.7, NAV1.8, NAV1.9, sphingosin-1-phosphate receptor (S1P1R), NMDA channel, transmembrane protein, multispan transmembrane protein, T-cell receptor motifs; T-cell alpha chains; T-cell β chains; T-cell γ chains; T-cell δ chains; CCR7; CD3; CD4; CD5; CD7; CD8; CD11b; CD11c; CD16; CD19; CD20; CD21 ; CD22; CD25; CD28; CD34; CD35; CD40; CD45RA; CD45RO; CD52; CD56; CD62L; CD68; CD80; CD95; CD117; CD127; CD133; CD137 (4-1 BB); CD163; F4/80; IL-4Ra; Sca-1 ; CTLA-4; GITR; GARP; LAP; granzyme B; LFA-1 ; transferrin receptor; NKp46, perforin, CD4+; Th1; Th2; Th17; Th40; Th22; Th9; Tfh, Canonical Treg. FoxP3+; Tr1; Th3; Treg17; TREG; CDCP1, NT5E, EpCAM, CEA, gpA33, Mucins, TAG-72, Carbonic anhydrase IX, PSMA, Folate binding protein, Gangliosides (e.g., CD2, CD3, GM2), Lewis-γ2, VEGF, VEGFR 1/2/3, αVβ3, α5β1, ErbB1/EGFR, ErbB1/HER2, ErB3, c-MET, IGF1R, EphA3, TRAIL-R1, TRAIL-R2, RANKL, FAP, Tenascin, PDL-1, BAFF, HDAC, ABL, FLT3, KIT, MET, RET, IL-1β, ALK, RANKL, mTOR, CTLA-4, IL-6, IL-6R, JAK3, BRAF, PTCH, Smoothened, PIGF, ANPEP, TIMP1, PLAUR, PTPRJ, LTBR, or ANTXR1, Folate receptor alpha (FRa), ERBB2 (Her2/neu), EphA2, IL-13Ra2, epidermal growth factor receptor (EGFR), Mesothelin, TSHR, CD19, CD123, CD22, CD30, CD171, CS-1, CLL-1, CD33, EGFRvIII , GD2, GD3, BCMA, MUC16 (CA125), L1CAM, LeY, MSLN, IL13R□1, L1-CAM, Tn Ag, prostate specific membrane antigen (PSMA), ROR1, FLT3, FAP, TAG72, CD38, CD44v6, CEA, EPCAM, B7H3, KIT, interleukin-11 receptor a (IL-11Ra), PSCA, PRSS21, VEGFR2, LewisY, CD24, platelet-derived growth factor receptor-beta (PDGFR-beta), SSEA-4, CD20, MUC1, NCAM, Prostase, PAP, ELF2M, Ephrin B2, IGF-1 receptor, CAIX, LMP2, gplOO, bcr-abl, tyrosinase, Fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, Folate receptor beta, TEM1/CD248, TEM7R, CLDN6, GPRC5D, CXORF61, CD97, CD179a, ALK, Polysialic acid, PLACl, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-la, MAGE-A1, legumain, HPV E6, E7, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, prostein, survivin, telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, Androgen receptor, Cyclin B1, MYCN, RhoC, TRP-2, CYPIB I, BORIS,

SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2, CD79a, CD79b, CD72, LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, GPC3, FCRL5, IGLL1, a neoantigen, CD133, CD15, CD184, CD24, CD56, CD26, CD29, CD44, HLA-A, HLA-B, HLA-C, (HLA-A,B,C) CD49f, CD151 CD340, CD200, tkrA, trkB, or trkC.

449. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome associates with and/or binds a target cell or a surface feature of a target cell.

450. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, causes or is capable of causing secretion of a protein from a target cell or ligand presentation on the surface of a target cell.

451. The fusosome, fusosome composition, fusosome preparation, or method of embodiment 450, wherein the fusosome and/or compositions or preparations thereof, causes or is capable of causing cell death of the target cell.

452. The fusosome, fusosome composition, fusosome preparation, or method of embodiment 450 or 451, wherein the fusosome is from a NK source cell.

453. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, senses and/or responses, or is capable of sending and/or responding to one or more local environment features, e.g., metabolite, interleukin, antigen, etc or combinations thereof.

454. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, is capable of chemotaxis, extravasation, and/or one or more metabolic activities (e.g., kyneurinine,

gluconeogenesis, prostaglandin fatty acid oxidation, adenosine metabolism, urea cycle, and thermogenic respiration).

455. The fusosome, fusosome composition, fusosome preparation, or method of embodiment 454, wherein the source cell is:

a) a neutrophil and the fusosome and/or compositions or preparations thereof, are capable of homing to a site of injury;

b) a macrophage and the fusosome and/or compositions or preparations thereof, are capable of phagocytosis or

c) a brown adipose tissue cell and the fusosome and/or compositions or preparations thereof, are capable of lipolysis.

456. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome:

a) the fusosome fuses at a higher rate with a target cell than with a non-target cell, e.g., by at least at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, e.g., in an assay of Example 54;

b) the fusosome fuses at a higher rate with a target cell than other fusosomes, e.g., by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, e.g., in an assay of Example 54; and/or

c) the fusosome fuses with target cells at a rate such that an agent in the fusosome is delivered to at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, of target cells after 24, 48, or 72 hours, e.g., in an assay of Example 54.

457. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome:

a) is present, per fusosome, at a copy number of at least, or no more than, 10, 50, 100, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000, 100,000,000, 500,000,000, or 1,000,000,000 copies, e.g., as measured by an assay of Example 29; or

b) is present at a copy number of at least 1,000 copies, e.g., as measured by an assay of

Example 29.

458. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% of the fusogen comprised by the fusosome is disposed in the cell membrane.

459. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome also comprises fusogen internally, e.g., in the cytoplasm or an organelle.

460. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome comprises:

a) a therapeutic agent (e.g., a therapeutic membrane payload agent, nuclear payload agent, or organellar payload agent) at a copy number per fusosome of at least, or no more than, 10, 50, 100, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000, 100,000,000, 500,000,000, or 1,000,000,000 copies, e.g., as measured by an assay of Example 43;

b) a protein therapeutic agent at a copy number of at least 10, 50, 100, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000, 100,000,000, 500,000,000, or 1,000,000,000 copies, e.g., as measured by an assay of Example 43;

c) a nucleic acid therapeutic agent at a copy number of at least 10, 50, 100, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000, 100,000,000, 500,000,000, or 1,000,000,000 copies;

d) a DNA therapeutic agent at a copy number of at least 10, 50, 100, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000, 100,000,000, 500,000,000, or 1,000,000,000 copies;

e) an RNA therapeutic agent at a copy number of at least 10, 50, 100, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000, 1,000,000, 5,000,000, 10,000,000,

50,000,000, 100,000,000, 500,000,000, or 1,000,000,000 copies;

f) a therapeutic agent that is exogenous relative to the source cell at a copy number of at least 10, 50, 100, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000, 100,000,000, 500,000,000, or 1,000,000,000 copies;

g) a protein therapeutic agent that is exogenous relative to the source cell at a copy number of at least 10, 50, 100, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000, 100,000,000, 500,000,000, or 1,000,000,000 copies; and/or

h) a nucleic acid (e.g., DNA or RNA) therapeutic agent that is exogenous relative to the source cell at a copy number of at least 10, 50, 100, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000, 100,000,000, 500,000,000, or 1,000,000,000 copies.

461. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the ratio of the copy number of the fusogen to the copy number of the therapeutic agent is between 1,000,000:1 and 100,000:1, 100,000:1 and 10,000:1, 10,000:1 and 1,000:1, 1,000:1 and 100:1, 100:1 and 50:1, 50:1 and 20:1, 20:1 and 10:1, 10:1 and 5:1, 5:1 and 2:1, 2:1 and 1:1, 1:1 and 1:2, 1:2 and 1:5, 1:5 and 1:10, 1:10 and 1:20, 1:20 and 1:50, 1:50 and 1:100, 1:100 and 1:1,000, 1:1,000 and 1:10,000, 1:10,000 and 1:100,000, or 1:100,000 and 1:1,000,000.

462. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome delivers to a target cell:

a) at least 10, 50, 100, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000, 100,000,000, 500,000,000, or 1,000,000,000 copies of a therapeutic agent (e.g., a therapeutic membrane payload agent, nuclear payload agent, or organellar payload agent);

b) at least 10, 50, 100, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000, 100,000,000, 500,000,000, or

1,000,000,000 copies of a protein therapeutic agent;

c) at least 10, 50, 100, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000, 100,000,000, 500,000,000, or 1,000,000,000 copies of a nucleic acid therapeutic agent;

d) at least 10, 50, 100, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000, 100,000,000, 500,000,000, or 1,000,000,000 copies of an RNA therapeutic agent; and/or

e) at least 10, 50, 100, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000, 100,000,000, 500,000,000, or 1,000,000,000 copies of a DNA therapeutic agent.

463. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, comprises:

a) 0.00000001 mg fusogen to 1 mg fusogen per mg of total protein in fusosome, e.g., 0.00000001 - 0.0000001, 0.0000001 - 0.000001, 0.000001 - 0.00001, 0.00001 - 0.0001, 0.0001 - 0.001, 0.001 - 0.01, 0.01 - 0.1, or 0.1 – 1 mg fusogen per mg of total protein in fusosome; or

b) 0.00000001 mg fusogen to 5 mg fusogen per mg of lipid in fusosome, e.g., 0.00000001 - 0.0000001, 0.0000001 - 0.000001, 0.000001 - 0.00001, 0.00001 - 0.0001, 0.0001 - 0.001, 0.001 - 0.01, 0.01 - 0.1, 0.1 – 1, or 1-5 mg fusogen per mg of lipid in fusosome.

464. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, is characterized by a lipid composition substantially similar to that of the source cell or wherein one or more of CL, Cer, DAG, HexCer, LPA, LPC, LPE, LPG, LPI, LPS, PA, PC, PE, PG, PI, PS, CE, SM and TAG is within 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50%, e.g., within 75%, of the corresponding lipid level in the source cell.

465. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, is

characterized by a ratio of cardiolipin: ceramide that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cardiolipin: ceramide in the source cell; or by a ratio of cardiolipin: diacylglycerol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cardiolipin: diacylglycerol in the source cell; or by a ratio of cardiolipin: hexosylceramide that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cardiolipin: hexosylceramide in the source cell; or by a ratio of cardiolipin:lysophosphatidate that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cardiolipin: lysophosphatidate in the source cell; or by a ratio of cardiolipin: lyso-phosphatidylcholine that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cardiolipin: lyso-phosphatidylcholine in the source cell; or by a ratio of cardiolipin: lyso-phosphatidylethanolamine that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cardiolipin: lyso-phosphatidylethanolamine in the source cell; or by a ratio of cardiolipin: lyso-phosphatidylglycerol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cardiolipin : lyso-phosphatidylglycerol in the source cell; or by a ratio of cardiolipin: lyso-phosphatidylinositol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cardiolipin : lyso-phosphatidylinositol in the source cell; or by a ratio of cardiolipin: lyso-phosphatidylserine that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cardiolipin : lyso-phosphatidylserine in the source cell; or by a ratio of cardiolipin: phosphatidate that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cardiolipin : phosphatidate in the source cell; or by a ratio of cardiolipin: phosphatidylcholine that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cardiolipin : phosphatidylcholine in the source cell; or by a ratio of cardiolipin: phosphatidylethanolamine that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cardiolipin : phosphatidylethanolamine in the source cell; or by a ratio of cardiolipin: phosphatidylglycerol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cardiolipin : phosphatidylglycerol in the source cell; or by a ratio of cardiolipin: phosphatidylinositol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cardiolipin : phosphatidylinositol in the source cell; or by a ratio of cardiolipin: phosphatidylserine that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cardiolipin : phosphatidylserine in the source cell; or by a ratio of cardiolipin: cholesterol ester that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cardiolipin : cholesterol ester in the source cell; or by a ratio of cardiolipin: sphingomyelin that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cardiolipin : sphingomyelin in the source cell; or by a ratio of cardiolipin: triacylglycerol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cardiolipin

: triacylglycerol in the source cell; or by a ratio of phosphatidylcholine: ceramide that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylcholine: ceramide in the source cell; or by a ratio of phosphatidylcholine: diacylglycerol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylcholine: diacylglycerol in the source cell; or by a ratio of phosphatidylcholine: hexosylceramide that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylcholine: hexosylceramide in the source cell; or by a ratio of phosphatidylcholine:lysophosphatidate that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylcholine: lysophosphatidate in the source cell; or by a ratio of phosphatidylcholine: lyso-phosphatidylcholine that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylcholine: lyso-phosphatidylcholine in the source cell; or by a ratio of phosphatidylcholine: lyso-phosphatidylethanolamine that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylcholine: lyso-phosphatidylethanolamine in the source cell; or by a ratio of phosphatidylcholine: lyso-phosphatidylglycerol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylcholine : lyso-phosphatidylglycerol in the source cell; or by a ratio of phosphatidylcholine: lyso-phosphatidylinositol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylcholine : lyso-phosphatidylinositol in the source cell; or by a ratio of phosphatidylcholine: lyso-phosphatidylserine that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylcholine : lyso-phosphatidylserine in the source cell; or by a ratio of phosphatidylcholine: phosphatidate that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cardiolipin : phosphatidate in the source cell; or by a ratio of phosphatidylcholine: phosphatidylethanolamine that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylcholine : phosphatidylethanolamine in the source cell; or by a ratio of cardiolipin: phosphatidylglycerol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylcholine : phosphatidylglycerol in the source cell; or by a ratio of phosphatidylcholine: phosphatidylinositol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylcholine : phosphatidylinositol in the source cell; or by a ratio of phosphatidylcholine: phosphatidylserine that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylcholine : phosphatidylserine in the source cell; or by a ratio of phosphatidylcholine: cholesterol ester that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylcholine : cholesterol ester in the source cell; or by a ratio of phosphatidylcholine: sphingomyelin that is within 10%, 20%, 30%,

40%, or 50% of the ratio of phosphatidylcholine : sphingomyelin in the source cell; or by a ratio of phosphatidylcholine: triacylglycerol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylcholine : triacylglycerol in the source cell; or by a ratio of phosphatidylethanolamine: ceramide that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylethanolamine: ceramide in the source cell; or by a ratio of phosphatidylethanolamine: diacylglycerol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylethanolamine: diacylglycerol in the source cell; or by a ratio of phosphatidylethanolamine: hexosylceramide that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylethanolamine: hexosylceramide in the source cell; or by a ratio of phosphatidylethanolamine:lysophosphatidate that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylethanolamine: lysophosphatidate in the source cell; or by a ratio of phosphatidylethanolamine: lyso-phosphatidylcholine that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylethanolamine: lyso-phosphatidylcholine in the source cell; or by a ratio of phosphatidylethanolamine: lyso-phosphatidylethanolamine that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylethanolamine: lyso-phosphatidylethanolamine in the source cell; or by a ratio of phosphatidylethanolamine: lyso-phosphatidylglycerol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylethanolamine : lyso-phosphatidylglycerol in the source cell; or by a ratio of phosphatidylethanolamine: lyso-phosphatidylinositol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylethanolamine : lyso-phosphatidylinositol in the source cell; or by a ratio of phosphatidylethanolamine: lyso-phosphatidylserine that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylethanolamine : lyso-phosphatidylserine in the source cell; or by a ratio of phosphatidylethanolamine: phosphatidate that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylethanolamine : phosphatidate in the source cell; or by a ratio of phosphatidylethanolamine: phosphatidylglycerol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylethanolamine : phosphatidylglycerol in the source cell; or by a ratio of phosphatidylethanolamine: phosphatidylinositol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylethanolamine : phosphatidylinositol in the source cell; or by a ratio of phosphatidylethanolamine: phosphatidylserine that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylethanolamine : phosphatidylserine in the source cell; or by a ratio of phosphatidylethanolamine: cholesterol ester that is within 10%, 20%, 30%, 40%, or 50% of the

ratio of phosphatidylethanolamine : cholesterol ester in the source cell; or by a ratio of phosphatidylethanolamine: sphingomyelin that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylethanolamine : sphingomyelin in the source cell; or by a ratio of phosphatidylethanolamine: triacylglycerol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylethanolamine : triacylglycerol in the source cell; or by a ratio of phosphatidylserine: ceramide that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylserine: ceramide in the source cell; or by a ratio of phosphatidylserine: diacylglycerol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylserine: diacylglycerol in the source cell; or by a ratio of phosphatidylserine: hexosylceramide that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylserine: hexosylceramide in the source cell; or by a ratio of phosphatidylserine:lysophosphatidate that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylserine: lysophosphatidate in the source cell; or by a ratio of phosphatidylserine: lyso-phosphatidylcholine that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylserine: lyso-phosphatidylcholine in the source cell; or by a ratio of phosphatidylserine: lyso-phosphatidylethanolamine that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylserine: lyso-phosphatidylethanolamine in the source cell; or by a ratio of phosphatidylserine: lyso-phosphatidylglycerol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylserine : lyso-phosphatidylglycerol in the source cell; or by a ratio of phosphatidylserine: lyso-phosphatidylinositol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylserine : lyso-phosphatidylinositol in the source cell; or by a ratio of phosphatidylserine: lyso-phosphatidylserine that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylserine : lyso-phosphatidylserine in the source cell; or by a ratio of phosphatidylserine: phosphatidate that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylserine : phosphatidate in the source cell; or by a ratio of phosphatidylserine: phosphatidylglycerol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylserine : phosphatidylglycerol in the source cell; or by a ratio of phosphatidylserine: phosphatidylinositol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylserine : phosphatidylinositol in the source cell; or by a ratio of phosphatidylserine: cholesterol ester that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylserine : cholesterol ester in the source cell; or by a ratio of phosphatidylserine: sphingomyelin that is within 10%, 20%, 30%,

40%, or 50% of the ratio of phosphatidylserine : sphingomyelin in the source cell; or by a ratio of phosphatidylserine: triacylglycerol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylserine : triacylglycerol in the source cell; or by a ratio of sphingomyelin: ceramide that is within 10%, 20%, 30%, 40%, or 50% of the ratio of sphingomyelin: ceramide in the source cell; or by a ratio of sphingomyelin: diacylglycerol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of sphingomyelin: diacylglycerol in the source cell; or by a ratio of sphingomyelin: hexosylceramide that is within 10%, 20%, 30%, 40%, or 50% of the ratio of sphingomyelin: hexosylceramide in the source cell; or by a ratio of sphingomyelin:lysophosphatidate that is within 10%, 20%, 30%, 40%, or 50% of the ratio of sphingomyelin: lysophosphatidate in the source cell; or by a ratio of sphingomyelin: lyso-phosphatidylcholine that is within 10%, 20%, 30%, 40%, or 50% of the ratio of sphingomyelin: lyso-phosphatidylcholine in the source cell; or by a ratio of sphingomyelin: lyso-phosphatidylethanolamine that is within 10%, 20%, 30%, 40%, or 50% of the ratio of sphingomyelin: lyso-phosphatidylethanolamine in the source cell; or by a ratio of sphingomyelin: lyso-phosphatidylglycerol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of sphingomyelin : lyso-phosphatidylglycerol in the source cell; or by a ratio of sphingomyelin: lyso-phosphatidylinositol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of sphingomyelin : lyso-phosphatidylinositol in the source cell; or by a ratio of sphingomyelin: lyso-phosphatidylserine that is within 10%, 20%, 30%, 40%, or 50% of the ratio of sphingomyelin : lyso-phosphatidylserine in the source cell; or by a ratio of sphingomyelin: phosphatidate that is within 10%, 20%, 30%, 40%, or 50% of the ratio of sphingomyelin : phosphatidate in the source cell; or by a ratio of sphingomyelin: phosphatidylglycerol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of sphingomyelin : phosphatidylglycerol in the source cell; or by a ratio of sphingomyelin: phosphatidylinositol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of sphingomyelin : phosphatidylinositol in the source cell; or by a ratio of sphingomyelin: cholesterol ester that is within 10%, 20%, 30%, 40%, or 50% of the ratio of sphingomyelin : cholesterol ester in the source cell; or by a ratio of sphingomyelin: triacylglycerol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of sphingomyelin : triacylglycerol in the source cell; or by a ratio of cholesterol ester: ceramide that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cholesterol ester: ceramide in the source cell; or by a ratio of cholesterol ester: diacylglycerol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cholesterol ester: diacylglycerol in the source cell;

or by a ratio of cholesterol ester: hexosylceramide that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cholesterol ester: hexosylceramide in the source cell; or by a ratio of cholesterol ester:lysophosphatidate that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cholesterol ester: lysophosphatidate in the source cell; or by a ratio of cholesterol ester: lyso-phosphatidylcholine that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cholesterol ester: lyso-phosphatidylcholine in the source cell; or by a ratio of cholesterol ester: lyso-phosphatidylethanolamine that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cholesterol ester: lyso-phosphatidylethanolamine in the source cell; or by a ratio of cholesterol ester: lyso-phosphatidylglycerol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cholesterol ester : lyso-phosphatidylglycerol in the source cell; or by a ratio of cholesterol ester: lyso-phosphatidylinositol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cholesterol ester : lyso-phosphatidylinositol in the source cell; or by a ratio of cholesterol ester: lyso-phosphatidylserine that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cholesterol ester : lyso-phosphatidylserine in the source cell; or by a ratio of cholesterol ester: phosphatidate that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cholesterol ester : phosphatidate in the source cell; or by a ratio of cholesterol ester: phosphatidylglycerol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cholesterol ester : phosphatidylglycerol in the source cell; or by a ratio of cholesterol ester: phosphatidylinositol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cholesterol ester : phosphatidylinositol in the source cell; or by a ratio of cholesterol ester: triacylglycerol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cholesterol ester : triacylglycerol in the source cell.

466. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, is characterized by:

a) a proteomic composition similar to that of the source cell, e.g., using an assay of Example 42;

b) ratio of lipids to proteins that is within 10%, 20%, 30%, 40%, or 50% of the corresponding ratio in the source cell, e.g., as measured using an assay of Example 49;

c) a ratio of proteins to nucleic acids (e.g., DNA or RNA) that is within 10%, 20%, 30%,

40%, or 50% of the corresponding ratio in the source cell, e.g., as measured using an assay of Example 50;

d) a ratio of proteins to DNA that is greater than the corresponding ratio in the source cell, e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% greater, e.g., as measured using an assay of Example 50;

e) a ratio of lipids to nucleic acids (e.g., DNA) that is within 10%, 20%, 30%, 40%, or 50% of the corresponding ratio in the source cell, e.g., as measured using an assay of Example 51; and/or

f) a ratio of lipids to nucleic acids (e.g., DNA) that is greater than the corresponding ratio in the source cell, e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% greater, e.g., as measured using an assay of Example 51.

467. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, is characterized by:

a) a half-life in a subject, e.g., in a mouse, that is within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% of the half life of a reference cell, e.g., the source cell, e.g., by an assay of Example 75; or

b) a half-life in a subject, e.g., in a mouse, that is at least 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 12 hours, or 24 hours, e.g., in a human subject or in a mouse, e.g., by an assay of Example 75.

468. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, transports or is capable of transporting glucose (e.g., labeled glucose, e.g., 2-NBDG) across a membrane, e.g., by at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% more than a negative control, e.g., an otherwise similar fusosome in the absence of glucose, e.g., as measured using an assay of Example 64.

469. The fusosome, fusosome composition, fusosome preparation, or method of any of the

preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, is characterized by:

a) esterase activity in the lumen that is within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of that of the esterase activity in a reference cell, e.g., the source cell or a mouse embryonic fibroblast, e.g., using an assay of Example 66;

b) a metabolic activity level (e.g., citrate synthase activity) that is within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the metabolic activity level in a reference cell, e.g., the source cell, e.g., as described in Example 68;

c) a metabolic activity level (e.g., citrate synthase activity) that is at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the metabolic activity level in a reference cell, e.g., the source cell, e.g., as described in Example 68;

d) a respiration level (e.g., oxygen consumption rate) that is within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the respiration level in a reference cell, e.g., the source cell, e.g., as described in Example 69;

e) a respiration level (e.g., oxygen consumption rate) that is at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the respiration level in a reference cell, e.g., the source cell, e.g., as described in Example 69; and/or

f) an Annexin-V staining level of at most 18,000, 17,000, 16,000, 15,000, 14,000, 13,000, 12,000, 11,000, or 10,000 MFI, e.g., using an assay of Example 70, or wherein the fusosome comprises an Annexin-V staining level at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% lower than the Annexin-V staining level of otherwise similar fusosomes, or a composition or preparation thereof, treated with menadione in the assay of Example 70.

470. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, wherein the fusosome comprises an Annexin-V staining level at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% lower than the Annexin-V staining level of a macrophage treated with menadione in the assay of Example 70.

471. The fusosome, fusosome composition, fusosome preparation, or method of any of the

preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, is characterized by:

a) a miRNA content level of at least at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or greater than that of the source cell, e.g., by an assay of Example 39;

b) a miRNA content level of at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or greater of the miRNA content level of the source cell (e.g., up to 100% of the miRNA content level of the source cell), e.g., by an assay of Example 39;

c) a total RNA content level of at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or greater of the total RNA content level of the source cell (e.g., up to 100% of the total RNA content level of the source cell), e.g., as measured by an assay of Example 108;

d) a soluble : non-soluble protein ratio is within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or greater than that of the source cell, e.g., within 1%-2%, 2%-3%, 3%-4%, 4%-5%, 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, or 80%-90% of that of the source cell, e.g., by an assay of Example 47; and/or

e) an LPS level less than 5%, 1%, 0.5%, 0.01%, 0.005%, 0.0001%, 0.00001% or less of the lipid content of fusosomes, e.g., as measured by an assay of Example 48.

472. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, is capable of signal transduction, e.g., transmitting an extracellular signal, e.g., AKT phosphorylation in response to insulin, or glucose (e.g., labeled glucose, e.g., 2-NBDG) uptake in response to insulin, e.g., by at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% more than a negative control, e.g., an otherwise similar fusosome in the absence of insulin, e.g., using an assay of Example 63.

473. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, wherein the fusosome targets a tissue, e.g., liver, lungs, heart, spleen, pancreas, gastrointestinal tract, kidney, testes, ovaries, brain, reproductive organs, central nervous system, peripheral

nervous system, skeletal muscle, endothelium, inner ear, or eye, when administered to a subject, e.g., a mouse, e.g., wherein at least 0.1%, 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 60%, 70%, 80%, or 90% of the fusosomes in a population of administered fusosomes are present in the target tissue after 24, 48, or 72 hours, e.g., by an assay of Example 87 or 100.

474. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, is characterized by:

a) a juxtacrine-signaling level of at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% greater than the level of juxtacrine signaling induced by a reference cell, e.g., the source cell or a bone marrow stromal cell (BMSC), e.g., by an assay of Example 71;

b) a juxtacrine-signaling level of at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% (e.g., up to100%) of the level of juxtacrine signaling induced by a reference cell, e.g., the source cell or a bone marrow stromal cell (BMSC), e.g., by an assay of Example 71;

c) a paracrine-signaling level of at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% greater than the level of paracrine signaling induced by a reference cell, e.g., the source cell or a macrophage, e.g., by an assay of Example 72;

d) a paracrine-signaling level of at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% (e.g., up to100%) of the level of paracrine signaling induced by a reference cell, e.g., the source cell or a macrophage, e.g., by an assay of Example 72;

e) polymerizing actin at a level within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% compared to the level of polymerized actin in a reference cell, e.g., the source cell or a C2C12 cell, e.g., by the assay of Example 73; and/or

f) a membrane potential within about 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% of the membrane potential of a reference cell, e.g., the source cell or a C2C12 cell, e.g., by an assay of Example 74, or wherein provided fusosomes, and/or compositions or preparations thereof, are characterized by a membrane potential of about -20 to -

150mV, -20 to -50mV, -50 to -100mV, or -100 to -150mV, or wherein the fusosome has a membrane potential of less than -1mv, -5mv, -10mv, -20mv, -30mv, -40mv, -50mv, -60mv, -70mv, -80mv, -90mv, -100mv.

475. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, is capable of extravasation from blood vessels, e.g., at a rate at least 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% the rate of extravasation of the source cell, e.g., using an assay of Example 57, e.g., wherein the source cell is a neutrophil, lymphocyte, B cell, macrophage, or NK cell, optionally wherein provided fusosomes, and/or compositions or preparations thereof, are capable of chemotaxis, e.g., of at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% (e.g., up to 100%) compared to a reference cell, e.g., a macrophage, e.g., using an assay of Example 58.

476. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, is capable of phagocytosis, e.g., at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% (e.g., up to 100%) compared to a reference cell, e.g., a macrophage, e.g., using an assay of Example 60.

477. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, is capable of crossing a cell membrane, e.g., an endothelial cell membrane or the blood brain barrier.

478. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, is capable of:

a) secreting a protein, e.g., at a rate at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% greater than a reference cell, e.g., a mouse embryonic fibroblast, e.g., using an assay of Example 62; or

b) secreting a protein, e.g., at a rate at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% (e.g., up to 100%) compared to a reference cell, e.g., a mouse embryonic fibroblast, e.g., using an assay of Example 62.

479. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, is not capable of:

a) transcription or have transcriptional activity of less than 1%, 2.5% 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of that of the transcriptional activity of a reference cell, e.g., the source cell, e.g., using an assay of Example 19; or

b) nuclear DNA replication or has nuclear DNA replication of less than 1%, 2.5% 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the nuclear DNA replication of a reference cell, e.g., the source cell, e.g., using an assay of Example 20.

480. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, lacks chromatin or has a chromatin content of less than 1%, 2.5% 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the of the chromatin content of a reference cell, e.g., the source cell, e.g., using an assay of Example 37.

481. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, meets a pharmaceutical or good manufacturing practices (GMP) standard or was made according to GMP.

482. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof:

a) is characterized by a pathogen level below a predetermined reference value, e.g., are substantially free of pathogens;

b) comprises a contaminant (e.g., nuclear component such as nuclear DNA) level below a

predetermined reference value, e.g., are substantially free of one or more specified contaminants; and/or

c) is characterized by low immunogenicity, e.g., as described herein.

483. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the source cell or target cell is an endothelial cell, a fibroblast, a blood cell (e.g., a macrophage, a neutrophil, a granulocyte, a leukocyte), a stem cell (e.g., a mesenchymal stem cell, an umbilical cord stem cell, bone marrow stem cell, a hematopoietic stem cell, an induced pluripotent stem cell e.g., an induced pluripotent stem cell derived from a subject's cells), an embryonic stem cell (e.g., a stem cell from embryonic yolk sac, placenta, umbilical cord, fetal skin, adolescent skin, blood, bone marrow, adipose tissue, erythropoietic tissue, hematopoietic tissue), a myoblast, a parenchymal cell (e.g., hepatocyte), an alveolar cell, a neuron (e.g., a retinal neuronal cell) a precursor cell (e.g., a retinal precursor cell, a myeloblast, myeloid precursor cells, a thymocyte, a meiocyte, a megakaryoblast, a promegakaryoblast, a melanoblast, a lymphoblast, a bone marrow precursor cell, a normoblast, or an angioblast), a progenitor cell (e.g., a cardiac progenitor cell, a satellite cell, a radial gial cell, a bone marrow stromal cell, a pancreatic progenitor cell, an endothelial progenitor cell, a blast cell), or an immortalized cell (e.g., HeLa, HEK293, HFF-1, MRC-5, WI-38, IMR 90, IMR 91, PER.C6, HT-1080, or BJ cell), optionally wherein the source cell is other than a 293 cell, HEK cell, human endothelial cell, or a human epithelial cell, monocyte, macrophage, dendritic cell, or stem cell, optionally wherein the source cell or target cell is a white blood cell or a stem cell, optionally wherein the source cell or target cell is selected from a neutrophil, a lymphocyte (e.g., a T cell, a B cell, a natural killer cell), a macrophage, a granulocyte, a mesenchymal stem cell, a bone marrow stem cell, an induced pluripotent stem cell, an embryonic stem cell, or a myeloblast.

484. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the source cell is:

a) a cell grown under adherent or suspension conditions

b) a primary cell, a cultured cell, an immortalized cell, or a cell line (e.g., myelobast cell line, e.g., C2C12);

c) allogeneic, e.g., obtained from a different organism of the same species as the target cell;
d) autologous, e.g., obtained from the same organism as the target cell; or
e) heterologous, e.g., obtained from an organism of a different species from the target cell.

485. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the source cell comprises a second agent that is exogenous to the source cell, e.g., a therapeutic agent, e.g., a protein or a nucleic acid (e.g., an RNA, e.g., an mRNA or miRNA).

486. The fusosome, fusosome composition, fusosome preparation, or method of embodiment 485, wherein the second agent is present at least, or no more than, 10, 20, 50, 100, 200, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000 or 1,000,000 copies comprised by the fusosome, or is present at an average level of at least, or no more than, 10, 20, 50, 100, 200, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000 or 1,000,000 copies per fusosome.

487. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the target cell is in an organism, e.g., a primary cell isolated from an organism.

488. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the targeting domain interacts with a target cell moiety on the target cell, e.g., a cell surface feature.

489. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome does not comprise said target cell moiety.

490. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome comprises a fusogen which interacts with a fusogen binding partner on the target cell, thereby allowing the fusosome to bind or fuse to the target cell,

optionally wherein the fusosome does not comprise said fusogen binding partner.

491. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the targeting domain is not part of the fusogen or wherein the fusogen comprises the targeting domain.

492. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusogen binding partner is or is a portion of a different entity from the target cell moiety or wherein the fusogen binding partner is or is a portion of the target cell moiety.

493. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome enters the target cell by endocytosis, e.g., wherein the level of agent (e.g., membrane payload agent, nuclear payload agent, or organellar payload agent, and optionally a second agent) delivered via an endocytic pathway is 0.01-0.6, 0.01-0.1, 0.1-0.3, or 0.3-0.6, or at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater than a chloroquine treated reference cell contacted with similar fusosomes, e.g., using an assay of Example 91.

494. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% of fusosomes in a fusosome composition or preparation that enter a target cell enter via a non-endocytic pathway, e.g., the fusosomes enter the target cell via fusion with the cell surface, optionally wherein at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% of fusosomes in a fusosome composition or preparation that enter a target cell enter the cytoplasm (e.g., do not enter an endosome or lysosome).

495. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein less than 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, 5%, 4%, 3%, 2%, or 1% of fusosomes in a fusosome composition or preparation that enter a target

cell enter an endosome or lysosome.

496. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome enters the target cell by a non-endocytic pathway, e.g., wherein the level of agent (e.g., membrane payload agent, nuclear payload agent, or organellar payload agent, and optionally a second agent) delivered is at least 90%, 95%, 98%, or 99% that of a chloroquine treated reference cell, e.g., using an assay of Example 91.

497. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome delivers an agent (e.g., membrane payload agent, nuclear payload agent, or organellar payload agent, and optionally a second agent) to a target cell via a dynamin mediated pathway, e.g., wherein the level of agent (e.g., membrane protein payload agent and/or second agent) delivered via a dynamin mediated pathway is in the range of 0.01-0.6, or at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater than Dynasore treated target cells contacted with similar fusosomes, e.g., as measured in an assay of Example 92.

498. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome delivers an agent (e.g., membrane payload agent, nuclear payload agent, or organellar payload agent, and optionally a second agent) to a target cell via micropinocytosis, e.g., the level of agent (e.g., membrane payload agent, nuclear payload agent, or organellar payload agent, and optionally a second agent) delivered via macropinocytosis is in the range of 0.01-0.6, or at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater than EIPA treated target cells contacted with similar fusosomes, e.g., as measured in an assay of Example 92.

499. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome delivers an agent (e.g., membrane payload agent, nuclear payload agent, or organellar payload agent, and optionally a second agent) to a target cell via an actin-mediated pathway, e.g., wherein a level of agent (e.g., membrane payload agent,

nuclear payload agent, or organellar payload agent, and optionally a second agent) delivered via an actin-mediated pathway will be in the range of 0.01-0.6, or at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater than Latrunculin B treated target cells contacted with similar fusosomes, e.g., as measured in an assay of Example 92.

500. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, has a density of <1, 1-1.1, 1.05-1.15, 1.1-1.2, 1.15-1.25, 1.2-1.3, 1.25-1.35, or >1.35 g/mL, e.g., by an assay of Example 33.

501. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, comprises less than 0.01%, 0.05%, 0.1%, 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 4%, 5%, or 10% source cells by protein mass or wherein less than 0.01%, 0.05%, 0.1%, 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 4%, 5%, or 10% of cells have a functional nucleus.

502. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% of fusosomes in the fusosome composition or preparation comprise an organelle, e.g., a mitochondrion.

503. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, comprises a therapeutic agent that is exogenous relative to the source cell.

504. The fusosome, fusosome composition, fusosome preparation, or method of embodiment 503, wherein:

a) the therapeutic agent is exogenous relative to the target cell; and/or

b) the exogenous therapeutic agent is chosen from one or more of a protein, e.g., a transmembrane protein, a cell surface protein, a secreted protein, a receptor, an antibody; a nucleic

acid, e.g., DNA, a chromosome (e.g. a human artificial chromosome), RNA, mRNA, siRNA, miRNA, or a small molecule.

505. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome enters the cell by endocytosis or a non-endocytic pathway.

506. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, does not comprise a nucleus.

507. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome is substantially free of nuclear DNA.

508. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof:

a) is refrigerated or frozen;

b) does not comprise a functional nucleus, and/or provided fusosome compositions or preparations comprise one or more fusosomes without a functional nucleus;

c) comprises less than 0.01%, 0.05%, 0.1%, 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 4%, 5%, or 10% source cells by protein mass or less than 0.01%, 0.05%, 0.1%, 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 4%, 5%, or 10% of cells have a functional nucleus;

d) has been maintained at said temperature for at least 1, 2, 3, 6, or 12 hours; 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, or 4 weeks; 1, 2, 3, or 6 months; or 1, 2, 3, 4, or 5 years; and/or

e) is characterized by an activity of at least 50%, 60%, 70%, 80%, 90%, 95%, or 99% of the activity of the population before maintenance at said temperature, e.g., by one or more of:

i) fusing at a higher rate with a target cell than with a non-target cell, e.g., by at least at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, e.g., in an assay of Example 54;

ii) fusing at a higher rate with a target cell than with other fusosomes, e.g., by

at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, e.g., in an assay of Example 54;

iii) fusing with target cells at a rate such that an agent in the fusosome is delivered to at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, of target cells after 24, 48, or 72 hours, e.g., in an assay of Example 54; or

iv) level of fusogen at a copy number of at least, or no more than, 10, 50, 100, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000 or 1,000,000 copies, e.g., as measured by an assay of Example 29.

509. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, and/or composition or preparation thereof, is stable at a temperature of:

a) less than 4 C for at least 1, 2, 3, 6, or 12 hours; 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, or 4 weeks; 1, 2, 3, or 6 months; or 1, 2, 3, 4, or 5 years;

b) less than -20°C for at least 1, 2, 3, 6, or 12 hours; 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, or 4 weeks; 1, 2, 3, or 6 months; or 1, 2, 3, 4, or 5 years; or

c) less than -80°C for at least 1, 2, 3, 6, or 12 hours; 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, or 4 weeks; 1, 2, 3, or 6 months; or 1, 2, 3, 4, or 5 years.

510. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein one or more of the following is true:

i) the source cell is other than a 293 cell;

ii) the source cell is not transformed or immortalized;

iii) the source cell is transformed or immortalized using a method other than adenovirus-mediated immortalization, e.g., immortalized by spontaneous mutation or telomerase expression;

iv) the fusogen is other than VSVG, a SNARE protein, or a secretory granule protein;

v) the therapeutic agent is other than Cre or GFP, e.g., EGFP;

vi) the therapeutic agent is a nucleic acid (e.g., RNA, e.g., mRNA, miRNA, or siRNA) or a protein exogenous to the source cell (e.g., an antibody, e.g., an antibody), e.g., in the lumen;

or

vii) the fusosome does not comprise mitochondria.

511. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein one or more of the following is true:

i) the source cell is other than a 293 or HEK cell;

ii) the source cell is not transformed or immortalized;

iii) the source cell is transformed or immortalized using a method other than adenovirus-mediated immortalization, e.g., immortalized by spontaneous mutation or telomerase expression;

iv) the fusogen is not a viral fusogen; or

v) the fusosome has a diameter of other than between 40 and 150 nm, e.g., greater than 150 nm, 200 nm, 300 n, 400 nm, or 500 nm.

512. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein one or more of the following is true:

i) the membrane protein is expressed by the source cell;

ii) the fusogen is other than TAT, TAT-HA2, HA-2, gp41, Alzheimer's beta-amyloid peptide, a Sendai virus protein, or amphipathic net-negative peptide (WAE 11);

iii) the fusogen is a mammalian fusogen;

iv) the fusosome comprises in its lumen a polypeptide selected from an enzyme, antibody, or anti-viral polypeptide;

v) the fusosome does not comprise a therapeutic transmembrane protein, e.g., a therapeutic transmembrane protein that is exogenous relative to the source cell; or

vi) the fusosome does not comprise CD63 or GLUT4.

513. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein one or more of the following is true:

i) does not comprise a virus, is not infectious, or does not propagate in a host cell;

ii) is not a VLP (virus like particle);

iii) does not comprise a viral structural protein, e.g., a viral capsid protein, e.g., a viral nucleocapsid protein, or wherein the amount of viral capsid protein is less than 10%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.2%, or 0.1% of total protein, e.g., by an assay of Example 53;

iv) does not comprise a viral matrix protein;

v) does not comprise a viral non-structural protein;

vi) comprises less than 10, 50, 100, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000, 100,000,000, 500,000,000, 1,000,000,000 copies per vesicle of a viral structural protein; or

vii) the fusosome is not a virosome.

514. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the ratio of the copy number of the fusogen to the copy number of viral structural protein on the fusosome is at least 1,000,000:1, 100,000:1, 10,000:1, 1,000:1, 100:1, 50:1 1, 20:1, 10:1, 5:1, or 1:1; or wherein the ratio of the copy number of the fusogen to the copy number of viral matrix protein on the fusosome is at least 1,000,000:1, 100,000:1, 10,000:1, 1,000:1, 100:1, 50:1, 20:1, 10:1, 5:1, or 1:1.

515. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein one or more of the following is true:

i) the fusosome does not comprise a water-immiscible droplet;

ii) the fusosome comprises an aqueous lumen and a hydrophilic exterior;

iii) the fusogen is a protein fusogen.

516. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein one or more of the following is true:

i) the fusogen is a mammalian fusogen or a viral fusogen;

ii) the fusosome was not made by loading the fusosome with a therapeutic or diagnostic substance;

iii) the source cell was not loaded with a therapeutic or diagnostic substance;

iv) the fusosome does not comprise doxorubicin, dexamethasone, cyclodextrin; polyethylene

glycol, a micro RNA e.g., miR125, VEGF receptor, ICAM-1, E-selectin, iron oxide, a fluorescent protein e.g., GFP or RFP, a nanoparticle, or an RNase, or does not comprise an exogenous form of any of the foregoing that is exogenous to the source cell; or

v) the fusosome further comprises a therapeutic agent that is exogenous to the source cell, having one or more post-translational modifications, e.g., glycosylation.

517. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome is unilamellar or multilamellar.

518. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, fusosome composition, or fusosome preparation is characterized by:

a) a diameter within about 0.01%, 0.05%, 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, of that of the source cell, e.g., as measured by an assay of Example 30;

b) a diameter that is less than about 0.01%, 0.05%, 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, of that of the source cell, e.g., as measured by an assay of Example 30;

c) a diameter within about 0.01%-0.05%, 0.05%-0.1%, 0.1%-0.5%, 0.5%- 1%, 1%-2%, 2%-3%, 3%-4%, 4%-5%, 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, or 80%-90% the diameter of the source cell, e.g., as measured by an assay of Example 30;

d) a diameter that is less than about 0.01%-0.05%, 0.05%-0.1%, 0.1%-0.5%, 0.5%- 1%, 1%-2%, 2%-3%, 3%-4%, 4%-5%, 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, or 80%-90% of the diameter of the source cell, e.g., as measured by an assay of Example 30;

e) a diameter that is at least about 10 nm, 20 nm, 30 nm, 40 nm, 50 nm, 60 nm, 70 nm, 80 nm, 90 nm, 100 nm, 150 nm, 200 nm, or 250 nm e.g., as measured by an assay of Example 32;

f) a diameter that is about 10 nm, 20 nm, 30 nm, 40 nm, 50 nm, 60 nm, 70 nm, 80 nm, 90 nm, 100 nm, 150 nm, 200 nm, or 250 nm (e.g., ±20%) e.g., as measured by an assay of Example

32;

g) a diameter that is at least about 500 nm, 750 nm, 1,000 nm, 1,500 nm, 2,000 nm, 2,500 nm, 3,000 nm, 5,000 nm, 10,000 nm, or 20,000 nm, e.g., as measured by an assay of Example 32;

h) a diameter that is about 500 nm, 750 nm, 1,000 nm, 1,500 nm, 2,000 nm, 2,500 nm, 3,000 nm, 5,000 nm, 10,000 nm, or 20,000 nm (e.g., ±20%), e.g., as measured by an assay of Example 32; and/or

i) a diameter that is greater than 5 μm, 6 μm, 7 μm, 8 μm, 10 μm, 20 μm, 50 μm, 100 μm, 150 μm, or 200 μm.

519. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, fusosome composition, or fusosome preparation has a volume that is less than about 0.01%-0.05%, 0.05%-0.1%, 0.1%-0.5%, 0.5%- 1%, 1%-2%, 2%-3%, 3%-4%, 4%-5%, 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, or 80%-90%% of the volume of the source cell.

520. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, fusosome composition, or fusosome preparation has a density of other than between 1.08 g/mL and 1.12 g/mL, optionally wherein the density is 1.25 g/mL +/- 0.05, e.g., as measured by an assay of Example 33, optionally wherein the density is <1, 1-1.1, 1.05-1.15, 1.1-1.2, 1.15-1.25, 1.2-1.3, 1.25-1.35, or >1.35 g/mL, e.g., by an assay of Example 33.

521. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein one or more of the following is true:

i) the fusosome is not an exosome;

ii) the fusosome is a microvesicle;

iii) the fusosome comprises a non-mammalian fusogen;

iv) the fusosome has been engineered to comprise a fusogen;

v) the fusosome comprises a fusogen that is exogenous relative to the source cell or an overexpressed fusogen;

vi) the fusosome has a diameter of at least 80 nm, 100 nm, 200 nm, 500 nm, 1000 nm, 1200 nm, 1400 nm, or 1500 nm, or a population or plurality of fusosomes has an average diameter of at least 80 nm, 100 nm, 200 nm, 500 nm, 1000 nm, 1200 nm, 1400 nm, or 1500 nm;

vii) the fusosome comprises one or more organelles, e.g., a mitochondrion, Golgi apparatus, lysosome, endoplasmic reticulum, vacuole, endosome, acrosome, autophagosome, centriole, glycosome, glyoxysome, hydrogenosome, melanosome, mitosome, cnidocyst, peroxisome, proteasome, vesicle, and stress granule;

viii) the fusosome comprises a cytoskeleton or a component thereof, e.g., actin, Arp2/3, formin, coronin, dystrophin, keratin, myosin, or tubulin;

ix) a preparation comprising a plurality of the fusosomes does not have a flotation density of 1.08–1.22 g/mL, or has a density of at least 1.18–1.25 g/mL, or 1.05–1.12 g/mL, e.g., in a sucrose gradient centrifugation assay, e.g., as described in Théry et al., "Isolation and characterization of exosomes from cell culture supernatants and biological fluids." Curr Protoc Cell Biol. 2006 Apr; Chapter 3:Unit 3.22;

x) the lipid bilayer is enriched for ceramides or sphingomyelins or a combination thereof compared to the source cell, or the lipid bilayer is not enriched (e.g., is depleted) for glycolipids, free fatty acids, or phosphatidylserine, or a combination thereof, compared to the source cell;

xi) the fusosome comprises Phosphatidyl serine (PS) or CD40 ligand or both of PS and CD40 ligand, e.g., when measured in an assay of Example 52;

xii) the fusosome is enriched for PS compared to the source cell, e.g., in a population of fusosomes at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% are positive for PS by an assay of Kanada M, et al. (2015) Differential fates of biomolecules delivered to target cells via extracellular vesicles. Proc Natl Acad Sci USA 112:E1433–E1442;

xiii) the fusosome is substantially free of acetylcholinesterase (AChE), or contains less than 0.001, 0.002, 0.005, 0.01,0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, 200, 500, or 1000 AChE activity units/μg of protein , e.g., by an assay of Example 67;

xiv) the fusosome is substantially free of a Tetraspanin family protein (e.g., CD63, CD9, or CD81), an ESCRT-related protein (e.g., TSG101, CHMP4A-B, or VPS4B), Alix, TSG101, MHCI, MHCII, GP96, actinin-4, mitofilin, syntenin-1, TSG101, ADAM10, EHD4, syntenin-1,

TSG101, EHD1, flotillin-1, heat-shock 70-kDa proteins (HSC70/HSP73, HSP70/HSP72), or any combination thereof, or contains less than 0.05%, 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 5%, or 10% of any individual exosomal marker protein and/or less than 0.05%, 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, or 25% of total exosomal marker proteins of any of said proteins, or is de-enriched for any one or more of these proteins compared to the source cell, or is not enriched for any one or more of these proteins, e.g., by an assay of Example 44;

xv) the fusosome comprises a level of Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) that is below 500, 250, 100, 50, 20, 10, 5, or 1 ng GAPDH/μg total protein or below the level of GAPDH in the source cell, e.g., less than 1%, 2.5%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, less than the level of GAPDH per total protein in ng/μg in the source cell, e.g., using an assay of Example 45;

xvi) the fusosome is enriched for one or more endoplasmic reticulum proteins (e.g., calnexin), one or more proteasome proteins, or one or more mitochondrial proteins, or any combination thereof, e.g., wherein the amount of calnexin is less than 500, 250, 100, 50, 20, 10, 5, or 1 ng Calnexin / μg total protein, or wherein the fusosome comprises less Calnexin per total protein in ng/μg compared to the source cell by 1%, 2.5%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, e.g., using an assay of Example 46;

xvii) the fusosome comprises an agent (e.g., protein, mRNA, or siRNA) that is exogenous relative to the source cell, e.g., as measured using an assay of Example 39 or 40; or

xviii) the fusosome can be immobilized on a mica surface by atomic force microscopy for at least 30 min, e.g., by an assay of Kanada M, et al. (2015) Differential fates of biomolecules delivered to target cells via extracellular vesicles. Proc Natl Acad Sci USA 112:E1433–E1442.

522. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein one or more of the following is true:

i) the fusosome is an exosome;

ii) the fusosome is not a microvesicle;

iii) the fusosome has a diameter of less than 80 nm, 100 nm, 200 nm, 500 nm, 1000 nm, 1200 nm, 1400 nm, or 1500 nm, or a population of fusosomes has an average diameter of at least 80 nm, 100 nm, 200 nm, 500 nm, 1000 nm, 1200 nm, 1400 nm, or 1500 nm;

iv) the fusosome does not comprise an organelles;

v) the fusosome does not comprise a cytoskeleton or a component thereof, e.g., actin, Arp2/3, formin, coronin, dystrophin, keratin, myosin, or tubulin;

vi) a preparation comprising a plurality of the fusosomes has a flotation density of 1.08–1.22 g/mL, e.g., in a sucrose gradient centrifugation assay, e.g., as described in Théry et al., "Isolation and characterization of exosomes from cell culture supernatants and biological fluids." Curr Protoc Cell Biol. 2006 Apr; Chapter 3:Unit 3.22;

vii) the lipid bilayer is not enriched (e.g., is depleted) for ceramides or sphingomyelins or a combination thereof compared to the source cell, or the lipid bilayer is enriched for glycolipids, free fatty acids, or phosphatidylserine, or a combination thereof, compared to the source cell;

viii) the fusosome does not comprise, or is depleted for relative to the source cell, Phosphatidyl serine (PS) or CD40 ligand or both of PS and CD40 ligand, e.g., when measured in an assay of Example 52;

ix) the fusosome is not enriched (e.g., is depleted) for PS compared to the source cell, e.g., in a population of fusosomes less than 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% are positive for PS by an assay of Kanada M, et al. (2015) Differential fates of biomolecules delivered to target cells via extracellular vesicles. Proc Natl Acad Sci USA 112:E1433–E1442;

x) the fusosome comprises acetylcholinesterase (AChE), e.g. at least 0.001, 0.002, 0.005, 0.01,0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, 200, 500, or 1000 AChE activity units/μg of protein , e.g., by an assay of Example 67;

xi) the fusosome comprises a Tetraspanin family protein (e.g., CD63, CD9, or CD81), an ESCRT-related protein (e.g., TSG101, CHMP4A-B, or VPS4B), Alix, TSG101, MHCI, MHCII, GP96, actinin-4, mitofilin, syntenin-1, TSG101, ADAM10, EHD4, syntenin-1, TSG101, EHD1, flotillin-1, heat-shock 70-kDa proteins (HSC70/HSP73, HSP70/HSP72), or any combination thereof, e.g., contains more than 0.05%, 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 5%, or 10% of any individual exosomal marker protein and/or less than 0.05%, 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, or 25% of total exosomal marker proteins of any of said proteins, or is enriched for any one or more of these proteins compared to the source cell, e.g., by an assay of Example 44;

xii) the fusosome comprises a level of Glyceraldehyde 3-phosphate dehydrogenase

(GAPDH) that is above 500, 250, 100, 50, 20, 10, 5, or 1 ng GAPDH/μg total protein or below the level of GAPDH in the source cell, e.g., at least 1%, 2.5%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, greater than the level of GAPDH per total protein in ng/μg in the source cell, e.g., using an assay of Example 45;

xiii) the fusosome is not enriched for (e.g., is depleted for) one or more endoplasmic reticulum proteins (e.g., calnexin), one or more proteasome proteins, or one or more mitochondrial proteins, or any combination thereof, e.g., wherein the amount of calnexin is less than 500, 250, 100, 50, 20, 10, 5, or 1 ng Calnexin / μg total protein, or wherein the fusosome comprises less Calnexin per total protein in ng/μg compared to the source cell by 1%, 2.5%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, e.g., using an assay of Example 46; or

xiv) the fusosome can not be immobilized on a mica surface by atomic force microscopy for at least 30 min, e.g., by an assay of Kanada M, et al. (2015) Differential fates of biomolecules delivered to target cells via extracellular vesicles. Proc Natl Acad Sci USA 112:E1433–E1442.

523. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein one or more of the following is true:

i) the fusosome does not comprise a VLP;

ii) the fusosome does not comprise a virus;

iii) the fusosome does not comprise a replication-competent virus;

iv) the fusosome does not comprise a viral protein, e.g., a viral structural protein, e.g., a capsid protein or a viral matrix protein;

v) the fusosome does not comprise a capsid protein from an enveloped virus;

vi) the fusosome does not comprise a nucleocapsid protein; or

vii) the fusogen is not a viral fusogen.

524. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome comprises cytosol.

525. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome comprises or is comprised by a cytobiologic.

526. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome comprises or is comprised by an enucleated cell.

527. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome comprises or is comprised by a chondrisome.

528. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein one or more of the following is true:

i) the fusosome or the source cell does not form a teratoma when implanted into subject, e.g., by an assay of Example 102;

ii) the fusosome and/or compositions or preparations thereof, are capable of chemotaxis, e.g., of within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or greater than a reference cell, e.g., a macrophage, e.g., using an assay of Example 58;

iii) the fusosome and/or compositions or preparations thereof, are capable of homing, e.g., at the site of an injury, wherein the cytobiologic is from a human cell, e.g., using an assay of Example 59, e.g., wherein the source cell is a neutrophil; or

iv) the fusosome and/or compositions or preparations thereof, are capable of phagocytosis, e.g., wherein phagocytosis by the fusosome is detectable within .5, 1, 2, 3, 4, 5, or 6 hours in using an assay of Example 60, e.g., wherein the source cell is a macrophage.

529. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, fusosome composition, or fusosome preparation retains one, two, three, four, five six or more of any of the characteristics for 5 days or less, e.g., 4 days or less, 3 days or less, 2 days or less, 1 day or less, e.g., about 12-72 hours, after administration into a subject, e.g., a human subject.

530. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome has one or more of the following characteristics:

a) comprises one or more endogenous proteins from a source cell, e.g., membrane proteins or cytosolic proteins;

b) comprises at least 10, 20, 50, 100, 200, 500, 1000, 2000, or 5000 different proteins;

c) comprises at least 1, 2, 5, 10, 20, 50, or 100 different glycoproteins;

d) at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% by mass of the proteins in the fusosome are naturally-occurring proteins;

e) comprises at least 10, 20, 50, 100, 200, 500, 1000, 2000, or 5000 different RNAs; or

f) comprises at least 2, 3, 4, 5, 10, or 20 different lipids, e.g., selected from CL, Cer, DAG, HexCer, LPA, LPC, LPE, LPG, LPI, LPS, PA, PC, PE, PG, PI, PS, CE, SM and TAG.

531. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome has been manipulated to have, or the fusosome is not a naturally occurring cell and has, or wherein the nucleus does not naturally have one, two, three, four, five or more of the following properties:

a) the partial nuclear inactivation results in a reduction of at least 50%, 60%, 70%, 80%, 90% or more in nuclear function, e.g., a reduction in transcription or DNA replication, or both, e.g., wherein transcription is measured by an assay of Example 19 and DNA replication is measured by an assay of Example 20;

b) the fusosome is not capable of transcription or has transcriptional activity of less than 1%, 2.5% 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of that of the transcriptional activity of a reference cell, e.g., the source cell, e.g., using an assay of Example 19;

c) the fusosome is not capable of nuclear DNA replication or has nuclear DNA replication of less than 1%, 2.5% 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the nuclear DNA replication of a reference cell, e.g., the source cell, e.g., using an assay of Example 20;

d) the fusosome lacks chromatin or has a chromatin content of less than 1%, 2.5% 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the of the chromatin content of a reference cell, e.g., the source cell, e.g., using an assay of Example 37;

e) the fusosome lacks a nuclear membrane or has less than 50%, 40%, 30%, 20%, 10%, 5%, 4%, 3%, 2%, or 1% the amount of nuclear membrane of a reference cell, e.g., the source

cell or a Jurkat cell, e.g., by an assay of Example 36;

f) the fusosome lacks functional nuclear pore complexes or has reduced nuclear import or export activity, e.g., by at least 50%, 40%, 30%, 20%, 10%, 5%, 4%, 3%, 2%, or 1% by an assay of Example 36, or the fusosome lacks on or more of a nuclear pore protein, e.g., NUP98 or Importin 7.

g) the fusosome does not comprise histones or has histone levels less than 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the histone level of the source cell (e.g., of H1, H2a, H2b, H3, or H4), e.g., by an assay of Example 37;

h) the fusosome comprises less than 20, 10, 5, 4, 3, 2, or 1 chromosome;

i) nuclear function is eliminated;

j) the fusosome is an enucleated mammalian cell;

k) the nucleus is removed or inactivated, e.g., extruded by mechanical force, by radiation or by chemical ablation; or

l) the fusosome is from a mammalian cell having DNA that is completely or partially removed, e.g., during interphase or mitosis.

532. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein fusosome comprises mtDNA or vector DNA, e.g., wherein the fusosome does not comprise DNA, or is substantially free of DNA.

533. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the source cell is a primary cell, immortalized cell or a cell line (e.g., myelobast cell line, e.g., C2C12).

534. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome is from a source cell having a modified genome, e.g., having reduced immunogenicity (e.g., by genome editing, e.g., to remove an MHC protein, e.g., MHC complex).

535. The fusosome, fusosome composition, fusosome preparation, or method of any of the

preceding embodiments, wherein the source cell is from a cell culture treated with an immunosuppressive agent.

536. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the source cell is substantially non-immunogenic, e.g., using an assay described herein

537. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the source cell comprises an exogenous agent, e.g., a therapeutic agent.

538. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the source cell is a recombinant cell.

539. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the source cell is from a cell culture treated with an anti-inflammatory signal.

540. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome further comprises an agent that is exogenous relative to the source cell, e.g., a therapeutic membrane payload agent, nuclear payload agent, or organellar payload agent, e.g., a protein or a nucleic acid (e.g., a DNA, a chromosome (e.g. a human artificial chromosome), an RNA, e.g., an mRNA or miRNA); e.g., wherein the exogenous agent is present at at least, or no more than, 10, 20, 50, 100, 200, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000, 100,000,000, 500,000,000, or 1,000,000,000 copies.

541. The fusosome, fusosome composition, fusosome preparation, or method of embodiment 381, wherein the fusosome has an altered, e.g., increased or decreased level of one or more endogenous molecule, e.g., protein or nucleic acid (e.g., in some embodiments, endogenous relative to the

source cell, and in some embodiments, endogenous relative to the target cell), e.g., due to treatment of the source cell, e.g., mammalian source cell with a siRNA or gene editing enzyme.

542. The fusosome, fusosome composition, fusosome preparation, or method of embodiment 540 or 541, wherein the endogenous molecule is:

a) present at at least, or no more than, 10, 20, 50, 100, 200, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000, 100,000,000, 500,000,000, or 1,000,000,000 copies;

b) present at a concentration of at least 1, 2, 3, 4, 5, 10, 20, 50, 100, 500, 10^3, 5.0 x 10^3, 10^4, 5.0 x 10^4, 10^5, 5.0 x 10^5, 10^6, 5.0 x 10^6, 1.0 x 10^7, 5.0 x 10^7, or 1.0 x 10^8, greater than its concentration in the source cell;

c) present at a concentration of at least 1, 2, 3, 4, 5, 10, 20, 50, 100, 500, 10^3, 5.0 x 10^3, 10^4, 5.0 x 10^4, 10^5, 5.0 x 10^5, 10^6, 5.0 x 10^6, 1.0 x 10^7, 5.0 x 10^7, or 1.0 x 10^8 less than its concentration in the source cell.

543. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusogen:

a) is a viral fusogen, e.g., HA, HIV-1 ENV, gp120, or VSV-G;

b) is a mammalian fusogen, e.g., a SNARE, a Syncytin, myomaker, myomixer, myomerger, or FGFRL1;

c) is active at a pH of 4-5, 5-6, 6-7, 7-8, 8-9, or 9-10;

d) is not active at a pH of 4-5, 5-6, 6-7, 7-8, 8-9, or 9-10;

e) fuses to a target cell at the surface of the target cell;

f) promotes fusion in a lysosome-independent manner;

g) is a protein fusogen;

h) is a lipid fusogen, e.g., oleic acid, glycerol mono-oleate, a glyceride, diacylglycerol, or a modified unsaturated fatty acid;

i) is a chemical fusogen, e.g., PEG; optionally wherein the fusogen is a small molecule fusogen, e.g., halothane, an NSAID such as meloxicam, piroxicam, tenoxicam, and chlorpromazine;

j) is recombinant;

k) is biochemically incorporated, e.g., the fusogen is provided as a purified protein and contacted with a lipid bilayer under conditions that allow for associate of the fusogen with the lipid bilayer; and/or

l) is biosynthetically incorporated, e.g. expressed in a source cell under conditions that allow the fusogen to associate with the lipid bilayer.

544. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusogen binds a target cell, e.g., a target cell other than a HeLa cell, e.g., a target cell that is not transformed or immortalized.

545. The fusosome, fusosome composition, fusosome preparation, or method of embodiment 544, wherein the cell that is not transformed displays contact inhibition and/or its growth is dependent on the same survival factors or growth factors as a normal cell of the same type.

546. The fusosome composition or fusosome preparation of any of the preceding embodiments, wherein the plurality of fusosomes are the same, or are different.

547. The fusosome composition or fusosome preparation of embodiment 546, wherein the plurality of fusosomes are:

a) from one, two or more types of source cells; and/or

b) the same if at least 0.01%-0.05%, 0.05%-0.1%, 0.1%-0.5%, 0.5%- 1%, 1%-2%, 2%-3%, 3%-4%, 4%-5%, 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, or 80%-90% of the fusosomes in the fusosome composition share at least one property selected from: comprise the same fusogen; produced using the same type of source cell; or comprise the same payload (e.g., membrane payload agent, nuclear payload agent, or organellar payload agent).

548. The fusosome composition or fusosome preparation of embodiment 546 or 547, wherein:

a) at least 50%, 60%, 70%, 80%, 90%, 95%, or 99% of fusosomes in the plurality have a

diameter within 10%, 20%, 30%, 40%, or 50% of the mean diameter of the fusosomes in the fusosome composition or preparation; or

b) at least 50%, 60%, 70%, 80%, 90%, 95%, or 99% of fusosomes in the plurality have a volume within 10%, 20%, 30%, 40%, or 50% of the mean volume of the fusosomes in the fusosome composition or preparation.

549. The fusosome composition or fusosome preparation of any of embodiments 546-548, wherein the fusosome composition or preparation has less than about 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, 5%, variability in diameter distribution within 10%, 50%, or 90% of the source cell population variability in diameter distribution, e.g., based on Example 31.

550. The fusosome composition or fusosome preparation of any of embodiments 546-549, wherein at least 50%, 60%, 70%, 80%, 90%, 95%, or 99% of fusosomes in the plurality have a copy number of the fusogen within 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the mean fusogen copy number in the fusosomes in the fusosome composition or preparation.

551. The fusosome composition or fusosome preparation of any of embodiments 546-550, wherein at least 50%, 60%, 70%, 80%, 90%, 95%, or 99% of fusosomes in the plurality have a copy number of the therapeutic agent within 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the mean therapeutic agent copy number in the fusosomes in the fusosome composition or preparation.

552. The fusosome composition or fusosome preparation of any of embodiments 546-551, wherein the fusosome composition or preparation comprises at least 105, 106, 107, 108, 109, or 1010 or fusosomes. In some embodiments, the fusosome composition or preparation is in a volume of at least 1 μL, 2 μL, 5 μL, 10 μL, 20 μL, 50 μL, 100 μL, 200 μL, 500 μL, 1 mL, 2 mL, 5 mL, or 10 mL.

553. The fusosome composition or fusosome preparation of any of the preceding embodiments, wherein the plurality of fusosomes comprises at least 0.01%-0.05%, 0.05%-0.1%, 0.1%-0.5%,

0.5%- 1%, 1%-2%, 2%-3%, 3%-4%, 4%-5%, 5%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, or 80%-90% fusosomes which have one or more of the following characteristics:

(i) do not comprise a nucleus or a functional nucleus;

(ii) are substantially free of nuclear DNA; or

(iii) do not comprise mitochondria or functional mitochondria.

554. The pharmaceutical composition of any of the preceding embodiments, wherein one or more of the following is true:

a) the pharmaceutical composition meets a pharmaceutical or good manufacturing practices (GMP) standard;

b) the pharmaceutical composition was made according to good manufacturing practices (GMP);

c) the pharmaceutical composition has a pathogen level below a predetermined reference value, e.g., is substantially free of pathogens;

d) the pharmaceutical composition has a contaminant level (e.g., nuclear DNA) below a predetermined reference value, e.g., is substantially free of contaminants; or

e) the pharmaceutical composition has low immunogenicity, e.g., as described herein.

555. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the biological function is selected from:

a) modulating, e.g., inhibiting or stimulating, an enzyme;

b) modulating, e.g., increasing or decreasing levels of, a molecule (e.g., a protein, nucleic acid, or metabolite, drug, or toxin) in the subject, e.g., by inhibiting or stimulating synthesis or by inhibiting or stimulating degradation of the factor;

c) modulating, e.g., increasing or decreasing, viability of a target cell or tissue; or

d) modulating a protein state, e.g., increasing or decreasing phosphorylation of the protein, or modulating the protein conformation;

e) promoting healing of an injury;

f) modulating, e.g., increasing or decreasing, an interaction between two cells;

g) modulating, e.g., promoting or inhibiting, cell differentiation;

h) altering distribution of a factor (e.g., a protein, nucleic acid, metabolite, drug, or toxin) in the subject;

i) modulating, e.g. increasing or decreasing, an immune response; or

j) modulating, e.g. increasing or decreasing, recruitment of cells to a target tissue.

556. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the subject has a cancer, an inflammatory disorder, autoimmune disease, a chronic disease, inflammation, damaged organ function, an infectious disease, metabolic disease, degenerative disorder, genetic disease (e.g., a genetic deficiency or a dominant genetic disorder), or an injury.

557. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the subject has an infectious disease and the fusosome comprises an antigen for the infectious disease.

558. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the subject has a genetic deficiency and the fusosome comprises a protein for which the subject is deficient, or a nucleic acid (e.g., a DNA, a gDNA, a cDNA, an RNA, a pre-mRNA, an mRNA, etc.) encoding the protein, or a DNA encoding the protein, or a chromosome encoding the protein, or a nucleus comprising a nucleic acid encoding the protein.

559. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the subject has a dominant genetic disorder, and the fusosome comprises a nucleic acid inhibitor (e.g., siRNA or miRNA) of the dominant mutant allele.

560. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the subject has a dominant genetic disorder, and the fusosome comprises a nucleic acid inhibitor (e.g., siRNA or miRNA) of the dominant mutant allele, and the fusosome also comprises an mRNA encoding a non-mutated allele of the mutated gene that is not

targeted by the nucleic acid inhibitor.

561. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the subject is in need of vaccination and/or is in need of regeneration, e.g., of an injured site.

562. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome comprises a nucleic acid which further comprises one or more sequences encoding one or more signal sequences, e.g., wherein a target cell translocates a protein comprising a signal sequence to the cell membrane of the target cell.

563. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome composition or preparation is:

a) administered to the subject at least 1, 2, 3, 4, or 5 times;

b) administered to the subject systemically (e.g., orally, parenterally, subcutaneously, intravenously, intramuscularly, intraperitoneally) or locally;

c) administered to the subject such that the fusosome composition or preparation reaches a target tissue selected from liver, lungs, heart, spleen, pancreas, gastrointestinal tract, kidney, testes, ovaries, brain, reproductive organs, central nervous system, peripheral nervous system, skeletal muscle, endothelium, inner ear, or eye;

d) co-administered with an immunosuppressive agent, e.g., a glucocorticoid, cytostatic, antibody, or immunophilin modulator; and/or

e) co-administered with an immunostimulatory agent, e.g., an adjuvant, interleukin, cytokine, or chemokine.

564. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein administration of the fusosome composition or preparation results in upregulation or downregulation of a gene in a target cell in the subject, e.g., wherein the fusosome comprises a transcriptional activator or repressor, a translational activator or repressor, or an epigenetic activator or repressor.

565. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome preparation comprises:

a) at least 105, 106, 107, 108, 109, 1010, 1011, 1012, 1013, 1014, or 1015 fusosomes; and/or

b) at least 10 mL, 20 mL, 50 mL, 100 mL, 200 mL, 500 mL, 1 L, 2 L, 5 L, 10 L, 20 L, or 50 L total volume.

566. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the first fusogen is not a lipopeptide.

567. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome, fusosome composition, or fusosome preparation has partial or complete nuclear inactivation (e.g., nuclear removal).

568. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the source cell is:

a) a cell grown under adherent or suspension conditions;

b) a primary cell, a cultured cell, an immortalized cell, or a cell line (e.g., myelobast cell line, e.g., C2C12);

c) allogeneic, e.g., obtained from a different organism of the same species as the target cell;

d) heterologous, e.g., obtained from an organism of a different species from the target cell.

569. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome is not captured by:

a) the scavenger system in circulation or by Kupffer cells in the sinus of the liver; or

b) the reticulo-endothelial system (RES) in a subject, e.g., by an assay of Example 76.

570. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein when a plurality of fusosomes are administered to a subject, less

than 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, of the plurality are not captured by the RES after 24 hours, e.g., by an assay of Example 76.

571. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome comprises a viral structural protein and/or a viral matrix protein.

572. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome is substantially free of, or has a lower number of one or more of the following organelles: a mitochondrion, Golgi apparatus, lysosome, endoplasmic reticulum, vacuole, endosome, acrosome, autophagosome, centriole, glycosome, glyoxysome, hydrogenosome, melanosome, mitosome, cnidocyst, peroxisome, proteasome, vesicle, and stress granule, e.g., as compared to the source cell.

573. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome does not comprise Cre or GFP, e.g., EGFP.

574. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome composition or pharmaceutical composition has been maintained at a predetermined temperature for at least 1, 2, 3, 6, or 12 hours; 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, or 4 weeks; 1, 2, 3, or 6 months; or 1, 2, 3, 4, or 5 years; and/or wherein the predetermined temperature is selected from about 4, 0, -4, -10, -12, -16, -20, -80, or -160°C.

575. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome composition or pharmaceutical composition, has an activity of at least 50%, 60%, 70%, 80%, 90%, 95%, or 99% of the activity of the plurality before maintenance at said temperature, e.g., by one or more of:

i) the fusosome fuses at a higher rate with a target cell than with a non-target cell, e.g., by at least at least 10%, e.g., in an assay of Example 54;

ii) the fusosome fuses at a higher rate with a target cell than with other fusosomes,

e.g., by at least 50%, e.g., in an assay of Example 54;

iii) the fusosome fuses with target cells at a rate such that an agent in the fusosome is delivered to at least 10% of target cells after 24 hours, e.g., in an assay of Example 54; or

iv) the fusogen is present at a copy number of at least 50%, 60%, 70%, 80%, 90%, 95%, or 99% of the fusogen copy number of the plurality before maintenance at said temperature, e.g., as measured by an assay of Example 29.

576. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome composition or pharmaceutical composition is considered stable if it has an activity of at least 50%, 60%, 70%, 80%, 90%, 95%, or 99% of the activity of the plurality before storage at said temperature for said time period, e.g., by one or more of:

i) the fusosome fuses at a higher rate with a target cell than with a non-target cell, e.g., by at least at least 10%, e.g., in an assay of Example 54;

ii) the fusosome fuses at a higher rate with a target cell than with other fusosomes, e.g., by at least 50%, e.g., in an assay of Example 54;

iii) the fusosome fuses with target cells at a rate such that an agent in the fusosome is delivered to at least 10% of target cells after 24 hours, e.g., in an assay of Example 54; or

iv) the fusogen is present at a copy number of at least 50%, 60%, 70%, 80%, 90%, 95%, or 99% of the fusogen copy number of the plurality before maintenance at said temperature, e.g., as measured by an assay of Example 29.

577. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the disease or disorder is selected from cancer, autoimmune disorder, or infectious disease.

578. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome comprises a neoantigen.

579. The fusosome, fusosome composition, fusosome preparation, or method of any of the

preceding embodiments, wherein the fusosome composition is administered:

a) to the subject at least 1, 2, 3, 4, or 5 times;

b) to the subject systemically (e.g., orally, parenterally, subcutaneously, intravenously, intramuscularly, intraperitoneally) or locally; and/or

c) to the subject such that the fusosome composition reaches a target tissue selected from liver, lungs, heart, spleen, pancreas, gastrointestinal tract, kidney, testes, ovaries, brain, reproductive organs, central nervous system, peripheral nervous system, skeletal muscle, endothelium, inner ear, or eye.

580. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the fusosome composition is co-administered with:

a) an immunosuppressive agent, e.g., a glucocorticoid, cytostatic, antibody, or immunophilin modulator; and/or

b) an immunostimulatory agent, e.g., an adjuvant, interleukin, cytokine, or chemokine.

581. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the plurality of fusosomes has a local, distal, or systemic effect.

582. The fusosome, fusosome composition, fusosome preparation, or method of any of the preceding embodiments, wherein the organism is a human, e.g., wherein the human has a disease, disorder, or condition.

583. The fusosome, fusosome composition, fusosome preparation, or method of embodiment 582, wherein presence of the membrane payload agent, nuclear payload agent, or organellar payload agent in the cell membrane lipid bilayer of the target cell improves one or more symptoms of the disease, disorder, or condition.

[0001] Any of the aspects herein, e.g., the fusosomes, fusosome compositions, preparations and methods above, can be combined with one or more of the embodiments herein, e.g., one or of the embodiments described herein.

[0002] Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

[0003] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. For example, all GenBank, Unigene, and Entrez sequences referred to herein, e.g., in any Table herein, are incorporated by reference. Unless otherwise specified, the sequence accession numbers specified herein, including in any Table herein, refer to the database entries current as of February 17, 2018. When one gene or protein references a plurality of sequence accession numbers, all of the sequence variants are encompassed. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

[0004] The following detailed description of the invention will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention, there are shown in the drawings described herein certain embodiments, which are presently exemplified. It should be understood, however, that the invention is not limited to the precise arrangement and instrumentalities of the embodiments shown in the drawings.

[0005] FIG. 1 quantifies staining of fusosomes with a dye for endoplasmic reticulum.

[0006] FIG. 2 quantifies staining of fusosomes with a dye for mitochondria.

[0007] FIG. 3 quantifies staining of fusosomes with a dye for lysosomes.

[0008] FIG. 4 quantifies staining of fusosomes with a dye for F-actin.

[0009] FIG. 5 is a graph showing recovery of GFP fluorescence after photobleaching of cells contacted with fusogens expressing Cre and GFP.

[00010] FIG. 6 is a graph showing the percentage of target cells expressing RFP after contacting with fusosomes or negative controls.

[00011] FIG. 7 is an image of a positive organelle delivery via fusion between donor and

recipient HeLa cells. The intracellular areas indicated in white indicate overlap between donor and recipient mitochondria. The intracellular regions in grey indicate where donor and recipient organelles do not overlap.

[00012] FIG. 8 is an image of a positive organelle delivery via fusion between donor and recipient HeLa cells. The intracellular areas indicated in white indicate overlap between donor and recipient mitochondria. The intracellular regions in grey indicate where donor and recipient organelles do not overlap.

[00013] FIG. 9 shows microscopy images of the indicated tissues from mice injected with fusosomes. White indicates represent RFP-fluorescent cells, indicating delivery of a protein cargo to the cells in vivo.

[00014] FIG. 10 is a series of images showing successful delivery of fusosomes to murine tissues in vivo by the indicated routes of administration, resulting in expression of luciferase by targeted cells.

[00015] FIG. 11 shows microscopy images of tdTomato fluorescence in murine muscle tissue, indicating delivery of a protein cargo to muscle cells by cytobiologics.

DETAILED DESCRIPTION

[00016] The invention describes fusosomes that include a membrane protein payload agent, and related methods.

Definitions

[00017] ***Agent:*** In general, the term "agent", as used herein, may be used to refer to a compound or entity including, for example, a peptide, a polypeptide, a nucleic acid (e.g., DNA, a chromosome (e.g. a human artificial chromosome), RNA, mRNA, siRNA, miRNA), a saccharide or a polysaccharide, a lipid, a small molecule, or a combination or complex thereof. The term may refer to an entity that is or comprises an organelle, or a fraction, extract, or component thereof.

[00018] ***Antibody:*** As used herein, the term "antibody" refers to a polypeptide that includes canonical immunoglobulin sequence elements sufficient to confer specific binding to a particular target antigen. For purposes of the present invention, in certain embodiments, any polypeptide or

complex of polypeptides that includes sufficient immunoglobulin domain sequences to confer specific binding to an antigen can be referred to and/or used as an "antibody", whether such polypeptide is naturally produced (e.g., generated by an organism reacting to an antigen), or produced by recombinant engineering, chemical synthesis, or other artificial system or methodology. In some embodiments, an antibody is polyclonal; in some embodiments, an antibody is monoclonal. In some embodiments, an antibody has constant region sequences that are characteristic of mouse, rabbit, primate, or human antibodies. In some embodiments, antibody sequence elements are humanized, primatized, chimeric, etc. In embodiments, an antibody utilized in accordance with the present invention is in a format selected from, but not limited to, intact IgA, IgG, IgE or IgM antibodies; bi- or multi- specific antibodies (e.g., Zybodies®, etc); antibody fragments such as Fab fragments, Fab' fragments, F(ab')2 fragments, Fd' fragments, Fd fragments, and isolated CDRs or sets thereof; single chain Fvs; polypeptide-Fc fusions; single domain antibodies (e.g., shark single domain antibodies such as IgNAR or fragments thereof); cameloid antibodies; masked antibodies (e.g., Probodies®); Small Modular ImmunoPharmaceuticals ("SMIPs™"); single chain or Tandem diabodies (TandAb®); VHHs; Anticalins®; Nanobodies®; minibodies; BiTE®s; ankyrin repeat proteins or DARPINs®; Avimers®; DARTs; TCR-like antibodies;, Adnectins®; Affilins®; Trans-bodies®; Affibodies®; TrimerX®; MicroProteins; Fynomers®, Centyrins®; and KALBITOR®s. In some embodiments, an antibody may lack a covalent modification (e.g., attachment of a glycan) that it would have if produced naturally. In some embodiments, an antibody may contain a covalent modification (e.g., attachment of a glycan, a payload [e.g., a detectable moiety, a therapeutic moiety, a catalytic moiety, etc.], or other pendant group [e.g., poly-ethylene glycol, etc.]. In some embodiments, an antibody of any of the above-described formats comprises one or more complement determining regions, e.g., CDR1, CD2, and/or CDR3.

[00019] ***Antigen binding domain:*** The term "antigen binding domain" as used herein refers to that portion of antibody or a chimeric antigen receptor which binds an antigen. In some embodiments, an antigen binding domain binds to a cell surface antigen of a cell. In some embodiments an antigen binding domain binds an antigen characteristic of a cancer, e.g., a tumor associated antigen in a neoplastic cell. In some embodiments, an antigen binding domain binds an antigen characteristic of an infectious disease, e.g. a virus associated antigen in a virus infected

cell. In some embodiments, an antigen binding domain binds an antigen characteristic of a cell targeted by a subject's immune system in an autoimmune disease, e.g., a self-antigen. In some embodiments, an antigen binding domain is or comprises an antibody or antigen-binding portion thereof. In some embodiments, an antigen binding domain is or comprises an scFv or Fab.

[00020] ***Associated with:*** In some embodiments, two or more entities are physically "associated" with one another if they interact, directly or indirectly, so that they are and/or remain in physical proximity with one another. In some embodiments, two or more entities that are physically associated with one another are covalently linked to one another; in some embodiments, two or more entities that are physically associated with one another are not covalently linked to one another but are non-covalently associated, for example by means of hydrogen bonds, van der Waals interaction, hydrophobic interactions, magnetism, and combinations thereof.

[00021] ***Cancer***: The terms "cancer", "malignancy", "neoplasm", "tumor", and "carcinoma", are used herein to refer to cells that exhibit relatively abnormal, uncontrolled, and/or autonomous growth, so that they exhibit an aberrant growth phenotype characterized by a significant loss of control of cell proliferation. In some embodiments, a tumor may be or comprise cells that are precancerous (e.g., benign), malignant, pre-metastatic, metastatic, and/or non-metastatic. The present disclosure specifically identifies certain cancers to which its teachings may be particularly relevant. In some embodiments, a relevant cancer may be characterized by a solid tumor. In some embodiments, a tumor may be a disperse tumor or a liquid tumor. In some embodiments, a relevant cancer may be characterized by a hematologic tumor. In general, examples of different types of cancers known in the art include, for example, leukemias, lymphomas (Hodgkin's and non-Hodgkin's), myelomas and myeloproliferative disorders; sarcomas, melanomas, adenomas, carcinomas of solid tissue, squamous cell carcinomas of the mouth, throat, larynx, and lung, liver cancer, genitourinary cancers such as prostate, cervical, bladder, uterine, and endometrial cancer and renal cell carcinomas, bone cancer, pancreatic cancer, skin cancer, cutaneous or intraocular melanoma, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, head and neck cancers, breast cancer, gastro-intestinal cancers and nervous system cancers, benign lesions such as papillomas, and the like.

[00022] **Cargo**: As used herein, "cargo" or "payload" comprises an agent which may be

delivered by a fusosome to a target cell. In some embodiments a cargo comprises one or more of a therapeutic agent, e.g., a therapeutic agent that is endogenous or exogenous to the source cell. In some embodiments, the therapeutic agent is chosen from one or more of a protein, e.g., an enzyme, a transmembrane protein, a receptor, an antibody; a nucleic acid, e.g., DNA, a chromosome (e.g. a human artificial chromosome), RNA, mRNA, siRNA, miRNA, or a small molecule. In some embodiments, a cargo is or comprises a membrane protein payload agent. In some embodiments, a cargo is or comprises an organelle.

[00023] ***CDR:*** As used herein, "CDR" refers to a complementarity determining region, e.g., which can be situated within an antibody variable region. There are three CDRs in each of the variable regions of the heavy chain and the light chain, which are designated CDR1, CDR2 and CDR3, for each of the variable regions. A "*set of CDRs*" or "*CDR set*" refers to a group of three or six CDRs that occur in either a single variable region capable of binding the antigen or the CDRs of cognate heavy and light chain variable regions capable of binding the antigen. Certain systems have been established in the art for defining CDR boundaries (e.g., Kabat, Chothia, etc.); those skilled in the art appreciate the differences between and among these systems and are capable of understanding CDR boundaries to the extent required to understand and to practice the claimed invention.

[00024] ***Cell Membrane***: As used herein, a "cell membrane" refers to a membrane derived from a cell, e.g., a source cell or a target cell.

[00025] ***Cytobiologic***: As used herein, "cytobiologic" refers to a portion of a cell that comprises a lumen and a cell membrane, or a cell having partial or complete nuclear inactivation. In some embodiments, the cytobiologic comprises one or more of a cytoskeleton component, an organelle, and a ribosome. In embodiments, the cytobiologic is an enucleated cell, a microvesicle, or a cell ghost.

[00026] ***Cytosol:*** As used herein, "cytosol" refers to the aqueous component of the cytoplasm of a cell. The cytosol may comprise proteins, RNA, metabolites, and ions.

[00027] ***Endogenous*:** As used herein, the term "endogenous" refers to an agent, e.g., a protein or lipid that is naturally found in a relevant system (e.g., cell, tissue, organism, source cell, or target cell, etc). For example, in some embodiments, a fusosome or a membrane-enclosed

preparation may be said to contain one or more "endogenous" lipids and/or proteins when the relevant lipids and/or proteins are naturally found in a source cell from which the fusosome or membrane-enclosed preparation is obtained or derived (e.g., the source cell of the fusosome or membrane-enclosed preparation). In some embodiments, an endogenous agent is overexpressed in a source cell.

[00028] ***Exogenous***: As used herein, the term "exogenous" refers to an agent (e.g., a protein or lipid) that is not naturally found in a relevant system (e.g., a cell, a tissue, an organism, a source cell or a target cell, etc.). In embodiments, the agent is engineered and/or introduced into the relevant system, For example, in some embodiments, a fusosome or a membrane-enclosed preparation may be said to contain one or more "exogenous" lipids and/or proteins when the relevant lipids and/or proteins are not naturally found in a source cell from which the fusosome or membrane-enclosed preparation is obtained or derived (e.g., the source cell of the fusosome or membrane-enclosed. In some embodiments, an exogenous agent is a variant of an endogenous agent, such as, for example, a protein variant that differs in one or more structural aspects such as amino acid sequence, post-translational modification, etc from a reference endogenous protein, etc).

[00029] ***Functional variant:*** The term "functional variant" refers to a polypeptide that has a substantially identical amino acid sequence to a reference amino acid sequence, or is encoded by a substantially identical nucleotide sequence, and is capable of having one or more activities of the reference amino acid sequence.

[00030] ***Fused Cell***: As used herein, a "fused cell" refers to a cell produced by the contacting of one or more fusosomes with a target cell. In some embodiments of the fused cell, at least a portion of the lipid bilayer of one or more fusosomes is associated with a membrane of the target cell.

[00031] ***Fusogen:*** As used herein, "fusogen" refers to an agent or molecule that creates an interaction between two membrane enclosed lumens. In embodiments, the fusogen facilitates fusion of the membranes. In other embodiments, the fusogen creates a connection, e.g., a pore, between two lumens (e.g., the lumen of the fusosome and a cytoplasm of a target cell). In some embodiments, the fusogen comprises a complex of two or more proteins, e.g., wherein neither

protein has fusogenic activity alone.

[00032] ***Fusogen binding partner***: As used herein, "fusogen binding partner" refers to an agent or molecule that interacts with a fusogen to facilitate fusion between two membranes. In some embodiments, a fusogen binding partner may be or comprise a surface feature of a cell.

[00033] ***Fusosome Composition:*** As used herein, "fusosome composition" refers to a composition comprising one or more fusosomes.

[00034] ***Membrane protein payload agent***: As used herein, "membrane protein payload agent" refers to a cargo that is or comprises a membrane protein and/or a nucleic acid encoding a membrane protein, which cargo may be included in a fusosome or membrane-enclosed preparation as described herein (e.g., for delivery to a target cell). A membrane protein is a protein which associates with (e.g., is localized in and/or on) or is capable of associating with a cell membrane. In some embodiments a membrane protein is a transmembrane protein. In some embodiments, a membrane protein comprises a domain that at least partially (e.g., completely) spans a membrane, e.g., cell membrane. In some embodiments, a membrane protein is associated with an interior (e.g., cytosolic) portion of a membrane lipid bilayer. In some embodiments a membrane protein is associated with an exterior portion of a membrane lipid bilayer (e.g., with a cell surface or with a surface of a fusosome or a membrane-enclosed preparation as described herein). In some embodiments, a membrane protein is associated with an exterior portion of a membrane lipid bilayer is a cell surface protein. In some embodiments a membrane protein passes through a membrane lipid bilayer and is secreted. In some embodiments a membrane protein is a naturally occurring protein. In some embodiments a membrane protein is an engineered and/or synthetic protein (e.g., a chimeric antigen receptor). In some embodiments a membrane protein is a therapeutic agent.

[00035] ***Nuclear payload agent***: As used herein, "nuclear payload agent" refers to a cargo that is or comprises or encodes an agent that localizes to the nucleus, which cargo may be included in a fusosome or membrane-enclosed preparation as described herein (e.g., for delivery to a target cell). In some embodimets, a nuclear payload agent becomes preferentially enriched in the nucleoplasm, nucleolus, perionucleolar region, a Cajal body, a clastosome, a gems nuclear body, a histone locus body (HLB), a nuclear speckle, a nuclear stress body, a paraspeckle, a PML body,

a polycomb body. In some embodiments a nuclear payload agent is a nuclear protein payload agent. In some embodiments, a nuclear payload agent comprises a functional nucleic acid and/or a non-coding nucleic acid.

[00036] ***Nuclear protein payload agent***: As used herein, "nuclear protein payload agent" refers to a cargo that is or comprises a nuclear protein and/or a nucleic acid encoding a nuclear protein, which cargo may be included in a fusosome or membrane-enclosed preparation as described herein (e.g., for delivery to a target cell). A nuclear protein is a protein which associates with or is capable of associating with the nucleus. In some embodimets, a nuclear protein becomes preferentially enriched in the nucleoplasm, nucleolus, perionucleolar region, a Cajal body, a clastosome, a gems nuclear body, a histone locus body (HLB), a nuclear speckle, a nuclear stress body, a paraspeckle, a PML body, a polycomb body. In some embodiments a nuclear protein is a naturally occurring protein. In some embodiments a nuclear protein is an engineered and/or synthetic protein. In some embodiments a nuclear protein is a therapeutic agent.

[00037] ***Organellar payload agent***: As used herein, "organellar payload agent" refers to a cargo that is or comprises or encodes an agent that localizes to a cytosolic organelle, which cargo may be included in a fusosome or membrane-enclosed preparation as described herein (e.g., for delivery to a target cell). The nucleus is not a cytosolic organelle. In some embodiments, the organelle is a membrane-bound organelle. On other embodiments, the organelle is not a membrane-bound organelle. In some embodiments, the cytosolic organelle is a mitochondriaon, Golgi apparatus, endoplasmic reticulum, vacuole, acrosome, autophagosome, centriole, glycosome, glyoxysome, hydrogenosome, melanosome, mitosome, cnidocyst, peroxisome, proteasome, vesicle, stress granule, lysosome, or endosome. In some embodiments an organellar payload agent is an organellar protein payload agent. In some embodiments, an organellar payload agent comprises a functional nucleic acid and/or a non-coding nucleic acid.

[00038] ***Organellar protein payload agent***: As used herein, "organellar protein payload agent" refers to a cargo that is or comprises an protein that becomes preferentially enriched in a cytosolic organelle, and/or a nucleic acid encoding said protein, which cargo may be included in a fusosome or membrane-enclosed preparation as described herein (e.g., for delivery to a target cell). In some embodiments the protein is an engineered and/or synthetic protein. In some embodiments

the protein is a therapeutic agent.

[00039]

[00040] ***Pharmaceutical composition:*** As used herein, the term "pharmaceutical composition" refers to an active agent, formulated together with one or more pharmaceutically acceptable carriers. In some embodiments, active agent is present in unit dose amount appropriate for administration in a therapeutic regimen to a relevant subject. In some embodiments, pharmaceutical compositions may be specially formulated for parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation.

[00041] ***Pharmaceutically acceptable carrier:*** As used herein, the term "pharmaceutically acceptable carrier" means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, or excipient.

[00042] ***Preferentially enriched:*** As used herein, an agent that is "preferentially enriched" in a particular region of a target cell refers to the agent being present at a higher concentration in the particular region compared to at least one reference region of the cell. In some embodients, the reference region is the cytosol. In some embodiments, an agent that is preferentially enriched in a particular region of the target cell is present at a higher concentration in the particular region compared to every other region of the cell. In some embodiments, the agent that is preferentially enriched in the particular region of the target cell is present at a concentration at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 2-fold, 5-fold, or 10-fold higher than the concentration in a reference region, e.g., the cysosol or plasma membrane. "Preferentially enriched" and "enriched" are used interchangeably herein.

[00043] ***Purified:*** As used herein, the term "purified" means altered or removed from the natural state. For example, a cell or cell fragment naturally present in a living animal is not "purified," but the same cell or cell fragment partially or completely separated from the coexisting materials of its natural state is "purified." A purified fusosome composition can exist in substantially pure form, or can exist in a non-native environment such as, for example, a culture medium such as a culture medium comprising cells.

[00044] ***Source cell:*** As used herein, a "source cell" refers to a cell from which a fusosome is derived, e.g., obtained. In some embodiments, derived includes obtaining a membrane enclosed preparation from a source cell and adding a fusogen.

[00045] ***Substantially identical:*** In the context of a nucleotide sequence, the term "substantially identical" is used herein to refer to a first nucleic acid sequence that contains a sufficient or minimum number of nucleotides that are identical to aligned nucleotides in a second nucleic acid sequence such that the first and second nucleotide sequences encode a polypeptide having common functional activity, or encode a common structural polypeptide domain or a common functional polypeptide activity, for example, nucleotide sequences having at least about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to a reference sequence, *e.g.*, a sequence provided herein. The compositions and methods herein encompass polypeptides and nucleic acids having the sequences specified, or sequences substantially identical or similar thereto, *e.g.*, sequences at least 85%, 90%, or 95% identical or higher to the sequence specified. In the context of an amino acid sequence, the term "substantially identical" is used herein to refer to a first amino acid sequence that contains a sufficient or minimum number of amino acid residues that are i) identical to, or ii) conservative substitutions of aligned amino acid residues in a second amino acid sequence such that the first and second amino acid sequences can have a common structural domain and/or common functional activity, for example, amino acid sequences that contain a common structural domain having at least about 85%, 90%. 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to a reference sequence, *e.g.*, a sequence provided herein.

[00046] ***Target cell moiety:*** As used herein, the term "target cell moiety" is used to refer to a feature of a cell (e.g., a target cell) which may be used to specifically (relative to at least one other cell in the relevant system) target a fusosome to the cell. In some embodiments, a target cell moiety is a surface feature of a target cell. In some embodiments, a target cell moiety is or is a portion of a protein associated with the cell membrane of a target cell. In some embodiments, a target cell moiety is, or is a portion of, a peptide or protein associated with the membrane of a target cell. In some embodiments, a target cell moiety is or is a portion of a lipid associated with the membrane of a target cell. In some embodiments, a target cell moiety is or is a portion of a saccharide associated with the membrane of a target cell.

[00047] ***Targeting domain***: As used herein, the term "targeting domain" is a feature of a fusosome which associates or interacts with a target cell moiety. In some embodiments, a targeting domain specifically (under conditions of exposure) associates or interacts with a target cell moiety. In some embodiments, a targeting domain specifically binds to a target cell moiety present on a target cell. In some embodiments, a targeting domain is or comprises a domain of a fusogen e.g., is covalently linked to a fusogen, e.g., is part of a fusogen polypeptide. In some embodiments, a targeting domain is is a separate entity from any fusogen, e.g., is not covalently linked to a fusogen, e.g., is not part of a fusogen polypeptide.

[00048] ***Stable:*** The term "stable," when applied to compositions herein, means that the compositions maintain one or more aspects of their physical structure and/or activity over a period of time under a designated set of conditions. In some embodiments, the designated conditions are under cold storage (e.g., at or below about 4 ºC, -20 ºC, or -80 ºC).

[00049] ***Target cell***: As used herein "target cell" refers to a cell which a fusosome fuses to.

[00050] ***Variant:*** The term "variant" refers to a polypeptide that has a substantially identical amino acid sequence to a reference amino acid sequence, or is encoded by a substantially identical nucleotide sequence. In some embodiments, the variant is a functional variant.

Fusosomes

[00051] The fusosome compositions and methods described herein comprise (a) a lipid bilayer, (b) a lumen (e.g., comprising cytosol) surrounded by the lipid bilayer; (c) a fusogen that is exogenous or overexpressed relative to the source cell , e.g., wherein the fusogen is disposed in the lipid bilayer, and (d) a membrane protein payload agent. In embodiments, the fusosome is derived from a non-plant cell, e.g., a mammalian cell, or derivative thereof (e.g., a mitochondrion, a chondrisome, an organelle, a vesicle, or an enucleated cell), and comprises a fusogen, e.g., protein, lipid or chemical fusogen.

Encapsulation

[00052] In some embodiments of the compositions and methods described herein include fusosomes, e.g., naturally derived bilayers of amphipathic lipids with a fusogen. Fusosomes may

comprise several different types of lipids, e.g., amphipathic lipids, such as phospholipids. Fusosomes may comprise a lipid bilayer as the outermost surface. Such compositions can surprisingly be used in the methods of the invention. In some instances, membranes may take the form of an autologous, allogeneic, xenogeneic or engineered cell such as is described in Ahmad et al. 2014 Miro1 regulates intercellular mitochondrial transport & enhances mesenchymal stem cell rescue efficacy. EMBO Journal. 33(9):994-1010. In some embodiments, the compositions include engineered membranes such as described in, e.g. in Orive. et al. 2015. Cell encapsulation: technical and clinical advances. Trends in Pharmacology Sciences; 36 (8):537-46; and in Mishra. 2016. Handbook of Encapsulation and Controlled Release. CRC Press. In some embodiments, the compositions include naturally occurring membranes (McBride et al. 2012. A Vesicular Transport Pathway Shuttles Cargo from mitochondria to lysosomes. Current Biology 22:135-141).

[00053] In some embodiments, a composition described herein includes a naturally derived membrane, e.g., membrane vesicles prepared from cells or tissues. In some embodiments, a fusosome is a vesicle derived from MSCs or astrocytes.

[00054] In some embodiments, a fusosome is an exosome.

[00055] Exemplary exosomes and other membrane-enclosed bodies are described, e.g., in US2016137716, which is herein incorporated by reference in its entirety. In some embodiments, the fusosome comprises a vesicle that is, for instance, obtainable from a cell, for instance a microvesicle, an exosome, an apoptotic body (from apoptotic cells), a microparticle (which may be derived from e.g. platelets), an ectosome (derivable from, e.g., neutrophiles and monocytes in serum), a prostatosome (obtainable from prostate cancer cells), a cardiosome (derivable from cardiac cells), and the like.

[00056] Exemplary exosomes and other membrane-enclosed bodies are also described in WO/2017/161010, WO/2016/077639, US20160168572, US20150290343, and US20070298118, each of which is incorporated by reference herein in its entirety. In some embodiments, the fusosome comprises an extracellular vesicle, nanovesicle, or exosome. In some embodiments a fusosome comprises an extracellular vesicle, e.g., a cell-derived vesicle comprising a membrane that encloses an internal space and has a smaller diameter than the cell from which it is derived. In embodiments the extracellular vesicle has a diameter from 20nm to 1000 nm. In embodiments the

fusosome comprises an apoptotic body, a fragment of a cell, a vesicle derived from a cell by direct or indirect manipulation, a vesiculated organelle, and a vesicle produced by a living cell (e.g., by direct plasma membrane budding or fusion of the late endosome with the plasma membrane). In embodiments the extracellular vesicle is derived from a living or dead organism, explanted tissues or organs, or cultured cells. In embodiments, the fusosome comprises a nanovesicle, e.g., a cell-derived small (e.g., between 20-250 nm in diameter, or 30-150 nm in diameter) vesicle comprising a membrane that encloses an internal space, and which is generated from said cell by direct or indirect manipulation. The production of nanovesicles can, in some instances, result in the destruction of the source cell. The nanovesicle may comprise a lipid or fatty acid and polypeptide. In embodiments, the fusosome comprises an exosome. In embodiments, the exosome is a cell-derived small (e.g., between 20-300 nm in diameter, or 40-200nm in diameter) vesicle comprising a membrane that encloses an internal space, and which is generated from said cell by direct plasma membrane budding or by fusion of the late endosome with the plasma membrane. In embodiments, production of exosomes does not result in the destruction of the source cell. In embodiments, the exosome comprises lipid or fatty acid and polypeptide.

[00057] Exemplary exosomes and other membrane-enclosed bodies are also described in US 20160354313, which is herein incorporated by reference in its entirety. In embodiments, the fusosome comprises a Biocompatible Delivery Module, an exosome (e.g., about 30 nm to about 200 nm in diameter), a microvesicle (e.g., about 100 nm to about 2000 nm in diameter) an apoptotic body (e.g., about 300 nm to about 2000 nm in diameter), a membrane particle, a membrane vesicle, an exosome-like vesicle, an ectosome-like vesicle, an ectosome, or an exovesicle.

[00058] In some embodiments, a fusosome is a microvesicle. In some embodiments, a fusosome is a cell ghost. In some embodiments, a vesicle is a plasma membrane vesicle, e.g. a giant plasma membrane vesicle.

[00059] Fusosomes can be made from several different types of lipids, e.g., amphipathic lipids, such as phospholipids. The fusosome may comprise a lipid bilayer as the outermost surface. This bilayer may be comprised of one or more lipids of the same or different type. Examples include without limitation phospholipids such as phosphocholines and phosphoinositols. Specific examples include without limitation DMPC, DOPC, and DSPC.

Fusogens

[00060] In some embodiments, the fusosome described herein (e.g., comprising a vesicle or a portion of a cell) includes one or more fusogens, e.g., to facilitate the fusion of the fusosome to a membrane, e.g., a cell membrane. Also these compositions may include surface modifications made during or after synthesis to include one or more fusogens. The surface modification may comprise a modification to the membrane, e.g., insertion of a lipid or protein into the membrane.

[00061] In some embodiments, the fusosomes comprise one or more fusogens on their exterior surface (e.g., integrated into the cell membrane) to target a specific cell or tissue type (e.g., cardiomyocytes). Fusosomes may comprise a targeting domain. Fusogens include without limitation protein based, lipid based, and chemical based fusogens. The fusogen may bind a partner, e.g., a feature on a target cells' surface. In some embodiments the partner on a target cells' surface is a target cell moiety. In some embodiments, the fusosome comprising the fusogen will integrate the membrane into a lipid bilayer of a target cell.

[00062] In some embodiments, one or more of the fusogens described herein may be included in the fusosome.

Protein Fusogens

[00063] In some embodiments, the fusogen is a protein fusogen, e.g., a mammalian protein or a homologue of a mammalian protein (e.g., having 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or greater identity), a non-mammalian protein such as a viral protein or a homologue of a viral protein (e.g., having 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or greater identity), a native protein or a derivative of a native protein, a synthetic protein, a fragment thereof, a variant thereof, a protein fusion comprising one or more of the fusogens or fragments, and any combination thereof.

[00064] In some embodiments, the fusogen results in mixing between lipids in the fusosome and lipids in the target cell. In some embodiments, the fusogen results in formation of one or more pores between the lumen of the fusosome and the cytosol of the target cell, e.g., the fusosome is, or comprises, a connexin as described herein.

(i) Mammalian Proteins

[00065] In some embodiments, the fusogen may include a mammalian protein, see Table 1. Examples of mammalian fusogens may include, but are not limited to, a SNARE family protein such as vSNAREs and tSNAREs, a syncytin protein such as Syncytin-1 (DOI: 10.1128/JVI.76.13.6442–6452.2002), and Syncytin-2, myomaker (biorxiv.org/content/early/2017/04/02/123158, doi.org/10.1101/123158, doi: 10.1096/fj.201600945R, doi:10.1038/nature12343), myomixer (www.nature.com/nature/journal/v499/n7458/full/nature12343.html, doi:10.1038/nature12343), myomerger (science.sciencemag.org/content/early/2017/04/05/science.aam9361, DOI: 10.1126/science.aam9361), FGFRL1 (fibroblast growth factor receptor-like 1), Minion (doi.org/10.1101/122697), an isoform of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (e.g., as disclosed in US 6,099,857A), a gap junction protein such as connexin 43, connexin 40, connexin 45, connexin 32 or connexin 37 (e.g., as disclosed in US 2007/0224176, Hap2, any protein capable of inducing syncytium formation between heterologous cells (see Table 2), any protein with fusogen properties (see Table 3), a homologue thereof, a fragment thereof, a variant thereof, and a protein fusion comprising one or more proteins or fragments thereof. In some embodiments, the fusogen is encoded by a human endogenous retroviral element (hERV) found in the human genome. Additional exemplary fusogens are disclosed in US 6,099,857A and US 2007/0224176, the entire contents of which are hereby incorporated by reference.

Table 1: Non-limiting examples of human and non-human fusogens.

Human and Non-Human Fusogen Classes		
Fusogen Class	**Uniprot Protein Family ID**	**# of sequences**
EFF-AFF	PF14884	191
SNARE	PF05739	5977
DC-STAMP	PF07782	633
ENV	PF00429	312

Table 2: Genes that encode proteins with fusogen properties.

Human genes with the gene ontology annotation of: Syncytium formation by plasma membrane fusion proteins	
ID	**Symbol**
A0A024R0I0	DYRK1B
A0A024R1N1	MYH9
A0A024R2D8	CAV3
A0A096LNV2	FER1L5
A0A096LPA8	FER1L5
A0A096LPB1	FER1L5
A0AVI2	FER1L5
A6NI61	TMEM8C (myomaker)
B3KSL7	-
B7ZLI3	FER1L5
H0YD14	MYOF
O43184	ADAM12
O60242	ADGRB3
O60500	NPHS1
O95180	CACNA1H
O95259	KCNH1
P04628	WNT1
P15172	MYOD1
P17655	CAPN2
P29475	NOS1
P35579	MYH9
P56539	CAV3
Q2NNQ7	FER1L5
Q4KMG0	CDON
Q53GL0	PLEKHO1
Q5TCZ1	SH3PXD2A
Q6YHK3	CD109
Q86V25	VASH2
Q99697	PITX2

Human genes with the gene ontology annotation of:	
Syncytium formation by plasma membrane fusion proteins	
ID	**Symbol**
Q9C0D5	TANC1
Q9H295	DCSTAMP
Q9NZM1	MYOF
Q9Y463	DYRK1B

Table 3: Human Fusogen Candidates

Fusogen Class	Gene ID
SNARE	O15400
	Q16623
	K7EQB1
	Q86Y82
	E9PN33
	Q96NA8
	H3BT82
	Q9UNK0
	P32856
	Q13190
	O14662
	P61266
	O43752
	O60499
	Q13277
	B7ZBM8
	A0AVG3
	Q12846
DC-STAMP	Q9H295
	Q5T1A1
	Q5T197
	E9PJX3

Fusogen Class	Gene ID
	Q9BR26
ENV	Q9UQF0
	Q9N2K0
	P60507
	P60608
	B6SEH9
	P60508
	B6SEH8
	P61550
	P60509
	Q9N2J8
Muscle Fusion (Myomaker)	H0Y5B2
	H7C1S0
	Q9HCN3
	A6NDV4
	K4DI83
Muscle Fusion (Myomixer)	NP_001302423.1
	ACT64390.1
	XP_018884517.1
	XP_017826615.1
	XP_020012665.1
	XP_017402927.1
	XP_019498363.1
	ELW65617.1
	ERE90100.1
	XP_017813001.1
	XP_017733785.1
	XP_017531750.1
	XP_020142594.1
	XP_019649987.1
	XP_019805280.1
	NP_001170939.1

Fusogen Class	Gene ID
	NP_001170941.1
	XP_019590171.1
	XP_019062106.1
	EPQ04443.1
	EPY76709.1
	XP_017652630.1
	XP_017459263.1
	OBS58441.1
	XP_017459262.1
	XP_017894180.1
	XP_020746447.1
	ELK00259.1
	XP_019312826.1
	XP_017200354.1
	BAH40091.1
HA	P03452
	Q9Q0U6
	P03460
GAP JUNCTION	P36382
	P17302
	P36383
	P08034
	P35212
Other	FGFRL1
	GAPDH

[00066] In some embodiments, the fusosome comprises a curvature-generating protein, e.g., Epsin1, dynamin, or a protein comprising a BAR domain. See, e.g., Kozlovet al, CurrOp StrucBio 2015, Zimmerberget al. Nat Rev 2006, Richard et al, Biochem J 2011.

(ii) Non-mammalian Proteins

Viral Proteins

[00067] In some embodiments, the fusogen may include a non-mammalian protein, e.g., a viral protein. In some embodiments, a viral fusogen is a Class I viral membrane fusion protein, a Class III viral membrane fusion protein, a viral membrane glycoprotein, or other viral fusion proteins, or a homologue thereof, a fragment thereof, a variant thereof, or a protein fusion comprising one or more proteins or fragments thereof.

[00068] In some embodiments, Class I viral membrane fusion proteins include, but are not limited to, Baculovirus F protein, e.g., F proteins of the nucleopolyhedrovirus (NPV) genera, e.g., Spodoptera exigua MNPV (SeMNPV) F protein and Lymantria dispar MNPV (LdMNPV).

[00069] In some embodiments, Class III viral membrane fusion proteins include, but are not limited to, rhabdovirus G (e.g., fusogenic protein G of the Vesicular Stomatatis Virus (VSV-G)), herpesvirus glycoprotein B (e.g., Herpes Simplex virus 1 (HSV-1) gB)), Epstein Barr Virus glycoprotein B (EBV gB), thogotovirus G, baculovirus gp64 (e.g., Autographa California multiple NPV (AcMNPV) gp64), and Borna disease virus (BDV) glycoprotein (BDV G).

[00070] Examples of other viral fusogens, e.g., membrane glycoproteins and viral fusion proteins, include, but are not limited to: viral syncytia proteins such as influenza hemagglutinin (HA) or mutants, or fusion proteins thereof; human immunodeficiency virus type 1 envelope protein (HIV-1 ENV), gp120 from HIV binding LFA-1 to form lymphocyte syncytium, HIV gp41, HIV gp160, or HIV Trans-Activator of Transcription (TAT); viral glycoprotein VSV-G, viral glycoprotein from vesicular stomatitis virus of the Rhabdoviridae family; glycoproteins gB and gH-gL of the varicella-zoster virus (VZV); murine leukaemia virus (MLV)-10A1; Gibbon Ape Leukemia Virus glycoprotein (GaLV); type G glycoproteins in Rabies, Mokola, vesicular stomatitis virus and Togaviruses; murine hepatitis virus JHM surface projection protein; porcine respiratory coronavirus spike- and membrane glycoproteins; avian infectious bronchitis spike glycoprotein and its precursor; bovine enteric coronavirus spike protein; the F and H, HN or G genes of Measles virus; canine distemper virus, Newcastle disease virus, human parainfluenza virus 3, simian virus 41, Sendai virus and human respiratory syncytial virus; gH of human herpesvirus 1 and simian varicella virus, with the chaperone protein gL; human, bovine and

cercopithicine herpesvirus gB; envelope glycoproteins of Friend murine leukaemia virus and Mason Pfizer monkey virus; mumps virus hemagglutinin neuraminidase, and glyoproteins F1 and F2; membrane glycoproteins from Venezuelan equine encephalomyelitis; paramyxovirus F protein; SIV gp160 protein; Ebola virus G protein; or Sendai virus fusion protein, or a homologue thereof, a fragment thereof, a variant thereof, and a protein fusion comprising one or more proteins or fragments thereof.

[00071] Non-mammalian fusogens include viral fusogens, homologues thereof, fragments thereof, and fusion proteins comprising one or more proteins or fragments thereof. Viral fusogens include class I fusogens, class II fusogens, class III fusogens, and class IV fusogens. In embodiments, class I fusogens such as human immunodeficiency virus (HIV) gp41, have a characteristic postfusion conformation with a signature trimer of α-helical hairpins with a central coiled-coil structure. Class I viral fusion proteins include proteins having a central postfusion six-helix bundle. Class I viral fusion proteins include influenza HA, parainfluenza F, HIV Env, Ebola GP, hemagglutinins from orthomyxoviruses, F proteins from paramyxoviruses (e.g. Measles, (Katoh et al. BMC Biotechnology 2010, 10:37)), ENV proteins from retroviruses, and fusogens of filoviruses and coronaviruses. In embodiments, class II viral fusogens such as dengue E glycoprotein, have a structural signature of β- sheets forming an elongated ectodomain that refolds to result in a trimer of hairpins. In embodiments, the class II viral fusogen lacks the central coiled coil. Class II viral fusogen can be found in alphaviruses (e.g., E1 protein) and flaviviruses (e.g., E glycoproteins). Class II viral fusogens include fusogens from Semliki Forest virus, Sinbis, rubella virus, and dengue virus. In embodiments, class III viral fusogens such as the vesicular stomatitis virus G glycoprotein, combine structural signatures found in classes I and II. In embodiments, a class III viral fusogen comprises α helices (e.g., forming a six-helix bundle to fold back the protein as with class I viral fusogens), and β sheets with an amphiphilic fusion peptide at its end, reminiscent of class II viral fusogens. Class III viral fusogens can be found in rhabdoviruses and herpesviruses. In embodiments, class IV viral fusogens are fusion-associated small transmembrane (FAST) proteins (doi:10.1038/sj.emboj.7600767, Nesbitt, Rae L., "Targeted Intracellular Therapeutic Delivery Using Liposomes Formulated with Multifunctional FAST proteins" (2012). Electronic Thesis and Dissertation Repository. Paper 388), which are encoded by nonenveloped reoviruses. In embodiments, the class IV viral fusogens are sufficiently small

that they do not form hairpins (doi: 10.1146/annurev-cellbio-101512-122422, doi:10.1016/j.devcel.2007.12.008).

[00072] Additional exemplary fusogens are disclosed in US 9,695,446, US 2004/0028687, US 6,416,997, US 7,329,807, US 2017/0112773, US 2009/0202622, WO 2006/027202, and US 2004/0009604, the entire contents of all of which are hereby incorporated by reference.

[00073] In some embodiments, a fusogen described herein comprises an amino acid sequence of Table 4, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to a portion of the sequence, e.g., a portion of 100, 200, 300, 400, 500, or 600 amino acids in length. For instance, in some embodiments, a fusogen described herein comprises an amino acid sequence having at least 80% identity to any amino acid sequence of Table 4. In some embodiments, a nucleic acid sequence described herein encodes an amino acid sequence of Table 4, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to a portion of the sequence, e.g., a portion of 40, 50, 60, 80, 100, 200, 300, 400, 500, or 600 amino acids in length.

[00074] In some embodiments, a fusogen described herein comprises an amino acid sequence set forth in any one of SEQ ID NOS: 627-683, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to a portion of the sequence, e.g., a portion of 100, 200, 300, 400, 500, or 600 amino acids in length. For instance, in some embodiments, a fusogen described herein comprises an amino acid sequence having at least 80% identity to an amino acid sequence set forth in any one of SEQ ID NOS: 627-683. In some embodiments, a nucleic acid sequence described herein encodes an amino acid sequence set forth in any one of SEQ ID NOS: 627-683, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to a portion of the sequence, e.g., a portion of 40, 50, 60, 80, 100, 200, 300, 400, 500, or 600 amino acids in length.

Table 4. Paramyxovirus F sequence clusters. Column 1, Genbank ID includes the Genbank ID of the whole genome sequence of the virus that is the centroid sequence of the cluster. Column 2, Nucleotides of CDS provides the nucleotides corresponding to the CDS of the gene in the whole genome. Column 3, Full Gene Name, provides the full name of the gene including Genbank ID, virus species, strain, and protein name. Column 4, Sequence, provides the amino acid sequence of the gene. Column 5, #Sequences/Cluster provides the number of sequences that cluster with this centroid sequence.

Genbank ID	Nucleotides of CDS	Full Gene Name	Sequence	#Sequences /Cluster	SEQ ID NO
KP317927	5630-7399	gb:KP317927:5630-7399|Organism:Human respiratory syncytial virus|Strain Name:Kilifi_9465_7_RSVB_2011|Protein Name:fusion glycoprotein|Gene Symbol:F	MIPQARTELNLGQITMELLIHRSSAIFL TLAINALYLTSSQNITEEFYQSTCSAVS RGYLSALRTGWYTSVITIELSNIKETK CNGTDTKVKLIKQELDKYKNAVTELQ LLMQNTPAANNRARREAPQYMNYTI NTTGSLNVSISKKRKRRFLGFLLGVGS AIASGIAVSKVLHLEGEVNKIKNALLS TNKAVVSLSNGVSVLTSKVLDLKNYI NNQLLPIVNQQSCRISNIETVIEFQQKN SRLLEITREFSVNAGVTTPLSTYMLTN SELLSLINDMPITNDQKKLMSSNVQIV RQQSYSIMSIIKEEVLAYVVQLPIYGVI DTPCWKLHTSPLCTTNIKEGSNICLTR TDRGWYCDNAGSVSFFPQADTCKVQ SNRVFCDTMNSLTLPSEVSLCNTDIFN SKYDCKIMTSKTDISSSVITSLGAIVSC YGKTKCTASNKNRGIIKTFSNGCDYV SNKGVDTVSVGNTLYYVNKLEGKNL YVKGEPIINYYDPLVFPSDEFDASISQV NEKINQSLAFIRRSDELLHNVNTGKST TNIMITAIIIVIIVVLLSLIAIGLLLYCKA KNTPVTLSKDQLSGINNIAFSK	993	627
AB524405	4556-6217	gb:AB524405:4556-	MDPKPSTSYLHAFPLIFVAISLVFMAG RASALDGRPLAAAGIVVTGDKAVNIY	418	628

		6217|Organism:Newcastle disease virus|Strain Name:Goose/Alaska/415/91|Protein Name:fusion protein|Gene Symbol:F	TSSQTGTIIIKLLPNMPKDKEQCAKSPL DAYNRTLTTLLAPLGDSIRRIQESVTT SGGERQERLVGAIIGGVALGVATAAQ ITAASALIQANQNAANILKLKESIAAT NEAVHEVTSGLSQLAVAVGKMQQFV NDQFNKTAQEIDCIKITQQVGVELNLY LTELTTVFGPQITSPALTQLTIQALYNL AGGNMDYMLTKLGVGNNQLSSLISS GLISGNPILYDSQTQLLGIQVTLPSVGN LNNMRATYLETLSVSTNKGFASALVP KVVTQVGSVIEELDTSYCIETDLDLYC TRIVTFPMSPGIFSCLGGNTSACMYSK TEGALTTPYMTLKGSVIANCKMTTCR CADPPGIISQNYGEAVSLIDKKVCNILT LDGITLRLSGEFDATYQKNISIQDSQV VITGNLDISTELGNVNNSISNALDKLE ESNSKLDKVNVRLTSTSALITYIVLTTI ALICGIVSLVLACYIMYKQKAQQKTL LWLGNNTLDQMRATTKM		
AF266286	4875-7247	gb:AF266286:4875-7247|Organism:Measles virus strain AIK-C|Strain Name:Measles virus strain Edmonston (AIK-C vaccine)|Protein	MSIMGLKVNVSAIFMAVLLTLQTPTG QIHWGNLSKIGVVGIGSASYKVMTRS SHQSLVIKLMPNITLLNNCTRVEIAEY RRLLRTVLEPIRDALNAMTQNIRPVQS VASSRRHKRFAGVVLAGAALGVATA AQITAGIALHQSMLNSQAIDNLRASLE TTNQAIEAIRQAGQEMILAVQGVQDY INNELIPSMNQLSCDLIGQKLGLKLLR YYTEILSLFGPSLRDPISAEISIQALSYA LGGDINKVLEKLGYSGGDLLGILESRG IKARITHVDTESYFIVLSIAYPTLSEIKG VIVHRLEGVSYNIGSQEWYTTVPKYV ATQGYLISNFDESSCTFMPEGTVCSQN ALYPMSPLLQECLRGYTKSCARTLVS GSFGNRFILSQGNLIANCASILCKCYTT	128	629

		Name:fusion protein|Gene Symbol:F	GTIINQDPDKILTYIAADNCPVVEVNG VTIQVGSRRYPDAVYLHRIDLGPPILL ERLDVGTNLGNAIAKLEDAKELLESS DQILRSMKGLSSTCIVYILIAVCLGGLI GIPALICCCRGRCNKKGEQVGMSRPG LKPDLTGTSKSYVRSL		
AB503857	3068-4687	gb:AB503857:3068-4687|Organism:Human metapneumovirus|Strain Name:Jpn03-1|Protein Name:fusion glycoprotein precursor|Gene Symbol:F	MSWKVVIIFSLLITPQHGLKESYLEES CSTITEGYLSVLRTGWYTNVFTLEVG DVENLTCSDGPSLIKTELDLTKSALRE LKTVSADQLAREEQIEKPRQSRFVLG AIALGVATAAAVTAGVAIAKTIRLESE VTAIKNALKTTNEAVSTLGNGVRVLA TAVRELKDFVSKNLTRAINKNKCDID DLKMAVSFSQFNRRFLNVVRQFSDNA GITPAISLDLMTDAELARAVSNMPTSA GQIKLMLENRAMVRRKGFGILIGVYG SSVIYMVQLPIFGVIDTPCWIVKAAPS CSEKKGNYACLLREDQGWYCQNAGS TVYYPNEKDCETRGDHVFCDTAAGIN VAEQSKECNINISTTNYPCKVSTGRHP ISMVALSPLGALVACYKGVSCSIGSNR VGIIKQLNKGCSYITNQDADTVTIDNT VYQLSKVEGEQHVIKGRPVSSSFDPIK FPEDQFNVALDQVFENIENSQALVDQ SNRILSSAEKGNTGFIIVIILIAVLGSSM ILVSIFIIIKKTKKPTGAPPELSGVTNNG FIPHS	125	630
EU277658	5078-6700	gb:EU277658:5078-6700|Organism:Bovine parainfluenza virus	MIIIVITMILSLTPSSLCQIDITKLQSVG VLVNSPKGIKISQNFETRYLILSLIPKIE DSHSCGNQQIDQYKKLLDRLIIPLYDG LKLQKDVIVVNHESHNNTNLRTKRFF GEIIGTIAIGIATSAQITAAVALVEAKQ ARSDIDKLKEAIKDTNKAVQSIQSSVG NLIVAVKSVQDYVNNEIVPSITRLGCE	93	631

		3|Strain Name:Q5592|Protein Name:fusion protein|Gene Symbol:F	AAGLQLGIALTQHYSELTNIFGDNIGT LGEKGVKLQGIASLYRTNITEVFTTST VDQYDIYDLLFTESIKMRVIDVDLSDY SITLQVRLPLLTKVSNTQIYKVDSISYN IQGKEWYIPLPHHIMTKGAFLGGADIK ECIESFSNYICPSDPGFILNHEMENCLS GNITQCPKTIVTSDIVPRYAFVDGGVI ANCIPTTCTCNGIDNRINQSPDQGIKIIT YKECQIVGINGMLFKTNQEGTLAKYT FDNIKLNNSVALNPIDISLELNKAKSD LEESKRWIEKSNQKLDSIGSWHQSSVT IIIIIVMIVVLLIINAIIIMIMIRYLRDRNR HLNNKDSEPYVLTNRQ		
AB040874	4546-6162	gb:AB040874:4546-6162|Organism:Mumps virus|Strain Name:Miyahara|Protein Name:fusion protein|Gene Symbol:F	MKVFLVTCLGFAVFSSSVCVNINILQQ IGYIKQQVRQLSYYSQSSSSYIVVKLL PNIQPTDNSCEFKSVTQYNKTLSNLLL PIAENINNIASPSSGSRRHKRFAGIAIGI AALGVATAAQVTAAVSLVQAQTNAR AIAAMKNSIQATNRAVFEVKEGTQRL AIAVQAIQDHINTIMNTQLNNMSCQIL DNQLATSLGLYLTELTTVFQPQLINPA LSPISIQALRSLLGSMTPAVVQATLSTS ISAAEILSAGLMEGQIVSVLLDEMQMI VKINIPTIVTQSNALVIDFYSISSFINNQ ESIIQLPDRILEIGNEQWSYPAKNCKLT RHHIFCQYNEAERLSLESKLCLAGNIS ACVFSPIAGSYMRRFVALDGTIVANC RSLTCLCKSPSYPIYQPDHHAVTTIDL TACQTLSLDGLDFSIVSLSNITYAENLT ISLSQTINTQPIDISTELSKVNASLQNA VKYIKESNHQLQSVNVNSKIGAIIVAA LVLSILSIIISLLFCCWAYVATKEIRRIN FKTNHINTISSSVDDLIRY	89	632
AB47509	4908-6923	gb:AB475	MNPHEQTIPMHEKIPKRSKTQTHTQQ	46	633

7		097:4908-6923|Organism:Canine distemper virus|Strain Name:M25CR|Protein Name:fusion protein|Gene Symbol:F	DLPQQHSTKSAESKTSRARHSITSAQRSTHYDPRTADWPDYYIMKRTRSCKQASYRSDNIPAHGDHDGIIHHTPESVSQGAKSRLKMGQSNAVKSGSQCTWLVLWCIGVASLFLCSKAQIHWNNLSTIGIIGTDSVHYKIMTRPSHQYLVIKLMPNVSLIDNCTKAELDEYEKLLSSILEPINQALTLMTKNVKPLQSVGSGRRQRRFAGVVLAGAALGVATAAQITAGIALHQSNLNAQAIQSLRTSLEQSNKAIEEIREATQETVIAVQGVQDYVNNELVPAMQHMSCELVGQRLGLKLLRYYTELLSIFGPSLRDPISAEISIQALSYALGGEIHKILEKLGYSGNDMIAILESRGIKTKITHVDLPGKFIILSVSYPTLSEVKGVIVHRLEAVSYNIGSQEWYTTVPRYVATNGYLISNFDESSCVFVSESAICSQNSLYPMSPLLQQCIRGDTSSCARTLVSGTMGNKFILSKGNIVANCASILCKCYSTSTIINQSPDKLLTFIASDTCPLVEIDGVTIQVGSRQYPDMVYESKVALGPAISLERLDVGTNLGNALKKLDDAKVLIDSSNQILETVRRSSFNFGSLLSVPILSCTALALLLLICCCKRRYQQTHKQNTKVDPTFKPDLTGTSRSYVRSL		
AJ849636	5526-7166	gb:AJ849636:5526-7166|Organism:Peste-des-petits-ruminants virus|Strain	MTRVAILTFLFLFPNAVACQIHWGNLSKIGIVGTGSASYKVMTRPSHQTLVIKLMPNITAIDNCTKSEIAEYKRLLITVLKPVEDALSVITKNVRPIQTLTPGRRTRRFAGAVLAGVALGVATAAQITAGVALHQSLMNSQAIESLKTSLEKSNQAIEEIRLANKETILAVQGVQDYINNELVPSVHRMSCELVGHKLGLKLLRYYTEILSIFGPSLRDPIAAEISIQALSYALGGDINRIL	34	634

		Name:Turkey 2000|Protein Name:fusion protein|Gene Symbol:F	DKLGYSGGDFLAILESKGIKARVTYV DTRDYFIILSIAYPTLSEIKGVIVHKIEA ITYNIGAQEWYTTIPKYVATQGYLISN FDETSCVFTPDGTVCSQNALYPMSPLL QECFQGSTKSCARTLVSGTISNRFILSK GNLIANCASVLCKCYTTETVISQDPDK LLTVVASDKCPVVEVDGVTIQVGSRE YPDSVYLHKIDLGPAISLEKLDVGTNL GNAVTRLENAKELLDASDQILKTVKG VPFGGNMYIALAACIGVSLGLVTLICC CKGRCKNKEVPISKINPGLKPDLTGTS KSYVRSL		
AF017149	6618-8258	gb:AF017149|Organism:Hendra virus|Strain Name:UNKNOWN-AF017149|Protein Name:fusion|Gene Symbol:F	MATQEVRLKCLLCGIIVLVLSLEGLGI LHYEKLSKIGLVKGITRKYKIKSNPLT KDIVIKMIPNVSNVSKCTGTVMENYK SRLTGILSPIKGAIELYNNNTHDLVGD VKLAGVVMAGIAIGIATAAQITAGVA LYEAMKNADNINKLKSSIESTNEAVV KLQETAEKTVYVLTALQDYINTNLVP TIDQISCKQTELALDLALSKYLSDLLF VFGPNLQDPVSNSMTIQAISQAFGGN YETLLRTLGYATEDFDDLLESDSIAGQ IVYVDLSSYYIIVRVYFPILTEIQQAYV QELLPVSFNNDNSEWISIVPNFVLIRNT LISNIEVKYCLITKKSVICNQDYATPM TASVRECLTGSTDKCPRELVVSSHVPR FALSGGVLFANCISVTCQCQTTGRAIS QSGEQTLLMIDNTTCTTVVLGNIIISLG KYLGSINYNSESIAVGPPVYTDKVDIS SQISSMNQSLQQSKDYIKEAQKILDTV NPSLISMLSMIILYVLSIAALCIGLITFIS FVIVEKKRGNYSRLDDRQVRPVSNGD LYYIGT	29	635
AB00579	4866-6563	gb:AB005	MATYIQRVQCISALLSVVLTTLVSCQI	23	636

5		795:4866-6563|Organism:Sendai virus|Strain Name:Ohital|Protein Name:fusion protein|Gene Symbol:F	PRDRLSNIGVIVDEGKSLKIAGSHESR YIVLSLVPGIDLENGCGTAQVIQYKSL LNRLLIPLRDALDLQEALITVTNDTMT GADVPQSRFFGAVIGTIALGVATSAQI TAGIALAEAREAKRDIALIKESMTKTH KSIELLQNAVGEQILALKTLQDFVNDE IKPAISELGCETAALRLGIKLTQHYSEL LTAFGSNFGTIGEKSLTLQALSSLYSA NITEIMTTIRTGQSNIYDVIYTEQIKGT VIDVDLERYMVTLSVKIPILSEVPGVLI HKASSISYNIDGEEWYVTVPSHILSRA SFLGGANIADCVESRLTYICPRDPAQLI PDSQQKCILGDTTRCPVTKVVDNIIPK FAFVNGGVVANCIASTCTCGTGRRPIS QDRSKGVVFLTHDNCGLIGVNGIELY ANRKGHDATWGVQNLTVGPAIAIRPV DISLNLAAATDFLQDSRAELEKARKIL SEVGRWYNSGATLITIIVVMIVVLVVII VIVIVLYRLRRSMLMSNPAGRISRDTY TLEPKIRHMYTNGGFDAMTEKR		
AF457102	5088-6755	gb:AF457102|Organism:Human parainfluenza virus 1 strain Washington/1964|Strain Name:Washington 1964|Protein Name:F	MQKSEILFLVYSSLLLSSSLCQIPVEKL SNVGVIINEGKLLKIAGSYESRYIVLSL VPSIDLQDGCGTTQIIQYKNLLNRLLIP LKDALDLQESLITITNDTTVTNDNPQT RFFGAVIGTIALGVATAAQITAGIALA EAREARKDIALIKDSIVKTHNSVELIQ RGIGEQIIALKTLQDFVNDEIRPAIGEL RCETTALKLGIKLTQHYSELATAFSSN LGTIGEKSLTLQALSSLYSANITEILST TKKDKSDIYDIIYTEQVKGTVIDVDLE KYMVTLLVKIPILSEIPGVLIYRASSISY NIEGEEWHVAIPNYIINKASSLGGADV TNCIESKLAYICPRDPTQLIPDNQQKCI LGDVSKCPVTKVINNLVPKFAFINGG	21	637

		glycoprotein|Gene Symbol:F	VVANCIASTCTCGTNRIPVNQDRSRG VTFLTYTNCGLIGINGIELYANKRGRD TTWGNQIIKVGPAVSIRPVDISLNLAS ATNFLEESKTELMKARAIISAVGGWH NTESTQIIMIIIVCILIIIICGILYYLYRVR RLLVMINSTHNSPVNAYTLESRMRNP YMGNNSN		
AB910309	4951-6582	gb:AB910309:4951-6582|Organism:Feline morbillivirus|Strain Name:SS1|Protein Name:fusion protein|Gene Symbol:F	MGKIRVIIISSLLLSNITTAQVGWDNLT SIGVISTKQYDYKITTLNTNQLMVIKM VPNISSIINCTKPELMKYRELVLGVIRP INESLELMNSYINMRAGSERFIGAVIA GVALGVATAAQITSGIALHNSIMNKR QIQELRKALSTTNKAIDEIRIAGERTLI AVQGVQDYINNIIIPMQDKLQCDILSS QLAIALLRYYTNILTVFGPSIRDPVTSII SIQALSQAFNGNLQALLDGLGYTGRD LRDLLESRSITGQIIHADMTDLFLVLRI NYPSITEMQGVTIYELNSITYHIGPEE WYTIMPNFIAVQGFLTSNFDERKCSIT KSSILCQQNSIYPMSTEMQRCIKGEIRF CPRSKAVGTLVNRFILTKGNLMANCL GVICRCYSSGQIITQDPSKLITIISQEEC KEVGVDGIRIMVGPRKLPDVIFNARLE VGVPISLSKLDVGTDLAIASAKLNNSK ALLEQSDKILDSMSKLDSINSRITGLIL AIMAIFIITVTIIWIIYKRCRNKDNKFST SIEPLYIPPSYNSPHSVVKSI	12	638
KT071755	4310-6070	gb:KT071755:4310-6070|Organism:Avian paramyxovirus	MIAALFISLFATCGALDNSVLAPVGIA SAQEWQLAAYTNTLSGTIAVRFVPVL PGNLSTCAQATLAEYNKTVTNILGPL KENLETLLSEPTKTAARFVGAIIGTVA LGVATSAQITAAVALNQAQENARNIW RLKESIRKTNEAVLELKDGLASTAIAL DKVQKFINEDIIPQIKEIDCQVVANKL	12	639

		2|Strain Name:APMV-2/Procardueliss nipalensis/China/Suiling/53/2013|Protein Name:fusion protein|Gene Symbol:F	GVYLSLYLTELTTIFGAQITNPALTPLS YQALYNLCGGDMGKLTELIGVKAKDI NSLYEANLITGQVIGYDSESQIILIQVS YPSVSEVTGVRATELVTVSVTTPKGE GRAIAPKYVAQSRVVTEELDTSTCRFS KTTLYCRSIITRPLPPLIANCLNGLYQD CQYTTEIGALSSRFITVNGGIIANCRAT ICKCVNPPKIIVQSDASSLTVIDSAICK DVVLDNVQLRLEGKLSAQYFTNITIDL SQITTSGSLDISSEIGSINNTVNKVEELI AESNAWLQAVNPHLVNNTSIIVLCVL AAIFVVWLVALTGCLAYYIKKSSATR MVGIGSSPAGNPYVAQSATKM		
AY029299	4598-6265	gb:AY029299|Organism:Avian paramyxovirus 6|Strain Name:APMV-6/duck/Taiwan/Y1/98|Protein Name:fusion protein|Gene Symbol:F	MGARLGPLAMAPGRYVIIFNLILLHRV VSLDNSRLLQQGIMSATEREIKVYTNS ITGSIAVRLIPNLPQEVLKCSAGQIKSY NDTLNRIFTPIKANLERLLATPSMLED NQNPAPEPRLIGAIIGTAALGLATAAQ VTAALALNQAQDNAKAILNLKESITK TNEAVLELKDATGQIAIALDKTQRFIN DNILPAINNLTCEVAGAKVGVELSLYL TELSTVFGSQITNPALSTLSIQALMSLC GNDFNYLLNLMGAKHSDLGALYEAN LINGRIIQYDQASQIMVIQVSVPSISSIS GLRLTELFTLSIETPVGEGKAVVPQFV VESGQLLEEIDTQACTLTDTTAYCTIV RTKPLPELVAQCLRGDESRCQYTTGIG MLESRFGVFDGLVIANCKATICRCLAP EMIITQNKGLPLTVISQETCKRILIDGV TLQIEAQVSGSYSRNITVGNSQIAPSGP LDISSELGKVNQSLSNVEDLIDQSNQL LNRVNPNIVNNTAIIVTIVLLVLLVLW CLALTISILYVSKHAVRMIKTVPNPYV	11	640

			MQAKSPGSATQF		
AY141760	5028-6665	gb:AY141760|Organism:Fer-de-Lance paramyxovirus|Strain Name:ATCC VR-895|Protein Name:fusion protein F|Gene Symbol:F	MTRITILQIILTLTLPVMCQVSFDNLEQVGVMFDKPKFLKITGPASTATMIIKLIPTLGTMESCGTSAVNEYKKTLDTILVPLRDTINKLSTDITVVEGTSNISNKREKRFVGIAIAVGAVALATSAQITAGIALSNTIKNAEAIESIKSSIQASNQAIQKVIDAQGRTVTVINGIQDHINSVINPALNQLGCDVAKNTLAISLTQYFSKLSLLFGPNLRNPVEQPLSVQAIAGLMDGDINAVVSQLGYTQSDLLDLLSTESIVGTVTAIDMVNYMIQIEMSFPQYITIPDTKVLEGHKITFNDKGSEWQTQVPSTIAVRDILIAGVDPDGCSITSTSYICKNDPTYAMSEVLTNCFRGNTQECPRARITSTFATRFAIARSTVIANCVAAVCLCGDPGIPVVQKAEVTLTAMTLDQCSLITVDGLQIKPSKSIANVTANFGNITLGPVVSVGDLDLSAELTKVQSDLKEAQDKLDESNAILQGINNKILTAPTSIALIVVSVVVILLIIGMISWLVWLTKAVRRSNTRSERVTPSAYNNLGFIK	8	641
EU877976	4330-6410	gb:EU877976:4330-6410|Organism:Avian paramyxovirus 4|Strain Name:APMV-4/KR/YJ/06|Protein	MRLSRTILTLILGTLTGYLMGAHSTNVNEGPKSEGIRGDLIPGAGIFVTQVRQLQIYQQSGYHDLVIRLLPLLPAELNDCQREVVTEYNNTVSQLLQPIKTNLDTLLADGGTRDADIQPRFIGAIIATGALAVATVAEVTAAQALSQSKTNAQNILKLRDSIQATNQAVFEISQGLEATATVLSKLQTELNENIIPSLNNLSCAAMGNRLGVSLSLYLTLMTTLFGDQITNPVLTPISYSTLSAMAGGHIGPVMSKILAGSVTSQLGAEQLIASGLIQSQVVGYDSQYQLLVIRVNLVRIQEVQNTRVVSLRTLAVNRDGG	8	642

		Name:fusion protein|Gene Symbol:F	LYRAQVPPEVVERSGIAERFYADDCV LTTTDYICSSIRSSRLNPELVKCLSGAL DSCTFERESALLSTPFFVYNKAVVAN CKAATCRCNKPPSIIAQYSASALVTITT DTCADLEIEGYRFNIQTESNSWVAPNF TVSTSQIVSVDPIDISSDIAKINSSIEAA REQLELSNQILSRINPRIVNDESLIAIIV TIVVLSLLVIGLIVVLGVMYKNLKKV QRAQAAMMMQQMSSSQPVTTKLGTP F		
AB176531	4793-6448	gb:AB176531:4793-6448|Organism:Human parainfluenza virus 2|Strain Name:Nishio|Protein Name:fusion protein|Gene Symbol:F	MHHLHPMIVCIFVMYTGIVGSDAIAG DQLLNIGVIQSKIRSLMYYTDGGASFI VVKLLPNLPPSNGTCNITSLDAYNVTL FKLLTPLIENLSKISTVTDTKTRQKRFA GVVVGLAALGVATAAQITAAVAIVK ANANAAAINNLASSIQSTNKAVSDVID ASRTIATAVQAIQDRINGAIVNGITSAS CRAHDALIGSILNLYLTELTTIFHNQIT NPALTPLSIQALRILLGSTLPIVIESKLN TNFNTAELLSSGLLTGQIISISPMYMQ MLIQINVPTFIMQPGAKVIDLIAISANH KLQEVVVQVPNRILEYANELQNYPAN DCVVTPNSVFCRYNEGSPIPESQYQCL RGNLNSCTFTPIIGNFLKRFAFANGVL YANCKSLLCRCADPPHVVSQDDTQGI SIIDIKRCSEMMLDTFSFRITSTFNATY VTDFSMINANIVHLSPLDLSNQINSINK SLKSAEDWIADSNFFANQARTAKTLY SLSAIALILSVITLVVVGLLIAYIIKLVS QIHQFRSLAATTMFHRENPAFFSKNN HGNIYGIS	7	643
BK005918	4677-6302	gb:BK005918|Organism:Porcin	MPQQQVAHTCVMLWGIISTVSGINTE ALSQYGVVVTNVRQLTYYTQAGSTY LAVRLLPSLASPDQSCALHSIINYNAT	7	644

		e rubulavirus|Strain Name:UNKNOWN-BK005918|Protein Name:fusion protein|Gene Symbol:F	LQAILSPIAENLNLISTALREQHRKKRF AGVAIGLTALGVATAAQATAAVALV RANKNAEKVEQLSQALGETNAAISDL IDATKNLGFAVQAIQNQINTAILPQIH NLSCQVIDAQLGNILSLYLTELTTVFQ PQLTNPALSPLTIQALRAVLGTTLPAL LSEKLKSNIPLGDLMSSGLLKGQLVGL NLQNMLMIIELYIPTLSTHSTAKVLDL VTISSHVNGREVEIQVPNRVLELGSEV LGYGGSECALTMSHILCPFNDARVLS TDMKYCLQGNITHCIFSPVVGSFLRRF ALVNGVVIANCADMSCVCFDPQEIIY QNFQEPTTVIDIKKCGKVQLDTLTFTIS TFANRTYGPPAYVPPDNIIQSEPLDISG NLIAVNNSLSSALNHLATSEILRNEQI WTSSLGISTIVALVIIGILIICLVVTWAA LWALLKEVRGLNSAVNSQLSSYVMG DKFIRY		
KC237063	4530-6185	gb:KC237063:4530-6185|Organism:Parainfluenza virus 5|Strain Name:08-1990|Protein Name:fusion protein|Gene Symbol:F|Segment:	MGTRIQFLMVSCLLAGTGSLDPAALM QIGVIPTNVRQLMYYTEASSAFIVVKL MPTIDSPISGCNITSISSYNATMTKLLQ PIGENLETIRYQLIPTRRRRRFVGVVIG LAALGVATAAQVTAAVALVKANKN AAAILNLKNAIQKTNAAVADVVQAT QSLGTAVQAVQDHINSVVSPAITAAN CKAQDAIIGSILNLYLTELTTIFHNQIT NPALSPITIQALRILLGSTLPTVVRKSF NTQISAAELLSSGLLTGQIVGLDLTYM QMVIKIELPTLTVQPATQIIDLVTISAFI NNREVMAQLPTRIIVTGSLIQAYPASQ CTITPNTVYCRYNDAQVLSDDTMACL QGNLTRCTFSPVVGSFLTRFVLFDGIV YANCRSMLCKCMQPAAVILQPSSSPV TVIDMHKCVSLQLDNLRFTITQLANIT	7	645

		4	YNSTIKLETSQILPIDPLDISQNLAAVN KSLSDALQHLAQSDTYLSAITSATTTS VLSIIAICLGSLGLILIILISVVVWKLLTI VAANRNRMENFVYHNSAFHHSRSDL SEKNQPATLGTR		
AY729016	5862-7523	gb:AY729016:5862-7523|Organism:Murine pneumonia virus|Strain Name:15; ATCC VR-25|Protein Name:fusion glycoprotein precursor| Gene Symbol:F	MIPGRIFLVLLVIFNTKPIHPNTLTEKF YESTCSVETAGYKSALRTGWHMTVM SIKLSQINIESCKSSNSLLAHELAIYSSA VDELRTLSSNALKSKRKKRFLGLILGL GAAVTAGVALAKTVQLESEIALIRDA VRNTNEAVVSLTNGMSVLAKVVDDL KNFISKELLPKINRVSCDVHDITAVIRF QQLNKRLLEVSREFSSNAGLTHTVSSF MLTDRELTSIVGGMAVSAGQKEIMLS SKAIMRRNGLAILSSVNADTLVYVIQL PLFGVMDTDCWVIRSSIDCHNIADKY ACLARADNGWYCHNAGSLSYFPSPT DCEIHNGYAFCDTLKSLTVPVTSRECN SNMYTTNYDCKISTSKTYVSTAVLTT MGCLVSCYGHNSCTVINNDKGIIRTLP DGCHYISNKGVDRVQVGNTVYYLSK EVGKSIVVRGEPLVLKYDPLSFPDDKF DVAIRDVEHSINQTRTFLKASDQLLDL SENRENKNLNKSYILTTLLFVVMLIIIM AVIGFILYKVLKMIRDNKLKSKSTPGL TVLS	6	646
AB543336	5174-6805	gb:AB543336:5174-6805|Organism:Human parainfluenza virus 4a|Strain	MGVKGLSLIMIGLLISPITNLDITHLMN LGTVPTAIRSLVYYTYTKPSYLTVDLI PNLKNLDQNCNYSSLNYYNKTALSLI QPIADNINRLTKPITSSEIQSRFFGAVIG TIALGVATAAQVTAAIGLAKAQENAK LILTLKKAATETNEAVRDLANSNKIV VKMISAIQNQINTIIQPAIDQINCQIKDL QVANILNLYLTEITTVFHNQLTNPALE	5	647

		Name:M-25|Protein Name:fusion protein|Gene Symbol:F	SISIQALKSLLGPTLPEVLSKLDLNNIS AASVMASGLIKGQIIAVDIPTMTLVLM VQIPSISPLRQAKIIDLTSITIHTNSQEV QAVVPARFLEIGSEILGFDGSVCQITK DTIFCPYNDAYELPIQQKRCLQGQTRD CVFTPVAGTFPRRFLTTYGTIVANCRD LVCSCLRPPQIIYQPDENPVTIIDKDLC TTLTLDSITIEIQKSINSTFRREVVLEST QVRSLTPLDLSTDLNQYNQLLKSAED HIQRSTDYLNSINPSIVNNNAIIILIILCI LLILTVTICIIWLKYLTKEVKNVARNQ RLNRDADLFYKIPSQIPVPR		
AF298895	4834-6450	gb:AF298895|Organism:Tioman virus|Strain Name:UNKNOWN-AF298895|Protein Name:fusion protein|Gene Symbol:F	MRIALTAVIVSIHFDLAFPMNKNSLLS VGLVHKSVKNLYFYSQGSPSYIVVKL VPTLGNVPGNCTLNSLVRYKSTVSSL LSPLAENLEYLQKTLTVSRGGRRRRF AGVAIGLAALGVAAAAQATAAVALV EARQNAAQIQSLSEAIQNTNLAVNEL KTAIGASATAIQAIQTQINEVINPAINR LSCEILDAQLASMLNLYLIHLTTVFQN QLTNPALTPLSIQSLQSLLQGTSSVLTN ITSSSKLALNDALVTGLITGQVVGLN MTSLQIVIAAYVPSVAKLSNAVVHNFI RITTSVNGTEVIIQSPTIIMEQNEVMYD LKTGHCTESDLNIYCPYVDAQLLSPG MTNCINGRLNDCTFSKVVGSFPTRFA AVEGAILANCKYLQCNCLTPPYIITPL NGEMISMIDLSKCQRLDLGTIVFDINN PVNVTFNGNYRADVGQMIVTNPLDIS AELNQINTSLSNAQGFLSKSDAWLHV SQWVTNSGTIFIILIIGLIVGIVYMIINT YVVVQIIKEINRMRTSDRAHLLKGSIS SIST	5	648
FJ215863	4499-6130	gb:FJ2158	MGQISVYLINSVLLLLVYPVNSIDNTLI	5	649

		63:4499-6130|Organism:Avian paramyxovirus 8|Strain Name:goose/Delaware/1053/76|Protein Name:fusion protein|Gene Symbol:F	APIGVASANEWQLAAYTTSLSGTIAV RFLPVLPDNMTTCLRETITTYNNTVN NILGPLKSNLDALLSSETYPQTRLIGA VIGSIALGVATSAQITAAVALKQAQD NARNILALKEALSKTNEAVKELSSGL QQTAIALGKIQSFVNEEILPSINQLSCE VTANKLGVYLSLYLTELTTIFGAQLTN PALTSLSYQALYNLCGGNMAMLTQKI GIKQQDVNSLYEAGLITGQVIGYDSQ YQLLVIQVNYPSISEVTGVRATELVTV SVTTDKGEGKAIVPQFVAESRVTIEEL DVASCKFSSTTLYCRQVNTRALPPLV ASCLRGNYDDCQYTTEIGALSSRYITL DGGVLVNCKSIVCRCLNPSKIISQNTN AAVTYVDATICKTIQLDDIQLQLEGSL SSVYARNISIEISQVTTSGSLDISSEIGNI NNTVNRVEDLIHQSEEWLAKVNPHIV NNTTLIVLCVLSALAVIWLAVLTAIIIY LRTKLKTISALAVTNTIQSNPYVNQTK RESKF		
JN689227	4689-6521	gb:JN689227:4689-6521|Organism:Tailam virus|Strain Name:TL8K|Protein Name:fusion protein|Gene Symbol:F	MKLSVVYTTLLVSTFYSDLARSQLAL SELTKIGVIPGRSYDLKISTQASYQYM VVKLIPNLTGLNNCTNGTIEAYKKML NRLLSPIDAALRKMKDAVNDKPPESV GNVKFWGAVIGGVALGVATSAQITA GVALHNSIQNANAILALKDSIRQSNKA IQELQTAMSTTVVVLNALQDQINNQL VPAINSLGCQVVANTLGLKLNQYFSEI SLVFGPNLRDPTSETLSIQALSRAFNG DFDSMLSKLKYDDSDFLDLLESDSIRG RIIDVSLSDYLITIQIEYPALLSIKDAVI QTFNLISYNTRGTEWISIFPKQLLVRGT YISNIDISQCVIAATSIICKSDTSTPISSA TWSCATGNITNCARTRVVNAHVPRFA	5	650

			LYGGVVFANCAPVVCKCQDPLYSINQ EPKVTNVMVDVDACKEMYLDGLYIT LGKTQISRAMYAEDVSLGGPISVDPID LGNEINSINSAINRSEEHLNHANELLD KVNPRIVNVKTFGVMIGLLVLVVLWC VITLVWLICLTKQLARTAYAGSMGSR ASTVNSLSGFVG		
JX857409	4831-6615	gb:JX857409:4831-6615|Organism:Porcine parainfluenza virus 1|Strain Name:S206N|Protein Name:fusion protein|Gene Symbol:F	MQVTTLRPAIILSIALLVTGQVPRDKL ANLGIIIKDSKALKIAGSYENRYIVLSL VPTIDNVNGCGSIQIAKYKEMLERLLI PIKDALDLQESLIVIDNETVNNNYSPQ YRFVGAIIGTIALGVATAAQVTAGVA LMEAREAKRDISMLKEAIEKTQNSIEK LQNSAGEQILALKMLQDYVNGEIKPA IEELGCETAALKLGIALTQHYTELTNA FGSNLGSIGEKSLTLQALSSLYKTNITN ILTATNLGKTDIYDIIYAEQVKGRVID VDLKRYMVTISVKIPILSEIPGVLIYEV SSISYNIDGAEWYAAVPDHILSKSAYI GGADISDCIESRLTYICPQDPAQIIADN QQQCFFGHLDKCPITKVIDNLVPKFAF INGGVVANCIASTCTCGEERIQVSQDR NKGVTFLTHNNCGLIGINGIEFHANKK GSDATWNVSPIGVGPAVSLRPVDISLQ IVAATNFLNSSRKDLMKAKEILNQVG NLKDLTTITIINIVIIIILLICVIGLGILYH QLRSALGMRDKMSVLNNSSYSLEPRT AQVQVIKPTSFMG	5	651
AY640317	2932-4571	gb:AY640317:2932-4571|Organism:Avian metapneu	MDVRICLLLFLISNPSSCIQETYNEESC STVTRGYKSVLRTGWYTNVFNLEIGN VENITCNDGPSLIDTELVLTKNALREL KTVSADQVAKESRLSSPRRRRFVLGAI ALGVATAAAVTAGVALAKTIRLEGEV KAIKNALRNTNEAVSTLGNGVRVLAT	4	652

		movirus|Strain Name:LAH A|Protein Name:F|Gene Symbol:F	AVNDLKEFISKKLTPAINQNKCNIADI KMAISFGQNNRRFLNVVRQFSDSAGI TSAVSLDLMTDDELVRAINRMPTSSG QISLMLNNRAMVRRKGFGILIGVYDG TVVYMVQLPIFGVIETPCWRVVAAPL CRKRRGNYACILREDQGWYCTNAGS TAYYPNKDDCEVRDDYVFCDTAAGI NVALEVDQCNYNISTSKYPCKVSTGR HPVSMVALTPLGGLVSCYESVSCSIGS NKVGIIKQLGKGCTHIPNNEADTITID NTVYQLSKVVGEQRTIKGAPVVNNFN PILFPVDQFNVALDQVFESIDRSQDLID KSNDLLGADAKSKAGIAIAIVVLVILG IFFLLAVIYYCSRVRKTKPKHDYPATT GHSSMAYVS		
KU646513	4641-6498	gb:KU646513:4641-6498|Organism:Avian paramyxovirus 13 goose/Kazakhstan/5751/2013|Strain Name:APMV-13/white fronted goose/Northern Kazakhstan/5751/20	MARFSWEIFRLSTILLIAQTCQGSIDGR LTLAAGIVPVGDRPISIYTSSQTGIIVV KLIPNLPDNKKDCAKQSLQSYNETLS RILTPLATAMSAIRGNSTTQVRENRLV GAIIGSVALGVATAAQITAATALIQAN QNAANIARLANSIAKTNEAVTDLTEG LGTLAIGVGKLQDYVNEQFNNTAVAI DCLTLESRLGIQLSLYLTELMGVFGNQ LTSPALTPITIQALYNLAGGNLNALLS RLGASETQLGSLINSGLIKGMPIMYDD ANKLLAVQVELPSIGKLNGARSTLLET LAVDTTRGPSSPIIPSAVIEIGGAMEEL DLSPCITTDLDMFCTKIISYPLSQSTLS CLNGNLSDCVFSRSEGVLSTPYMTIKG KIVANCKQVICRCMDPPQILSQNYGE ALLLIDENTCRSLELSGVILKLAGTYE SEYTRNLTVDPSQVIITGPLDISAELSK VNQSIDSAKENIAESNKFLSQVNVKLL SSSAMITYIVATVVCLIIAITGCVIGIYT	4	653

		13|Protein Name:fusion protein|Gene Symbol:F	LTKLKSQQKTLLWLGNNAEMHGSRSKTSF		
AF326114	4818-6482	gb:AF326114|Organism:Menangle virus|Strain Name:UNKNOWN-AF326114|Protein Name:fusion protein|Gene Symbol:F	MMPRVLGMIVLYLTHSQILCINRNTLYQIGLIHRSVKKVNFYSQGSPSYIVVKLVPTLAAIPPNCSIKSLQRYKETVTSLVQPISDNLGYLQDKLVTGQSRRRRRFAGVAIGLAALGVAAAAQATAAVALVETRENAGKIQALSESIQNTNQAVHSLKTALGFSATAIQAIQNQVNEVINPAINKLSCEVLDSQLASMLNLYLIHLTTVFQTQLTNPALTPLSIQALTSVLQGTSGVLMNSTNSTLTQPIDLLATGLITGQIISVNMTSLQLIIATFMPSIAELPNAVLHSFFRITTSVNLTEVMIQSPEFIMEQNGVFYDFNTAHCQLGDNNVYCPYIDAARLSSMMTNCINGNLGECVFSRVIGSFPSRFVSLNGAILANCKFMRCNCLSPEKIITPLDGEMISLIDLRVCQKLTLGTITFEISQPVNVSFQGGFVANAGQIIVTNPFDISAELGQINNSLNDAQGFLDQSNNWLKVSGWINNSGSLFIAGIVVIGLIVLCIVIIIYINVQIIREVNRLRSFIYRDYVLDHDKAPYSPESSSPHRKSLKTVS	3	654
GU206351	5441-7468	gb:GU206351:5441-7468|Organism:Avian paramyxovirus	MLQLPLTILLSILSAHQSLCLDNSKLIHAGIMSTTEREVNVYAQSITGSIVVRLIPNIPSNHKSCATSQIKLYNDTLTRLLTPIKANLEGLISAVSQDQSQNSGKRKKRFVGAVIGAAALGLATAAQVTATVALNQAQENARNILRLKNSIQKTNEAVMELKDAVGQTAVAIDKTQAFINNQILPAIS	3	655

		5|Strain Name:budgerigar/Kunitachi/74|Protein Name:fusion protein|Gene Symbol:F	NLSCEVLGNKIGVQLSLYLTELTTVFG NQLTNPALTTLSLQALYNLCGDDFNY LINLLNAKNRNLASLYEANLIQGRITQ YDSMNQLLIIQVQIPSISTVSGMRVTEL FTLSVDTPIGEGKALVPKYVLSSGRIM EEVDLSSCAITSTSVFCSSIISRPLPLETI NCLNGNVTQCQFTANTGTLESRYAVI GGLVIANCKAIVCRCLNPPGVIAQNLG LPITIISSNTCQRINLEQITLSLGNSILST YSANLSQVEMNLAPSNPLDISVELNR VNTSLSKVESLIKESNSILDSVNPQILN VKTVIILAVIIGLIVVWCFILTCLIVRGF MLLVKQQKFKGLSVQNNPYVSNNSH		
JQ001776	6129-8166	gb:JQ001776:6129-8166|Organism:Cedar virus|Strain Name:CG1a|Protein Name:fusion glycoprotein|Gene Symbol:F	MSNKRTTVLIIISYTLFYLNNAAIVGFD FDKLNKIGVVQGRVLNYKIKGDPMTK DLVLKFIPNIVNITECVREPLSRYNETV RRLLLPIHNMLGLYLNNTNAKMTGL MIAGVIMGGIAIGIATAAQITAGFALY EAKKNTENIQKLTDSIMKTQDSIDKLT DSVGTSILILNKLQTYINNQLVPNLELL SCRQNKIEFDLMLTKYLVDLMTVIGP NINNPVNKDMTIQSLSLLFDGNYDIM MSELGYTPQDFLDLIESKSITGQIIYVD MENLYVVIRTYLPTLIEVPDAQIYEFN KITMSSNGGEYLSTIPNFILIRGNYMSN IDVATCYMTKASVICNQDYSLPMSQN LRSCYQGETEYCPVEAVIASHSPRFAL TNGVIFANCINTICRCQDNGKTITQNIN QFVSMIDNSTCNDVMVDKFTIKVGKY MGRKDINNINIQIGPQIIIDKVDLSNEIN KMNQSLKDSIFYLREAKRILDSVNISLI SPSVQLFLIIISVLSFIILLIIIVYLYCKSK HSYKYNKFIDDPDYYNDYKRERINGK ASKSNNIYYVGD	3	656

LC168749	4869-7235	gb:LC168749:4869-7235|Organism:Rinderpest morbillivirus|Strain Name:Lv|Protein Name:F protein|Gene Symbol:F	MGILFAALLAMTNPHLATGQIHWGNLSKIGVVGTGSASYKVMTQSSHQSLVIKLMPNVTAIDNCTKTEIMEYKRLLGTVLKPIREALNAITKNIKPIQSSTTSRRHKRFAGVVLAGAALGVATAAQITAGIALHQSMMNSQAIESLKASLETTNQAIEEIRQAGQEMVLAVQGVQDYINNELVPAMGQLSCEIVGQKLGLKLLRYYTEILSLFGPSLRDPVSAELSIQALSYALGGDINKILEKLGYSGSDLLAILESKGIKAKITYVDIESYFIVLSIAYPSLSEIKGVIVHRLESVSYNIGSQEWYTTVPRYVATQGYLISNFDDTPCAFTPEGTICSQNALYPMSPLLQECFRGSTRSCARTLVSGSIGNRFILSKGNLIANCASILCKCYTTGSIISQDPDKILTYIAADQCPVVEVGGVTIQVGSREYSDAVYLHEIDLGPPISLEKLDVGTNLWNAVTKLEKAKDLLDSSDLILENIKGVSVTNTGYILVGVGLIAVVGILIITCCCKKRRSDNKVSTMVLNPGLRPDLTGTSKSYVRSL	2	657
LC187310	6250-7860	gb:LC187310:6250-7860|Organism:Avian paramyxovirus 10|Strain Name:rAPMV-10-FI324/YmHA|Protein	MTRTRLLFLLTCYIPGAVSLDNSILAPAGIISASERQIAIYTQTLQGTIALRFIPVLPQNLSSCAKDTLESYNSTVSNLLLPIAENLNALLKDADKPSQRIIGAIIGSVALGVATTAQVTAALAMTQAQQNARNIWKLKESIKNTNQAVLELKDGLQQSAIALDKVQSFINSEILPQINQLGCEVAANKLGIFLSLYLTEITTVFKNQITNPALSTLSYQALYNLCGGNMAALTKQIGIKDTEINSLYEAELITGQVIGYDSADQILLIQVSYPSVSRVQGVRAVELLTVSVATPKGEGKAIAPSFIAQSNIIAEELDTQPCKFSKTTLYCRQVNTRTLPVRVANCLKGK	2	658

		Name:fusion protein|Gene Symbol:F	YNDCQYTTEIGALASRYVTITNGVVANCRSIICRCLDPEGIVAQNSDAAITVIDRSTCKLIQLGDITLRLEGKLSSSYSKNITIDISQVTTSGSLDISSELGSINNTITKVEDLISKSNDWLSKVNPTLISNDTIIALCVIAGIVVIWLVIITILSYYILIKLKNVALLSTMPKKDLNPYVNNTKF		
NC_005283	5277-6935	gb:NC_005283:5277-6935|Organism:Dolphin morbillivirus|Strain Name:UNKNOWN-NC_005283|Protein Name:fusion protein|Gene Symbol:F	MAASNGGVMYQSFLTIIILVIMTEGQIHWGNLSKIGIVGTGSASYKVMTRPNHQYLVIKLMPNVTMIDNCTRTEVTEYRKLLKTVLEPVKNALTVITKNIKPIQSLTTSRRSKRFAGVVLAGVALGVATAAQITAGVALHQSIMNSQSIDNLRTSLEKSNQAIEEIRQASQETVLAVQGVQDFINNELIPSMHQLSCEMLGQKLGLKLLRYYTEILSIFGPSLRDPVSAEISIQALSYALGGDINKILEKLGYSGADLLAILESRGIKAKVTHVDLEGYFIVLSIAYPTLSEVKGVIVHKLEAVSYNLGSQEWYTTLPKYVATNGYLISNFDESSCAFMSEVTICSQNALYPMSPLLQQCLRGSTASCARSLVSGTIGNRFILSKGNLIANCASVLCKCYSTGTIISQDPDKLLTFVAADKCPLVEVDGITIQVGSREYPDSVYVSRIDLGPAISLEKLDVGTNLGSALTKLDNAKDLLDSSNQILENVRRSSFGGAMYIGILVCAGALVILCVLVYCCRRHCRKRVQTPPKATPGLKPDLTGTTKSYVRSL	2	659
NC_005339	5374-7602	gb:NC_005339:5374-7602|Organism:Mosman	MSNYFPARVIIIVSLITAVSCQISFQNLSTIGVFKFKEYDYRVSGDYNEQFLAIKMVPNVTGVENCTASLIDEYRHVIYNLLQPINTTLTASTSNVDPYAGNKKFFGAVIAGVALGVATAAQVTAGVALYEARQNAAAIAEIKESLHYTHKAIESLQISQ	2	660

		virus|Strain Name:UNKNOWN-NC_005339|Protein Name:fusion protein|Gene Symbol:F	KQTVVAIQGIQDQINTNIIPQINALTCEIANQRLRLMLLQYYTEMLSSFGPIIQDPLSGHITVQALSQAAGGNITGLMRELGYSSKDLRYILSVNGISANIIDADPEIGSIILRIRYPSMIKIPDVAVMELSYLAYHAAGGDWLTVGPRFILKRGYSLSNLDITSCTIGEDFLLCSKDVSSPMSLATQSCLRGDTQMCSRTAVQDREAPRFLLLQGNLIVNCMSVNCKCEDPEETITQDPAYPLMVLGSDTCKIHYIDGIRIKLGKVQLPPITVLNTLSLGPIVVLNPIDVSNQLSLVETTVKESEDHLKNAIGALRSQSRVGGVGIVAIVGLIIATVSLVVLVISGCCLVKYFSRTATLESSLTTIEHGPTLAPKSGPIIPTYINPVYRHD		
NC_007454	4635-6384	gb:NC_007454:4635-6384|Organism:J-virus|Strain Name:UNKNOWN-NC_007454|Protein Name:fusion protein|Gene Symbol:F	MKPVALIYLTILAFTVKVRSQLALSDLTKIGIIPAKSYELKISTQAAQQLMVIKLIPNVNGLTNCTIPVMDSYKKMLDRILKPIDDALNHVKNAIQDKQGDGVPGVRFWGAIIGGVALGVATSAQITAGVALHNSIQNANAILQLKESIRNSNKAIEELQAGLQSTVLVINALQDQINSQLVPAINTLGCSVIANTLGLRLNQYFSEISLVFGPNLRDPTSQTLSIQAIAKAFNGDFDSMMKKMHYTDSDFLDLLESDSIRGRIISVSLEDYLIIIQIDYPGLTTIPNSVVQTFNLITYNYKGTEWESIFPRELLIRGSYISNIDISQCVGTSKSMICKSDTSTTISPATWACATGNLTSCARTRVVNSHSTRFALSGGVLFANCAPIACRCQDPQYSINQEPKTTNVMVTSEDCKELYIDGFYLTLGKKMLDRAMYAEDVALGGSVSVDPIDIGNELNSINESINKSHEYLDKANELLEQVNPNIVNVSSFSFILVISILLIIWFIVTLVWL	2	661

			IYLTKHMNFIVGKVAMGSRSSTVNSL SGFVG		
NC_009489	4620-6500	gb:NC_009489:4620-6500|Organism:Mapuera virus|Strain Name:BeAnn 370284|Protein Name:fusion protein|Gene Symbol:F	MRSSLFLVLTLLVPFAHSIDSITLEQYG TVITSVRSLAYFLETNPTYISVRLMPAI QTDSSHCSYHSIENYNLTLTKLLLPLQ ENLHQITDSLSSRRRKKRFAGVAVGL AALGVATAAQVTAAIAVVKAKENSA KIAQLTSAISETNRAVQDLIEGSKQLA VAVQAIQDQINNVIQPQLTNLSCQVA DAQVGTILNMYLTELTTVFHPQITNSA LTPITIQALRSLLGSTLPQVVTSTIKTD VPLQDLLTSGLLKGQIVYLDLQSMIM VVSVSVPTIALHSMAKVYTLKAISAH VNNAEVQMQVPSRVMELGSEIMGYD IDQCEETSRYLFCPYNGGSILSATMKM CLNGNISQCVFTPIYGSFLQRFVLVDG VIVANCRDMTCACKSPSKIITQPDSLP VTIIDSTSCSNLVLDTLELPIISINNATY RPVQYVGPNQIIFSQPLDLLSQLGKINS SLSDAIEHLAKSDEILEQIQWDSPQGY TLIALTSVLAFVVVAIVGLLISTRYLIF EIRRINTTLTQQLSSYVLSNKIIQY	2	662
NC_017937	4534-6330	gb:NC_017937:4534-6330|Organism:Nariva virus|Strain Name:UNKNOWN-NC_017937|Protein	MAEQEKTPLRYKILLIIIVINHYNITNV FGQIHLANLSSIGVFVTKTLDYRTTSD PTEQLLVINMLPNISNIQDCAQGVVNE YKHLISSLLTPINDTLDLITSNINPYSGR NKLFGEIIAGAALTVATSAQITAGVAL YEARQNAKDIAAIKESLGYAYKAIDK LTTATREITVVINELQDQINNRLIPRIN DLACEVWATRLQAMLLQYYAEIFSVI GPNLQDPLSGKISIQALARAAGGNIKL MVDELNYSGQDLSRLVKVGAIKGQII DADPSLGVVIIKMRYPNIIKIPNVAISE LSYVSYSSDGQDWITTGPNYIVTRGYS	2	663

		Name:fusion protein|Gene Symbol:F	IANIQTSSCSVGDDFVLCDRDMTYPM SQVTQDCLRGNIALCSRMVVRDREAP RYLILQGNMVANCMSITCRCEEPESEI YQSPDQPLTLLTRDTCDTHVVDGIRIR LGVRKLPTISVINNITLGPIITTDPIDVS NQLNAVVSTIDQSAELLHQAQRVLSE RARGARDHILATAAIVICVVLAVLILV LLIGLVYLYRTQNEILVKTTMLEQVPT FAPKSFPMESQIYSGKTNKGYDPAE		
NC_025256	6865-8853	gb:NC_025256:6865-8853|Organism:Bat Paramyxovirus Eid_hel/GH-M74a/GHA/2009|Strain Name:Bat PV/Eid_hel/GH-M74a/GHA/2009|Protein Name:fusion protein|Gene Symbol:F	MKKKTDNPTISKRGHNHSRGIKSRAL LRETDNYSNGLIVENLVRNCHHPSKN NLNYTKTQKRDSTIPYRVEERKGHYP KIKHLIDKSYKHIKRGKRRNGHNGNII TIILLLILILKTQMSEGAIHYETLSKIGLI KGITREYKVKGTPSSKDIVIKLIPNVTG LNKCTNISMENYKEQLDKILIPINNIIE LYANSTKSAPGNARFAGVIIAGVALG VAAAAQITAGIALHEARQNAERINLL KDSISATNNAVAELQEATGGIVNVITG MQDYINTNLVPQIDKLQCSQIKTALDI SLSQYYSEILTVFGPNLQNPVTTSMSI QAISQSFGGNIDLLLNLLGYTANDLLD LLESKSITGQITYINLEHYFMVIRVYYP IMTTISNAYVQELIKISFNVDGSEWVS LVPSYILIRNSYLSNIDISECLITKNSVI CRHDFAMPMSYTLKECLTGDTEKCPR EAVVTSYVPRFAISGGVIYANCLSTTC QCYQTGKVIAQDGSQTLMMIDNQTCS IVRIEEILISTGKYLGSQEYNTMHVSV GNPVFTDKLDITSQISNINQSIEQSKFY LDKSKAILDKINLNLIGSVPISILFIIAIL SLILSIITFVIVMIIVRRYNKYTPLINSDP SSRRSTIQDVYIIPNPGEHSIRSAARSID RDRD	2	664

NC_025347	4471-6386	gb:NC_025347:4471-6386|Organism:Avian paramyxovirus 7|Strain Name:APMV-7/dove/Tennessee/4/75|Protein Name:fusion protein|Gene Symbol:F	MRVRPLIIILVLLVLLWLNILPVIGLDNSKIAQAGIISAQEYAVNVYSQSNEAYIALRTVPYIPPHNLSCFQDLINTYNTTIQNIFSPIQDQITSITSASTLPSSRFAGLVVGAIALGVATSAQITAAVALTKAQQNAQEIIRLRDSIQNTINAVNDITVGLSSIGVALSKVQNYLNDVINPALQNLSCQVSALNLGIQLNLYLTEITTIFGPQITNPSLTPLSIQALYTLAGDNLMQFLTRYGYGETSVSSILESGLISAQIVSFDKQTGIAILYVTLPSIATLSGSRVTKLMSVSVQTGVGEGSAIVPSYVIQQGTVIEEFIPDSCIFTRSDVYCTQLYSKLLPDSILQCLQGSMADCQFTRSLGSFANRFMTVAGGVIANCQTVLCRCYNPVMIIPQNNGIAVTLIDGSLCKELELEGIRLTMADPVFASYSRDLIINGNQFAPSDALDISSELGQLNNSISSATDNLQKAQESLNKSIIPAATSSWLIILLFVLVSISLVIGCISIYFIYKHSTTNRSRNLSSDIISNPYIQKAN	2	665
NC_025348	4790-6570	gb:NC_025348:4790-6570|Organism:Tuhoko virus 2|Strain Name:UNKNOWN-NC_025348|Protein Name:fusion protein|Gene	MAPCVLFLSSLLLISTISPSHGINQPALRRIGAIVSSVKQLKFYSKTKPNYIIVKLLPTINLSKSNCNLTSINRYKESVIEIIKPLADNIDNLNQKLLPKNRRKRMAGVAIGLAALGVAAAAQATAAVALVEARKNTQMIQSLADSIQDTNAAVQAVNIGLQNSAVAIQAIQNQINNVINPALDRLNCEVLDAQIASILNLYLIKSVTIFQNQLTNPALQQLSIQMLSIVMQDTAKILGNFTIGDKFDQHDLLGSGLITGQVVGVNLTNLQLIIAAFIPSIAPLPQAYIIDLISITISVNDTEAVIQIPERIMEHGSSIYQFGGKQCVYGQFSAYCPFSDAVLMTQDLQLCMKGNIEHCIFSSVLGSFPNRFASVDGVFY	2	666

		ne Symbol:F	ANCKYMSCACSDPLQVIHQDDSVNL MVIDSSVCRSLTLGHVTFPIIAFSNVSY QMKTNISIEQMIVTSPLDLSTELKQINN SVNIANTFLDSSNRALKTSIFGTSSQIIL IVLLIFTCLLILYVIFLTYIIKILIKEVKR LRDGNSRTGSKLSFINPDV		
NC_025350	4663-6428	gb:NC_025350:4663-6428|Organism:Tuhoko virus 3|Strain Name:UNKNOWN-NC_025350|Protein Name:fusion protein|Gene Symbol:F	MLWLTILIALVGNHESTCMNINFLQSL GQINSQKRFLNFYTQQPPSYMVIRLVP TLQLSANNCTLGSIVRYRNAIKELIQP MDENLRWLSSNLIPQRRGKRFAGVAV GLAALGVAVAAQATAAVALVEARAN AEKIASMSQSIQETNKAVTSLSQAVSA SGIAIQAIQNEINNVIHPILNQVQCDVL DARVGNILNLYLIKVTTIFQNQLTNPA LQRLSTQALSMLMQSTSSYLRNLSSSE SAINADLSMTNLIEAQIVGINMTNLQL VLAVFIPSIARLNGALLYDFISITISSNQ TEVMLQIPHRVLEIGNSLYTFEGTQCE MTKLNAYCLYSDAIPVTESLRDCMNG LFSQCGFVRIIGSFANRFASVNGVIYA NCKHLTCSCLQPDEIITQDTNVPLTIID TKRCTKISLGHLTFTIREYANVTYSLR TEIANSQITVVSPLDLSSQLTTINNSLA DATNHIMNSDRILDRLNSGLYSKWVII FLICASIVSLIGLVFLGFLIRGLILELRS KHRSNLNKASTYSIDSSIGLT	2	667
NC_025352	5950-8712	gb:NC_025352:5950-8712|Organism:Mojiang virus|Strain	MALNKNMFSSLFLGYLLVYATTVQSS IHYDSLSKVGVIKGLTYNYKIKGSPST KLMVVKLIPNIDSVKNCTQKQYDEYK NLVRKALEPVKMAIDTMLNNVKSGN NKYRFAGAIMAGVALGVATAATVTA GIALHRSNENAQAIANMKSAIQNTNE AVKQLQLANKQTLAVIDTIRGEINNNI IPVINQLSCDTIGLSVGIRLTQYYSEIIT	2	668

		Name:Tonggguan1|Protein Name:fusion protein|Gene Symbol:F	AFGPALQNPVNTRITIQAISSVFNGNF DELLKIMGYTSGDLYEILHSELIRGNII DVDVDAGYIALEIEFPNLTLVPNAVV QELMPISYNIDGDEWVTLVPRFVLTRT TLLSNIDTSRCTITDSSVICDNDYALPM SHELIGCLQGDTSKCAREKVVSSYVP KFALSDGLVYANCLNTICRCMDTDTP ISQSLGATVSLLDNKRCSVYQVGDVLI SVGSYLGDGEYNADNVELGPPIVIDKI DIGNQLAGINQTLQEAEDYIEKSEEFL KGVNPSIITLGSMVVLYIFMILIAIVSVI ALVLSIKLTVKGNVVRQQFTYTQHVP SMENINYVSH		
NC_025363	4622-6262	gb:NC_025363:4622-6262|Organism:Avian paramyxovirus 12|Strain Name:Wigeon/Italy/3920_1/2005|Protein Name:fusion protein|Gene Symbol:F	MAIPVPSSTALMIFNILVSLAPASALD GRLLLGAGIVPTGDRQVNVYTSSQTGI IALKLLPNLPKDKENCAEVSIRSYNET LTRILTPLAQSMAAIRGNSTVSTRGRE PRLVGAIIGGVALGVATAAQITAATAL IQANQNAENIARLAKGLAATNEAVTD LTKGVGSLAIGVGKLQDYVNEQFNRT GEAIECLTIESRVGVQLSLYLTEVIGVF GDQITSPALSDISIQALYNLAGGNLNV LLQKMGIEGTQLGSLINSGLIKGRPIM YDDGNKILGIQVTLPSVGRINGARATL LEAIAVATPKGNASPLIPRAVISVGSLV EELDMTPCVLTPTDIFCTRILSYPLSDS LTTCLKGNLSSCVFSRTEGALSTPYVS VHGKIVANCKSVVCRCVEPQQIISQN YGEALSLIDESLCRILELNGVILKMDG QFTSEYTKNITIDPVQVIISGPIDISSELS QVNQSLDSALENIKESNSYLSKVNVK LISSSAMITYIVITVICLILTFVALVLGI YSYTKIRSQQKTLIWMGNNIARSKEG NRF	2	669

NC_025373	4617-6582	gb:NC_025373:4617-6582|Organism:Avian paramyxovirus 3|Strain Name:turkey/Wisconsin/68|Protein Name:fusion protein|Gene Symbol:F	MASPMVPLLIITVVPALISSQSANIDKLIQAGIIMGSGKELHIYQESGSLDLYLRLLPVIPSNLSHCQSEVITQYNSTVTRLLSPIAKNLNHLLQPRPSGRLFGAVIGSIALGVATSAQISAAIALVRAQQNANDILALKAAIQSSNEAIKQLTYGQEKQLLAISKIQKAVNEQVIPALTALDCAVLGNKLAAQLNLYLIEMTTIFGDQINNPVLTPIPLSYLLRLTGSELNDVLLQQTRSSLSLIHLVSKGLLSGQIIGYDPSVQGIIIRIGLIRTQRIDRSLVFXPYVLPITISSNIATPIIPDCVVKKGVIIEGMLKSNCIELERDIICKTINTYQITKETRACLQGNITMCKYQQSRTQLSTPFITYNGVVIANCDLVSCRCIRPPMIITQVKGYPLTIINRNLCTELSVDNLILNIETNHNFSLNPTIIDSQSRLIATSPLEIDALIQDAQHHAAAALLKVEESNAHLLRVTGLGSSSWHIILILTLLVCTIAWLIGLSIYVCRIKNDDSTDKEPTTQSSNRGIGVGSIQYMT	2	670
NC_025386	5548-7206	gb:NC_025386:5548-7206|Organism:Salem virus|Strain Name:UNKNOWN-NC_025386|Protein Name:fusion	MNPLNQTLIAKVLGFLLLSSSFTVGQIGFENLTRIGVHQVKQYGYKLAHYNSHQLLLIRMIPTVNGTHNCTHQVITRYREMVREIITPIKGALDIMKKAVSPDLVGARIFGAIVAGAALGIATSAQITAGVALHRTKLNGQEISKLKEAVSLTNEAVEQLQYSQGKSILAIQGIQDFINFNVVPLLEEHTCGIAKLHLEMALMEYFQKLILVFGPNLRDPIGSTIGIQALATLFQNNMFEVSLRLGYAGDDLEDVLQSNSIRANIIEAEPDSGFIVLAIRYPTLTLVEDQVITELAHITFNDGPQEWVATIPQFVTYRGLVLANIDVSTCTFTERNVICARDQTYPMIIDLQLCMRGNIAKCGRTRVTGSTASRF	2	671

		protein|Gene Symbol:F	LLKDGNMYANCIATMCRCMSSSSIINQEPSHLTTLIVKETCSEVMIDTIRITLGERKHPPIDYQTTITLGQPIALAPLDVGTELANAVSYLNKSKVLLEHSNEVLSSVSTAHTSLTATIVLGIVVGGLAILIVVMFLFLEAQVIKVQRAMMLCPITNHGYLPNEDLLTRGHSIPTIG		
NC_025390	4805-6460	gb:NC_025390:4805-6460|Organism:Avian paramyxovirus 9|Strain Name:duck/New York/22/1978|Protein Name:fusion protein|Gene Symbol:F	MGYFHLLLILTAIAISAHLCYTTTLDGRKLLGAGIVITEEKQVRVYTAAQSGTIVLRSFRVVSLDRYSCMESTIESYNKTVYNILAPLGDAIRRIQASGVSVERIREGRIFGAILGGVALGVATAAQITAAIALIQANENAKNILRIKDSITKTNEAVRDVTNGVSQLTIAVGKLQDFVNKEFNKTTEAINCVQAAQQLGVELSLYLTEITTVFGPQITSPALSKLTIQALYNLAGVSLDVLLGRLGADNSQLSSLVSSGLITGQPILYDSESQILALQVSLPSISDLRGVRATYLDTLAVNTAAGLASAMIPKVVIQSNNIVEELDTTACIAAEADLYCTRITTFPIASAVSACILGDVSQCLYSKTNGVLTTPYVAVKGKIVANCKHVTCRCVDPTSIISQNYGEAATLIDDQLCKVINLDGVSIQLSGTFESTYVRNVSISANKVIVSSSIDISNELENVNSSLSSALEKLDESDAALSKVNVHLTSTSAMATYIVLTVIALILGFVGLGLGCFAMIKVKSQAKTLLWLGAHADRSYILQSKPAQSST	2	672
NC_025403	4826-6649	gb:NC_025403:4826-6649|Organism:Achimota virus	MWIMIILSLFQIIPGVTPINSKVLTQLGVITKHTRQLKFYSHSTPSYLVVKLVPTINTESTVCNFTSLSRYKDSVRELITPLAKNIDNLNSILTIPKRRKRMAGVVIGLAALGVAAAAQATAAVALIEAKKNTEQIQALSESIQNTNKAVSSIEKGLSSAAIA	2	673

		1|Strain Name:UNKNOWN-NC_025403|Protein Name:fusion protein|Gene Symbol:F	VQAIQNQINNVINPALTALDCGVTDA QLGNILNLYLIKTLTVFQKQITNPALQ PLSIQALNIIMQETSSVLRNFTKTDEIE HTDLLTSGLITGQVVGVNLTNLQLIIA AFIPSIAPLNQAYILDFIRITVNINNSES MIQIPERIMEHGISLYQFGGDQCTFSD WSAYCPYSDATLMAPGLQNCFRGQA ADCVFSTVMGSFPNRFVSVQGVFYVN CKFIRCACTQPQRLITQDDSLSLTQIDA KTCRMLTLGFVQFSINEYANVTYSFK NNVTAGQLIMTNPIDLSTEIKQMNDS VDEAARYIEKSNAALNKLMYGGRSDI VTTVLLVGFILLVVYVIFVTYILKILM KEVARLRNSNHPDLIKPYNYPM		
NC_025404	4772-6647	gb:NC_025404:4772-6647|Organism:Achimota virus 2|Strain Name:UNKNOWN-NC_025404|Protein Name:fusion protein|Gene Symbol:F	MLNSFYQIICLAVCLTTYTVISIDQHNL LKAGVIVKSIKGLNFYSRGQANYIIVK LIPNVNVTDTDCDIGSIKRYNETVYSLI KPLADNIDYLRTQFAPTKRKKRFAGV AIGLTALGVATAAQVTAAVALVKAQ ENARKLDALADSIQATNEAVQDLSTG LQAGAIAIQAIQSEINHVINPALERLSC EIIDTRVASILNLYLIRLTTVFHRQLVN PALTPLSIQALNHLLQGETEGLVKNES KMTDSKIDLLMSGLITGQVVGVNIKH MQLMIAVFVPTTAQLPNAYVINLLTIT ANINNSEVLVQLPNQILERSGIIYQFRG KDCVSSPNHMYCPYSDASILSPELQLC LQGRLEMCLFTQVVGSFPTRFASDKGI VYANCRHLQCACSEPEGIIYQDDTSAI TQIDASKCSTLKLDMLTFKLSTYANK TFDASFSVGKDQMLVTNLLDLSAELK TMNASVAHANKLIDKSNLLIQSNALIG HSNTIFIVVIVILAVMVLYLIIVTYIIKVI MVEVSRLKRMNIYSIDK	2	674

NC_025410	4958-6751	gb:NC_025410:4958-6751|Organism:Tuhoko virus 1|Strain Name:UNKNOWN-NC_025410|Protein Name:fusion protein|Gene Symbol:F	MVTIIKPLILLVTVILQISGHIDTTALTSIGAVIASSKEIMYYAQSTPNYIVIKLIPNLPNIPSQCNFSSIAYYNKTLLDLFTPISDNINMLHQRLSNTGRNRRFAGVAIGLAALGVATAAQVTAAFALVEAKSNTAKIAQIGQAIQNTNAAINSLNAGIGGAVTAIQAIQTQINGIITDQINAATCTALDAQIGTLLNMYLLQLTTTFQPQIQNPALQPLSIQALHRIMQGTSIVLSNLTDSSKYGLNDALSAGLITGQIVSVDLRLMQITIAANVPTLSRLENAIAHDIMRITTNVNNTEVIVQLPETIMEHAGRLYQFNKDHCLSSTQRFFCPYSDAKLLTSKISSCLSGIRGDCIFSPVVGNFATRFISVKGVIIANCKFIRCTCLQPEGIISQLDDHTLTVIDLKLCNKLDLGLIQFDLQVLSNISYEMTLNTSQNQLILTDPLDLSSELQTMNQSINNAANFIEKSNSLLNSSTYEFNRSVALLVALILLSLTILYVIVLTCVVKLLVHEVSKNRRHIQDLESHHK	2	675
NC_028249	4850-7055	gb:NC_028249:4850-7055|Organism:Phocine distemper virus|Strain Name:PDV/Wadden_Sea.NLD/1988|Protein	MTRVKKLPVPTNPPMHHSLDSPFLNPEHATGKISITDDTSSQLTNFLYHKYHKTTINHLSRTISGTDPPSAKLNKFGSPILSTYQIRSALWWIAMVILVHCVMGQIHWTNLSTIGIIGTDSSHYKIMTRSSHQYLVLKLMPNVSIIDNCTKAELDEYEKLLNSVLEPINQALTLMTKNVKSLQSLGSGRRQRRFAGVVIAGAALGVATAAQITAGVALYQSNLNAQAIQSLRASLEQSNKAIDEVRQASQNIIIAVQGVQDYVNNEIVPALQHMSCELIGQRLGLKLLRYYTELLSVFGPSLRDPVSAEISIQALSYALGGEIHKILEKLGYSGNDMVAILETKGIRAKITHVDLSGKFIVLSISYPTLSEVKGV	2	676

		Name:fusion protein|Gene Symbol:F	VVHRLEAVSYNIGSQEWYTTVPRYVATNGYLISNFDESSCVFVSESAICSQNSLYPMSPILQQCLRGETASCARTLVSGTLGNKFILSKGNIIANCASILCKCHSTSKIINQSPDKLLTFIASDTCSLVEIDGVTIQVGSRQYPDVVYASKVILGPAISLERLDVGTNLGSALKKLNDAKVLIESSDQILDTVKNSYLSLGTLIALPVSIGLGLILLLLICCCKKRYQHLFSQSTKVAPVFKPDLTGTSKSYVRSL		
NC_028362	5217-6842	gb:NC_028362:5217-6842|Organism:Caprine parainfluenza virus 3|Strain Name:JS2013|Protein Name:fusion protein|Gene Symbol:F	MIKKIICIFSMPILLSFCQVDIIKLQRVGILVSKPKSIKISQNFETRYLVLNLIPNIENAQSCGDQQIKQYKKLLDRLIIPLYDGLRLQQDIIVVDNNLKNNTNHRAKRFFGEIIGTIALGVATSAQITAAVALVEAKQARSDIERVKNAVRDTNKAVQSIQGSVGNLIVAVKSVQDYVNNEIVPSIKRLGCEAAGLQLGIALTQHYSELTNIFGDNIGTLKEKGIKLQGIASLYHTNITEIFTTSTVDQYDIYDLLFTESIKMRVIDVDLNDYSITLQVRLPLLTKISDAQIYNVDSVSYNIGGTEWYIPLPRNIMTKGAFLGGANLQDCIESFSDYICPSDPGFILNRDIENCLSGNITQCPKTLVISDIVPRYAFVDGGVIANCLSTTCTCNGIDNRINQAPDQGIKIITYKDCQTIGINGMLFKTNQEGTLAAYTPVDITLNNSVNLDPIDLSIELNRARSDLAESKEWIKRSEAKLDSVGSWYQSSTTEIIQIVMIIVLFIINIIVLIVLIKYSRSQNQSMNNHMNEPYILTNKVQ	2	677
AF079780	5919-7580	gb:AF079780|Organism:Tupaia	MASLLKTICYIYLITYAKLEPTPKSQLDLDSLASIGVVDAGKYNYKLMTTGSEKLMVIKLVPNITYATNCNLTAHTAYTKMIERLLTPINQSLYEMRSVITERDGG	1	678

		paramyxovirus|Strain Name:UNKNOWN-AF079780|Protein Name:fusion protein|Gene Symbol:F	TIFWGAIIAGAALGVATAAAITAGVALHRAEQNARNIAALKDALRNSNEAIQHLKDAQGHTVLAIQGLQEQINNNIIPKLKESHCLGVNNQLGLLLNQYYSEILTVFGPNLQNPVSASLTIQAIAKAFNGDFNSLMTNLNYDPTDLLDILESNSINGRIIDVNLNEKYIALSIEIPNFITLTDAKIQTFNRITYGYGSNEWLTLIPDNILEYGNLISNVDLTSCVKTKSSYICNQDTSYPISSELTRCLRGDTSSCPRTPVVNSRAPTFALSGGHIYANCAKAACRCEKPPMAIVQPATSTLTFLTEKECQEVVIDQINIQLAPNRLNKTIITDGIDLGPEVIINPIDVSAELGNIELEMDKTQKALDRSNKILDSMITEVTPDKLLIAMIVVFGILLLWLFGVSYYAFKIWSKLHFLDSYVYSLRNPSHHRSNGHQNHSFSTDISG		
EU403085	4664-6585	gb:EU403085:4664-6585|Organism:Avian paramyxovirus 3|Strain Name:APMV3/PKT/Netherland/449/75|Protein Name:fusion protein|Gene	MQPGSALHLPHLYIIIALVSDGTLGQTAKIDRLIQAGIVLGSGKELHISQDSGTLDLFVRLLPVLPSNLSHCQLEAITQYNKTVTRLLAPIGKNLEQVLQARPRGRLFGPIIGSIALGVATSAQITAAIALVRAQQNANDILALKNALQSSNEAIRQLTYGQDKQLLAISKIQKAVNEQILPALDQLDCAVLGTKLAVQLNLYLIEMTTIFGEQINNPVLATIPLSYILRLTGAELNNVLMKQARSSLSLVQLVSKGLLSGQVIGYDPSVQGLIIRVNLMRTQKIDRALVYQPYVLPITLNSNIVTPIAPECVIQKGTIIEGMSRKDCTELEQDIICRTVTTYTLARDTRLCLQGNISSCRYQQSGTQLHTPFITYNGAVIANCDLVSCRCLRPPMIITQVKGYPLTIITRSVCQELSVDNLVLNIETHHNFSLNPTIIDPLTRVIATTPLEIDSLIQEAQDHA		

		Symbol:F	NAALAKVEESDKYLRAVTGGNYSNW YIVLVIVLLFGNLGWSLLLTVLLCRSR KQQRRYQQDDSVGSERGVGVGTIQY MS		
KX258200	4443-6068	gb:KX258200:4443-6068|Organism:Avian paramyxovirus 14|Strain Name:APMV14/duck/Japan/11OG0352/2011|Protein Name:fusion protein|Gene Symbol:F	MEKGTVLFLAALTLYNVKALDNTKL LGAGIASGKEHELKIYQSSVNGYIAVK LIPFLPSTKRECYNEQLKNYNATINRL MGPINDNIKLVLSGVKTRTREGKLIGA IIGTAALGLATAAQVTAAIALEQAQD NARAILTLKESIRNTNNAVSELKTGLS EVSIALSKTQDYINTQIMPALSNLSCEI VGLKIGIQLSQYLTEVTAVFGNQITNP ALQPLSMQALYQLCGGDFSLLLDKIG ADRNELESLYEANLVTGRIVQYDTAD QLVIIQVSIPSVSTLSGYRVTELQSISV DMDHGEGKAVIPRYIVTSGRVIEEMDI SPCVLTATAVYCNRLLTTSLPESVLKC LDGDHSSCTYTSNSGVLETRYIAFDG MLIANCRSIVCKCLDPPYIIPQNKGKPL TIISKEVCKKVTLDGITLLIDAEFTGEY GLNITIGPDQFAPSGALDISTELGKLNN SINKAEDYIDKSNELLNRVNVDIVNDT AVIVLCVMSALVVVWCIGLTVGLIYV SKNTLRAVAIKGTSIENPYVSSGKHAK NSS	1	680
KY511044	4592-6247	gb:KY511044:4592-6247|Organism:Avian paramyxovirus UPO216|Strain	MIFTMYHVTVLLLLSLLTLPLGIQLAR ASIDGRQLAAAGIVVTGEKAINLYTSS QTGTIVVKLLPNVPQGREACMRDPLT SYNKTLTSLLSPLGEAIRRIHESTTETA GLVQARLVGAIIGSVALGVATSAQITA AAALIQANKNAENILKLKQSIAATNE AVHEVTDGLSQLAVAVGKMQDFINT QFNNTAQEIDCIRISQQLGVELNLYLT ELTTVFGPQITSPALSPLSIQALYNLAG	1	681

		Name:APMV-15/WB/Kr/UPO216/2014|Protein Name:fusion protein|Gene Symbol:F	GNLDVLLSKIGVGNNQLSALISSGLISGSPILYDSQTQLLGIQVTLPSVSSLNNMRAIFLETLSVSTDKGFAAALIPKVVTTVGTVTEELDTSYCIETDIDLFCTRIVTFPMSPGIYACLNGNTSECMYSKTQGALTTPYMSVKGSIVANCKMTTCRCADPASIISQNYGEAVSLIDSSVCRVITLDGVTLRLSGSFDSTYQKNITIRDSQVIITGSLDISTELGNVNNSINNALDKIEESNQILESVNVSLTSTNALIVYIICTALALICGITGLILSCYIMYKMRSQQKTLMWLGNNTLDQMRAQTKM		
NC_025360	6104-8123	gb:NC_025360:6104-8123|Organism:Atlantic salmon paramyxovirus|Strain Name:ASPV/Yrkje371/95|Protein Name:fusion protein|Gene Symbol:F	MDGPKFRFVLLILLTAPARGQVDYDKLLKVGIFEKGTANLKISVSSQQRYMVIKMMPNLGPMNQCGIKEVNLYKESILRLITPISTTLNYIKSEIQVEREVALQPNGTIVRFFGLIVAAGALTLATSAQITAGIALHNSLENAKAIKGLTDAIKESNLAIQKIQDATAGTVIALNALQDQVNTNIIPAINTLGCTAAGNTLGIALTRYYSELIMIFGPSLGNPVEAPLTIQALAGAFNGDLHGMIREYGYTPSDIEDILRTNSVTGRVIDVDLVGMNIVLEINLPTLYTLRDTKIVNLGKITYNVDGSEWQTLVPEWLAIRNTLMGGVDLSRCVVSSRDLICKQDPVFSLDTSIISCLNGNTESCPRNRVVNSVAPRYAVIRGNILANCISTTCLCGDPGVPIIQKGDNTLTAMSINDCKLVGVDGYVFRPGPKAVNVTFNLPHLNLGPEVNVNPVDISGALGKVEQDLASSRDHLAKSEKILSGINPNIINTEMVLVAVILSLVCAMVVIGIVCWLSILTKWVRSCRADCRRPNKGPDLGPIMSSQDNLSF	1	682
UniProt		FUS_NIP	MVVILDKRCYCNLLILILMISECSVGIL		683

ID: Q9IH63		AV Fusion glycoprotein F0 OS=Nipah virus	HYEKLSKIGLVKGVTRKYKIKSNPLT KDIVIKMIPNVSNMSQCTGSVMENYK TRLNGILTPIKGALEIYKNNTHDLVGD VRLAGVIMAGVAIGIATAAQITAGVA LYEAMKNADNINKLKSSIESTNEAVV KLQETAEKTVYVLTALQDYINTNLVP TIDKISCKQTELSLDLALSKYLSDLLFV FGPNLQDPVSNSMTIQAISQAFGGNYE TLLRTLGYATEDFDDLLESDSITGQIIY VDLSSYYIIVRVYFPILTEIQQAYIQEL LPVSFNNDNSEWISIVPNFILVRNTLIS NIEIGFCLITKRSVICNQDYATPMTNN MRECLTGSTEKCPRELVVSSHVPRFA LSNGVLFANCISVTCQCQTTGRAISQS GEQTLLMIDNTTCPTAVLGNVIISLGK YLGSVNYNSEGIAIGPPVFTDKVDISS QISSMNQSLQQSKDYIKEAQRLLDTV NPSLISMLSMIILYVLSIASLCIGLITFIS FIIVEKKRNTYSRLEDRRVRPTSSGDL YYIGT		

[00075] In some embodiments, a fusogen described herein comprises an amino acid sequence of Table 5, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to a portion of the sequence, e.g., a portion of 100, 200, 300, 400, 500, or 600 amino acids in length. For instance, in some embodiments, a fusogen described herein comprises an amino acid sequence having at least 80% identity to any amino acid sequence of Table 5. In some embodiments, a nucleic acid sequence described herein encodes an amino acid sequence of Table 5, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to a portion of the sequence, e.g., a portion of 40, 50, 60, 80, 100, 200, 300, 400, 500, or 600 amino acids in length.

[00076] In some embodiments, a fusogen described herein comprises an amino acid sequence set forth in any one of SEQ ID NOS: 684-759, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to a portion of the sequence, e.g., a portion of 100, 200, 300, 400, 500, or 600 amino acids in length. For instance, in some embodiments, a fusogen described herein comprises an amino acid sequence having at least 80% identity to an amino acid sequence set forth in any one of SEQ ID NOS: 684-759. In some embodiments, a nucleic acid sequence described herein encodes an amino acid sequence set forth in any one of SEQ ID NOS: 684-759, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to a portion of the sequence, e.g., a portion of 40, 50, 60, 80, 100, 200, 300, 400, 500, or 600 amino acids in length.

Table 5. Paramyxovirus protein G, H, and HN sequence clusters. Column 1, Genbank ID includes the Genbank ID of the whole genome sequence of the virus that is the centroid sequence of the cluster. Column 2, nucleotides of CDS provides the nucleotides corresponding to the CDS of the gene in the whole genome. Column 3, Full Gene Name, provides the full name of the gene including Genbank ID, virus species, strain, and protein name. Column 4, Sequence, provides the amino acid sequence of the gene. Column 5, #Sequences/ Cluster, provides the number of sequences that cluster with this centroid sequence.

Genbank ID	**Nucleotides of CDS**	**Full sequence ID**	**Sequence**	**#Sequences /Cluster**	**SEQ ID NO**
KU950686	4643-5638	gb:KU950686:4643-5638|Organism:Human respiratory syncytial virus|Strain Name:RSVA/H	MSKTKDQRTAKTLERTWDTLNHLLFISSC LYKLNLKSIAQITLSILAMIISTSLIIAAIIFIA SANHKVTLTTAIIQDATNQIKNTTPTYLTQ NPQLGISFSNLSGTTLQSTTILASTTPSAEST PQSTTVKIINTTTTQILPSKPTTKQRQNKPQ NKPNNDFHFEVFNFVPCSICSNNPTCWAIC KRIPNKKPGKKTTTKPTKKPTLKTTKKDP KPQTTKPKEALTTKPTGKPTINTTKTNIRTT	706	684

		omo sapiens/USA/TH_10506/2014|Protein Name:attachment glycoprotein|Gene Symbol:G	LLTSNTKGNPEHTSQEETLHSTTSEGYLSPSQVYTTSGQEETLHSTTSEGYLSPSQVYTTSEYLSQSLSSSNTTK		
AB524405	6424-8274	gb:AB524405:6424-8274|Organism:Newcastle disease virus|Strain Name:Goose/Alaska/415/91|Protein Name:hemagglutinin-neuraminidase protein|Gene Symbol:HN	MERGVSQVALENDEREAKNTWRLVFRVTVLFLTIVTLAISAAALAFSMNASTPQDLEGIPVAISKVEDKITSALGASQDVMDRIYKQVALESPLALLNTESTIMNALTSLSYQINGAANASGCGAPVPDPDYIGGIGKELIVDDTSDVTSFYPSAFQEHLNFIPAPTTGSGCTRIPSFDMSATHYCYTHNVILSGCRDHSHSHQYLALGVLRTSATGRVFFSTLRSINLDDTQNRKSCSVSATPLGCDMLCSKVTETEEEDYQSTDPTLMVHGRLGFDGQYHERDLDVHTLFGDWVANYPGVGGGSFINNRVWFPVYGGLKPGSPTDKRQEGQYAIYKRYNDTCPDDQEYQVRMAKSAYKPNRFGGKRVQQAILSIGVSTTLADDPVLTVTSNTITLMGAEGRVMTVGTSHYLYQRGSSYYSPAILYPLTIANKTATLQDPYKFNAFTRPGSVPCQASARCPNSCVTGVYTDPYPIVFHKNHTLRGVFGTMLDDEQARLNPVSAVFDSIARSRVTRVSSSSTKAAYTTSTCFKVVKTGKVYCLSIAEISNTLFGEFRIVPLLVEILRDEGRSEARSALTTQGHPGWNDEVVDPIFCAVTNQTDHRQKLEEYAQSWP	418	685
JQ582844	4686-5636	gb:JQ582844:4686-5636|Organism:Human respiratory	MSKNKNQRTARTLEKTWDTLNHLIVISSCLYKLNLKSIAQIALSVLAMIISTSLIIAAIIFIISANHKVTLTTVTVQTIKNHTEKNITTYLTQVSPERVSPSKQPTTTPPIHTNSATISPNTKSETHHTTAQTKGRTTTPTQNNKPSTKPRPKN	278	686

		syncytial virus|Strain Name:NH1067|Protein Name:receptor-binding glycoprotein|Gene Symbol:G	PPKKPKDDYHFEVFNFVPCSICGNNQLCKS ICKTIPNNKPKKKPTTKPTNKPPTKTTNKR DPKTPAKTLKKETTINPTTKKPTPKTTERD TSTPQSTVLDTTTSKHTERDTSTPQSTVLD TTTSKHTIQQQSLHSITPENTPNSTQTPTAS EPSTSNSTQKL		
AB2544556	7271-9136	gb:AB254456:7271-9136|Organism:Measles virus|Strain Name:SSPE-Kobe-1|Protein Name:Hemagglutinin|Gene Symbol:H	MSPHRDRINAFYRDNPHPKGSRIVINREHL MIDRPYVLLAVLFVMFLSLIGLLAIAGIRL HRAAIYTAEIHKSLSTNLDVTNSIEHQVKD VLTPLFKIIGDEVGLRTPQRFTDLVKFISDK IKFLNPDREYDFRDLTWCINPPERIKLDYD QYCADVAAEELMNALVNSTLLEARATNQ FLAVSKGNCSGPTTIRGQFSNMSLSLLDLY LSRGYNVSSIVTMTSQGMYGGTYLVGKPN LSSKGSELSQLSMHRVFEVGVIRNPGLGAP VFHMTNYFEQPVSNDFSNCMVALGELRFA ALCHREDSVTVPYQGSGKGVSFQLVKLGV WKSPTDMQSWVPLSTDDPVIDRLYLSSHR GVIADNQAKWAVPTTRTDDKLRMETCFQ QACKGKNQALCENPEWAPLKDNRIPSYG VLSVNLSLTVELKIKIASGFGPLITHGSGM DLYKTNHDNVYWLTIPPMKNLALGVINTL EWIPRFKVSPNLFTVPIKEAGEDCHAPTYL PAEVDGDVKLSSNLVILPGQDLQYVLATY DTSRVEHAVVYYVYSPSRSFSYFYPFRLPI KGVPIELQVECFTWDQKLWCRHFCVLADS ESGGHITHSGMVGMGVSCTVTREDGTNR RQGCQ	128	687
AB0408874	6614-8362	gb:AB040874:6614-8362|Organism:Mumps	MEPSKLFTMSDNATFAPGPVINAADKKTF RTCFRILVLSVQAVTLILVIVTLGELVRMIN DQGLSNQLSSIADKIRESATMIASAVGVM NQVIHGVTVSLPLQIEGNQNQLLSTLATIC	87	688

		virus|Strain Name:Miyahara|Protein Name:hemagglutinin-neuraminidase|Gene Symbol:HN	TGKKQVSNCSTNIPLVNDLRFINGINKFIIE DYATHDFSIGHPLNMPSFIPTATSPNGCTRI PSFSLGKTHWCYTHNVINANCKDHTSSNQ YISMGILVQTASGYPMFKTLKIQYLSDGLN RKSCSIATVPDGCAMYCYVSTQLETDDYA GSSPPTQKLTLLFYNDTVTERTISPTGLEGN WATLVPGVGSGIYFENKLIFPAYGGVLPNS SLGVKSAREFFRPVNPYNPCSGPQQDLDQ RALRSYFPSYFSNRRVQSAFLVCAWNQIL VTNCELVVPSNNQTLMGAEGRVLLINNRL LYYQRSTSWWPYELLYEISFTFTNSGQSSV NMSWIPIYSFTRPGSGNCSGENVCPTACVS GVYLDPWPLTPYSHQSGINRNFYFTGALL NSSTTRVNPTLYVSALNNLKVLAPYGNQG LFASYTTTTCFQDTGDASVYCVYIMELAS NIVGEFQILPVLTRLTIT		
AB736166	6709-8427	gb:AB736166:6709-8427|Organism:Human respirovirus 3|Strain Name:ZMLS/2011|Protein Name:hemagglutinin-neuraminidase|Gene Symbol:HN	MEYWKHTNHGKDAGNELETATATHGNR LTNKITYILWTITLVLLSIVFIIVLINSIKSEK AHESLLQDINNEFMEVTEKIQVASDNTND LIQSGVNTRLLTIQSHVQNYIPISLTQQISDL RKFISEITIRNDNQEVPPQRITHDVGIKPLNP DDFWRCTSGLPSLMRTPKIRLMPGPGLLA MPTTVDGCVRTPSLVINDLIYAYTSNLITR GCQDIGKSYQVLQIGIITVNSDLVPDLNPRI SHTFNINDNRKSCSLALLNTDVYQLCSTPK VDERSDYASSGIEDIVLDIVNYDGSISTTRF KNNNISFDQPYAALYPSVGPGIYYKGKIIFL GYGGLEHPINENAICNTTGCPGKTQRDCN QASHSPWFSDRRMVNSIIVVDKGLNSVPK LKVWTISMRQNYWGSEGRLLLLGNKIYIY TRSTSWHSKLQLGIIDITDYSDIRIKWTWH NVLSRPGNNECPWGHSCPDGCITGVYTDA YPLNPTGSIVSSVILDSQKSRVNPVITYSTA TERVNELAIRNKTLSAGYTTTSCITHYNKG	78	689

			YCFHIVEINHKSLNTFQPMLFKTEIPKSCS		
KJ627396	6166-6885	gb:KJ627396:6166-6885|Organism:Human metapneumovirus|Strain Name:HMPV/Homo sapiens/PER/FLI1305/2010/A|Protein Name:attachment glycoprotein G|Gene Symbol:G	MEVKVENIRAIDMLKARVKNRVARSKCF KNASLILIGITTLSIALNIYLIINYTIQKTTSE SEHHTSSPPTESNKETSTIPIDNPDITPNSQH PTQQSTESLTLYPASSMSPSETEPASTPGIT NRLSLADRSTTQPSESRTKTNSTVHKKNK KNISSTISRTQSPPRTTAKAVSRTTALRMSS TGERPTTTSVQSDSSTTAQNHEETGPANPQ ASVSTM	71	690
AB475097	7079-8902	gb:AB475097:7079-8902|Organism:Canine distemper virus|Strain Name:M25CR|Protein Name:hemagglutinin|Gene Symbol:H	MLSYQDKVGAFYKDNARANSSKLSLVTE EQGGRRPPYLLFVLLILLVGILALLAIAGVR FRQVSTSNVEFGRLLKDDLEKSEAVHHQV MDVLTPLFKIIGDEIGLRLPQKLNEIKQFIL QKTNFFNPNREFDFRDLHWCINPPSKIKVN FTNYCDAIGVRKSIASAANPILLSALSGGR GDIFPPYRCSGATTSVGRVFPLSVSLSMSLI SKTSEIISMLTAISDGVYGKTYLLVPDYIER EFDTQKIRVFEIGFIKRWLNDMPLLQTTNY MVLPENSKAKVCTIAVGELTLASLCVDES TVLLYHDSNGSQDSILVVTLGIFGATPMNQ VEEVIPVAHPSVERIHITNHRGFIKDSVAT WMVPALVSEQQEGQKNCLESACQRKSYP MCNQTSWEPFGGVQLPSYGRLTLPLDASI DLQLNISFTYGPVILNGDGMDYYENPLLDS GWLTIPPKNGTILGLINKASRGDQFTVTPH VLTFAPRESSGNCYLPIQTSQIMDKDVLTE SNLVVLPTQNFRYVVATYDISRENHAIVY	45	691

			YVYDPIRTISYTYPFRLTTKGRPDFLRIECF VWDDDLWCHQFYRFESDITNSTTSVEDLV RIRFSCNRSKP		
AJ8 496 36	7326- 9155	gb:AJ849636:7 326- 9155|Organism :Peste-des- petits- ruminants virus|Strain Name:Turkey 2000|Protein Name:haemagg lutinin|Gene Symbol:H	MSAQRERINAFYKDNPHNKNHRVILDRER LVIERPYILLGVLLVMFLSLIGLLAIAGIRL HRATVGTSEIQSRLNTNIELTESIDHQTKD VLTPLFKIIGDEVGIRIPQKFSDLVKFISDKI KFLNPDREYDFRDLRWCMNPPERVKINFD QFCEYKAAVKSIEHIFESPLNKSKKLQSLT LGPGTGCLGRTVTRAHFSELTLTLMDLDL EMKHNVSSVFTVVEEGLFGRTYTVWRSD ARDPSTDLGIGHFLRVFEIGLVRDLGLGPP VFHMTNYLTVNMSDDYRRCLLAVGELKL TALCSSSETVTLGERGVPKREPLVVVILNL AGPTLGGELYSVLPTSDLMVEKLYLSSHR GIIKDDEANWVVPSTDVRDLQNKGECLVE ACKTRPPSFCNGTGSGPWSEGRIPAYGVIR VSLDLASDPGVVITSVFGPLIPHLSGMDLY NNPFSRAVWLAVPPYEQSFLGMINTIGFPN RAEVMPHILTTEIRGPRGRCHVPIELSRRV DDDIKIGSNMVILPTIDLRYITATYDVSRSE HAIVYYIYDTGRSSSYFYPVRLNFKGNPLS LRIECFPWRHKVWCYHDCLIYNTITDEEV HTRGLTGIEVTCNPV	34	692
AB0 057 95	6693- 8420	gb:AB005795: 6693- 8420|Organism :Sendai virus|Strain Name:Ohita|Pr otein Name:hemaggl utinin- neuraminidase	MDGDRSKRDSYWSTSPGGSTTKLVSDSER SGKVDTWLLILAFTQWALSIATVIICIVIAA RQGYSMERYSMTVEALNTSNKEVKESLTS LIRQEVITRAANIQSSVQTGIPVLLNKNSRD VIRLIEKSCNRQELTQLCDSTIAVHHAEGIA PLEPHSFWRCPAGEPYLSSDPEVSLLPGPSL LSGSTTISGCVRLPSLSIGEAIYAYSSNLITQ GCADIGKSYQVLQLGYISLNSDMFPDLNP VVSHTYDINDNRKSCSVVATGTRGYQLCS MPIVDERTDYSSDGIEDLVLDILDLKGRTK	23	693

		protein|Gene Symbol:HN	SHRYSNSEIDLDHPFSALYPSVGSGIATEGS LIFLGYGGLTTPLQGDTKCRIQGCQQVSQD TCNEALKITWLGGKQVVSVLIQVNDYLSE RPRIRVTTIPITQNYLGAEGRLLKLGDQVYI YTRSSGWHSQLQIGVLDVSHPLTISWTPHE ALSRPGNEDCNWYNTCPKECISGVYTDAY PLSPDAANVATVTLYANTSRVNPTIMYSN TTNIINMLRIKDVQLEAAYTTTSCITHFGK GYCFHIIEINQKSLNTLQPMLFKTSIPKLCK AES		
AF4571 02	6903-8630	gb:AF457102|Organism:Human parainfluenza virus 1 strain Washington/1964|Strain Name:Washington 1964|Protein Name:HN glycoprotein|Gene Symbol:HN	MAEKGKTNSSYWSTTRNDNSTVNTHINTP AGRTHIWLLIATTMHTVLSFIIMILCIDLIIK QDTCMKTNIMTVSSMNESAKIIKETITELIR QEVISRTINIQSSVQSGIPILLNKQSRDLTQL IEKSCNRQELAQICENTIAIHHADGISPLDP HDFWRCPVGEPLLSNNPNISLLPGPSLLSG STTISGCVRLPSLSIGDAIYAYSSNLITQGC ADIGKSYQVLQLGYISLNSDMYPDLNPVIS HTYDINDNRKSCSVIAAGTRGYQLCSLPTV NETTDYSSEGIEDLVFDILDLKGKTKSHRY KNEDITFDHPFSAMYPSVGSGIKIENTLIFL GYGGLTTPLQGDTKCVINRCTNVNQSVCN DALKITWLKKRQVVNVLIRINNYLSDRPKI VVETIPITQNYLGAEGRLLKLGKKIYIYTRS SGWHSNLQIGSLDINNPMTIKWAPHEVLS RPGNQDCNWYNRCPRECISGVYTDAYPLS PDAVNVATTTLYANTSRVNPTIMYSNTSEI INMLRLKNVQLEAAYTTTSCITHFGKGYC FHIVEINQASLNTLQPMLFKTSIPKICKITS	21	694
KJ6273 97	6146-6888	gb:KJ627397:6146-6888|Organism:Human metapneumovir	MEVRVENIRAIDMFKAKMKNRIRSSKCYR NATLILIGLTALSMALNIFLIIDYATLKNMT KVEHCVNMPPVEPSKKSPMTSAADLNTKL NPQQATQLTTEDSTSLAATSENHLHTETTP TSDATISQQATDEHTTLLRPINRQTTQTTTE	21	695

		us|Strain Name:HMPV/ Homo sapiens/PER/FPP00098/2010/B|Protein Name:attachment glycoprotein G|Gene Symbol:G	KKPTGATTKKDKEKETTTRTTSTAATQTLNTTNQTSNGREATTTSARSRNGATTQNSDQTIQAADPSSKPYHTQTNTTTAHNTDTSSLSS		
AF0171749	8913-10727	gb:AF017149|Organism:Hendra virus|Strain Name:UNKNOWN-AF017149|Protein Name:glycoprotein|Gene Symbol:G	MMADSKLVSLNNNLSGKIKDQGKVIKNYYGTMDIKKINDGLLDSKILGAFNTVIALLGSIIIIVMNIMIIQNYTRTTDNQALIKESLQSVQQQIKALTDKIGTEIGPKVSLIDTSSTITIPANIGLLGSKISQSTSSINENVNDKCKFTLPPLKIHECNISCPNPLPFREYRPISQGVSDLVGLPNQICLQKTTSTILKPRLISYTLPINTREGVCITDPLLAVDNGFFAYSHLEKIGSCTRGIAKQRIIGVGEVLDRGDKVPSMFMTNVWTPPNPSTIHHCSSTYHEDFYYTLCAVSHVGDPILNSTSWTESLSLIRLAVRPKSDSGDYNQKYIAITKVERGKYDKVMPYGPSGIKQGDTLYFPAVGFLPRTEFQYNDSNCPIIHCKYSKAENCRLSMGVNSKSHYILRSGLLKYNLSLGGDIILQFIEIADNRLTIGSPSKIYNSLGQPVFYQASYSWDTMIKLGDVDTVDPLRVQWRNNSVISRPGQSQCPRFNVCPEVCWEGTYNDAFLIDRLNWVSAGVYLNSNQTAENPVFAVFKDNEILYQVPLAEDDTNAQKTITDCFLLENVIWCISLVEIYDTGDSVIRPKLFAVKIPAQCSES	14	696
AF2123102	8943-10751	gb:AF212302|Organism:Nipah virus|Strain	MPAENKKVRFENTTSDKGKIPSKVIKSYYGTMDIKKINEGLLDSKILSAFNTVIALLGSIVIIVMNIMIIQNYTRSTDNQAVIKDALQGIQ	14	697

		Name:UNKNOWN-AF212302|Protein Name:attachment glycoprotein|Gene Symbol:G	QQIKGLADKIGTEIGPKVSLIDTSSTITIPANIGLLGSKISQSTASINENVNEKCKFTLPPLKIHECNISCPNPLPFREYRPQTEGVSNLVGLPNNICLQKTSNQILKPKLISYTLPVVGQSGTCITDPLLAMDEGYFAYSHLERIGSCSRGVSKQRIIGVGEVLDRGDEVPSLFMTNVWTPPNPNTVYHCSAVYNNEFYYVLCAVSTVGDPILNSTYWSGSLMMTRLAVKPKSNGGGYNQHQLALRSIEKGRYDKVMPYGPSGIKQGDTLYFPAVGFLVRTEFKYNDSNCPITKCQYSKPENCRLSMGIRPNSHYILRSGLLKYNLSDGENPKVVFIEISDQRLSIGSPSKIYDSLGQPVFYQASFSWDTMIKFGDVLTVNPLVVNWRNNTVISRPGQSQCPRFNTCPEICWEGVYNDAFLIDRINWISAGVFLDSNQTAENPVFTVFKDNEILYRAQLASEDTNAQKTITNCFLLKNKIWCISLVEIYDTGDNVIRPKLFAVKIPEQCT		
EU439428	6751-8638	gb:EU439428:6751-8638|Organism:Swine parainfluenza virus 3|Strain Name:92-7783_ISU-92|Protein Name:hemagglutinin-neuraminidase HN|Gene Symbol:HN	MEYWKHTNSTKDTNNELGTTRDRHSSKATNIIMYIFWTTTSTILSVIFIMILINLIQENNHNKLMLQEIKKEFAVIDTKIQKTSDDISTSIQSGINTRLLTIQSHVQNYIPLSLTQQMSDLRKFINDLTTKREHQEVPIQRMTHDSGIEPLNPDKFWRCTSGNPSLTSSPKIRLIPGPGLLATSTTVNGCIRIPSLAINNLIYAYTSNLITQGCQDIGKSYQVLQIGIITINSDLVPDLNPRVTHTFNIDDNRKSCSLALLNTDVYQLCSTPKVDERSDYASTGIEDIVLDIVTSNGLIITTRFTNNNITFDKPYAALYPSVGPGIYYKDKVIFLGYGGLEHEENGDVICNTTGCPGKTQRDCNQASYSPWFSNRRMVNSIIVVDKSIDTTFSLRVWTIPMRQNYWGSEGRLLLLGDRIYIYTRSTSWHSKLQLGVIDISDYNNIRINWTWHNVLSRPGNDECPWGHSCPDGCITGVYTDAY	14	698

			PLNPSGSVVSSVILDSQKSRENPIITYSTAT NRVNELAIYNRTLPAAYTTTNCITHYDKG YCFHIVEINHRSLNTFQPMLFKTEVPKNCS		
KF5 301 64	6157-6906	gb:KF530164:6 157-6906|Organism :Human metapneumovir us|Strain Name:HMPV/ AUS/17283278 8/2004/B|Protei n Name:attachme nt glycoprotein G|Gene Symbol:G	MEVRVENIRAIDMFKAKIKNRIRSSRCYRN ATLILIGLTALSMALNIFLIIDHATLRNMIK TENCANMPSAEPSKKTPMTSTAGPSTKPN PQQATQWTTENSTSPAATLEGHPYTGTTQ TPDTTAPQQTTDKHTALPKSTNEQITQTTT EKKTTRATTQKREKRKENTNQTTSTAATQ TTNTTNQTRNASETITTSDGPRIDTTTQSSE QTARATEPGSSPYHARRGAGPR	14	699
AB9 103 09	6960-8747	gb:AB910309: 6960-8747|Organism :Feline morbillivirus|St rain Name:SS1|Prot ein Name:hemaggl utinin protein|Gene Symbol:H	MKNINIKYYKDSNRYLGKILDEHKIVNSQL YSLSIKVITIIAIIVSLIATIMTIINATSGRTTL NSNTDILLNQRDEIHSIHEMIFDRVYPLITA MSTELGLHIPTLLDELTKAIDQKIKIMNPPV DTVTSDLSWCIKPPNGIIIDPKGYCESMELS KTYKLLLDQLDVSRKKSLTINRKNINQCQ LVDDSEIIFATVNIQSTPRFLNFGHTVSNQR ITFGQGTYSSTYILTIQEDGITDVQYRVFEI GYISDQFGVFPSLIVSRVLPIRMVLGMESC TLTSDRQGGYFLCMNTLTRSIYDYVNIRDL KSLYITLPHYGKVNYTYFNFGKIRSPHEID KLWLTSDRGQIISGYFAAFVTITIRNYNNY PYKCLNNPCFDNSENYCRGWYKNITGTDD VPILAYLLVEMYDEEGPLITLVAIPPYNYT APSHNSLYYDDKINKLIMTTSHIGYIQINEV HEVIVGDNLKAILLNRLSDEHPNLTACRLN QGIKEQYKSDGMIISNSALIDIQERMYITVK	12	700

			AIPPVGNYNFTVELHSRSNTSYILLPKQFN AKYDKLHLECFNWDKSWWCALIPQFSLS WNESLSVDTAIFNLINCK		
AB759118	7116-8957	gb:AB759118:7116-8957|Organism:Avian paramyxovirus 6|Strain Name:red-necked stint/Japan/8KS0813/2008|Protein Name:hemagglutinin-neuraminidase|Gene Symbol:HN	MASPSELNRSQATLYEGDPNSKRTWRTVY RASTLILDLAILCVSIVAIVRMSTLTPSDVT DSISSSITSLSDTYQSVWSDTHQKVNSIFKE VGISIPVTLDKMQVEMGTAVNIITDAVRQL QGVNGSAGFSITNSPEYSGGIDALIYPQKSL NGKSLAISDLLEHPSFIPAPTTSHGCTRIPTF HLGYRHWCYSHNTIESGCHDAGESIMYLS MGAVGVGHQGKPVFTTSAAVILDDGKNR KSCSVVANPNGCDVLCSLVKQTEDQDYA DPTPTPMIHGRLHFNGTYTESMLDQSLFTG HWVAQYPAVGSGSVSHGRLFFPLYGGISK SSSLFPKLRAHAYFTHNEELECKNLTSKQR EDLFNAYMPGKIAGSLWAQGIVICNLTTL ADCKIAVANTSTMMMAAEGRLQLVQDK VVLYQRSSSWWPVLIYYDILVSELVNARH LDIVNWVPYPQSKFPRPTWTKGLCEKPSIC PAVCVTGVYQDVWVVSVGDFSNETVVIG GYLEAASERKDPWIAAANQYNWLTRRQL FTAQTEAAYSSTTCFRNTHQDKVFCLTIME VTDNLLGDWRIAPLLYEVTVVDRQQSSRK AVAMSEAHRTRFKYYSPENKFTPQH	11	701
AY141760	6791-8485	gb:AY141760|Organism:Fer-de-Lance paramyxovirus|Strain Name:ATCC VR-895|Protein Name:hemagglutinin-neuraminidase	MDPKSYYCNEDLRSDGGEKSPGGDLYKGI ILVSTVISLIIAIISLAFIIDNKINIQSLDPLRG LEDSYLVPIKDKSESISQDIQEGIFPRLNLIT AATTTTIPRSIAIQTKDLSDLIMNRCYPSVV NNDTSCDVLAGAIHSNLFSQLDPSTYWTC SSGTPTMNQTVKLLPDNSQIPGSTYSTGCV RIPTFSLGSMIYSYSHNVIYEGCNDHSKSSQ YWQLGYISTSKTGEPLQQVSRTLTLNNGL NRKSCSTVAQGRGAYLLCTNVVEDERTD YSTEGIQDLTLDYIDIFGAERSYRYTNNEV	8	702

		protein HN|Gene Symbol:HN	DLDRPYAALYPSVGSGTVYNDRILFLGYG GLMTPYGDQAMCQAPECTSATQEGCNSN QLIGYFSGRQIVNCIIEIITVGTEKPIIRVRTI PNSQVWLGAEGRIQTLGGVLYLYIRSSGW HALAQTGIILTLDPIRISWIVNTGYSRPGNG PCSASSRCPAQCITGVYTDIFPLSQNYGYL ATVTLLSGVDRVNPVISYGTSTGRVADSQ LTSSSQVAAYTTTTCFTFNQKGYCYHIIEL SPATLGIFQPVLVVTEIPKICS		
EU8779776	6248-8161	gb:EU877976:6248-8161|Organism:Avian paramyxovirus 4|Strain Name:APMV-4/KR/YJ/06|Protein Name:hemagglutinin-neuraminidase| Gene Symbol:HN	MQGNMEGSRDNLTVDDELKTTWRLAYR VVSLLLMVSALIISIVILTRDNSQSIITAINQS SDADSKWQTGIEGKITSIMTDTLDTRNAAL LHIPLQLNTLEANLLSALGGNTGIGPGDLE HCRYPVHDTAYLHGVNRLLINQTADYTAE GPLDHVNFIPAPVTTTGCTRIPSFSVSSSIW CYTHNVIETGCNDHSGSNQYISMGVIKRA GNGLPYFSTVVSKYLTDGLNRKSCSVAAG SGHCYLLCSLVSEPEPDDYVSPDPTPMRLG VLTWDGSYTEQAVPERIFKNIWSANYPGV GSGAIVGNKVLFPFYGGVRNGSTPEVMNR GRYYYIQDPNDYCPDPLQDQILRAEQSYY PTRFGRRMVMQGVLACPVSNNSTIASQCQ SYYFNNSLGFIGAESRIYYLNGNIYLYQRS SSWWPHPQIYLLDSRIASPGTQNIDSGVNL KMLNVTVITRPSSGFCNSQSRCPNDCLFGV YSDIWPLSLTSDSIFAFTMYLQGKTTRIDPA WALFSNHAIGHEARLFNKEVSAAYSTTTC FSDTIQNQVYCLSILEVRSELLGAFKIVPFL YRVL	8	703
AB176531	6821-8536	gb:AB176531:6821-8536|Organism:Human parainfluenza	MEDYSNLSLKSIPKRTCRIIFRTATILGICTL IVLCSSILHEIIHLDVSSGLMDSDDSQQGIIQ PIIESLKSLIALANQILYNVAIIIPLKIDSIETV IFSALKDMHTGSMSNTNCTPGNLLLHDAA YINGINKFLVLKSYNGTPKYGPLLNIPSFIP	7	704

		virus 2|Strain Name:Nishio|Protein Name:hemagglutinin-neuraminidase protein|Gene Symbol:HN	SATSPNGCTRIPSFSLIKTHWCYTHNVMLG DCLDFTTSNQYLAMGIIQQSAAAFPIFRTM KTIYLSDGINRKSCSVTAIPGGCVLYCYVA TRSEKEDYATTDLAELRLAFYYYNDTFIER VISLPNTTGQWATINPAVGSGIYHLGFILFP VYGGLISGTPSYNKQSSRYFIPKHPNITCAG NSSEQAAAARSSYVIRYHSNRLIQSAVLIC PLSDMHTARCNLVMFNNSQVMMGAEGR LYVIDNNLYYYQRSSSWWSASLFYRINTD FSKGIPPIIEAQWVPSYQVPRPGVMPCNAT SFCPANCITGVYADVWPLNDPEPTSQNAL NPNYRFAGAFLRNESNRTNPTFYTASASA LLNTTGFNNTNHKAAYTSSTCFKNTGTQK IYCLIIIEMGSSLLGEFQIIPFLRELIP		
AF0527 55	6584-8281	gb:AF052755|Organism:Parainfluenza virus 5|Strain Name:W3A|Protein Name:hemagglutinin-neuraminidase protein|Gene Symbol:HN	MVAEDAPVRATCRVLFRTTTLIFLCTLLAL SISILYESLITQKQIMSQAGSTGSNSGLGSIT DLLNNILSVANQIIYNSAVALPLQLDTLEST LLTAIKSLQTSDKLEQNCSWSAALINDNRY INGINQFYFSIAEGRNLTLGPLLNMPSFIPT ATTPEGCTRIPSFSLTKTHWCYTHNVILNG CQDHVSSNQFVSMGIIEPTSAGFPFFRTLKT LYLSDGVNRKSCSISTVPGGCMMYCFVST QPERDDYFSAAPPEQRIIIMYYNDTIVERII NPPGVLDVWATLNPGTGSGVYYLGWVLF PIYGGVIKGTSLWNNQANKYFIPQMVAAL CSQNQATQVQNAKSSYYSSWFGNRMIQS GILACPLRQDLTNECLVLPFSNDQVLMGA EGRLYMYGDSVYYYQRSNSWWPMTMLY KVTITFTNGQPSAISAQNVPTQQVPRPGTG DCSATNRCPGFCLTGVYADAWLLTNPSST STFGSEATFTGSYLNTATQRINPTMYIANN TQIISSQQFGSSGQEAAYGHTTCFRDTGSV MVYCIYIIELSSSLLGQFQIVPFIRQVTLS	7	705
BK0	6560-	gb:BK005918|	MSQLGTDQIMHLAQPAIARRTWRLCFRIF	7	706

05918	8290	Organism:Porcine rubulavirus|Strain Name:UNKNOWN-BK005918|Protein Name:attachment protein|Gene Symbol:HN	ALFILIAIVITQIFMLTFDHTLLTTTQFLTSIGNLQSTITSWTPDVQAMLSISNQLIYTTSITLPLKISTTEMSILTAIRDHCHCPDCSSACPTRQMLLNDPRYMSGVNQFIGAPTESINITFGPLFGIPSFIPTSTTTQGCTRIPSFALGPSHWCYTHNFITAGCADGGHSNQYLAMGTIQSASDGSPLLITARSYYLSDGVNRKSCSIAVVPGGCAMYCYVATRSETDYYAGNSPPQQLLTLVFSNDTIIERTIHPTGLANGWVMLVPGVGSGTLYNEYLLFPAYGGMQQILANQSGEINQFFTPYNATVRCAMAQPQFSQRAAASYYPRYFSNRWIRSAIVACPYRAIYQTQCTLIPLPNRMVMMGSEGRIFTLGDRLFYYQRSSSWWPYPLLYQVGLNFLTTPPSVSSMTQVPLEHLARPGKGGCPGNSHCPATCVTGVYADVWPLTDPRSGVGGTSLVAAGGLDSTSERMAPVNYLAIGESLLSKTYLLSKTQPAAYTTTTCFRDTDTGKIYCITIAELGKVLLGEFQIVPFLREIKIQSRY		
EU338414	6015-7913	gb:EU338414:6015-7913|Organism:Avian paramyxovirus 2|Strain Name:APMV-2/Chicken/California/Yucaipa/56|Protein Name:hemagglutinin-neuraminidase|Gene Symbol:HN	MDFPSRENLAAGDISGRKTWRLLFRILTLSIGVVCLAINIATIAKLDHLDNMASNTWTTTEADRVISSITTPLKVPVNQINDMFRIVALDLPLQMTSLQKEITSQVGFLAESINNVLSKNGSAGLVLVNDPEYAGGIAVSLYQGDASAGLNFQPISLIEHPSFVPGPTTAKGCIRIPTFHMGPSHWCYSHNIIASGCQDASHSSMYISLGVLKASQTGSPIFLTTASHLVDDNINRKSCSIVASKYGCDILCSIVIETENEDYRSDPATSMIIGRLFFNGSYTESKINTGSIFSLFSANYPAVGSGIVVGDEAAFPIYGGVKQNTWLFNQLKDFGYFTHNDVYKCNRTDIQQTILDAYRPPKISGRLWVQGILLCPVSLRPDPGCRLKVFNTSNVMMGAEARLIQVGSTVYLYQRSSSWWVVGLTYKLDVSEITSQTGNTLNHVDPIAHTK	7	707

			FPRPSFRRDACARPNICPAVCVSGVYQDIW PISTATNNSNIVWVGQYLEAFYSRKDPRIG IATQYEWKVTNQLFNSNTEGGYSTTTCFR NTKRDKAYCVVISEYADGVFGSYRIVPQLI EIRTTTGKSE		
KC4039073	6234-6964	gb:KC403973:6234-6964|Organism:Human metapneumovirus|Strain Name:HMPV/USA/TN-82-518/1982/A|Protein Name:attachment glycoprotein G|Gene Symbol:G|Segment: 8	MEVKVENIRTIDMLKARVKNRVARSKCFK NASLILIGITTLSIALNIYLIINYTMQENTSES EHHTSSSPMESSRETPTVPIDNSDTNPSSQY PTQQSTEGSTLYFAASASSPETEPTSTPDTT SRPPFVDTHTTPPSASRTKTSPAVHTKNNP RISSRTHSPPWAMTRTVRRTTTLRTSSIRK RSSTASVQPDSSATTHKHEEASPVSPQTSA STTRPQRKSMEASTSTTYNQTS	6	708
KF015281	4511-5844	gb:KF015281:4511-5844|Organism:Canine pneumovirus|Strain Name:dog/Bari/100-12/ITA/2012|Protein Name:attachment protein|Gene Symbol:G	MRPAEQLIQENYKLTSLSMGRNFEVSGST TNLNFERTQYPDTFRAVVKVNQMCKLIAG VLTSAAVAVCVGVIMYSVFTSNHKANSM QNATIRNSTSAPPQPTAGPPTTEQGTTPKFT KPPTKTTTHHEITEPAKMVTPSEDPYQCSS NGYLDRPDLPEDFKLVLDVICKPPGPEHHS TNCYEKREINLGSVCPDLVTMKANMGLN NGGGEEAAPYIEVITLSTYSNKRAMCVHN GCDQGFCFFLSGLSTDQKRAVLELGGQQA IMELHYDSYWKHYWSNSNCVVPRTNCNL TDQTVILFPSFNNKNQSQCTTCADSAGLD NKFYLTCDGLSRNLPLVGLPSLSPQAHKA ALKQSTGTTTAPTPETRNPTPAPRRSKPLS RKKRALCGVDSSREPKPTMPYWCPMLQL	6	709

			FPRRSNS		
KF9733339	4624-5310	gb:KF973339:4624-5310|Organism:Respiratory syncytial virus type A|Strain Name:RSV-A/US/BID-V7358/2002|Protein Name:truncated attachment glycoprotein|Gene Symbol:G	MSKTKDQRAAKTLEKTWDTLNHLLFISSCLYKSNLKSIAQITLSILAMTIPTSLIIVATTFIASANNKVTPTTAIIQDATSQIKNTTPTHLTQNPQPGISFFNLSGTISQTTAILAPTTPSVEPILQSTTVKTKNTTTTQIQPSKLTTKQRQNKPPNKPNDDFHFEVFNFVPCSICSNNPTCWAICKRIPSKKPGKKTTTKPTKKQTIKTTKKDLKPQTTKPKEAPTT	6	710
FJ215864	6383-8116	gb:FJ215864:6383-8116|Organism:Avian paramyxovirus 8|Strain Name:pintail/Wakuya/20/78|Protein Name:hemagglutinin-neuraminidase protein|Gene Symbol:HN	MSNIASSLENIVEQDSRKTTWRAIFRWSVLLITTGCLALSIVSIVQIGNLKIPSVGDLADEVVTPLKTTLSDTLRNPINQINDIFRIVALDIPLQVTSIQKDLASQFSMLIDSLNAIKLGNGTNLIIPTSDKEYAGGIGNPVFTVDAGGSIGFKQFSLIEHPSFIAGPTTTRGCTRIPTFHMSESHWCYSHNIIAAGCQDASASSMYISMGVLHVSSSGTPIFLTTASELIDDGVNRKSCSIVATQFGCDILCSIVIEKEGDDYWSDTPTPMRHGRFSFNGSFVETELPVSSMFSSFSANYPAVGSGEIVKDRILFPIYGGIKQTSPEFTELVKYGLFVSTPTTVCQSSWTYDQVKAAYRPDYISGRFWAQVILSCALDAVDLSSCIVKIMNSSTVMMAAEGRIIKIGIDYFYYQRSSSWWPLAFVTKLDPQELADTNSIWLTNSIPIPQSKFPRPSYSENYCTKPAVCPATCVTGVYSDIWPLTSSSSLPSIIWIGQYLDAPVGRTYPRFGIANQSHWYLQEDILPTSTASAYSTTTCFKNTARNRVFCVTIAEFADGLFGEYRITPQLYELVRN	5	711

			N		
JX857409	6619-8542	gb:JX857409:6619-8542|Organism:Porcine parainfluenza virus 1|Strain Name:S206N|Protein Name:haemagglutinin protein|Gene Symbol:H	MEETKVKTSEYWARSPQIHATNHPNVQN REKIKEILTILISFISSLSLVLVIAVLIMQSLH NGTILRCKDVGLESINKSTYSISNAILDVIK QELITRIINTQSSVQVALPILINKKIQDLSLII EKSSKVHQNSPTCSGVAALTHVEGIKPLDP DDYWRCPSGEPYLEDELTLSLIPGPSMLAG TSTIDGCVRLPSLAIGKSLYAYSSNLITKGC QDIGKSYQVLQLGIITLNSDLHPDLNPIISH TYDINDNRKSCSVAVSETKGYQLCSMPRV NEKTDYTSDGIEDIVFDVLDLKGSSRSFKF SNNDINFDHPFSALYPSVGSGIIWKNELYF LGYGALTTALQGNTKCNLMGCPGATQDN CNKFISSSWLYSKQMVNVLIQVKGYLSSK PSIIVRTIPITENYVGAEGKLVGTRERIYIYT RSTGWHTNLQIGVLNINHPITITWTDHRVL SRPGRSPCAWNNKCPRNCTTGVYTDAYPI SPDANYVATVTLLSNSTRNNPTIMYSSSDR VYNMLRLRNTELEAAYTTTSCIVHFDRGY CFHIIEINQKELNTLQPMLFKTAIPKACRIS NL	5	712
KF908238	7510-9249	gb:KF908238:7510-9249|Organism:Human parainfluenza virus 4b|Strain Name:QLD-01|Protein Name:hemagglutinin-neuraminidase protein|Gene Symbol:HN	MQDSRGNTQIFSQANSMVKRTWRLLFRIV TLILLISIFVLSLIIVLQSTPGNLQSDVDIIRK ELDELMENFETTSKSLLSVANQITYDVSVL TPIRQEATETNIIAKIKDHCKDRVVKGEST CTLGHKPLHDVSFLNGFNKFYFTYRDNVQ IRLNPLLDYPNFIPTATTPHGCIRIPSFSLSQ THWCYTHNTILRGCEDTASSKQYVSLGTL QTLENGDPYFKVEYSHYLNDRKNRKSCSV VAVLDGCLLYCVIMTKNETENFKDPQLAT QLLTYISYNGTIKERIINPPGSSRDWVHISP GVGSGILYSNYIIFPLYGGLMENSMIYNNQ SGKYFFPNSTKLPCSNKTSEKITGAKDSYTI TYFSKRLIQSAFLICDLRQFLSEDCEILIPSN	5	713

			DHMLVGAEGRLYNIENNIFYYQRGSSWW PYPSLYRIKLNSNKKYPRIIEIKFTKIEIAPRP GNKDCPGNKACPKECITGVYQDIWPLSYP NTAFPHKKRAYYTGFYLNNSLARRNPTFY TADNLDYHQQERLGKFNLTAGYSTTTCFK QTTTARLYCLYILEVGDSVIGDFQIFPFLRS IDQAIT		
KT0 717 57	6066-7962	gb:KT071757:6 066-7962|Organism :Avian paramyxovirus 2|Strain Name:APMV-2/Emberiza spodocephala/China/Daxing'anling/974/2013|Protein Name:hemagglutinin-neuraminidase protein|Gene Symbol:HN	MDALSRENLTEISQGGRRTWRMLFRILTL VLTLVCLAINIATIAKLDSIDTSKVQTWTTT ESDRVIGSLTDTLKIPINQVNDMFRIVALDL PLQMTTLQKEIASQVGFLAESINNFLSKNG SAGSVLVNDPEYAGGIGTSLFHGDSASGL DFEAPSLIEHPSFIPGPTTAKGCIRIPTFHMS ASHWCYSHNIIASGCQDAGHSSMYISMGV LKATQAGSPSFLTTASQLVDDKLNRKSCSI ISTTYGCDILCSLVVENEDADYRSDPPTDM ILGRLFFNGTYSESKLNTSAIFQLFSANYPA VGSGIVLGDEIAFPVYGGVKQNTWLFNQL KDYGYFAHNNVYKCNNSNIHQTVLNAYR PPKISGRLWSQVVLICPMRLFINTDCRIKVF NTSTVMMGAEARLIQVGSDIYLYQRSSSW WVVGLTYKLDFQELSSKTGNILNNVSPIA HAKFPRPSYSRDACARPNICPAVCVSGVY QDIWPISTAHNLSQVVWVGQYLEAFYARK DPWIGIATQYDWKKNVRLFNANTEGGYS TTTCFRNTKRDKAFCVIISEYADGVFGSYR IVPQLIEIRTTSKKGLPS	5	714
LC0 411 32	6605-8437	gb:LC041132:6 605-8437|Organism :Avian paramyxovirus goose/Shimane/67/2000|Strain	MQPGISEVSFVNDERSERGTWRLLFRILTI VLCLTSIGIGIPALIYSKEAATSGDIDKSLEA VKTGMSTLSSKIDESINTEQKIYRQVILEAP VSQLNMESNILSAITSLSYQIDGTSNSSGCG SPMHDQDFVGGINKEIWTTDNVNLGEITL TPFLEHLNFIPAPTTGNGCTRIPSFDLGLTH WCYTHNVILSGCQDYSSSFQYIALGVLKIS	4	715

		Name:goose/Shimane/67/2000|Protein Name:hemagglutinin-neuraminidase|Gene Symbol:HN	ATGHVFLSTMRSINLDDERNRKSCSISATSIGCDIICSLVTEREVDDYNSPAATPMIHGRLDFSGKYNEVDLNVGQLFGDWSANYPGVGGGSFLNGRVWFPIYGGVKEGTPTFKENDGRYAIYTRYNDTCPDSESEQVSRAKSSYRPSYFGGKLVQQAVLSIKIDDTLGLDPVLTISNNSITLMGAESRVLQIEEKLYFYQRGTSWFPSLIMYPLTVDDKMVRFEPPTIFDQFTRPGNHPCSADSRCPNACVTGVYTDGYPIVFHNNHSIAAVYGMQLNDVTNRLNPRSAVWYGVSMSNVIRVSSSTTKAAYTTSTCFKVKKTQRVYCLSIGEIGNTLFGEFRIVPLLLEVYSEKGKSLKSSFDGWEDISINNPLRPLDNHRVDPILISNYTSSWP
AF092942	4705-5478	gb:AF092942|Organism:Bovine respiratory syncytial virus|Strain Name:ATue51908|Protein Name:attachment glycoprotein|Gene Symbol:G	MSNHTHHLKFKTLKRAWKASKYFIVGLSCLYKFNLKSLVQTALTTLAMITLTSLVITAIIYISVGNAKAKPTSKPTIQQTQQPQNHTSPFFTEHNYKSTHTSIQSTTLSQLPNTDTTRETTYSHSINETQNRKIKSQSTLPATRKPPINPSGSNPPENHQDHNNSQTLPYVPCSTCEGNLACLSLCQIGPERAPSRAPTITLKKTPKPKTTKKPTKTTIHHRTSPEAKLQPKNNTAAPQQGILSSPEHHTNQSTTQI	3	716
AF326114	6691-847	gb:AF326114|Organism:Mengle virus|Strain Name:UNKNOWN-AF326114|Protein Name:attachme	MWNSIPQLVSDHEEAKGKFTDIPLQDDTDSQHPSGSKSTCRTLFRTVSIILSLVILVLGVTSTMFSAKYSGGCATNSQLLGVSNLINQIQKSIDSLISEVNQVSITTAVTLPIKIMDFGKSVTDQVTQMIRQCNTVCKGPGQKPGSQNVRIMPSNNLSTFQNINMSARGIAYQDVPLTFVRPIKNPQSCSRFPSYSVSFGVHCFANAVTDQTCELNQNTFYRVVLSVSKGNISDPSSLETKAETRTPKGTPVRTCSIISSVYGCYLLCSK	3	717

		nt protein|Gene Symbol:HN	ATVPESEEMKTIGFSQMFILYLSMDSKRIIY DNIVSSTSAIWSGLYPGEGAGIWHMGQLF FPLWGGIPFLTPLGQKILNSTLDIPEVGSKC KSDLTSNPAKTKDMLFSPYYGENVMVFGF LTCYLLSNVPTNCHADYLNSTVLGFGSKA QFYDYRGIVYMYIQSAGWYPFTQIFRITLQ LKQNRLQAKSIKRIEVTSTTRPGNRECSVL RNCPYICATGLFQVPWIVNSDAITSKEVDN MVFVQAWAADFTEFRKGILSLCSQVSCPI NDLLSKDNSYMRDTTTYCFPQTVPNILSCT SFVEWGGDSGNPINILEIHYEVIFVAS		
GU2063651	7500-9714	gb:GU206351:7500-9714|Organism:Avian paramyxovirus 5|Strain Name:budgerigar/Kunitachi/74|Protein Name:hemagglutinin neuraminidase protein|Gene Symbol:HN	MDKSYYTEPEDQRGNSRTWRLLFRLIVLT LLCLIACTSVSQLFYPWLPQVLSTLISLNSSI ITSSNGLKKEILNQNIKEDLIYREVAINIPLT LDRVTVEVGTAVNQITDALRQLQSVNGSA AFALSNSPDYSGGIEHLVFQRNTLINRSVS VSDLIEHPSFIPTPTTQHGCTRIPTFHLGTRH WCYSHNIIGQGCADSGASMMYISMGALG VSSLGTPTFTTSATSILSDSLNRKSCSIVATT EGCDVLCSIVTQTEDQDYADHTPTPMIHG RLWFNGTYTERSLSQSLFLGTWAAQYPAV GSGIMTPGRVIFPFYGGVIPNSPLFLDLERF ALFTHNGDLECRNLTQYQKEAIYSAYKPP KIRGSLWAQGFIVCSVGDMGNCSLKVINT STVMMGAEGRLQLVGDSVMYYQRSSSW WPVGILYRLSLVDIIARDIQVVINSEPLPLS KFPRPTWTPGVCQKPNVCPAVCVTGVYQ DLWAISAGETLSEMTFFGGYLEASTQRKD PWIGVANQYSWFMRRRLFKTSTEAAYSSS TCFRNTRLDRNFCLLIFELTDNLLGDWRIV PLLFELTIV	3	718
JQ0017776	8170-10275	gb:JQ001776:8170-10275|Organis	MLSQLQKNYLDNSNQQGDKMNNPDKKLS VNFNPLELDKGQKDLNKSYYVKNKNYNV SNLLNESLHDIKFCIYCIFSLLIIITIINIITISIV	3	719

		m:Cedar virus|Strain Name:CG1a|Protein Name:attachment glycoprotein|Gene Symbol:G	ITRLKVHEENNGMESPNLQSIQDSLSSLTN MINTEITPRIGILVTATSVTLSSSINYVGTKT NQLVNELKDYITKSCGFKVPELKLHECNIS CADPKISKSAMYSTNAYAELAGPPKIFCKS VSKDPDFRLKQIDYVIPVQQDRSICMNNPL LDISDGFFTYIHYEGINSCKKSDSFKVLLSH GEIVDRGDYRPSLYLLSSHYHPYSMQVINC VPVTCNQSSFVFCHISNNTKTLDNSDYSSD EYYITYFNGIDRPKTKKIPINNMTADNRYI HFTFSGGGGVCLGEEFIIPVTTVINTDVFTH DYCESFNCSVQTGKSLKEICSESLRSPTNSS RYNLNGIMIISQNNMTDFKIQLNGITYNKL SFGSPGRLSKTLGQVLYYQSSMSWDTYLK AGFVEKWKPFTPNWMNNTVISRPNQGNC PRYHKCPEICYGGTYNDIAPLDLGKDMYV SVILDSDQLAENPEITVFNSTTILYKERVSK DELNTRSTTTSCFLFLDEPWCISVLETNRF NGKSIRPEIYSYKIPKYC		
KP271123	6644-8431	gb:KP271123:6644-8431|Organism:Teviot virus|Strain Name:Geelong|Protein Name:attachment protein|Gene Symbol:HN	MWSTQASKHPAMVNSATNLVDIPLDHPSS AQFPINRKRTGRLIYRLFSILCNLILISILISL VVIWSRSSRDCAKSDGLSSVDNQLSSLSRS INSLITEVNQISVTTAINLPIKLSEFGKSVVD QVTQMIRQCNAACKGPGEKPGIQNVRINIP NNFSTYSELNRTANSLNFQSRTALFARPNP YPKTCSRFPSYSVYFGIHCFSHAVTDSSCE LSDSTYYRLVIGVADKNLSDPADVKYIGE TTTPVRVQTRGCSVVSSIYGCYLLCSKSNQ DYQDDFREQGFHQMFILFLSRELKTTFFDD MVSSTTVTWNGLYPGEGSGIWHMGHLVF PLWGGIRFGTHASEGILNSTLELPPVGPSC KRSLADNGLINKDVLFSPYFGDSVMVFAY LSCYMLSNVPTHCQVETMNSSVLGFGSRA QFYDLKGIVYLYIQSAGWFSYTQLFRLSLQ SKGYKLSVKQIKRIPISSTSRPGTEPCDIIHN	3	720

			CPYTCATGLFQAPWIVNGDSIRDRDVRNM AFVQAWSGAINTFQRPFMSICSQYSCPLSE LLDSESSIMRSTTTYCFPSLTESILQCVSFIE WGGPVGNPISINEVYSSISFRPD		
AY2864 09	7644-9542	gb:AY286409| Organism:Mossman virus|Strain Name:UNKNOWN-AY286409|Protein Name:attachment glycoprotein|Gene Symbol:G	MVDPPAVSYYTGTGRNDRVKVVTTQSTN PYWAHNPNQGLRRLIDMVVNVIMVTGVIF ALINIILGIVIISQSAGSRQDTSKSLDIIQHVD SSVAITKQIVMENLEPKIRSILDSVSFQIPKL LSSLLGPGKTDPPIALPTKASTPVIPTEYPSL NTTTCLRIEESVTQNAAALFNISFDLKTVM YELVTRTGGCVTLPSYSELYTRVRTFSTAI RNPKTCQRAGQETDLNLIPAFIGTDTGILIN SCVRQPVIATGDGIYALTYLTMRGTCQDH RHAVRHFEIGLVRRDAWWDPVLTPIHHFT EPGTPVFDGCSLTVQNQTALALCTLTTDG PETDIHNGASLGLALVHFNIRGEFSKHKVD PRNIDTQNQGLHLVTTAGKSAVKKGILYS FGYMVTRSPEPGDSKCVTEECNQNNQEKC NAYSKTTLDPDKPRSMIIFQIDVGAEYFTV DKVVVVPRTQYYQLTSGDLFYTGEENDLL YQLHNKGWYNKPIRGRVTFDGQVTLHEH SRTYDSLSNQRACNPRLGCPSTCELTSMAS YFPLDKDFKAAVGVIALRNGMTPIITYSTD DWRNHWKYIKNADLEFSESSLSCYSPNPP LDDYVLCTAVITAKVMSNTNPQLLATSW YQYDKCHT	2	721
AY9000 01	7809-9938	gb:AY900001| Organism:J-virus|Strain Name:UNKNOWN-AY900001|Protein Name:attachme	MNPVAMSNFYGINQADHLREKGDQPEKG PSVLTYVSLITGLLSLFTIIALNVTNIIYLTG SGGTMATIKDNQQSMSGSMRDISGMLVE DLKPKTDLINSMVSYTIPSQISAMSAMIKN EVLRQCTPSFMFNNTICPIAEHPVHTSYFEE VGIEAISMCTGTNRKLVVNQGINFVEYPSF IPGSTKPGGCVRLPSFSLGLEVFAYAHAIT QDDCTSSSTPDYYFSVGRIADHGTDVPVFE	2	722

		nt glycoprotein|Gene Symbol:G	TLAEWFLDDKMNRRSCSVTAAGKGGWL GCSILVGSFTDELTSPEVNRISLSYMDTFGK KKDWLYTGSEVRADQSWSALFFSVGSGV VIGDTVYFLVWGGLNHPINVDAMCRAPG CQSPTQSLCNYAIKPQEWGGNQIVNGILHF KHDTNEKPTLHVRTLSPDNNWMGAEGRL FHFHNSGKTFIYTRSSTWHTLPQVGILTLG WPLSVQWVDITSISRPGQSPCEYDNRCPHQ CVTGVYTDLFPLGVSYEYSVTAYLDQVQS RMNPKIALVGAQEKIYEKTITTNTQHADY TTTSCFAYKLRVWCVSIVEMSPGVITTRQP VPFLYHLNLGCQDTSTGSLTPLDAHGGTY LNTDPVGNKVDCYFVLHEGQIYFGMSVGP INYTYSIVGRSREIGANMNVSLNQLCHSVY TEFLKEKEHPGTRNNIDVEGWLLKRIETLN GTKIFGLDDLEGSGPGHQSGPEDPSIAPIGH N		
EF1997 72	6150-6944	gb:EF199772:6150-6944|Organism:Avian metapneumovirus|Strain Name:PL-2|Protein Name:attachment glycoprotein|Gene Symbol:G	MEVKVENVGKSQELKVKVKNFIKRSDCK KKLFALILGLVSFELTMNIMLSVMYVESNE ALSLCRIQGTPAPRDNKTNTENATKETTLH TTTTTRDPEVRETKTTKPQANEGATNPSR NLTTKGDKHQTTRATTEAELEKQSKQTTE PGTSTQKHTPARPSSKSPTTTQATAQPTTP TAPKASTAPKNRQATTKKTETDTTTASRA RNTNNPTETATTTPKATTETGKGKEGPTQ HTTKEQPETTARETTTPQPRRTAGASPRAS	2	723
JF424833	5981-7156	gb:JF424833:5981-7156|Organism:Avian metapneumovir	MGSKLYMVQGTSAYQTAVGFWLDIGRRY ILAIVLSAFGLTCTVTIALTVSVIVEQSVLE ECRNYNGGDRDWWSTTQEQPTTAPSATP AGNYGGLQTARTRKSESCLHVQISYGDM YSRSDTVLGGFDCMGLLVLCKSGPICQRD	2	724

		us|Strain Name:IT/Ty/A/259-01/03|Protein Name:attachment protein|Gene Symbol:G	NQVDPTALCHCRVDLSSVDCCKVNKISTN SSTTSEPQKTNPAWPSQDNTDSDPNPQGIT TSTATLLSTSLGLMLTSKTGTHKSGPPQAL PGSNTNGKTTTDRELGSTNQPNSTTNGQH NKHTQRMTLPPSYDNTRTILQHTTPWEKT FSTYKPTHSPTNESDQSLPTTQNSINCEHFD PQGKEKICYRVGSYNSNITKQCRIDVPLCS TYNTVCMKTYYTEPFNCWRRIWRCLCDD GVGLVEWCCTS		
JN689227	7918-12444	gb:JN689227:7918-12444|Organism:Tailam virus|Strain Name:TL8K|Protein Name:attachment glycoprotein|Gene Symbol:G	MSQLAAHNLAMSNFYGIHQGGQSTSQKE EEQPVQGVIRYASMIVGLLSLFTIIALNVTN IIYMTESGGTMQSIKNAQGSIDGSMKDLSG TIMEDIKPKTDLINSMVSYNIPAQLSMIHQI IKNDVLKQCTPSFMFNNTICPLAENPTHSR YFEEVNLDSISECSGNEMSLELGTEPEFIEY PSFAPGSTKPGSCVRLPSFSLSSTVFAYTHT IMGHGCSELDVGDHYLAIGRIADAGHEIPQ FETISSWFINDKINRRSCTVAAGVMETWM GCVIMTETFYDDLDSLDTGKITISYLDVFG RKKEWIYTRSEILYDYTYTSVYFSIGSGVV VGDTVYFLLWGSLSSPIEETAYCYAPGCSN YNQRMCNEAQRPAKFGHRQMANAILRFK TNSMGKPSISVRTLSPTVIPFGTEGRLIYSD FTKIIYLYLRSTSWYVLPLTGLLILGPPVSIS WVTQEAVSRPGEYPCGASNRCPKDCITGV YTDLFPLGARYEYAVTVYLNAETYRVNPT LALIDRSKIIARKKITTESQKAGYTTTTCFV FKLRIWCMSVVELAPATMTAFEPVPFLYQ LDLTCKRNNGTTAMQFSGQDGMYKSGRY KSPRNECFFEKVSNKYYFVVSTPEGIQPYE VRDLTPERVSHVIMYISDVCAPALSAFKKL IPAMRPITTLTIGNWQFRPVDISGGLRVNIY RNLTRYGDLSMSAPEDPGTDTFPGTHAPS KGHEEVGHYTLPNEKLSEVTTAAVKTKES	2	725

			LNLIPDTKDTRGEEENGSGLNEIITGHTTPG HIKTHPAETKVTKHTVIIPQIEEDGSGATTS TELQDETGYHTEDYNTTNTNGSLTAPNER NNYTSGDHTVSGEDITHTITVSDRTKTTQT LPTDNTFNQTPTKIQEGSPKSESTPKDYTAI ESEDSHFTDPTLIRSTPEGTIVQVIGDQFHS AVTQLGESNAIGNSEPIDQGNNLIPTTDRG TMDNTSSQSHSSTTSTQGSHSAGHGSQSN MNLTALADTDSVTDQSTSTQEIDHEHENV SSILNPLSRHTRVMRDTVQEALTGAWGFIR GMIP		
KC5 622 42	6178-6926	gb:KC562242: 6178-6926|Organism :Human metapneumovir us|Strain Name:HMPV/ USA/C1-334/2004/B|Pro tein Name:attachme nt glycoprotein G|Gene Symbol:G	MEVRVENIRAIDMFKAKIKNRIRNSRCYR NATLILIGLTALSMALNIFLIIDHATLRNMI KTENCANMPSAEPSKKTPMTSIAGPSTKPN PQQATQWTTENSTSPAATLEGHPYTGTTQ TPDTTAPQQTTDKHTALPKSTNEQITQTTT EKKTTRATTQKRKKEKKTQTKPQVQLQP KQPTPPTKSEMQVRQSQHPTDPELTPLPKA VNRQPGQQNQAPHHIMHGEVQDPGERNT QVSHPSS	2	726
KC9 150 36	6154-7911	gb:KC915036: 6154-7911|Organism :Avian metapneumovir us type C|Strain Name:GDY|Pr otein Name:attachme	MEVKIENVGKSQELRVKVKNFIKRSDCKK KLFALILGLISFDITMNIMLSVMYVESNEA LSSCRVQGTPAPRDNRTNTENTAKETTLH TMTTTRNTEAGGTKTTKPQADERATSPSK NPTIGADKHKTTRATTEAEQEKQSKQTTE PGTSTPKHIPARPSSKSPATTKTTTQPTTPT VAKGGTAPKNRQTTTKKTEADTPTTSRAK QTNKPTGTETTPPRATTETDKDKEGPTQH TTKEQPETTAGGTTTPQPRRTTSRPAPTTN	2	727

		nt glycoprotein|Gene Symbol:G	TKEGAETTGTRTTKSTQTSASPPRPTRSTPS KTATGTNKRATTTKGPNTASTDRRQQTRT TPKQDQQTQTKAKTTTNKAHAKAATTPE HNTDTTDSMKENSKEDKTTRDPSSKATTK QENTSKGTTATNLGNNTEAGARTPPTTTP TRHTTEPATSTAGGHTKARTTRWKSTAAR QPTRNNTTADTKTAQSKQTTPAQLGNNTT PENTTPPDNKSNSQTNVAPTEEIEIGSSLWR RRYVYGPCRENALEHPMNPCLKDNTTWI YLDNGRNLPAGYYDSKTDKIICYGIYRGN SYCYGRIECTCKNGTGLLSYCCNSYNWS		
LC1687049	7239-9196	gb:LC168749:7239-9196|Organism:Rinderpest morbillivirus|Strain Name:Lv|Protein Name:H protein|Gene Symbol:H	MSSPRDRVNAFYKDNLQFKNTRVVLNKE QLLIERPYMLLAVLFVMFLSLVGLLAIAGI RLHRAAVNTAEINSGLTTSIDITKSIEYQVK DVLTPLFKIIGDEVGLRTPQRFTDLTKFISD KIKFLNPDKEYDFRDINWCISPPERIKINYD QYCAHTAAEELITMLVNSSLAGTAVLRTS LVNLGRSCTGSTTTKGQFSNMSLALSGIYS GRGYNISSMITITEKGMYGSTYLVGKHNQ GARRPSTAWQRDYRVFEVGIIRELGVGTP VFHMTNYLELPRQPELEICMLALGEFKLA ALCLADNSVALHYGGLRDDHKIRFVKLG VWPSPADSDTLATLSAVDPTLDGLYITTHR GIIAAGKAVWAVPVTRTDDQRKMGQCRR EACREKPPPFCNSTDWEPLEAGRIPAYGIL TIRLGLADKPEIDIISEFGPLITHDSGMDLYT PLDGNEYWLTIPPLQNSALGTVNTLVLEPS LKISPNILTLPIRSGGGDCYTPTYLSDLADD DVKLSSNLVILPSRNLQYVSATYDTSRVEH AIVYYIYSTGRLSSYYYPVKLPIKGDPVSL QIGCFPWGLKLWCHHFCSVIDSGTGKQVT HTGAVGIEITCNSR	2	728
LC1873	8144-9871	gb:LC187310:8144-	MDSSQMNILDAMDRESSKRTWRGVFRVT TIIMVVTCVVLSAITLSKVAHPQGFDTNEL	2	729

10		9871|Organism:Avian paramyxovirus 10|Strain Name:rAPMV-10-FI324/YmHA|Protein Name:hemagglutinin-neuraminidase|Gene Symbol:HN	GNGIVDRVSDKITEALTVPNNQIGEIFKIVA LDLHVLVSSSQQAIAGQIGMLAESINSILSQ NGSASTILSSSPEYAGGIGVPLFSNKLTNGT VIKPITLIEHPSFIPGPTTIGGCTRIPTFHMAS SHWCYSHNIIEKGCKDSGISSMYISLGVLQ VLKKGTPVFLVTASAVLSDDRNRKSCSIIS SRFGCEILCSLVTEAESDDYKSDTPTGMVH GRLYFNGTYREGLVDTETIFRDFSANYPG VGSGEIVEGHIHFPIYGGVKQNTGLYNSLT PYWLDAKNKYDYCKLPYTNQTIQNSYKPP FIHGRFWAQGILSCELDLFNLGNCNLKIIRS DKVMMGAESRLMLVGSKLLMYQRASSW WPLGITQEIDIAELHSSNTTILREVKPILSSK FPRPSYQPNYCTKPSVCPAVCVTGVYTDM WPISITGNISDYAWISHYLDAPTSRQQPRIG IANQYFWIHQTTIFPTNTQSSYSTTTCFRNQ VRSRMFCLSIAEFADGVFGEFRIVPLLYEL RV		
NC_004074	6590-8563	gb:NC_004074:6590-8563|Organism:Tioman virus|Strain Name:UNKNOWN-NC_004074|Protein Name:attachment protein|Gene Symbol:HN	MWATSESKAPIPANSTLNLVDVPLDEPQTI TKHRKQKRTGRLVFRLLSLVLSLMTVILV LVILASWSQKINACATKEGFNSLDLQISGL VKSINSLITEVNQISITTAINLPIKLSDFGKSI VDQVTQMIRQCNAVCKGPGEKPGIQNIRI NIPNNFSTYLELNNTVKSIELQRRPALLARP NPIPKSCSRFPSYSVNFGIHCFAHAITDQSC ELSDKTYYRLAIGISDKNLSDPSDVKYIGE AFTPMGLQARGCSVISSIYGCYLLCSKSNQ GYEADFQTQGFHQMYILFLSRDLKTTLFN DMISSTTVVWNGLYPGEGAGIWHMGYLIF PLWGGIKIGTPASTSILNSTLDLPLVGPSCK STLEENNLINKDVLFSPYFGESVMVFGFLS CYMLSNVPTHCQVEVLNSSVLGFGSRSQL MDLKGIVYLYIQSAGWYSYTQLFRLSLQS RGYKLTVKQIRRIPISSTTRPGTAPCDVVH	2	730

			NCPYTCATGLFQAPWIVNGDSILDRDVRN LVFVQAWSGNFNTFQKGLISICNQYTCPLT TLLDNDNSIMRSTTTYCYPSLSEYNLQCQS FIEWGGPVGNPIGILEVHYIIKFK		
NC_005283	7091-8905	gb:NC_005283:7091-8905|Organism:Dolphin morbillivirus|Strain Name:UNKNOWN-NC_005283|Protein Name:haemagglutinin protein|Gene Symbol:H	MSSPRDKVDAFYKDIPRPRNNRVLLDNER VIIERPLILVGVLAVMFLSLVGLLAIAGVRL QKATTNSIEVNRKLSTNLETTVSIEHHVKD VLTPLFKIIGDEVGLRMPQKLTEIMQFISNK IKFLNPDREYDFNDLHWCVNPPDQVKIDY AQYCNHIAAEELIVTKFKELMNHSLDMSK GRIFPPKNCSGSVITRGQTIKPGLTLVNIYT TRNFEVSFMVTVISGGMYGKTYFLKPPEP DDPFEFQAFRIFEVGLVRDVGSREPVLQMT NFMVIDEDEGLNFCLLSVGELRLAAVCVR GRPVVTKDIGGYKDEPFKVVTLGIIGGGLS NQKTEIYPTIDSSIEKLYITSHRGIIRNSKAR WSVPAIRSDDKDKMEKCTQALCKSRPPPS CNSSDWEPLTSNRIPAYAYIALEIKEDSGLE LDITSNYGPLIIHGAGMDIYEGPSSNQDWL AIPPLSQSVLGVINKVDFTAGFDIKPHTLTT AVDYESGKCYVPVELSGAKDQDLKLESNL VVLPTKDFGYVTATYDTSRSEHAIVYYVY DTARSSSYFFPFRIKARGEPIYLRIECFPWS RQLWCHHYCMINSTVSNEIVVVDNLVSIN MSCSR	2	731
NC_007803	7978-12504	gb:NC_007803:7978-12504|Organism:Beilong virus|Strain Name:Li|Protein Name:attachment	MSQLAAHNLAMSNFYGTHQGDLSGSQKG EEQQVQGVIRYVSMIVSLLSLFTIIALNVTN IIYMTESGGTMQSIKTAQGSIDGSMREISG VIMEDVKPKTDLINSMVSYNIPAQLSMIHQ IIKNDVPKQCTPSFMFNNTICPLAENPTHSR YFEEVNLDSISECSGPDMHLGLGVNPEFIE FPSFAPGSTKPGSCVRLPSFSLSTTVFAYTH TIMGHGCSELDVGDHYFSVGRIADAGHEIP QFETISSWFINDKINRRSCTVAAGAMEAW	2	732

		glycoprotein|Gene Symbol:G	MGCVIMTETFYDDRNSLDTGKLTISYLDV FGRKKEWIYTRSEILYDYTYTSVYFSVGSG VVVGDTVYFLIWGSLSSPIEETAYCFAPDC SNYNQRMCNEAQRPSKFGHRQMVNGILK FKTTSTGKPLLSVGTLSPSVVPFGSEGRLM YSEITKIIYLYLRSTSWHALPLTGLFVLGPP TSISWIVQRAVSRPGEFPCGASNRCPKDCV TGVYTDLFPLGSRYEYAATVYLNSETYRV NPTLALINQTNIIASKKVTTESQRAGYTTTT CFVFKLRVWCISVVELAPSTMTAYEPIPFL YQLDLTCKGKNGSLAMRFAGKEGTYKSG RYKSPRNECFFEKVSNKYYFIVSTPEGIQP YEIRDLTPDRMPHIIMYISDVCAPALSAFK KLLPAMRPITTLTIGNWQFRPVEVSGGLRV NIGRNLTKEGDLTMSAPEDPGSNTFPGNHI PGNGILDAGYYTVEYPKE		
NC_009489	6559-8512	gb:NC_009489:6559-8512|Organism:Mapuera virus|Strain Name:BeAnn 370284|Protein Name:attachment protein|Gene Symbol:HN	MASLQSEPGSQKPHYQSDDQLVKRTWRSF FRFSVLVVTITSLALSIITLIGVNRISTAKQIS NAFAAIQANILSSIPDIRPINSLLNQLVYTSS VTLPLRISSLESNVLAAIQEACTYRDSQSSC SATMSVMNDQRYIEGIQVYSGSFLDLQKH TLSPPIAFPSFIPTSTTTVGCTRIPSFSLTKTH WCYTHNYIKTGCRDATQSNQYIALGTIYT DPDGTPGFSTSRSQYLNDGVNRKSCSISAV PMGCALYCFISVKEEVDYYKGTVPPAQTLI LFFFNGTVHEHRIVPSSMNSEWVMLSPGV GSGVFYNNYIIFPLYGGMTKDKAEKRGEL TRFFTPKNSRSLCKMNDSVFSNAAQSAYY PPYFSSRWIRSGLLACNWNQIITTNCEILTF SNQVMMMGAEGRLILINDDLFYYQRSTS WWPRPLVYKLDIELNYPDSHIQRVDQVEV TFPTRPGWGGCVGNNFCPMICVSGVYQD VWPVTNPVNTTDSRTLWVGGTLLSNTTRE NPASVVTSGGSISQTVSWFNQTVPGAYSTT	2	733

			TCFNDQVQGRIFCLIIFEVGGGLLGEYQIVP FLKELKYQGAVHA		
NC_ 017 937	6334- 8544	gb:NC_017937 :6334- 8544|Organism :Nariva virus|Strain Name:UNKNO WN- NC_017937|Pr otein Name:attachme nt protein|Gene Symbol:H	MAPINYPASYYTNNAERPVVITTKSTESKG QRPLPLGNARFWEYFGHVCGTLTFCMSLI GIIVGIIALANYSSDKDWKGRIGGDIQVTR MATEKTVKLILEDTTPKLRNILDSVLFQLP KMLASIASKINTQTPPPPTTSGHSTALATQ CSSNCENRPEIGYDYLRQVEQSLQRITNISI QLLEASEIHSMAGAYPNALYKIRTQDSWS VTAKECPLQAFQPNLNLIPAMIGTATGALI RNCVRQPVIVVDDGVYMLTYLAMRGSCQ DHQKSVRHFEMGVITSDPFGDPVPTPLRH WTKRALPAYDGCALAVKGHAGFALCTET SVGPLRDRTAKRKPNIVLFKASLVGELSER VIPPQSWLSGFSFFSVYTVAGKGYAYHSKF HAFGNVVRVGQSEYQAKCRGTGCPTANQ DDCNTAQRVSQEDNTYLHQAILSVDIDSVI DPEDVVYVIERDQYYQASAGDLYRVPETG EILYNLHNGGWSNEVQVGRIQPSDRFYMR EIQLTSTRVPAPNGCNRVKGCPGGCVAVIS PAFTPMHPEFNVGVGIFPMNQPHNPSIMH VQQQTELFWKPIVGGNITLHESSIACYSTV PPNPSYDLCIGVMTLLLHQGQLPQFQALS WYQPTMCNGNAPQNRRALIPVIVEDSKA MSVSSDAPRTP	2	734
NC_ 025 256	9117- 1101 5	gb:NC_025256 :9117- 11015|Organis m:Bat Paramyxovirus Eid_hel/GH- M74a/GHA/20 09|Strain Name:BatPV/E	MPQKTVEFINMNSPLERGVSTLSDKKTLN QSKITKQGYFGLGSHSERNWKKQKNQND HYMTVSTMILEILVVLGIMFNLIVLTMVYY QNDNINQRMAELTSNITVLNLNLNQLTNKI QREIIPRITLIDTATTITIPSAITYILATLTTRI SELLPSINQKCEFKTPTLVLNDCRINCTPPL NPSDGVKMSSLATNLVAHGPSPCRNFSSV PTIYYYRIPGLYNRTALDERCILNPRLTISST KFAYVHSEYDKNCTRGFKYYELMTFGEIL	2	735

		id_hel/GH-M74a/GHA/2009|Protein Name:glycoprotein|Gene Symbol:G	EGPEKEPRMFSRSFYSPTNAVNYHSCTPIV TVNEGYFLCLECTSSDPLYKANLSNSTFHL VILRHNKDEKIVSMPSFNLSTDQEYVQIIPA EGGGTAESGNLYFPCIGRLLHKRVTHPLC KKSNCSRTDDESCLKSYYNQGSPQHQVVN CLIRIRNAQRDNPTWDVITVDLTNTYPGSR SRIFGSFSKPMLYQSSVSWHTLLQVAEITD LDKYQLDWLDTPYISRPGGSECPFGNYCP TVCWEGTYNDVYSLTPNNDLFVTVYLKSE QVAENPYFAIFSRDQILKEFPLDAWISSART TTISCFMFNNEIWCIAALEITRLNDDIIRPIY YSFWLPTDCRTPYPHTGKMTRVPLRSTYN Y		
NC_025347	6398-8418	gb:NC_025347:6398-8418|Organism:Avian paramyxovirus 7|Strain Name:APMV-7/dove/Tennessee/4/75|Protein Name:hemagglutinin-neuraminidase|Gene Symbol:HN	MESIGKGTWRTVYRVLTILLDVVIIILSVIA LISLGLKPGERIINEVNGSIHNQLVPLSGITS DIQAKVSSIYRSNLLSIPLQLDQINQAISSSA RQIADTINSFLALNGSGTFIYTNSPEFANGF NRAMFPTLNQSLNMLTPGNLIEFTNFIPTPT TKSGCIRIPSFSMSSSHWCYTHNIIASGCQD HSTSSEYISMGVVEVTDQAYPNFRTTLSIT LADNLNRKSCSIAATGFGCDILCSVVTETE NDDYQSPEPTQMIYGRLFFNGTYSEMSLN VNQMFADWVANYPAVGSGVELADFVIFP LYGGVKITSTLGASLSQYYYIPKVPTVNCS ETDAQQIEKAKASYSPPKVAPNIWAQAVV RCNKSVNLANSCEILTFNTSTMMMGAEGR LLMIGKNVYFYQRSSSYWPVGIIYKLDLQE LTTFSSNQLLSTIPIPFEKFPRPASTAGVCSK PNVCPAVCQTGVYQDLWVLYDLGKLENT TAVGLYLNSAVGRMNPFIGIANTLSWYNT TRLFAQGTPASYSTTTCFKNTKIDTAYCLSI LELSDSLLGSWRITPLLYNITLSIMS	2	736
NC_025	6590-8548	gb:NC_025348:6590-	MPPVPTVSQSIDEGSFTDIPLSPDDIKHPLS KKTCRKLFRIVTLIGVGLISILTIISLAQQTGI	2	737

348		8548|Organism :Tuhoko virus 2|Strain Name:UNKNOWN-NC_025348|Protein Name:hemagglutinin-neuraminidase| Gene Symbol:HN	LRKVDSSDFQSYVQESFKQVLNLMKQFSS NLNSLIEITSVTLPFRIDQFGTDIKTQVAQL VRQCNAVCRGPIKGPTTQNIVYPALYETSL NKTLETKNVRIQEVRQEVDPVPGPGLSNG CTRNPSFSVYHGVWCYTHATSIGNCNGSL GTSQLFRIGNVLEGDGGAPYHKSLATHLL TTRNVSRQCSATASYYGCYFICSEPVLTER DDYETPGIEPITIFRLDPDGNWVVFPNINRF TEYSLKALYPGIGSGVLFQGKLIFPMYGGI DKERLSALGLGNIGLIERRMADTCNHTEK ELGRSFPGAFSSPYYHDAVMLNFLLICEMI ENLPGDCDLQILNPTNMSMGSESQLSVLD NELFLYQRSASWWPYTLIYRLNMRYTGK YLKPKSIIPMVIKSNTRPGYEGCNHERVCP KVCVTGVFQAPWILSIGRDHKERVSNVTY MVAWSMDKSDRTYPAVSVCGSDTCKLTV PLGDSKVHSAYSVTRCYLSRDHMSAYCLV IFELDARPWAEMRIQSFLYKLILT		
NC_025350	6451-8341	gb:NC_025350:6451-8341|Organism :Tuhoko virus 3|Strain Name:UNKNOWN-NC_025350|Protein Name:hemagglutinin-neuraminidase| Gene Symbol:HN	MHNRTQSVSSIDTSSDVYLPRRKKAVTKF TFKKIFRVLILTLLLSIIIIIAVIFPKIDHIRETC DNSQILETITNQNSEIKNLINSAITNLNVLLT STTVDLPIKLNNFGKSIVDQVTMMVRQCN AVCRGPGDRPTQNIELFKGLYHTSPPSNTS TKLSMITEASNPDDIVPRPGKLLGCTRFPSF SVHYGLWCYGHMASTGNCSGSSPSVQIIRI GSIGTNKDGTPKYVIIASASLPETTRLYHCS VTMTSIGCYILCTTPSVSETDDYSTMGIEK MSISFLSLDGYLTQLGQPTGLDNQNLYAL YPGPGSGVIFRDFLIFPMMGGIRLMDAQK MLNRNITYRGFPPSETCTESELKLKQEVAN MLTSPYYGEVLVLNFLYVCSLLDNIPGDCS VQLIPPDNMTLGAESRLYVLNGSLIMYKR GSSWWPYTELYQINYRVNNRAFRVRESVR INTTSTSRPGVQGCNLEKVCPKVCVSGIYQ	2	738

			SPGIISAPVNPTRQEEGLLYFLVWTSSMSSR TGPLSSLCDHSTCRITYPIGDDTIFIGYTDSS CFMSSIKEGIYCIAFLELDNQPYSMMAIRSL SYIIN		
NC_025352	8716-11257	gb:NC_025352:8716-11257|Organism:Mojiang virus|Strain Name:Tonggua n1|Protein Name:attachme nt glycoprotein|G ene Symbol:G	MATNRDNTITSAEVSQEDKVKKYYGVET AEKVADSISGNKVFILMNTLLILTGAIITITL NITNLTAAKSQQNMLKIIQDDVNAKLEMF VNLDQLVKGEIKPKVSLINTAVSVSIPGQIS NLQTKFLQKYVYLEESITKQCTCNPLSGIF PTSGPTYPPTDKPDDDTTDDDKVDTTIKPI EYPKPDGCNRTGDHFTMEPGANFYTVPNL GPASSNSDECYTNPSFSIGSSIYMFSQEIRK TDCTAGEILSIQIVLGRIVDKGQQGPQASPL LVWAVPNPKIINSCAVAAGDEMGWVLCS VTLTAASGEPIPHMFDGFWLYKLEPDTEV VSYRITGYAYLLDKQYDSVFIGKGGGIQK GNDLYFQMYGLSRNRQSFKALCEHGSCL GTGGGGYQVLCDRAVMSFGSEESLITNAY LKVNDLASGKPVIIGQTFPPSDSYKGSNGR MYTIGDKYGLYLAPSSWNRYLRFGITPDIS VRSTTWLKSQDPIMKILSTCTNTDRDMCP EICNTRGYQDIFPLSEDSEYYTYIGITPNNG GTKNFVAVRDSDGHIASIDILQNYYSITSA TISCFMYKDEIWCIAITEGKKQKDNPQRIY AHSYKIRQMCYNMKSATVTVGNAKNITIR RY	2	739
NC_025363	6503-8347	gb:NC_025363:6503-8347|Organism:Avian paramyxovirus 12|Strain Name:Wigeon/ Italy/3920_1/20	MESATSQVSFENDKTSDRRTWRAVFRVL MIILALSSLCVTVAALIYSAKAAIPGNIDAS EQRILSSVEAVQVPVSRLEDTSQKIYRQVIL EAPVTQLNMETNILNAITSLSYQIDASANS SGCGAPVHDSDFTGGVGRELLQEAEVNLT IIRPSKFLEHLNFIPAPTTGNGCTRIPSFDLG QTHWCYTHNVVLNGCRDRGHSFQYVALG ILRTSATGSVFLSTLRSVNLDDDRNRKSCS	2	740

		05|Protein Name:hemagglutinin-neuraminidase|Gene Symbol:HN	VSATPIGCEMLCSLVTETEEGDYDSIDPTP MVHGRLGFDGKYREVDLSEKEIFADWRA NYPAVGGGAFFGNRVWFPVYGGLKEGTQ SERDAEKGYAIYKRFNNTCPDDNTTQIAN AKASYRPSRFGGRFIQQGILSFKVEGNLGS DPILSLTDNSITLMGAEARVMNIENKLYLY QRGTSWFPSALVYPLDVANTAVKVRAPYI FDKFTRPGGHPCSASSRCPNVCVTGVYTD AYPLVFSRSHDIVAVYGMQLAAGTARLDP QAAIWYGNEMSTPTKVSSSTTKAAYTTST CFKVTKTKRIYCISIAEIGNTLFGEFRIVPLL IEVQKTPLTRRSELRQQMPQPPIDLVIDNPF CAPSGNLSRKNAIDEYANSWP		
NC_025373	6619-8605	gb:NC_025373:6619-8605|Organism:Avian paramyxovirus 3|Strain Name:turkey/Wisconsin/68|Protein Name:hemagglutinin|Gene Symbol:HN	MEPTGSKVDIVPSQGTKRTCRTFYRLLILIL NLIIIILTIISIYVSISTDQHKLCNNEADSLLH SIVEPITVPLGTDSDVEDELREIRRDTGINIP IQIDNTENIILTTLASINSNIARLHNATDESP TCLSPVNDPRFIAGINKITKGSMIYRNFSNL IEHVNFIPSPTTLSGCTRIPSFSLSKTHWCYS HNVISTGCQDHAASSQYISIGIVDTGLNNE PYLRTMSSRLLNDGLNRKSCSVTAGAGVC WLLCSVVTESESADYRSRAPTAMILGRFN FYGDYTESPVPASLFSGRFTANYPGVGSGT QLNGTLYFPIYGGVVNDSDIELSNRGKSFR PRNPTNPCPDPEVTQSQRAQASYYPTRFGR LLIQQAILACRISDTTCTDYYLLYFDNNQV MMGAEARIYYLNNQMYLYQRSSSWWPH PLFYRFSLPHCEPMSVCMITDTHLILTYATS RPGTSICTGASRCPNNCVDGVYTDVWPLT EGTTQDPDSYYTVFLNSPNRRISPTISIYSY NQKISSRLAVGSEIGAAYTTSTCFSRTDTG ALYCITIIEAVNTIFGQYRIVPILVQLISD	2	741
NC_025	7541-9403	gb:NC_025386:7541-	MKAMHYYKNDFADPGTNDNSSDLTTNPFI SNQIKSNLSPPVLAEGHLSPSPIPKFRKILLT	2	742

386		9403|Organism :Salem virus|Strain Name:UNKNOWN-NC_025386|Protein Name:attachment glycoprotein|Gene Symbol:G	ISFVSTIVVLTVILLVLTIRILTIIEASAGDEK DIHTILSSLLNTFMNEYIPVFKNLVSIISLQIP QMLIDLKTSSTQMMQSLKTFPRDLETLST VTQSVAVLLEKAKSTIPDINKFYKNVGKV TFNDPNIKVLTLEVPAWLPIVRQCLKQDFR QVISNSTGFALIGALPSQLFNEFEGYPSLAI VSEVYAITYLKGVMFENQENFLYQYFEIG TISPDGYNKPYFLRHTSVMLSTFKLSGKCT AAVDYRGGIFLCTPSPKIPKILQNPPDLPTL TVVSIPFDGRYTIRNISLMLTDEADIIYDLD TLQGRGVLQAMRFYALVRVISSSSPRHFPF CKNSWCPTADDKICDQSRRLGADGNYPV MYGLISIPAHSSYQGNVSLKLIDPKYYAYT RDASLFYNSMTDTYHYSFGTRGWVSRPII GELLLGDDIVLTRYTVRSVSRATAGDCTT VSMCPQACSGGMNSIFYPLNFDKPQVTGV AIRQYERQQEGIIVVTMNDHYYYSVPIIKN GTLLISSVTDCFWLMGDLWCMSLMEKNN LPLGVRSLAHLTWNIHWSCS		
NC_025390	6647-8386	gb:NC_025390:6647-8386|Organism :Avian paramyxovirus 9|Strain Name:duck/New York/22/1978|Protein Name:hemagglutinin-neuraminidase|Gene Symbol:HN	MESGISQASLVNDNIELRNTWRTAFRVVS LLLGFTSLVLTACALHFALNAATPADLSSI PVAVDQSHHEILQTLSLMSDIGNKIYKQVA LDSPVALLNTESTLMSAITSLSYQINNAAN NSGCGAPVHDKDFINGVAKELFVGSQYNA SNYRPSRFLEHLNFIPAPTTGKGCTRIPSFD LAATHWCYTHNVILNGCNDHAQSYQYISL GILKVSATGNVFLSTLRSINLDDDENRKSC SISATPLGCDLLCAKVTEREEADYNSDAAT RLVHGRLGFDGVYHEQALPVESLFSDWV ANYPSVGGGSYFDNRVWFGVYGGIRPGS QTDLLQSEKYAIYRRYNNTCPDNNPTQIER AKSSYRPQRFGQRLVQQAILSIRVEPSLGN DPKLSVLDNTVVLMGAEARIMTFGHVAL MYQRGSSYFPSALLYPLSLTNGSAAASKPF	2	743

			IFEQYTRPGSPPCQATARCPNSCVTGVYTD AYPLFWSEDHKVNGVYGMMLDDITSRLN PVAAIFDRYGRSRVTRVSSSSTKAAYTTNT CFKVVKTKRVYCLSIAEIENTLFGEFRITPL LSEIIFDPNLEPSDTSRN		
NC_025403	6692-8645	gb:NC_025403:6692-8645|Organism:Achimota virus 1|Strain Name:UNKNOWN-NC_025403|Protein Name:attachment protein|Gene Symbol:HN	MATNLSTITNGKFSQNSDEGSLTELPFFEH NRKVATTKRTCRFVFRSVITLCNLTILIVTV VVLFQQAGFIKRTESNQVCETLQNDMHGV VTMSKGVITTLNNLIEITSVNLPFQMKQFG QGIVTQVTQMVRQCNAVCKGPTIGPDIQNI VYPASYESMIKHPVNNSNILLSEIRQPLNFV PNTGKLNGCTRTPSFSVYNGFWCYTHAES DWNCNGSSPYMQVFRVGVVTSDYDYNVI HKTLHTKTSRLANVTYQCSTISTGYECYFL CSTPNVDEITDYKTPGIESLQIYKIDNRGTF AKFPITDQLNKELLTALYPGPGNGVLYQG RLLFPMHGGMQSSELNKVNLNNTVLSQFN DNKGCNATEIKLESEFPGTFTSPYYSNQVM LNYILICEMIENLPGNCDLQIVAPKNMSMG SESQLYSINNKLYLYQRSSSRWPYPLIYEV GTRLTNRQFRLRAINRFLIKSTTRPGSEGC NIYRVCPKVCVTGVYQAPWILHVSKAGSQ SIAKVLYAVAWSKDHMSRKGPLFSICDND TCFLTKSLASEHVHSGYSITRCYLENSERHI ICVVIMELDASPWAEMRIQSVIYNITLPS	2	744
NC_025404	6655-8586	gb:NC_025404:6655-8586|Organism:Achimota virus 2|Strain Name:UNKNOWN-NC_025404|Protein	MDNSMSISTISLDAQPRIWSRHESRRTWRN IFRITSLVLLGVTVIICIWLCCEVARESELEL LASPLGALIMAINTIKSSVVKMTTELNQVT FTTSIILPNKVDQFGQNVVSQVAQLVKQC NAVCRGHQDTPELEQFINQKNPTWILQPN YTTKLTNLHEIDSIIPLVDYPGFSKSCTRFPS FSEGSKFWCFTYAVVKEPCSDISSSIQVVK YGAIKANHSDGNPYLVLGTKVLDDGKFR RGCSITSSLYGCYLLCSTANVSEVNDYAHT	2	745

		Name:attachment protein|Gene Symbol:HN	PAYPLTLELISKDGITTDLSPTYTVQLDKW SALYPGIGSGVIFKGYLMFPVYGGLPFKSP LISASWVGPGNKWPVDFSCSEDQYSTFNF SNPYSALYSPHFSNNIVVSALFVCPLNENL PYSCEVQVLPQGNLTIGAEGRLYVIDQDL YYYQRSTSWWPYLQLYKLNIRITNRVFRV RSLSLLPIKSTTRPGYGNCTYFKLCPHICVT GVYQSPWLISIRDKRPHEEKNILYFIGWSP DEQIRQNPLVSLCHETACFINRSLATNKTH AGYSESHCVQSFERNKLTCTVFYELTAKP WAEMRVQSLLFQVDFL		
NC_025410	6799-8869	gb:NC_025410:6799-8869|Organism:Tuhoko virus 1|Strain Name:UNKNOWN-NC_025410|Protein Name:hemagglutinin-neuraminidase|Gene Symbol:HN	MDSRSDSFTDIPLDNRIERTVTSKKTWRSIF RVTAIILLIICVVVSSISLNQHNDAPLNGAG NQATSGFMDAIKSLEKLMSQTINELNQVV MTTSVQLPNRITKFGQDILDQVTQMVRQC NAVCRGPGVGPSIQNYVIQGHAPTVSFDPI SAEYQKFVFGITEKTLITAYHNPWECLRFP SQHLFDTTWCVSYQILTQNCSDHGPRITVI QLGEIMIANNLSTVFRDPVIKYIRHHIWLRS CSVVAYYSQCTIFCTSTNKSEPSDYADTGY EQLFLATLQSDGTFTEHSMHGVNIVHQWN AIYGGVGNGVIIGRNMLIPLYGGINYYDHN TTIVQTVDLRPYPIPDSCSQTDNYQTNYLP SMFTNSYYGTNLVVSGYLSCRLMAGTPTS CSIRVIPIENMTMGSEGQFYLINNQLYYYK RSSNWIRDTQVYLLSYSDKGNIIEITSAERY IFKSVTSPDEGDCVTNHGCPSNCIGGLFQA PWILNDFKLCGSNITCPKIVTVWADQPDK RSNPMLSIAETDKLLLHKSYINYHTAVGYS TVLCFDSPKLNLKTCVVLQELMSDDKLLI RISYSIVSIMVE	2	746
NC_028249	7059-9010	gb:NC_028249:7059-9010|Organism	MFSHQDKVGAFYKNNARANSSKLSLVTD EVEERRSPWFLSILLILLVGILILLAITGIRFH QVVKSNLEFNKLLIEDMEKTKAVHHQVK	2	747

		:Phocine distemper virus|Strain Name:PDV/Wadden_Sea.NLD/1988|Protein Name:hemagglutinin protein|Gene Symbol:H	DVLTPLFKIIGDEVGLRLPQKLNEIKQFIVQ KTNFFNPNREFDFRELHWCINPPSKVKVNF TQYCEITEFKEATRSVANSILLLTLYRGRD DIFPPYKCRGATTSMGNVFPLAVSLSMSLI SKPSEVINMLTAISEGIYGKTYLLVTDDTE ENFETPEIRVFEIGFINRWLGDMPLFQTTN YRIISNNSNTKICTIAVGELALASLCTKESTI LLNLGDEESQNSVLVVILGLFGATHMDQL EEVIPVAHPSIEKIHITNHRGFIKDSVATWM VPALALSEQGEQINCLRSACKRRTYPMCN QTSWEPFGDKRLPSYGRLTLSLDVSTDLSI NVSVAQGPIIFNGDGMDYYEGTLLNSGWL TIPPKNGTILGLINQASKGDQFIVTPHILTFA PRESSTDCHLPIQTYQIQDDDVLLESNLVV LPTQSFEYVVATYDVSRSDHAIVYYVYDP ARTVSYTYPFRLRTKGRPDILRIECFVWDG HLWCHQFYRFQLDATNSTSVVENLIRIRFS CDRLDP		
NC_028362	6951-8675	gb:NC_028362:6951-8675|Organism:Caprine parainfluenza virus 3|Strain Name:JS2013|Protein Name:hemagglutinin-neuraminidase|Gene Symbol:HN	MEYWGHTNNPDKINRKVGVDQVRDRSKT LKIITFIISMMTSIMSTVALILILIMFIQNNNN NRIILQELRDETDAIEARIQKASNDIGVSIQS GINTRLLTIQNHVQNYIPLALTQQVSSLRES INDVITKREETQSKMPIQRMTHDDGIEPLIP DNFWKCPSGIPTISASPKIRLIPGPGLLATST TINGCIRLPSLVINNLIYAYTSNLITQGCQDI GKSYQVLQIGIITINSDLVPDLNPRITHTFDI DDNRKSCSLALRNADVYQLCSTPKVDERS DYSSIGIEDIVLDIVTSEGTVSTTRFTNNNIT FDKPYAALYPSVGPGIYYDNKIIFLGYGGL EHEENGDVICNITGCPGKTQHDCNQASYS PWFSNRRMVNAIILVNKGLNKVPSLQVWT IPMRQNYWGSEGRLLLLGNKIYIYTRSTS WHSKLQLGTLDISNYNDIRIRWTHHDVLS RPGSEECPWGNTCPRGCITGVYNDAYPLN	2	748

			PSGSVVSSVILDSRTSRENPIITYSTDTSRVN ELAIRNNTLSAAYTTTNCVTHYGKGYCFH IIEINHKSLNTLQPMLFKTEIPKSCN		
AB5 484 28	5999-7261	gb:AB548428:5999-7261|Organism:Avian metapneumovirus|Strain Name:VCO3/60616|Protein Name:attachment glycoprotein|Gene Symbol:G	MGSELYIIEGVSSSEIVLKQVLRRSKKILLG LVLSALGLTLTSTIVISICISVEQVKLRQCV DTYWAENGSLHPGQSTENTSTRGKTTTKD PRRLQATGAGKFESCGYVQVVDGDMHDR SYAVLGGVDCLGLLALCESGPICQGDTWS EDGNFCRCTFSSHGVSCCKKPKSKATTAQ RNSKPANSKSTPPVHSDRASKEHNPSQGE QPRRGPTSSKTTIASTPSTEDTAKPTISKPK LTIRPSQRGPSGSTKAASSTPSHKTNTRGTS KTTDQRPRTGPTPERPRQTHSTATPPPTTPI HKGRAPTPKPTTDLKVNPREGSTSPTAIQK NPTTQSNLVDCTLSDPDEPQRICYQVGTY NPSQSGTCNIEVPKCSTYGHACMATLYDT PFNCWRRTRRCICDSGGELIEWCCTSQ	1	749
AF0 797 80	8118-10115	gb:AF079780|Organism:Tupaia paramyxovirus|Strain Name:UNKNOWN-AF079780|Protein Name:hemagglutinin|Gene Symbol:H	MDYHSHTTQTGSNETLYQDPLQSQSGSRD TLDGPPSTLQHYSNPPPYSEEDQGIDGPQR SQPLSTPHQYDRYYGVNIQHTRVYNHLGT IYKGLKLAFQILGWVSVIITMIITVTTLKKM SDGNSQDSAMLKSLDENFDAIQEVANLLD NEVRPKLGVTMTQTTFQLPKELSEIKRYLL RLERNCPVCGTEATPQGSKGNASGDTAFC PPCLTRQCSEDSTHDQGPGVEGTSRNHKG KINFPHILQSDDCGRSDNLIVYSINLVPGLS FIQLPSGTKHCIIDVSYTFSDTLAGYLIVGG VDGCQLHNKAIIYLSLGYYKTKMIYPPDYI AIATYTYDLVPNLRDCSIAVNQTSLAAICT SKKTKENQDFSTSGVHPFYIFTLNTDGIFT VTVIEQSQLKLDYQYAALYPATGPGIFIGD HLVFLMWGGLMTKAEGDAYCQASGCND AHRTSCNIAQMPSAYGHRQLVNGLLMLPI KELGSHLIQPSLETISPKINWAGGHGRLYY	1	750

			NWEINTTYIYIEGKTWRSRPNLGIISWSKPL SIRWIDHSVARRPGARPCDSANDCPEDCL VGGYYDMFPMSSDYKTAITIIPTHHQWPSS PALKLFNTNREVRVVMILRPPNNVKKTTIS CIRIMQTNWCLGFIIFKEGNNAWGQIYSYI YQVESTCPNTK		
AY590688	6138-7935	gb:AY590688:6138-7935|Organism:Avian metapneumovirus|Strain Name:Colorado|Protein Name:attachment glycoprotein|Gene Symbol:G	MEVKVENVGKSQELKVKVKNFIKRSDCK KKLFALILGLVSFELTMNIMLSVMYVESNE ALSLCRIQGTPAPRDNKTNTENATKETTLH TTTTTRDPEVRETKTTKPQANEGATNPSR NLTTKGDKHQTTRATTEAELEKQSKQTTE PGTSTQKHTPTRPSSKSPTTTQAIAQLTTPT TPKASTAPKNRQATTKKTETDTTTASRAR NTNNPTETATTTPKATTETGKSKEGPTQHT TKEQPETTAGETTTPQPRRTASRPAPTTKIE EEAETTKTRTTKSTQTSTGPPRPTGGAPSG AATEGSGRAAAAGGPSAASAGGRRRTEA AAERDRRTRAGAGPTAGGARARTAAASE RGADTAGSAGGGPGGDGATGGLSGGAPA EREDASGGTAAAGPGDGTEADGRAPPAA ALAGRTTESAAGAAGDSGRAGTAGWGSA ADGRSTGGNAAAEAGAAQSGRAAPRQPS GGTAPESTAPPNSGGSGRADAAPTEEVGV GSGLWRGRYVCGPCGESVPEHPMNPCFG DGTAWICSDDGGSLPAGCYDGGTDGVVC CGVCGGNSCCCGRVECTCGGGAGLLSCC CGSYSWS	1	751
EU403085	6620-8593	gb:EU403085:6620-8593|Organism:Avian paramyxovirus 3|Strain Name:APMV3/	MESPPSGKDAPAFREPKRTCRLCYRATTLS LNLTIVVLSIISIYVSTQTGANNSCVNPTIVT PDYLTGSTTGSVEDLADLESQLREIRRDTG INLPVQIDNTENLILTTLASINSNLRFLQNA TTESQTCLSPVNDPRFVAGINRIPAGSMAY NDFSNLIEHVNFIPSPTTLSGCTRIPSFSLSK THWCYTHNVISNGCLDHAASSQYISIGIVD	1	752

		PKT/Netherland/449/75|Protein Name:hemagglutinin-neuraminidase protein|Gene Symbol:HN	TGLNNEPYFRTMSSKSLNDGLNRKSCSVT AAANACWLLCSVVTEYEAADYRSRTPTA MVLGRFDFNGEYTEIAVPSSLFDGRFASNY PGVGSGTQVNGTLYFPLYGGVLNGSDIET ANKGKSFRPQNPKNRCPDSEAIQSFRAQDS YYPTRFGKVLIQQAIIACRISNKSCTDFYLL YFDNNRVMMGAEARLYYLNNQLYLYQR SSSWWPHPLFYSISLPSCQALAVCQITEAH LTLTYATSRPGMSICTGASRCPNNCVDGV YTDVWPLTKNDAQDPNLFYTVYLNNSTR RISPTISLYTYDRRIKSKLAVGSDIGAAYTT STCFGRSDTGAVYCLTIMETVNTIFGQYRI VPILLRVTSR		
FJ977568	6139-7936	gb:FJ977568:6139-7936|Organism:Avian metapneumovirus|Strain Name:aMPV/MN/turkey/2a/97|Protein Name:attachment glycoprotein|Gene Symbol:G	MEVKVENVGKSQELKVKVKNFIKRSDCK KKLFALILGLVSFELTMNIMLSVMYVESNE ALSLCRIQGTPAPRDNKTNTENATKETTLH TTTTTRDPEVRETKTTKPQANEGATNPSR NLTTKGDKHQTTRATTEAELEKQSKQTTE PGTSTQKHTPARPSSKSPTTTQATAQPTTP TAPKASTAPKNRQATTKKTETDTTTASRA RNTNNPTETATTTPKATTETGKGKEGPTQ HTTKEQPETTARETTTPQPRRTASRPAPTT KIEEEAETTKTRTTKNTQTSTGPPRPTRSTP SKTATENNKRTTTTKRPNTASTDSRQQTR TTAEQDQQTQTRAKPTTNGAHPQTTTTPE HNTDTTNSTKGSPKEDKTTRDPSSKTPTEQ EDASKGTAAANPGGSAEADRRAPPATTPT GRTTESAAGTTGDDSGAETTRRRSAADRR PTGGSTAAEAGTAQSGRATPKQPSGGTAA GNTAPPNNESSGRADAAPAEEAGVGPSIR RGRHACGPRRESAPEHPTNPCPGDGTAWT RSDGGGNLPAGRHDSGADGAARRGARGG NPRRRGRAERTRGGGAGPPSCRCGSHNRS	1	753
HG9	5997-	gb:HG934339:	MGAKLYAISGASDAQLMKKTCAKLLEKV	1	754

34339	7166	5997-7166|Organism:Avian metapneumovirus type D|Strain Name:Turkey/1985/Fr85.1|Protein Name:attachment glycoprotein|Gene Symbol:G	VPIIILAVLGITGTTTIALSISISIERAVLSDCTTQLRNGTTSGSLSNPTRSTTSTAVTTRDIRGLQTTRTRELKSCSNVQIAYGYLHDSSNPVLDSIGCLGLLALCESGPFCQRNYNPRDRPKCRCTLRGKDISCCKEPPTAVTTSKTTPWGTEVHPTYPTQVTPQSQPATMAHQTATANQRSSTTEPVGSQGNTTSSNPEQQTEPPPSPQHPPTTTSQDQSTETADGQEHTPTRKTPTATSNRRSPTPKRQETGRATPRNTATTQSGSSPPHSSPPGVDANMEGQCKELQAPKPNSVCKGLDIYREALPRGCDKVLPLCKTSTIMCVDAYYSKPPICFGYNQRCFCMETFGPIEFCCKS		
JN032116	4659-5252	gb:JN032116:4659-5252|Organism:Respiratory syncytial virus|Strain Name:B/WI/629-12/06-07|Protein Name:attachment glycoprotein|Gene Symbol:G	MSKNKNQRTARTLEKTWDTLNHLIVISSCLYKLNLKSIAQIALSVLAMIISTSLIIAAIIFIISANHKVTLTTVTVQTIKNHTEKNITTYLTQVSPERVSPSKQPTTTPPIHTNSATISPNTKSEIHHTTAQTKGRTSTPTQNNKPNTKPRPKNPPKKDDYHFEVFNFVPCSICGNNQLCKSICKTIPSNKPRKNQP	1	755
KX258200	6254-7996	gb:KX258200:6254-7996|Organism:Avian paramyxovirus 14|Strain Name:APMV14/duck/Japan/1	MEGSRTVIYQGDPNEKNTWRLVFRTLTLILNLAILSVTIASIIITSKITLSEVTTLKTEGVEEVITPLMATLSDSVQQEKMIYKEVAISIPLVLDKIQTDVGTSVAQITDALRQIQGVNGTQAFALSNAPEYSGGIEVPLFQIDSFVNKSMSISGLLEHASFIPSPTTLHGCTRIPSFHLGPRHWCYTHNIIGSRCRDEGFSSMYISIGAITVNRDGNPLFITTASTILADDNNRKSCSIIASSYG	1	756

		1OG0352/2011|Protein Name:hemagglutinin-neuraminidase protein|Gene Symbol:HN	CDLLCSIVTESENDDYANPNPTKMVHGRF LYNGSYVEQALPNSLFQDKWVAQYPGVG SGITTHGKVLFPIYGGIKKNTQLFYELSKY GFFAHNKELECKNMTEEQIRDIKAAYLPS KTSGNLFAQGIIYCNISKLGDCNVAVLNTS TTMMGAEGRLQMMGEYVYYYQRSSSWW PVGIVYKKSLAELMNGINMEVLSFEPIPLS KFPRPTWTAGLCQKPSICPDVCVTGVYTD LFSVTIGSTTDKDTYFGVYLDSATERKDP WVAAADQYEWRNRVRLFESTTEAAYTTS TCFKNTVNNRVFCVSIVELRENLLGDWKI VPLLFQIGVSQGPPPK		
KX940961	7978-12504	gb:KX940961:7978-12504|Organism:Beilong virus|Strain Name:ERN081008_1S|Protein Name:attachment glycoprotein|Gene Symbol:G	MSQLAAHNLAMSNFYGTHQGDLSGSQKG EEQQVQGVIRYVSMIVGLLSLFTIIALNVT NIIYMTESGGTMQSIKTAQGSIDGSMREIS GVIMEDVKPKTDLINSMVSYNIPAQLSMIH QIIKNDVLKQCTPSFMFNNTICPLAENPTHS RYFEEVNLDSISECSGPDMHLGLGVNPEFI EFPSFAPGSTKPGSCVRLPSFSLSTTVFAYT HTIMGHGCSELDVGDHYFSVGRIADAGHE IPQFETISSWFINDKINRRSCTVAAGAMEA WMGCVIMTETFYDDLNSLDTGKLTISYLD VFGRKKEWIYTRSEILYDYTYTSVYFSVGS GVVVGDTVYFLIWGSLSSPIEETAYCFAPD CSNYNQRMCNEAQRPSKFGHRQMVNGIL KFKTTSTGKPLLSVGTLSPSVVPFGSEGRL MYSEITKIIYLYLRSTSWHALPLTGLFVLGP PTSISWIVQRAVSRPGEFPCGASNRCPKDC VTGVYTDLFPLGSRYEYAATVYLNSETYR VNPTLALINQTNIIASKKVTTESQRAGYTT TTCFVFKLRVWCISVVELAPSTMTAYEPIP FLYQLDLTCKGKNGSLAMRFTGKEGTYKS GRYKSPRNECFFEKVSNKYYFIVSTPEGIQ PYEIRDLTPDRMPHIIMYISDVCAPALSAFK	1	757

			KLLPAMRPITTLTIGNWQFRPVEVSGGLRV SIGRNLTKEGDLTMSAPEDPGSNTFPGGHI PGNGLFDAGYYTVEYPKEWKQTTPKPSEG GNIIDKNKTPVIPSRDNPTSDSSIPHRESIEP VRPTREVLKSSDYVTIVSTDSGSGSGDFAT GVPWTGVSPKAPQNGINLPGTELPHPTVL DRINTPAPSDPKVSADSDHTRDTIDPTALS KPLNHDTTGDTDTRINTGTATYGFTPGRE ATSSGKLANDLTNSTSVPSEAHPSASTSEA SKPEKNTDNRVTQDPTSGTAERPTTNAPV DGKHSTQLTDARPNTADPERTSQHSSSTTR DEVKPSLPSTTEASTHQRTEAATPPELVNN TLNPPSTQVRSVRSLMQDAIAQAWNFVRG VTP		
KY5 110 44	6454- 8310	gb:KY511044: 6454- 8310|Organism :Avian paramyxovirus UPO216|Strain Name:APMV- 15/WB/Kr/UP O216/2014|Prot ein Name:hemaggl utinin- neuraminidase protein|Gene Symbol:HN	MERGISEVALANDRTEEKNTWRLIFRITVL VVSVITLGLTAASLVYSMNAAQPADFDGII PAVQQVGTSLTNSIGGMQDVLDRTYKQV ALESPLTLLNMESTIMNAITSLSYKINNGG NSSGCGAPIHDPEYIGGIGKELLIDDNVDV TSFYPSAFKEHLNFIPAPTTGAGCTRIPSFD LSATHYCYTHNVILSGCQDHSHSHQYIAL GVLKLSDTGNVFFSTLRSINLDDTANRKSC SISATPLGCDILCSKVTETELEDYKSEEPTP MVHGRLSFDGTYSEKDLDVNNLFSDWTA NYPSVGGGSYIGNRVWYAVYGGLKPGSN TDQSQRDKYVIYKRYNNTCPDPEDYQINK AKSSYTPSYFGSKRVQQAILSIAVSPTLGSD PVLTPLSNDVVLMGAEGRVMHIGGYTYL YQRGTSYYSPALLYPLNIQDKSATASSPYK FDAFTRPGSVPCQADARCPQSCVTGVYTD PYPLIFAKDHSIRGVYGMMLNDVTARLNPI AAVFSNISRSQITRVSSSSTKAAYTTSTCFK VIKTNRIYCMSIAEISNTLFGEFRIVPLLVEI LSNGGNTARSAGGTPVKESPKGWSDAIAE	1	758

			PLFCTPTNVTRYNADIRRYAYSWP		
NC_025360	8127-10158	gb:NC_025360:8127-10158|Organism:Atlantic salmon paramyxovirus|Strain Name:ASPV/Yrkje371/95|Protein Name:hemagglutinin-neuraminidase protein|Gene Symbol:HN	MPPAPSPVHDPSSFYGSSLFNEDTASRKGT SEEIHLLGIRWNTVLIVLGLILAIIGIGIGASS FSASGITGNTTKEIRLIVEEMSYGLVRISDS VRQEISPKVTLLQNAVLSSIPALVTTETNTII NAVKNHCNSPPTPPPPTEAPLKKHETGMA PLDPTTYWTCTSGTPRFYSSPNATFIPGPSP LPHTATPGGCVRIPSMHIGSEIYAYTSNLIA SGCQDIGKSYQNVQIGVLDRTPEGNPEMS PMLSHTFPINDNRKSCSIVTLKRAAYIYCS QPKVTEFVDYQTPGIEPMSLDHINANGTTK TWIYSPTEVVTDVPYASMYPSVGSGVVID GKLVFLVYGGLLNGIQVPAMCLSPECPGID QAACNASQYNQYLSGRQVVNGIATVDLM NGQKPHISVETISPSKNWFGAEGRLVYMG GRLYIYIRSTGWHSPIQIGVIYTMNPLAITW VTNTVLSRPGSAGCDWNNRCPKACLSGV YTDAYPISPDYNHLATMILHSTSTRSNPVM VYSSPTNMVNYAQLTTTAQIAGYTTTSCF TDNEVGYCATALELTPGTLSSVQPILVMT KIPKECV	1	759

(iii) Other Proteins

[00077] In some embodiments, the fusogen may include a pH dependent (e.g., as in cases of ischemic injury) protein, a homologue thereof, a fragment thereof, and a protein fusion comprising one or more proteins or fragments thereof. Fusogens may mediate membrane fusion at the cell surface or in an endosome or in another cell-membrane bound space.

[00078] In some embodiments, the fusogen includes a EFF-1, AFF-1, gap junction protein, e.g., a connexin (such as Cn43, GAP43, CX43) (DOI: 10.1021/jacs.6b05191), other tumor connection proteins, a homologue thereof, a fragment thereof, a variant thereof, and a protein fusion comprising one or more proteins or fragments thereof.

Modifications to Protein Fusogens

[00079] In some embodiments protein fusogens can be altered to reduce immunoreactivity. For instance, protein fusogens may be decorated with molecules that reduce immune interactions, such as PEG (DOI: 10.1128/JVI.78.2.912–921.2004). Thus, in some embodiments, the fusogen comprises PEG, e.g., is a PEGylated polypeptide. Amino acid residues in the fusogen that are targeted by the immune system may be altered to be unrecognized by the immune system (doi: 10.1016/j.virol.2014.01.027, doi:10.1371/journal.pone.0046667). In some embodiments the protein sequence of the fusogen is altered to resemble amino acid sequences found in humans (humanized). In some embodiments the protein sequence of the fusogen is changed to a protein sequence that binds MHC complexes less strongly. In some embodiments, the protein fusogens are derived from viruses or organisms that do not infect humans (and which humans have not been vaccinated against), increasing the likelihood that a patient's immune system is naïve to the protein fusogens (e.g., there is a negligible humoral or cell-mediated adaptive immune response towards the fusogen) (doi:10.1006/mthe.2002.0550, doi:10.1371/journal.ppat.1005641, doi:10.1038/gt.2011.209, DOI 10.1182/blood-2014-02-558163). Without wishing to be bound by theory, in some embodiments, a protein fusogen derived from a virus or organism that do not infect humans does not have a natural fusion targets in patients, and thus has high specificity.

Lipid Fusogens

[00080] In some embodiments, the fusosome may be treated with fusogenic lipids, such as saturated fatty acids. In some embodiments, the saturated fatty acids have between 10-14 carbons. In some embodiments, the saturated fatty acids have longer-chain carboxylic acids. In some embodiments, the saturated fatty acids are mono-esters.

[00081] In some embodiments, the fusosome may be treated with unsaturated fatty acids. In some embodiments, the unsaturated fatty acids have between C16 and C18 unsaturated fatty acids. In some embodiments, the unsaturated fatty acids include oleic acid, glycerol mono-oleate, glycerides, diacylglycerol, modified unsaturated fatty acids, and any combination thereof.

[00082] Without wishing to be bound by theory, in some embodiments negative curvature lipids promote membrane fusion. In some embodiments, the fusosome comprises one or more negative curvature lipids, e.g., negative curvature lipids that are exogenous relative to the source

cell, in the membrane. In embodiments, the negative curvature lipid or a precursor thereof is added to media comprising source cells or fusosomes. In embodiments, the source cell is engineered to express or overexpress one or more lipid synthesis genes. The negative curvature lipid can be, e.g., diacylglycerol (DAG), cholesterol, phosphatidic acid (PA), phosphatidylethanolamine (PE), or fatty acid (FA).

[00083] Without wishing to be bound by theory, in some embodiments positive curvature lipids inhibit membrane fusion. In some embodiments, the fusosome comprises reduced levels of one or more positive curvature lipids, e.g., exogenous positive curvature lipids, in the membrane. In embodiments, the levels are reduced by inhibiting synthesis of the lipid, e.g., by knockout or knockdown of a lipid synthesis gene, in the source cell. The positive curvature lipid can be, e.g., lysophosphatidylcholine (LPC), phosphatidylinositol (PtdIns), lysophosphatidic acid (LPA), lysophosphatidylethanolamine (LPE), or monoacylglycerol (MAG).

Chemical Fusogens

[00084] In some embodiments, the fusosome may be treated with fusogenic chemicals. In some embodiments, the fusogenic chemical is polyethylene glycol (PEG) or derivatives thereof.

[00085] In some embodiments, the chemical fusogen induces a local dehydration between the two membranes that leads to unfavorable molecular packing of the bilayer. In some embodiments, the chemical fusogen induces dehydration of an area near the lipid bilayer, causing displacement of aqueous molecules between cells and allowing interaction between the two membranes together.

[00086] In some embodiments, the chemical fusogen is a positive cation. Some nonlimiting examples of positive cations include Ca2+, Mg2+, Mn2+, Zn2+, La3+, Sr3+, and H+.

[00087] In some embodiments, the chemical fusogen binds to the target membrane by modifying surface polarity, which alters the hydration-dependent intermembrane repulsion.

[00088] In some embodiments, the chemical fusogen is a soluble lipid soluble. Some nonlimiting examples include oleoylglycerol, dioleoylglycerol, trioleoylglycerol, and variants and derivatives thereof.

[00089] In some embodiments, the chemical fusogen is a water-soluble chemical. Some

nonlimiting examples include polyethylene glycol, dimethyl sulphoxide, and variants and derivatives thereof.

[00090] In some embodiments, the chemical fusogen is a small organic molecule. A nonlimiting example includes n-hexyl bromide.

[00091] In some embodiments, the chemical fusogen does not alter the constitution, cell viability, or the ion transport properties of the fusogen or target membrane.

[00092] In some embodiments, the chemical fusogen is a hormone or a vitamin. Some nonlimiting examples include abscisic acid, retinol (vitamin A1), a tocopherol (vitamin E), and variants and derivatives thereof.

[00093] In some embodiments, the fusosome comprises actin and an agent that stabilizes polymerized actin. Without wishing to be bound by theory, stabilized actin in a fusosome can promote fusion with a target cell. In embodiments, the agent that stabilizes polymerized actin is chosen from actin, myosin, biotin-streptavidin, ATP, neuronal Wiskott–Aldrich syndrome protein (N-WASP), or formin See, e.g., Langmuir. 2011 Aug 16;27(16):10061-71 and Wen et al., Nat Commun. 2016 Aug 31;7. In embodiments, the fusosome comprises actin that is exogenous or overexpressed relative to the source cell, e.g., wild-type actin or actin comprising a mutation that promotes polymerization. In embodiments, the fusosome comprises ATP or phosphocreatine, e.g., exogenous ATP or phosphocreatine.

Small Molecule Fusogens

[00094] In some embodiments, the fusosome may be treated with fusogenic small molecules. Some nonlimiting examples include halothane, nonsteroidal anti-inflammatory drugs (NSAIDs) such as meloxicam, piroxicam, tenoxicam, and chlorpromazine.

[00095] In some embodiments, the small molecule fusogen may be present in micelle-like aggregates or free of aggregates.

Fusogen Modifications

[00096] In some embodiments, the fusogen is linked to a cleavable protein. In some cases, a cleavable protein may be cleaved by exposure to a protease. An engineered fusion protein may

bind any domain of a transmembrane protein. The engineered fusion protein may be linked by a cleavage peptide to a protein domain located within the intermembrane space. The cleavage peptide may be cleaved by one or a combination of intermembrane proteases (e.g. HTRA2/OMI which requires a non-polar aliphatic amino acid - valine, isoleucine or methionine are preferred - at position P1, and hydrophilic residues - arginine is preferred - at the P2 and P3 positions).

[00097] In some embodiments, fusogen proteins are engineered by any methods known in the art or any method described herein to comprise a proteolytic degradation sequence, e.g., a mitochondrial or cytosolic degradation sequence. Fusogen proteins may be engineered to include, but is not limited to a proteolytic degradation sequence, e.g., a Caspase 2 protein sequence (e.g., Val-Asp-Val-Ala-Asp-l-)(SEQ ID NO: 604) or other proteolytic sequences (see, for example, Gasteiger et al., The Proteomics Protocols Handbook; 2005: 571-607), a modified proteolytic degradation sequence that has at least 75%, 80%, 85%, 90%, 95% or greater identity to the wildtype proteolytic degradation sequence, a cytosolic proteolytic degradation sequence, e.g., ubiquitin, or a modified cytosolic proteolytic degradation sequence that has at least 75%, 80%, 85%, 90%, 95% or greater identity to the wildtype proteolytic degradation sequence. In some embodiments, a composition comprises mitochondria in a source cell or chondrisome comprising a protein modified with a proteolytic degradation sequence, e.g., at least 75%, 80%, 85%, 90%, 95% or greater identity to the wildtype proteolytic degradation sequence, a cytosolic proteolytic degradation sequence, e.g., ubiquitin, or a modified cytosolic proteolytic degradation sequence that has at least 75%, 80%, 85%, 90%, 95% or greater identity to the wildtype proteolytic degradation sequence.

[00098] In some embodiments, the fusogen may be modified with a protease domain that recognizes specific proteins, e.g., over-expression of a protease, e.g., an engineered fusion protein with protease activity. For example, a protease or protease domain from a protease, such as MMP, mitochondrial processing peptidase, mitochondrial intermediate peptidase, inner membrane peptidase.

[00099] See, Alfonzo, J.D. & Soll, D. Mitochondrial tRNA import – the challenge to understand has just begun. Biological Chemistry 390: 717-722. 2009; Langer, T. et al. Characterization of Peptides Released from Mitochondria. THE JOURNAL OF BIOLOGICAL

CHEMISTRY. Vol. 280, No. 4. 2691–2699, 2005; Vliegh, P. et al. Synthetic therapeutic peptides: science and market. Drug Discovery Today. 15(1/2). 2010; Quiros P.M.m et al., New roles for mitochondrial proteases in health, ageing and disease. Nature Reviews Molecular Cell Biology. V16, 2015; Weber-Lotfi, F. et al. DNA import competence and mitochondrial genetics. Biopolymers and Cell. Vol. 30. N 1. 71–73, 2014.

Non-Endocytyic Entry into Target Cells

[000100] In some embodiments, a fusosome or fusosome composition described herein delivers a cargo to a target cell via a non-endocytic pathway. Without wishing to be bound by theory, a non-endocytic delivery route can improve the amount or percentage of cargo delivered to the cell, e.g., to the desired compartment of the cell.

[000101] Accordingly, in some embodiments, a plurality of fusosomes described herein, when contacted with a target cell population in the presence of an inhibitor of endocytosis, and when contacted with a reference target cell population not treated with the inhibitor of endocytosis, delivers the cargo to at least 30%, 40%, 50%, 60%, 70%, or 80% of the number of cells in the target cell population compared to the reference target cell population.

[000102] In some embodiments, less than 10% of cargo enters the cell by endocytosis.

[000103] In some embodiments, the inhibitor of endocytosis is an inhibitor of lysosomal acidification, e.g., bafilomycin A1.

[000104] In some embodiments, cargo delivered is determined using an endocytosis inhibition assay, e.g., an assay of Example 125.

[000105] In some embodiments, cargo enters the cell through a dynamin-independent pathway or a lysosomal acidification-independent pathway, a macropinocytosis-independent pathway, or an actin-independent pathway.

[000106] In some embodiments (e.g., embodiments for assaying non-endocytic delivery of cargo) cargo delivery is assayed using one or more of (e.g., all of) the following steps: (a) placing 30,000 HEK-293T target cells into a first well of a 96-well plate comprising 100 nM bafilomycin A1, and placing a similar number of similar cells into a second well of a 96-well plate lacking bafilomycin A1, (b) culturing the target cells for four hours in DMEM media at 37°C and 5% CO_2,

(c) contacting the target cells with 10 ug of fusosomes that comprise cargo, (d) incubating the target cells and fusosomes for 24 hrs at 37ºC and 5% CO2, and (e) determining the percentage of cells in the first well and in the second well that comprise the cargo. Step (e) may comprise detecting the cargo using microscopy, e.g., using immunofluorescence. Step (e) may comprise detecting the cargo indirectly, e.g., detecting a downstream effect of the cargo, e.g., presence of a reporter protein. In some embodiments, one or more of steps (a)-(e) above is performed as described in Example 125.

[000107] In some embodiments, an inhibitor of endocytosis (e.g., chloroquine or bafilomycin A1) inhibits inhibits endosomal acidification. In some embodiments, cargo delivery is independent of lysosomal acidification. In some embodiments, an inhibitor of endocytosis (e.g., Dynasore) inhibits dynamin. In some embodiments, cargo delivery is independent of dynamin activity.

[000108] In some embodiments, the fusosome enters the target cell by endocytosis, e.g., wherein the level of therapeutic agent delivered via an endocytic pathway is 0.01-0.6, 0.01-0.1, 0.1-0.3, or 0.3-0.6, or at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater than a chloroquine treated reference cell contacted with similar fusosomes, e.g., using an assay of Example 91. In some embodiments, at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% of fusosomes in a fusosome composition that enter a target cell enter via a non-endocytic pathway, e.g., the fusosomes enter the target cell via fusion with the cell surface. In some embodiments, the level of a therapeutic agent delivered via a non-endocytic pathway for a given fusosome is 0.1-0.95, 0.1-0.2, 0.2-0.3, 0.3-0.4, 0.4-0.5, 0.5-0.6, 0.6-0.7, 0.7-0.8, 0.8-0.9, 0.9-0.95, or at least at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater than a chloroquine treated reference cell, e.g., using an assay of Example 90. In some embodiments, at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% of fusosomes in a fusosome composition that enter a target cell enter the cytoplasm (e.g., do not enter an endosome or lysosome). In some embodiments, less than 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, 5%, 4%, 3%, 2%, or 1% of fusosomes in a fusosome composition that enter a target cell enter an endosome or lysosome. In some embodiments, the fusosome enters the target cell by a non-endocytic pathway, e.g., wherein the level of therapeutic agent delivered is at least 90%, 95%, 98%, or 99% that of a chloroquine treated reference cell, e.g.,

using an assay of Example 91. In an embodiment, a fusosome delivers an agent to a target cell via a dynamin mediated pathway. In an embodiment, the level of agent delivered via a dynamin mediated pathway is in the range of 0.01-0.6, or at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater than Dynasore treated target cells contacted with similar fusosomes, e.g., as measured in an assay of Example 92. In an embodiment, a fusosome delivers an agent to a target cell via macropinocytosis. In an embodiment, the level of agent delivered via macropinocytosis is in the range of 0.01-0.6, or at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater than EIPA treated target cells contacted with similar fusosomes, e.g., as measured in an assay of Example 92. In an embodiment, a fusosome delivers an agent to a target cell via an actin-mediated pathway. In an embodiment, the level of agent delivered via an actin-mediated pathway will be in the range of 0.01-0.6, or at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater than Latrunculin B treated target cells contacted with similar fusosomes, e.g., as measured in an assay of Example 92.

[000109] In some embodiments, the cargo delivered to the target cell is determined using an endocytosis inhibition assay, e.g., an assay of Example 90, 92, or 125.

[000110] In some embodiments, cargo enters the target cell through a dynamin-independent pathway or a lysosomal acidification-independent pathway, a macropinocytosis-independent pathway (e.g., wherein the inhibitor of endocytosis is an inhibitor of macropinocytosis, e.g., 5-(N-ethyl-N-isopropyl)amiloride (EIPA), e.g., at a concentration of 25 μM), or an actin-independent pathway (e.g., wherein the inhibitor of endocytosis is an inhibitor of actin polymerization is, e.g., Latrunculin B, e.g., at a concentration of 6 μM).

[000111] In some embodiments, the fusosome, when contacted with a target cell population, delivers cargo to a target cell location other than an endosome or lysosome, e.g., to the cytosol, an organelle, or the cell membrane. In embodiments, less 50%, 40%, 30%, 20%, or 10% of the cargo is delivered to an endosome or lysosome.

Specific Delivery to Target Cells

[000112] In some embodiments, a fusosome composition described herein delivers cargo preferentially to a target cell compared to a non-target cell. Accordingly, in certain embodiments,

a fusosome described herein has one or both of the following properties: (i) when the plurality of fusosomes are contacted with a cell population comprising target cells and non-target cells, the cargo is present in at least 2-fold, 5-fold, 10-fold, 20-fold, 50-fold, or 100-fold more target cells than non-target cells, or (ii) the fusosomes of the plurality fuse at a higher rate with a target cell than with a non-target cell by at least at least 50%.

[000113] In some embodiments, presence of cargo is measured by microscopy, e.g., using an assay of Example 126. In some embodiments, fusion is measured by microscopy, e.g., using an assay of Example 54. In some embodiments, the targeting moiety is specific for a cell surface marker on the target cell. In some embodiments, the cell surface marker is a cell surface marker of a skin cell, cardiomyocyte, hepatocyte, intestinal cell (e.g., cell of the small intestine), pancreatic cell, brain cell, prostate cell, lung cell, colon cell, or bone marrow cell.

[000114] In some embodiments (e.g., embodiments for specific delivery of cargo to a target cell versus a non-target cell), cargo delivery is assayed using one or more of (e.g., all of) the following steps: (a) placing 30,000 HEK-293T target cells that over-express CD8a and CD8b into a first well of a 96-well plate and placing 30,000 HEK-293T non-target cells that do not over-express CD8a and CD8b into a second well of a 96-well plate, (b) culturing the cells for four hours in DMEM media at 37°C and 5% CO2, (c) contacting the target cells with 10 ug of fusosomes that comprise cargo, (d) incubating the target cells and fusosomes for 24 hrs at 37°C and 5% CO_2, and (e) determining the percentage of cells in the first well and in the second well that comprise the cargo. Step (e) may comprise detecting the cargo using microscopy, e.g., using immunofluorescence. Step (e) may comprise detecting the cargo indirectly, e.g., detecting a downstream effect of the cargo, e.g., presence of a reporter protein. In some embodiments, one or more of steps (a)-(e) above is performed as described in Example 126.

[000115] In some embodiments, the fusosome fuses at a higher rate with a target cell than with a non-target cell, e.g., by at least at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 20-fold, 50-fold, or 100-fold, e.g., in an assay of Example 54. In some embodiments, the fusosome fuses at a higher rate with a target cell than with other fusosomes, e.g., by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, e.g., in an assay of Example 54. In some embodiments, the fusosome fuses with target cells

at a rate such that an agent in the fusosome is delivered to at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, of target cells after 24, 48, or 72 hours, e.g., in an assay of Example 54. In embodiments, the amount of targeted fusion is about 30%-70%, 35%-65%, 40%-60%, 45%-55%, or 45%-50%, e.g., about 48.8% e.g., in an assay of Example 54. In embodiments, the amount of targeted fusion is about 20%-40%, 25%-35%, or 30%-35%, e.g., about 32.2% e.g., in an assay of Example 55.

[000116] In some embodiments, the fusosome composition delivers at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of the cargo to the target cell population compared to the reference target cell population or to a non-target cell population. In some embodiments, the fusosome composition delivers at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% more of the cargo to the target cell population compared to the reference target cell population or to a non-target cell population.

Fusosome Generation

Fusosomes Generated from Cells

[000117] Compositions of fusosomes may be generated from cells in culture, for example cultured mammalian cells, e.g., cultured human cells. The cells may be progenitor cells or non-progenitor (e.g., differentiated) cells. The cells may be primary cells or cell lines (e.g., a mammalian, e.g., human, cell line described herein). In embodiments, the cultured cells are progenitor cells, e.g., bone marrow stromal cells, marrow derived adult progenitor cells (MAPCs), endothelial progenitor cells (EPC), blast cells, intermediate progenitor cells formed in the subventricular zone, neural stem cells, muscle stem cells, satellite cells, liver stem cells, hematopoietic stem cells, bone marrow stromal cells, epidermal stem cells, embryonic stem cells, mesenchymal stem cells, umbilical cord stem cells, precursor cells, muscle precursor cells, myoblast, cardiomyoblast, neural precursor cells, glial precursor cells, neuronal precursor cells, hepatoblasts.

[000118] In some embodiments, the source cell is an endothelial cell, a fibroblast, a blood cell (e.g., a macrophage, a neutrophil, a granulocyte, a leukocyte), a stem cell (e.g., a mesenchymal

stem cell, an umbilical cord stem cell, bone marrow stem cell, a hematopoietic stem cell, an induced pluripotent stem cell e.g., an induced pluripotent stem cell derived from a subject's cells), an embryonic stem cell (e.g., a stem cell from embryonic yolk sac, placenta, umbilical cord, fetal skin, adolescent skin, blood, bone marrow, adipose tissue, erythropoietic tissue, hematopoietic tissue), a myoblast, a parenchymal cell (e.g., hepatocyte), an alveolar cell, a neuron (e.g., a retinal neuronal cell) a precursor cell (e.g., a retinal precursor cell, a myeloblast, myeloid precursor cells, a thymocyte, a meiocyte, a megakaryoblast, a promegakaryoblast, a melanoblast, a lymphoblast, a bone marrow precursor cell, a normoblast, or an angioblast), a progenitor cell (e.g., a cardiac progenitor cell, a satellite cell, a radial gial cell, a bone marrow stromal cell, a pancreatic progenitor cell, an endothelial progenitor cell, a blast cell), or an immortalized cell (e.g., HeLa, HEK293, HFF-1, MRC-5, WI-38, IMR 90, IMR 91, PER.C6, HT-1080, or BJ cell).

[000119] The cultured cells may be from epithelial, connective, muscular, or nervous tissue or cells, and combinations thereof. Fusosome can be generated from cultured cells from any eukaryotic (e.g., mammalian) organ system, for example, from the cardiovascular system (heart, vasculature); digestive system (esophagus, stomach, liver, gallbladder, pancreas, intestines, colon, rectum and anus); endocrine system (hypothalamus, pituitary gland, pineal body or pineal gland, thyroid, parathyroids, adrenal glands); excretory system (kidneys, ureters, bladder); lymphatic system (lymph, lymph nodes, lymph vessels, tonsils, adenoids, thymus, spleen); integumentary system (skin, hair, nails); muscular system (e.g., skeletal muscle); nervous system (brain, spinal cord, nerves); reproductive system (ovaries, uterus, mammary glands, testes, vas deferens, seminal vesicles, prostate); respiratory system (pharynx, larynx, trachea, bronchi, lungs, diaphragm); skeletal system (bone, cartilage), and combinations thereof. In embodiments, the cells are from a highly mitotic tissue (e.g., a highly mitotic healthy tissue, such as epithelium, embryonic tissue, bone marrow, intestinal crypts). In embodiments, the tissue sample is a highly metabolic tissue (e.g., skeletal tissue, neural tissue, cardiomyocytes).

[000120] In some embodiments a cell is a suspension cell. In some embodiments a cell is an adherent cell.

[000121] In some embodiments, the cells are from a young donor, e.g., a donor 25 years, 20 years, 18 years, 16 years, 12 years, 10 years, 8 years of age, 5 years of age, 1 year of age, or less.

In some embodiments, the cells are from fetal tissue.

[000122] In some embodiments, the cells are derived from a subject and administered to the same subject or a subject with a similar genetic signature (e.g., MHC-matched).

[000123] In certain embodiments, the cells have telomeres of average size greater than 3000, 4000, 5000, 6000, 7000, 8000, 9000, or 10000 nucleotides in length (e.g., between 4,000-10,000 nucleotides in length, between 6,000-10,000 nucleotides in length).

[000124] Fusosomes may be generated from cells generally cultured according to methods known in the art. In some embodiments, the cells may be cultured in 2 or more "phases", e.g., a growth phase, wherein the cells are cultured under conditions to multiply and increase biomass of the culture, and a "production" phase, wherein the cells are cultured under conditions to alter cell phenotype (e.g., to maximize mitochondrial phenotype, to increase number or diameter of mitochondria, to increase oxidative phosphorylation status). There may also be an "expression" phase, wherein the cells are cultured under conditions to maximize expression of protein fusogens or agents exogenous relative to the source cell, on the cell membrane and to restrict unwanted fusion in other phases.

[000125] In some embodiments, fusosomes are generated from cells synchronized, e.g., during a growth phase or the production phase. For example, cells may be synchronized at G1 phase by elimination of serum from the culture medium (e.g., for about 12- 24 hours) or by the use in the culture media of DNA synthesis inhibitors such as thymidine, aminopterin, hydroxyurea and cytosine arabinoside. Additional methods for mammalian cell cycle synchronization are known and disclosed, e.g., in Rosner et al. 2013. Nature Protocols 8:602–626 (specifically Table 1 in Rosner).

[000126] In some embodiments, the cells can be evaluated and optionally enriched for a desirable phenotype or genotype for use as a source for fusosome composition as described herein. For example, cells can be evaluated and optionally enriched, e.g., before culturing, during culturing (e.g., during a growth phase or a production phase) or after culturing but before fusosome production, for example, for one or more of: membrane potential (e.g., a membrane potential of -5 to -200 mV; cardiolipin content (e.g., between 1-20% of total lipid); cholesterol, phosphatidylethanolamine (PE), diglyceride (DAG), phosphatidic acid (PA), or fatty acid (FA)

content; genetic quality > 80%, >85%, > 90%; fusogen expression or content; cargo expression or content.

[000127] In some embodiments, fusosomes are generated from a cell clone identified, chosen, or selected based on a desirable phenotype or genotype for use as a source for fusosome composition described herein. For example, a cell clone is identified, chosen, or selected based on low mitochondrial mutation load, long telomere length, differentiation state, or a particular genetic signature (e.g., a genetic signature to match a recipient).

[000128] A fusosome composition described herein may be comprised of fusosomes from one cellular or tissue source, or from a combination of sources. For example, a fusosome composition may comprise fusosomes from xenogeneic sources (e.g., animals, tissue culture of the aforementioned species' cells), allogeneic, autologous, from specific tissues resulting in different protein concentrations and distributions (liver, skeletal, neural, adipose, etc.), from cells of different metabolic states (e.g., glycolytic, respiring). A composition may also comprise fusosomes in different metabolic states, e.g. coupled or uncoupled, as described elsewhere herein.

[000129] In some embodiments, fusosomes are generated from source cells expressing a fusogen, e.g., a fusogen described herein. In some embodiments, the fusogen is disposed in a membrane of the source cell, e.g., a lipid bilayer membrane, e.g., a cell surface membrane, or a subcellular membrane (e.g., lysosomal membrane). In some embodiments, fusosomes are generated from source cells with a fusogen disposed in a cell surface membrane.

[000130] In some embodiments, fusosomes are generated by inducing budding of an exosome, microvesicle, membrane vesicle, extracellular membrane vesicle, plasma membrane vesicle, giant plasma membrane vesicle, apoptotic body, mitoparticle, pyrenocyte, lysosome, or other membrane enclosed vesicle.

[000131] In some embodiments, fusosomes are generated by inducing cell enucleation. Enucleation may be performed using assays such as genetic, chemical (e.g., using Actinomycin D, see Bayona-Bafaluyet al., "A chemical enucleation method for the transfer of mitochondrial DNA to ρ° cells" Nucleic Acids Res. 2003 Aug 15; 31(16): e98), mechanical methods (e.g., squeezing or aspiration, see Lee et al., "A comparative study on the efficiency of two enucleation methods in pig somatic cell nuclear transfer: effects of the squeezing and the aspiration methods." Anim

Biotechnol. 2008;19(2):71-9), or combinations thereof. Enucleation refers not only to a complete removal of the nucleus but also the displacement of the nucleus from its typical location such that the cell contains the nucleus but it is non-functional.

[000132] In embodiments, making a fusosome comprises producing cell ghosts, giant plasma membrane vesicle, or apoptotic bodies. In embodiments, a fusosome composition comprises one or more of cell ghosts, giant plasma membrane vesicle, and apoptotic bodies.

[000133] In some embodiments, fusosomes are generated by inducing cell fragmentation. In some embodiments, cell fragmentation can be performed using the following methods, including, but not limited to: chemical methods, mechanical methods (e.g., centrifugation (e.g., ultracentrifugation, or density centrifugation), freeze-thaw, or sonication), or combinations thereof.

[000134] In some embodiments, a fusosome can be generated from a source cell expressing a fusogen, e.g., as described herein, by any one, all of, or a combination of the following methods:

i) inducing budding of a mitoparticle, exosome, or other membrane enclosed vesicle;
ii) inducing nuclear inactivation, e.g., enucleation, by any of the following methods or a combination thereof:

a) a genetic method;

b) a chemical method, e.g., using Actinomycin D; or

c) a mechanical method, e.g., squeezing or aspiration; or

iii) inducing cell fragmentation, e.g., by any of the following methods or a combination thereof:

a) a chemical method;

b) a mechanical method, e.g., centrifugation (e.g., ultracentrifugation or density centrifugation); freeze thaw; or sonication.

[000135] For avoidance of doubt, it is understood that in many cases the source cell actually used to make the fusosome will not be available for testing after the fusosome is made. Thus, a comparison between a source cell and a fusosome does not need to assay the source cell that was actually modified (e.g., enucleated) to make the fusosome. Rather, cells otherwise similar to the source cell, e.g., from the same culture, the same genotype same tissue type, or any combination thereof, can be assayed instead.

Modifications to Cells Prior to Fusosome Generation

[000136] In some aspects, a modification is made to a cell, such as modification of a subject, tissue or cell, prior to fusosome generation. Such modifications can be effective to, e.g., improve fusion, fusogen expression or activity, structure or function of the cargo, or structure or function of the target cell.

(i) Physical Modifications

[000137] In some embodiments, a cell is physically modified prior to generating the fusosome. For example, as described elsewhere herein, a fusogen may be linked to the surface of the cell.

[000138] In some embodiments, a cell is treated with a chemical agent prior to generating the fusosome. For example, the cell may be treated with a chemical or lipid fusogen, such that the chemical or lipid fusogen non-covalently or covalently interacts with the surface of the cell or embeds within the surface of the cell. In some embodiments, the cell is treated with an agent to enhance fusogenic properties of the lipids in the cell membrane.

[000139] In some embodiments, the cell is physically modified prior to generating the fusosome with one or more covalent or non-covalent attachment sites for synthetic or endogenous small molecules or lipids on the cell surface that enhance targeting of the fusosome to an organ, tissues, or cell-type.

[000140] In embodiments, a fusosome comprises increased or decreased levels of an endogenous molecule. For instance, the fusosome may comprise an endogenous molecule that also naturally occurs in the naturally occurring source cell but at a higher or lower level than in the fusosome. In some embodiments, the polypeptide is expressed from an exogenous nucleic acid in the source cell or fusosome. In some embodiments, the polypeptide is isolated from a source and loaded into or conjugated to a source cell or fusosome.

[000141] In some embodiments, a cell is treated with a chemical agent, e.g., small molecule, prior to generating the fusosome to increase the expression or activity of an endogenous fusogen in the cell (e.g., in some embodiments, endogenous relative to the source cell, and in some embodiments, endogenous relative to the target cell). In some embodiments, a small molecule

may increase expression or activity of a transcriptional activator of the endogenous fusogen. In some embodiments, a small molecule may decrease expression or activity of a transcriptional repressor of the endogenous fusogen. In some embodiments, a small molecule is an epigenetic modifier that increases expression of the endogenous fusogen.

[000142] In some embodiments, fusosomes are generated from cells treated with fusion arresting compounds, e.g., lysophosphatidylcholine. In some embodiments, fusosomes are generated from cells treated with dissociation reagents that do not cleave fusogens, e.g., Accutase.

[000143] In some embodiments, a source cell is physically modified with, e.g., CRISPR activators, prior to generating a fusosome to add or increase the concentration of fusogens.

[000144] In some embodiments, the cell is physically modified to increase or decrease the quantity, or enhance the structure or function of organelles, e.g., mitochondria, Golgi apparatus, endoplasmic reticulum, intracellular vesicles (such as lysosomes, autophagosomes).

(ii) Genetic Modifications

[000145] In some embodiments, a cell is genetically modified prior to generating the fusosome to increase the expression of an endogenous fusogen in the cell (e.g., in some embodiments, endogenous relative to the source cell, and in some embodiments, endogenous relative to the target cell . In some embodiments, a genetic modification may increase expression or activity of a transcriptional activator of the endogenous fusogen. In some embodiments, a genetic modification may decrease expression or activity of a transcriptional repressor of the endogenous fusogen. In some embodiments the activator or repressor is a nuclease-inactive cas9 (dCas9) linked to a transcriptional activator or repressor that is targeted to the endogenous fusogen by a guide RNA. In some embodiments, a genetic modification epigenetically modifies an endogenous fusogen gene to increase its expression. In some embodiments the epigenetic activator a nuclease-inactive cas9 (dCas9) linked to an epigenetic modifier that is targeted to the endogenous fusogen by a guide RNA.

[000146] In some embodiments, a cell is genetically modified prior to generating the fusosome to increase the expression of an exogenous fusogen in the cell, e.g., delivery of a

transgene. In some embodiments, a nucleic acid, e.g., DNA, mRNA or siRNA, is transferred to the cell prior to generating the fusosome, e.g., to increase or decrease the expression of a cell surface molecule (protein, glycan, lipid or low molecular weight molecule) used for organ, tissue, or cell targeting. In some embodiments, the nucleic acid targets a repressor of a fusogen, e.g., an shRNA, siRNA construct. In some embodiments, the nucleic acid encodes an inhibitor of a fusogen repressor.

[000147] In some embodiments, the method comprises introducing a nucleic acid , that is exogenous relative to the source cell encoding a fusogen into a source cell. The exogenous nucleic acid may be, e.g., DNA or RNA. In some embodiments the exogenous nucleic acid may be e.g., a DNA, a gDNA, a cDNA, an RNA, a pre-mRNA, an mRNA, an miRNA, an siRNA, etc. In some embodiments, the exogenous DNA may be linear DNA, circular DNA, or an artificial chromosome. In some embodiments the DNA is maintained episomally. In some embodiments the DNA is integrated into the genome. The exogenous RNA may be chemically modified RNA, e.g., may comprise one or more backbone modification, sugar modifications, noncanonical bases, or caps. Backbone modifications include, e.g., phosphorothioate, N3′ phosphoramidite, boranophosphate, phosphonoacetate, thio-PACE, morpholino phosphoramidites, or PNA. Sugar modifications include, e.g., 2′-O-Me, 2′F, 2′F-ANA, LNA, UNA, and 2′-O-MOE. Noncanonical bases include, e.g., 5-bromo-U, and 5-iodo-U, 2,6-diaminopurine, C-5 propynyl pyrimidine, difluorotoluene, difluorobenzene, dichlorobenzene, 2-thiouridine, pseudouridine, and dihydrouridine. Caps include, e.g., ARCA. Additional modifications are discussed, e.g., in Deleavey et al., "Designing Chemically Modified Oligonucleotides for Targeted Gene Silencing" Chemistry & Biology Volume 19, Issue 8, 24 August 2012, Pages 937-954, which is herein incorporated by reference in its entirety.

[000148] In some embodiments, a cell is treated with a chemical agent, e.g. a small molecule, prior to generating the fusosome to increase the expression or activity of a fusogen that is exogenous relative to the source cell in the cell. In some embodiments, a small molecule may increase expression or activity of a transcriptional activator of the exogenous fusogen. In some embodiments, a small molecule may decrease expression or activity of a transcriptional repressor of the exogenous fusogen. In some embodiments, a small molecule is an epigenetic modifier that

increases expression of the exogenous fusogen.

[000149] In some embodiments, the nucleic acid encodes a modified fusogen. For example, a fusogen that has regulatable fusogenic activity, e.g., specific cell-type, tissue-type or local microenvironment activity. Such regulatable fusogenic activity may include, activation and/or initiation of fusogenic activity by low pH, high pH, heat, infrared light, extracellular enzyme activity (eukaryotic or prokaryotic), or exposure of a small molecule, a protein, or a lipid. In some embodiments, the small molecule, protein, or lipid is displayed on a target cell.

[000150] In some embodiments, a cell is genetically modified prior to generating the fusosome to alter (i.e., upregulate or downregulate) the expression of signaling pathways (e.g., the Wnt/Beta-catenin pathway). In some embodiments, a cell is genetically modified prior to generating the fusosome to alter (e.g., upregulate or downregulate) the expression of a gene or genes of interest. In some embodiments, a cell is genetically modified prior to generating the fusosome to alter (e.g., upregulate or downregulate) the expression of a nucleic acid (e.g. a miRNA or mRNA) or nucleic acids of interest. In some embodiments, nucleic acids, e.g., DNA, mRNA or siRNA, are transferred to the cell prior to generating the fusosome, e.g., to increase or decrease the expression of signaling pathways, genes, or nucleic acids. In some embodiments, the nucleic acid targets a repressor of a signaling pathway, gene, or nucleic acid, or represses a signaling pathway, gene, or nucleic acid. In some embodiments, the nucleic acid encodes a transcription factor that upregulates or downregulates a signaling pathway, gene, or nucleic acid. In some embodiments the activator or repressor is a nuclease-inactive cas9 (dCas9) linked to a transcriptional activator or repressor that is targeted to the signaling pathway, gene, or nucleic acid by a guide RNA. In some embodiments, a genetic modification epigenetically modifies an endogenous signaling pathway, gene, or nucleic acid to its expression. In some embodiments the epigenetic activator a nuclease-inactive cas9 (dCas9) linked to a epigenetic modifier that is targeted to the signaling pathway, gene, or nucleic acid by a guide RNA. In some embodiments, a cell's DNA is edited prior to generating the fusosome to alter (e.g., upregulate or downregulate) the expression of signaling pathways (e.g. the Wnt/Beta-catenin pathway), gene, or nucleic acid. In some embodiments, the DNA is edited using a guide RNA and CRISPR-Cas9/Cpf1 or other gene editing technology.

[000151] A cell may be genetically modified using recombinant methods. A nucleic acid sequence coding for a desired gene can be obtained using recombinant methods, such as, for example by screening libraries from cells expressing the gene, by deriving the gene from a vector known to include the same, or by isolating directly from cells and tissues containing the same, using standard techniques. Alternatively, a gene of interest can be produced synthetically, rather than cloned.

[000152] Expression of natural or synthetic nucleic acids is typically achieved by operably linking a nucleic acid encoding the gene of interest to a promoter, and incorporating the construct into an expression vector. The vectors can be suitable for replication and integration in eukaryotes. Typical cloning vectors contain transcription and translation terminators, initiation sequences, and promoters useful for expression of the desired nucleic acid sequence.

[000153] In some embodiments, a cell may be genetically modified with one or more expression regions, e.g., a gene. In some embodiments, the cell may be genetically modified with an exogenous gene (e.g., capable of expressing an exogenous gene product such as an RNA or a polypeptide product) and/or an exogenous regulatory nucleic acid. In some embodiments, the cell may be genetically modified with an exogenous sequence encoding a gene product that is endogenous to a target cell and/or an exogenous regulatory nucleic acid capable of modulating expression of an endogenous gene. In some embodiments, the cell may be genetically modified with an exogenous gene and/or a regulatory nucleic acid that modulates expression of an exogenous gene. In some embodiments, the cell may be genetically modified with an exogenous gene and/or a regulatory nucleic acid that modulates expression of an endogenous gene. It will be understood by one of skill in the art that the cell described herein may be genetically modified to express a variety of exogenous genes that encode proteins or regulatory molecules, which may, e.g., act on a gene product of the endogenous or exogenous genome of a target cell. In some embodiments, such genes confer characteristics to the fusosome, e.g., modulate fusion with a target cell. In some embodiments, the cell may be genetically modified to express an endogenous gene and/or regulatory nucleic acid. In some embodiments, the endogenous gene or regulatory nucleic acid modulates the expression of other endogenous genes. In some embodiments, the cell may be genetically modified to express an endogenous gene and/or regulatory nucleic acid which is

expressed differently (e.g., inducibly, tissue-specifically, constitutively, or at a higher or lower level) than a version of the endogenous gene and/or regulatory nucleic acid on other chromosomes.

[000154] The promoter elements, e.g., enhancers, regulate the frequency of transcriptional initiation. Typically, these are located in the region 30-110 bp upstream of the start site, although a number of promoters have recently been shown to contain functional elements downstream of the start site as well. The spacing between promoter elements frequently is flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. In the thymidine kinase (tk) promoter, the spacing between promoter elements can be increased to 50 bp apart before activity begins to decline. Depending on the promoter, it appears that individual elements can function either cooperatively or independently to activate transcription.

[000155] One example of a suitable promoter is the immediate early cytomegalovirus (CMV) promoter sequence. This promoter sequence is a strong constitutive promoter sequence capable of driving high levels of expression of any polynucleotide sequence operatively linked thereto. Another example of a suitable promoter is Elongation Growth Factor-1α (EF-1α). However, other constitutive promoter sequences may also be used, including, but not limited to the simian virus 40 (SV40) early promoter, mouse mammary tumor virus (MMTV), human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter, MoMuLV promoter, an avian leukemia virus promoter, an Epstein-Barr virus immediate early promoter, a Rous sarcoma virus promoter, as well as human gene promoters such as, but not limited to, the actin promoter, the myosin promoter, the hemoglobin promoter, and the creatine kinase promoter.

[000156] Further, the invention should not be limited to the use of constitutive promoters. Inducible promoters are also contemplated as part of the invention. The use of an inducible promoter provides a molecular switch capable of turning on expression of the polynucleotide sequence which it is operatively linked when such expression is desired, or turning off the expression when expression is not desired. Examples of inducible promoters include, but are not limited to a tissue-specific promoter, metallothionine promoter, a glucocorticoid promoter, a progesterone promoter, and a tetracycline promoter. In some embodiments, expression of a fusogen is upregulated before fusosomes are generated, e.g., 3, 6, 9, 12, 24, 26, 48, 60, or 72 hours before fusosomes are generated.

[000157] The expression vector to be introduced into the source can also contain either a selectable marker gene or a reporter gene or both to facilitate identification and selection of expressing cells from the population of cells sought to be transfected or infected through viral vectors. In other aspects, the selectable marker may be carried on a separate piece of DNA and used in a co-transfection procedure. Both selectable markers and reporter genes may be flanked with appropriate regulatory sequences to enable expression in the host cells. Useful selectable markers include, for example, antibiotic-resistance genes, such as neo and the like.

[000158] Reporter genes may be used for identifying potentially transfected cells and for evaluating the functionality of regulatory sequences. In general, a reporter gene is a gene that is not present in or expressed by the recipient source and that encodes a polypeptide whose expression is manifested by some easily detectable property, e.g., enzymatic activity. Expression of the reporter gene is assayed at a suitable time after the DNA has been introduced into the recipient cells. Suitable reporter genes may include genes encoding luciferase, beta-galactosidase, chloramphenicol acetyl transferase, secreted alkaline phosphatase, or the green fluorescent protein gene (e.g., Ui-Tei et al., 2000 FEBS Letters 479: 79-82). Suitable expression systems are well known and may be prepared using known techniques or obtained commercially. In general, the construct with the minimal 5' flanking region showing the highest level of expression of reporter gene is identified as the promoter. Such promoter regions may be linked to a reporter gene and used to evaluate agents for the ability to modulate promoter-driven transcription.

[000159] In some embodiments, a cell may be genetically modified to alter expression of one or more proteins. Expression of the one or more proteins may be modified for a specific time, e.g., development or differentiation state of the source. In some embodiments, fusosomes are generated from a source of cells genetically modified to alter expression of one or more proteins, e.g., fusogen proteins or non-fusogen proteins that affect fusion activity, structure or function. Expression of the one or more proteins may be restricted to a specific location(s) or widespread throughout the source.

[000160] In some embodiments, the expression of a fusogen protein is modified. In some embodiments, fusosomes are generated from cells with modified expression of a fusogen protein, e.g., an increase or a decrease in expression of a fusogen by at least 10%, 15%, 20%, 30%, 40%,

50%, 60%, 75%, 80%, 90% or more.

[000161] In some embodiments, cells may be engineered to express a cytosolic enzyme (e.g., proteases, phosphatases, kinases, demethylases, methyltransferases, acetylases) that targets a fusogen protein. In some embodiments, the cytosolic enzyme affects one or more fusogens by altering post-translational modifications. Post-translational protein modifications of proteins may affect responsiveness to nutrient availability and redox conditions, and protein–protein interactions. In some embodiments, a fusosome comprises fusogens with altered post-translational modifications, e.g., an increase or a decrease in post-translational modifications by at least 10%, 15%, 20%, 30%, 40%, 50%, 60%, 75%, 80%, 90% or more.

[000162] Methods of introducing a modification into a cell include physical, biological and chemical methods. See, for example, Geng. & Lu, Microfluidic electroporation for cellular analysis and delivery. Lab on a Chip. 13(19):3803-21. 2013; Sharei, A. et al. A vector-free microfluidic platform for intracellular delivery. PNAS vol. 110 no. 6. 2013; Yin, H. et al., Non-viral vectors for gene-based therapy. Nature Reviews Genetics. 15: 541–555. 2014. Suitable methods for modifying a cell for use in generating the fusosomes described herein include, for example, diffusion, osmosis, osmotic pulsing, osmotic shock, hypotonic lysis, hypotonic dialysis, ionophoresis, electroporation, sonication, microinjection, calcium precipitation, membrane intercalation, lipid mediated transfection, detergent treatment, viral infection, receptor mediated endocytosis, use of protein transduction domains, particle firing, membrane fusion, freeze-thawing, mechanical disruption, and filtration.

[000163] Confirming the presence of a genetic modification includes a variety of assays. Such assays include, for example, molecular biological assays, such as Southern and Northern blotting, RT-PCR and PCR; biochemical assays, such as detecting the presence or absence of a particular peptide, e.g., by immunological means (ELISAs and Western blots) or by assays described herein.

Fusosome Modifications

[000164] In some aspects, a modification is made to the fusosome. Such modifications can be effective to, e.g., improve targeting, function, or structure.

[000165] In some embodiments, the fusosome is treated with a fusogen, e.g., a chemical fusogen described herein, that may non-covalently or covalently link to the surface of the membrane. In some embodiments, the fusosome is treated with a fusogen, e.g., a protein or a lipid fusogen, that may non-covalently or covalently link or embed itself in the membrane.

[000166] In some embodiments, a ligand is conjugated to the surface of the fusosome via a functional chemical group (carboxylic acids, aldehydes, amines, sulfhydryls and hydroxyls) that is present on the surface of the fusosome.

[000167] Such reactive groups include without limitation maleimide groups. As an example, fusosomes may be synthesized to include maleimide conjugated phospholipids such as without limitation DSPE-MaL-PEG2000.

[000168] In some embodiments, a small molecule or lipid, synthetic or native, may be covalently or non-covalent linked to the surface of the fusosome. In some embodiments, a membrane lipid in the fusosome may be modified to promote, induce, or enhance fusogenic properties.

[000169] In some embodiments, the fusosome is modified by loading with modified proteins (e.g., enable novel functionality, alter post-translational modifications, bind to the mitochondrial membrane and/or mitochondrial membrane proteins, form a cleavable protein with a heterologous function, form a protein destined for proteolytic degradation, assay the agent's location and levels, or deliver the agent as a carrier). In some embodiments, a fusosome is loaded with one or more modified proteins.

[000170] In some embodiments, a protein exogenous relative to the source cell is non-covalently bound to the fusosome. The protein may include a cleavable domain for release. In some embodiments, the invention includes a fusosome comprising an exogenous protein with a cleavable domain.

[000171] In some embodiments, the fusosome is modified with a protein destined for proteolytic degradation. A variety of proteases recognize specific protein amino acid sequences and target the proteins for degradation. These protein degrading enzymes can be used to specifically degrade proteins having a proteolytic degradation sequence. In some embodiments, a

fusosome comprises modulated levels of one or more protein degrading enzymes, e.g., an increase or a decrease in protein degrading enzymes by at least 10%, 15%, 20%, 30%, 40%, 50%, 60%, 75%, 80%, 90% or more.

[000172] As described herein, non-fusogen additives may be added to the fusosome to modify their structure and/or properties. For example, either cholesterol or sphingomyelin may be added to the membrane to help stabilize the structure and to prevent the leakage of the inner cargo. Further, membranes can be prepared from hydrogenated egg phosphatidylcholine or egg phosphatidylcholine, cholesterol, and dicetyl phosphate. (see, e.g., Spuch and Navarro, Journal of Drug Delivery, vol. 2011, Article ID 469679, 12 pages, 2011. doi:10.1155/2011/469679 for review).

[000173] In some embodiments, the fusosome comprises one or more targeting groups (e.g., a targeting protein) on the exterior surface to target a specific cell or tissue type (e.g., cardiomyocytes). These targeting groups include without limitation receptors, ligands, antibodies, and the like. These targeting groups bind their partner on the target cells' surface. In embodiments, the targeting protein is specific for a cell surface marker on a target cell described herein, e.g., a skin cell, cardiomyocyte, hepatocyte, intestinal cell (e.g., cell of the small intestine), pancreatic cell, brain cell, prostate cell, lung cell, colon cell, or bone marrow cell.

[000174] In some embodiments, the fusosome described herein is functionalized with a diagnostic agent. Examples of diagnostic agents include, but are not limited to, commercially available imaging agents used in positron emissions tomography (PET), computer assisted tomography (CAT), single photon emission computerized tomography, x-ray, fluoroscopy, and magnetic resonance imaging (MRI); and contrast agents. Examples of suitable materials for use as contrast agents in MRI include gadolinium chelates, as well as iron, magnesium, manganese, copper, and chromium.

[000175] Another example of introducing functional groups to the fusosome is during post-preparation, by direct crosslinking fusosome and ligands with homo- or heterobifunctional crosslinkers. This procedure may use a suitable chemistry and a class of crosslinkers (CDI, EDAC, glutaraldehydes, etc. as discussed herein) or any other crosslinker that couples a ligand to the fusosome surface via chemical modification of the fusosome surface after preparation. This also

includes a process whereby amphiphilic molecules such as fatty acids, lipids or functional stabilizers may be passively adsorbed and adhered to the fusosome surface, thereby introducing functional end groups for tethering to ligands.

Cargo

[000176] In some embodiments, a fusosome described herein includes a cargo that is or comprises a membrane protein payload agent. In some embodiments, the membrane protein payload agent may be or may encode a therapeutic protein. A fusosome may additionally include other cargo, e.g., in some embodiments, a fusosome described herein includes a cargo that is or comprises a therapeutic agent. In some embodiments, a fusosome described herein includes a plurality of membrane payload agents. In some embodiments, a fusosome described herein includes a cargo that is or comprises a plurality of therapeutic agents. In some embodiments, a fusosome comprises a cargo comprising one or more membrane protein payload agents and one or more therapeutic agents. In some embodiments, a cargo may be a therapeutic agent that is exogenous or endogenous relative to the source cell.

[000177] In some embodiments a fusosome comprises a cargo associated with the fusosome lipid bilayer. In some embodiments a fusosome comprises a cargo disposed within the lumen of the fusosome. In some embodiments, a fusosome comprises a cargo associated with the fusosome lipid bilayer and a cargo disposed within the lumen of the fusosome.

[000178] In some embodiments, a cargo is not expressed naturally in a cell from which the fuososme is derived. In some embodiments, a cargo is expressed naturally in the cell from which a fusosome is derived. In some embodiments, a cargo is a mutant of a wild type nucleic acid or protein expressed naturally in a cell from which the fusosome is derived or is a wild type of a mutant expressed naturally in a cell from which a fusosome is derived.

[000179] In some embodiments, a cargo is loaded into a fusosome via expression in a cell from which the fusosome is derived (e.g. expression from DNA introduced via transfection, transduction, or electroporation). In some embodiments, a cargo is expressed from DNA integrated into the genome of the cell from which the fusosome is derived or maintained episosomally in the cell from which the fusosome is derived. In some embodiments, expression of a cargo is constitutive in the cell from which the fusosome is derived. In some embodiments, expression of

a cargo in the cell from which the fusosome is derived is induced. In some embodiments, expression of the cargo is induced in the cell from which the fusosome is derived immediately prior to generating the fusosome. In some embodiments, expression of a cargo in the cell from which the fusosome is derived is induced at the same time as expression of the fusogen in the cell from which the fusosome is derived.

[000180] In some embodiments, a cargo is loaded into a fusosome via electroporation into the fusosome itself or into a cell from which the fusosome is derived. In some embodiments, a cargo is loaded into a fusosome via transfection into the fusosome itself or into a cell from which the fusosome is derived.

[000181] In some aspects, the disclosure provides a fusosome composition (e.g., a pharmaceutical composition) comprising: (i) one or more of a chondrisome (e.g., as described in international application, PCT/US16/64251), a mitochondrion, an organelle (e.g., Mitochondria, Lysosomes, nucleus, cell membrane, cytoplasm, endoplasmic reticulum, ribosomes, vacuoles, endosomes, spliceosomes, polymerases, capsids, acrosome, autophagosome, centriole, glycosome, glyoxysome, hydrogenosome, melanosome, mitosome, myofibril, cnidocyst, peroxisome, proteasome, vesicle, stress granule, and networks of organelles), or an enucleated cell, e.g., an enucleated cell comprising any of the foregoing, and (ii) a fusogen, e.g., a myomaker protein.

[000182] In embodiments, the fusogen is present in a lipid bilayer external to the mitochondrion or chondrisome. In embodiments, the chondrisome has one or more of the properties as described, for example, in international application, PCT/US16/64251, which is herein incorporated by reference in its entirety, including the Examples and the Summary of the Invention.

[000183] In some embodiments, the cargo may include one or more nucleic acid sequences, one or more polypeptides, a combination of nucleic acid sequences and/or polypeptides, one or more organelles, and any combination thereof. In some embodiments, the cargo may include one or more cellular components. In some embodiments, the cargo includes one or more cytosolic and/or nuclear components.

[000184] In some embodiments, the cargo includes a nucleic acid, e.g., DNA, nDNA (nuclear DNA), mtDNA (mitochondrial DNA), protein coding DNA, gene, operon, chromosome, genome,

transposon, retrotransposon, viral genome, intron, exon, modified DNA, mRNA (messenger RNA), tRNA (transfer RNA), modified RNA, microRNA, siRNA (small interfering RNA), tmRNA (transfer messenger RNA), rRNA (ribosomal RNA), mtRNA (mitochondrial RNA), snRNA (small nuclear RNA), small nucleolar RNA (snoRNA), SmY RNA (mRNA trans-splicing RNA), gRNA (guide RNA), TERC (telomerase RNA component), aRNA (antisense RNA), cis-NAT (Cis-natural antisense transcript), CRISPR RNA (crRNA), lncRNA (long noncoding RNA), piRNA (piwi-interacting RNA), shRNA (short hairpin RNA), tasiRNA (trans-acting siRNA), eRNA (enhancer RNA), satellite RNA, pcRNA (protein coding RNA), dsRNA (double stranded RNA), RNAi (interfering RNA), circRNA (circular RNA), reprogramming RNAs, aptamers, and any combination thereof. In some embodiments, the nucleic acid is a wild-type nucleic acid. In some embodiments, the protein is a mutant nucleic acid. In some embodiments the nucleic acid is a fusion or chimera of multiple nucleic acid sequences.

[000185] In some embodiments, DNA in the fusosome or DNA in the cell that the fusosome is derived from is edited to correct a genetic mutation using a gene editing technology, e.g. a guide RNA and CRISPR-Cas9/Cpf1, or using a different targeted endonuclease (e.g., Zinc-finger nucleases, transcription-activator-like nucleases (TALENs)). In some embodiments, the genetic mutation is linked to a disease in a subject. Examples of edits to DNA include small insertions/deletions, large deletions, gene corrections with template DNA, or large insertions of DNA. In some embodiments, gene editing is accomplished with non-homologous end joining (NHEJ) or homology directed repair (HDR). In some embodiments, the edit is a knockout. In some embodiments, the edit is a knock-in. In some embodiments, both alleles of DNA are edited. In some embodiments, a single allele is edited. In some embodiments, multiple edits are made. In some embodiments, the fusosome or cell is derived from a subject, or is genetically matched to the subject, or is immunologically compatible with the subject (e.g. having similar MHC).

[000186] In some embodiments, the cargo may include a nucleic acid. For example, the cargo may comprise RNA to enhance expression of an endogenous protein (e.g., in some embodiments, endogenous relative to the source cell, and in some embodiments, endogenous relative to the target cell), or a siRNA or miRNA that inhibits protein expression of an endogenous protein. For example, the endogenous protein may modulate structure or function in the target

cells. In some embodiments, the cargo may include a nucleic acid encoding an engineered protein that modulates structure or function in the target cells. In some embodiments, the cargo is a nucleic acid that targets a transcriptional activator that modulate structure or function in the target cells.

[000187] In some embodiments, the cargo comprises a self-replicating RNA, e.g., as described herein. In some embodiments, the self-replicating RNA is single stranded RNA and/or linear RNA. In some embodiments, the self-replicating RNA encodes one or more proteins, e.g., a protein described herein, e.g., a membrane protein, a secreted protein, a nuclear protein, or an organellar protein. In some embodiments, the self-replicating RNA comprises a partial or complete genome from arterivirus or alphavirus, or a variant thereof.

[000188] In some embodiments, the cargo can comprise an RNA that can be delivered into a target cell, and RNA is replicated inside the target cell. Replication of the self-replicating RNA can involve RNA replication machinery that is exogenous to the host cell, and/or RNA replication machinery that is endogenous to the host cell.

[000189] In some embodiments, the self-replicating RNA comprises a viral genome, or a self-replicating portion or analog thereof. In some embodiments, the self-replicating RNA is from a positive-sense single-stranded RNA virus. In some embodiments, the self-replicating RNA comprises a partial or complete arterivirus genome, or a variant thereof. In some embodiments, the arterivirus comprises Equine arteritis virus (EAV), Porcine respiratory and reproductive syndrome virus (PRRSV), Lactate dehydrogenase elevating virus (LDV), and Simian hemorrhagic fever virus (SHFV). In some embodiments, the self-replicating RNA comprises a partial or complete alphavirus genome, or a variant thereof. In some embodiments, the alphavirus belongs to the VEEV/EEEV group (e.g., Venezuelan equine encephalitis virus), the SF group, or the SIN group.

[000190] In some embodiments, the fusosome that comprises the self-replicating RNA further comprises: (i) one or more proteins that promote replication of the RNA, or (ii) a nucleic acid encoding one or more proteins that promote replication of the RNA, e.g., as part of the self-replicating RNA or in a separate nucleic acid molecule.

[000191] In some embodiments, the self-replicating RNA lacks at least one functional gene encoding one or more viral structural protein relative to the corresponding wild-type genome. For

instance, in some embodiments the self-replicating RNA fully lacks one or more genes for viral structural proteins or comprises a non-functional mutant gene for a viral structural protein. In some embodiments, the self-replicating RNA does not comprise any genes for viral structural proteins.

[000192] In some embodiments, the self-replicating RNA comprises a viral capsid enhancer, e.g., as described in International Application WO2018/106615, which is hereby incorporated by reference in its entirety. In some embodiments, the viral capsid enhancer is an RNA structure that increases translation of a coding sequence in cis, e.g., by allowing eIF2alpha independent translation of the coding sequence. In some embodiments, a host cell has impaired translation, e.g., due to PKR-mediated phosphorylation of eIF2alpha. In embodiments, the viral capsid enhancer comprises a Downstream Loop (DLP) from a viral capsid protein, or a variant of the DLP. In some embodiments, the viral capsid enhancer is from a virus belonging to the Togaviridae family, e.g., the Alphavirus genus of the Togaviridae family. In some embodiments, the viral capsid enhancer has a sequence of SEQ ID NO: 1 of WO2018/106615 (which sequence is herein incorporated by reference in its entirety), or a sequence having at least 70%, 80%, 85%, 90%, 95%, or 99% identity thereto. In some embodiments, the sequence has the same secondary structure shown in Fig. 1 of WO2018/106615.

[000193] In some embodiments, the self-replicating RNA comprises one or more arterivirus sequences, e.g., as described in International Application WO2017/180770, which is hereby incorporated by reference in its entirety. In some embodiments, the self-replicating RNA comprises ORF7 (or a functional fragment or variant thereof) and/or the self-replicating RNA lacks a functional ORF2a (e.g., fully lacks ORF2a, or comprises a non-functional mutant of ORF2a) of an arterivirus. In some embodiments, the self-replicating RNA lacks a functional ORF2b, ORF3, ORF4, ORF5a, ORF5, or ORF6 or any combination thereof (e.g., fully lacks the sequence(s) or comprises a non-functional mutant of the sequence(s)). In some embodiments, the self-replicating RNA lacks a portion of one or more of ORF2a, ORF2b, ORF3, ORF4, ORF5a, ORF5, or ORF6. In some embodiments, the self-replicating RNA comprises one or more subgenomic (sg) promoters, e.g., situated at a non-native site. In some embodiments, the promoter comprises sg promoter 1, sg promoter 2, sg promoter 3, sg promoter 4, sg promoter 5, sg promoter 6, sg promoter

7, or a functional fragment or variant thereof. In some embodiments, the self-replicating RNA comprises one or more transcriptional termination signals, e.g., T7 transcriptional termination signals, e.g., a mutant T7 transcription termination signal, e.g., a mutant T7 transcription termination signal comprising one or more of (e.g., any two of, or all of) T9001G, T3185A, or G3188A.

[000194] In some embodiments, the self-replicating RNA comprises a 5' UTR, e.g., a mutant alphavirus 5' UTR, e.g., as described in International Application WO2018/075235, which is hereby incorporated by reference in its entirety. In some embodiments, the mutant alphavirus 5' UTR comprises one or more nucleotide substitutions at position 1, 2, 4, or a combination thereof. In some embodiments, the mutant alphavirus 5' UTR comprises a U-> G substitution at position 2.

[000195] In some embodiments, the cargo includes a polypeptide, e.g., enzymes, structural polypeptides, signaling polypeptides, regulatory polypeptides, transport polypeptides, sensory polypeptides, motor polypeptides, defense polypeptides, storage polypeptides, transcription factors, antibodies, cytokines, hormones, catabolic polypeptides, anabolic polypeptides, proteolytic polypeptides, metabolic polypeptides, kinases, transferases, hydrolases, lyases, isomerases, ligases, enzyme modulator polypeptides, protein binding polypeptides, lipid binding polypeptides, membrane fusion polypeptides, cell differentiation polypeptides, epigenetic polypeptides, cell death polypeptides, nuclear transport polypeptides, nucleic acid binding polypeptides, reprogramming polypeptides, DNA editing polypeptides, DNA repair polypeptides, DNA recombination polypeptides, DNA integration polypeptides, targeted endonucleases (e.g. Zinc-finger nucleases, transcription-activator-like nucleases (TALENs), cas9 and homologs thereof), recombinases, and any combination thereof. In some embodiments the protein targets a protein in the cell for degredation. In some embodiments the protein targets a protein in the cell for degredation by localizing the protein to the proteasome. In some embodiments, the protein is a wild-type protein. In some embodiments, the protein is a mutant protein. In some embodiments the protein is a fusion or chimeric protein.

[000196] In some embodiments, the cargo includes a small molecule, e.g., ions (e.g. Ca^{2+}, Cl^{-}, Fe^{2+}), carbohydrates, lipids, reactive oxygen species, reactive nitrogen species, isoprenoids,

signaling molecules, heme, polypeptide cofactors, electron accepting compounds, electron donating compounds, metabolites, ligands, and any combination thereof. In some embodiments the small molecule is a pharmaceutical that interacts with a target in the cell. In some embodiments the small molecule targets a protein in the cell for degredation. In some embodiments the small molecule targets a protein in the cell for degredation by localizing the protein to the proteasome. In some embodiments that small molecule is a proteolysis targeting chimera molecule (PROTAC).

[000197] In some embodiments, the cargo includes a mixture of proteins, nucleic acids, or metabolites, e.g., multiple polypeptides, multiple nucleic acids, multiple small molecules; combinations of nucleic acids, polypeptides, and small molecules; ribonucleoprotein complexes (e.g. Cas9-gRNA complex); multiple transcription factors, multiple epigenetic factors, reprogramming factors (e.g. Oct4, Sox2, cMyc, and Klf4); multiple regulatory RNAs; and any combination thereof.

[000198] In some embodiments, the cargo includes one or more organelles, e.g., chondrisomes, mitochondria, lysosomes, nucleus, cell membrane, cytoplasm, endoplasmic reticulum, ribosomes, vacuoles, endosomes, spliceosomes, polymerases, capsids, acrosome, autophagosome, centriole, glycosome, glyoxysome, hydrogenosome, melanosome, mitosome, myofibril, cnidocyst, peroxisome, proteasome, vesicle, stress granule, networks of organelles, and any combination thereof.

[000199] In some embodiments, the cargo is enriched at the fusosome or cell membrane. In some embodiments, the cargo is enriched by targeting to the membrane via a peptide signal sequence. In some embodiments, the cargo is enriched by binding with a membrane associated protein, lipid, or small molecule. In some embodiments, the cargo is enriched by dimerizing with a membrane associated protein, lipid, or small molecule. In some embodiments the cargo is chimeric (e.g. a chimeric protein, or nucleic acid) and comprises a domain that mediates binding or dimerization with a membrane associated protein, lipid, or small molecule. Membrane-associated proteins of interest include, but are not limited to, any protein having a domain that stably associates, e.g., binds to, integrates into, etc., a cell membrane (i.e., a membrane-association domain), where such domains may include myristoylated domains, farnesylated domains, transmembrane domains, and the like. Specific membrane-associated proteins of interest include,

but are not limited to: myristoylated proteins, e.g., p 60 v-src and the like; farnesylated proteins, e.g., Ras, Rheb and CENP-E,F, proteins binding specific lipid bilayer components e.g. AnnexinV, by binding to phosphatidyl-serine, a lipid component of the cell membrane bilayer and the like; membrane anchor proteins; transmembrane proteins, e.g., transferrin receptors and portions thereof; and membrane fusion proteins. In some embodiments, the membrane associated protein contains a first dimerization domain. The first dimerization domain may be, e.g., a domain that directly binds to a second dimerization domain of a cargo or binds to a second dimerization domain via a dimerization mediator. In some embodiments the cargo contains a second dimerization domain. The second dimerization domain may be, e.g., a domain that dimerizes (e.g., stably associates with, such as by non-covalent bonding interaction, either directly or through a mediator) with the first dimerization domain of the membrane associated protein either directly or through a dimerization mediator. With respect to the dimerization domains, these domains are domains that participate in a binding event, either directly or via a dimerization mediator, where the binding event results in production of the desired multimeric, e.g., dimeric, complex of the membrane associated and target proteins. The first and second dimerization domains may be homodimeric, such that they are made up of the same sequence of amino acids, or heterodimeric, such that they are made up of differing sequences of amino acids. Dimerization domains may vary, where domains of interest include, but are not limited to: ligands of target biomolecules, such as ligands that specifically bind to particular proteins of interest (e.g., protein:protein interaction domains), such as SH2 domains, Paz domains, RING domains, transcriptional activator domains, DNA binding domains, enzyme catalytic domains, enzyme regulatory domains, enzyme subunits, domains for localization to a defined cellular location, recognition domains for the localization domain, the domains listed at URL: pawsonlab.mshri.on.ca/index.php?option=com_content&task=view&id=30&Itemid=63/, etc. In some embodiments the first dimerization domain binds nucleic acid (e.g. mRNA, miRNA, siRNA, DNA) and the second dimerization domain is a nucleic acid sequence present on the cargo (e.g. the first dimerization domain is MS2 and the second dimerization domain is the high affinity binding loop of MS2 RNA). Any convenient compound that functions as a dimerization mediator may be employed. A wide variety of compounds, including both naturally occurring and synthetic substances, can be used as dimerization mediators. Applicable and readily observable or

measurable criteria for selecting a dimerization mediator include: (A) the ligand is physiologically acceptable (i.e., lacks undue toxicity towards the cell or animal for which it is to be used); (B) it has a reasonable therapeutic dosage range; (C) it can cross the cellular and other membranes, as necessary (where in some instances it may be able to mediate dimerization from outside of the cell), and (D) binds to the target domains of the chimeric proteins for which it is designed with reasonable affinity for the desired application. A first desirable criterion is that the compound is relatively physiologically inert, but for its dimerization mediator activity. In some instances, the ligands will be non-peptide and non-nucleic acid. Additional dimerization domains are described, e.g., in US20170087087 and US20170130197, each of which is herein incorporated by reference in its entirety.

Payload Agents

[000200] The methods and compositions described herein can be used to target payloads (e.g., payload agents). For instance, payload agents can be targeted to a cellular membrane, e.g., through the use of a co-translational endoplasmic reticulum (ER) signal. The cellular membrane can be, e.g., an ER membrane, a plasma membrane, membrane of secreted and/or secretory vesicles, or lysosomal membrane. In some embodiments, payload agents are targeted for secretion. In some embodiments, the methods and compositions described herein can be used to target payloads to the lumen of an organelle (e.g. a Golgi apparatus, secretory vesicle, or lysosome) after translation in the ER. In some embodiments, payload agents can be targeted to the nucleus, e.g., through the use of a nuclear localization signal. In another example, payload agents can be targeted to the mitochondria or peroxisome, e.g., through the use of a mitochondria or peroxisome localization signal, respectively.

[000201] A protein payload agent (e.g., a membrane protein payload agent or a secreted protein payload agent) may be or comprise, e.g., a protein, or a nucleic acid encoding a protein, selected from: a perinuclear membrane protein, an inner nuclear membrane protein, a nucleoplasm protein, a nucleolus protein, a perionucleolar protein, a cajal body protein, a clastosome protein, a gems nuclear bodies protein, a histone body protein, a nuclear speckles protein, a nuclear stress body protein, a paraspeckle protein, a PML bodies protein, a polycomb body protein, a transmembrane protein, a cell surface protein, a protein associated with the cytosolic side of a

membrane, an endoplasmic reticulum protein, a lysosome protein, a Golgi apparatus protein, a secreted protein, a secretory vesicle protein, a mitochondrial membrane protein, an outer mitochondrial membrane protein, an inner mitochondrial membrane protein, a mitochondrial inner boundary membrane protein, a mitochondrial cristal membrane protein, a mitochondrial DNA protein, a mitochondrial intermembrane space protein, a mitochondrial matrix protein, a mitochondrial matrix granule protein, a mitochondrial cristae protein, a mitochondrial ribosome protein, a mitochondrial intracristal protein, a mitochondrial peripheral space protein, a peroxisomal membrane protein, a peroxisomal crystalloid core protein, a peroxisomal matrix protein, a peroxin, or an endosomal protein, or a combination thereof.

[000202] In some embodiments, the membrane protein payload agent is an exogenous version of a protein that is naturally present in or targeted to the target membrane. In some embodiments, the membrane protein payload agent is not naturally present or targeted to the target membrane. In some embodiments, a protein payload agent (e.g., a membrane protein payload agent or a secreted protein payload agent) may be or comprise, e.g., a protein, or a nucleic acid encoding a protein, selected from: a cell surface receptor protein, a transporter, an ion channel, membrane associated enzyme, a cell adhesion protein, an immunoglobulin, a T cell receptor, an endoplasmic reticulum protein, a lysosome protein, a Golgi apparatus protein, a secreted protein, a secretory vesicle protein, an endosomal protein. A membrane protein payload agent may be, e.g., a recombinant version of a naturally occurring membrane protein, or a synthetic protein, e.g., a protein having a sequence not found in nature or domains not found together in nature, e.g., a chimeric membrane protein, e.g., a transmembrane protein having an extracellular domain derived from a first naturally occurring protein and a transmembrane domain and/or intracellular domain derived from a second naturally occurring protein, e.g., a chimeric antigen receptor.

[000203] In some embodiments, the nuclear protein payload agent is an exogenous version of a protein that is naturally present in or targeted to the nucleus. In some embodiments, the nuclear protein payload agent is not naturally present or targeted to the nucleus. In some embodiments, a protein payload agent (e.g., a nuclear protein payload agent) may be or comprise, e.g., a protein, or a nucleic acid encoding a protein, selected from: a transcription factor, a nuclease, a recombinase, an epigenetic factor, a post-transcriptional RNA modification factor (e.g. an

mRNA splicing factor), a non-coding RNA (e.g. a snRNA or snoRNA) or ribonucleic protein (e.g. a snRNP or snoRNP), a structural protein (e.g. a lamin or karysokeletal protein). A nuclear protein payload agent may be, e.g., a recombinant version of a naturally occurring nuclear protein, or a synthetic protein, e.g., a protein having a sequence not found in nature or domains not found together in nature, e.g., a chimeric nuclear protein, e.g., a nuclear protein composed of a translational fusion or association between a DNA binding domain derived from a zinc-finger, a TAL, or an RNA-guided protein, or other DNA binding domains known in the art, and a nucleic acid modifying domain from a second protein, e.g., a nuclease, a recombinase, a base editor (e.g. a deaminase), a nickase, a transcription factor (e.g. an activator or a repressor), a viral motif that alters DNA regulation (e.g. VP16 or VP64), an epigenetic modifying factor (e.g. a demethylase), or nucleic acid modifying domain known in the art.

[000204] An organellar protein payload agent may be, e.g., a recombinant version of a naturally occurring nuclear protein, or a synthetic protein, e.g., a protein having a sequence not found in nature or domains not found together in nature, e.g., a chimeric mitochondria or peroxisome protein, e.g., a mitochondria or peroxisome protein composed of a translational fusion or association between a mtDNA binding domain derived from a zinc-finger, a TAL, or an RNA-guided protein, or other mtDNA binding domains known in the art, and a nucleic acid modifying domain from a second protein, e.g., a nuclease, a recombinase, a base editor (e.g. a deaminase), a nickase, a transcription factor (e.g. an activator or a repressor), or nucleic acid modifying domain known in the art.

[000205] In some embodiments, the mitochondria or perixosome protein payload agent is an exogenous version of a protein that is naturally present in or targeted to the mitochondria or peroxisome. In some embodiments, the mitochondria or peroxisome protein payload agent is not naturally present or targeted to the mitochondria or peroxisome. In some embodiments, a protein payload agent (e.g., a mitochondria or peroxisome protein payload agent) may be or comprise, e.g., a protein, or a nucleic acid encoding a protein, selected from: a mitochondrial membrane protein, a outer mitochondrial membrane protein, an inner mitochondrial membrane protein, a mitochondrial inner boundary membrane protein, a mitochondrial cristal membrane protein, a mitochondrial DNA protein, a mitochondrial intermembrane space protein, a mitochondrial matrix

protein, a mitochondrial matrix granule protein, a mitochondrial cristae protein, a mitochondrial ribosome protein, a mitochondrial intracristal protein, a mitochondrial peripheral space protein, a peroxisomal membrane protein, a peroxisomal crystalloid core protein, a peroxisomal matrix protein, a peroxin, a transcription factor, a nuclease, a recombinase, an epigenetic factor, a non-coding RNA or ribonucleic protein, a structural protein, an enzyme, a transporter, a respiration complex, a fusion or fission protein, a cytochrome, a signaling protein, an apoptosis protein or factor, a mitochondrial import protein, a mitochondrial export protein, or an electron transport protein.

[000206] In some embodiments, the payload agent (e.g., membrane protein payload agent, nuclear protein payload agent, mitochondria protein payload agent, or perixosome protein payload agent) comprises a protein (e.g., a synthetic protein) comprising an effector domain linked to a localization domain (e.g., TAL, ZF, Cas9, or meganuclease). Exemplary effector domains include, without limitation, nucleases, recombinases, integrases, base editors (e.g., deaminases), transcription factors, and epigenetic modifiers.

[000207] In some embodiments, a fusosome comprises a protein payload agent (e.g., a membrane protein payload agent, nuclear protein payload agent, organellar protein payload agent, mitochondria protein payload agent, or peroxisome protein payload agent, e.g., as described herein, or a secreted protein payload agent). In some embodiments, a protein payload agent is a protein and/or a nucleic acid that encodes it. In some embodiments the protein is expressed in a cell line and then incorporated into a fusosome. A person of ordinary skill will appreciate that to the extent any such protein is expressed by the cell line, the cell line is capable of any post-translational processing necessary to make the protein. In some embodiments post-translational processing comprises one or more of protein splicing, protein cleavage, protein folding, protein glycosylation, dimerization, etc.

[000208] In some embodiments, the protein (e.g., membrane protein, nuclear protein, organellar protein payload agent, mitochondria protein payload agent, or peroxisome protein payload agent, e.g., as described herein, or secreted protein) is expressed by the source cell from which a fusosome is derived. A person of ordinary skill will appreciate that to the extent any such protein is expressed by the source cell, the source cell is capable of any post-translational

processing necessary to make the protein. In some embodiments post-translational processing comprises one or more of protein splicing, protein cleavage, protein folding, protein glycosylation, dimerization, etc. In some embodiments a protein payload agent is a nucleic acid. In some embodiments the nucleic acid encodes a cell surface or nuclear protein. In some embodiments the nucleic acid encodes an outer nuclear membrane protein, a perinuclear membrane protein, an inner nuclear membrane protein, a nucleoplasm protein, a nucleolus protein, an endoplasmic reticulum protein, a lysosome protein, a Golgi apparatus protein, a secreted protein, a secretory vesicle protein, organellar protein, mitochondria protein, or peroxisome protein, or an endosomal protein. In some embodiments a protein payload agent, e.g., membrane protein payload agent, nuclear protein payload agent, or organellar protein payload agent, e.g., as described herein, is a protein or nucleic acid encoding a protein selected from the cell surface antigens described herein. In some embodiments the nucleic acid encodes an engineered cell surface protein. In some embodiments an engineered cell surface protein is a chimeric antigen receptor. In some embodiments, the nucleic acid encodes a mitochondrial membrane protein, a outer mitochondrial membrane protein, an inner mitochondrial membrane protein, a mitochondrial inner boundary membrane protein, a mitochondrial cristal membrane protein, a mitochondrial DNA protein, a mitochondrial intermembrane space protein, a mitochondrial matrix protein, a mitochondrial matrix granule protein, a mitochondrial cristae protein, a mitochondrial ribosome protein, a mitochondrial intracristal protein, a mitochondrial peripheral space protein, a peroxisomal membrane protein, a peroxisomal crystalloid core protein, a peroxisomal matrix protein, or a peroxinin. In some embodiments a protein payload agent, e.g., mitochondria or peroxisome protein payload agent, is a protein or nucleic acid encoding a protein selected from the mitochondria or peroxisome proteins described herein.

[000209] In some embodiments, a protein payload agent (e.g., a membrane protein payload agent, nuclear protein payload agent, organellar protein payload agent, mitochondria protein payload agent, or peroxisome protein payload agent, or a secreted protein payload agent, e.g., as described herein) comprises a nucleic acid which is expressed by the fused target cell. A person of ordinary skill will appreciate that to the extent any protein produced by expression of a nucleic acid protein payload agent (e.g., membrane protein payload agent, nuclear protein payload agent, organellar protein payload agent, mitochondria protein payload agent, or peroxisome protein

payload agent, e.g., as described herein) requires post-translational processing, such post-translational processing will be performed in the fused target cell. In some embodiments post-translational may comprise one or more of protein splicing, protein cleavage, protein folding, protein glycosylation, dimerization, etc. In some embodiments, the post-translational modification is a covalent attachment of a lipid, such as a fatty acid, isoprenoid, sterol, phospholipid, glycosylphosphatidyl inositol (GPI), cholesterol, farnesyl, geranylgeranyl, myristoyl, palmitoyl, which in some embodiments targets the protein to the plasma membrane. In some embodiments, the post-translational modification is direct phosphorylation of a nuclear localization sequence.

[000210] In some embodiments, the protein payload agent (e.g., a membrane protein payload agent, nuclear protein payload agent, organellar protein payload agent, mitochondria protein payload agent, or peroxisome protein payload agent, or a secreted protein payload agent, e.g., as described herein) comprises a nucleic acid, e.g., RNA or DNA. In some embodiments, the nucleic acid is, comprises, or consists of one or more natural nucleic acid residues. In some embodiments, the nucleic acid is, comprises, or consists of one or more nucleic acid analogs. In some embodiments, the nucleic acid has a nucleotide sequence that encodes a functional gene product such as an RNA or protein. In some embodiments, the nucleic acid includes one or more introns. In some embodiments, nucleic acids are prepared by one or more of isolation from a natural source, enzymatic synthesis by polymerization based on a complementary template (*in vivo* or *in vitro*), reproduction in a recombinant cell or system, and chemical synthesis. In some embodiments, the nucleic acid is at least 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 1 10, 120, 130, 140, 150, 160, 170, 180, 190, 20, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000 or more residues long. In some embodiments, the nucleic acid is partly or wholly single stranded; in some embodiments, the nucleic acid is partly or wholly double stranded. In some embodiments the nucleic acid has a nucleotide sequence comprising at least one element that encodes, or is the complement of a sequence that encodes, a polypeptide. The nucleic acid may incude variants, e.g., having an overall sequence identity with a reference nucleic acid of at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99%. In some embodiments, a variant nucleic acid does not share at least one characteristic sequence element with a reference nucleic acid. In some embodiments, a variant nucleic acid shares one or more of

the biological activities of the reference nucleic acid. In some embodiments, a nucleic acid variant has a nucleic acid sequence that is identical to that of the reference but for a small number of sequence alterations at particular positions. In some embodiments, fewer than about 20%, about 15%, about 10%, about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, or about 2% of the residues in a variant are substituted, inserted, or deleted, as compared to the reference. In some embodiments, a variant nucleic acid comprises about 10, about 9, about 8, about 7, about 6, about 5, about 4, about 3, about 2, or about 1 substituted residues as compared to a reference. In some embodiments, a variant nucleic acid comprises a very small number (e.g., fewer than about 5, about 4, about 3, about 2, or about 1) number of substituted, inserted, or deleted, functional residues that participate in a particular biological activity relative to the reference. In some embodiments, a variant nucleic acid comprises not more than about 15, about 12, about 9, about 3, or about 1 addition or deletion, and, in some embodiments, comprises no additions or deletions, as compared to the reference. In some embodiments, a variant nucleic acid comprises fewer than about 27, about 24, about 21, about 18, about 15, about 12, about 9, about 6, about 3, or fewer than about 9, about 6, about 3, or about 2 additions or deletions as compared to the reference.

[000211] In some embodiments, the protein payload agent (e.g., a membrane protein payload agent, nuclear protein payload agent, organellar protein payload agent, mitochondria, protein payload agent, or peroxisome protein payload agent, or a secreted protein payload agent e.g., as described herein) comprises a protein. The protein may include moieties other than amino acids (*e.g.*, may be glycoproteins, proteoglycans, etc.) and/or may be otherwise processed or modified. The protein can sometimes include more than one polypeptide chain, for example linked by one or more disulfide bonds or associated by other means. The protein may contain L-amino acids, D-amino acids, or both and may contain any of a variety of amino acid modifications or analogs. In some embodiments, proteins may comprise natural amino acids, non-natural amino acids, synthetic amino acids, and combinations thereof. In some embodiments, proteins are antibodies, antibody fragments, biologically active portions thereof, and/or characteristic portions thereof. A polypeptide may include its variants, e.g., having an overall sequence identity with a reference polypeptide of at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99%. In some embodiments, a variant polypeptide does not share at least one characteristic

sequence element with a reference polypeptide. In some embodiments, a variant polypeptide shares one or more of the biological activities of the reference polypeptide. In some embodiments, a polypeptide variant has an amino acid sequence that is identical to that of the reference but for a small number of sequence alterations at particular positions. In some embodiments, fewer than about 20%, about 15%, about 10%, about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, or about 2% of the residues in a variant are substituted, inserted, or deleted, as compared to the reference. In some embodiments, a variant polypeptide comprises about 10, about 9, about 8, about 7, about 6, about 5, about 4, about 3, about 2, or about 1 substituted residues as compared to a reference. In some embodiments, a variant polypeptide comprises a very small number (e.g., fewer than about 5, about 4, about 3, about 2, or about 1) number of substituted, inserted, or deleted, functional that participate in a particular biological activity relative to the reference. In some embodiments, a variant polypeptide comprises not more than about 5, about 4, about 3, about 2, or about 1 addition or deletion, and, in some embodiments, comprises no additions or deletions, as compared to the reference. In some embodiments, a variant polypeptide comprises fewer than about 25, about 20, about 19, about 18, about 17, about 16, about 15, about 14, about 13, about 10, about 9, about 8, about 7, about 6, and commonly fewer than about 5, about 4, about 3, or about 2 additions or deletions as compared to the reference.

Signal Sequences

[000212] In some embodiments, a protein payload agent (e.g., a membrane protein payload agent, nuclear protein payload agent, organellar protein payload agent, mitochondria protein payload agent, or peroxisome protein payload agent, or a secreted protein payload agent, e.g., as described herein) is a protein (or nucleic acid encoding it) that includes or included a signal sequence directing the protein to a particular site or location (e.g., to the cell surface, nucleus, an organelle, mitochondria, or peroxisome). Those skilled in the art will appreciate that, in certain instances, a cell uses "sorting signals" which are amino acid motifs that are at least temporarily part of a protein (e.g., when initially produced), to target the protein to particular subcellular location (e.g., to a particular organelle or surface membrane of a target cell). In some embodiments a sorting signal is a signal sequence, a signal peptide, or a leader sequence, which directs a protein to an organelle called the endoplasmic reticulum (ER), mitochondria, or peroxisome; in some such

embodiments, the protein is then delivered to the plasma membrane. See US20160289674A1. In some such embodiments, the protein is then secreted. In some such embodiments, the protein is then trafficked to the lysosome. In some such embodiments, the protein is then trafficked to the Golgi apparatus. In some such embodiments, the protein is then trafficked to a secretory vesicle, and may then be secreted from the cell. In some such embodiments, the protein is then trafficked to an endosome. In some embodiments a sorting signal is a signal sequence, a signal peptide, or a leader sequence, which directs a protein the nucleus (e.g., a nuclear localization sequence); in some such embodiments, the protein is then delivered to the nucleus.

[000213] In some embodiments, protein targeting to the ER is cotranslational. In some embodiments protein translocation and membrane insertion are coupled to protein synthesis. In some embodiments a signal sequence may be hydrophobic. In some embodiments a signal sequence may be partially hydrophobic. In some embodiments a signal sequence is recognized by a signal recognition particle (SRP). In some embodiments the SRP recognizing a signal sequence as it emerges from a ribosome. In some embodiments, a nascent peptide chain-ribosome complex is targeted to the ER by binding to an SRP receptor. In some embodiments a signal sequence interacts with an Sec61α subunit of a translocon and initiates translocation of a membrane protein or partial chain of said membrane protein.

[000214] In some embodiments, protein targeting to the mitochondria is through a mitochondria localization sequence (also known as a mitochondria signal sequence or presequence) that enables translocation into the mitochondria. Canonical mitochondrial import pathways often require the presence of Tom40 protein, which constitutes a channel of the translocase of the outer membrane (TOM) complex. Apart from the channel itself, the TOM complex contains a number of receptor proteins, such as Tom20, Tom22, and Tom70, that participate in recognition of the incoming precursor proteins. Import through the TOM complex depends on interactions between precursor proteins with receptor domains that are exposed by subunits of the TOM complex. Certain classes of precursor proteins differentially interact with the TOM complex. Precursor proteins that are targeted to mitochondria by the cleavable mitochondrial targeting signal, called a presequence, are first recognized by Tom20 before they interact with Tom22. Hydrophobic precursors of carrier proteins that contain an integral targeting signal require

the presence of the Tom70 receptor. On their way through the channel of Tom40, both types of protein precursors interact with the pore, but the pattern of interacting residues of Tom40 is different for the presequence and carrier precursors. In some embodiments the mitochondrial protein is transported post-translationally or co-translationally. Mitochondrial targeting elements can exist as either individual or multiple units scattered along the length of the precursor and vary markedly in terms of sequence, structure and location, reflecting their role in alternative mitochondrial sorting routes. The "classical" mitochondrial targeting signal is a cleavable N-terminal positively charged sequence, termed a presequence, which directs proteins to the mitochondrial matrix, inner membrane and in a few cases the mitochondrial intermembrane space. In some embodiments, a mitochondrial presequence comprises an N-terminal extension that is an alpha-helical segment with net positive charge a length between 15 and 55 amino acids. However, the greater part of mitochondrial proteins residing in the outer membrane, intermembrane space and inner membrane lack the classical presequence, but rather contain internal cryptic targeting sequences within the mature protein. In some embodiments, the mitochondrial localization sequence is recognized by the TOM complex. In some embodiments, the mitochondrial protein is further transplanted into the mitochondrial outer membrane with the help of a TIM chaperone or mitochondrial outer membrane insertase. In some embodiments, the mitochondrial protein is further transported into the mitochondrial intermembrane space by the mitochondrial intermembrane space import and assembly machinery. In some embodiments, the mitochondrial protein is further transported into the TIM23 complex into the mitochondrial matrix or inner membrane. In some embodiments, the mitochondrial protein is further transported into the TIM22 complex into the mitochondrial matrix or inner membrane.

[000215] In some embodiments, protein targeting to the peroxisome is through peroxisome localization sequence (also known as a peroxisome targeting signal) that enables translocation into the peroxisome. There are two main classes of peroxisome localization sequences. Peroxisome targeting signal 1 is characterized by a c-terminal tripeptide with a consensus sequence (S/A/C)-(K/R/H)-(L/A). The common is serine-lysine-leucine (SKL). Peroxisome targeting sequence 2 is characterized by a nonapeptide located near the N-terminus with a consensus sequence (R/K)-(L/V/I)-XXXXX-(H/Q)-(L/A/F) where X is any amino acid. Some proteins contain neither of these signals and their transport is based on association with a protein that does contain a

peroxisomal targeting signal. In some embodiments the peroxisome localization sequence interacts with a PEX gene. In some embodiments the peroxisome localization sequence interacts with the PTS1 receptor encoded by the PEX5 gene. In some embodiments the peroxisome localization sequence interacts with the PTS2 receptor encoded by the PEX7 gene. In some embodiments the peroxisomal localization sequence is a "mPTS" motif, which is more poorly defined and may consist of discontinuous subdomains. One of these usually is a cluster of basic amino acids (arginines and lysines) within a loop of protein (i.e., between membrane spans) that will face the matrix.

[000216] In some embodiments, a membrane protein payload agent comprises an in-frame fusion of a protein of interest to the coding sequence of a transmembrane protein, or an in-frame fusion of a protein of interest to the transmembrane domain or membrane-anchoring domain of a protein (e.g. fusion to the transferrin receptor membrane anchor domain). See, e.g., Winndard, P, et al. Development of novel chimeric transmembrane proteins for multimodality imaging of cancer cells, Cancer Biology & Therapy. 12:1889-1899 (2007).

[000217] In some embodiments a sorting signal or signal peptide is appended to the N or C terminus of a protein (e.g., membrane protein or secreted protein). See Goder, V. & Spiess, M., Topogenesis of membrane proteins: determinants and dynamics. FEBS Letters. 504(3): 87-93 (2001). In some embodiments the protein is a natural protein. In some embodiments the membrane protein is a synthetic protein.

[000218] In some embodiments, a mitochondrial or peroxisome protein payload agent comprises an in-frame fusion of a protein of interest to the coding sequence of a mitochondrial or peroxisome protein, or an in-frame fusion of a protein of interest to the mitochondrial or peroxisome localization sequence of a protein.

[000219] In some embodiments a mitochondrial or peroxisome localization sequence is appended to the N or C terminus of a protein (e.g., mitochondrial or peroxisome protein). In some embodiments the protein is a natural protein. In some embodiments the mitochondrial or peroxisome protein is a synthetic protein.

[000220] In some embodiments, a signal emerges from a ribosome only after translation of a transcript has reached a stop codon. In some embodiments insertion of a membrane protein is

post-transational.

[000221] In some embodiments a signal sequence is selected from Table 6. In some embodiments a signal sequence comprises a sequence selected from Table 6. In some embodiments a signal sequence of Table 6 may be appended to the N-terminus of a protein, e.g., a membrane protein or secreted protein. In some embodiments a signal sequence of Table 6 may be appended to the C-terminus of a protein, e.g., a membrane protein or secreted protein. A person of ordinary skill will appreciate that the signal sequences below are not limited for use with their respective naturally associated proteins.

Table 6: Exemplary signal sequences.

Signal Sequence	Naturally Associated Protein	Location Associated Protein is Directed	SEQ ID NO
MRVKEKYQHL WRWGWKWGTM LLGILMICSA TE	HIV-1 gp41	Plasma membrane	1
CAAL	p21ras	Plasma membrane	2
KKKKKK	p21ras	Plasma membrane	3
RRRRR	p21ras	Plasma membrane	4
MRLLLALLGV LLSVPGPPVL S	FGFR4	Plasma membrane	5
CSIMNLMCGS TC	ROP7 GTPase	Plasma membrane	6

Signal Sequence	Naturally Associated Protein	Location Associated Protein is Directed	SEQ ID NO
GHKSEEKREK MKRTLLKDWK TRLSYFLQNS STPGKPKTGK KSKQQ	RGS2	Plasma membrane	7
RSTLKLTTLQ CQYSTVMD	LHR	Plasma membrane \ basolateral cell surface	8
RQGLHNMEDV YELIENSH	TSHR	Plasma membrane \ basolateral cell surface	9
MDCRKMARFS YSVIWIMAIS KVFELGLVAG	TDGF1	Plasma membrane	10
MPAWGALFLL WATAEA	(GP)IX	Plasma membrane	11
RDYR	VPAC2	Plasma membrane	12
KMALRVALNN KQSGQITVKT SSSDHLSLAI AGLVPIALSI YQKFKPGVSP SYSIY	Toc159	Plasma membrane	13

Signal Sequence	Naturally Associated Protein	Location Associated Protein is Directed	SEQ ID NO
MGSKIVQVFL MLALFATSAL A	Classical arabinogalactan protein 4	Plasma membrane	14
MNSKAMQALI FLGFLATSCL A	Classical arabinogalactan protein 2	Plasma membrane	15
MGAAASIQTT VN	L1R	Plasma membrane	16
SVM	GTP-binding protein Rheb	Plasma membrane	17
FALLGTHGAS G	CD147	Plasma membrane	18
RRRTFLK	PlcH	Plasma membrane	19
MGGKWSKSSV	Nef	Plasma membrane	20
DDPERE	Nef	Plasma membrane	21
EEANTGENNS LLHPMS	HIV-1 NA7	Plasma membrane	22
SRRGLV	DmsA	Plasma membrane	23

Signal Sequence	Naturally Associated Protein	Location Associated Protein is Directed	SEQ ID NO
SRRRFL	TorA/ TorA-MalE	Plasma membrane	24
SRRQFI	SufI	Plasma membrane \ periplasm	25
QRRDFL	YacK	Plasma membrane \ periplasm	26
MNKIYSIKYS AATGGLIAVS ELAKKVICKT NRKISAALLS LAVISYTNII YA	Pet (Serine protease pet autotransporter)	Plasma membrane	27
MNPNQKIITI GSICMVIGIV SLMLQIGNII SIWVSHSIQT	Influenza A Neuraminidase	Plasma Membrane	28
LRCLACSCFR TPVWPR	prRDH	Plasma Membrane	29
MGCGCSSHPE	Lck	Plasma Membrane	30
MPFVNKQFN	BoNT/ A-LC	Plasma Membrane	31
DEQNAKNAAQ DRNSNKSSKG FFSKLGCC	Yck2p	Plasma Membrane	32

Signal Sequence	Naturally Associated Protein	Location Associated Protein is Directed	SEQ ID NO
MLCCMRRTKQ	GAP-43	Plasma Membrane	33
VTNGSTYILV PLSH	FSHR	Plasma Membrane	34
AETENFV	M3 mAChR	Plasma Membrane	35
RARHRRNVDR VSIGSYRT	pIgR	Plasma Membrane	36
YEDQ	RhBG	Plasma Membrane	37
LLVTSLLLCELPHPAFL IP	GM-CSF Receptor (GM_CSFR)	Plasma membrane	38
DSWGILFSHP	Prdx6	Lysosome	39
[DE]x{3}L[LI]	OCA2/ GLUT8/ p40/ ZIP1	Lysosome \ Melanosome	40
DETQPLLI	OCA2/ GLUT8/ p40/ ZIP1 motif	Lysosome \ Melanosome	605
Ex{3}LL	GLUT8	Lysosome	41
ETQPLL	GLUT8 motif	Lysosome	606
EEEx{8}LI	CLN3	Lysosome	42
EEEAESAARQPLI	CLN3 motif	Lysosome	607

Signal Sequence	Naturally Associated Protein	Location Associated Protein is Directed	SEQ ID NO
YTPL	HLA-DM/ CD8	Lysosome	43
NQPLLT	gp-75	Lysosome \ Melanosome	44
RRPGQRRPPP PPPPPPLPPP PPPPPLPPLP LPPL	FasL	Lysosome	45
RGQGSMDEGT ADERAPLIRT	Limp II	Lysosome	46
Yx{2}[VILF WCM]	LAMP1/ Ptc1/ Endocytosed GalR1/ CLN7/ tyrosinase/ SPPL2a	Lysosome	47
EKITLL	Insulin receptor	Lysosome	48
SSLKILSKG	CXCR4	Lysosome \ Endosome	49
PNQALLRILK ETEFKKIKVL GSG	EGF receptor	Lysosome	50
YLLNT	Nramp2	Lysosome	51
YGSI	Nramp1 (Slc11a1)	Lysosome	52
GYDQL	Cystinosin	Lysosome	53
YFPQA	Cystinosin	Lysosome	54
LLDLLDE	ClC-3	Lysosome \ Endosome	55

Signal Sequence	Naturally Associated Protein	Location Associated Protein is Directed	SEQ ID NO
LKQETEVELY NEFPEPMKLD KN	rab7	Lysosome \ Endosome	56
RRRAVVKEPL LPPEDDTRDN	E-cadherin	Lysosome	57
PGYRHV	LAP	Lysosome	58
GYx{2}I	lgp120	Lysosome	59
GTEKCVWGPS YWCQNME	prosaposin	Lysosome	60
Kx{3}Q	ARD1	Lysosome	61
KLMDQ	ARD1 motif	Lysosome	608
EQEPLL	CLN7	Lysosome	62
YPAF	LAPTM4	Lysosome	63
YEMA	LAPTM4	Lysosome	64
GYx{2}[VIL FWCM]	Cystinosis	Lysosome	65
SLLKGRQGIY	OA1	Lysosome \ Melanosome	66
ETERLL	Mucolipin-1	Lysosome	67
EEHSLL	Mucolipin-1	Lysosome	68
DDSDEDLL	mannose 6-phosphate/ insulin-like growth factor II receptor	Lysosome	69

Signal Sequence	Naturally Associated Protein	Location Associated Protein is Directed	SEQ ID NO
EQERLL	p40	Lysosome	70
CINCCKVL	RhoB	Lysosome	71
EFFDAx{1}E	Opi1p	Endoplasmic Reticulum	72
Dx{1}E	VSV-G/ GONST1/ CASP	Endoplasmic Reticulum	73
DIE	VSV-G/ GONST1/ CASP motif	Endoplasmic Reticulum	609
SKK	FV glycoprotein	Endoplasmic Reticulum	74
MAGARRGWRL APVRRGVCGP RARPLMRPLW LLFAVSFFGW TGALDG	NHE6	Endoplasmic Reticulum	75
MGSLWSKISQ LFVDA	E protein (PRRSV)	Endoplasmic Reticulum	76
MRVKEKYQHL WRWGWRWGTM LLGMLMIC	p-gp160 (HIV-1 envelope protein)	Endoplasmic Reticulum	77
MRLSWFRVLT VLSICLSAVA T	FKBP-13	Endoplasmic Reticulum	78

Signal Sequence	Naturally Associated Protein	Location Associated Protein is Directed	SEQ ID NO
MDPVVVLGLC LSCLLLLSLW KQSYGGGKL	P450 2C1	Endoplasmic Reticulum	79
HALSYWKPFL VNMCVATVLT AGAYLCYRFL FNSNT	PTP-1B	Endoplasmic Reticulum	80
SPRGSRPSWG PTDPRRRSRN LGKVIDTLTC GFADLMGYIP LVGA	hepatitis C virus glycoprotein E1	Endoplasmic Reticulum	81
MALWMRFLPL LALLVLWEPK PAQA	proinsulin	Endoplasmic Reticulum	82
MEAMWLLCVA LAVLAWG	GlcNAc-PI	Endoplasmic Reticulum	83
IPHDLCHNGE KSKKPSKIKS LFKKKSK	STIM2	Endoplasmic Reticulum	84
RPSNNNTTTT TTTDATQPHH HHHHHRHRDA GVKNVTNNSK TTESSSSSSA AKEKPKHKKG LLHKLKKKL	Ist2	Endoplasmic Reticulum	85
[HK]x{1}K	p11/ TASK-1	Endoplasmic Reticulum	86

Signal Sequence	Naturally Associated Protein	Location Associated Protein is Directed	SEQ ID NO
VVQAITFIFK SLGLKCVQFL PQVMPTFLNV IRVCDGAIRE	mTOR	Endoplasmic Reticulum	87
TFCSTALLIT ALALVCTLLY L	E3/ 19K (adenovirus)	Endoplasmic Reticulum	88
GVMLGSIFCA LITMLGHI	Cosmc	Endoplasmic Reticulum	89
FLAAFVVMLC ITIVTIVVYI YLV	gp36.5/ m164	Endoplasmic Reticulum	90
WGVLAGIAYF SMVGNWAKVL VVLLLFAGV	E1 (HCV)	Endoplasmic Reticulum	91
KKSQ	Tapasin (tpn)	Endoplasmic Reticulum	92
WWQLTLGAIC ALPLAGLLAC CA	E1 (Rubella virus)	Endoplasmic Reticulum	93
Yx{4}LL	B5R envelope protein	Golgi	94
Lx{2}KN	ABCA	Golgi (early post -Golgi comparments)	95
LLWKN	ABCA motif	Golgi (early post -Golgi comparments)	610

Signal Sequence	Naturally Associated Protein	Location Associated Protein is Directed	SEQ ID NO
CPSDSEEDEG	Furin	Golgi	96
YKGL	Furin	Golgi	97
MAKTAMAYKE KMKELSMLSL ICSCFYPEPR NINI	SCG10	Golgi	98
YAGL	gpI	Golgi	99
DDFEDSESTD TEEE	gpI	Golgi	100
MASPGSGFWS FGSEDGSGDS ENPGTAR	GAD65	Golgi	101
QV[SA]PA	rhodopsin	Golgi	102
RLLDSSNATW QRDQPDTHRL SRLDAHVMSM	G1 membrane glycoprotein	Golgi	103
WEK[IM]x{2 }FF	SopD2	Golgi	104
YKNL	ARD1 (ARF domain)	Golgi	105
YEGL	ARD1 (ARF domain)	Golgi	106
MKFREPLLGG SAAMPGASLQ RACRLLVAVC ALHLGVTLVY YLA	"beta-1,4-GT"	Golgi	107

Signal Sequence	Naturally Associated Protein	Location Associated Protein is Directed	SEQ ID NO
MKELTMLSLS FFFVSALLAA AANPLLSAA	miraculin (STI)	Secretory Pathway	108
MRISKPHLRS ISIQCYLCLL LNSHFLTEAG IHVFILGCFS AGLPKTEA	IL -15 (LSP)	Secretory Pathway	109
MNKIYSLKYS HITGGLIAVS ELSGRVSSRA TGKKKHKRIL ALCFLGLLQS SYSFA	EspP (E. coli autotransporter)	Secretory Pathway	110
MARQGCLGSF QVISLFTFAI SVNICLG	ENPP2	Secretory Pathway	111
MQRARPTLWA AALTLLVLLR GPPVARA	IGFBP3	Secretory Pathway	112
MKSVYFVAGL FIMLAQGSWQ	proglucagon	Secretory Pathway	113
MNIKKFAKQA TVLTFTTALL AGGATQAFA	Levansucrase	Secretory Pathway	114

Signal Sequence	Naturally Associated Protein	Location Associated Protein is Directed	SEQ ID NO
MQQDGTQQDR IKQSPAPLNG MSRRGFLGGA GTLALATASG LLLPGTAHA	XlnC	Secretory Pathway	115
MGSYALPRSG VRRSIRVLLL ALVVGVLGTA TALIAPPGAH A	xylanase A	Secretory Pathway	116
MNKVFKVIWC KTSQTWIAVS ELSKAFSLST TTDIPKKTKI FIAAAPLLFL SFNTNA	EmaA	Secretory Pathway	117
SDPRLRQFLQ KSK	prosomatostatin	Secretory Pathway	118
CQDLTTESNL LAC	POMC (pro-opiomelanocortin)	Secretory Pathway	119
LPTDTTTFKR IFLKRM	Prorenin	Secretory Pathway	120
HDEL	invertase enzyme	Endoplasmic Reticulum	121
KTEL	AGR2	Endoplasmic Reticulum	122

Signal Sequence	Naturally Associated Protein	Location Associated Protein is Directed	SEQ ID NO
HNEL	LRP's receptor-associatedprotein(RAP)	Endoplasmic Reticulum	123
KEDL	XP124L	Endoplasmic Reticulum (pre-golgi compartments)	124
KDEL	ERp59/ PDI/ ABP1	Endoplasmic Reticulum	125
KEEL	ERp72	Endoplasmic Reticulum	126
QEDL	ERp61	Endoplasmic Reticulum	127

[000222] In some embodiments, protein targeting to the nucleus is through a nuclear localization sequence (also known as a nuclear localization signal) that enables translocation through the nuclear envelope via nuclear pore complexes. The nuclear pore complex is composed of nucleoporins. Nucleoporins interact with transport molecules known as karyopherins. Karyophins bind to proteins containing a nuclear localization sequence and transport the protein across the nuclear pore complex. In some embodiments a nuclear localization sequence consists of one or more short (e.g., <50 amino-acid residues) sequence of basic amino acids. In some embodiments a nuclear localization sequence consists of one or more short (e.g., <50 amino-acid residues) sequence of lysines or arginines. In some embodiments the nuclear localization sequence is monopartitie or bipartite. In some embodiments the nuclear localization sequence is at the N or C terminus of a protein payload agent. In some embodiments the nuclear localization sequence is in the middle of the amino acid sequence of a protein payload agent and is exposed on the protein

surface. In some embodiments a nuclear localization sequence is recognized by a karyophin. In some embodiment the nuclear localization sequence interacts with one or more karyopherin. In some embodiments the karyophin recognizes a nuclear localization sequence as it emerges from a ribosome. In some embodiments the karyophin recognizes a nuclear localization sequence on a fully translated protein.

[000223] In some embodiments, a nuclear protein payload agent comprises an in-frame fusion of a protein of interest to the coding sequence of a nuclear protein, or an in-frame fusion of a protein of interest to the nuclear localization sequence of a protein.

[000224] In some embodiments a nuclear localization sequence is appended to the N or C terminus of a protein (e.g., nuclear protein). In some embodiments the protein is a natural protein. In some embodiments the nuclear protein is a synthetic protein.

[000225] In some embodiments a nuclear localization sequence is selected from Table 6-1. In some embodiments a nuclear localization sequence comprises a sequence selected from Table 6-1. In some embodiments a nuclear localization sequence of Table 6-1 may be appended to the N-terminus of a protein, e.g., a nuclear protein. In some embodiments a signal sequence of Table 6-1 may be appended to the C-terminus of a protein, e.g., a nuclear protein. A person of ordinary skill will appreciate that the signal sequences below are not limited for use with their respective naturally associated proteins. In some embodiments, the nuclear localization sequence is defined as the nuclear localization sequence from the proteins listed in Table 6 of US 2015-0246139, which is incorporated by reference herein. In some embodiments, the nuclear localization signal has the sequence of amino acids set forth in any one of SEQ ID NOS: 128-507 and 605-626.

Table 6-1: Exemplary Nuclear Localization Sequences

Signal	**Reported Protein(s)**	**Localization**	**SEQ ID NO**
TAKRS	Yap1	Nucleus	128
KKKGSKTS	Yap1	Nucleus	129
GKISKHWTGI	PLSCR1	Nucleus	130

SYNDFGNYNN QSSNFGPMKG GNFGGRSSGP YGGGGQYFAK PRNQGGYGG	hnRNP A1	Nucleus	131
SNFGPMKGGN FGGRSSGPY	hnRNP A1	Nucleus	132
PPTKKIR	NUCKS	Nucleus	133
KNQKQGKGKT CKKGKK	SA2L (L for longer isoform)	Nucleus	134
KRKRGRPGRP PSTNKKPRK	SA1/ STAG1	Nucleus	135
RKRR	TWIST	Nucleus	136
KRGKK	TWIST	Nucleus	137
RKKRR	Daxx	Nucleus	138
KKSRKEKK	Daxx	Nucleus	139
RSGGNHRRNG RGGRGGYNRR NNGYHPY	Hrp1	Nucleus	140
SKKRSLED	Ty1 Integrase	Nucleus	141
PPRSKKRI	Ty1 Integrase	Nucleus	142
KSAKISKPLH	Ste12	Nucleus	143
KNKEISMP	Ste12	Nucleus	144
KKMIEEKKAV LKRKMK	OCR-2	Nucleus	145
NFNHIHKRIR RVADKYLSG	PI4K230	Nucleus	146
CNKRKYSLN	NF-AT	Nucleus	147
GKRKK	NF-AT	Nucleus	148
EED	cytoskeletal	Nucleus	149

	red cell protein 4.1R		
KKKRER	cytoskeletal red cell protein 4.1R	Nucleus	150
EEKRKR	NF-kB p65	Nucleus	151
GDRRAAPARP	Maize R protein	Nucleus	152
MISEALRKAI GKR	Maize R protein	Nucleus	153
MSERKRREKL	Maize R protein	Nucleus	154
GGGx{3}KNR Rx{6}RGGRN	Nab2	Nucleus	155
GNLDIQRPKR KRKNSRVTFS EDDEI	NIPP1	Nucleus	156
INPEDVDPSV GRFRNMVQTA VVPVKKKRVE G	NIPP1	Nucleus	157
GSGQIQLWQF LLELLSDSAN ASCITWEGTN GEFKMTDPDE VARRWGERKS KPNMNYDKLS RALRYYYDKN IMTKVHGKRY AYKF	Fli1	Nucleus	158
HRKYEAPRHx {6}PRKR	L3	Nucleus	159
HSAELALNVI TKKRKGYEDD	Cyclin H	Nucleus	160
DYVSKKSKHE EEEWTDDDLV ESL	Cyclin H	Nucleus	161
KGRKR	IGFBP-3	Nucleus	162
KGVKRKADTT TP	BET family	Nucleus	163
KILKKRHI	cGMP-PK	Nucleus	164

KKIR	PSR	Nucleus	165
KKYR	PSR	Nucleus	166
KKKKRK	BRD2	Nucleus	167
KKQALKEKEL GNDAYKKK	mSTI1/ Hop	Nucleus	168
KKSTALSREL GKIMRRR	Mcm10	Nucleus	169
KKKTVINDLL HYKKEK	Mcm10	Nucleus	170
KKx{15}KKR K	DNAhelicaseQ1	Nucleus	171
KNLEAIVSVI AERNKKK	5-lipoxygenase	Nucleus	172
KRKx{11}KK KSKK	hpoly(ADP)poly	Nucleus	173
KRKx{22}KE LQKQITK	HIV-1	Nucleus	174
KRKVEDPYC	HIV-1 motif	Nucleus	611
KRKKKPYC	HIV-1 motif	Nucleus	612
KRQQLKENNK LRR	dHSF	Nucleus	175
KRVAKRKLIE QNRERRRK	Thyroid hormone receptor (TR)	Nucleus	176
KRx{9}KTKK	NP of Thogoto virus	Nucleus	177
KSKKQK	Chicken v-rel	Nucleus	178
KSRKRKL	v-jun	Nucleus	179
KTLETVPLER KKREK	RNP B1	Nucleus	180
KVLKKRRL	IL-1a	Nucleus	181
Kx{2}Kx{16 }Kx{2}K	Ror1	Nucleus	182
KIYKG HLYLPGMDHA	Ror1 motif	Nucleus	613

QLVAIKTLK			
"Lx{30}R{1 ,3}x{55}R{ 1,3}"	cytotoxic ribonuclease	Nucleus	183
NKIPIKDLLN PQ	MATa2	Nucleus	184
MEQRGQNAPA ASGARKRHGP GPREARGARP GPRVPKTLVL VVAAVLLLVS AESA	TNFRSF10B	Nucleus	185
MGTLYARHVN AGSTGPIKST IRIYFKPKHV KAWIPRPPRL CQYEKAKNVN FQPSGVTTTR QSI	VP1 capsid protein (CVB3)	Nucleus	186
CNGCRNMLLY PRGATNVCCA LC	PsLSD1	Nucleus	187
CANCRTTLMY PYGAPSVKCA VC	PsLSD1	Nucleus	188
CGGCRTLLMY TRGATSVRCS CC	PsLSD1	Nucleus	189
MVQLRPRASR	NOL7	Nucleus	190
KRFKRRWMVR KMKTKK	NOL7	Nucleus	191
RRDKTLLKEK RKRREELFIE QKKRK	NOL7	Nucleus	192
NYKKPKL	hFGF-1a/ b	Nucleus	193
PDGKRKRK	TR?1	Nucleus	194
PEEVKKRKKA V	Cofilin	Nucleus	195
PKKKSRKSS	Basonuclin	Nucleus	196
PKRRTARKQL PKR	h DNA ligase I	Nucleus	197

PLKRKKL	CDC6	Nucleus	198
PPx{2}R	Sam68	Nucleus	199
PPTVR	Sam68	Nucleus	614
PRPR	bovine herpesvirus 1 VP22	Nucleus	200
REKKKFLKRR	Mx1 (mouse)	Nucleus	201
REKKRQFEMK RKLH	phosphatase inhibitor 2 (Inh2)	Nucleus	202
RESGKKRKRK RLKPT	PDGF-A	Nucleus	203
RKCSQTQCPR KVIK	PML	Nucleus	204
RKRKLPCDTP GQGLTCSGEK RRR	RAC3	Nucleus	205
RKRPVRRR	FGF	Nucleus	206
RKx{10}RKx {10}KK	glucocorticoid receptor	Nucleus	207
RLKKLKCSKx {19}KTKR	GAL4	Nucleus	208
RLKKLK CSKEKPKCAK CLKNNWECRY SPKTKR	GAL4	Nucleus	615
RLRRDAGGRG GVYEHLGGAP RRRK	FGF3	Nucleus	209
GKGRPRRGFK TRRTQKSS	FGF3	Nucleus	210
RRERx{4}RP RKIPR	BDV-P	Nucleus	211
RRGLKR	SRF	Nucleus	212
RRKRQR	Dorsal	Nucleus	213

RRKRR	TTF-1	Nucleus	214
RRRGL	Angiogenin	Nucleus	215
RRRR	EBV gp110	Nucleus	216
RSRARSASLG TTTQGWDPPP LRRPSRARRR	cytomegalovirus kinase pUL97	Nucleus	217
RGGRKRPLRP PLVSLARTPL CRRR	cytomegalovirus kinase pUL97	Nucleus	218
RVTIRTVRVR RPPKGKHRK	PDGF-B	Nucleus	219
[ST]Px{1}K R[LI]	Cdc6	Nucleus	220
SGVVLPSTGV VKSSGGGGGG DSDHSDLEAS VVKEAESSRV VDPEKRPRKR GRKPANG	CrMYC2	Nucleus	221
DVKIIGREAM IRVQSSKNNH PAARVMGALK DLDLEL	CrMYC2	Nucleus	222
GREEPLNHVE AERQRREKLN QRFYALRAVV PNVSKMDKAS LLGDAISYIN ELKAK	CrMYC2	Nucleus	223
SHAAGSKQHE SIPGKAKKPK VKKKEKGKKE KGKKKEAPH	H2RSP	Nucleus	224
SKLCLGWLWG M	Hepatitis B virus core protein	Nucleus	225
TKKQKT	prothymosin alpha	Nucleus	226
VRILESWFAK NIENPYLDT	Matalpha2	Nucleus	227
VRVNVKREYD HMNGSRESPV DCSVSKCNKL VGGGEANPMN	Fli1	Nucleus	228

YNSYMDEKNG PPPPNMTTNE RRVIV			
YHYGLLTCES CKGFFKRTVQ NQKRYTCIEN QNCQIDKTQR KRCPYCRFKK CI	LRH1	Nucleus	229
DVGMKLEAVR ADRMRGGRNK FGPMYKRDRA LKQQKK	LRH1	Nucleus	230
YNDFGNYNNQ SSNFGPMKGG NFGGRSSGPY	hnRNP A1	Nucleus	231
LRLTLLELVR RLNGNG	Borna Disease Virus p10	Nucleus	232
RRRKVEAGRT KLAHFRQRKT KGDSSHSEKK TAKRK	Pericentrin	Nucleus	233
RRRHIVRKRT LRR	EGFR/ ErbB2/ ErbB3/ ErbB4	Nucleus	234
KRRQQKIRKY TMRR	ErbB2	Nucleus	235
RRWKEELDEE LQRKR	NPK1	Nucleus	236
KKLKKKK	SMAD1	Nucleus	237
KKYIDGQKKK CGEERRRV	IL-5	Nucleus	238
RKRKL	SRC-3	Nucleus	239
KRRR	SRC-3	Nucleus	240
KRSAEGGNPP KPLKKLR	p110RB1	Nucleus	241
PKKLRPP	IGFBP-2	Nucleus	242
RRx{26}RKG FYKRKQCKPS	IGFBP-5	Nucleus	243

RGRKRG			
RRHMEASL QELKASPRMV PRAVYLPNCD RKGFYKRKQC KPSRGRKRG	IGFBP-5 motif	Nucleus	616
EGKVTKRKHD NEGSGSKRPK V	Ku70	Nucleus	244
Rx{11}HRGF YRKRQCRSSQ GQRRG	IGFBP-6	Nucleus	245
R GAQTLYVPNC DHRGFYRKRQ CRSSQGQRRG	IGFBP-6	Nucleus	617
PEKKKEKQEK K	UL84	Nucleus	246
GRKKRRQRRR A	HIV-1 Tat	Nucleus	247
MRKTKLAPT	FMDV 3D	Nucleus	248
SHSSMAKRAK KESQDFI	RecQ5	Nucleus	249
AVEGKPLSRA AQLRERRKDL HVSGKPSPRY ALKKRALEAK KNK	CT311	Nucleus	250
SGYGKVSRRG GHQNSYKPY	hnRNP D	Nucleus	251
RDRGYDKADR EEGKERRHHR REELAPY	PQBP-1	Nucleus	252
GEGERPAQNE KRKEKNIKRG GNRFEPYANP TKR	hnRNP M	Nucleus	253
FKSSQEEVRS YSDPPLKFMS VQRPGPY	hnRNP F	Nucleus	254

SPS	Egr-1	Nucleus	255
[RK]x{2}Lx {1}[VY]x{2 }[VI]x{1}[KR]x{3}[KR]	Asr1p (recognized by five yeast importins)	Nucleus	256
KKKRKR	Ire1p	Nucleus	257
KRGSRGGKKG RK	Ire1p	Nucleus	258
KKGR	Ire1p	Nucleus	259
KHSQSRR	Pin1	Nucleus	260
KNRRAKCRQQ R	Crx	Nucleus	261
KPNKRK	HDGF	Nucleus	262
KRRAGDLLED SPKRPK	HDGF	Nucleus	263
KRRK	UL80 Proteins	Nucleus	264
RARKRLK	UL80 Proteins	Nucleus	265
KRRRER	CMV pAP	Nucleus	266
KARKRLK	CMV pAP	Nucleus	267
MEEAVTMAPA AVSSAVVGDP MEYNAILRRK LEEDLE	Opaque2 (O2)	Nucleus	268
MPTEERVRKK ESNRESARRS RYRKAAHLKE L	Opaque2 (O2)	Nucleus	269
KRKGDEVDGV DEVAKKKSKK	PARP	Nucleus	270
GKKNQKHKLK M	plant potyviral NIa	Nucleus	271
KRKGTTRGMG AKSRKFINMY GFDPTDFSYI	plant potyviral NIa	Nucleus	272

KKQRYMEIQE TLKK	NPR1	Nucleus	273
KRK	AIRE (Autoimmune regulator)/ MSH6	Nucleus	274
FKRKHKKDIS QNKRAVRR	HSP70	Nucleus	275
KRKx{8}KKx {1}K	VP1 capsid protein (simian virus 40)	Nucleus	276
MAPKRRRRRK	VP1 capsid protein (simian virus 40) motif	Nucleus	618
NVRQLLSGEQ GADILMVQEA GSLPSSAVRT SRVIQHGGTP IEEYTWNLGT RSRPNMVYIY YSRLDVGANR VNLAIVS	CDTB	Nucleus	277
KKRR	MSH6/ BRCA2	Nucleus	278
RKRKR	MSH6	Nucleus	279
KRPR	hEXO1	Nucleus	280
RKQVSSHIQV LARRKLR	TEA/ ATTS protein scalloped	Nucleus	281
KRCFKKGAR	BPV-1 E2	Nucleus	282
KCYRFRVKKN HRHR	BPV-1 E2	Nucleus	283
RPRK	HPV type 16 E6	Nucleus	284
KQRHLDKKQR	HPV type 16 E7	Nucleus	285
KCLKFYSK	HPV type 16 E8	Nucleus	286
KRKKRK	HPV16 L1	Nucleus	287
KRKx{12}KR	HPV16 L1	Nucleus	288

KRKKRK	HPV16 L1 motif	Nucleus	619
LKCLRYRFKK H	HPV16 E2	Nucleus	289
KRPRSPSS	EBNA-1	Nucleus	290
KRKVLGSSQN SSGSEASETP VKRRK	bovine papillomavirus type 1 E1	Nucleus	291
PDRKRLRK	U69 protein kinase (HHV-6)	Nucleus	292
PRGSGPRRAA ST	VP19C (HSV-1)	Nucleus	293
RRKTTPSYSG QYRTARR	ORF9 (VZV)	Nucleus	294
ARRPSCSPEQ HGGKVARLQP PPTKAQPA	ICP27 (HSV-1 type 1)	Nucleus	295
RGGRRGRRRG RGRGG	ICP27 (HSV-1 type 1)	Nucleus	296
PAAKRVKLD	c-myc	Nucleus	297
RQRRNELKRS	c-myc	Nucleus	298
MHGDTPTLHE YMLDLQPETT DLYCYEQLSD SSEEEDE	HPV16 E7	Nucleus	299
MRHKRSAKRT KR	HPV16 L2	Nucleus	300
RKRRKR	HPV16 L2	Nucleus	301
IASRRGLVRY SRIGQRGSMH	L2 (human papillomavirus type 6B)	Nucleus	302
MNKIPIKDLL NPQ	repressor alpha 2	Nucleus	303
VRILESWFAK NIENPY	repressor alpha 3	Nucleus	304

GRRKAAKRKW AAQ	Nuclear myosin I (NM1)	Nucleus	305
KKRKRR	Nk2.2	Nucleus	306
RYKMKRAR	Nk2.2	Nucleus	307
RGRKSSGFPK SVKSR	5-lipoxygenase (5-LO)	Nucleus	308
LRDGRAKLAR D	5-lipoxygenase	Nucleus	309
KCHKDLPR	5-lipoxygenase	Nucleus	310
ARKRKPSP	IRF2BP2	Nucleus	311
REPADLEMNL TVPVRR	CDT	Nucleus	312
RRTQISSDHF PVGVSRR	CDT	Nucleus	313
RSKR	BLM	Nucleus	314
RRPALRSPPL GTRKRK	ICP22	Nucleus	315
DIPPRPKRAR VNLR	ICP22	Nucleus	316
PIKKK	BRCA2	Nucleus	317
GSIIRKWN	hPLSCR4	Nucleus	318
KRKx{18}KR RKx{3}KRR	BPV1 E1	Nucleus	319
FDYTPNSKRQ RCGM	U27 (HHV-7)	Nucleus	320
KKKKKEEEGE GKKK	act/ inh betaA	Nucleus	321
KRPRP	adenovE1a	Nucleus	322
RGRRRRQR	Amida	Nucleus	323
RKRRR	Amida	Nucleus	324
PPRIYPQLPS APT	BDV-P	Nucleus	325

PRPRKIPR	BDV-P	Nucleus	326
HSHKKKKIRT SPTFRRPKTL RLRRQPKYPR KSAPRRNKLD HY	BIB	Nucleus	327
PKKNRLRR	BRCA1	Nucleus	328
KRKRRP	BRCA1	Nucleus	329
RRRx{11}KR RK	CBP80	Nucleus	330
RRRHSDEN DGGQPHKRRK	CBP80 motif	Nucleus	620
SKRVAKRKL	c-erb-A	Nucleus	331
RRERNKMAAA KCRNRRR	cFOS	Nucleus	332
KRMRNRIAAS KCRKRKL	cJUN	Nucleus	333
PLLKKIKQ	c-myb	Nucleus	334
PAKRARRG	VP1 (CPV)	Nucleus	335
KVNSRKRRKE VPGPNGATEE D	CTP	Nucleus	336
LKDVRKRKLG PGH	DNAseEBV	Nucleus	337
YKRPCKRSFI RFI	DNAseEBV	Nucleus	338
KKEKKKSKK	dyskerin	Nucleus	339
GKKRSKA	H2B	Nucleus	340
RAIKRRPGLD FDDDGEGNSK FLR	ARNT	Nucleus	341
RKRKKMPASQ RSKRRKT	BLM	Nucleus	342
KRPACTLKPE CVQQLLVCSQ	cytidine deaminase	Nucleus	343

EAKK			
PRGRRQPIPK ARQP	HCV	Nucleus	344
PRRGPR	HCV	Nucleus	345
KKSKKGRQEA LERLKKA	DNA pol alpha (human)	Nucleus	346
RKEWLTNFME DRRQRKL	Topo II (human)	Nucleus	347
KKQTTLAFKP IKKGKKR	Topo II (human)	Nucleus	348
RKCLQAGMNL EARKTKK	Glucocorticoid	Nucleus	349
GKKKYKLKH	HIV-1	Nucleus	350
KKKYKLK	HIV1422	Nucleus	351
KSKKKAQ	HIV1423	Nucleus	352
RQARRNRRRR WR	HIV-1Rev	Nucleus	353
KRAAEDDEDD DVDTKKQK	prothymosin alpha	Nucleus	354
RRKGKEK	Huntington disease protein	Nucleus	355
RRSMKRK	hVDR	Nucleus	356
RKRAFHGDDP FGEGPPDKK	ICP-8	Nucleus	357
MAPSAKATAA KKAVVKGTNG KKALKVRTSA TFRLPKTLKL AR	L25	Nucleus	358
KTRKHRG	L29 (ribosomal protein)	Nucleus	359
KHRKHPG	L29	Nucleus	360

KKKKRKREK	LEF-1	Nucleus	361
NQSSNFGPMK GGNFGGRSSG PYGGGGQYFA KPRNQGGY	M9	Nucleus	362
YNNQSSNFGP MKGGN	M9	Nucleus	363
MNKIPIKDLL NPG	Mat-alpha	Nucleus	364
PQSRKKLR	Max	Nucleus	365
KRQRx{20}K KSKK	Mitosin	Nucleus	366
VNEAFETLKR C	MyoD	Nucleus	367
CKRKTTNADR RKA	MyoD	Nucleus	368
QRKRQK	NF-kB	Nucleus	369
HRIEEKRKRT YETFKSI	NF-kB	Nucleus	370
PPQKKIKS	N-myc	Nucleus	371
Sx{1}GTKRS Yx{2}M	NPI-1	Nucleus	372
TKRSx{3}M	NPI-3	Nucleus	373
PPRKKRTVV	NS5A	Nucleus	374
KRKKEMANKS APEAKKKK	Nucleolin	Nucleus	375
KRx{10}KKK L	Nucleoplasmin	Nucleus	376
KRPAATKKAG QAKKKK	Nucloplasmin	Nucleus	377
PQPKKKP	p53-(NLS2)	Nucleus	378
KRx{7,9}PQ PKKKP	p53-(NLS1)	Nucleus	379
KRALPN NTSSSPQPKK KP	p53-(NLS1) motif	Nucleus	621
RIRKKLR	p54	Nucleus	380

LKRKLQR	Pax-QNR	Nucleus	381
RRMKWKK	PDX-1	Nucleus	382
KVTKNKS	Pho4	Nucleus	383
KRRGKPGP	Pho4	Nucleus	384
PYLNKRKGKP	Pho4p	Nucleus	385
PKKARED	polyoma virus large-T	Nucleus	386
VSRKRPR	polyoma virus large-T	Nucleus	387
APKRKSGVSK C	polyomaVP1	Nucleus	388
EEDGPQKKKR RL	polyomaVP2	Nucleus	389
TEKK[QG]KS ILYD	Hsc3 (proteasomal)	Nucleus	390
REKKEKEQKE K	Hsc9 (proteasomal)	Nucleus	391
LEKKVKKKFD W	Hsc iota (proteasomal)	Nucleus	392
YLTQETNKVE TYKEQPLKTP GKKKKGKP	PTHrP	Nucleus	393
RVHPYQR	QKI-5	Nucleus	394
SDREDGNYCP PVKRERTS	RanBP3	Nucleus	395
KKKRERLD	RCP	Nucleus	396
MPKTRRRPRR SQRKRPPT	HTLV-1 Rex	Nucleus	397
PRRRK	SOX9	Nucleus	398
KRPMNAFMVW AQAARRK	SOX9	Nucleus	399
RPRRK	SRY	Nucleus	400
KRPMNAFIVW SRDQRRK	SRY	Nucleus	401

APTKRKGS	SV40(VP1)	Nucleus	402
PNKKKRK	SV40(VP2)	Nucleus	403
PKKKRKV	SV40 large T antigen	Nucleus	404
SDKKVRSRLI E	alpha (Ta)	Nucleus	405
KKKRRSREK	TCF-1	Nucleus	406
RKRIREDRKA TTAQKVQQMK QRLNENERKR KR	TCPTP	Nucleus	407
KDCVINKHHR NRCQYCRLQR	TR2	Nucleus	408
GRKRKKRT	Tst1/ Oct6	Nucleus	409
PKRPRDRHDG ELGGRKRARG	VirD2 (C-terminal)	Nucleus	410
EYLSRKGKLE L	VirD2 (N-terminal)	Nucleus	411
KAKRQR	v-Rel	Nucleus	412
IKYFKKFPKD	yeast SKI3	Nucleus	413
RKRKK	yeast DNApol alpha	Nucleus	414
MASQGTKRSY EQM	vRNPs (influenza A)	Nucleus	415
KRGINDRNFW RGENGRKTR	vRNPs (influenza A)	Nucleus	416
RRMLGAPLRQ RRVR	SOCS1	Nucleus	417
VRTTKGKRKR IDV	Lamin L1	Nucleus	418
RSSRGKRRRI E	Lamin B2	Nucleus	419
KRKx{9}KIR	HSF2 (human)	Nucleus	420
KRK VSSSKPEENK IR	HSF2 (human) motif	Nucleus	622
KRKRx{9}KK	HSF2 (human)	Nucleus	421

KRKRPL LLNTNGAQKK	HSF2 (human) motif	Nucleus	623
RLRKx{10}R KFKK	Progesterone receptor (human)	Nucleus	422
RKDRRGGRML KHKRQRDDGE GRGEVGSAGD MRAANLWPSP LMIKRSKK	Estrogen receptor (human)	Nucleus	423
KRx{11}KRS R	hnRNP K	Nucleus	424
KRPAEDMEEE QAFKRSR	hnRNP K motif	Nucleus	624
RQKRx{13}K RRSSPSKDGR	FHF-1	Nucleus	425
RQKRQARESN SDRVSASKRR SSPSKDGR	FHF-1 motif	Nucleus	625
KRx{11}KKT R	NO38	Nucleus	426
KRKx{8}KKA KV	xnf7	Nucleus	427
KRKLEE PEPEPKKAKV	xnf7 motif	Nucleus	626
RKKRKx{12} KKSK	N1/ N2	Nucleus	428
KKx{10}RKR GRPRK	SWI5	Nucleus	429
KKx{11}RLR KK	TGA-1A	Nucleus	430
KKRARx{11} RQRKK	TGA-1B	Nucleus	431
KLRx{12}RR EIQKR	VirE2	Nucleus	432
RAIKTKx{7} KLKSK	VirE2	Nucleus	433
RKx{10}RKL KK	Androgen receptor	Nucleus	434
GKRQVLIRP	S22	Nucleus	435
GIIKPISKH	S25	Nucleus	436

KKRK	CUL4B	Nucleus	437
KKLKK	Smad3	Nucleus	438
DNPGKKRKST DDNEGPSPKR RVIT	DNA polymerase (AcMNPV)	Nucleus	439
CSVKRKRDDD	DNA polymerase (AcMNPV)	Nucleus	440
SKPFSCGRSG KGHKRKTPFG NTERKTPVKK L	MSL1	Nucleus	441
KRELELLKHN PKRRKIT	USP22	Nucleus	442
RRRK	Calpain 5	Nucleus	443
YIFEVKKPED EVLICIQQRP KRSTRREG	Calpain 5	Nucleus	444
MADAFEVHYI ADWADISSEY PKLHILTTSG RVLSVLAELS	Muscovy duck reovirus p10.8	Nucleus	445
MMPSSVSWGI LLAGLCCLVP VSLA	α-1-antitrypsin	Nucleus	446
MAGPATQSPM KLMALQLLLW HSALWTVQEA	G-CSF	Nucleus	447
MQRVNMIMAE SPSLITICLLGY LLSAECTVFLD HENANKILNRP KR	Factor IX	Nucleus	448
MKGSLLLLLV	Prolactin	Nucleus	449

SNLLLCQSVAP			
MKWVTFISLLF LFSSAYSRG VFRR	Albumin	Nucleus	450
MWWRLWWLL LLLLLLPMWA	HMMSP38	Nucleus	451
MLFNLRILLNN AAFRNGHNFM VRNFRCGQPLQ	ornithine carbamoyl-transferase	Nucleus	452
MSVLTPLLLRG LTGSARRLPVP RAKIHSL	Cytochrome C Oxidase subunit 8A	Nucleus	453
MSVLTPLLLRG LTGSARRLPVP RAKIHSL	Cytochrome C Oxidase subunit 8A	Nucleus	454
MVTKITLSPQN FRIQKQETTLL KEKSTEKNSLA KSILAVKNHFI ELRSKLSERFIS HKNT	Type III, bacterial	Nucleus	455
MLSFVDTRTLL LLAVTSCLATCQ	Viral source	Nucleus	456
MGSSQAPRMG SVGGHGLMAL LMAGLILPGILA	Viral source	Nucleus	457
MAGIFYFLFSF LFGICD	Viral source	Nucleus	458

MENRLLRVFL VWAALTMDG ASA	Viral source	Nucleus	459
MARQGCFGSY QVISLFTFAIGV NLCLG	Viral source	Nucleus	460
MSRLPVLLLLQ LLVRPGLQ	Bacillus source	Nucleus	461
MKQQKRLYAR LLTLLFALIFLL PHSSASA	Bacillus source	Nucleus	462
MATPLPPPSPR HLRLLRLLLSG	Secretion signal	Nucleus	463
MKAPGRLVLII LCSVVFS	Secretion signal	Nucleus	464
MLQLWKLLCG VLT	Secretion signal	Nucleus	465
MLYLQGWSM PAVA	Secretion signal	Nucleus	466
MDNVQPKIKH RPFCFSVKGHV KMLRLDIINSL VTTVFMLIVSV LALIP	Secretion signal	Nucleus	467
MPCLDQQLTV HALPCPAQPSS LAFCQVGFLTA	Secretion signal	Nucleus	468
MKTLFNPAPAI	Secretion signal	Nucleus	469

ADLDPQFYTLS DVFCCNESEAE ILTGLTVGSAADA			
MKPLLVVFVF LFLWDPVLA	Secretion signal	Nucleus	470
MSCSLKFTLIVI FFTCTLSSS	Secretion signal	Nucleus	471
MVLTKPLQRN GSMMSFENVK EKSREGGPHA HTPEEELCFVV THTPQVQTTL NLFFHIFKVLT QPLSLLWG	Secretion signal	Nucleus	472
MATPPFRLIRK MFSFKVSRWM GLACFRSLAAS	Secretion signal	Nucleus	473
MSFFQLLMKR KELIPLVVFMT VAAGGASS	Secretion signal	Nucleus	474
MVSALRGAPLI RVHSSPVSSPS VSGPAALVSCL SSQSSALS	Secretion signal	Nucleus	475
MMGSPVSHLL AGFCVWVVLG	Secretion signal	Nucleus	476
MASMAAVLT WALALLSAFS ATQA	Secretion signal	Nucleus	477

MVLMWTSGD AFKTAYFLLK GAPLQFSVCGL LQVLVDLAILG QATA	Secretion signal	Nucleus	478
MDFVAGAIGG VCGVAVGYPL DTVKVRIQTEP LYTGIWHCVR DTYHRERVWG FYRGLSLPVCT VSLVSS	Secretion signal	Nucleus	479
MEKPLFPLVPL HWFGFGYTAL VVSGGIVGYV KTGSVPSLAA GLLFGSLA	Secretion signal	Nucleus	480
MGLLLPLALCI LVLC	Secretion signal	Nucleus	481
MGIQTSPVLLA SLGVGLVTLL GLAVG	Secretion signal	Nucleus	482
MSDLLLLGLIG GLTLLLLLTLL AFA	Secretion signal	Nucleus	483
METVVIVAIGV LATIFLASFAA LVLVCRQ	Secretion signal	Nucleus	484
MAGSVECTWG	Secretion signal	Nucleus	485

WGHCAPSPLL LWTLLLFAAPF GLLG			
MMPSRTNLAT GIPSSKVKYSR LSSTDDGYIDL QFKKTPPKIPY KAIALATVLFLIGA	Secretion signal	Nucleus	486
MALPQMCDGS HLASTLRYCM TVSGTVVLVA GTLCFA	Secretion signal	Nucleus	487
MKKWFVAAGI GAGLLMLSSAA	Vrg-6	Nucleus	488
MKQSTIALALL PLLFTPVTKA	PhoA	Nucleus	489
MKKTAIAIAV ALAGFATVAQA	OmpA	Nucleus	490
MKKLMLAIFFS VLSFPSFSQS	STI	Nucleus	491
MKKNIAFLLAS MFVFSIATNAYA	STII	Nucleus	492
MFAKRFKTSL LPLFAGFLLLF HLVLAGPAAAS	Amylase	Nucleus	493
MRFPSIFTAVL FAASSALA	Alpha Factor	Nucleus	494
MRFPSIFTTVL	Alpha Factor	Nucleus	495

FAASSALA			
MRFPSIFTSVLF AASSALA	Alpha Factor	Nucleus	496
MRFPSIFTHVL FAASSALA	Alpha Factor	Nucleus	497
MRFPSIFTIVLF AASSALA	Alpha Factor	Nucleus	498
MRFPSIFTFVLF AASSALA	Alpha Factor	Nucleus	499
MRFPSIFTEVL FAASSALA	Alpha Factor	Nucleus	500
MRFPSIFTGVL FAASSALA	Alpha Factor	Nucleus	501
MRSSPLLRSAV VAALPVLALA	Endoglucanase V	Nucleus	502
MGAAAVRWH LCVLLALGTR GRL	Secretion Signal	Nucleus	503
MRSSLVLFFVS AWTALA	Fungal source	Nucleus	504
MLRGPGPGRL LLLAVLCLGTS VRCTETGKSKR	Fibronectin	Nucleus	505
MLRGPGPGLL LLAVQCLGTA VPSTGA	Fibronectin	Nucleus	506

MRRGALTGLL LVLCLSVVLR AAPSATSKKRR	Fibronectin	Nucleus	507

[000226] In some embodiments the nuclear protein payload agent localizes a target protein or target nucleic acid to the nucleus. In some embodiments the nuclear protein payload agent is composed of protein containing a nuclear localization sequence and a target binding domain. In some embodiments the target binding domain is an antibody, a nanobody, an scFv, or any other protein binding domain. In some embodiments the target binding domain is a nucleic acid binding domain. In some embodiments the nuclear protein payload agent increases localization in the nucleus of the target protein or target nucleic acid over a cell that does not have the nuclear protein payload agent.

[000227] In some embodiments a mitochondrial or peroxisome localization sequence is selected from Table 6-2. In some embodiments a nuclear localization sequence comprises a sequence selected from Table 6-2. In some embodiments a mitochondrial or peroxisome localization sequence of Table 6-2 may be appended to the N-terminus of a protein, e.g., a mitochondrial or peroxisome protein. In some embodiments a signal sequence of Table 6-2 may be appended to the C-terminus of a protein, e.g., a mitochondrial or peroxisome protein. A person of ordinary skill will appreciate that the signal sequences below are not limited for use with their respective naturally associated proteins.

Table 6-2: Exemplary mitochondrial or peroxisomal localization sequences

Signal Sequence	**Naturally Associated Protein**	**Location Associated Protein is Directed**	**SEQ ID NO**
GRV	PaxG	Peroxisomes	508
SKV	CSS1 and Gibberellin regulated family protein	Peroxisomes	509

SLM	EDA8/ MEE46 and Aspartyl protease family protein	Peroxisomes	510
SKL	Cit1p/ MLYCD	Peroxisomes	511
SKI	Hydoxyacid oxidase 1/ catalase (H. polymorpha)	Peroxisomes	512
HRM	IPP isomerase(rat)	Peroxisomes	513
SRL	PMvK/ PEPCK/ Urate oxidase	Peroxisomes	514
CKL	HMG-CoA	Peroxisomes	515
NKL	DAS	Peroxisomes	516
ARF	MOX	Peroxisomes	517
AKL	SCPx/ pre-nsLTP	Peroxisomes	518
SQL	AGT (Alanine--glyoxylate aminotransferase)	Peroxisomes	519
QKL	AA-CoA thiolase	Peroxisomes	520
ANL	Rat liver catalase	Peroxisomes	521
AKI	HDE	Peroxisomes	522
SSL	gPGK	Peroxisomes	523
Kx{6}[IL]x {1}[LFI]LK [LFI]	PEX2	Peroxisomes	524

KVFFSRLIQI LKIMVPRTFC	PMP70	Peroxisomes	525
MTVPYLNSNR NVASYLQSNS	Cit2p	Peroxisomes	526
SVx{5}QL	HMG-CoA synthase/ MPPD	Peroxisomes	527
MNGDQNSDVY AQEKQDFVQH	FPP	Peroxisomes	528
MHRLQVVLGH LAGRPESSSA LQAAPC	3-ketoacyl-CoA thiolase B	Peroxisomes	529
EALREALQHC PKNKL	SPT	Peroxisomes	530
LSSNSKF	Catalase A	Peroxisomes	531
GFATKFYTEE GNLDWVYNNT PVFFI	Catalase A	Peroxisomes	532
MERLRQIASQ ATAASA	amine oxidase	Peroxisomes	533
KANL	human catalase	Peroxisomes	534
RPSI	cottonseed catalase (Ccat)	Peroxisomes	535
APKI	PpPox1p	Peroxisomes	536
MDLLPPKPKY	p46Shc/ p66Shc	Mitochondria	537

NPLRNESLSS LEEGASGSTP PE			
MSFFNISRRF YSQIVKKSVK IKR	GlyRS	Mitochondria	538
MFAAVARRTA TGTLPGAVTL FGRKSFEGYY RDYPLHPRNF GVMASLF	TgSOD2	Mitochondria	539
LVPRYRGLFH HISKLDGGVR FL	MLYCD	Mitochondria	540
MIFPVARYAL RWLRRPEDRA FSRAAMEMAL RGVR	Top3alpha	Mitochondria	541
MAGRLLGKAL AAVSLSLALA SVTIRSSRCR GIQAFRNSFS SSWFHL	NUDT9	Mitochondria	542
PNNSGFIYQQ HGGQQKRARF DHQ	HCF	Mitochondria	543
MKSFITRNKT AI	70 kD mitochondrial outer membrane protein	Mitochondria	544
MFRMLAKASV	SPTm/ glyoxylate	Mitochondria	545

TLGSRAASWV RNM	aminotransferase 1 (AGT)		
MFSNLSKRWA QRTLSKSFYS TATGAASKSG KLTQKLVTAG VAAAGITAST LLYADSLTAE A	cytochrome c1	Mitochondria	546
MLSSSFERN	SCS-beta	Mitochondria \ Hydrogenosome	547
MAFWGWRAAA ALRLWGRVVE RVEAGGGVGP FQACGCRLVL GGRDDVSAGL RGSHGARGEP LDP	SIRT3	Mitochondria	548
MRRMPRCGIR LPLLRPS	PARG	Mitochondria	549
MVDRGLGRHL WRLTRRGPPA AADAVAPRPL MGFYEAATQN QADCQLWALL RRGLTTASTL R	UL12 (herpes virus) or UL12.5	Mitochondria	550
MAFLRSMWGV LSALGRSGAE LCTGCGSRLR	TFAM	Mitochondria	551

SPFSFVYLPR WF			
HSLLPALCDS KIRSKALGSD HCPITLYLAL	APE1	Mitochondria	552
AIRILVCEGS GLLAAANDIL AARAQRPAAR GSTSGGESRL RGERARP	VP22	Mitochondria	553
RGFWANLMQI LRDITGVTAI CSFLYTKLGI APFGDPVTMF	GGNNV	Mitochondria	554
MRTVFSQIPR FKQVNQYIRM	Cdc17	Mitochondria	555
MAAACRSVKG L	human 17bHSD10/ SCHAD	Mitochondria	556
MISASRAAAA RLVGTAASRS PAAARPQDGW NGLSHEAFRF VSRRDY	PBP74	Mitochondria	557
RHFWADSKSS	Omb (Outer mitochondrial membrane cytochrome b5)	Mitochondria	558
MTVPYLNSNR	Cit2p	Mitochondria	559

NVASYLQSNS			
MYRYLAKALL PSRAGPAALG SAANHSAALL GRGRGQPAAA SQPGLALAAR RHY	hGDH	Mitochondria	560
MFIRNFVNII GSQKSITKTI ARNYFSDNSK LIIPRHG	PfHslV	Mitochondria	561
MLRFTNCSCK TFVKSSYKLN IRRMNSSFRT ETDAFGEIHV PADKYWGAQT QRSFQNFKIG GARERMPLPL VHAFGVLKKS AAIVNESLGG LD	fumarase	Mitochondria	562
RHSTRTQHLS VETSY	BCS1	Mitochondria	563
VVRVTRTWVP KGL	CYP1A1	Mitochondria	564
MLATRALSLI GKRAISTSVC LRAHGSVVKS	CYP2E1	Mitochondria	565
VRGHPKSRGN FPP	cytochrome P4502B1	Mitochondria	566

MLARGLPLRS ALVKACPPIL STVGEGWGHH RVGTGEGAG	CYP11A1	Mitochondria	567
FSRSFSTPLP LSTKTLISLS PPHRTFAVRA	GR	Mitochondria	568
TRTWVPKGLK SPP	P450MT2	Mitochondria	569
VTHGYNVQRY NQLRKNFGFY RAYSSLRGCK SVFRRI	Hmi1p	Mitochondria	570
MEFIYIYMNF FFFFSVGRKF KKFNKVRVVR	PR-M (Progesterone Receptor)	Mitochondria	571
MVGRNSAIAA GVCGALFIGY CIYFDRKRRS DPNFK	Tom 20	Mitochondria	572
LLRLIGLTTI FSATALGFLA HKRGLLVRV	monoamine oxidase B	Mitochondria	573
MALLRGVFIV AAKRTP	3-oxoacyl-CoA thiolase	Mitochondria	574
MSTPSIVIAS ARTA	bacterial thiolase	Mitochondria	575

KTLLSLALVG ACITLGAYLG HK	Bcl-2	Mitochondria	576
MKSFITRNKT AILATVAATG TAIGAYYYY	Tom 70/ Mas70p	Mitochondria	577
MLSLRQSIRF FK	cytochrome c oxidase subunit IV	Mitochondria	578
MGRTVVVLGG GISGLAASYH LSRAPCPP	PPOX	Mitochondria	579
MLKYKPLLKI SKNCEAAILR ASKTRLNTIR AYGSTVPKSK SFEQDSRKRT QSWTALRVGA ILAATSSVAY LNWHNGQIDN	cytochrome b2	Mitochondria	580
CLRRSWNSVT YFQNIGRISL	KMO	Mitochondria	581
MVLPRLYTAT SRAAFKAAKQ SAPLLST	F1-ATPase beta-subunit	Mitochondria	582
MQRSIFARF	delta-aminolevulinate synthase	Mitochondria	583

MLSNLRILLN KAALRKAHTS MVRNFRYGKP VQ	OTC	Mitochondria	584
MATSASSHLS KAIKHMYMKL PQG	GS	Mitochondria	585
VAVHNFLNEG AWNEIVEWER RFGKGLM	CCHL	Mitochondria	586
PKDMTPKAAL LQVLGRINSK YATEPPFDRH DWYV	CCHL	Mitochondria	587
ESRKGQERFN RWFLTGMTVA GVVLLGSLFS RK	Bcl-x(L)	Mitochondria	588
MANLMMRLPI SLRSFSVSAS SS	AtSrx	Mitochondria \ Chloroplast	589
MWRCGGRQGL GVLRR	MP1	Mitochondria	590
MAVFRSGLLV LTTPLASLAP RLASILTSAA	CoAsy	Mitochondria	591
RALAHGVRVL	HCV	Mitochondria	592
MRSYEFHLAR	Protein A (AHNV)	Mitochondria	593

MSGATLCVVT GYRLLTSKWL ADRIEDYRQR VI			
RPTGFWENLK RILRDITGIT ALCGFLYNKL GMAPFG	Protein A (AHNV)	Mitochondria	594
TELLVGIATV SGCGAVVYCI SK	Protein A (FHV)	Mitochondria	595
RYSLKVLLSY HGWMFAEHGK MSRST	CPT1	Mitochondria	596
MSVLTPLLL RGLTGSARR LPVPRAKIHSL	Cytochrome C Oxidase subunit 8A	Mitochondria	597

[000228] *Membrane Protein Payload Agents*

[000229] In some embodiments a membrane protein payload agent is a protein (or a nucleic acid that encodes it) that is naturally found on a membrane surface of a cell (e.g., on a surface of a plasma membrane).

[000230] Exemplary membrane proteins (and/or nucleic acids encoding them) can be found, for example, in U.S. Patent Publication No. 2016/0289674, the contents of which are hereby incorporated by reference. In some embodiments, a membrane protein payload agent (and/or a nucleic acid that encodes it) has a sequence as set forth in any one of SEQ ID NOs: 8144-16131 of U.S. Patent Publication No. 2016/0289674, or in a functional fragment tereof. In some embodiments, a membrane protein payload agent is a plasma membrane protein (nucleic acid

encoding it) as set forth in any one of SEQ ID NOs: 8144-16131 of U.S. Patent Publication No. 2016/0289674, or a fragment, variant, or homolog thereof (or nucleic acid that encodes it) of a plasma membrane protein of.

[000231] In some embodiments, a membrane protein relevant to the present disclosure is a therapeutic membrane protein. In some embodiments, a membrane protein relevant to the present disclosure is or comprises a cell surface receptor, a membrane transport protein (e.g., an active or passive transport protein such as, for example, an ion channel protein, a pore-forming protein [e.g., a toxin protein], etc), a membrane enzyme, and/or a cell adhesion protein).

[000232] In some embodiments a membrane protein is a single spanning membrane protein. In some embodiments a single-spanning membrane protein may assume a final topology with a cytoplasmic N- and an exoplasmic C-terminus (N_{cyt}/C_{exo}) or with the opposite orientation (N_{exo}/C_{cyt}).

[000233] In some embodiments a membrane protein is a Type I membrane protein comprising an N-terminal cleavable signal sequence and stop-transfer sequence (N_{exo}/C_{cyt}). In some embodiments a signal is at the C terminus. In some embodiments the N-terminal cleavable signal sequence targets nascent peptide to the ER. In some embodiments an N-terminal cleavable signal sequence comprises a hydrophobic stretch of typically 7–15 predominantly apolar residues. In some embodiments a Type I membrane protein comprises a stop-transfer sequence which halts the further translocation of the polypeptide and acts as a transmembrane anchor. In some embodiments a stop transfer sequence comprises an amino acid sequence of about 20 hydrophobic residues. In some embodiments the N-terminus of the Type I membrane protein is extracellular and the C-terminus is cytoplasmic. In some embodiments a Type I membrane protein may be a glycophorin or an LDL receptor.

[000234] In some embodiments a membrane protein is a Type II membrane protein comprising a signal-anchor sequence (N_{cyt}/C_{exo}). In some embodiments a signal is at the C terminus. In some embodiments a signal-anchor sequence is responsible for both insertion and anchoring of a Type II membrane protein. In some embodiments a signal-anchor sequence comprises about 18-25 predominantly apolar residues. In some embodiments a signal-anchor sequence lacks a signal peptidase cleavage site. In some embodiments a signal-anchor sequence

may be positioned internally within a polypeptide chain. In some embodiments a signal-anchor sequence induces translocation of the C-terminal end of a protein across a cell membrane. In some embodiments the C-terminus of the Type II membrane protein is extracellular and the N-terminus is cytoplasmic. In some embodiments a Type II membrane protein may be a transferrin receptor or a galactosyl transferase receptor.

[000235] In some embodiments a membrane protein is a Type III membrane protein comprising a reverse signal-anchor sequence (N_{exo}/C_{cyt}). In some embodiments a signal is at the N terminus. In some embodiments a reverse signal-anchor sequence is responsible for both insertion and anchoring of a Type III membrane protein. In some embodiments a reverse signal-anchor sequence comprises about 18-25 predominantly apolar residues. In some embodiments a signal-anchor sequence lacks a signal peptidase cleavage site. In some embodiments a signal-anchor sequence may be positioned internally within a polypeptide chain. In some embodiments a signal-anchor sequence induces translocation of the N-terminal end of a protein across a cell membrane. In some embodiments the N-terminus of the Type III membrane protein is extracellular and the C-terminus is cytoplasmic. In some embodiments a Type I membrane protein may be a synaptogamin, neuregulin, or cytochrome P-450.

[000236] In some embodiments, Type I, Type II, or Type III membrane proteins are inserted into a cell membraned via a cellular pathway comprising SRP, SRP receptor and Sec61 translocon.

[000237] In some embodiments a membrane protein is predominantly exposed to cytosol and anchored to a membrane by a C-terminal signal sequence, but which does not interact with an SRP. In some embodiments a protein is cytochrome b_5, or a SNARE protein (e.g., synaptobrevin).

[000238] In some embodiments a membrane protein payload agent comprises a signal sequence which localizes the payload membrane protein to the cell membrane. In some embodiments a membrane protein payload agent is a nucleic acid wherein the nucleic acid encodes a signal sequence which localizes a payload membrane protein encoded by the nucleic acid to the cell membrane.

(i) Integrin Membrane Protein Payloads

[000239] In some embodiments, a membrane protein payload agent is or compromises an

integrin or functional fragment, variant, or homolog thereof, or a nucleic acid encoding it. In some embodiments a membrane protein payload agent is or compromises an integrin selected from Table 7, or functional fragment, variant, or homolog thereof, or a nucleic acid encoding it. In embodiments, a membrane protein payload agent comprises a protein having a sequence at least least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99% identical to the polypeptide sequence of a protein of Table 7, or a nucleic acid encoding the same. In embodiments, the membrane protein payload agent comprises a nucleic acid having a sequence at least least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99% identical to the nucleic acid sequence of a gene of Table 7.

Table 7: Exemplary integrin proteins

UniProt ID	Entry name	Gene names
P05556	ITB1_HUMAN	ITGB1 FNRB MDF2 MSK12
P05106	ITB3_HUMAN	ITGB3 GP3A
P06756	ITAV_HUMAN	ITGAV MSK8 VNRA VTNR
P05107	ITB2_HUMAN	ITGB2 CD18 MFI7
P17301	ITA2_HUMAN	ITGA2 CD49B
P23229	ITA6_HUMAN	ITGA6
P26006	ITA3_HUMAN	ITGA3 MSK18
P16144	ITB4_HUMAN	ITGB4
P08514	ITA2B_HUMAN	ITGA2B GP2B ITGAB
P08648	ITA5_HUMAN	ITGA5 FNRA
P13612	ITA4_HUMAN	ITGA4 CD49D
P20701	ITAL_HUMAN	ITGAL CD11A
P11215	ITAM_HUMAN	ITGAM CD11B CR3A
P26010	ITB7_HUMAN	ITGB7
P20702	ITAX_HUMAN	ITGAX CD11C
P56199	ITA1_HUMAN	ITGA1
Q9UKX5	ITA11_HUMAN	ITGA11 MSTP018
P18084	ITB5_HUMAN	ITGB5
Q13683	ITA7_HUMAN	ITGA7 UNQ406/PRO768
P53708	ITA8_HUMAN	ITGA8

UniProt ID	Entry name	Gene names
P18564	ITB6_HUMAN	ITGB6
P26012	ITB8_HUMAN	ITGB8
P38570	ITAE_HUMAN	ITGAE
O75578	ITA10_HUMAN	ITGA10 UNQ468/PRO827
Q13349	ITAD_HUMAN	ITGAD
Q13797	ITA9_HUMAN	ITGA9

(ii) Ion Channel Proteins

[000240] In some embodiments, a membrane protein payload agent is or compromises an ion channel protein or functional fragment, variant, or homolog thereof, or a nucleic acid encoding it. In some embodiments a membrane protein payload agent is or compromises an ion channel protein selected from Table 8, or functional fragment, variant, or homolog thereof, or a nucleic acid encoding it. In embodiments, a membrane protein payload agent comprises a protein having a sequence at least least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99% identical to the polypeptide sequence of a protein of Table 8, or a nucleic acid encoding the same. In embodiments, the membrane protein payload agent comprises a nucleic acid having a sequence at least least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99% identical to the nucleic acid sequence of a gene of Table 8.

Table 8: Exemplary ion channel proteins

Uniprot ID	Entry name	Gene names
P46098	5HT3A_HUMAN	HTR3A 5HT3R HTR3
O95264	5HT3B_HUMAN	HTR3B
Q8WXA8	5HT3C_HUMAN	HTR3C
Q70Z44	5HT3D_HUMAN	HTR3D
A5X5Y0	5HT3E_HUMAN	HTR3E
P02708	ACHA_HUMAN	CHRNA1 ACHRA CHNRA
P11230	ACHB_HUMAN	CHRNB1 ACHRB CHRNB
Q07001	ACHD_HUMAN	CHRND ACHRD
Q04844	ACHE_HUMAN	CHRNE ACHRE

Uniprot ID	Entry name	Gene names
P07510	ACHG_HUMAN	CHRNG ACHRG
P78348	ASIC1_HUMAN	ASIC1 ACCN2 BNAC2
Q16515	ASIC2_HUMAN	ASIC2 ACCN ACCN1 BNAC1 MDEG
Q9UHC3	ASIC3_HUMAN	ASIC3 ACCN3 SLNAC1 TNAC1
Q96FT7	ASIC4_HUMAN	ASIC4 ACCN4
Q9NY37	ASIC5_HUMAN	ASIC5 ACCN5 HINAC
P37088	SCNNA_HUMAN	SCNN1A SCNN1
P51168	SCNNB_HUMAN	SCNN1B
P51172	SCNND_HUMAN	SCNN1D DNACH
P51170	SCNNG_HUMAN	SCNN1G
P48048	KCNJ1_HUMAN	KCNJ1 ROMK1
P78508	KCJ10_HUMAN	KCNJ10
Q14654	KCJ11_HUMAN	KCNJ11
Q14500	KCJ12_HUMAN	KCNJ12 IRK2 KCNJN1
Q9UNX9	KCJ14_HUMAN	KCNJ14 IRK4
Q99712	KCJ15_HUMAN	KCNJ15 KCNJ14
Q15842	KCNJ8_HUMAN	KCNJ8
Q9UGQ2	FLOWR_HUMAN	CACFD1 C9orf7 PSEC0107 PSEC0248 UNQ3071/PRO9903
Q12791	KCMA1_HUMAN	KCNMA1 KCNMA SLO
Q8NEC5	CTSR1_HUMAN	CATSPER1
Q96P56	CTSR2_HUMAN	CATSPER2
Q86XQ3	CTSR3_HUMAN	CATSPER3
Q7RTX7	CTSR4_HUMAN	CATSPER4
P29973	CNGA1_HUMAN	CNGA1 CNCG CNCG1
Q96S66	CLCC1_HUMAN	CLCC1 KIAA0761 MCLC
P35523	CLCN1_HUMAN	CLCN1 CLC1
P51788	CLCN2_HUMAN	CLCN2

Uniprot ID	Entry name	Gene names
P51800	CLCKA_HUMAN	CLCNKA
P51801	CLCKB_HUMAN	CLCNKB
O00299	CLIC1_HUMAN	CLIC1 G6 NCC27
O15247	CLIC2_HUMAN	CLIC2
O95833	CLIC3_HUMAN	CLIC3
Q9Y696	CLIC4_HUMAN	CLIC4
Q9NZA1	CLIC5_HUMAN	CLIC5
Q96NY7	CLIC6_HUMAN	CLIC6 CLIC1L
P51797	CLCN6_HUMAN	CLCN6 KIAA0046
Q494W8	CRFM7_HUMAN	CHRFAM7A
Q16281	CNGA3_HUMAN	CNGA3 CNCG3
Q8IV77	CNGA4_HUMAN	CNGA4
Q14028	CNGB1_HUMAN	CNGB1 CNCG2 CNCG3L CNCG4 RCNC2
Q9NQW8	CNGB3_HUMAN	CNGB3
Q16280	CNGA2_HUMAN	CNGA2 CNCA CNCA1 CNCG2
P48549	KCNJ3_HUMAN	KCNJ3 GIRK1
P48051	KCNJ6_HUMAN	KCNJ6 GIRK2 KATP2 KCNJ7
Q92806	KCNJ9_HUMAN	KCNJ9 GIRK3
P48544	KCNJ5_HUMAN	KCNJ5 GIRK4
P14867	GBRA1_HUMAN	GABRA1
P47869	GBRA2_HUMAN	GABRA2
P34903	GBRA3_HUMAN	GABRA3
P48169	GBRA4_HUMAN	GABRA4
P31644	GBRA5_HUMAN	GABRA5
Q16445	GBRA6_HUMAN	GABRA6
P18505	GBRB1_HUMAN	GABRB1
P47870	GBRB2_HUMAN	GABRB2
P28472	GBRB3_HUMAN	GABRB3
O14764	GBRD_HUMAN	GABRD
P78334	GBRE_HUMAN	GABRE
Q8N1C3	GBRG1_HUMAN	GABRG1

Uniprot ID	Entry name	Gene names
P18507	GBRG2_HUMAN	GABRG2
Q99928	GBRG3_HUMAN	GABRG3
O00591	GBRP_HUMAN	GABRP
P24046	GBRR1_HUMAN	GABRR1
P28476	GBRR2_HUMAN	GABRR2
A8MPY1	GBRR3_HUMAN	GABRR3
Q9UN88	GBRT_HUMAN	GABRQ
P42261	GRIA1_HUMAN	GRIA1 GLUH1 GLUR1
P42262	GRIA2_HUMAN	GRIA2 GLUR2
P42263	GRIA3_HUMAN	GRIA3 GLUR3 GLURC
P48058	GRIA4_HUMAN	GRIA4 GLUR4
Q9ULK0	GRID1_HUMAN	GRID1 KIAA1220
O43424	GRID2_HUMAN	GRID2 GLURD2
P39086	GRIK1_HUMAN	GRIK1 GLUR5
Q13002	GRIK2_HUMAN	GRIK2 GLUR6
Q13003	GRIK3_HUMAN	GRIK3 GLUR7
Q16099	GRIK4_HUMAN	GRIK4 GRIK
Q16478	GRIK5_HUMAN	GRIK5 GRIK2
Q05586	NMDZ1_HUMAN	GRIN1 NMDAR1
Q12879	NMDE1_HUMAN	GRIN2A NMDAR2A
Q13224	NMDE2_HUMAN	GRIN2B NMDAR2B
Q14957	NMDE3_HUMAN	GRIN2C NMDAR2C
O15399	NMDE4_HUMAN	GRIN2D GluN2D NMDAR2D
Q8TCU5	NMD3A_HUMAN	GRIN3A KIAA1973
O60391	NMD3B_HUMAN	GRIN3B

Uniprot ID	Entry name	Gene names
P23415	GLRA1_HUMAN	GLRA1
P23416	GLRA2_HUMAN	GLRA2
O75311	GLRA3_HUMAN	GLRA3
Q5JXX5	GLRA4_HUMAN	GLRA4
P48167	GLRB_HUMAN	GLRB
P51790	CLCN3_HUMAN	CLCN3
P51793	CLCN4_HUMAN	CLCN4
P51795	CLCN5_HUMAN	CLCN5 CLCK2
P51798	CLCN7_HUMAN	CLCN7
O15554	KCNN4_HUMAN	KCNN4 IK1 IKCA1 KCA4 SK4
O60928	KCJ13_HUMAN	KCNJ13
Q9NPI9	KCJ16_HUMAN	KCNJ16
B7U540	KCJ18_HUMAN	KCNJ18
P63252	KCNJ2_HUMAN	KCNJ2 IRK1
P48050	KCNJ4_HUMAN	KCNJ4 IRK3
Q9GZZ6	ACH10_HUMAN	CHRNA10 NACHRA10
Q15822	ACHA2_HUMAN	CHRNA2
P32297	ACHA3_HUMAN	CHRNA3 NACHRA3
P43681	ACHA4_HUMAN	CHRNA4 NACRA4
P30532	ACHA5_HUMAN	CHRNA5 NACHRA5
Q15825	ACHA6_HUMAN	CHRNA6
P36544	ACHA7_HUMAN	CHRNA7 NACHRA7
Q9UGM1	ACHA9_HUMAN	CHRNA9 NACHRA9
P17787	ACHB2_HUMAN	CHRNB2
Q05901	ACHB3_HUMAN	CHRNB3
P30926	ACHB4_HUMAN	CHRNB4
O00180	KCNK1_HUMAN	KCNK1 HOHO1 KCNO1 TWIK1
P57789	KCNKA_HUMAN	KCNK10 TREK2
Q9HB15	KCNKC_HUMAN	KCNK12
Q9HB14	KCNKD_HUMA	KCNK13

Uniprot ID	Entry name	Gene names
	N	
Q9H427	KCNKF_HUMAN	KCNK15 TASK5
Q96T55	KCNKG_HUMAN	KCNK16 TALK1
Q96T54	KCNKH_HUMAN	KCNK17 TALK2 TASK4 UNQ5816/PRO19634
Q7Z418	KCNKI_HUMAN	KCNK18 TRESK TRIK
O95069	KCNK2_HUMAN	KCNK2 TREK TREK1
O14649	KCNK3_HUMAN	KCNK3 TASK TASK1
Q9NYG8	KCNK4_HUMAN	KCNK4 TRAAK
O95279	KCNK5_HUMAN	KCNK5 TASK2
Q9Y257	KCNK6_HUMAN	KCNK6 TOSS TWIK2
Q9Y2U2	KCNK7_HUMAN	KCNK7
Q9NPC2	KCNK9_HUMAN	KCNK9 TASK3
Q5JUK3	KCNT1_HUMAN	KCNT1 KIAA1422
Q6UVM3	KCNT2_HUMAN	KCNT2 SLICK
A8MYU2	KCNU1_HUMAN	KCNU1 KCNMA3 KCNMC1 SLO3
Q09470	KCNA1_HUMAN	KCNA1
Q16322	KCA10_HUMAN	KCNA10
P16389	KCNA2_HUMAN	KCNA2
P22001	KCNA3_HUMAN	KCNA3 HGK5
P22459	KCNA4_HUMAN	KCNA4 KCNA4L
P22460	KCNA5_HUMAN	KCNA5
P17658	KCNA6_HUMAN	KCNA6
Q96RP8	KCNA7_HUMAN	KCNA7
Q14721	KCNB1_HUMAN	KCNB1
Q92953	KCNB2_HUMAN	KCNB2
P48547	KCNC1_HUMAN	KCNC1
Q96PR1	KCNC2_HUMAN	KCNC2
Q14003	KCNC3_HUMAN	KCNC3
Q03721	KCNC4_HUMAN	KCNC4 C1orf30

Uniprot ID	Entry name	Gene names
Q9NSA2	KCND1_HUMAN	KCND1
Q9NZV8	KCND2_HUMAN	KCND2 KIAA1044
Q9UK17	KCND3_HUMAN	KCND3
P15382	KCNE1_HUMAN	KCNE1
A0A087WTH5	KCE1B_HUMAN	KCNE1B
Q9Y6J6	KCNE2_HUMAN	KCNE2
Q9Y6H6	KCNE3_HUMAN	KCNE3
Q8WWG9	KCNE4_HUMAN	KCNE4
Q9UJ90	KCNE5_HUMAN	KCNE5 AMMECR2 KCNE1L
Q9H3M0	KCNF1_HUMAN	KCNF1
Q9UIX4	KCNG1_HUMAN	KCNG1
Q9UJ96	KCNG2_HUMAN	KCNG2 KCNF2
Q8TAE7	KCNG3_HUMAN	KCNG3
Q8TDN1	KCNG4_HUMAN	KCNG4 KCNG3
O95259	KCNH1_HUMAN	KCNH1 EAG EAG1
Q12809	KCNH2_HUMAN	KCNH2 ERG ERG1 HERG
Q9ULD8	KCNH3_HUMAN	KCNH3 KIAA1282
Q9UQ05	KCNH4_HUMAN	KCNH4
Q8NCM2	KCNH5_HUMAN	KCNH5 EAG2
Q9H252	KCNH6_HUMAN	KCNH6 ERG2
Q9NS40	KCNH7_HUMAN	KCNH7 ERG3
Q96L42	KCNH8_HUMAN	KCNH8
P51787	KCNQ1_HUMAN	KCNQ1 KCNA8 KCNA9 KVLQT1
O43526	KCNQ2_HUMAN	KCNQ2
O43525	KCNQ3_HUMAN	KCNQ3
P56696	KCNQ4_HUMAN	KCNQ4
Q9NR82	KCNQ5_HUMAN	KCNQ5
Q96KK3	KCNS1_HUMAN	KCNS1
Q9ULS6	KCNS2_HUMAN	KCNS2 KIAA1144
Q9BQ31	KCNS3_HUMAN	KCNS3
Q6PIU1	KCNV1_HUMAN	KCNV1

Uniprot ID	Entry name	Gene names
Q8TDN2	KCNV2_HUMAN	KCNV2
O60741	HCN1_HUMAN	HCN1 BCNG1
Q9UL51	HCN2_HUMAN	HCN2 BCNG2
Q9P1Z3	HCN3_HUMAN	HCN3 KIAA1535
Q9Y3Q4	HCN4_HUMAN	HCN4
Q92952	KCNN1_HUMAN	KCNN1 SK
Q9H2S1	KCNN2_HUMAN	KCNN2
Q9UGI6	KCNN3_HUMAN	KCNN3 K3
P35498	SCN1A_HUMAN	SCN1A NAC1 SCN1
Q9Y5Y9	SCNAA_HUMAN	SCN10A
Q9UI33	SCNBA_HUMAN	SCN11A SCN12A SNS2
Q99250	SCN2A_HUMAN	SCN2A NAC2 SCN2A1 SCN2A2
Q9NY46	SCN3A_HUMAN	SCN3A KIAA1356 NAC3
P35499	SCN4A_HUMAN	SCN4A
Q14524	SCN5A_HUMAN	SCN5A
Q01118	SCN7A_HUMAN	SCN7A SCN6A
Q9UQD0	SCN8A_HUMAN	SCN8A MED
Q15858	SCN9A_HUMAN	SCN9A NENA
Q07699	SCN1B_HUMAN	SCN1B
O60939	SCN2B_HUMAN	SCN2B UNQ326/PRO386
Q9NY72	SCN3B_HUMAN	SCN3B KIAA1158
Q8IWT1	SCN4B_HUMAN	SCN4B
Q8IZF0	NALCN_HUMAN	NALCN VGCNL1
Q9ULQ1	TPC1_HUMAN	TPCN1 KIAA1169 TPC1
Q8NHX9	TPC2_HUMAN	TPCN2 TPC2
P54289	CA2D1_HUMAN	CACNA2D1 CACNL2A CCHL2A MHS3
Q9NY47	CA2D2_HUMAN	CACNA2D2 KIAA0558
Q8IZS8	CA2D3_HUMAN	CACNA2D3
Q7Z3S7	CA2D4_HUMAN	CACNA2D4
Q13936	CAC1C_HUMAN	CACNA1C CACH2 CACN2 CACNL1A1 CCHL1A1

Uniprot ID	Entry name	Gene names
Q01668	CAC1D_HUMAN	CACNA1D CACH3 CACN4 CACNL1A2 CCHL1A2
O60840	CAC1F_HUMAN	CACNA1F CACNAF1
Q13698	CAC1S_HUMAN	CACNA1S CACH1 CACN1 CACNL1A3
Q02641	CACB1_HUMAN	CACNB1 CACNLB1
Q08289	CACB2_HUMAN	CACNB2 CACNLB2 MYSB
P54284	CACB3_HUMAN	CACNB3 CACNLB3
O00305	CACB4_HUMAN	CACNB4 CACNLB4
Q00975	CAC1B_HUMAN	CACNA1B CACH5 CACNL1A5
O00555	CAC1A_HUMAN	CACNA1A CACH4 CACN3 CACNL1A4
Q15878	CAC1E_HUMAN	CACNA1E CACH6 CACNL1A6
O43497	CAC1G_HUMAN	CACNA1G KIAA1123
O95180	CAC1H_HUMAN	CACNA1H
Q9P0X4	CAC1I_HUMAN	CACNA1I KIAA1120
Q96D96	HVCN1_HUMAN	HVCN1 VSOP UNQ578/PRO1140
Q14722	KCAB1_HUMAN	KCNAB1 KCNA1B
Q13303	KCAB2_HUMAN	KCNAB2 KCNA2B KCNK2
O43448	KCAB3_HUMAN	KCNAB3 KCNA3B
Q5VU97	CAHD1_HUMAN	CACHD1 KIAA1573 VWCD1
Q401N2	ZACN_HUMAN	ZACN L2 LGICZ LGICZ1 ZAC

(iii) Pore Forming Proteins

[000241] In some embodiments, a membrane protein payload agent is or compromises a pore forming protein or functional fragment, variant, or homolog thereof, or a nucleic acid encoding it. In some embodiments, a pore forming protein may be a hemolysin or functional fragment, variant, or homolog thereof, or a nucleic acid encoding it. In some embodiments a membrane protein payload agent is or compromises a hemolysin selected from Table 9, or functional fragment, variant, or homolog thereof, or a nucleic acid encoding it. In some embodiments, a pore forming protein may be a colicin or functional fragment, variant, or homolog thereof, or a nucleic acid encoding it. In some embodiments a membrane protein payload agent is or compromises a colicin selected from Table 10, or functional fragment, variant, or homolog thereof, or a nucleic acid encoding it.

[000242] In embodiments, a membrane protein payload agent comprises a protein having a sequence at least least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99% identical to the polypeptide sequence of a protein of Table 9, or a nucleic acid encoding the same. In embodiments, the membrane protein payload agent comprises a nucleic acid having a sequence at least least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99% identical to the nucleic acid sequence of a gene of Table 9.

[000243] In embodiments, a membrane protein payload agent comprises a protein having a sequence at least least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99% identical to the polypeptide sequence of a protein of Table 10, or a nucleic acid encoding the same. In embodiments, the membrane protein payload agent comprises a nucleic acid having a sequence at least least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99% identical to the nucleic acid sequence of a gene of Table 10.

Table 9: Exemplary hemolysin proteins

Uniprot ID	Entry name	Gene names
P19247	VVHA_VIBVU	vvhA VV2_0404
P09545	HLYA_VIBCH	hlyA VC_A0219
Q08677	HLY4_AERSA	ash4
P55870	HLY1_AERHH	ahh1 AHA_1512
Q4UK99	HLYC_RICFE	tlyC RF_1185
Q68W10	HLYC_RICTY	tlyC RT0725
O05961	HLYC_RICPR	tlyC RP740
A8GTI4	HLYC_RICRS	tlyC A1G_06280
Q92GI2	HLYC_RICCN	tlyC RC1141
A8F2M1	HLYC_RICM5	tlyC RMA_1168
Q93RR6	HLYE_SALPA	hlyE clyA sheA SPA1306
Q9REB3	HLYE_ECO57	hlyE clyA sheA Z1944 ECs1677
Q8Z727	HLYE_SALTI	hlyE clyA sheA STY1498 t1477
P77335	HLYE_ECOLI	hlyE clyA hpr sheA ycgD b1182 JW5181
P14711	HLYT_GRIHO	
A8GUH1	HLYC_RICB8	tlyC A1I_00305
A8EZU0	HLYC_RICCK	tlyC A1E_04760

Uniprot ID	Entry name	Gene names
A8GPR9	HLYC_RICAH	tlyC A1C_05795
Q1RGX2	HLYC_RICBR	tlyC RBE_1311
Q9RCT3	HLYEL_SHIFL	SF1171 S1259
Q8FI27	HLYEL_ECOL6	c1630
P28030	HLY_VIBMI	tdh
P28031	HLY1_GRIHO	tdh
P19249	HLY1_VIBPA	tdh1 tdh VPA1378
P19250	HLY2_VIBPA	tdh2 tdh trh VPA1314
P28029	HLY3_VIBPH	tdh3 tdh/I tdhX

Table 10: Exemplary colicin proteins

Uniprot ID	Entry name	Gene names
Q47500	CE05_ECOLX	cfa
Q47125	CE10_ECOLX	cta
P04480	CEA_CITFR	caa
Q47108	CEA_ECOLX	caa
P02978	CEA1_ECOLX	cea
P21178	CEA1_SHISO	cea
P04419	CEA2_ECOLX	col ceaB
P00646	CEA3_ECOLX	ceaC
P18000	CEA5_ECOLX	col
P17999	CEA6_ECOLX	
Q47112	CEA7_ECOLX	colE7 cea
P09882	CEA8_ECOLX	col
P09883	CEA9_ECOLX	col cei
P05819	CEAB_ECOLX	cba
P00645	CEAC_ECOLX	ccl
P17998	CEAD_ECOLX	cda
Q47502	CEAK_ECOLX	cka
P08083	CEAN_ECOLX	cna
P06716	CEIA_ECOLX	cia

Uniprot ID	Entry name	Gene names
P04479	CEIB_ECOLX	cib
P22520	CVAB_ECOLX	cvaB
Q06583	PYS1_PSEAI	pys1
Q06584	PYS2_PSEAE	pys2 PA1150

(iv) Toll-Like Receptors

[000244] In some embodiments, a membrane protein payload agent is or compromises a toll-like receptor (TLR) or functional fragment, variant, or homolog thereof, or a nucleic acid encoding it. In some embodiments a membrane protein payload agent is or compromises a toll-like receptor selected from Table 11, or functional fragment, variant, or homolog thereof, or a nucleic acid encoding it. In embodiments, a membrane protein payload agent comprises a protein having a sequence at least least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99% identical to the polypeptide sequence of a protein of Table 11, or a nucleic acid encoding the same. In embodiments, the membrane protein payload agent comprises a nucleic acid having a sequence at least least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99% identical to the nucleic acid sequence of a gene of Table 11.

Table 11: Exemplary toll-like receptors

UniProt ID	Entry name	Gene names (primary)
Q86XR7	TCAM2_HUMAN	TICAM2
Q9BXR5	TLR10_HUMAN	TLR10
Q15399	TLR1_HUMAN	TLR1
O60603	TLR2_HUMAN	TLR2
O15455	TLR3_HUMAN	TLR3
O00206	TLR4_HUMAN	TLR4
O60602	TLR5_HUMAN	TLR5
Q9Y2C9	TLR6_HUMAN	TLR6
Q9NYK1	TLR7_HUMAN	TLR7
Q9NR97	TLR8_HUMAN	TLR8
Q9NR96	TLR9_HUMAN	TLR9

[000245] In some embodiments, a membrane protein payload agent is or compromises an interleukin receptor or functional fragment, variant, or homolog thereof, or a nucleic acid encoding it. In some embodiments a membrane protein payload agent is or compromises an interleukin receptor selected from Table 12 or functional fragment, variant, or homolog thereof, or a nucleic acid encoding it. In embodiments, a membrane protein payload agent comprises a protein having a sequence at least least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99% identical to the polypeptide sequence of a protein of Table 12, or a nucleic acid encoding the same. In embodiments, the membrane protein payload agent comprises a nucleic acid having a sequence at least least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99% identical to the nucleic acid sequence of a gene of Table 12.

(v) Interleukin Receptor Payloads

Table 12: Exemplary interleukin receptors

Uniprot ID	Entry name	Gene names (primary)
Q5EG05	CAR16_HUMAN	CARD16
Q92851	CASPA_HUMAN	CASP10
P25024	CXCR1_HUMAN	CXCR1
P25025	CXCR2_HUMAN	CXCR2
P31785	IL2RG_HUMAN	IL2RG
Q01167	FOXK2_HUMAN	FOXK2
Q8IU57	INLR1_HUMAN	IFNLR1
Q8IU54	IFNL1_HUMAN	IFNL1
Q8IZJ0	IFNL2_HUMAN	IFNL2
Q8IZI9	IFNL3_HUMAN	IFNL3
Q12905	ILF2_HUMAN	ILF2
Q12906	ILF3_HUMAN	ILF3
P01583	IL1A_HUMAN	IL1A
P01584	IL1B_HUMAN	IL1B
Q8WWZ1	IL1FA_HUMAN	IL1F10
Q9NPH3	IL1AP_HUMAN	IL1RAP
Q9NZN1	IRPL1_HUMAN	IL1RAPL1
P18510	IL1RA_HUMAN	IL1RN

Uniprot ID	Entry name	Gene names (primary)
P14778	IL1R1_HUMAN	IL1R1
P27930	IL1R2_HUMAN	IL1R2
P51617	IRAK1_HUMAN	IRAK1
Q5VVH5	IKBP1_HUMAN	IRAK1BP1
Q9Y616	IRAK3_HUMAN	IRAK3
Q9NWZ3	IRAK4_HUMAN	IRAK4
O43187	IRAK2_HUMAN	IRAK2
Q01638	ILRL1_HUMAN	IL1RL1
Q9HB29	ILRL2_HUMAN	IL1RL2
P22301	IL10_HUMAN	IL10
Q13651	I10R1_HUMAN	IL10RA
Q08334	I10R2_HUMAN	IL10RB
P20809	IL11_HUMAN	IL11
Q14626	I11RA_HUMAN	IL11RA
P42701	I12R1_HUMAN	IL12RB1
Q99665	I12R2_HUMAN	IL12RB2
P29459	IL12A_HUMAN	IL12A
P29460	IL12B_HUMAN	IL12B
P35225	IL13_HUMAN	IL13
P78552	I13R1_HUMAN	IL13RA1
Q14627	I13R2_HUMAN	IL13RA2
P40933	IL15_HUMAN	IL15
Q13261	I15RA_HUMAN	IL15RA
Q96F46	I17RA_HUMAN	IL17RA
Q9NRM6	I17RB_HUMAN	IL17RB
Q8NAC3	I17RC_HUMAN	IL17RC
Q8NFM7	I17RD_HUMAN	IL17RD
Q8NFR9	I17RE_HUMAN	IL17RE
Q16552	IL17_HUMAN	IL17A
Q9UHF5	IL17B_HUMAN	IL17B
Q9P0M4	IL17C_HUMAN	IL17C
Q8TAD2	IL17D_HUMAN	IL17D

Uniprot ID	Entry name	Gene names (primary)
Q96PD4	IL17F_HUMAN	IL17F
Q13478	IL18R_HUMAN	IL18R1
O95256	I18RA_HUMAN	IL18RAP
O95998	I18BP_HUMAN	IL18BP
P60568	IL2_HUMAN	IL2
P01589	IL2RA_HUMAN	IL2RA
P14784	IL2RB_HUMAN	IL2RB
Q9NYY1	IL20_HUMAN	IL20
Q9UHF4	I20RA_HUMAN	IL20RA
Q6UXL0	I20RB_HUMAN	IL20RB
Q9HBE4	IL21_HUMAN	IL21
Q9HBE5	IL21R_HUMAN	IL21R
Q9GZX6	IL22_HUMAN	IL22
Q8N6P7	I22R1_HUMAN	IL22RA1
Q969J5	I22R2_HUMAN	IL22RA2
Q5VWK5	IL23R_HUMAN	IL23R
Q9NPF7	IL23A_HUMAN	IL23A
Q9H293	IL25_HUMAN	IL25
Q9NPH9	IL26_HUMAN	IL26
Q6UWB1	I27RA_HUMAN	IL27RA
Q8NEV9	IL27A_HUMAN	IL27
Q14213	IL27B_HUMAN	EBI3
P08700	IL3_HUMAN	IL3
P26951	IL3RA_HUMAN	IL3RA
Q6EBC2	IL31_HUMAN	IL31
Q8NI17	IL31R_HUMAN	IL31RA
O95760	IL33_HUMAN	IL33
Q6ZMJ4	IL34_HUMAN	IL34
Q9UHA7	IL36A_HUMAN	IL36A
Q9NZH7	IL36B_HUMAN	IL36B
Q9NZH8	IL36G_HUMAN	IL36G
Q9UBH0	I36RA_HUMAN	IL36RN

Uniprot ID	Entry name	Gene names (primary)
Q9NZH6	IL37_HUMAN	IL37
P05112	IL4_HUMAN	IL4
P24394	IL4RA_HUMAN	IL4R
P05113	IL5_HUMAN	IL5
Q01344	IL5RA_HUMAN	IL5RA
P05231	IL6_HUMAN	IL6
P08887	IL6RA_HUMAN	IL6R
P40189	IL6RB_HUMAN	IL6ST
P13232	IL7_HUMAN	IL7
P16871	IL7RA_HUMAN	IL7R
P10145	IL8_HUMAN	CXCL8
P15248	IL9_HUMAN	IL9
Q01113	IL9R_HUMAN	IL9R
Q16649	NFIL3_HUMAN	NFIL3
Q99650	OSMR_HUMAN	OSMR
Q8NDX1	PSD4_HUMAN	PSD4
Q14005	IL16_HUMAN	IL16
Q6ZVW7	I17EL_HUMAN	IL17REL
O43353	RIPK2_HUMAN	RIPK2
Q6IA17	SIGIR_HUMAN	SIGIRR
Q8IUC6	TCAM1_HUMAN	TICAM1
Q86XR7	TCAM2_HUMAN	TICAM2
Q9Y4K3	TRAF6_HUMAN	TRAF6
P58753	TIRAP_HUMAN	TIRAP
Q15399	TLR1_HUMAN	TLR1
O60603	TLR2_HUMAN	TLR2
O60602	TLR5_HUMAN	TLR5
Q8TDR0	MIPT3_HUMAN	TRAF3IP1
Q13445	TMED1_HUMAN	TMED1
Q08881	ITK_HUMAN	ITK
Q9NP60	IRPL2_HUMAN	IL1RAPL2

(vi) Cell Adhesion Protein Payloads

[000246] In some embodiments, a membrane protein payload agent is or compromises a cell adhesion protein or functional fragment, variant, or homolog thereof, or a nucleic acid encoding it. In some embodiments a membrane protein payload agent is or compromises a cell adhesion protein selected from Table 13, or functional fragment, variant, or homolog thereof, or a nucleic acid encoding it. In some embodiments, a cell adhesion protein may be a cadherin or functional fragment, variant, or homolog thereof, or a nucleic acid encoding it. In some embodiments a membrane protein payload agent is or compromises a cadherin selected from Table 14, or functional fragment, variant, or homolog thereof, or a nucleic acid encoding it. In some embodiments, a cell adhesion protein may be a selectin or functional fragment, variant, or homolog thereof, or a nucleic acid encoding it. In some embodiments a membrane protein payload agent is or compromises a selectin selected from Table 15, or functional fragment, variant, or homolog thereof, or a nucleic acid encoding it. In some embodiments, a cell adhesion protein may be a mucin or functional fragment, variant, or homolog thereof, or a nucleic acid encoding it. In some embodiments a membrane protein payload agent is or compromises a mucin selected from Table 16, or functional fragment, variant, or homolog thereof, or a nucleic acid encoding it.

[000247] In embodiments, a membrane protein payload agent comprises a protein having a sequence at least least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99% identical to the polypeptide sequence of a protein of Table 13, or a nucleic acid encoding the same. In embodiments, the membrane protein payload agent comprises a nucleic acid having a sequence at least least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99% identical to the nucleic acid sequence of a gene of Table 13.

[000248] In embodiments, a membrane protein payload agent comprises a protein having a sequence at least least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99% identical to the polypeptide sequence of a protein of Table 14, or a nucleic acid encoding the same. In embodiments, the membrane protein payload agent comprises a nucleic acid having a sequence at least least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99% identical to the nucleic acid sequence of a gene of Table 14.

[000249] In embodiments, a membrane protein payload agent comprises a protein having a

sequence at least least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99% identical to the polypeptide sequence of a protein of Table 15, or a nucleic acid encoding the same. In embodiments, the membrane protein payload agent comprises a nucleic acid having a sequence at least least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99% identical to the nucleic acid sequence of a gene of Table 15.

[000250] In embodiments, a membrane protein payload agent comprises a protein having a sequence at least least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99% identical to the polypeptide sequence of a protein of Table 16, or a nucleic acid encoding the same. In embodiments, the membrane protein payload agent comprises a nucleic acid having a sequence at least least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99% identical to the nucleic acid sequence of a gene of Table 16.

Table 13: Exemplary intercellular adhesion molecule proteins

UniProt ID	Entry name	Gene names (primary)
P05362	ICAM1_HUMAN	ICAM1
P13598	ICAM2_HUMAN	ICAM2
P32942	ICAM3_HUMAN	ICAM3
Q14773	ICAM4_HUMAN	ICAM4
Q9UMF0	ICAM5_HUMAN	ICAM5

Table 14: Exemplary cadherin proteins

Uniprot ID	Entry name	Gene names (primary)
P07949	RET_HUMAN	RET
P12830	CADH1_HUMAN	CDH1
P55290	CAD13_HUMAN	CDH13
Q9H251	CAD23_HUMAN	CDH23
P19022	CADH2_HUMAN	CDH2
O60716	CTND1_HUMAN	CTNND1
P35221	CTNA1_HUMAN	CTNNA1
P22223	CADH3_HUMAN	CDH3
P33151	CADH5_HUMAN	CDH5
Q9NYQ6	CELR1_HUMAN	CELSR1

Uniprot ID	Entry name	Gene names (primary)
Q14126	DSG2_HUMAN	DSG2
Q9HBB8	CDHR5_HUMAN	CDHR5
Q96JQ0	PCD16_HUMAN	DCHS1
O94985	CSTN1_HUMAN	CLSTN1
Q02487	DSC2_HUMAN	DSC2
Q6V0I7	FAT4_HUMAN	FAT4
Q02413	DSG1_HUMAN	DSG1
Q14517	FAT1_HUMAN	FAT1
Q9BYE9	CDHR2_HUMAN	CDHR2
P55291	CAD15_HUMAN	CDH15
A7KAX9	RHG32_HUMAN	ARHGAP32
Q12864	CAD17_HUMAN	CDH17
P55287	CAD11_HUMAN	CDH11
Q9NYQ7	CELR3_HUMAN	CELSR3
Q14574	DSC3_HUMAN	DSC3
Q9NYQ8	FAT2_HUMAN	FAT2
P55283	CADH4_HUMAN	CDH4
P55285	CADH6_HUMAN	CDH6
P55286	CADH8_HUMAN	CDH8
Q9Y6N8	CAD10_HUMAN	CDH10
P55289	CAD12_HUMAN	CDH12
Q13634	CAD18_HUMAN	CDH18
Q9UJ99	CAD22_HUMAN	CDH22
O75309	CAD16_HUMAN	CDH16
Q9H159	CAD19_HUMAN	CDH19
Q9HBT6	CAD20_HUMAN	CDH20
Q8IXH8	CAD26_HUMAN	CDH26
Q9ULB5	CADH7_HUMAN	CDH7
Q9ULB4	CADH9_HUMAN	CDH9
Q6ZTQ4	CDHR3_HUMAN	CDHR3
Q9HCU4	CELR2_HUMAN	CELSR2
Q86UP0	CAD24_HUMAN	CDH24

Uniprot ID	Entry name	Gene names (primary)
Q96JP9	CDHR1_HUMAN	CDHR1
A6H8M9	CDHR4_HUMAN	CDHR4
Q9UI47	CTNA3_HUMAN	CTNNA3
A4D0V7	CPED1_HUMAN	CPED1
Q08554	DSC1_HUMAN	DSC1
P32926	DSG3_HUMAN	DSG3
Q86SJ6	DSG4_HUMAN	DSG4
Q8TDW7	FAT3_HUMAN	FAT3
Q9NPG4	PCD12_HUMAN	PCDH12
O60330	PCDGC_HUMAN	PCDHGA12
Q6V1P9	PCD23_HUMAN	DCHS2
Q9UN71	PCDGG_HUMAN	PCDHGB4
Q08174	PCDH1_HUMAN	PCDH1

Table 15: Exemplary selectin proteins

UniProt ID	Entry name	Gene names (primary)
P16109	LYAM3_HUMAN	SELP
P16581	LYAM2_HUMAN	SELE
Q14242	SELPL_HUMAN	SELPLG
P14151	LYAM1_HUMAN	SELL

Table 16: Exemplary mucin proteins

UniProt ID	Entry name	Gene names (primary)
P15941	MUC1_HUMAN	MUC1
Q99102	MUC4_HUMAN	MUC4
Q8TDQ0	HAVR2_HUMAN	HAVCR2
Q9HC84	MUC5B_HUMAN	MUC5B
P98088	MUC5A_HUMAN	MUC5AC
Q685J3	MUC17_HUMAN	MUC17
Q9UJU6	DBNL_HUMAN	DBNL
Q9HBB8	CDHR5_HUMAN	CDHR5
Q8WXI7	MUC16_HUMAN	MUC16
Q9UHX3	AGRE2_HUMAN	ADGRE2

UniProt ID	Entry name	Gene names (primary)
Q02817	MUC2_HUMAN	MUC2
Q8TAX7	MUC7_HUMAN	MUC7
Q96D42	HAVR1_HUMAN	HAVCR1
Q9H3R2	MUC13_HUMAN	MUC13
Q8N307	MUC20_HUMAN	MUC20
Q6W4X9	MUC6_HUMAN	MUC6
Q02505	MUC3A_HUMAN	MUC3A
Q7L513	FCRLA_HUMAN	FCRLA
Q14246	AGRE1_HUMAN	ADGRE1
Q86WA6	BPHL_HUMAN	BPHL
Q7Z5P9	MUC19_HUMAN	MUC19
Q9UKN1	MUC12_HUMAN	MUC12
Q5SSG8	MUC21_HUMAN	MUC21
Q9BY15	AGRE3_HUMAN	ADGRE3
Q6UWI2	PARM1_HUMAN	PARM1
O95395	GCNT3_HUMAN	GCNT3
Q86SQ3	AGRE4_HUMAN	ADGRE4P
Q6BAA4	FCRLB_HUMAN	FCRLB
Q96DR8	MUCL1_HUMAN	MUCL1
E2RYF6	MUC22_HUMAN	MUC22
Q9H195	MUC3B_HUMAN	MUC3B
Q9ULC0	MUCEN_HUMAN	EMCN
Q8N387	MUC15_HUMAN	MUC15
Q12889	OVGP1_HUMAN	OVGP1
E2RYF7	PBMU2_HUMAN	HCG22
Q96H15	TIMD4_HUMAN	TIMD4

(vii) Transport Protein Payloads

In some embodiments, a membrane protein payload agent is or compromises a transport protein or functional fragment, variant, or homolog thereof, or a nucleic acid encoding it. In some embodiments a membrane protein payload agent is or compromises a transport protein selected

from Table 17, or functional fragment, variant, or homolog thereof, or a nucleic acid encoding it. In embodiments, a membrane protein payload agent comprises a protein having a sequence at least least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99% identical to the polypeptide sequence of a protein of Table 17, or a nucleic acid encoding the same. In embodiments, the membrane protein payload agent comprises a nucleic acid having a sequence at least least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99% identical to the nucleic acid sequence of a gene of Table 17.

Table 17: Exemplary transport proteins

UniProt ID	Entry name	Gene names (primary)
Q9NRA8	4ET_HUMAN	EIF4ENIF1
Q9NS82	AAA1_HUMAN	SLC7A10
Q15758	AAAT_HUMAN	SLC1A5
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Q9BZC7	ABCA2_HUMAN	ABCA2
Q99758	ABCA3_HUMAN	ABCA3
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Q8IZY2	ABCA7_HUMAN	ABCA7
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O75027	ABCB7_HUMAN	ABCB7
Q9NP78	ABCB9_HUMAN	ABCB9
Q9NRK6	ABCBA_HUMAN	ABCB10
Q9UG63	ABCF2_HUMAN	ABCF2
P45844	ABCG1_HUMAN	ABCG1
Q9UNQ0	ABCG2_HUMAN	ABCG2
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Q12864	CAD17_HUMAN	CDH17
Q53S99	CB083_HUMAN	C2orf83
P51790	CLCN3_HUMAN	CLCN3
P51793	CLCN4_HUMAN	CLCN4

UniProt ID	Entry name	Gene names (primary)
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P51798	CLCN7_HUMAN	CLCN7
Q9NRU3	CNNM1_HUMAN	CNNM1
Q9H8M5	CNNM2_HUMAN	CNNM2
Q8NE01	CNNM3_HUMAN	CNNM3
Q6P4Q7	CNNM4_HUMAN	CNNM4
O15431	COPT1_HUMAN	SLC31A1
O15432	COPT2_HUMAN	SLC31A2
Q8WWI5	CTL1_HUMAN	SLC44A1
Q8IWA5	CTL2_HUMAN	SLC44A2
Q8N4M1	CTL3_HUMAN	SLC44A3
Q53GD3	CTL4_HUMAN	SLC44A4
Q8NCS7	CTL5_HUMAN	SLC44A5
P30825	CTR1_HUMAN	SLC7A1
P52569	CTR2_HUMAN	SLC7A2
Q8WY07	CTR3_HUMAN	SLC7A3
O43246	CTR4_HUMAN	SLC7A4
P43003	EAA1_HUMAN	SLC1A3
P43004	EAA2_HUMAN	SLC1A2
P43005	EAA3_HUMAN	SLC1A1
P48664	EAA4_HUMAN	SLC1A6
O00341	EAA5_HUMAN	SLC1A7
P55899	FCGRN_HUMAN	FCGRT
Q9UPI3	FLVC2_HUMAN	FLVCR2
Q96A29	FUCT1_HUMAN	SLC35C1
O43826	G6PT1_HUMAN	SLC37A4
O95528	GTR10_HUMAN	SLC2A10
Q9BYW1	GTR11_HUMAN	SLC2A11
Q8TD20	GTR12_HUMAN	SLC2A12
Q8TDB8	GTR14_HUMAN	SLC2A14
P11166	GTR1_HUMAN	SLC2A1
P11168	GTR2_HUMAN	SLC2A2

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P11169	GTR3_HUMAN	SLC2A3
P14672	GTR4_HUMAN	SLC2A4
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Q6PXP3	GTR7_HUMAN	SLC2A7
Q9NY64	GTR8_HUMAN	SLC2A8
Q9NRM0	GTR9_HUMAN	SLC2A9
Q9HCP6	HHATL_HUMAN	HHATL
Q6P1K1	HRG1_HUMAN	SLC48A1
Q12756	KIF1A_HUMAN	KIF1A
Q6ZP29	LAAT1_HUMAN	PQLC2
Q15012	LAP4A_HUMAN	LAPTM4A
Q01650	LAT1_HUMAN	SLC7A5
Q9GIP4	LAT1L_HUMAN	SLC7A5P2
Q8MH63	LAT1N_HUMAN	SLC7A5P1
Q9UHI5	LAT2_HUMAN	SLC7A8
O75387	LAT3_HUMAN	SLC43A1
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Q9H0U3	MAGT1_HUMAN	MAGT1
Q8N8R3	MCATL_HUMAN	SLC25A29
Q96MC6	MF14A_HUMAN	MFSD14A
Q9NYZ2	MFRN1_HUMAN	SLC25A37
Q96A46	MFRN2_HUMAN	SLC25A28
Q14728	MFS10_HUMAN	MFSD10
Q5TF39	MFS4B_HUMAN	MFSD4B
Q6N075	MFSD5_HUMAN	MFSD5
Q9H2D1	MFTC_HUMAN	SLC25A32
Q8N4V1	MMGT1_HUMAN	MMGT1
Q8TF71	MOT10_HUMAN	SLC16A10
Q8NCK7	MOT11_HUMAN	SLC16A11
Q6ZSM3	MOT12_HUMAN	SLC16A12

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Q7RTX9	MOT14_HUMAN	SLC16A14
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O60669	MOT2_HUMAN	SLC16A7
O95907	MOT3_HUMAN	SLC16A8
O15427	MOT4_HUMAN	SLC16A3
O15374	MOT5_HUMAN	SLC16A4
O15375	MOT6_HUMAN	SLC16A5
O15403	MOT7_HUMAN	SLC16A6
P36021	MOT8_HUMAN	SLC16A2
Q7RTY1	MOT9_HUMAN	SLC16A9
P33527	MRP1_HUMAN	ABCC1
Q92887	MRP2_HUMAN	ABCC2
O15438	MRP3_HUMAN	ABCC3
O15439	MRP4_HUMAN	ABCC4
O15440	MRP5_HUMAN	ABCC5
O95255	MRP6_HUMAN	ABCC6
Q9HD23	MRS2_HUMAN	MRS2
Q7RTP0	NIPA1_HUMAN	NIPA1
Q8N8Q9	NIPA2_HUMAN	NIPA2
Q6NVV3	NIPA3_HUMAN	NIPAL1
Q0D2K0	NIPA4_HUMAN	NIPAL4
P49281	NRAM2_HUMAN	SLC11A2
Q12908	NTCP2_HUMAN	SLC10A2
Q9Y619	ORNT1_HUMAN	SLC25A15
Q9BXI2	ORNT2_HUMAN	SLC25A2
Q86UW1	OSTA_HUMAN	SLC51A
Q86UW2	OSTB_HUMAN	SLC51B
Q04671	P_HUMAN	OCA2
Q96NT5	PCFT_HUMAN	SLC46A1
O75915	PRAF3_HUMAN	ARL6IP5
Q02094	RHAG_HUMAN	RHAG

UniProt ID	Entry name	Gene names (primary)
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Q9UBD6	RHCG_HUMAN	RHCG
Q92681	RSCA1_HUMAN	RSC1A1
Q9BXP2	S12A9_HUMAN	SLC12A9
Q8WWT9	S13A3_HUMAN	SLC13A3
Q86YT5	S13A5_HUMAN	SLC13A5
P46059	S15A1_HUMAN	SLC15A1
Q16348	S15A2_HUMAN	SLC15A2
Q8IY34	S15A3_HUMAN	SLC15A3
Q8N697	S15A4_HUMAN	SLC15A4
Q6NT16	S18B1_HUMAN	SLC18B1
P41440	S19A1_HUMAN	SLC19A1
O60779	S19A2_HUMAN	SLC19A2
Q9BZV2	S19A3_HUMAN	SLC19A3
Q8WUM9	S20A1_HUMAN	SLC20A1
Q08357	S20A2_HUMAN	SLC20A2
O15245	S22A1_HUMAN	SLC22A1
O15244	S22A2_HUMAN	SLC22A2
O75751	S22A3_HUMAN	SLC22A3
Q9H015	S22A4_HUMAN	SLC22A4
O76082	S22A5_HUMAN	SLC22A5
Q4U2R8	S22A6_HUMAN	SLC22A6
Q9Y694	S22A7_HUMAN	SLC22A7
Q8TCC7	S22A8_HUMAN	SLC22A8
Q8IVM8	S22A9_HUMAN	SLC22A9
Q63ZE4	S22AA_HUMAN	SLC22A10
Q9NSA0	S22AB_HUMAN	SLC22A11
Q96S37	S22AC_HUMAN	SLC22A12
Q9Y226	S22AD_HUMAN	SLC22A13
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Q8IZD6	S22AF_HUMAN	SLC22A15
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Q6T423	S22AP_HUMAN	SLC22A25
Q9UHI7	S23A1_HUMAN	SLC23A1
Q9UGH3	S23A2_HUMAN	SLC23A2
Q6PIS1	S23A3_HUMAN	SLC23A3
Q86VD7	S2542_HUMAN	SLC25A42
Q86WA9	S2611_HUMAN	SLC26A11
Q9H2B4	S26A1_HUMAN	SLC26A1
P50443	S26A2_HUMAN	SLC26A2
O43511	S26A4_HUMAN	SLC26A4
Q9BXS9	S26A6_HUMAN	SLC26A6
Q8TE54	S26A7_HUMAN	SLC26A7
Q96RN1	S26A8_HUMAN	SLC26A8
Q7LBE3	S26A9_HUMAN	SLC26A9
O00337	S28A1_HUMAN	SLC28A1
O43868	S28A2_HUMAN	SLC28A2
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Q14542	S29A2_HUMAN	SLC29A2
Q9BZD2	S29A3_HUMAN	SLC29A3
Q7RTT9	S29A4_HUMAN	SLC29A4
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Q9Y2D2	S35A3_HUMAN	SLC35A3
Q96G79	S35A4_HUMAN	SLC35A4
Q9BS91	S35A5_HUMAN	SLC35A5
P78383	S35B1_HUMAN	SLC35B1
Q8TB61	S35B2_HUMAN	SLC35B2
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Q495M3	S36A2_HUMAN	SLC36A2
Q495N2	S36A3_HUMAN	SLC36A3
Q6YBV0	S36A4_HUMAN	SLC36A4
Q9H2H9	S38A1_HUMAN	SLC38A1
Q96QD8	S38A2_HUMAN	SLC38A2
Q99624	S38A3_HUMAN	SLC38A3
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Q8IZM9	S38A6_HUMAN	SLC38A6
Q9NVC3	S38A7_HUMAN	SLC38A7
A6NNN8	S38A8_HUMAN	SLC38A8
Q8NBW4	S38A9_HUMAN	SLC38A9
Q9HBR0	S38AA_HUMAN	SLC38A10
Q08AI6	S38AB_HUMAN	SLC38A11
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Q9NP94	S39A2_HUMAN	SLC39A2
Q9BRY0	S39A3_HUMAN	SLC39A3
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Q92504	S39A7_HUMAN	SLC39A7
Q9C0K1	S39A8_HUMAN	SLC39A8
Q9NUM3	S39A9_HUMAN	SLC39A9
Q9ULF5	S39AA_HUMAN	SLC39A10
Q8N1S5	S39AB_HUMAN	SLC39A11
Q504Y0	S39AC_HUMAN	SLC39A12
Q96H72	S39AD_HUMAN	SLC39A13
Q15043	S39AE_HUMAN	SLC39A14
Q9NP59	S40A1_HUMAN	SLC40A1

UniProt ID	Entry name	Gene names (primary)
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Q9NWF4	S52A1_HUMAN	SLC52A1
Q9HAB3	S52A2_HUMAN	SLC52A2
Q9NQ40	S52A3_HUMAN	SLC52A3
P48066	S6A11_HUMAN	SLC6A11
P48065	S6A12_HUMAN	SLC6A12
Q9NSD5	S6A13_HUMAN	SLC6A13
Q9UN76	S6A14_HUMAN	SLC6A14
Q9H2J7	S6A15_HUMAN	SLC6A15
Q9GZN6	S6A16_HUMAN	SLC6A16
Q9H1V8	S6A17_HUMAN	SLC6A17
Q96N87	S6A18_HUMAN	SLC6A18
Q695T7	S6A19_HUMAN	SLC6A19
Q9NP91	S6A20_HUMAN	SLC6A20
Q8TCU3	S7A13_HUMAN	SLC7A13
Q8TBB6	S7A14_HUMAN	SLC7A14
Q70HW3	SAMC_HUMAN	SLC25A26
P43007	SATT_HUMAN	SLC1A4
P53794	SC5A3_HUMAN	SLC5A3
Q9Y289	SC5A6_HUMAN	SLC5A6
Q9GZV3	SC5A7_HUMAN	SLC5A7
Q8N695	SC5A8_HUMAN	SLC5A8
Q8WWX8	SC5AB_HUMAN	SLC5A11
Q1EHB4	SC5AC_HUMAN	SLC5A12
P30531	SC6A1_HUMAN	SLC6A1
P23975	SC6A2_HUMAN	SLC6A2
Q01959	SC6A3_HUMAN	SLC6A3
P31645	SC6A4_HUMAN	SLC6A4
Q9Y345	SC6A5_HUMAN	SLC6A5

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P31641	SC6A6_HUMAN	SLC6A6
Q99884	SC6A7_HUMAN	SLC6A7
P48029	SC6A8_HUMAN	SLC6A8
P48067	SC6A9_HUMAN	SLC6A9
P46721	SO1A2_HUMAN	SLCO1A2
Q9Y6L6	SO1B1_HUMAN	SLCO1B1
Q9NPD5	SO1B3_HUMAN	SLCO1B3
G3V0H7	SO1B7_HUMAN	SLCO1B7
Q9NYB5	SO1C1_HUMAN	SLCO1C1
Q92959	SO2A1_HUMAN	SLCO2A1
O94956	SO2B1_HUMAN	SLCO2B1
Q9UIG8	SO3A1_HUMAN	SLCO3A1
Q96BD0	SO4A1_HUMAN	SLCO4A1
Q6ZQN7	SO4C1_HUMAN	SLCO4C1
Q9H2Y9	SO5A1_HUMAN	SLCO5A1
Q86UG4	SO6A1_HUMAN	SLCO6A1
Q3KNW5	SOAT_HUMAN	SLC10A6
O95149	SPN1_HUMAN	SNUPN
Q8N434	SVOPL_HUMAN	SVOPL
Q9BRV3	SWET1_HUMAN	SLC50A1
Q03518	TAP1_HUMAN	TAP1
Q03519	TAP2_HUMAN	TAP2
Q13454	TUSC3_HUMAN	TUSC3
Q13336	UT1_HUMAN	SLC14A1
Q15849	UT2_HUMAN	SLC14A2
Q16572	VACHT_HUMAN	SLC18A3
Q9P2U7	VGLU1_HUMAN	SLC17A7
Q9P2U8	VGLU2_HUMAN	SLC17A6
Q8NDX2	VGLU3_HUMAN	SLC17A8
Q9H598	VIAAT_HUMAN	SLC32A1
P54219	VMAT1_HUMAN	SLC18A1
Q05940	VMAT2_HUMAN	SLC18A2

UniProt ID	Entry name	Gene names (primary)
Q9UPY5	XCT_HUMAN	SLC7A11
Q9UM01	YLAT1_HUMAN	SLC7A7
Q92536	YLAT2_HUMAN	SLC7A6
Q6XR72	ZNT10_HUMAN	SLC30A10
Q9Y6M5	ZNT1_HUMAN	SLC30A1
Q9BRI3	ZNT2_HUMAN	SLC30A2
Q99726	ZNT3_HUMAN	SLC30A3
O14863	ZNT4_HUMAN	SLC30A4
Q8TAD4	ZNT5_HUMAN	SLC30A5
Q6NXT4	ZNT6_HUMAN	SLC30A6
Q8NEW0	ZNT7_HUMAN	SLC30A7
Q8IWU4	ZNT8_HUMAN	SLC30A8
Q6PML9	ZNT9_HUMAN	SLC30A9

(viii) Chimeric Antigen Receptor Payloads

[000251] In some embodiments a membrane protein payload agent is or comprises a CAR, e.g., a first generation CAR or a nucleic acid encoding a first generation CAR. In some embodiments, a first generation CAR comprises an antigen binding domain, a transmembrane domain, and signaling domain. In some embodiments a signaling domain mediates downstream signaling during T-cell activation.

[000252] In some embodiments a membrane protein payload agent is or comprises a second generation CAR or a nucleic acid encoding a second generation CAR. In some embodiments a second generation CAR comprises an antigen binding domain, a transmembrane domain, and two signaling domains. In some embodiments a signaling domain mediates downstream signaling during T-cell activation. In some embodiments a signaling domain is a costimulatory domain. In some embodiments, a costimulatory domain enhances cytokine production, CAR T-cell proliferation, and or CAR T-cell persistence during T cell activation.

[000253] In some embodiments a membrane protein payload agent is or comprises a third generation CAR or a nucleic acid encoding a third generation CAR. In some embodiments, a third generation CAR comprises an antigen binding domain, a transmembrane domain, and at least three

signaling domains. In some embodiments a signaling domain mediates downstream signaling during T-cell activation. In some embodiments a signaling domain is a costimulatory domain. In some embodiments, a costimulatory domain enhances cytokine production, CAR T-cell proliferation, and or CAR T-cell persistence during T cell activation. In some embodiments, a third generation CAR comprises at least two costimulatory domains. In some embodiments, the at least two costimulatory domains are not the same.

[000254] In some embodiments a membrane protein payload agent is or comprises a fourth generation CAR or a nucleic acid encoding a fourth generation CAR. In some embodiments a fourth generation CAR comprises an antigen binding domain, a transmembrane domain, and at least two, three, or four signaling domains. In some embodiments a signaling domain mediates downstream signaling during T-cell activation. In some embodiments a signaling domain is a costimulatory domain. In some embodiments, a costimulatory domain enhances cytokine production, CAR T-cell proliferation, and or CAR T-cell persistence during T cell activation.

[000255] In some embodiments, a first, second, third, or fourth generation CAR further comprises a domain which upon successful signaling of the CAR induces expression of a cytokine gene. In some embodiments, a cytokine gene is endogenous or exogenous to a target cell comprising a CAR which comprises a domain which upon successful signaling of the CAR induces expression of a cytokine gene. In some embodiments a cytokine gene encodes a pro-inflammatory cytokine. In some embodiments a cytokine gene encodes IL-1, IL-2, IL-9, IL-12, IL-18, TNF, or IFN-gamma, or functional fragment thereof. In some embodiments a domain which upon successful signaling of the CAR induces expression of a cytokine gene is or comprises a transcription factor or functional domain or fragment thereof. In some embodiments a domain which upon successful signaling of the CAR induces expression of a cytokine gene is or comprises a transcription factor or functional domain or fragment thereof. In some embodiments a transcription factor or functional domain or fragment thereof is or comprises a nuclear factor of activated T cells (NFAT), an NF-kB, or functional domain or fragment thereof. See, e.g., Zhang. C. et al., Engineering CAR-T cells. Biomarker Research. 5:22 (2017); WO 2016126608; Sha, H. et al. Chimaeric antigen receptor T-cell therapy for tumour immunotherapy. Bioscience Reports Jan 27, 2017, 37 (1).

(a) CAR Antigen Binding Domains

[000256] In some embodiments, a CAR antigen binding domain is or comprises an antibody or antigen-binding portion thereof. In some embodiments, a CAR antigen binding domain is or comprises an scFv or Fab. In some embodiments a CAR antigen binding domain comprises an scFv or Fab fragment of a T-cell alpha chain antibody; T-cell β chain antibody; T-cell γ chain antibody; T-cell δ chain antibody; CCR7 antibody; CD3 antibody; CD4 antibody; CD5 antibody; CD7 antibody; CD8 antibody; CD11b antibody; CD11c antibody; CD16 antibody; CD19 antibody; CD20 antibody; CD21 antibody; CD22 antibody; CD25 antibody; CD28 antibody; CD34 antibody; CD35 antibody; CD40 antibody; CD45RA antibody; CD45RO antibody; CD52 antibody; CD56 antibody; CD62L antibody; CD68 antibody; CD80 antibody; CD95 antibody; CD117 antibody; CD127 antibody; CD133 antibody; CD137 (4-1 BB) antibody; CD163 antibody; F4/80 antibody; IL-4Ra antibody; Sca-1 antibody; CTLA-4 antibody; GITR antibody GARP antibody; LAP antibody; granzyme B antibody; LFA-1 antibody; or transferrin receptor antibody.

[000257] In some embodiments, an antigen binding domain binds to a cell surface antigen of a cell. In some embodiments, a cell surface antigen is characteristic of one type of cell. In some embodiments, a cell surface antigen is characteristic of more than one type of cell.

[000258] In some embodiments a CAR antigen binding domain binds a cell surface antigen characteristic of a T-cell. In some embodiments, an antigen characteristic of a T-cell may be a cell surface receptor, a membrane transport protein (e.g., an active or passive transport protein such as, for example, an ion channel protein, a pore-forming protein, etc.), a transmembrane receptor, a membrane enzyme, and/or a cell adhesion protein characteristic of a T-cell. In some embodiments, an antigen characteristic of a T-cell may be a G protein-coupled receptor, receptor tyrosine kinase, tyrosine kinase associated receptor, receptor-like tyrosine phosphatase, receptor serine/ threonine kinase, receptor guanylyl cyclase, or histidine kinase associated receptor.

[000259] In some embodiments, an antigen characteristic of a T-cell may be a T-cell receptor. In some embodiments, a T-cell receptor may be AKT1; AKT2; AKT3; ATF2; BCL10; CALM1; CD3D (CD3δ); CD3E (CD3ε); CD3G (CD3γ); CD4; CD8; CD28; CD45; CD80 (B7-1); CD86 (B7-2); CD247 (CD3ζ); CTLA4 (CD152); ELK1; ERK1 (MAPK3); ERK2; FOS; FYN; GRAP2 (GADS); GRB2; HLA-DRA; HLA-DRB1; HLA-DRB3; HLA-DRB4; HLA-DRB5; HRAS;

IKBKA (CHUK); IKBKB; IKBKE; IKBKG (NEMO); IL2; ITPR1; ITK; JUN; KRAS2; LAT; LCK; MAP2K1 (MEK1); MAP2K2 (MEK2); MAP2K3 (MKK3); MAP2K4 (MKK4); MAP2K6 (MKK6); MAP2K7 (MKK7); MAP3K1 (MEKK1); MAP3K3; MAP3K4; MAP3K5; MAP3K8; MAP3K14 (NIK); MAPK8 (JNK1); MAPK9 (JNK2); MAPK10 (JNK3); MAPK11 (p38β); MAPK12 (p38γ); MAPK13 (p38δ); MAPK14 (p38α); NCK; NFAT1; NFAT2; NFKB1; NFKB2; NFKBIA; NRAS; PAK1; PAK2; PAK3; PAK4; PIK3C2B; PIK3C3 (VPS34); PIK3CA; PIK3CB; PIK3CD; PIK3R1; PKCA; PKCB; PKCM; PKCQ; PLCY1; PRF1 (Perforin); PTEN; RAC1; RAF1; RELA; SDF1; SHP2; SLP76; SOS; SRC; TBK1; TCRA; TEC; TRAF6; VAV1; VAV2; or ZAP70.

[000260] In some embodiments a CAR antigen binding domain binds an antigen characteristic of a cancer. In some embodiments an antigen characteristic of a cancer is selected from a cell surface receptor, an ion channel-linked receptor, an enzyme-linked receptor, a G protein-coupled receptor, receptor tyrosine kinase, tyrosine kinase associated receptor, receptor-like tyrosine phosphatase, receptor serine/ threonine kinase, receptor guanylyl cyclase, histidine kinase associated receptor, Epidermal Growth Factor Receptors (EGFR) (including ErbB1/EGFR, ErbB2/HER2, ErbB3/HER3, and ErbB4/HER4), Fibroblast Growth Factor Receptors (FGFR) (including FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, FGF7, FGF18, and FGF21) Vascular Endothelial Growth Factor Receptors (VEGFR) (including VEGF-A, VEGF-B, VEGF-C, VEGF-D, and PIGF), RET Receptor and the Eph Receptor Family (including EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, EphA8, EphA9, EphA10, EphB1, EphB2. EphB3, EphB4, and EphB6), CXCR1, CXCR2, CXCR3, CXCR4, CXCR6, CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR8, CFTR, CIC-1, CIC-2, CIC-4, CIC-5, CIC-7, CIC-Ka, CIC-Kb, Bestrophins, TMEM16A, GABA receptor, glycin receptor, ABC transporters, NAV1.1, NAV1.2, NAV1.3, NAV1.4, NAV1.5, NAV1.6, NAV1.7, NAV1.8, NAV1.9, sphingosin-1-phosphate receptor (S1P1R), NMDA channel, transmembrane protein, multispan transmembrane protein, T-cell receptor motifs; T-cell alpha chains; T-cell β chains; T-cell γ chains; T-cell δ chains; CCR7; CD3; CD4; CD5; CD7; CD8; CD11b; CD11c; CD16; CD19; CD20; CD21 ; CD22; CD25; CD28; CD34; CD35; CD40; CD45RA; CD45RO; CD52; CD56; CD62L; CD68; CD80; CD95; CD117; CD127; CD133; CD137 (4-1 BB); CD163; F4/80; IL-4Ra; Sca-1 ; CTLA-4; GITR; GARP; LAP; granzyme B; LFA-1 ; transferrin receptor; NKp46, perforin, CD4+; Th1; Th2; Th17; Th40; Th22; Th9; Tfh,

Canonical Treg. FoxP3+; Tr1; Th3; Treg17; $T_{RE}G$; CDCP1, NT5E, EpCAM, CEA, gpA33, Mucins, TAG-72, Carbonic anhydrase IX, PSMA, Folate binding protein, Gangliosides (e.g., CD2, CD3, GM2), Lewis-γ^2, VEGF, VEGFR 1/2/3, $\alpha V\beta 3$, $\alpha 5\beta 1$, ErbB1/EGFR, ErbB1/HER2, ErB3, c-MET, IGF1R, EphA3, TRAIL-R1, TRAIL-R2, RANKL, FAP, Tenascin, PDL-1, BAFF, HDAC, ABL, FLT3, KIT, MET, RET, IL-1β, ALK, RANKL, mTOR, CTLA-4, IL-6, IL-6R, JAK3, BRAF, PTCH, Smoothened, PIGF, ANPEP, TIMP1, PLAUR, PTPRJ, LTBR, or ANTXR1, Folate receptor alpha (FRa), ERBB2 (Her2/neu), EphA2, IL-13Ra2, epidermal growth factor receptor (EGFR), Mesothelin, TSHR, CD19, CD123, CD22, CD30, CD171, CS-1, CLL-1, CD33, EGFRvIII , GD2, GD3, BCMA, MUC16 (CA125), L1CAM, LeY, MSLN, IL13Rα1, L1-CAM, Tn Ag, prostate specific membrane antigen (PSMA), ROR1, FLT3, FAP, TAG72, CD38, CD44v6, CEA, EPCAM, B7H3, KIT, interleukin-11 receptor a (IL-11Ra), PSCA, PRSS21, VEGFR2, LewisY, CD24, platelet-derived growth factor receptor-beta (PDGFR-beta), SSEA-4, CD20, MUC1, NCAM, Prostase, PAP, ELF2M, Ephrin B2, IGF-1 receptor, CAIX, LMP2, gplOO, bcr-abl, tyrosinase, Fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, Folate receptor beta, TEM1/CD248, TEM7R, CLDN6, GPRC5D, CXORF61, CD97, CD179a, ALK, Polysialic acid, PLACl, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-la, MAGE-A1, legumain, HPV E6, E7, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, prostein, survivin, telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, Androgen receptor, Cyclin B1, MYCN, RhoC, TRP-2, CYPIB I, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2, CD79a, CD79b, CD72, LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, GPC3, FCRL5, IGLL1, a neoantigen, CD133, CD15, CD184, CD24, CD56, CD26, CD29, CD44, HLA-A, HLA-B, HLA-C, (HLA-A,B,C) CD49f, CD151 CD340, CD200, tkrA, trkB, or trkC, or an antigenic fragment or antigenic portion thereof.

[000261] In some embodiments a CAR antigen binding domain binds an antigen characteristic of an infectious disease (e.g. a viral infection or a bacterial infection). In some embodiments an antigen is characteristic of an infectious disease selected from HIV, hepatitis B virus, hepatitis C virus, Human herpes virus, Human herpes virus 8 (HHV-8, Kaposi sarcoma-

associated herpes virus (KSHV)), Human T-lymphotrophic virus-1 (HTLV-1), Merkel cell polyomavirus (MCV), Simian virus 40 (SV40), Eptstein-Barr virus, CMV, human papillomavirus. In some embodiments an antigen characteristic of an infectious disease is selected from a cell surface receptor, an ion channel-linked receptor, an enzyme-linked receptor, a G protein-coupled receptor, receptor tyrosine kinase, tyrosine kinase associated receptor, receptor-like tyrosine phosphatase, receptor serine/ threonine kinase, receptor guanylyl cyclase, or histidine kinase associated receptor. In some embodiments, a CAR antigen binding domain binds an antigen characteristic of an infectious disease, wherein the antigen is selected from HIV Env, gpl20, or CD4-induced epitope on HIV-1 Env. See, e.g., WO2015/077789, the contents of which are herein incorporated by reference. In some embodiments, a CAR antigen binding domain comprises CD4 or an HIV binding fragment thereof.

[000262] In some embodiments a CAR antigen binding domain binds an antigen characteristic of an autoimmune or inflammatory disorder. In some embodiments the antigen is characteristic of an autoimmune or inflammatory disorder selected from chronic graft-vs-host disease (GVHD), lupus, arthritis, immune complex glomerulonephritis, goodpasture, uveitis, hepatitis, systemic sclerosis or scleroderma, type I diabetes, multiple sclerosis, cold agglutinin disease, Pemphigus vulgaris, Grave's disease, autoimmune hemolytic anemia, Hemophilia A, Primary Sjogren's Syndrome, thrombotic thrombocytopenia purrpura, neuromyelits optica, Evan's syndrome, IgM mediated neuropathy, cyroglobulinemia, dermatomyositis, idiopathic thrombocytopenia, ankylosing spondylitis, bullous pemphigoid, acquired angioedema, chronic urticarial, antiphospholipid demyelinating polyneuropathy, and autoimmune thrombocytopenia or neutropenia or pure red cell aplasias, while exemplary non-limiting examples of alloimmune diseases include allosensitization (see, for example, Blazar et al., 2015, Am. J. Transplant, 15(4):931-41) or xenosensitization from hematopoietic or solid organ transplantation, blood transfusions, pregnancy with fetal allosensitization, neonatal alloimmune thrombocytopenia, hemolytic disease of the newborn, sensitization to foreign antigens such as can occur with replacement of inherited or acquired deficiency disorders treated with enzyme or protein replacement therapy, blood products, and gene therapy. In some embodiments an antigen characteristic of an autoimmune or inflammatory disorder is selected from a cell surface receptor, an ion channel-linked receptor, an enzyme-linked receptor, a G protein-coupled receptor, receptor

tyrosine kinase, tyrosine kinase associated receptor, receptor-like tyrosine phosphatase, receptor serine/ threonine kinase, receptor guanylyl cyclase, or histidine kinase associated receptor. In some embodiments, a CAR antigen binding domain binds to a ligand expressed on B cells, plasma cells, or plasmablasts. In some embodiments, a CAR antigen binding domain binds an antigen characteristic of an autoimmune or inflammatory disorder, wherein the antigen is selected from CD10, CD19, CD20, CD22, CD24, CD27, CD38, CD45R, CD138, CD319, BCMA, CD28, TNF, interferon receptors, GM-CSF, ZAP-70, LFA-1, CD3 gamma, CD5 or CD2. See US 2003/0077249; WO 2017/058753; WO 2017/058850, the contents of which are herein incorporated by reference.

(b) CAR Transmembrane Domains

[000263] In some embodiments a CAR comprises a transmembrane domain. In some embodiments a CAR comprises at least a transmembrane region of the alpha, beta or zeta chain of a T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, or functional variant thereof. In some embodiments a CAR comprises at least a transmembrane region of CD8α, CD8β, 4-1BB/CD137, CD28, CD34, CD4, FcεRIγ, CD16, OX40/CD134, CD3ζ, CD3ε, CD3γ, CD3δ, TCRα, TCRβ, TCRζ, CD32, CD64, CD64, CD45, CD5, CD9, CD22, CD37, CD80, CD86, CD40, CD40L/CD154, VEGFR2, FAS, and FGFR2B, or functional variant thereof.

(c) CAR Signaling Domains

[000264] In some embodiments a CAR comprises a signaling domain of one or more of B7-1/CD80; B7-2/CD86; B7-H1/PD-L1; B7-H2; B7-H3; B7-H4; B7-H6; B7-H7; BTLA/CD272; CD28; CTLA-4; Gi24/VISTA/B7-H5; ICOS/CD278; PD-1; PD-L2/B7-DC; PDCD6); 4-1BB/TNFSF9/CD137; 4-1BB Ligand/TNFSF9; BAFF/BLyS/TNFSF13B; BAFF R/TNFRSF13C; CD27/TNFRSF7; CD27 Ligand/TNFSF7; CD30/TNFRSF8; CD30 Ligand/TNFSF8; CD40/TNFRSF5; CD40/TNFSF5; CD40 Ligand/TNFSF5; DR3/TNFRSF25; GITR/TNFRSF18; GITR Ligand/TNFSF18; HVEM/TNFRSF14; LIGHT/TNFSF14; Lymphotoxin-alpha/TNF-beta; OX40/TNFRSF4; OX40 Ligand/TNFSF4; RELT/TNFRSF19L; TACI/TNFRSF13B; TL1A/TNFSF15; TNF-alpha; TNF RII/TNFRSF1B); 2B4/CD244/SLAMF4; BLAME/SLAMF8; CD2; CD2F-10/SLAMF9; CD48/SLAMF2; CD58/LFA-3; CD84/SLAMF5; CD229/SLAMF3;

CRACC/SLAMF7; NTB-A/SLAMF6; SLAM/CD150); CD2; CD7; CD53; CD82/Kai-1; CD90/Thy1; CD96; CD160; CD200; CD300a/LMIR1; HLA Class I; HLA-DR; Ikaros; Integrin alpha 4/CD49d; Integrin alpha 4 beta 1; Integrin alpha 4 beta 7/LPAM-1; LAG-3; TCL1A; TCL1B; CRTAM; DAP12; Dectin-1/CLEC7A; DPPIV/CD26; EphB6; TIM-1/KIM-1/HAVCR; TIM-4; TSLP; TSLP R; lymphocyte function associated antigen-1 (LFA-1); NKG2C, a CD3 zeta domain, an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof.

[000265] In some embodiments a CAR comprises a signaling domain which is a costimulatory domain. In some embodiments a CAR comprises a second costimulatory domain. In some embodiments a CAR comprises at least two costimulatory domains. In some embodiments a CAR comprises at least three costimulatory domains. In some embodiments a CAR comprises a costimulatory domain selected from one or more of CD27, CD28, 4-1BB, CD134/OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83.

[000266] In some embodiments, a CAR comprises a CD3 zeta domain or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof. In some embodiments, a CAR comprises a (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; and (ii) a CD28 domain, or a 4-1BB domain, or functional variant thereof. In some embodiments, a CAR comprises a (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; (ii) a CD28 domain or functional variant thereof; and (iii) a 4-1BB domain, or a CD134 domain, or functional variant thereof. In some embodiments, a CAR comprises a (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; (ii) a CD28 domain or functional variant thereof; (iii) a 4-1BB domain, or a CD134 domain, or functional variant thereof; and (iv) a cytokine or costimulatory ligand transgene.

(d) CAR Spacers

[000267] In some embodiments a CAR comprises one or more spacers. In some embodiments a CAR comprises a spacer between the antigen binding domain and the transmembrane domain. In some embodiments a CAR comprises a spacer between the transmembrane domain and the intracellular signaling domain.

(e) CAR Membrane Protein Payload Agents

[000268] In addition to the CARs described herein, various chimeric antigen receptors and nucleotide sequences encoding the same are known in the art and would be suitable for fusosomal delivery and reprogramming of target cells in vivo and in vitro as described herein. See, e.g., WO2013040557; WO2012079000; WO2016030414; Smith T, et al., Nature Nanotechnology. 2017. DOI: 10.1038/NNANO.2017.57, the disclosures of which are herein incorporated by reference.

[000269] In some embodiments a fusosome comprising a membrane protein payload agent which is or comprises a CAR or a nucleic acid encoding a CAR (e.g., a DNA, a gDNA, a cDNA, an RNA, a pre-MRNA, an mRNA, an miRNA, an siRNA, etc.) is delivered to a target cell. In some embodiments the target cell is an effector cell, e.g., a cell of the immune system that expresses one or more Fc receptors and mediates one or more effector functions. In some embodiments, a target cell may include, but may not be limited to, one or more of a monocyte, macrophage, neutrophil, dendritic cell, eosinophil, mast cell, platelet, large granular lymphocyte, Langerhans' cell, natural killer (NK) cell, T-lymphocyte (e.g., T-cell), a Gamma delta T cell, B-lymphocyte (e.g., B-cell) and may be from any organism including but not limited to humans, mice, rats, rabbits, and monkeys.

Nuclear Protein Payload Agents

[000270] In some embodiments, the payload agent is or compromises a nuclear protein payload agent or functional fragment, variant, or homolog thereof, or a nucleic acid encoding it. In some embodiments a nuclear protein payload agent is or compromises a protein selected from Table 17-1, or functional fragment, variant, or homolog thereof, or a nucleic acid encoding it. In embodiments, a nuclear protein payload agent comprises a protein having a sequence at least least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99% identical to the polypeptide sequence of a protein of Table 17-1, or a nucleic acid encoding the same. In embodiments, the nuclear protein payload agent comprises a nucleic acid having a sequence at least least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99% identical to the nucleic acid sequence of a gene of Table 17-1. In some embodiments, the payload

agent is or comprises a protein that localizes to the nucleolus or a nuclear body, or a nucleic acid encoding the same. In some embodiments, the payload agent is or comprises a structural protein, transcriptional activator, transcriptional repressor, epigenetic modifier, histone acetyltransferase, histone deacetylase, histone methyltransferase, histone demethylase, DNA modifying enzyme, RNA splicing factor, or genome homeostasis protein, or a nucleic acid encoding the same.

Table 17-1: Exemplary nuclear proteins

UniProt ID	Entry name	Gene names	Localization/Subtype
P22087	fibrillarin	FBL	Nucleolus
Q8N9T8	KRI1 homolog	KRI1	Nucleolus
Q9H8H0	nucleolar protein 11	NOL11	Nucleolus
P17480	upstream binding transcription factor	UBTF	Nucleolus
Q99848	EBNA1 binding protein 2	EBNA1BP2	Nucleolus
P38432	coilin	COIL	Nuclear Bodies
P29590	promyelocytic leukemia	PML	Nuclear Bodies
Q9Y3Y2	chromatin target of PRMT1	CHTOP	Nuclear Bodies
Q9BSI4	TERF1 interacting nuclear factor 2	TINF2	Nuclear Bodies
P00519	ABL proto-oncogene 1, non-receptor tyrosine kinase	ABL1	Nuclear Bodies
P20700	lamin B1	LMNB1	Structural Proteins
P07199	centromere protein B	CENPB	Structural Proteins
P07305	H1 histone family member 0	H1F0	Structural Proteins
Q8NG31	kinetochore scaffold 1	KNL1	Structural Proteins
Q16576	RB binding protein 7, chromatin remodeling factor	RbAp46	Structural Proteins

UniProt ID	Entry name	Gene names	Localization/Subtype
P40763	signal transducer and activator of transcription 3	STAT3	Transcriptional Activator
Q9BZS1	forkhead box P3	FOXP3	Transcriptional Activator
P41235	hepatocyte nuclear factor 4 alpha	HNF4	Transcriptional Activator
Q01196	runt related transcription factor 1	RUNX1	Transcriptional Activator
Q9UJU2	lymphoid enhancer binding factor 1	LEF1	Transcriptional Activator
Q9H334	forkhead box P1	FOXP1	Transcriptional Repressor
O14529	cut like homeobox 2	CUX2	Transcriptional Repressor
P14859	POU class 2 homeobox 1	POU2F1	Transcriptional Repressor
O95343	SIX homeobox 3	SIX3	Transcriptional Repressor
Q04206	RELA proto-oncogene, NF-kB subunit	RELA	Transcriptional Repressor
Q12824	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1	SMARCB1	Epigenetic Modifier
Q9P2D1	chromodomain helicase DNA binding protein 7	CHD7	Epigenetic Modifier
Q9NRG0	chromatin accessibility complex subunit 1	CHRAC1	Epigenetic Modifier

UniProt ID	Entry name	Gene names	Localization/Subtype
Q9NQ94	APOBEC1 complementation factor	ACF	Epigenetic Modifier
O60885	bromodomain containing 4	BRD4	Epigenetic Modifier
Q99966	Cbp/p300 interacting transactivator with Glu/Asp rich carboxy-terminal domain 1	CITED1	Histone Acetyltransferase
Q92830	lysine acetyltransferase 2A	KAT2A	Histone Acetyltransferase
O14929	histone acetyltransferase 1	HAT1	Histone Acetyltransferase
O75529	TATA-box binding protein associated factor 5 like	TAF5L	Histone Acetyltransferase
Q92993	lysine acetyltransferase 5	KAT5	Histone Acetyltransferase
Q96EB6	sirtuin 1	SIRT1	Histone Deacetylase
Q8IXJ6	sirtuin 2	SIRT2	Histone Deacetylase
Q96DB2	histone deacetylase 11	HDAC11	Histone Deacetylase
O15379	histone deacetylase 3	HDAC3	Histone Deacetylase
Q9UBN7	histone deacetylase 6	HDAC6	Histone Deacetylase
Q9NR48	ASH1 like histone lysine methyltransferase	ASH1L	Histone Methyltransferase
Q8TEK3	DOT1 like histone lysine methyltransferase	DOT1L	Histone Methyltransferase
Q96L73	nuclear receptor binding SET domain protein 1	NSD1	Histone Methyltransferase
Q01105	SET nuclear proto-	SET	Histone

UniProt ID	Entry name	Gene names	Localization/Subtype
	oncogene		Methyltransferase
Q03164	lysine methyltransferase 2A	KMT2A	Histone Methyltransferase
Q9Y2K7	lysine demethylase 2A	KDM2A	Histone Demethylase
O60341	lysine demethylase 1A	KDM1A	Histone Demethylase
Q9Y4C1	lysine demethylase 3A	KDM3A	Histone Demethylase
O94953	lysine demethylase 4B	KDM4B	Histone Demethylase
P41229	lysine demethylase 5C	KDM5C	Histone Demethylase
P26358	DNA methyltransferase 1	DNMT1	DNA Modifying Enzymes
Q9Y6K1	DNA methyltransferase 3 alpha	DNMT3A	DNA Modifying Enzymes
Q9NZK5	adenosine deaminase 2	ADA2	DNA Modifying Enzymes
P55265	adenosine deaminase, RNA specific	ADAR	DNA Modifying Enzymes
P41238	apolipoprotein B mRNA editing enzyme catalytic subunit 1	APOBEC1	DNA Modifying Enzymes
Q15020	spliceosome associated factor 3, U4/U6 recycling protein	SART3	RNA splicing
Q9BWU1	cyclin dependent kinase 19	CDK11	RNA splicing
Q9UK58	cyclin L1	CCNL1	RNA splicing
Q8WWY3	pre-mRNA processing factor 31	PRPF31	RNA splicing
Q12874	splicing factor 3a subunit 3	SF3A3	RNA splicing
Q14676	mediator of DNA	MDC1	Genome Homeostasis

UniProt ID	Entry name	Gene names	Localization/Subtype
	damage checkpoint 1		
Q13315	ATM serine/threonine kinase	ATM	Genome Homeostasis
P09874	poly(ADP-ribose) polymerase 1	PARP1	Genome Homeostasis
P11387	DNA topoisomerase I	TOP1	Genome Homeostasis
P49917	DNA ligase 4	LIG4	Genome Homeostasis

Organellar Protein Payload Agents

[000271] In some embodiments, the payload agent is or compromises an organellar protein payload agent or functional fragment, variant, or homolog thereof, or a nucleic acid encoding it. In some embodiments an organellar protein payload agent is or compromises a protein selected from Table 17-2, or functional fragment, variant, or homolog thereof, or a nucleic acid encoding it. In embodiments, an organellar protein payload agent comprises a protein having a sequence at least least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99% identical to the polypeptide sequence of a protein of Table 17-2, or a nucleic acid encoding the same. In embodiments, the organellar protein payload agent comprises a nucleic acid having a sequence at least least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99% identical to the nucleic acid sequence of a gene of Table 17-2. In embodiments, the organellar protein payload agent localizes to an intracellular compartment, e.g., as described herein (e.g., as listed in Table 17-2). In embodiments, the organellar protein payload agent localizes to the endoplasmic reticulum, mitochondria, peroxisome, Golgi apparatus, lipid droplets, plasma membrane, stress granule, or cytoskeleton. In embodiments, the organellar protein payload agent comprises a localization signal for an intracellular compartment, e.g., as described herein (e.g., as listed in Table 17-2). In embodiments, the organellar protein payload agent compises a localization signal for one or more of (e.g., 1, 2, 3, or 4 of) the endoplasmic reticulum, mitochondria, peroxisome, Golgi apparatus, lipid droplets, plasma membrane, stress granule, or cytoskeleton.

Table 17-2: Exemplary organellar proteins

UniProt ID	Entry name	Gene names	Localization/Subtype
P51572	B cell receptor associated protein 31	BCAP31	Endoplasmic Reticulum
P27824	calnexin	CANX	Endoplasmic Reticulum
O43731	KDEL endoplasmic reticulum protein retention receptor 3	KDELR3	Endoplasmic Reticulum
Q9NQC3	reticulon 4	RTN4	Endoplasmic Reticulum
P61619	Sec61 translocon alpha 1 subunit	SEC61A1	Endoplasmic Reticulum
O14983	Sarcoplasmic/endoplasmic reticulum calcium ATPase 1	ATP2A1	Endoplasmic Reticulum
Q9GZP9	Derlin-2	DERL2	Endoplasmic Reticulum
Q8N6R1	Stress-associated endoplasmic reticulum protein 2	SERP2	Endoplasmic Reticulum
Q14257	Reticulocalbin-2	RCN2	Endoplasmic Reticulum
Q6DD88	Atlastin-3	ATL3	Endoplasmic Reticulum
P99999	cytochrome c, somatic	CYCS	Mitochondrion
O43615	translocase of inner mitochondrial membrane 44	TIMM44	Mitochondrion
O96008	translocase of outer mitochondrial membrane 40	TOMM40	Mitochondrion
P45880	voltage dependent anion channel 2	VDAC2	Mitochondrion

UniProt ID	Entry name	Gene names	Localization/Subtype
Q96D53	coenzyme Q8B	COQ8B	Mitochondrion
P24752	Acetyl-CoA acetyltransferase, mitochondrial	ACAT1	Mitochondrion
O75390	Citrate synthase, mitochondrial	CS	Mitochondrion
O14548	Cytochrome c oxidase subunit 7A-related protein, mitochondrial	COX7A2L	Mitochondrion
P07954	Fumarate hydratase, mitochondrial	FH	Mitochondrion
P22570	NADPH:adrenodoxin oxidoreductase, mitochondrial	FDXR	Mitochondrion
Q07820	Induced myeloid leukemia cell differentiation protein Mcl-1	MCL1	Mitochondrion
Q9P0J0	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 13	NDUFA13	Mitochondrion
Q00059	Transcription factor A, mitochondrial	TFAM	Mitochondrion
Q8WVX9	fatty acyl-CoA reductase 1	FAR1	Peroxisome
P09110	acetyl-CoA acyltransferase 1	ACAA1	Peroxisome
P28288	ATP binding cassette subfamily D member 3	ABCD3	Peroxisome
P51659	hydroxysteroid 17-beta dehydrogenase 4	HSD17B4	Peroxisome
O75381	peroxisomal biogenesis	PEX14	Peroxisome

UniProt ID	Entry name	Gene names	Localization/Subtype
	factor 14		
P22307	Non-specific lipid-transfer protein	SCP2	Peroxisome
P40855	Peroxisomal biogenesis factor 19	PEX19	Peroxisome
O75521	Enoyl-CoA delta isomerase 2	ECI2	Peroxisome
Q5T8D3	Acyl-CoA-binding domain-containing protein 5	ACBD5	Peroxisome
Q92805	golgin A1	GOLGA1	Golgi apparatus
O00461	golgi integral membrane protein 4	GOLIM4	Golgi apparatus
Q9HD26	golgi associated PDZ and coiled-coil motif containing	GOPC	Golgi apparatus
Q96ET8	trans-golgi network vesicle protein 23 homolog C	TVP23C	Golgi apparatus
Q9NXF8	zinc finger DHHC-type containing 7	ZDHHC7	Golgi apparatus
P48444	Coatomer subunit delta	ARCN1	Golgi apparatus
Q9NW68	BSD domain-containing protein 1	BSDC1	Golgi apparatus
P16671	Platelet glycoprotein 4	CD36	Golgi apparatus
P83436	Conserved oligomeric Golgi complex subunit 7	COG7	Golgi apparatus
O14579	Coatomer subunit epsilon	COPE	Golgi apparatus
Q92520	Protein FAM3C	FAM3C	Golgi apparatus
Q9BYC5	Alpha-(1,6)-fucosyltransferase	FUT8	Golgi apparatus
O60763	General vesicular	USO1	Golgi apparatus

UniProt ID	Entry name	Gene names	Localization/Subtype
	transport factor p115		
Q9HC07	Transmembrane protein 165	TMEM165	Golgi apparatus
Q9P1Q0	Vacuolar protein sorting-associated protein 54	VPS54	Golgi apparatus
Q9GZM5	Protein YIPF3	YIPF3	Golgi apparatus
Q96CS3	Fas associated factor family member 2	FAF2	Lipid Droplets
Q7L5N7	lysophosphatidylcholine acyltransferase 2	LPCAT2	Lipid Droplets
Q15738	NAD(P) dependent steroid dehydrogenase-like	NSDHL	Lipid Droplets
O60664	perilipin 3	PLIN3	Lipid Droplets
Q96AD5	patatin like phospholipase domain containing 2	PNPLA2	Lipid Droplets
P55283	cadherin 4	CDH4	Plasma membrane
P35222	catenin beta 1	CTNNB1	Plasma membrane
P00533	epidermal growth factor receptor	EGFR	Plasma membrane
P15311	ezrin	EZR	Plasma membrane
Q12846	syntaxin 4	STX4	Plasma membrane
Q9UNQ0	ATP-binding cassette sub-family G member 2	ABCG2	Plasma membrane
Q8WXE0	Caskin-2	CASKIN2	Plasma membrane
P14923	Junction plakoglobin	JUP	Plasma membrane
P13591	Neural cell adhesion molecule 1	NCAM	Plasma membrane
Q96RD7	Pannexin-1	PANX1	Plasma membrane
P11166	Solute carrier family 2, facilitated glucose transporter member 1	SLC2A1	Plasma membrane

UniProt ID	Entry name	Gene names	Localization/Subtype
Q8TAV4	Stomatin-like protein 3	STOML3	Plasma membrane
P35637	FUS RNA binding protein	FUS	Stress granule
Q13148	TAR DNA binding protein	TARDBP	Stress granule
Q92804	TATA-box binding protein associated factor 15	TAF15	Stress granule
Q01844	EWS RNA binding protein 1	EWSR1	Stress granule
Q9NQI0	DEAD-box helicase 4	DDX4	Stress granule
Q13148	TAR DNA-binding protein 43	TDP-43	Stress granule
P09651	Heterogeneous nuclear ribonucleoprotein A1	hnRNPA1	Stress granule
P22626	Heterogeneous nuclear ribonucleoproteins A2/B1	hnRNPA2	Stress granule
P31483	Nucleolysin TIA-1 isoform p40	TIA-1	Stress granule
Q9UPV0	centrosomal protein 164	CEP164	Cytoskeleton
Q9UHG0	doublecortin domain containing 2	DCDC2	Cytoskeleton
P05787	keratin 8	KRT8	Cytoskeleton
P35580	myosin heavy chain 10	MYH10	Cytoskeleton
P60709	actin beta	ACTB	Cytoskeleton
Q49A88	Coiled-coil domain-containing protein 14	CCDC14	Cytoskeleton
P05783	Keratin, type I cytoskeletal 18	KRT18	Cytoskeleton
O95613	Pericentrin	PCNT	Cytoskeleton
Q71U36	Tubulin alpha-1A chain	TUBA1A	Cytoskeleton
P68133	Actin, alpha skeletal muscle	ACTA1	Cytoskeleton

Secreted Payload Agents, e.g., Secreted Protein Payload Agents

[000272] Payload agents, e.g., protein payload agents, can also be targeted for secretion. In some embodiments, the methods and compositions described herein can be used to target payloads to the lumen of an organelle (e.g. a Golgi apparatus, secretory vesicle) after translation in the ER. In some embodiments, a secreted protein payload agent comprises a secreted protein or a nucleic acid encoding it.

Chondrisomes

[000273] In some embodiments, a fusosome or fusosome composition further comprises a chondrisome or chondrisome preparation. In some embodiments, a fusosome or fusosome composition comprises a modified chondrisome preparation derived from a cellular source of mitochondria. In some embodiments, a fusosome or fusosome composition comprises a chondrisome preparation expressing an exogenous protein. In some embodiments, the exogenous protein is exogenous to said mitochondria. In some embodiments, the exogenous protein is exogenous to said cellular source of mitochondria. Additional features and embodiments including chondrisomes, chondrisome preparations, methods, and uses are contemplated by the invention, e.g., as described in international application, PCT/US16/64251.

Immunogenicity

[000274] In some embodiments of any of the aspects described herein, the fusosome composition is substantially non-immunogenic. Immunogenicity can be quantified, e.g., as described herein.

[000275] In some embodiments, the fusosome composition has membrane symmetry of a cell which is, or is known to be, substantially non-immunogenic, e.g., a stem cell, mesenchymal stem cell, induced pluripotent stem cell, embryonic stem cell, sertoli cell, or retinal pigment epithelial cell. In some embodiments, the fusosome has an immunogenicity no more than 5%, 10%, 20%, 30%, 40%, or 50% greater than the immunogenicity of a stem cell, mesenchymal stem cell, induced pluripotent stem cell, embryonic stem cell, sertoli cell, or retinal pigment epithelial

cell as measured by an assay described herein.

[000276] In some embodiments, the fusosome composition comprises elevated levels of an immunosuppressive agent as compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, or a Jurkat cell. In some embodiments, the elevated level is at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 2-fold, 3-fold, 5-fold, 10-fold, 20-fold, 50-fold, or 100-fold. In some embodiments, the fusosome composition comprises an immunosuppressive agent that is absent from the reference cell. In some embodiments, the fusosome composition comprises reduced levels of an immune activating agent as compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, or a Jurkat cell. In some embodiments, the reduced level is at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% compared to the reference cell. In some embodiments, the immune activating agent is substantially absent from the fusosome.

[000277] In some embodiments, the fusosome composition comprises a membrane with composition substantially similar, e.g., as measured by proteomics, to that of a source cell, e.g., a substantially non-immunogenic source cell. In some embodiments, the fusosome composition comprises a membrane comprising at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, or 100% of the membrane proteins of the source cell. In some embodiments, the fusosome composition comprises a membrane comprising membrane proteins expressed at, at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%,95%, 99%, or 100% of the level of expression of the membrane proteins on a membrane of the source cell.

[000278] In some embodiments, the fusosome composition, or the source cell from which the fusosome composition is derived from, has one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, or more of the following characteristics:

a. less than 50%, 40%, 30%, 20%, 15%, 10%, or 5% or lesser expression of MHC class I or MHC class II, compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, or a HeLa cell;

b. less than 50%, 40%, 30%, 20%, 15%, 10%, or 5% or lesser expression of one or more co-stimulatory proteins including but not limited to: LAG3, ICOS-L, ICOS, Ox40L, OX40,

CD28, B7, CD30, CD30L 4-1BB, 4-1BBL, SLAM, CD27, CD70, HVEM, LIGHT, B7-H3, or B7-H4, compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, or a reference cell described herein;

c. expression of surface proteins which suppress macrophage engulfment e.g., CD47, e.g., detectable expression by a method described herein, e.g., more than 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, or more expression of the surface protein which suppresses macrophage engulfment, e.g., CD47, compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, or a Jurkat cell;

d. expression of soluble immunosuppressive cytokines, e.g., IL-10, e.g., detectable expression by a method described herein, e.g., more than 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, or more expression of soluble immunosuppressive cytokines, e.g., IL-10, compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, or a Jurkat cell;

e. expression of soluble immunosuppressive proteins, e.g., PD-L1, e.g., detectable expression by a method described herein, e.g., more than 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, or more expression of soluble immunosuppressive proteins, e.g., PD-L1, compared to a reference cell e.g., an unmodified cell otherwise similar to the source cell, or a Jurkat cell;

f. less than 50%, 40%, 30%, 20%, 15%, 10%, or 5% or lesser expression of soluble immune stimulating cytokines, e.g., IFN-gamma or TNF-a, compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, or a U-266 cell;

g. less than 50%, 40%, 30%, 20%, 15%, 10%, or 5% or lesser expression of endogenous immune-stimulatory antigen, e.g., Zg16 or Hormad1, compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, or an A549 cell or a SK-BR-3 cell;

h. expression of, e.g., detectable expression by a method described herein, HLA-E or HLA-G, compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, or a Jurkat cell;

i. surface glycosylation profile, e.g., containing sialic acid, which acts to, e.g., suppress NK cell activation;

j. less than 50%, 40%, 30%, 20%, 15%, 10%, or 5% or lesser expression of TCRα/β, compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, or a Jurkat cell;

k. less than 50%, 40%, 30%, 20%, 15%, 10%, or 5% or lesser expression of ABO blood groups, compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, or a HeLa cell;

l. less than 50%, 40%, 30%, 20%, 15%, 10%, or 5% or lesser expression of Minor Histocompatibility Antigen (MHA), compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, or a Jurkat cell; or

m. has less than 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less, of mitochondrial MHAs, compared to a reference cell e.g., an unmodified cell otherwise similar to the source cell, or a Jurkat cell, or has no detectable mitochondrial MHAs.

[000279] In embodiments, the co-stimulatory protein is 4-1BB, B7, SLAM, LAG3, HVEM, or LIGHT, and the ref cell is HDLM-2. In some embodiments, the co-stimulatory protein is BY-H3 and the reference cell is HeLa. In some embodiments, the co-stimulatory protein is ICOSL or B7-H4, and the reference cell is SK-BR-3. In some embodiments, the co-stimulatory protein is ICOS or OX40, and the reference cell is MOLT-4. In some embodiments, the co-stimulatory protein is CD28, and the reference cell is U-266. In some embodiments, the co-stimulatory protein is CD30L or CD27, and the reference cell is Daudi.

[000280] In some embodiments, the fusosome composition does not substantially elicit an immunogenic response by the immune system, e.g., innate immune system. In embodiments, an immunogenic response can be quantified, e.g., as described herein. In some embodiments, the an immunogenic response by the innate immune system comprises a response by innate immune cells including, but not limited to NK cells, macrophages, neutrophils, basophils, eosinophils, dendritic cells, mast cells, or gamma/delta T cells. In some embodiments, an immunogenic response by the innate immune system comprises a response by the complement system which includes soluble blood components and membrane bound components.

[000281] In some embodiments, the fusosome composition does not substantially elicit an immunogenic response by the immune system, e.g., adaptive immune system. In embodiments, an immunogenic response can be quantified, e.g., as described herein. In some embodiments, an immunogenic response by the adaptive immune system comprises an immunogenic response by an adaptive immune cell including, but not limited to a change, e.g., increase, in number or activity

of T lymphocytes (e.g., CD4 T cells, CD8 T cells, and or gamma-delta T cells), or B lymphocytes. In some embodiments, an immunogenic response by the adaptive immune system includes increased levels of soluble blood components including, but not limited to a change, e.g., increase, in number or activity of cytokines or antibodies (e.g., IgG, IgM, IgE, IgA, or IgD).

[000282] In some embodiments, the fusosome composition is modified to have reduced immunogenicity. Immunogenicity can be quantified, e.g., as described herein. In some embodiments, the fusosome composition has an immunogenicity less than 5%, 10%, 20%, 30%, 40%, or 50% lesser than the immunogenicity of a reference cell, e.g., an unmodified cell otherwise similar to the source cell, or a Jurkat cell.

[000283] In some embodiments of any of the aspects described herein, the fusosome composition is derived from a source cell, e.g., a mammalian cell, having a modified genome, e.g., modified using a method described herein, to reduce, e.g., lessen, immunogenicity. Immunogenicity can be quantified, e.g., as described herein.

[000284] In some embodiments, the fusosome composition is derived from a mammalian cell depleted of, e.g., with a knock out of, one, two, three, four, five, six, seven or more of the following:

a. MHC class I, MHC class II or MHA;
b. one or more co-stimulatory proteins including but not limited to: LAG3, ICOS-L, ICOS, Ox40L, OX40, CD28, B7, CD30, CD30L 4-1BB, 4-1BBL, SLAM, CD27, CD70, HVEM, LIGHT, B7-H3, or B7-H4;
c. soluble immune-stimulating cytokines e.g., IFN-gamma or TNF-a;
d. endogenous immune-stimulatory antigen, e.g., Zg16 or Hormad1;
e. T-cell receptors (TCR);
f. The genes encoding ABO blood groups, e.g., *ABO* gene;
g. transcription factors which drive immune activation, e.g., NFkB;
h. transcription factors that control MHC expression e.g., class II trans-activator (CIITA), regulatory factor of the Xbox 5 (RFX5), RFX-associated protein (RFXAP), or RFX ankyrin repeats (RFXANK; also known as RFXB); or
i. TAP proteins, e.g., TAP2, TAP1, or TAPBP, which reduce MHC class I expression.

[000285] In some embodiments, the fusosome is derived from a source cell with a genetic

modification which results in increased expression of an immunosuppressive agent, e.g., one, two, three or more of the following (e.g., wherein before the genetic modification the cell did not express the factor):

a. surface proteins which suppress macrophage engulfment, e.g., CD47; e.g., increased expression of CD47 compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, or a Jurkat cell;

b. soluble immunosuppressive cytokines, e.g., IL-10, e.g., increased expression of IL-10 compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, or a Jurkat cell;

c. soluble immunosuppressive proteins, e.g., PD-1, PD-L1, CTLA4, or BTLA; e.g., increased expression of immunosuppressive proteins compared to a reference cell, e.g., an unmodified cell otherwise similar to the cell source, or a Jurkat cell; or

d. a tolerogenic protein, e.g., an ILT-2 or ILT-4 agonist, e.g., HLA-E or HLA-G or any other endogenous ILT-2 or ILT-4 agonist, e.g., increased expression of HLA-E, HLA-G, ILT-2 or ILT-4 compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, or a Jurkat cell.

In some embodiments, the increased expression level is at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 2-fold, 3-fold, 5-fold, 10-fold, 20-fold, 50-fold, or 100-fold higher as compared to a reference cell.

In some embodiments, the fusosome is derived from a source cell modified to have decreased expression of an immune activating agent, e.g., one, two, three, four, five, six, seven, eight or more of the following:

a. less than 50%, 40%, 30%, 20%, 15%, 10%, or 5% or lesser expression of MHC class I or MHC class II, compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, or a HeLa cell;

b. less than 50%, 40%, 30%, 20%, 15%, 10%, or 5% or lesser expression of one or more co-stimulatory proteins including but not limited to: LAG3, ICOS-L, ICOS, Ox40L, OX40, CD28, B7, CD30, CD30L 4-1BB, 4-1BBL, SLAM, CD27, CD70, HVEM, LIGHT, B7-H3, or B7-H4, compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, or a reference cell described herein;

c. less than 50%, 40%, 30%, 20%, 15%, 10%, or 5% or lesser expression of soluble immune stimulating cytokines, e.g., IFN-gamma or TNF-a, compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, or a U-266 cell;

d. less than 50%, 40%, 30%, 20%, 15%, 10%, or 5% or lesser expression of endogenous immune-stimulatory antigen, e.g., Zg16 or Hormad1, compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, or an A549 cell or a SK-BR-3 cell;

e. less than 50%, 40%, 30%, 20%, 15%, 10%, or 5% or lesser expression of T-cell receptors (TCR) compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, or a Jurkat cell;

f. less than 50%, 40%, 30%, 20%, 15%, 10%, or 5% or lesser expression of ABO blood groups, compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, or a HeLa cell;

g. less than 50%, 40%, 30%, 20%, 15%, 10%, or 5% or lesser expression of transcription factors which drive immune activation, e.g., NFkB; compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, or a Jurkat cell

h. less than 50%, 40%, 30%, 20%, 15%, 10%, or 5% or lesser expression of transcription factors that control MHC expression, e.g., class II trans-activator (CIITA), regulatory factor of the Xbox 5 (RFX5), RFX-associated protein (RFXAP), or RFX ankyrin repeats (RFXANK; also known as RFXB) compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, or a Jurkat cell; or

i. less than 50%, 40%, 30%, 20%, 15%, 10%, or 5% or lesser expression of TAP proteins, e.g., TAP2, TAP1, or TAPBP, which reduce MHC class I expression compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, or a HeLa cell.

[000286] In some embodiments, a fusosome composition derived from a mammalian cell, e.g., a mesenchymal stem cell, modified using shRNA expressing lentivirus to decrease MHC Class I expression, has lesser expression of MHC Class I compared to an unmodified cell, e.g., a mesenchymal stem cell that has not been modified. In some embodiments, a fusosome composition derived from a mammalian cell, e.g., a mesenchymal stem cell, modified using lentivirus expressing HLA-G to increase expression of HLA-G, has increased expression of HLA-G compared to an unmodified cell, e.g., a mesenchymal stem cell that has not been modified.

[000287] In some embodiments, the fusosome composition is derived from a source cell, e.g., a mammalian cell, which is not substantially immunogenic, wherein the source cells stimulate, e.g., induce, T-cell IFN-gamma secretion, at a level of 0 pg/mL to >0 pg/mL, e.g., as assayed i*n vitro,* by IFN-gamma ELISPOT assay.

[000288] In some embodiments, the fusosome composition is derived from a source cell, e.g., a mammalian cell, wherein the mammalian cell is from a cell culture treated with an immunosuppressive agent, e.g., a glucocorticoid (e.g., dexamethasone), cytostatic (e.g., methotrexate), antibody (e.g., Muromonab-CD3), or immunophilin modulator (e.g., Ciclosporin or rapamycin).

[000289] In some embodiments, the fusosome composition is derived from a source cell, e.g., a mammalian cell, wherein the mammalian cell comprises an exogenous agent, e.g., a therapeutic agent.

[000290] In some embodiments, the fusosome composition is derived from a source cell, e.g., a mammalian cell, wherein the mammalian cell is a recombinant cell.

[000291] In some embodiments, the fusosome is derived from a mammalian cell genetically modified to express viral immunoevasins, e.g., hCMV US2, or US11.

[000292] In some embodiments, the surface of the fusosome, or the surface of the mammalian cell the fusosome is derived from, is covalently or non-covalently modified with a polymer, e.g., a biocompatible polymer that reduces immunogenicity and immune-mediated clearance, e.g., PEG.

[000293] In some embodiments, the surface of the fusosome, or the surface of the mammalian cell the fusosome is derived from is covalently or non-covalently modified with a sialic acid, e.g., a sialic acid comprising glycopolymers, which contain NK-suppressive glycan epitopes.

[000294] In some embodiments, the surface of the fusosome, or the surface of the mammalian cell the fusosome is derived from is enzymatically treated, e.g., with glycosidase enzymes, e.g., α-N-acetylgalactosaminidases, to remove ABO blood groups

[000295] In some embodiments, the surface of the fusosome, or the surface of the mammalian cell the fusosome is derived from is enzymatically treated, to give rise to, e.g., induce expression

of, ABO blood groups which match the recipient's blood type.

Parameters for assessing immunogenicity

[000296] In some embodiments, the fusosome composition is derived from a source cell, e.g., a mammalian cell which is not substantially immunogenic, or modified, e.g., modified using a method described herein, to have a reduction in immunogenicity. Immunogenicity of the source cell and the fusosome composition can be determined by any of the assays described herein.

[000297] In some embodiments, the fusosome composition has an increase, e.g., an increase of 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more, in in vivo graft survival compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell. In some embodiments, graft survival is determined by an assay measuring in vivo graft survival as described herein, in an appropriate animal model, e.g., an animal model described herein.

[000298] In some embodiments, the fusosome composition has an increase, e.g., an increase of 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more in teratoma formation compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell. In some embodiments, teratroma formation is determined by an assay measuring teratoma formation as described herein, in an appropriate animal model, e.g., in an animal model described herein.

[000299] In some embodiments, the fusosome composition has an increase, e.g., an increase of 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more in teratoma survival compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell. In some embodiments, the fusosome composition survives for one or more days in an assay of teratoma survival. In some embodiments, teratroma survival is determined by an assay measuring teratoma survival as described herein, in an appropriate animal model, e.g., in an animal model described herein. In an embodiments, teratoma formation is measured by imaging analysis, e.g., IHC staining, fluorescent staining or H&E, of fixed tissue, e.g., frozen or formalin fixed, as described in the Examples. In some embodiments, fixed tissue can be stained with any one or all of the following antibodies: anti-human CD3, anti-human CD4, or anti-human CD8.

[000300] In some embodiments, the fusosome composition has a reduction, e.g., a reduction of 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more in CD8+ T cell infiltration

into a graft or teratoma compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell. In some embodiments, CD8 T cell infiltration is determined by an assay measuring CD8+ T cell infiltration as described herein, e.g., histological analysis, in an appropriate animal model, e.g., an animal model described herein. In some embodiments, teratomas derived from the fusosome composition have CD8+ T cell infiltration in 0%, 0.1%, 1% 5%, 10%, 20%, 30%, 40% 50%, 60%, 70%, 80%, 90%, or 100% of 50x image fields of a histology tissue section.

[000301] In some embodiments, a fusosome composition has a reduction, e.g., a reduction of 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more in CD4+ T cell infiltration into a graft or teratoma compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell. In some embodiments, CD4 T cell infiltration is determined by an assay measuring CD4+ T cell infiltration as described herein, , e.g., histological analysis, in an appropriate animal model, e.g., an animal model described herein. In some embodiments, teratomas derived from the fusosome composition have CD4+ T cell infiltration in 0%, 0.1%, 1% 5%, 10%, 20%, 30%, 40% 50%, 60%, 70%, 80%, 90%, or 100% of 50x image fields of a histology tissue section.

[000302] In some embodiments, a fusosome composition has a reduction, e.g., a reduction of 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more in CD3+ NK cell infiltration into a graft or teratoma compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell. In some embodiments, CD3+ NK cell infiltration is determined by an assay measuring CD3+ NK cell infiltration as described herein, e.g., histological analysis, in an appropriate animal model, e.g., an animal model described herein. In some embodiments, teratomas derived from the fusosome composition have CD3+ NK T cell infiltration in 0%, 0.1%, 1% 5%, 10%, 20%, 30%, 40% 50%, 60%, 70%, 80%, 90%, or 100% of 50x image fields of a histology tissue section.

[000303] In some embodiments, the fusosome composition has a reduction in immunogenicity as measured by a reduction in humoral response following one or more implantation of the fusosome derived into an appropriate animal model, e.g., an animal model described herein, compared to a humoral response following one or more implantation of a reference cell, e.g., an unmodified cell otherwise similar to the source cell, into an appropriate animal model, e.g., an animal model described herein. In some embodiments, the reduction in

humoral response is measured in a serum sample by an anti-cell antibody titre, e.g., anti-fusosome antibody titre, e.g., by ELISA. In some embodiments, the serum sample from animals administered the fusosome composition has a reduction of 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more of an anti-cell antibody titer compared to the serum sample from animals administered an unmodified cell. In some embodiments, the serum sample from animals administered the fusosome composition has an increased anti-cell antibody titre, e.g., increased by 1%, 2%, 5%, 10%, 20%, 30%, or.40% from baseline, e.g., wherein baseline refers to serum sample from the same animals before administration of the fusosome composition.

[000304] In some embodiments, the fusosome composition has a reduction in macrophage phagocytosis, e.g., a reduction of 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more in macrophage phagocytosis compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, wherein the reduction in macrophage phagocytosis is determined by assaying the phagocytosis index *in vitro*, e.g., as described in Example 82. In some embodiments, the fusosome composition has a phagocytosis index of 0, 1, 10, 100, or more, e.g., as measured by an assay of Example 82, when incubated with macrophages in an *in vitro* assay of macrophage phagocytosis.

[000305] In some embodiments, the source cell has a reduction in cytotoxicity mediated cell lysis by PBMCs, e.g., a reduction of 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more in cell lysis compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell or a mesenchymal stem cells, e.g., using an assay of Example 83. In embodiments, the source cell expresses exogenous HLA-G.

[000306] In some embodiments, the fusosome composition has a reduction in NK-mediated cell lysis, e.g., a reduction of 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more in NK-mediated cell lysis compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, wherein NK-mediated cell lysis is assayed i*n vitro,* by a chromium release assay or europium release assay.

[000307] In some embodiments, the fusosome composition has a reduction in CD8+ T-cell mediated cell lysis, e.g., a reduction of 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more in CD8 T cell mediated cell lysis compared to a reference cell, e.g., an unmodified

cell otherwise similar to the source cell, wherein CD8 T cell mediated cell lysis is assayed i*n vitro,* by a chromium release assay or europium release assay. In embodiments, activation and/or proliferation is measured as described in Example 85.

[000308] In some embodiments, the fusosome composition has a reduction in CD4+ T-cell proliferation and/or activation, e.g., a reduction of 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, wherein CD4 T cell proliferation is assayed i*n vitro* (*e.g.* co-culture assay of modified or unmodified mammalian source cell, and CD4+T-cells with CD3/CD28 Dynabeads), e.g., as described in Example 86.

[000309] In some embodiments, the fusosome composition has a reduction in T-cell IFN-gamma secretion, e.g., a reduction of 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more in T-cell IFN-gamma secretion compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, wherein T-cell IFN-gamma secretion is assayed i*n vitro, e.g.,* by IFN-gamma ELISPOT.

[000310] In some embodiments, the fusosome composition has a reduction in secretion of immunogenic cytokines, e.g., a reduction of 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more in secretion of immunogenic cytokines compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, wherein secretion of immunogenic cytokines is assayed i*n vitro* using ELISA or ELISPOT.

[000311] In some embodiments, the fusosome composition results in increased secretion of an immunosuppressive cytokine, e.g., an increase of 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more in secretion of an immunosuppressive cytokine compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, wherein secretion of the immunosuppressive cytokine is assayed i*n vitro* using ELISA or ELISPOT.

[000312] In some embodiments, the fusosome composition has an increase in expression of HLA-G or HLA-E, e.g., an increase in expression of 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more of HLA-G or HLA-E, compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, wherein expression of HLA-G or HLA-E is assayed i*n vitro* using flow cytometry, e.g., FACS. In some embodiments, the fusosome composition is

derived from a source cell which is modified to have an increased expression of HLA-G or HLA-E, e.g., compared to an unmodified cell, e.g., an increased expression of 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more of HLA-G or HLA-E, wherein expression of HLA-G or HLA-E is assayed *in vitro* using flow cytometry, e.g., FACS. In some embodiments, the fusosome composition derived from a modified cell with increased HLA-G expression demonstrates reduced immunogenicity, e.g., as measured by reduced immune cell infiltration, in a teratoma formation assay, e.g., a teratoma formation assay as described herein.

[000313] In some embodiments, the fusosome composition has an increase in expression of T cell inhibitor ligands (e.g. CTLA4, PD1, PD-L1), e.g., an increase in expression of 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more of T cell inhibitor ligands as compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, wherein expression of T cell inhibitor ligands is assayed *in vitro* using flow cytometry, e.g., FACS.

[000314] In some embodiments, the fusosome composition has a decrease in expression of co-stimulatory ligands, e.g., a decrease of 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more in expression of co-stimulatory ligands compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, wherein expression of co-stimulatory ligands is assayed i*n vitro* using flow cytometry, e.g., FACS.

[000315] In some embodiments, the fusosome composition has a decrease in expression of MHC class I or MHC class II, e.g., a decrease in expression of 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more of MHC Class I or MHC Class II compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell or a HeLa cell, wherein expression of MHC Class I or II is assayed *in vitro* using flow cytometry, e.g., FACS.

[000316] In some embodiments, the fusosome composition is derived from a cell source, e.g., a mammalian cell source, which is substantially non-immunogenic. In some embodiments, immunogenicity can be quantified, e.g., as described herein. In some embodiments, the mammalian cell source comprises any one, all or a combination of the following features:

a. wherein the source cell is obtained from an autologous cell source; e.g., a cell obtained from a recipient who will be receiving, e.g., administered, the fusosome composition;

b. wherein the source cell is obtained from an allogeneic cell source which is of

matched, e.g., similar, gender to a recipient, e.g., a recipient described herein who will be receiving, e.g., administered; the fusosome composition;

c. wherein the source cell is obtained is from an allogeneic cell source is which is HLA matched with a recipient's HLA, e.g., at one or more alleles;

d. wherein the source cell is obtained is from an allogeneic cell source which is an HLA homozygote;

e. wherein the source cell is obtained is from an allogeneic cell source which lacks (or has reduced levels compared to a reference cell) MHC class I and II; or

f. wherein the source cell is obtained is from a cell source which is known to be substantially non-immunogenic including but not limited to a stem cell, a mesenchymal stem cell, an induced pluripotent stem cell, an embryonic stem cell, a sertoli cell, or a retinal pigment epithelial cell .

[000317] In some embodiments, the subject to be administered the fusosome composition has, or is known to have, or is tested for, a pre-existing antibody (e.g., IgG or IgM) reactive with a fusosome. In some embodiments, the subject to be administered the fusosome composition does not have detectable levels of a pre-existing antibody reactive with the fusosome. Tests for the antibody are described, e.g., in Example 78.

[000318] In some embodiments, a subject that has received the fusosome composition has, or is known to have, or is tested for, an antibody (e.g., IgG or IgM) reactive with a fusosome. In some embodiments, the subject that received the fusosome composition (e.g., at least once, twice, three times, four times, five times, or more) does not have detectable levels of antibody reactive with the fusosome. In embodiments, levels of antibody do not rise more than 1%, 2%, 5%, 10%, 20%, or 50% between two timepoints, the first timepoint being before the first administration of the fusosome, and the second timepoint being after one or more administrations of the fusosome. Tests for the antibody are described, e.g., in Example 79.

Additional therapeutic agents

[000319] In some embodiments, the fusosome composition is co-administered with an additional agent, e.g., a therapeutic agent, to a subject, e.g., a recipient, e.g., a recipient described herein. In some embodiments, the co-administered therapeutic agent is an immunosuppressive

agent, e.g., a glucocorticoid (e.g., dexamethasone), cytostatic (e.g., methotrexate), antibody (e.g., Muromonab-CD3), or immunophilin modulator (e.g., Ciclosporin or rapamycin). In embodiments, the immunosuppressive agent decreases immune mediated clearance of fusosomes. In some embodiments the fusosome composition is co-administered with an immunostimulatory agent, e.g., an adjuvant, an interleukin, a cytokine, or a chemokine.

[000320] In some embodiments, the fusosome composition and the immunosuppressive agent are administered at the same time, e.g., contemporaneously administered. In some embodiments, the fusosome composition is administered before administration of the immunosuppressive agent. In some embodiments, the fusosome composition is administered after administration of the immunosuppressive agent.

[000321] In some embodiments, the immunosuppressive agent is a small molecule such as ibuprofen, acetaminophen, cyclosporine, tacrolimus, rapamycin, mycophenolate, cyclophosphamide, glucocorticoids, sirolimus, azathriopine, or methotrexate.

[000322] In some embodiments, the immunosuppressive agent is an antibody molecule, including but not limited to: muronomab (anti-CD3), Daclizumab (anti-IL12), Basiliximab, Infliximab (Anti-TNFa), or rituximab (Anti-CD20).

[000323] In some embodiments, co-administration of the fusosome composition with the immunosuppressive agent results in enhanced persistence of the fusosome composition in the subject compared to administration of the fusosome composition alone. In some embodiments, the enhanced persistence of the fusosome composition in the co-administration is at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or longer, compared to persistence of the fusosome composition when administered alone. In some embodiments, the enhanced persistence of the fusosome composition in the co-administration is at least 1, 2, 3, 4, 5, 6, 7, 10, 15, 20, 25, or 30 days or longer, compared to survival of the fusosome composition when administered alone.

Delivery

[000324] In some embodiments, a fusogen (e.g., protein, lipid, or chemical fusogen) or a fusogen binding partner is delivered to a target cell or tissue prior to, at the same time, or after the delivery of a fusosome.

[000325] In some embodiments, a fusogen (e.g., protein, lipid, or chemical fusogen) or a fusogen binding partner is delivered to a non-target cell or tissue prior to, at the same time, or after the delivery of a fusosome.

[000326] In some embodiments, a nucleic acid that encodes a fusogen (e.g., protein or lipid fusogen) or a fusogen binding partner is delivered to a target cell or tissue prior to, at the same time, or after the delivery of a fusosome.

[000327] In some embodiments, a polypeptide, nucleic acid, ribonucleoprotein, or small-molecule that upregulates or downregulates expression of a fusogen (e.g., protein, lipid, or chemical fusogen) or a fusogen binding partner is delivered to a target cell or tissue prior to, at the same time, or after the delivery of a fusosome.

[000328] In some embodiments, a polypeptide, nucleic acid, ribonucleoprotein, or small-molecule that upregulates or downregulates expression of a fusogen (e.g., protein, lipid, or chemical fusogen) or a fusogen binding partner is delivered to a non-target cell or tissue prior to, at the same time, or after the delivery of a fusosome.

[000329] In some embodiments, the target cell or tissue is modified by (e.g., inducing stress or cell division) to increase the rate of fusion prior to, at the same time, or after the delivery of a fusosome. Some nonlimiting examples include, inducing ischemia, treatment with chemotherapy, antibiotic, irradiation, toxin, inflammation, inflammatory molecules, anti-inflammatory molecules, acid injury, basic injury, burn, polyethylene glycol, neurotransmitters, myelotoxic drugs, growth factors, or hormones, tissue resection, starvation, and/or exercise.

[000330] In some embodiments, the target cell or tissue is treated with a vasodilator (e.g. nitric oxide (NO), carbon monoxide, prostacyclin (PGI2), nitroglycerine, phentolamine) or vasoconstrictors (e.g. angiotensin (AGT), endothelin (EDN), norepinephrine)) to increase the rate of fusosome transport to the target tissue.

[000331] In some embodiments, the target cell or tissue is treated with a chemical agent, e.g., a chemotherapeutic. In such embodiments, the chemotherapeutic induces damage to the target cell or tissue that enhances fusogenic activity of target cells or tissue.

[000332] In some embodiments, the target cell or tissue is treated with a physical stress, e.g.,

electrofusion. In such embodiments, the physical stress destabilizes the membranes of the target cell or tissue to enhance fusogenic activity of target cells or tissue.

[000333] In some embodiments, the target cell or tissue may be treated with an agent to enhance fusion with a fusosome. For example, specific neuronal receptors may be stimulated with an anti-depressant to enhance fusogenic properties.

[000334] Compositions comprising the fusosomes described herein may be administered or targeted to the circulatory system, hepatic system, renal system, cardio-pulmonary system, central nervous system, peripheral nervous system, musculoskeletal system, lymphatic system, immune system, sensory nervous systems (sight, hearing, smell, touch, taste), digestive system, endocrine systems (including adipose tissue metabolic regulation), and reproductive system.

[000335] In embodiments, a fusosome composition described herein is delivered ex-vivo to a cell or tissue, e.g., a human cell or tissue. In some embodiments, the composition is delivered to an ex vivo tissue that is in an injured state (e.g., from trauma, disease, hypoxia, ischemia or other damage).

[000336] In some embodiments, the fusosome composition is delivered to an ex-vivo transplant (e.g., a tissue explant or tissue for transplantation, e.g., a human vein, a musculoskeletal graft such as bone or tendon, cornea, skin, heart valves, nerves; or an isolated or cultured organ, e.g., an organ to be transplanted into a human, e.g., a human heart, liver, lung, kidney, pancreas, intestine, thymus, eye). The composition improves viability, respiration, or other function of the transplant. The composition can be delivered to the tissue or organ before, during and/or after transplantation.

[000337] In some embodiments, a fusosome composition described herein is delivered ex-vivo to a cell or tissue derived from a subject. In some embodiments the cell or tissue is readministered to the subject (i.e., the cell or tissue is autologous).

[000338] The fusosomes may fuse with a cell from any mammalian (e.g., human) tissue, e.g., from epithelial, connective, muscular, or nervous tissue or cells, and combinations thereof. The fusosomes can be delivered to any eukaryotic (e.g., mammalian) organ system, for example, from the cardiovascular system (heart, vasculature); digestive system (esophagus, stomach, liver,

gallbladder, pancreas, intestines, colon, rectum and anus); endocrine system (hypothalamus, pituitary gland, pineal body or pineal gland, thyroid, parathyroids, adrenal glands); excretory system (kidneys, ureters, bladder); lymphatic system (lymph, lymph nodes, lymph vessels, tonsils, adenoids, thymus, spleen); integumentary system (skin, hair, nails); muscular system (e.g., skeletal muscle); nervous system (brain, spinal cord, nerves)'; reproductive system (ovaries, uterus, mammary glands, testes, vas deferens, seminal vesicles, prostate); respiratory system (pharynx, larynx, trachea, bronchi, lungs, diaphragm); skeletal system (bone, cartilage), and combinations thereof.

[000339] In embodiments, the fusosome targets a tissue, e.g., liver, lungs, heart, spleen, pancreas, gastrointestinal tract, kidney, testes, ovaries, brain, reproductive organs, central nervous system, peripheral nervous system, skeletal muscle, endothelium, inner ear, adipose tissue (e.g., brown adipose tissue or white adipose tissue) or eye, when administered to a subject, e.g., wherein at least 0.1%, 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the fusosomes in a population of administered fusosomes are present in the target tissue after 24, 48, or 72 hours, e.g., by an assay of Example 87 or 100.

[000340] In embodiments, the fusosomes may fuse with a cell from a source of stem cells or progenitor cells, e.g., bone marrow stromal cells, marrow-derived adult progenitor cells (MAPCs), endothelial progenitor cells (EPC), blast cells, intermediate progenitor cells formed in the subventricular zone, neural stem cells, muscle stem cells, satellite cells, liver stem cells, hematopoietic stem cells, bone marrow stromal cells, epidermal stem cells, embryonic stem cells, mesenchymal stem cells, umbilical cord stem cells, precursor cells, muscle precursor cells, myoblast, cardiomyoblast, neural precursor cells, glial precursor cells, neuronal precursor cells, hepatoblasts.

Fusogen Binding Partners, e.g., for landing pad embodiments

[000341] In certain aspects, the disclosure provides a method of delivering a fusosome to a target cell in a subject. In some embodiments, the method comprises administering to a subject a fusosome comprising a nucleic acid encoding a fusogen, e.g., a myomaker protein, wherein the nucleic acid is not present or is not expressed (e.g., is present but is not transcribed or not translated) within a cell, under conditions that allow the fusogen to be expressed on the surface of

the fusosome in the subject. In some embodiments, the method further comprises administering to the subject a composition comprising an agent, e.g., a therapeutic agent, and a fusogen binding partner, optionally, comprising a carrier, e.g., a membrane, under conditions that allow fusion of the fusogen on the fusosome, and the fusogen binding partner. In some embodiments, the carrier comprises a membrane, e.g., a lipid bilayer, e.g., the agent is disposed within a lipid bilayer. In some embodiments, the lipid bilayer fuses with the target cell, thereby delivering the agent to the target cell in the subject.

[000342] In some embodiments, a fusogen binding partner is a moiety, e.g., a protein molecule, disposed in a membrane (e.g., a lipid bilayer), of a target cell, e.g., a target cell disclosed herein. In some embodiments, the membrane can be a cell surface membrane, or a subcellular membrane of an organelle, e.g., a mitochondrion, lysosome, or Golgi apparatus. In some embodiments, a fusogen binding partner can be endogenously expressed, overexpressed, or exogenously expressed (e.g., by a method described herein). In some embodiments, the fusogen binding partner can cluster with other fusogen binding partners at the membrane.

[000343] In some embodiments, the presence of a fusogen binding partner, or a plurality of fusogen binding partners, in a membrane of a target cell, creates an interface that can facilitate the interaction, e.g., binding, between a fusogen binding partner on a target cell (e.g., a cell described herein), and a fusogen on a fusosome (e.g., a fusosome described herein). In some embodiments, the fusogen on a fusosome interacts with, e.g., binds to, a fusogen binding partner on target cell, e.g., on the membrane (e.g., lipid bilayer), of a target cell, to induce fusion of the fusosome with the target membrane. In some embodiments, the fusogen interacts with, e.g., binds to, a fusogen binding partner on a landing pad on a subcellular organelle, including a mitochondrion, to induce fusion of the fusosome with the subcellular organelle.

[000344] A fusogen binding partner can be introduced in a target cell, e.g., a target cell disclosed herein, by any of the methods discussed below.

[000345] In some embodiments, a method of introducing a fusogen binding partner to a target cell comprises removal, e.g., extraction, of a target cell (e.g., via apheresis or biopsy), from a subject (e.g., a subject described herein), and administration of, e.g., exposure to, a fusogen binding partner under conditions that allow the fusogen binding partner to be expressed on a membrane of

the target cell. In some embodiments, a method comprises contacting the target cell expressing a fusogen binding partner *ex vivo* with a fusosome comprising a fusogen to induce fusion of the fusosome with the target cell membrane. In some embodiments, a target cell fused to the fusosome is reintroduced into the subject, e.g., intravenously.

[000346] In some embodiments, a target cell expressing a fusogen binding partner is reintroduced into the subject, e.g., intravenously. In some embodiments, a method comprises administering to the subject a fusosome comprising a fusogen to allow interaction, e.g., binding, of the fusogen on the fusosome with the fusogen binding partner on the target cell, and fusion of the fusosome with the target cell membrane.

[000347] In some embodiments, the target cells are treated with an epigenetic modifier, e.g., a small molecule epigenetic modifier, to increase or decrease expression of an endogenous cell surface molecule (e.g.,in some embodiments, endogenous relative to the target cell), e.g., a fusogen binding partner, e.g., an organ, tissue, or cell targeting molecule, where the cell surface molecule is a protein, glycan, lipid or low molecular weight molecule. In some embodiments, a target cell is genetically modified to increase the expression of an endogenous cell surface molecule, e.g., a fusogen binding partner, e.g., an organ, tissue, or cell targeting molecule, where the cell surface molecule is a protein, glycan, lipid or low molecular weight molecule. In some embodiments, a genetic modification may decrease expression of a transcriptional activator of the endogenous cell surface molecule, e.g., a fusogen binding partner.

[000348] In some embodiments, a target cell is genetically modified to express, e.g., overexpress, an exogenous cell surface molecule, e.g., a fusogen binding partner, where the cell surface molecule is a protein, glycan, lipid or low molecular weight molecule.

[000349] In some embodiments, the target cell is genetically modified to increase the expression of an exogenous fusogen in the cell, e.g., delivery of a transgene. In some embodiments, a nucleic acid, e.g., DNA, mRNA or siRNA, is transferred to the target cell, e.g., to increase or decrease the expression of a cell surface molecule (protein, glycan, lipid or low molecular weight molecule). In some embodiments, the nucleic acid targets a repressor of a fusogen binding partner, e.g., an shRNA, or siRNA construct. In some embodiments, the nucleic acid encodes an inhibitor of a fusogen binding partner repressor.

Methods of Use

[000350] The administration of a pharmaceutical composition described herein may be by way of oral, inhaled, transdermal or parenteral (including intravenous, intratumoral, intraperitoneal, intramuscular, intracavity, and subcutaneous) administration. The fusosomes may be administered alone or formulated as a pharmaceutical composition.

[000351] The fusosomes may be administered in the form of a unit-dose composition, such as a unit dose oral, parenteral, transdermal or inhaled composition. Such compositions are prepared by admixture and are suitably adapted for oral, inhaled, transdermal or parenteral administration, and as such may be in the form of tablets, capsules, oral liquid preparations, powders, granules, lozenges, reconstitutable powders, injectable and infusable solutions or suspensions or suppositories or aerosols.

[000352] In some embodiments, delivery of a membrane protein payload agent via a fusosome composition described herein may induce or block cellular differentiation, de-differentiation, or trans-differentiation. The target mammalian cell may be a precursor cell. Alternatively, the target mammalian cell may be a differentiated cell, and the cell fate alteration includes driving de-differentiation into a pluripotent precursor cell, or blocking such de-differentiation. In situations where a change in cell fate is desired, effective amounts of a fusosome described herein encoding a cell fate inductive molecule or signal is introduced into a target cell under conditions such that an alteration in cell fate is induced. In some embodiments, a fusosome described herein is useful to reprogram a subpopulation of cells from a first phenotype to a second phenotype. Such a reprogramming may be temporary or permanent. Optionally, the reprogramming induces a target cell to adopt an intermediate phenotype.

[000353] Also provided are methods of reducing cellular differentiation in a target cell population. For example, a target cell population containing one or more precursor cell types is contacted with a fusosome composition described herein, under conditions such that the composition reduces the differentiation of the precursor cell. In certain embodiments, the target cell population contains injured tissue in a mammalian subject or tissue affected by a surgical procedure. The precursor cell is, e.g., a stromal precursor cell, a neural precursor cell, or a

mesenchymal precursor cell.

[000354] A fusosome composition described herein, comprising a membrane protein payload agent may be used to deliver such agent to a cell tissue or subject. Delivery of a membrane protein payload agent by administration of a fusosome composition described herein may modify cellular protein expression levels. In certain embodiments, the administered directs upregulation of (via expression in the cell, delivery in the cell, or induction within the cell) of one or more membrane protein payload agent (e.g., a polypeptide or nucleic acid) that provide a functional activity which is substantially absent or reduced in the cell in which the membrane protein payload agent is delivered. For example, the missing functional activity may be enzymatic, structural, signaling or regulatory in nature. In related embodiments, the administered composition directs up-regulation of one or more membrane protein payload agent that increases (e.g., synergistically) a functional activity which is present but substantially deficient in the cell in which the membrane protein payload agent is upregulated. In related embodiments, the administered composition directs down-regulation of one or more polypeptides that decreases (e.g., synergistically) a functional activity which is present or upregulated in the cell in which the polypeptide is downregulated. In certain embodiments, the administered composition directs upregulation of certain functional activities and downregulation of other functional activities.

[000355] In embodiments, the fusosome composition mediates an effect on a target cell, and the effect lasts for at least 1, 2, 3, 4, 5, 6, or 7 days, 2, 3, or 4 weeks, or 1, 2, 3, 6, or 12 months. In some embodiments (e.g., wherein the fusosome composition comprises an exogenous protein), the effect lasts for less than 1, 2, 3, 4, 5, 6, or 7 days, 2, 3, or 4 weeks, or 1, 2, 3, 6, or 12 months.

Ex-vivo Applications

[000356] In embodiments, the fusosome composition described herein is delivered ex-vivo to a cell or tissue, e.g., a human cell or tissue. In embodiments, the composition improves function of a cell or tissue ex-vivo, e.g., improves cell viability, signaling, respiration, or other function (e.g., another function described herein).

[000357] In some embodiments, the composition is delivered to an ex vivo tissue that is in an injured state (e.g., from trauma, disease, hypoxia, ischemia or other damage).

[000358] In some embodiments, the composition is delivered to an ex-vivo transplant (e.g., a tissue explant or tissue for transplantation, e.g., a human vein, a musculoskeletal graft such as bone or tendon, cornea, skin, heart valves, nerves; or an isolated or cultured organ, e.g., an organ to be transplanted into a human, e.g., a human heart, liver, lung, kidney, pancreas, intestine, thymus, eye). The composition can be delivered to the tissue or organ before, during and/or after transplantation.

[000359] In some embodiments, the composition is delivered, administered or contacted with a cell, e.g., a cell preparation. The cell preparation may be a cell therapy preparation (a cell preparation intended for administration to a human subject). In embodiments, the cell preparation comprises cells expressing a chimeric antigen receptor (CAR), e.g., expressing a recombinant CAR. The cells expressing the CAR may be, e.g., T cells, Natural Killer (NK) cells, cytotoxic T lymphocytes (CTL), regulatory T cells. In embodiments, the cell preparation is a neural stem cell preparation. In embodiments, the cell preparation is a mesenchymal stem cell (MSC) preparation. In embodiments, the cell preparation is a hematopoietic stem cell (HSC) preparation. In embodiments, the cell preparation is an islet cell preparation.

In Vivo Uses

[000360] The fusosome compositions described herein can be administered to a subject, e.g., a mammal, e.g., a human. In such embodiments, the subject may be at risk of, may have a symptom of, or may be diagnosed with or identified as having, a particular disease or condition (e.g., a disease or condition described herein). In one embodiment, the subject has cancer. In one embodiment, the subject has an infectious disease.

[000361] In some embodiments, the source of fusosomes is from the same subject that is administered a fusosome composition. In other embodiments, they are different. For example, the source of fusosomes and recipient tissue may be autologous (from the same subject) or heterologous (from different subjects). In either case, the donor tissue for fusosome compositions described herein may be a different tissue type than the recipient tissue. For example, the donor tissue may be muscular tissue and the recipient tissue may be connective tissue (e.g., adipose tissue). In other embodiments, the donor tissue and recipient tissue may be of the same or different type, but from different organ systems.

[000362] A fusosome composition described herein may be administered to a subject having a cancer, an autoimmune disease, an infectious disease, a metabolic disease, a neurodegenerative disease, or a genetic disease (e.g., enzyme deficiency). In some embodiments, a tissue of the subject is in need of regeneration.

[000363] In some embodiments, a therapeutically effective amount of a fusosome composition described herein is administered to a subject. In some embodiments, a therapeutically effective amount of a substance is an amount that is sufficient, when administered to a subject who has or is susceptible to a disease, disorder, and/or condition, to treat, and/or delay the onset of the disease, disorder, and/or condition. For example, in embodiments the effective amount of a fusosome in a formulation to treat a disease, disorder, and/or condition is the amount that alleviates, ameliorates, relieves, inhibits, delays onset of, reduces severity of and/or reduces incidence of one or more symptoms or features of the disease, disorder, and/or condition.

[000364] In some embodiments, a subject is treated with a fusosome composition. In some embodimetns, the treatment partially or completely alleviates, ameliorates, relieves, inhibits, delays onset of, reduces severity of, and/or reduces incidence of one or more symptoms, features, and/or causes of a particular disease, disorder, and/or condition. In some embodiments, treatment may be of a subject who has been diagnosed as suffering from the relevant disease, disorder, and/or condition. In some embodiments, treatment may be of a subject known to have one or more susceptibility factors that are statistically correlated with increased risk of development of the relevant disease, disorder, and/or condition. In some embodiments, treatment partially or completely ameliorates the root cause of the relevant disease, disorder, and/or condition.

[000365] In some embodiments, the fusosome composition is effective to treat the disease, e.g., cancer. In some embodiments, the fusosome composition is effective to reduce the number of cancer cells in the subject compared to the number of cancer cells in the subject before administration. In some embodiments, the fusosome composition is effective to reduce the number of cancer cells in the subject compared to the expected course of disease without treatment. In some embodiments, the subject experiences a complete response or partial response after administration of the fusosome composition.

[000366] In some embodiments, the fusosome is co-administered with an inhibitor of a

protein that inhibits membrane fusion. For example, Suppressyn is a human protein that inhibits cell-cell fusion (Sugimoto et al., "A novel human endogenous retroviral protein inhibits cell-cell fusion" Scientific Reports 3:1462 DOI: 10.1038/srep01462). Thus, in some embodiments, the fusosome is co-administered with an inhibitor of sypressyn, e.g., a siRNA or inhibitory antibody.

Non-human Applications

[000367] Compositions described herein may also be used to similarly modulate the cell or tissue function or physiology of a variety of other organisms including but not limited to: farm or working animals (horses, cows, pigs, chickens etc.), pet or zoo animals (cats, dogs, lizards, birds, lions, tigers and bears etc.), aquaculture animals (fish, crabs, shrimp, oysters etc.), plants species (trees, crops, ornamentals flowers etc), fermentation species (saccharomyces etc.). Fusosome compositions described herein can be made from such non-human sources and administered to a non-human target cell or tissue or subject.

[000368] Fusosome compositions can be autologous, allogeneic or xenogeneic to the target.

[000369] All references and publications cited herein are hereby incorporated by reference.

[000370] The following examples are provided to further illustrate some embodiments of the present invention, but are not intended to limit the scope of the invention; it will be understood by their exemplary nature that other procedures, methodologies, or techniques known to those skilled in the art may alternatively be used.

EXAMPLES

Example 1. Generating enucleated fusogenic cells via chemical treatment (PEG)

[000371] Mito-DsRed (a mitochondrial specific targeted dye) expressing donor HeLa cells were trypsinized with 0.25% trypsin, collected, spun at 500xg for 5min, washed once in PBS and counted. $10x10^6$ cells were subsequently resuspended in 3ml of 12.5% ficoll in complete MEM-alpha (+10% FBS, + 1% penicillin/streptomycin, + glutamine) supplemented with 10ug/mL cytochalasin-B for 15 min. To enucleate cells, they were transferred to a discontinuous ficoll gradient consisting of the following ficoll fractions (from top to bottom): 2 mL 12.5% ficoll, 0.5

mL 15% ficoll, 0.5 mL 16% ficoll, 2 mL 17% ficoll gradient, 2 mL 25% ficoll. All ficoll gradient fractions were made in complete DMEM supplemented with 10ug/mL cytochalasin-B. Gradients were spun on a Beckman SW-40 ultracentrifuge, Ti-70 rotor at 107971xg for 1h at 37°C. Following centrifugation, enucleated HeLa cells were collected from the 12.5%, 15%, 16%, and 1/2 of the 17% ficoll fractions and resuspended in complete DMEM (+10% FBS, + 1% penicillin/streptomycin, + glutamine), and spun at 500xg for 5min to pellet. Enucleated Mito-DsRed donor cells were washed 2x in DMEM. Simultaneously, Mito-GFP (a mitochondrial specific targeted dye) expressing recipient HeLa cells were trypsinized, counted, and prepared for fusion.

[000372] For fusion, enucleated Mito-DsRed donor HeLa cells were combined at a 1:1 ratio with Mito-GFP recipient HeLa cells (200,000 each) in a 50% polyethylene glycol solution (50% PEG by w/v prepared in DMEM complete w/10% DMSO) for 1 minute at 37°C. Cells were subsequently washed 3x in 10mL complete DMEM and plated on 35mm glass-bottom quadrant imaging dishes at density of 50k cells/quadrant, with each quadrant having an area of 1.9 cm^2.

Example 2. Generating nucleated fusogenic cells via chemical treatment (PEG)

[000373] Mito-DsRed (a mitochondrial specific targeted dye) expressing donor HeLa cells were trypsinized with 0.25% trypsin, collected, spun at 500xg for 5min, washed once in PBS and counted. $2x10^6$ cells were subsequently resuspended in complete DMEM (+10% FBS, + 1% penicillin/streptomycin, + glutamine), counted, and prepared for fusion.

[000374] Mito-DsRed donor cells were washed 3x in DMEM. Simultaneously, Mito-GFP (a mitochondrial specific targeted dye) expressing recipient HeLa cells were trypsinized, counted, and prepared for fusion.

[000375] For fusion, Mito-DsRed donor HeLa cells were combined at a 1:1 ratio with Mito-GFP recipient HeLa cells (200,000 each) in a 50% polyethylene glycol solution (50% PEG by w/v prepared in DMEM complete w/10% DMSO) with for 1 minute at 37°C. Cells were subsequently washed 3X in 10ml complete DMEM and plated on 35 mm glass-bottom quadrant imaging dishes at density of 50k cells/quadrant, with each quadrant having an area of 1.9 cm^2.

Example 3. Creation of HeLa cells expressing exogenous fusogens

[000376] This example describes the creation of tissue culture cells expressing an exogenous fusogen. The following example is equally applicable to any protein based fusogen and is equally applicable to production in primary cells (in suspension or adherent) and tissue. In certain cases, a fusogen pair can be used to induce fusion (delineated as fusogen and a fusogen binding partner).

[000377] The fusogen gene, fusion failure 1 (EFF-1), is cloned into pIRES2-AcGFP1 vector (Clontech), and this construct is then transfected into HeLa cells (CCL-2™, ATCC) using the Lipofectamine 2000 transfection reagent (Invitrogen). The fusogen binding partner gene, anchor-cell fusion failure 1 (AFF-1), is cloned into pIRES2 DsRed-Express 2 vector (Clontech), and this construct is then transfected into HeLa cells (CCL-2™, ATCC) using the Lipofectamine 2000 transfection reagent (Invitrogen). Transfected HeLa cells are kept at 37 °C, 5% CO2 in Dulbecco's Modified Eagle Medium (DMEM) supplemented with GlutaMAX (GIBCO), 10% fetal calf serum (GIBCO) and 500 mg/mL zeocin. EFF-1 expressing cells are isolated by sorting fluorescent activated cell sorting (FACS) to get a pure population of GFP+ Hela cells expressing EFF-1 fusogen. AFF-1 expressing cells are isolated by sorting fluorescent activated cell sorting (FACS) to get a pure population of DSRED+ Hela cells expressing AFF-1 fusogen binding partner.

Example 4. Organelle delivery via chemically enhanced fusogenic enucleated cells

[000378] Fusogenic cells (Mito-DsRed donor enucleated cells and Mito-GFP recipient HeLa cells) produced and fused as described in Example 1 were imaged on a Zeiss LSM 780 inverted confocal microscope at 63X magnification 24h following deposition in the imaging dish. Cells expressing only Mito-DsRed alone and Mito-GFP alone were imaged separately to configure acquisition settings in such a way as to ensure no signal overlap between the two channels in conditions where both Mito-DsRed and Mito-GFP were both present and acquired simultaneously. Ten regions of interest were chosen in a completely unbiased manner, with the only criteria being that a minimum of 10 cells be contained within each ROI, such that a minimum of 100 cells were available for downstream analysis. A given pixel in these images was determined to be positive for mitochondria if its intensity for either channel (mito-DsRed and mito-GFP) was greater than 10% of the maximum intensity value for each respective channel across all three ROIs.

[000379] Fusion events with organelle delivery were identified based on the criteria that

>50% of the mitochondria (identified by all pixels that are either mito-GFP+ or mito-Ds-Red+) in a cell were positive for both mitoDs-Red and mito-GFP based on the above indicated threshold, indicating that organelles (in this case mitochondria) containing these proteins have been delivered, fused and their contents intermingled. At the 24-hour time point multiple cells exhibited positive organelle delivery via fusion as indicated in Figure 7. This is the image of a positive organelle delivery via fusion between donor and recipient HeLa cells. The intracellular areas indicated in white indicate overlap between donor and recipient mitochondria. The intracellular regions in grey indicate where donor and recipient organelles do not overlap.

Example 5. Organelle delivery via chemically enhanced fusogenic nucleated cells

[000380] Fusogenic cells (Mito-DsRed donor cells and Mito-GFP recipient HeLa cells) produced and combined as described in example 2 were imaged on a Zeiss LSM 780 inverted confocal microscope at 63X magnification 24h following deposition in the imaging dish. Cells expressing only Mito-DsRed alone and Mito-GFP alone were imaged separately to configure acquisition settings in such a way as to ensure no signal overlap between the two channels in conditions where both Mito-DsRed and Mito-GFP were both present and acquired simultaneously. Ten regions of interest were chosen in a completely unbiased manner, with the only criteria being that a minimum of 10 cells be contained within each ROI, such that a minimum of 100 cells were available for downstream analysis. A given pixel in these images was determined to be positive for mitochondria if it's intensity for either channel (mito-DsRed and mito-GFP) was greater than 20% of the maximum intensity value for each respective channel across all three ROIs.

[000381] Fusion events with organelle delivery were identified based on the criteria that >50% of the mitochondria (identified by all pixels that are either mito-GFP+ or mito-Ds-Red+) in a cell were positive for both mitoDs-Red and mito-GFP based on the above indicated threshold, indicating that organelles (in this case mitochondria) containing these proteins have been delivered, fused and their contents intermingled. At the 24-hour time point multiple cells exhibited positive organelle delivery via fusion as indicated in Figure 8. This is the image of a positive organelle delivery via fusion between donor and recipient HeLa cells. The intracellular areas indicated in white indicate overlap between donor and recipient mitochondria. The intracellular regions in grey indicate where donor and recipient organelles do not overlap.

Example 6. Delivery of mitochondria via protein enhanced fusogenic enucleated cells

[000382] Fusogenic cells produced and combined as described in Example 3 are imaged on a Zeiss LSM 780 inverted confocal microscope at 63X magnification 24h following deposition in the imaging dish. Cells expressing only Mito-DsRed alone and Mito-GFP alone are imaged separately to configure acquisition settings in such a way as to ensure no signal overlap between the two channels in conditions where both Mito-DsRed and Mito-GFP are both present and acquired simultaneously. Ten regions of interest are chosen in a completely unbiased manner, with the only criteria being that a minimum of 10 cells be contained within each ROI, such that a minimum number of cells are available for downstream analysis. A given pixel in these images is determined to be positive for mitochondria if it's intensity for either channel (mito-DsRed and mito-GFP) is greater than 10% of the maximum intensity value for each respective channel across all three ROIs.

[000383] Fusion events with organelle delivery will be identified based on the criteria that >50% of the mitochondria (identified by all pixels that are either mito-GFP+ or mito-Ds-Red+) in a cell are positive for both mitoDs-Red and mito-GFP based on the above indicated threshold, which will indicate that organelles (in this case mitochondria) containing these proteins are delivered, fused and their contents intermingled. At the 24-hour time point multiple cells are expected to exhibit positive organelle delivery via fusion.

Example 7: Generation of fusosomes through nucleic acid electroporation

[000384] This example describes fusosome generation through electroporation of cells or vesicles with nucleic acids (e.g., mRNA or DNA) that encode a fusogen.

[000385] Transposase vectors (System Biosciences, Inc.) that include the open reading frame of the Puromycin resistance gene together with an open reading frame of a cloned fragment (e.g. Glycoprotein from Vesicular stomatitis virus [VSV-G], Oxford Genetics # OG592) are electroporated into 293Ts using an electroporator (Amaxa) and a 293T cell line specific nuclear transfection kit (Lonza).

[000386] Following selection with 1 μg/μL puromycin for 3-5 days in DMEM containing 20% Fetal Bovine Serum and 1x Penicillin/Streptomycin, the cells are then washed with 1xPBS,

ice-cold lysis buffer (150 mM NaCl, 0.1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris-HCl, pH 8.0 and protease inhibitor cocktail (Abcam, ab201117)), sonicated 3 times, 10-15 secs per time and centrifuged at 16,000 x g for 20min. A western blot is conducted on the recovered supernatant fraction with a probe specific to VSV-G to determine the non-membrane specific concentration of VSV-G from the fusosomes prepared from stably transfected cells or control cells and compared to the standard of VSV-G protein.

[000387] In embodiments, the fusosomes from stably transfected cells will have more VSV-G than fusosomes generated from cells that were not stably transfected.

Example 8: Generation of fusosomes through protein electroporation

[000388] This example describes electroporation of fusogens to generate fusosomes.

[000389] Approximately 5×10^6 cells or vesicles are used for electroporation using an electroporation transfection system (Thermo Fisher Scientific). To set up a master mix, 24 µg of purified protein fusogens is added to resuspension buffer (provided in the kit). The mixture is incubated at room temperature for 10 min. Meanwhile, the cells or vesicles are transferred to a sterile test tube and centrifuged at $500 \times g$ for 5 min. The supernatant is aspirated and the pellet is resuspended in 1 mL of PBS without Ca^{2+} and Mg^{2+}. The buffer with the fusogens is then used to resuspend the pellet of cells or vesicles. A cell or vesicle suspension is also used for optimization conditions, which vary in pulse voltage, pulse width and the number of pulses. After electroporation, the electroporated cells or vesicles with fusogens are washed with PBS, resuspended in PBS, and kept on ice.

[000390] See, for example, Liang et al., Rapid and highly efficiency mammalian cell engineering via Cas9 protein transfection, *Journal of Biotechnology* 208: 44-53, 2015.

Example 9: Generating and isolating fusosomes through vesicle formation and centrifugation

[000391] This example describes fusosome generation and isolation via vesiculation and centrifugation. This is one of the methods by which fusosomes may be isolated.

[000392] Fusosomes are prepared as follows. Approximately 4 x 106 HEK-293T cells are seeded in a 10 cm dish in complete media (DMEM + 10% FBS + Pen/Strep). One day after seeding,

15 μg of fusogen expressing plasmid or virus is delivered to cells. After a sufficient period of time for fusogen expression, medium is carefully replaced by fresh medium supplemented with 100 μM ATP. Supernatants are harvested 48-72 hours after fusogen expression, clarified by filtration through a 0.45 μm filter, and ultracentrifuged at 150,000 x g for 1 h. Pelleted material is resuspended overnight in ice cold PBS. Fusosomes are resuspended in desired buffer for experimentation.

[000393] See for example, Mangeot et al., Molecular Therapy, vol. 19 no. 9, 1656–1666, Sept. 2011

Example 10: Generating and isolating giant plasma membrane fusosomes

[000394] This example describes fusosome generation and isolation via vesiculation and centrifugation. This is one of the methods by which fusosomes may be isolated. Fusosomes are prepared as follows.

[000395] Briefly, HeLa cells that express a fusogen are washed twice in buffer (10 mM HEPES, 150 mM NaCl, 2 mM $CaCl_2$, pH 7.4), resuspended in a solution (1 mM DTT, 12.5 mM Paraformaldehyde, and 1 mM N-ethylmaleimide in GPMV buffer), and incubated at 37 °C for 1 h. Fusosomes are clarified from cells by first removing cells by centrifugation at 100 x g for 10 minutes, and then harvesting fusosomes at 20,000 x g for 1 h at 4 °C. The fusosomes are resuspended in desired buffer for experimentation.

[000396] See for example, Sezgin E *et al.* Elucidating membrane structure and protein behavior using giant membrane plasma vesicles. *Nat. Protocols.* 7(6):1042-51 2012.

Example 11: Generating and isolating fusosome ghosts

[000397] This example describes fusosome generation and isolation via hypotonic treatment and centrifugation. This is one of the methods by which fusosomes may be produced.

[000398] First, fusosomes are isolated from mesenchymal stem cells expressing fusogens (10^9 cells) primarily by using hypotonic treatment such that the cell ruptures and fusosomes are formed. Cells are resuspended in hypotonic solution, Tris-magnesium buffer (TM, e.g., pH 7.4 or pH 8.6 at 4^0C, pH adjustment made with HCl). Cell swelling is monitored by phase-contrast

microscopy. Once the cells swell and fusosomes are formed, the suspension is placed in a homogenizer. Typically, about 95% cell rupture is sufficient as measured through cell counting and standard AOPI staining. The membranes/fusosomes are then placed in sucrose (0.25 M or higher) for preservation. Alternatively, fusosomes can be formed by other approaches known in the art to lyse cells, such as mild sonication (Arkhiv anatomii, gistologii i embriologii; 1979, Aug, 77(8) 5-13; PMID: 496657), freeze-thaw (Nature. 1999, Dec 2;402(6761):551-5; PMID: 10591218), French-press (Methods in Enzymology, Volume 541, 2014, Pages 169-176; PMID: 24423265), needle-passaging (www.sigmaaldrich.com/technical-documents/protocols/biology/nuclear-protein-extraction.html) or solublization in detergent-containing solutions (www.thermofisher.com/order/catalog/product/89900).

[000399] To avoid adherence, the fusosomes are placed in plastic tubes and centrifuged. A laminated pellet is produced in which the topmost lighter gray lamina includes mostly fusosomes. However, the entire pellet is processed, to increase yields. Centrifugation (e.g., 3,000 rpm for 15 min at 4°C) and washing (e.g., 20 volumes of Tris magnesium/TM-sucrose pH 7.4) may be repeated.

[000400] In the next step, the fusosome fraction is separated by floatation in a discontinuous sucrose density gradient. A small excess of supernatant is left remaining with the washed pellet, which now includes fusosomes, nuclei, and incompletely ruptured whole cells. An additional 60% w/w sucrose in TM, pH 8.6, is added to the suspension to give a reading of 45% sucrose on a refractometer. After this step, all solutions are TM pH 8.6. 15 mL of suspension are placed in SW-25.2 cellulose nitrate tubes and a discontinuous gradient is formed over the suspension by adding 15 mL layers, respectively, of 40% and 35% w/w sucrose, and then adding 5 mL of TM-sucrose (0.25 M). The samples are then centrifuged at 20,000 rpm for 10 min, 4°C. The nuclei sediment form a pellet, the incompletely ruptured whole cells are collected at the 40%-45% interface, and the fusosomes are collected at the 35%-40% interface. The fusosomes from multiple tubes are collected and pooled. See for example, International patent publication, WO2011024172A2.

Example 12: Generating fusosomes through extrusion

[000401] This example describes fusosome manufacturing by extrusion through a membrane.

[000402] Briefly, hematopoietic stem cells that express fusogens are in a 37°C suspension at

a density of 1 x 10^6 cells/mL in serum-free media containing protease inhibitor cocktail (Set V, Calbiochem 539137-1ML). The cells are aspirated with a luer lock syringe and passed once through a disposable 5 mm syringe filter into a clean tube. If the membrane fouls and becomes clogged, it is set aside and a new filter is attached. After the entire cell suspension has passed through the filter, 5 mL of serum-free media is passed through all filters used in the process to wash any remaining material through the filter(s). The solution is then combined with the extruded fusosomes in the filtrate.

[000403] Fusosomes may be further reduced in diameter by continued extrusion following the same method with increasingly smaller filter pore sizes, ranging from 5 mm to 0.2 mm. When the final extrusion is complete, suspensions are pelleted by centrifugation (time and speed required vary by size) and resuspended in media.

[000404] Additionally, this process can be supplemented with the use of an actin cytoskeleton inhibitor in order to decrease the influence of the existing cytoskeletal structure on extrusion. Briefly, a 1 x 10^6 cell/mL suspension is incubated in serum-free media with 500 nM Latrunculin B (ab144291, Abcam, Cambridge, MA) and incubated for 30 minutes at 37 °C in the presence of 5% CO_2. After incubation, protease inhibitor cocktail is added and cells are aspirated into a luer lock syringe, with the extrusion carried out as previously described.

[000405] Fusosomes are pelleted and washed once in PBS to remove the cytoskeleton inhibitor before being resuspended in media.

Example 13: Generation of fusosomes through chemical treatment with protein

[000406] This example describes chemical-mediated delivery of fusogens to generate fusosomes. Approximately 5 × 10^6 cells or vesicles are used for chemical-mediated delivery of fusogens. The cells or vesicles are suspended in 50 µL of Opti-MEM medium. To set up a master mix, 24 µg of purified protein fusogens is mixed with 25 µL of Opti-MEM medium, followed by the addition of 25 µL of Opti-MEM containing 2 µL of lipid transfection reagent 3000. The cells or vesicles and fusogen solutions are mixed by gently swirling the plate and incubating at 37 C for 6 hours, such that the fusogen will be incorporated into the cell or vesicle membrane. Fusosomes are then washed with PBS, resuspended in PBS, and kept on ice.

[000407] See, also, Liang et al., Rapid and highly efficiency mammalian cell engineering via Cas9 protein transfection, *Journal of Biotechnology* 208: 44-53, 2015.

Example 14: Generation of fusosomes through treatment with fusogen-containing liposomes

[000408] This example describes liposome-mediated delivery of fusogens to a source cell to generate fusosomes. Approximately 5×10^6 cells or vesicles are used for liposome-mediated delivery of fusogens. The cells or vesicles are suspended in 50 µL of Opti-MEM medium. The fusogen protein is purified from cells in the presence of n-octyl b-D-glucopyranoside. n-octyl b-D-glucopyranoside is a mild detergent used to solubilize integral membrane proteins. The fusogen protein is then reconstituted into large (400nm diameter) unilamellar vesicles (LUVs) by mixing n-octyl b-D-glucopyranoside -suspended protein with LUVs presaturated with n-octyl b-D-glucopyranoside, followed by removal of n-octyl b-D-glucopyranoside, as described in Top et al., *EMBO* 24: 2980-2988, 2005. To set up a master mix, a mass of liposomes that contains 24 µg of total fusogen protein is mixed with 50 µL of Opti-MEM medium. The solutions of liposomes and source cells or vesicles are then combined, and the entire solution is mixed by gently swirling the plate and incubating at 37 C for 6 hours under conditions that allow fusion of the fusogen-containing liposomes and the source cells or vesicle, such that the fusogen protein will be incorporated into the source cell or vesicle membrane. Fusosomes are then washed with PBS, resuspended in PBS, and kept on ice. See, also, Liang et al., Rapid and highly efficiency mammalian cell engineering via Cas9 protein transfection, *Journal of Biotechnology* 208: 44-53, 2015.

Example 15: Isolating fusogenic microvesicles freely released from cells

[000409] This example describes isolation of fusosomes via centrifugation. This is one of the methods by which fusosomes may be isolated.

[000410] Fusosomes are isolated from cells expressing fusogens by differential centrifugation. Culture media (DMEM + 10% fetal bovine serum) is first clarified of small particles by ultracentrifugation at >100,000 *x g* for 1 h. Clarified culture media is then used to grow Mouse Embryonic Fibroblasts expressing fusogens. The cells are separated from culture media by centrifugation at 200 *x g* for 10 minutes. Supernatants are collected and centrifuged

sequentially twice at 500 x g for 10 minutes, once at 2,000 x g for 15 minutes, once at 10,000 x g for 30 min, and once at 70,000 x g for 60 minutes. Freely released fusosomes are pelleted during the final centrifugation step, resuspended in PBS and repelleted at 70,000 x g. The final pellet is resuspended in PBS.

[000411] See also, Wubbolts R *et al.* Proteomic and Biochemical Analyses of Human B Cell-derived Exosomes: Potential Implications for their Function and Multivesicular Body Formation. *J. Biol. Chem.* 278:10963-10972 2003.

Example 16: Physical enucleation of fusosomes

[000412] This example describes enucleation of fusosomes via cytoskeletal inactivation and centrifugation. This is one of the methods by which fusosomes may be modified.

[000413] Fusosomes are isolated from mammalian primary or immortalized cell lines that express a fusogen. The cells are enucleated by treatment with an actin skeleton inhibitor and ultracentrifugation. Briefly, C2C12 cells are collected, pelleted, and resuspended in DMEM containing 12.5% Ficoll 400 (F2637, Sigma, St. Louis MO) and 500 nM Latrunculin B (ab144291, Abcam, Cambridge, MA) and incubated for 30 minutes at 37 °C + 5% CO_2. Suspensions are carefully layered into ultracentrifuge tubes containing increasing concentrations of Ficoll 400 dissolved in DMEM (15%, 16%, 17%, 18%, 19%, 20%, 3 mL per layer) that have been equilibrated overnight at 37 °C in the presence of 5% CO_2. Ficoll gradients are spun in a Ti-70 rotor (Beckman-Coulter, Brea, CA) at 32,300 RPM for 60 minutes at 37 C. After ultracentrifugation, fusosomes found between 16 – 18% Ficoll are removed, washed with DMEM, and resuspended in DMEM.

[000414] Staining for nuclear content with Hoechst 33342 as described in Example 35 followed by the use of flow cytometry and/or imaging will be performed to confirm the ejection of the nucleus.

Example 17: Modifying fusosomes via irradiation

[000415] The following example describes modifying fusosomes with gamma irradiation. Without being bound by theory, gamma irradiation may cause double stranded breaks in the DNA and drive cells to undergo apoptosis.

[000416] First, cells expressing fusogens are cultured in a monolayer on tissue culture flasks or plates below a confluent density (e.g. by culturing or plating cells). Then the medium is removed from confluent flasks, cells are rinsed with Ca2+ and Mg2+ free HBSS, and trypsinized to remove the cells from the culture matrix. The cell pellet is then resuspended in 10ml of tissue-culture medium without penicillin/streptomycin and transferred to a 100 mm Petri dish. The number of cells in the pellet should be equivalent to what would be obtained from 10-15 confluent MEF cultures on 150 cm^2 flasks. The cells are then exposed to 4000 rads from a γ-radiation source to generate fusosomes. The fusosomes are then washed and resuspended in the final buffer or media to be used.

Example 18: Modifying fusosomes via chemical treatment

[000417] The following example describes modifying fusosomes with mitomycin C treatment. Without being bound by any particular theory, mitomycin C treatment modifies fusosomes by inactivating the cell cycle.

[000418] First, cells expressing fusogens are cultured from a monolayer in tissue culture flasks or plates at a confluent density (e.g. by culturing or plating cells). One mg/mL mitomycin C stock solution is added to the medium to a final concentration of 10 μg/mL. The plates are then returned to the incubator for 2 to 3 hours. Then the medium is removed from confluent flasks, cells are rinsed with Ca2+ and Mg2+ free HBSS, and trypsinized to remove the cells from the culture matrix. The cells are then washed and resuspended in the final buffer or media to be used.

[000419] See for example, Mouse Embryo Fibroblast (MEF) Feeder Cell Preparation, Current Protocols in Molecular Biology. David A. Conner 2001.

Example 19: Lack of transcriptional activity in fusosomes

[000420] This Example quantifies transcriptional activity in fusosomes compared to parent cells, e.g., source cells, used for fusosome generation. In some embodiments, transcriptional activity will be low or absent in fusosomes compared to the parent cells, e.g., source cells.

[000421] Fusosomes are a chassis for the delivery of therapeutic agent. Therapeutic agents, such as miRNA, mRNAs, proteins and/or organelles that can be delivered to cells or local tissue environments with high efficiency could be used to modulate pathways that are not normally active

or active at pathological low or high levels in recipient tissue. In some embodiments, observation that fusosomes are not capable of transcription, or that fusosomes have transcriptional activity of less than their parent cell, will demonstrate that removal of nuclear material has sufficiently occurred.

[000422] Fusosomes are prepared by any one of the methods described in previous Examples. A sufficient number of fusosomes and parent cells used to generate the fusosomes are then plated into a 6 well low-attachment multiwell plate in DMEM containing 20% Fetal Bovine Serum, 1x Penicillin/Streptomycin and the fluorescent-taggable alkyne–nucleoside EU for 1hr at 37°C and 5% CO_2. For negative controls, a sufficient number of fusosomes and parent cells are also plated in multiwell plate in DMEM containing 20% Fetal Bovine Serum, 1x Penicillin/Streptomycin but with no alkyne–nucleoside EU.

[000423] After the 1 hour incubation the samples are processed following the manufacturer's instructions for an imaging kit (ThermoFisher Scientific). The cell and fusosome samples including the negative controls are washed thrice with 1xPBS buffer and resuspended in 1xPBS buffer and analyzed by flow cytometry (Becton Dickinson, San Jose, CA, USA) using a 488nm argon laser for excitation, and the 530+/-30nm emission. BD FACSDiva software was used for acquisition and analysis. The light scatter channels are set on linear gains, and the fluorescence channels on a logarithmic scale, with a minimum of 10,000 cells analyzed in each condition.

[000424] In some embodiments, transcriptional activity as measured by 530+/-30nm emission in the negative controls will be null due to the omission of the alkyne–nucleoside EU. In some embodiments, the fusosomes will have less than about 70%, 60%, 50%, 40%, 30%, 20%, 10%, 5%, 4%, 3%, 2%, 1% or less transcriptional activity than the parental cells.

[000425] See also, Proc Natl Acad Sci U S A, 2008, Oct 14;105(41):15779-84. doi: 10.1073/pnas.0808480105. Epub 2008 Oct 7.

Example 20: Lack of DNA replication or replication activity

[000426] This Example quantifies DNA replication in fusosomes. In some embodiments, fusosomes will replicate DNA at a low rate compared to cells.

[000427] Fusosomes are prepared by any one of the methods described in previous Examples.

Fusosome and parental cell DNA replication activity is assessed by incorporation of a fluorescent-taggable nucleotide (ThermoFisher Scientific # C10632). Fusosomes and an equivalent number of cells are incubated with EdU at a final concentration of 10 μM for 2hr, after preparation of an EdU stock solution with in dimethylsulfoxide. The samples are then fixed for 15 min using 3.7% PFA, washed with 1xPBS buffer, pH 7.4 and permeabilized for 15 min in 0.5% detergent solution in 1xPBS buffer, pH 7.4.

[000428] After permeabilization, fusosomes and cells in suspension in PBS buffer containing 0.5% detergent are washed with 1xPBS buffer, pH 7.4 and incubated for 30 min at 21°C in reaction cocktail, 1xPBS buffer, CuSO4 (Component F), azide-fluor 488, 1x reaction buffer additive.

[000429] A negative control for fusosome and cell DNA replication activity is made with samples treated the same as above but with no azide-fluor 488 in the 1x reaction cocktail.

[000430] The cell and fusosome samples are then washed and resuspended in 1xPBS buffer and analyzed by flow cytometry. Flow cytometry is done with a FACS cytometer (Becton Dickinson, San Jose, CA, USA) with 488nm argon laser excitation, and a 530+/-30nm emission spectrum is collected. FACS analysis software is used for acquisition and analysis. The light scatter channels are set on linear gains, and the fluorescence channels on a logarithmic scale, with a minimum of 10,000 cells analyzed in each condition. The relative DNA replication activity is calculated based on the median intensity of azide-fluor 488 in each sample. All events are captured in the forward and side scatter channels (alternatively, a gate can be applied to select only the fusosome population). The normalized fluorescence intensity value for the fusosomes is determined by subtracting from the median fluorescence intensity value of the fusosome the median fluorescence intensity value of the respective negative control sample. Then the normalized relative DNA replication activity for the fusosomes samples is normalized to the respective nucleated cell samples in order to generate quantitative measurements for DNA replication activity.

[000431] In some embodiments, fusosomes have less DNA replication activity than parental cells.

[000432] See, also, Salic, 2415–2420, doi: 10.1073/pnas.0712168105.

Example 21: Electroporation to modify fusosome with nucleic acid cargo

This example describes electroporation of fusosomes with nucleic acid cargo.

[000433] Fusosomes are prepared by any one of the methods described in a previous Example. Approximately 10^9 fusosomes and 1 μg of nucleic acids, e.g., RNA, are mixed in electroporation buffer (1.15 mM potassium phosphate pH 7.2, 25 mM potassium chloride, 60% iodixanol w/v in water). The fusosomes are electroporated using a single 4 mm cuvette using an electroporation system (BioRad, 165-2081). The fusosomes and nucleic acids are electroporated at 400 V, 125 μF and ∞ ohms, and the cuvette is immediately transferred to ice. After electroporation, fusosomes are washed with PBS, resuspended in PBS, and kept on ice.

[000434] See, for example, Kamerkar et al., Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer, *Nature,* 2017

[000435]

[000436] Example 22: Electroporation to modify fusosome with protein cargo

[000437] This example describes electroporation of fusosomes with protein cargo.

[000438] Fusosomes are prepared by any one of the methods described in a previous Example. Approximately 5×10^6 fusosomes are used for electroporation using an electroporation transfection system (Thermo Fisher Scientific). To set up a master mix, 24 μg of purified protein cargo is added to resuspension buffer (provided in the kit). The mixture is incubated at room temperature for 10 min. Meanwhile, fusosomes are transferred to a sterile test tube and centrifuged at $500 \times g$ for 5 min. The supernatant is aspirated and the pellet is resuspended in 1 mL of PBS without Ca^{2+} and Mg^{2+}. The buffer with the protein cargo is then used to resuspend the pellet of fusosomes. A fusosome suspension is then used for optimization conditions, which vary in pulse voltage, pulse width and the number of pulses. After electroporation, fusosomes are washed with PBS, resuspended in PBS, and kept on ice.

[000439] See, for example, Liang et al., Rapid and highly efficiency mammalian cell engineering via Cas9 protein transfection, *Journal of Biotechnology* 208: 44-53, 2015.

Example 23: Chemical treatment of fusosomes to modify with nucleic acid cargo

[000440] This example describes loading of nucleic acid cargo into a fusosome via chemical treatments.

[000441] Fusosomes are prepared by any one of the methods described in previous Examples. Approximately 10^6 fusosomes are pelleted by centrifugation at 10,000g for 5min at 4°C. The pelleted fusosomes are then resuspended in TE buffer (10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA) with 20µg DNA. The fusosome:DNA solution is treated with a mild detergent to increase DNA permeability across the fusosome membrane (Reagent B, Cosmo Bio Co., LTD, Cat# ISK-GN-001-EX). The solution is centrifuged again and the pellet is resuspended in buffer with a positively-charged peptide, such as protamine sulfate, to increase affinity between the DNA loaded fusosomes and the target recipient cells (Reagent C, Cosmo Bio Co., LTD, Cat# ISK-GN-001-EX). After DNA loading, the loaded fusosomes are kept on ice before use.

See, also, Kaneda, Y., et al., New vector innovation for drug delivery: development of fusigenic non-viral particles. *Curr. Drug Targets*, 2003

Example 24: Chemical treatment of fusosomes to modify with protein cargo

[000442] This example describes loading of protein cargo into a fusosome via chemical treatments.

[000443] Fusosomes are prepared by any one of the methods described in previous Examples. Approximately 10^6 fusosomes are pelleted by centrifugation at 10,000g for 5min at 4°C. The pelleted fusosomes are then resuspended in buffer with positively-charged peptides, such as protamine sulfate, to increase the affinity between the fusosomes and the cargo proteins (Reagent A, Cosmo Bio Co., LTD, Cat# ISK-GN-001-EX). Next 10µg of cargo protein is added to the fusosome solution followed by addition of a mild detergent to increase protein permeability across the fusosome membrane (Reagent B, Cosmo Bio Co., LTD, Cat# ISK-GN-001-EX). The solution is centrifuged again and the pellet is resuspended in buffer with the positively-charged peptide, such as protamine sulfate, to increase affinity between the protein loaded fusosomes and the target recipient cells (Reagent C, Cosmo Bio Co., LTD, Cat# ISK-GN-001-EX). After protein loading, the loaded fusosomes are kept on ice before use.

[000444] See, also, Yasouka, E., et al., Needleless intranasal administration of HVJ-E containing allergen attenuates experimental allergic rhinitis. *J. Mol. Med.*, 2007

Example 25: Transfection of fusosomes to modify with nucleic acid cargo

[000445] This example describes transfection of nucleic acid cargo into a fusosome. Fusosomes are prepared by any one of the methods described in previous Examples.

[000446] 5×10^6 fusosomes are maintained in Opti-Mem. 0.5 µg of nucleic acid is mixed with 25 µL of Opti-MEM medium, followed by the addition of 25 µL of Opti-MEM containing 2 µL of lipid transfection reagent 2000. The mixture of nucleic acids, Opti-MEM, and lipid transfection reagent is maintained at room temperature for 15 minutes, then is added to the fusosomes. The entire solution is mixed by gently swirling the plate and incubating at 37 C for 6 hours. Fusosomes are then washed with PBS, resuspended in PBS, and kept on ice.

[000447] See, also, Liang et al., Rapid and highly efficiency mammalian cell engineering via Cas9 protein transfection, *Journal of Biotechnology* 208: 44-53, 2015.

Example 26: Transfection of fusosomes to modify with protein cargo

[000448] This example describes transfection of protein cargo into a fusosome.

[000449] Fusosomes are prepared by any one of the methods described in previous Examples. 5×10^6 fusosomes are maintained in Opti-Mem. 0.5 µg of purified protein is mixed with 25 µL of Opti-MEM medium, followed by the addition of 25 µL of Opti-MEM containing 2 µL of lipid transfection reagent 3000. The mixture of protein, Opti-MEM, and lipid transfection reagent is maintained at room temperature for 15 minutes, then is added to the fusosomes. The entire solution is mixed by gently swirling the plate and incubating at 37 C for 6 hours. Fusosomes are then washed with PBS, resuspended in PBS, and kept on ice.

[000450] See, also, Liang et al., Rapid and highly efficiency mammalian cell engineering via Cas9 protein transfection, *Journal of Biotechnology* 208: 44-53, 2015.

Example 27: Fusosomes with lipid bilayer structure

[000451] This example describes the composition of fusosomes. In some embodiments, a fusosome composition will comprise a lipid bilayer structure, with a lumen in the center.

[000452] Without wishing to be bound by theory, the lipid bilayer structure of a fusosome promotes fusion with a target cell, and allows fusosomes to load different therapeutics.

[000453] Fusosomes are freshly prepared using the methods described in the previous Examples. The positive control is the native cell line (HEK293), and the negative control is cold DPBS and membrane-disrupted HEK293 cell prep, which has been passed through 36 gauge needles for 50 times.

[000454] Samples are spin down in Eppendorf tube, and the supernatant is carefully removed. Then a pre-warmed fixative solution (2.5% glutaraldehyde in 0.05 M cacodylate buffer with 0.1M NaCl, pH 7.5; keep at 37 °C for 30 min before use) is added to the sample pellet and kept at room temperature for 20 minutes. The samples are washed twice with PBS after fixation. Osmium tetroxide solution is added to the sample pellet and incubated 30 minutes. After rinsing once with PBS, 30%, 50%, 70% and 90% hexylene glycol is added and washed with swirling, 15 minutes each. Then 100% hexylene glycol is added with swirling, 3 times, 10 minutes each.

[000455] Resin is combined with hexylene glycol at 1:2 ratio, and then added to the samples and incubated at room temperature for 2 hours. After incubation, the solution is replaced with 100% resin and incubated for 4-6 hours. This step is repeated one more time with fresh 100% resin. Then it is replaced with 100% fresh resin, the level is adjusted to ~1-2 mm in depth, and baked for 8-12 hours. The Eppendorf tube is cut and pieces of epoxy cast with the sample is baked for an additional 16-24 hours. The epoxy cast is then cut into small pieces making note of the side with the cells. Pieces are glued to blocks for sectioning, using commercial 5-minute epoxy glue. A transmission electron microscope (JOEL, USA) is used to image the samples at a voltage of 80kV.

[000456] In some embodiments, fusosomes will show a lipid bilayer structure similar to the positive control (HEK293 cells), and no obvious structure is observed in the DPBS control. In some embodiments no lumenal structures will be observed in the disrupted cell preparation.

Example 28: Detecting fusogen expression

[000457] This example quantifies fusogen expression in fusosomes.

[000458] Transposase vectors (System Biosciences, Inc.) that include the open reading frame

of the Puromycin resistance gene together with an open reading frame of a cloned fragment (e.g. Glycoprotein from Vesicular stomatitis virus [VSV-G], Oxford Genetics # OG592) are electroporated into 293Ts using an electroporator (Amaxa) and a 293T cell line specific nuclear transfection kit (Lonza).

[000459] Following selection with 1 μg/μL puromycin for 3-5 days in DMEM containing 20% Fetal Bovine Serum and 1x Penicillin/Streptomycin, fusosomes are prepared from the stably expressing cell line or from control cells by any one of the methods described in previous Examples.

[000460] The fusosomes are then washed with 1xPBS, ice-cold lysis buffer (150 mM NaCl, 0.1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris-HCl, pH 8.0 and Protease Inhibitor Cocktail III (Abcam, ab201117)), sonicated 3 times, 10-15 seconds each time and centrifuged at 16,000 x g for 20min. A western blot is conducted on the recovered supernatant fraction with a probe specific to VSV-G to determine the non-membrane specific concentration of VSV-G from the fusosomes prepared from stably transfected cells or control cells and compared to the standard of VSV-G protein.

[000461] In some embodiments, fusosomes from stably transfected cells will have more VSV-G than fusosomes generated from cells that were not stably transfected.

Example 29: Quantification of fusogens

[000462] This example describes quantification of the absolute number of fusogens per fusosome.

[000463] A fusosome composition is produced by any one of the methods described in the previous Examples, except the fusosome is engineered as described in a previous Example to express a fusogen (VSV-G) tagged with GFP. In addition, a negative control fusosome is engineered with no fusogen (VSV-G) or GFP present.

[000464] The fusosomes with the GFP-tagged fusogen and the negative control(s) are then assayed for the absolute number of fusogens as follows. Commercially acquired recombinant GFP is serially diluted to generate a calibration curve of protein concentration. The GFP fluorescence of the calibration curve and a sample of fusosomes of known quantity is then measured in a

fluorimeter using a GFP light cube (469/35 excitation filter and a 525/39 emission filter) to calculate the average molar concentration of GFP molecules in the fusosome preparation. The molar concentration is then converted to the number of GFP molecules and divided by the number of fusosomes per sample to achieve an average number of GFP-tagged fusogen molecules per fusosome and thus provides a relative estimate of the number of fusogens per fusosome.

[000465] In some embodiments, GFP fluorescence will be higher in the fusosomes with GFP tag as compared to the negative controls, where no fusogen or GFP is present. In some embodiments, GFP fluorescence is relative to the number of fusogen molecules present.

[000466] Alternatively, individual fusosomes are isolated using a single cell prep system (Fluidigm) per manufacturer's instructions, and qRT-PCR is performed using a commercially available probeset (Taqman) and master mix designed to quantify fusogen or GFP cDNA levels based upon the C_t value. A RNA standard of the same sequence as the cloned fragment of the fusogen gene or the GFP gene is generated by synthesis (Amsbio) and then added to single cell prep system qRT-PCR experimental reaction in serial dilutions to establish a standard curve of C_t vs concentration of fusogen or GFP RNA.

[000467] The C_t value from fusosomes is compared to the standard curve to determine the amount of fusogen or GFP RNA per fusosome.

[000468] In some embodiments, fusogen and GFP RNA will be higher in the fusosomes with engineered to express the fusogens as compared to the negative controls, where no fusogen or GFP is present.

[000469] Fusogens may further be quantified in the lipid bilayer by analyzing the lipid bilayer structure as previously described and quantifying fusogens in the lipid bilayer by LC-MS as described in other Examples herein.

Example 30: Measuring the average diameter of fusosomes

[000470] This Example describes measurement of the average diameter of fusosomes.

[000471] Fusosomes are prepared by any one of the methods described in previous Examples. The fusosomes measured to determine the average diameter using commercially available systems (iZON Science). The system is used with software according to manufacturer's instructions and a

nanopore designed to analyze particles within the 40 nm to 10 µm diameter range. Fusosomes and parental cells are resuspended in phosphate-buffered saline (PBS) to a final concentration range of 0.01-0.1 µg protein/mL. Other instrument settings are adjusted as indicated in the following table:

Table 18: Fusosome measurement parameters and settings

Measurement Parameter	Setting
Pressure	6
Nanopore type	NP300
Calibration sample	CPC400_6P
Gold standard analysis	no
Capture assistant	none

[000472] All fusosomes are analyzed within 2 hours of isolation. In some embodiments, fusosomes will have a diameter within about 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or greater than parental source cells.

Example 31: Measuring the average diameter distribution of fusosomes

[000473] This Example describes measurement of the diameter distribution of fusosomes.

[000474] Fusosomes are generated by any one of the methods described in previous Examples, and are tested to determine the average diameter of particles using a commercially available system, such as described in a previous Example. In some embodiments, diameter thresholds for 10%, 50%, and 90% of the fusosomes centered around the median are compared to parental cells to assess fusosome diameter distribution.

[000475] In some embodiments, fusosomes will have less than about 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, 5%, or less of the parental cell's variability in diameter distribution within 10%, 50%, or 90% of the sample.

Example 32: Average volume of fusosomes

[000476] This example describes measurement of the average volume of fusosomes. Without wishing to be bound by theory, varying the size (e.g., diameter, volume, surface area, etc.) of fusosomes can make them versatile for distinct cargo loading, therapeutic design or application.

[000477] Fusosomes are prepared as described in previous Examples. The positive control is HEK293 cells or polystyrene beads with a known size. The negative control is HEK293 cells that are passed through a 36 gauge needle approximately 50 times.

[000478] Analysis with a transmission electron microscope, as described in a previous Example, is used to determine the size of the fusosomes. The diameter of the fusosome is measured and volume is then calculated.

[000479] In some embodiments, fusosomes will have an average size of approximately 50nm or greater in diameter.

Example 33: Average density of fusosomes

[000480] Fusosome density is measured via a continuous sucrose gradient centrifugation assay as described in Théry et al., Curr Protoc Cell Biol. 2006 Apr; Chapter 3:Unit 3.22. Fusosomes are obtained as described in previous Examples.

[000481] First, a sucrose gradient is prepared. A 2 M and a 0.25 sucrose solution are generated by mixing 4 mL HEPES/sucrose stock solution and 1 mL HEPES stock solution or 0.5 mL HEPES/sucrose stock solution and 4.5 mL HEPES stock solution, respectively. These two fractions are loaded into the gradient maker with all shutters closed, the 2 M sucrose solution in the proximal compartment with a magnetic stir bar, and the 0.25 M sucrose solution in the distal compartment. The gradient maker is placed on a magnetic stir plate, the shutter between proximal and distal compartments is opened and the magnetic stir plate is turned on. HEPES stock solution is made as follows: 2.4 g N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES; 20mMfinal), 300 H2O, adjust pH to 7.4 with 10 N NaOH and finally adjust volume to 500 mL with H2O. HEPES/sucrose stock solution is made as follows: 2.4 g hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES; 20 mM final), 428 g protease-free sucrose (ICN; 2.5 M final), 150 mL H2O, adjust pH to 7.4 with 10 N NaOH and finally adjust volume to 500 mL with H2O.

[000482] The fusosomes are resuspended in 2 mL of HEPES/sucrose stock solution and are poured on the bottom of an SW 41 centrifuge tube. The outer tubing is placed in the SW 41 tube, just above the 2 mL of fusosomes. The outer shutter is opened, and a continuous 2 M (bottom) to 0.25 M (top) sucrose gradient is slowly poured on top of the fusosomes. The SW 41 tube is lowered

as the gradient is poured, so that the tubing is always slightly above the top of the liquid.

[000483] All tubes with gradients are balanced with each other, or with other tubes having the same weight of sucrose solutions. The gradients are centrifuged overnight (≥14 hr) at 210,000 x g, 4°C, in the SW 41 swinging-bucket rotor with the brake set on low.

[000484] With a micropipettor, eleven 1-mL fractions, from top to bottom, are collected and placed in a 3-mL tube for the TLA-100.3 rotor. The samples are set aside and, in separate wells of a 96-well plate, 50 µl of each fraction is used to measure the refractive index. The plate is covered with adhesive foil to prevent evaporation and stored for no more than 1 hour at room temperature. A refractometer is used to measure the refractive index (hence the sucrose concentration, and the density) of 10 to 20 µl of each fraction from the material saved in the 96-well plate.

[000485] A table for converting the refractive index into g/mL is available in the ultracentrifugation catalog downloadable from the Beckman website.

[000486] Each fraction is then prepared for protein content analysis. Two milliliters of 20 mM HEPES, pH 7.4, is added to each 1-mL gradient fraction, and mixed by pipetting up and down two to three times. One side of each tube is marked with a permanent marker, and the tubes are placed marked side up in a TLA-100.3 rotor.

[000487] The 3 mL-tubes with diluted fractions are centrifuged for 1 hr at 110,000 x g, 4°C. The TLA-100.3 rotor holds six tubes, so two centrifugations for each gradient is performed with the other tubes kept at 4°C until they can be centrifuged.

[000488] The supernatant is aspirated from each of the 3-mL tubes, leaving a drop on top of the pellet. The pellet most probably is not visible, but its location can be inferred from the mark on the tube. The invisible pellet is resuspended and transferred to microcentrifuge tubes. Half of each resuspended fraction is used for protein contentment analysis by bicinchoninic acid assay, described in another Example. This provides a distribution across the various gradient fractions of the fusosome preparation. This distribution is used to determine the average density of the fusosomes. The second half volume fraction is stored at -80°C and used for other purposes (e.g. functional analysis, or further purification by immunoisolation) once protein analysis has revealed the fusosome distribution across fractions.

[000489] In some embodiments, using this assay or an equivalent, the average density of the preparation comprising a plurality of fusosomes will be 1.25 g/mL +/- 0.05 standard deviation. In some embodiments, the average density of the preparation will be in the range of 1-1.1, 1.05-1.15, 1.1-1.2, 1.15-1.25, 1.2-1.3, or 1.25-1.35 g/mL. In some embodiments, average density of the preparation will be less than 1 or more than 1.35.

Example 34: Measuring organelle content in fusosomes

[000490] This Example describes detection of organelles in fusosomes.

[000491] Fusosomes were prepared as described herein. For detection of endoplasmic reticulum (ER) and mitochondria, fusosomes or C2C12 cells were stained with 1 μM ER stain (E34251, Thermo Fisher, Waltham, MA) and 1 μM mitochondria stain (M22426, Thermo Fisher Waltham, MA). For detection of lysosomes, fusosomes or cells were stained with 50 nM lysosome stain (L7526, Thermo Fisher, Waltham, MA).

[000492] Stained fusosomes were run on a flow cytometer (Thermo Fisher, Waltham, MA) and fluorescence intensity was measured for each dye according to the table below. Validation for the presence of organelles was made by comparing fluorescence intensity of stained fusosomes to unstained fusosomes (negative control) and stained cells (positive control).

[000493] Fusosomes stained positive for endoplasmic reticulum (Figure 1), mitochondria (Figure 2), and lysosomes (Figure 3) 5 hours post-enucleation.

Table 19: Fusosome stains

Stain	Attune Laser/Filter	Laser Wavelength	Emission Filter (nm)
Hoechst 33342	VL1	405	450/40
ER-Tracker Green	BL1	488	530/30
MitoTracker Deep Red FM	RL1	638	670/14
LysoTracker Green	BL1	488	530/30

Example 35: Measuring nuclear content in fusosomes

[000494] This Example describes measuring nuclear content in a fusosome. To validate that fusosomes do not contain nuclei, fusosomes are stained with 1 $\mu g \cdot mL^{-1}$ Hoechst 33342 and 1 μM CalceinAM (C3100MP, Thermo Fisher, Waltham, MA) and the stained fusosomes are run on an Attune NXT Flow Cytometer (Thermo Fisher, Waltham, MA) to determine the fluorescence intensity of each dye according to the table below. In some embodiments, validation for the presence of cytosol (CalceinAM) and the absence of a nucleus (Hoechst 33342) will be made by comparing the mean fluorescence intensity of stained fusosomes to unstained fusosomes and stained cells.

Table 20: Flow cytometer settings

Stain	Attune Laser/Filter	Laser Wavelength	Emission Filter (nm)
Hoechst 33342	VL1	405	450/40
Calcein AM	BL1	488	530/30

Example 36: Measuring nuclear envelope content

[000495] This Example describes a measurement of the nuclear envelope content in enucleated fusosomes. The nuclear envelope isolates DNA from the cytoplasm of the cell.

[000496] In some embodiments, a purified fusosome composition comprises a mammalian cell, such as HEK-293Ts (293 [HEK-293] (ATCC® CRL-1573™), that has been enucleated as described herein. This Example describes the quantification of different nuclear membrane proteins as a proxy to measure the amount of intact nuclear membrane that remains after fusosome generation.

[000497] In this Example, $10x10^6$ HEK-293Ts and the equivalent amount of fusosomes prepared from $10x10^6$ HEK-293Ts are fixed for 15 min using 3.7% PFA, washed with 1xPBS buffer, pH 7.4 and permeabilized simultaneously, and then blocked for 15 min using 1xPBS buffer containing 1% Bovine Serum Albumin and 0.5% Triton® X-100, pH 7.4. After permeabilization, fusosomes and cells are incubated for 12 hours at 4°C with different primary antibodies, e.g. (anti-RanGAP1 antibody [EPR3295] (Abcam - ab92360), anti-NUP98 antibody [EPR6678] - nuclear

pore marker (Abcam - ab124980), anti-nuclear pore complex proteins antibody [Mab414] - (Abcam- ab24609), anti-importin 7 antibody (Abcam - ab213670), at manufacturer suggested concentrations diluted in 1xPBS buffer containing 1% bovine serum albumin and 0.5% Triton® X-100, pH 7.4. Fusosomes and cells are then washed with 1xPBS buffer, pH 7.4, and incubated for 2hr at 21°C with an appropriate fluorescent secondary antibody that detects the previous specified primary antibody at manufacturer suggested concentrations diluted in 1xPBS buffer containing 1% bovine serum albumin and 0.5% detergent, pH 7.4. Fusosomes and cells are then washed with 1xPBS buffer, re-suspended in 300µL of 1xPBS buffer, pH 7.4 containing 1 µg/mL Hoechst 33342, filtered through a 20 µm FACS tube and analyzed by flow cytometry.

[000498] Negative controls are generated using the same staining procedure but with no primary antibody added. Flow cytometry is performed on a FACS cytometer (Becton Dickinson, San Jose, CA, USA) with 488nm argon laser excitation, and a 530+/-30nm emission spectrum is collected. FACS acquisition software is used for acquisition and analysis. The light scatter channels are set on linear gains, and the fluorescence channels on a logarithmic scale, with a minimum of 10,000 cells analyzed in each condition. The relative intact nuclear membrane content is calculated based on the median intensity of fluorescence in each sample. All events are captured in the forward and side scatter channels.

[000499] The normalized fluorescence intensity value for the fusosomes is determined by subtracting from the median fluorescence intensity value of the fusosome the median fluorescence intensity value of the respective negative control sample. Then the normalized fluorescence for the fusosomes samples is normalized to the respective nucleated cell samples in order to generate quantitative measurements of intact nuclear membrane content.

[000500] In some embodiments, enucleated fusosomes will comprise less than 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% fluorescence intensity or nuclear envelope content compared to the nucleated parental cells.

Example 37: Measuring chromatin levels

[000501] This Example describes measurement of chromatin in enucleated fusosomes.

[000502] DNA can be condensed into chromatin to allow it to fit inside the nucleus. In some

embodiments, a purified fusosome composition as produced by any one of the methods described herein will comprise low levels of chromatin.

[000503] Enucleated fusosomes prepared by any of the methods previously described and positive control cells (e.g., parental cells) are assayed for chromatin content using an ELISA with antibodies that are specific to histone protein H3 or histone protein H4. Histones are the chief protein component of chromatin, with H3 and H4 the predominant histone proteins.

[000504] Histones are extracted from the fusosome preparation and cell preparation using a commercial kit (e.g. Abcam Histone Extraction Kit (ab113476)) or other methods known in the art. These aliquots are stored at -80°C until use. A serial dilution of standard is prepared by diluting purified histone protein (either H3 or H4) from 1 to 50 ng/μL in a solution of the assay buffer. The assay buffer may be derived from a kit supplied by a manufacturer (e.g. Abcam Histone H4 Total Quantification Kit (ab156909) or Abcam Histone H3 total Quantification Kit (ab115091)). The assay buffer is added to each well of a 48- or 96-well plate, which is coated with an anti-histone H3 or anti-H4 antibody and sample or standard control is added to the well to bring the total volume of each well to 50 μL. The plate is then covered and incubated at 37 degrees for 90 to 120 minutes.

[000505] After incubation, any histone bound to the anti-histone antibody attached to the plate is prepared for detection. The supernatant is aspirated and the plate is washed with 150 μL of wash buffer. The capture buffer, which includes an anti-histone H3 or anti-H4 capture antibody, is then added to the plate in a volume of 50 μL and at a concentration of 1 μg/mL. The plate is then incubated at room temperature on an orbital shaker for 60 minutes.

[000506] Next, the plate is aspirated and washed 6 times using wash buffer. Signal reporter molecule activatable by the capture antibody is then added to each well. The plate is covered and incubated at room temperature for 30 minutes. The plate is then aspirated and washed 4 times using wash buffer. The reaction is stopped by adding stop solution. The absorbance of each well in the plate is read at 450 nm, and the concentration of histones in each sample is calculated according to the standard curve of absorbance at 450 nm vs. concentration of histone in standard samples.

[000507] In some embodiments, fusosome samples will comprise less than 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% the histone concentration of the

nucleated parental cells.

Example 38: Measuring DNA content in fusosomes

[000508] This example describes quantification of the amount of DNA in a fusosome relative to nucleated counterparts. In some embodiments, fusosomes will have less DNA than nucleated counterparts. Nucleic acid levels are determined by measuring total DNA or the level of a specific house-keeping gene. In some embodiments, fusosomes having reduced DNA content or substantially lacking DNA will be unable to replicate, differentiate, or transcribe genes, ensuring that their dose and function is not altered when administered to a subject.

[000509] Fusosomes are prepared by any one of the methods described in previous Examples. Preparations of the same mass as measured by protein of fusosomes and source cells are used to isolate total DNA (e.g. using a kit such as Qiagen DNeasy catalog #69504), followed by determination of DNA concentration using standard spectroscopic methods to assess light absorbance by DNA (e.g. with Thermo Scientific NanoDrop).

[000510] In some embodiments, concentration of DNA in enucleated fusosomes will be less than about 50%, 40%, 30%, 20%, 10%, 5%, 4%, 3%, 2%, 1% or less than in parental cells.

[000511] Alternatively, the concentration of a specific house-keeping gene, such as GAPDH, can be compared between nucleated cells and fusosomes with semi-quantitative real-time PCR (RT-PCR). Total DNA is isolated from parental cells and fusosome and DNA concentration is measured as described herein. RT-PCR is carried out with a PCR kit (Applied Biosystems, catalog #4309155) using the following reaction template:

SYBR Green Master Mix:	10 µL
0.45 µM Forward Primer:	1 µL
0.45 µM Reverse Primer:	1 µL
DNA Template:	10 ng
PCR-Grade	Water: Variable

[000512] Forward and reverse primers are acquired from Integrated DNA Technologies. The table below details the primer pairs and their associated sequences:

Table 21: Primer sequences

Target	Forward Primer Sequence (5'→3') (SEQ ID NO)	Reverse Primer Sequence (5'→3') (SEQ ID NO)
Human nDNA (GAPDH)	GGAGTCCACTGGCGTCTTCAC (SEQ ID NO:598)	GAGGCATTGCTGATGATCTTGAGG (SEQ ID NO:599)

[000513] A real-time PCR system (Applied Biosystems) is used to perform the amplification and detection with the following protocol:

Denaturation, 94°C	2 min
40 Cycles of the following sequence:	
Denaturation, 94°C	15 sec
Annealing, Extension, 60°C	1 min

[000514] A standard curve of the C_t vs. DNA concentration is prepared with serial dilutions of GAPDH DNA and used to normalize the Ct nuclear value from fusosome PCR results to a specific amount (ng) of DNA.

[000515] In some embodiments, concentration of GAPDH DNA in enucleated fusosomes will be less than about 50%, 40%, 30%, 20%, 10%, 5%, 4%, 3%, 2%, 1% or less than in parental cells.

Example 39: Measuring miRNA content in fusosomes

[000516] This example describes quantification of microRNAs (miRNAs) in fusosomes. In some embodiments, a fusosome comprises miRNAs.

[000517] MiRNAs are regulatory elements that, among other activities, control the rate by which messenger RNAs (mRNAs) are translated into proteins. In some embodiments, fusosomes carrying miRNA may be used to deliver the miRNA to target sites.

[000518] Fusosomes are prepared by any one of the methods described in previous Examples. RNA from fusosomes or parental cells is prepared as described previously. At least one miRNA gene is selected from the Sanger Center miRNA Registry at www.sanger.ac.uk/Software/Rfam/mirna/index.shtml. miRNA is prepared as described in Chen *et*

al, *Nucleic Acids Research*, 33(20), 2005. All TaqMan miRNA assays are available through Thermo Fisher (A25576, Waltham, MA).

[000519] qPCR is carried out according to manufacturer's specifications on miRNA cDNA, and C_T values are generated and analyzed using a real-time PCR system as described herein.

[000520] In some embodiments, miRNA content of fusosomes will be at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or greater than that of their parental cells.

Example 40: Quantifying expression of an endogenous RNA or synthetic RNA in fusosomes

[000521] This example describes quantification of levels of endogenous RNA with altered expression, or a synthetic RNA that is expressed in a fusosome.

[000522] The fusosome or parental cell is engineered to alter the expression of an endogenous or synthetic RNA that mediates a cellular function to the fusosomes.

[000523] Transposase vectors (System Biosciences, Inc.) includes the open reading frame of the Puromycin resistance gene together with an open reading frame of a cloned fragment of a protein agent. The vectors are electroporated into 293Ts using an electroporator (Amaxa) and a 293T cell line specific nuclear transfection kit (Lonza).

[000524] Following selection with puromycin for 3-5 days in DMEM containing 20% Fetal Bovine Serum and 1x Penicillin/Streptomycin, fusosomes are prepared from the stably expressing cell line by any one of the methods described in previous Examples.

[000525] Individual fusosomes are isolated and protein agent or RNA per fusosome is quantified as described in a previous Example.

[000526] In some embodiments, fusosomes will have at least 1, 2, 3, 4, 5, 10, 20, 50, 100, 500, 10^3, 5.0 x 10^3, 10^4, 5.0 x 10^4, 10^5, 5.0 x 10^5, 10^6, 5.0 x 10^6, or more of the RNA per fusosome.

Example 41: Measuring lipid composition in fusosomes

[000527] This Example describes quantification of the lipid composition of fusosomes. In some embodiments, lipid composition of fusosomes is similar to the cells that they are derived

from. Lipid composition affects important biophysical parameters of fusosomes and cells, such as size, electrostatic interactions, and colloidal behavior.

[000528] The lipid measurements are based on mass spectrometry. Fusosomes are prepared by any one of the methods described in previous Examples.

[000529] Mass spectrometry-based lipid analysis is performed at a lipid analysis service (Dresden, Germany) as described (Sampaio, et al., Proc Natl Acad Sci, 2011, Feb 1;108(5):1903–7). Lipids are extracted using a two-step chloroform/methanol procedure (Ejsing, et al., Proc Natl Acad Sci, 2009, Mar 17;106(7):2136–41). Samples are spiked with an internal lipid standard mixture of: cardiolipin 16:1/15:0/15:0/15:0 (CL), ceramide 18:1;2/17:0 (Cer), diacylglycerol 17:0/17:0 (DAG), hexosylceramide 18:1;2/12:0 (HexCer), lysophosphatidate 17:0 (LPA), lyso-phosphatidylcholine 12:0 (LPC), lyso-phosphatidylethanolamine 17:1 (LPE), lyso-phosphatidylglycerol 17:1 (LPG), lyso-phosphatidylinositol 17:1 (LPI), lyso-phosphatidylserine 17:1 (LPS), phosphatidate 17:0/17:0 (PA), phosphatidylcholine 17:0/17:0 (PC), phosphatidylethanolamine 17:0/17:0 (PE), phosphatidylglycerol 17:0/17:0 (PG), phosphatidylinositol 16:0/16:0 (PI), phosphatidylserine 17:0/17:0 (PS), cholesterol ester 20:0 (CE), sphingomyelin 18:1;2/12:0;0 (SM) and triacylglycerol 17:0/17:0/17:0 (TAG).

[000530] After extraction, the organic phase is transferred to an infusion plate and dried in a speed vacuum concentrator. The first step dry extract is resuspended in 7.5 mM ammonium acetate in chloroform/methanol/propanol (1:2:4, V:V:V) and the second step dry extract is resuspended in 33% ethanol solution of methylamine in chloroform/methanol (0.003:5:1; V:V:V). All liquid handling steps are performed using a robotic platform for organic solvent with an anti-droplet control feature (Hamilton Robotics) for pipetting.

[000531] Samples are analyzed by direct infusion on a mass spectrometer (Thermo Scientific) equipped with an ion source (Advion Biosciences). Samples are analyzed in both positive and negative ion modes with a resolution of Rm/z=200=280000 for MS and Rm/z=200=17500 for tandem MS/MS experiments, in a single acquisition. MS/MS is triggered by an inclusion list encompassing corresponding MS mass ranges scanned in 1 Da increments (Surma, et al., Eur J lipid Sci Technol, 2015, Oct;117(10):1540–9). Both MS and MS/MS data are combined to monitor CE, DAG and TAG ions as ammonium adducts; PC, PC O-, as acetate

adducts; and CL, PA, PE, PE O-, PG, PI and PS as deprotonated anions. MS only is used to monitor LPA, LPE, LPE O-, LPI and LPS as deprotonated anions; Cer, HexCer, SM, LPC and LPC O- as acetate.

[000532] Data are analyzed with in-house developed lipid identification software as described in the following references (Herzog, et al., Genome Biol, 2011, Jan 19;12(1):R8; Herzog, et al., PLoS One, 2012, Jan;7(1):e29851). Only lipid identifications with a signal-to-noise ratio >5, and a signal intensity 5-fold higher than in corresponding blank samples are considered for further data analysis.

[000533] Fusosome lipid composition is compared to parental cells' lipid composition. In some embodiments, fusosomes and parental cells will have a similar lipid composition if >50% of the identified lipids in the parental cells are present in the fusosomes, and of those identified lipids, the level in the fusosome will be >25% of the corresponding lipid level in the parental cell.

Example 42: Measuring proteomic composition in fusosomes

[000534] This Example describes quantification of the protein composition of fusosomes. In some embodiments, protein composition of fusosomes will be similar to the cells that they are derived from.

[000535] Fusosomes are prepared by any one of the methods described in previous Examples. Fusosomes are resuspended in lysis buffer (7M Urea, 2M Thiourea, 4% (w/v) Chaps in 50 mM Tris pH 8.0) and incubated for 15 minutes at room temperature with occasional vortexing. Mixtures are then lysed by sonication for 5 minutes in an ice bath and spun down for 5 minutes at 13,000 RPM. Protein content is determined by a colorimetric assay (Pierce) and protein of each sample is transferred to a new tube and the volume is equalized with 50 mM Tris pH 8.

[000536] Proteins are reduced for 15 minutes at 65 Celsius with 10 mM DTT and alkylated with 15 mM iodoacetamide for 30 minutes at room temperature in the dark. Proteins are precipitated with gradual addition of 6 volumes of cold (-20°Celsius) acetone and incubated overnight at -80°C. Protein pellets are washed 3 times with cold (-20°Celsius) methanol. Proteins are resuspended in 50 mM Tris pH 8.3.

[000537] Next, trypsin/lysC is added to the proteins for the first 4h of digestion at 37 Celsius

with agitation. Samples are diluted with 50mM Tris pH 8 and 0.1% sodium deoxycholate is added with more trypsin/lysC for digestion overnight at 37 Celsius with agitation. Digestion is stopped and sodium deoxycholate is removed by the addition of 2% v/v formic acid. Samples are vortexed and cleared by centrifugation for 1 minute at 13,000 RPM. Peptides are purified by reversed phase solid phase extraction (SPE) and dried down. Samples are reconstituted in 20 µl of 3% DMSO, 0.2% formic acid in water and analyzed by LC-MS.

[000538] To have quantitative measurements, a protein standard is also run on the instrument. Standard peptides (Pierce, equimolar, LC-MS grade, #88342) are diluted to 4, 8, 20, 40 and 100 fmol/µL and are analyzed by LC-MS/MS. The average AUC (area under the curve) of the 5 best peptides per protein (3 MS/MS transition/peptide) is calculated for each concentration to generate a standard curve.

[000539] Acquisition is performed with a high resolution mass spectrometer (ABSciex, Foster City, CA, USA) equipped with an electrospray interface with a 25 µm iD capillary and coupled with micro-ultrahigh performance liquid chromatography (µUHPLC) (Eksigent, Redwood City, CA, USA). Analysis software is used to control the instrument and for data processing and acquisition. The source voltage is set to 5.2 kV and maintained at 225°C, curtain gas is set at 27 psi, gas one at 12 psi and gas two at 10 psi. Acquisition is performed in Information Dependent Acquisition (IDA) mode for the protein database and in SWATH acquisition mode for the samples. Separation is performed on a reversed phase column 0.3 µm i.d., 2.7 µm particles, 150mm long (Advance Materials Technology, Wilmington, DE) which is maintained at 60°C. Samples are injected by loop overfilling into a 5µL loop. For the 120 minute (samples) LC gradient, the mobile phase includes the following: solvent A (0.2% v/v formic acid and 3% DMSO v/v in water) and solvent B (0.2% v/v formic acid and 3% DMSO in EtOH) at a flow rate of 3 µL/min.

[000540] For the absolute quantification of the proteins, a standard curve (5 points, R2>0.99) is generated using the sum of the AUC of the 5 best peptides (3 MS/MS ion per peptide) per protein. To generate a database for the analysis of the samples, the DIAUmpire algorithm is run on each of the 12 samples and combined with the output MGF files into one database. This database is used with software (ABSciex) to quantify the proteins in each of the samples, using 5

transition/peptide and 5 peptide/protein maximum. A peptide is considered as adequately measured if the score computed is superior to 1.5 or had a FDR < 1%. The sum of the AUC of each of the adequately measured peptides is mapped on the standard curve, and is reported as fmol.

[000541] The resulting protein quantification data is then analyzed to determine protein levels and proportions of known classes of proteins as follows: enzymes are identified as proteins that are annotated with an Enzyme Commission (EC) number; ER associated proteins are identified as proteins that had a Gene Ontology (GO; http://www.geneontology.org) cellular compartment classification of ER and not mitochondria; exosome associated proteins are identified as proteins that have a Gene Ontology cellular compartment classification of exosomes and not mitochondria; and mitochondrial proteins are identified as proteins that are identified as mitochondrial in the MitoCarta database (Calvo et al., NAR 2015l doi:10.1093/nar/gkv1003). The molar ratios of each of these categories are determined as the sum of the molar quantities of all the proteins in each class divided by the sum of the molar quantities of all identified proteins in each sample.

[000542] Fusosome proteomic composition is compared to parental cell proteomic composition. In some embodiments, similar proteomic compositions between fusosomes and parental cells will be observed when >50% of the identified proteins are present in the fusosome, and of those identified proteins the level is >25% of the corresponding protein level in the parental cell.

Example 43: Quantifying an endogenous or synthetic protein level per fusosome

[000543] This example describes quantification of an endogenous or synthetic protein cargo in fusosomes. In some embodiments, fusosomes comprise an endogenous or synthetic protein cargo.

[000544] The fusosome or parental cell is engineered to alter the expression of an endogenous protein or express a synthetic cargo that mediates a therapeutic or novel cellular function.

[000545] Transposase vectors (System Biosciences, Inc.) that include the open reading frame of the puromycin resistance gene together with an open reading frame of a cloned fragment of a protein agent, optionally translationally fused to the open reading frame of a green fluorescent

protein (GFP). The vectors are electroporated into 293Ts using an electroporator (Amaxa) and a 293T cell line specific nuclear transfection kit (Lonza).

[000546] Following selection with puromycin for 3-5 days in DMEM containing 20% fetal bovine serum and 1x penicillin/streptomycin, fusosomes are prepared from the stably expressing cell line by any one of the methods described in previous Examples.

[000547] Altered expression levels of an endogenous protein or expression levels of a synthetic protein that are not fused to GFP are quantified by mass spectrometry as described above. In some embodiments, fusosomes will have at least 1, 2, 3, 4, 5, 10, 20, 50, 100, 500, 10^3, 5.0 x 10^3, 10^4, 5.0 x 10^4, 10^5, 5.0 x 10^5, 10^6, 5.0 x 10^6, or more protein agent molecules per fusosome.

[000548] Alternatively, purified GFP is serially diluted in DMEM containing 20% fetal bovine serum and 1x Penicillin/Streptomycin to generate a standard curve of protein concentration. GFP fluorescence of the standard curve and a sample of fusosomes is measured in a fluorimeter (BioTek) using a GFP light cube (469/35 excitation filter and a 525/39 emission filter) to calculate the average molar concentration of GFP molecules in the fusosomes. The molar concentration is then converted to number of GFP molecules and divided by the number of fusosomes per sample to achieve an average number of protein agent molecules per fusosome.

[000549] In some embodiments, fusosomes will have at least 1, 2, 3, 4, 5, 10, 20, 50, 100, 500, 10^3, 5.0 x 10^3, 10^4, 5.0 x 10^4, 10^5, 5.0 x 10^5, 10^6, 5.0 x 10^6, or more protein agent molecules per fusosome.

Example 44: Measuring markers of exosomal proteins in fusosomes

[000550] This assay describes quantification of the proteomics makeup of the sample preparation, and quantifies the proportion of proteins that are known to be specific markers of exosomes.

[000551] Fusosomes are pelleted and shipped frozen to the proteomics analysis center per standard biological sample handling procedures.

[000552] The fusosomes are thawed for protein extraction and analysis. First, they are resuspended in lysis buffer (7M urea, 2M thiourea, 4% (w/v) chaps in 50 mM Tris pH 8.0) and incubated for 15 minutes at room temperature with occasional vortexing. The mixtures are then

lysed by sonication for 5 minutes in an ice bath and spun down for 5 minutes at 13,000 RPM. Total protein content is determined by a colorimetric assay (Pierce) and 100 µg of protein from each sample is transferred to a new tube and the volume is adjusted with 50 mM Tris pH 8.

[000553] The proteins are reduced for 15 minutes at 65° Celsius with 10 mM DTT and alkylated with 15 mM iodoacetamide for 30 minutes at room temperature in the dark. The proteins are then precipitated with gradual addition of 6 volumes of cold (-20° Celsius) acetone and incubated over night at -80° Celsius.

[000554] The proteins are pelleted, washed 3 times with cold (-20° Celsius) methanol, and resuspended in 50 mM Tris pH 8. 3.33µg of trypsin/lysC is added to the proteins for a first 4h of digestion at 37° Celsius with agitation. The samples are diluted with 50 mM Tris pH 8 and 0.1 % sodium deoxycholate is added with another 3.3 µg of trypsin/lysC for digestion overnight at 37° Celsius with agitation. Digestion is stopped and sodium deoxycholate is removed by the addition of 2% v/v formic acid. Samples are vortexed and cleared by centrifugation for 1 minute at 13,000 RPM.

[000555] The proteins are purified by reversed phase solid phase extraction (SPE) and dried down. The samples are reconstituted in 3% DMSO, 0.2% formic acid in water and analyzed by LC-MS as described previously.

[000556] The resulting protein quantification data is analyzed to determine protein levels and proportions of know exosomal marker proteins. Specifically: tetraspanin family proteins (CD63, CD9, or CD81), ESCRT-related proteins (TSG101, CHMP4A-B, or VPS4B), Alix, TSG101, MHCI, MHCII, GP96, actinin-4, mitofilin, syntenin-1, TSG101, ADAM10, EHD4, syntenin-1, TSG101, EHD1, flotillin-1, heat-shock 70-kDa proteins (HSC70/HSP73, HSP70/HSP72). The molar ratio these exosomal marker proteins relative to all proteins measured is determined as the molar quantity of each specific exosome marker protein listed above divided by the sum of the molar quantities of all identified proteins in each sample and expressed as a percent.

[000557] Similarly, the molar ratio for all exosomal marker proteins relative to all proteins measured is determined as the sum of the molar quantity of all specific exosome marker protein listed above divided by the sum of the molar quantities of all identified proteins in each sample and expressed as a percent of the total.

[000558] In some embodiments, a sample will comprise less than 5% of any individual exosomal marker protein and less than 15% of total exosomal marker proteins.

[000559] In some embodiments, an individual exosomal marker protein will be present at less than 0.05%, 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, , or 10%.

[000560] In some embodiments, the sum of all exosomal marker proteins will be less than 0.05%, 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, or 25%.

Example 45: Measuring GAPDH in fusosomes

[000561] This assay describes quantification of the level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) in the fusosomes, and the relative level of GAPDH in the fusosomes compared to the parental cells.

[000562] GAPDH is measured in the parental cells and the fusosomes using a standard commercially available ELISA for GAPDH (ab176642, Abcam) per the manufacturer's directions.

[000563] Total protein levels are similarly measured via bicinchoninic acid assay as previously described in the same volume of sample used to measure GAPDH. In embodiments, using this assay, the level of GAPDH per total protein in the fusosomes will be <100ng GAPDH / µg total protein. Similarly, in embodiments, the decrease in GAPDH levels relative to total protein from the parental cells to the fusosomes will be greater than a 10% decrease.

[000564] In some embodiments, GAPDH content in the preparation in ng GAPDH / µg total protein will be less than 500, less than 250, less than 100, less than 50, less than 20, less than 10, less than 5, or less than1.

[000565] In some embodiments, a decrease in GAPDH per total protein in ng/µg from the parent cell to the preparation will be more than 1%, more than 2.5%, more than 5%, more than 10%, more than 15%, more than 20%, more than 30%, more than 40%, more than 50%, more than 60%, more than 70%, more than 80%, or more than 90%.

[000566]

Example 46: Measuring calnexin in fusosomes

[000567] This assay describes quantification of the level of calnexin (CNX) in the fusosomes,

and the relative level of CNX in the fusosomes compared to the parental cells.

[000568] Calnexin is measured in the starting cells and the preparation using a standard commercially available ELISA for calnexin (MBS721668, MyBioSource) per the manufacturer's directions.

[000569] Total protein levels are similarly measured via bicinchoninic acid assay as previously described in the same volume of sample used to measure calnexin. In embodiments, using this assay, the level of calnexin per total protein in the fusosomes will be <100ng calnexin / µg total protein. Similarly, in embodiments, the increase in calnexin levels relative to total protein from the parental cell to the fusosomes will be greater than a 10% increase.

[000570] In some embodiments, calnexin content in the preparation in ng calnexin / µg total protein will be less than 500, 250, 100, 50, 20, 10, 5, or 1.

[000571] In some embodiments, a decrease in calnexin per total protein in ng/µg from the parent cell to the preparation will be more than 1%, 2.5%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%.

Example 47: Comparison of soluble to insoluble protein mass

[000572] This Example describes quantification of the soluble:insoluble ratio of protein mass in fusosomes. In some embodiments, a soluble:insoluble ratio of protein mass in fusosomes will be similar to nucleated cells.

[000573] Fusosomes are prepared by any one of the methods described in previous Examples. The fusosome preparation is tested to determine the soluble : insoluble protein ratio using a standard bicinchoninic acid assay (BCA) (e.g. using the commercially available Pierce™ BCA Protein Assay Kit, Thermo Fischer product# 23225). Soluble protein samples are prepared by suspending the prepared fusosomes or parental cells at a concentration of $1x10^7$ cells or fusosomes/mL in PBS and centrifuging at 1600g to pellet the fusosomes or cells. The supernatant is collected as the soluble protein fraction.

[000574] The fusosomes or cells in the pellet are lysed by vigorous pipetting and vortexing in PBS with 2% Triton-X-100. The lysed fraction represents the insoluble protein fraction.

[000575] A standard curve is generated using the supplied BSA, from 0 to 20 µg of BSA per well (in triplicate). The fusosome or cell preparation is diluted such that the quantity measured is within the range of the standards. The fusosome preparation is analyzed in triplicate and the mean value is used. The soluble protein concentration is divided by the insoluble protein concentration to yield the soluble:insoluble protein ratio.

[000576] In some embodiments, a fusosome soluble:insoluble protein ratio will be within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or greater compared to the parental cells.

Example 48: Measuring LPS in fusosomes

[000577] This example describes quantification of levels of lipopolysaccharides (LPS) in fusosomes as compared to parental cells. In some embodiments, fusosomes will have lower levels of LPS compared to parental cells.

[000578] LPS are a component of bacterial membranes and potent inducer of innate immune responses.

[000579] The LPS measurements are based on mass spectrometry as described in the previous Examples.

[000580] In some embodiments, less than 5%, 1%, 0.5%, 0.01%, 0.005%, 0.0001%, 0.00001% or less of the lipid content of fusosomes will be LPS.

Example 49: Ratio of lipids to proteins in fusosomes

[000581] This Example describes quantification of the ratio of lipid mass to protein mass in fusosomes. In some embodiments, fusosomes will have a ratio of lipid mass to protein mass that is similar to nucleated cells.

[000582] Total lipid content is calculated as the sum of the molar content of all lipids identified in the lipidomics data set outlined in a previous Example. Total protein content of the fusosomes is measured via bicinchoninic acid assay as described herein.

[000583] Alternatively, the ratio of lipids to proteins can be described as a ratio of a particular lipid species to a specific protein. The particular lipid species is selected from the lipidomics data

produced in a previous Example. The specific protein is selected from the proteomics data produced in a previous Example. Different combinations of selected lipid species and proteins are used to define specific lipid:protein ratios.

Example 50: Ratio of proteins to DNA in fusosomes

[000584] This Example describes quantification of the ratio of protein mass to DNA mass in fusosomes. In some embodiments, fusosomes will have a ratio of protein mass to DNA mass that is much greater than cells.

[000585] Total protein content of the fusosomes and cells is measured as described in in a previous Example. The DNA mass of fusosomes and cells is measured as described in a previous Example. The ratio of proteins to total nucleic acids is then determined by dividing the total protein content by the total DNA content to yield a ratio within a given range for a typical fusosome preparation.

[000586] Alternatively, the ratio of proteins to nucleic acids is determined by defining nucleic acid levels as the level of a specific house-keeping gene, such as GAPDH, using semi-quantitative real-time PCR (RT-PCR).

[000587] The ratio of proteins to GAPDH nucleic acids is then determined by dividing the total protein content by the total GAPDH DNA content to define a specific range of protein:nucleic acid ratio for a typical fusosome preparation.

Example 51: Ratio of lipids to DNA in fusosomes

[000588] This Example describes quantification of the ratio of lipids to DNA in fusosomes compared to parental cells. In some embodiments, fusosomes will have a greater ratio of lipids to DNA compared to parental cells.

[000589] This ratio is defined as total lipid content (outlined in an Example above) or a particular lipid species. In the case of a particular lipid species, the range depends upon the particular lipid species selected. The particular lipid species is selected from the lipidomics data produced in the previously described Example. Nucleic acid content is determined as described in the previously described Example.

[000590] Different combinations of selected lipid species normalized to nucleic acid content are used to define specific lipid:nucleic acid ratios that are characteristic of a particular fusosome preparation.

Example 52: Analyzing surface markers on fusosomes

[000591] This assay describes identification of surface markers on the fusosomes.

[000592] Fusosomes are pelleted and shipped frozen to the proteomics analysis center per standard biological sample handling procedures.

[000593] To identify surface marker presence or absence on the fusosomes, they are stained with markers against phosphatidyl serine and CD40 ligand and analyzed by flow cytometry using a FACS system (Becton Dickinson). For detection of surface phosphatidylserine, the product is analyzed with an annexin V assay (556547, BD Biosciences) as described by the manufacturer.

[000594] Briefly, the fusosomes are washed twice with cold PBS and then resuspended in 1X binding buffer at a concentration of 1 x 10^6 fusosomes/mL. 10% of the resuspension is transferred to a 5 mL culture tube and 5 µl of FITC annexin V is added. The cells are gently vortexed and incubated for 15 min at room temperature (25°C) in the dark.

[000595] In parallel, a separate 10% of the resuspension is transferred to a different tube to act as an unstained control. 1X binding buffer is added to each tube. The samples are analyzed by flow cytometry within 1 hr.

[000596] In some embodiments, using this assay, the mean of the population of the stained fusosomes will be determined to be above the mean of the unstained cells indicating that the fusosomes comprise phosphatidyl serine.

[000597] Similarly, for the CD40 ligand, the following monoclonal antibody is added to another 10% of the washed fusosomes: PE-CF594 mouse anti-human CD154 clone TRAP1 (563589, BD Pharmigen) as per the manufacturer's directions. Briefly, saturating amounts of the antibody are used. In parallel, a separate 10% of the fusosomes are transferred to a different tube to act as an unstained control. The tubes are centrifuged for 5 min at 400 × g, at room temperature. The supernatant is decanted and the pellet is washed twice with flow cytometry wash solution. 0.5 mL of 1% paraformaldehyde fixative is added to each tube. Each is briefly vortexed and stored at

4°C until analysis on the flow cytometer.

[000598] In some embodiments, using this assay or equivalent, the mean of the population of the stained fusosomes will be above the mean of the unstained cells indicating that the fusosomes comprise CD40 ligand.

Example 53: Analysis of viral capsid proteins in fusosomes

[000599] This assay describes analysis of the makeup of the sample preparation and assesses the proportion of proteins that are derived from viral capsid sources.

[000600] Fusosomes are pelleted and shipped frozen to a proteomics analysis center per standard biological sample handling procedures.

[000601] The fusosomes are thawed for protein extraction and analysis. First, they are resuspended in lysis buffer (7M urea, 2M thiourea, 4% (w/v) chaps in 50 mM Tris pH 8.0) and incubated for 15 minutes at room temperature with occasional vortexing. The mixtures are then lysed by sonication for 5 minutes in an ice bath and spun down for 5 minutes at 13,000 RPM. Total protein content is determined by a colorimetric assay (Pierce) and 100 µg of protein from each sample is transferred to a new tube and the volume is adjusted with 50 mM Tris pH 8.

[000602] The proteins are reduced for 15 minutes at 65° Celsius with 10 mM DTT and alkylated with 15 mM iodoacetamide for 30 minutes at room temperature in the dark. The proteins are then precipitated with gradual addition of 6 volumes of cold (-20° Celsius) acetone and incubated over night at -80° Celsius.

[000603] The proteins are pelleted, washed 3 times with cold (-20° Celsius) methanol, and resuspended in 50 mM Tris pH 8. 3.33µg of trypsin/lysC is added to the proteins for a first 4h of digestion at 37° Celsius with agitation. The samples are diluted with 50 mM Tris pH 8 and 0.1 % sodium deoxycholate is added with another 3.3 µg of trypsin/lysC for digestion overnight at 37° Celsius with agitation. Digestion is stopped and sodium deoxycholate is removed by the addition of 2% v/v formic acid. Samples are vortexed and cleared by centrifugation for 1 minute at 13,000 RPM.

[000604] The proteins are purified by reversed phase solid phase extraction (SPE) and dried down. The samples are reconstituted in 3% DMSO, 0.2% formic acid in water and analyzed by

LC-MS as described previously.

[000605] The molar ratio of the viral capsid proteins relative to all proteins measured is determined as the molar quantity of all viral capsid proteins divided by the sum of the molar quantities of all identified proteins in each sample and expressed as a percent.

[000606] In some embodiments, using this approach or an equivalent, the sample will comprise less than 10% viral capsid protein. In some embodiments, a sample will comprise less than 0.5%, 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, or 90% viral capsid protein.

Example 54: Measuring fusion with a target cell

[000607] This example describes quantification of fusosome fusion with a target cell compared to a non-target cell.

[000608] In some embodiments, fusosome fusion with a target cell allows the cell-specific delivery of a cargo, carried within the lumen of the fusosome, to the cytosol of the recipient cell. Fusosomes produced by the herein described methods are assayed for fusion rate with a target cell as follows.

[000609] In this example, the fusosome comprises a HEK293T cell expressing Myomaker on its plasma membrane. In addition, the fusosome expresses mTagBFP2 fluorescent protein and Cre recombinase. The target cell is a myoblast cell, which expresses both Myomaker and Myomixer, and the non-target cell is a fibroblast cell, which expresses neither Myomaker nor Myomixer. A Myomaker-expressing fusosome is predicted to fuse with the target cell that expresses both Myomaker and Myomixer but not the non-target cell (Quinn et al., 2017, Nature Communications, 8, 15665. doi.org/10.1038/ncomms15665) (Millay et al., 2013, Nature, 499(7458), 301–305. doi.org/10.1038/nature12343). Both the target and non-target cell types are isolated from mice and stably-express "LoxP-stop-Loxp-tdTomato" cassette under a CMV promoter, which upon recombination by Cre turns on tdTomato expression, indicating fusion.

[000610] The target or non-target recipient cells are plated into a black, clear-bottom 96-well plate. Both target and non-target cells are plated for the different fusion groups. Next, 24 hours after plating the recipient cells, the fusosomes expressing Cre recombinase protein and Myomaker

are applied to the target or non-target recipient cells in DMEM media. The dose of fusosomes is correlated to the number of recipient cells plated in the well. After applying the fusosomes, the cell plate is centrifuged at 400g for 5 minutes to help initiate contact between the fusosomes and the recipient cells.

[000611] Starting at four hours after fusosome application, the cell wells are imaged to positively identify RFP-positive cells versus GFP-positive cells in the field or well.

[000612] In this example, cell plates are imaged using an automated microscope (www.biotek.com/products/imaging-microscopy-automated-cell-imagers/lionheart-fx-automated-live-cell-imager/). The total cell population in a given well is determined by first staining the cells with Hoechst 33342 in DMEM media for 10 minutes. Hoechst 33342 stains cell nuclei by intercalating into DNA and therefore is used to identify individual cells. After staining, the Hoechst media is replaced with regular DMEM media.

[000613] The Hoechst is imaged using the 405 nm LED and DAPI filter cube. GFP is imaged using the 465 nm LED and GFP filter cube, while RFP is imaged using 523 nm LED and RFP filter cube. Images of target and non-target cell wells are acquired by first establishing the LED intensity and integration times on a positive-control well; i.e., recipient cells treated with adenovirus coding for Cre recombinase instead of fusosomes.

[000614] Acquisition settings are set so that RFP and GFP intensities are at the maximum pixel intensity values but not saturated. The wells of interest are then imaged using the established settings. Wells are imaged every 4 hours to acquire time-course data for rates of fusion activity.

[000615] Analysis of GFP and RFP-positive wells is performed with software provided with the fluorescent microscope or other software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, rsb.info.nih.gov/ij/, 1997–2007).

[000616] The images are pre-processed using a rolling ball background subtraction algorithm with a 60 μm width. The total cell mask is set on the Hoechst-positive cells. Cells with Hoechst intensity significantly above background intensities are thresholded and areas too small or large to be Hoechst-positive cells are excluded.

[000617] Within the total cell mask, GFP and RFP-positive cells are identified by again

thresholding for cells significantly above background and extending the Hoechst (nuclei) masks for the entire cell area to include the entire GFP and RFP cellular fluorescence. The number of RFP-positive cells identified in control wells containing target or non-target recipient cells is used to subtract from the number of RFP-positive cells in the wells containing fusosome (to subtract for non-specific Loxp recombination). The number of RFP-positive cells (fused recipient cells) is then divided by the sum of the GFP-positive cells (recipient cells that have not fused) and RFP-positive cells at each time point to quantify the rate of fusosome fusion within the recipient cell population. The rate is normalized to the given dose of fusosome applied to the recipient cells. For rates of targeted fusion (fusosome fusion to targeted cells), the rate of fusion to the non-target cell is subtracted from the rate of fusion to the target cell in order to quantify rates of targeted fusion.

In some embodiments, average rate of fusion for the fusosomes with the target cells will be in the range of 0.01-4.0 RFP/GFP cells per hour for target cell fusion or at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater than non-target recipient cells with fusosomes. In some embodiments, groups with no fusosome applied will show a background rate of <0.01 RFP/GFP cells per hour.

Example 55: In vitro fusion to deliver a membrane protein

[000618] This example describes fusosome fusion with a cell *in vitro*. In some embodiments, fusosome fusion with a cell in vitro results in delivery of an active membrane protein to the recipient cell.

[000619] In this example, the fusosomes are generated from a HEK293T cell expressing the Sendai virus HVJ-E protein (Tanaka et al., 2015, Gene Therapy, 22(October 2014), 1–8. doi.org/10.1038/gt.2014.12). In some embodiments, fusosomes are generated to express the membrane protein, GLUT4, which is found primarily in muscle and fat tissues and is responsible for the insulin-regulated transport of glucose into cells. Fusosomes with and without GLUT4 are prepared from HEK293T cells as described by any of the methods described in a previous Example.

[000620] Muscles cells, such as, C2C12 cells, are then treated with fusosomes expressing GLUT4, fusosomes that do not express GLUT4, PBS (negative control), or insulin (positive control). The activity of GLUT4 on C2C12 cells is measured by the uptake of the fluorescent 2-

deoxyglucose analog, 2-[N-(7-nitrobenz-2-oxa-1,3-diaxol-4-yl)amino]-2-deoxyglucose (2-NBDG). The fluorescence of C2C12 cells is assessed via microscopy using methods described in previous Examples.

[000621] In some embodiments, C2C12 cells that are treated with fusosomes that express GLUT4 and insulin are expected to demonstrate increased fluorescence compared to C2C12 cells treated with PBS or fusosomes not expressing GLUT4. See, also, Yang et al., *Advanced Materials* 29, 1605604, 2017.

Example 56: In vivo delivery of membrane protein

[000622] This example describes fusosome fusion with a cell *in vivo*. In some embodiments, fusosome fusion with a cell in vivo results in delivery of an active membrane protein to the recipient cell.

[000623] In this example, the fusosomes are generated from a HEK293T cell expressing the Sendai virus HVJ-E protein as in the previous Example. In some embodiments, fusosomes are generated to express the membrane protein, GLUT4. Fusosomes with and without GLUT4 are prepared from HEK293T cells as described by any of the methods described in a previous Example.

[000624] BALB/c-nu mice are administered fusosomes expressing GLUT4, fusosomes that do not express GLUT4, or PBS (negative control). Mice are injected intramuscularly in the tibialis anterior muscle with fusosomes or PBS. Immediately prior to fusosome administration, mice are fasted for 12 hours and injected with [18F] 2-fluoro-2deoxy-d-glucose (18F-FDG), which is an analog of glucose that enables positron emission tomography (PET imaging). Mice are injected with 18F-FDG via the tail vein under anesthesia (2% isoflurane). PET imaging is performed using a nanoscale imaging system (1T, Mediso, Hungary). Imaging is conducted 4 hours after administration of fusosomes. Immediately after imaging, mice are sacrificed and the tibialis anterior muscle is weighed. PET images are reconstructed using a 3D imaging system in full detector mode, with all corrections on, high regularization, and eight iterations. Three-dimensional volume of interest (VOI) analysis of the reconstructed images is performed using the imaging software package (Mediso, Hungary) and applying standard uptake value (SUV) analysis. VOI fixed with a diameter of 2 mm sphere, is drawn for the tibialis anterior muscle site. The SUV of

each VOI sites is calculated using the following formula: SUV = (radioactivity in volume of interest, measured as Bq/cc × body weight)/ injected radioactivity.

[000625] In some embodiments, mice that are administered fusosomes expressing GLUT4 are expected to demonstrate an increased radioactive signal in VOI as compared to mice administered PBS or fusosomes that do not express GLUT4. See, also, Yang et al., *Advanced Materials* 29, 1605604, 2017.

Example 57: Measuring extravasation from blood vessels

[000626] This Example describes quantification of fusosome extravasation across an endothelial monolayer as tested with an in vitro microfluidic system (J.S Joen et al. 2013, journals.plos.org/plosone/article?id=10.1371/journal.pone.0056910).

[000627] Cells extravasate from the vasculature into surrounding tissue. Without wishing to be bound by theory, extravasation is one way for fusosomes to reach extravascular tissues.

[000628] The system includes three independently addressable media channels, separated by chambers into which an ECM-mimicking gel can be injected. In brief, the microfluidics system has molded PDMS (poly-dimethyl siloxane; Silgard 184; Dow Chemical, MI) through which access ports are bored and bonded to a cover glass to form microfluidic channels. Channel cross-sectional dimensions are 1 mm (width) by 120 μm (height). To enhance matrix adhesion, the PDMS channels are coated with a PDL (poly-D-lysine hydrobromide; 1 mg/mL; Sigma-Aldrich, St. Louis, MO) solution.

[000629] Next, collagen type I (BD Biosciences, San Jose, CA, USA) solution (2.0 mg/mL) with phosphate-buffered saline (PBS; Gibco) and NaOH is injected into the gel regions of the device via four separate filling ports and incubated for 30 min to form a hydrogel. When the gel is polymerized, endothelial cell medium (acquired from suppliers such as Lonza or Sigma) is immediately pipetted into the channels to prevent dehydration of the gel. Upon aspirating the medium, diluted hydrogel (BD science) solution (3.0 mg/mL) is introduced into the cell channel and the excess hydrogel solution is washed away using cold medium.

[000630] Endothelial cells are introduced into the middle channel and allowed to settle to form an endothelium. Two days after endothelial cell seeding, fusosomes or macrophage cells

(positive control) are introduced into the same channel where endothelial cells had formed a complete monolayer. The fusosomes are introduced so they adhere to and transmigrate across the monolayer into the gel region. Cultures are kept in a humidified incubator at 37°C and 5% CO_2. A GFP-expressing version of the fusosome is used to enable live-cell imaging via fluorescent microscopy. On the following day, cells are fixed and stained for nuclei using DAPI staining in the chamber, and multiple regions of interest are imaged using confocal microscope to determine how many fusosomes passed through the endothelial monolayer.

[000631] In some embodiments, DAPI staining will indicate that fusosomes and positive control cells are able to pass through the endothelial barrier after seeding.

Example 58: Measuring chemotactic cell mobility

[000632] This Example describes quantification of fusosome chemotaxis. Cells can move towards or away from a chemical gradient via chemotaxis. In some embodiments, chemotaxis will allow fusosomes to home to a site of injury, or track a pathogen. A purified fusosome composition as produced by any one of the methods described in previous Examples is assayed for its chemotactic abilities as follows.

[000633] A sufficient number of fusosomes or macrophage cells (positive control) are loaded in a micro-slide well according to the manufacturer's provided protocol in DMEM media (ibidi.com/img/cms/products/labware/channel_slides/S_8032X_Chemotaxis/IN_8032X_Chemotaxis.pdf). Fusosomes are left at 37°C and 5% CO_2 for 1h to attach. Following cell attachment, DMEM (negative control) or DMEM containing MCP1 chemoattractant is loaded into adjacent reservoirs of the central channel and the fusosomes are imaged continuously for 2 hours using a Zeiss inverted widefield microscope. Images are analyzed using ImageJ software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/, 1997–2007). Migration co-ordination data for each observed fusosome or cell is acquired with the manual tracking plugin (Fabrice Cordelières, Institut Curie, Orsay, France). Chemotaxis plots and migration velocities is determined with the Chemotaxis and Migration Tool (ibidi).

[000634] In some embodiments, an average accumulated distance and migration velocity of fusosomes will be within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or greater than the response of the positive control cells to chemokine. The response

of cells to a chemokine is described, e.g., in Howard E. Gendelman et al., *Journal of Neuroimmune Pharmacology*, 4(1): 47-59, 2009.

Example 59: Measuring homing potential

[000635] This Example describes homing of fusosomes to a site of injury. Cells can migrate from a distal site and/or accumulate at a specific site, e.g., home to a site. Typically, the site is a site of injury. In some embodiments, fusosomes will home to, e.g., migrate to or accumulate at, a site of injury.

[000636] Eight week old C57BL/6J mice (Jackson Laboratories) are dosed with notexin (NTX) (Accurate Chemical & Scientific Corp), a myotoxin, in sterile saline by intramuscular (IM) injection using a 30G needle into the right tibialis anterior (TA) muscle at a concentration of 2 μg/mL. The skin over the tibialis anterior (TA) muscle is prepared by depilating the area using a chemical hair remover for 45 seconds, followed by 3 rinses with water. This concentration is chosen to ensure maximum degeneration of the myofibers, as well as minimal damage to their satellite cells, the motor axons and the blood vessels.

[000637] On day 1 after NTX injection, mice receive an IV injection of fusosomes or cells that express firefly luciferase. Fusosomes are produced from cells that stably express firefly luciferase by any one of the methods described in previous Examples. A bioluminescent imaging system (Perkin Elmer) is used to obtain whole animal images of bioluminescence at 0, 1, 3, 7, 21, and 28 post injection.

[000638] Five minutes before imaging, mice receive an intraperitoneal injection of bioluminescent substrate (Perkin Elmer) at a dose of 150mg/kg in order to visualize luciferase. The imaging system is calibrated to compensate for all device settings. The bioluminescent signal is measured using Radiance Photons, with Total Flux used as a measured value. The region of interest (ROI) is generated by surrounding the signal of the ROI in order to give a value in photons/second. An ROI is assessed on both the TA muscle treated with NTX and on the contralateral TA muscle, and the ratio of photons/second between NTX-treated and NTX-untreated TA muscles is calculated as a measure of homing to the NTX-treated muscle.

[000639] In some embodiments, a ratio of photons/second between NTX-treated and NTX-

untreated TA muscles in fusosomes and cells will be greater than 1 indicating site specific accumulation of luciferase-expressing fusosomes at the injury.

[000640] See, for example, Plant et al., *Muscle Nerve* 34(5)L 577-85, 2006.

Example 60: Measuring phagocytic activity

[000641] This Example demonstrates phagocytic activity of fusosomes. In some embodiments, fusosomes have phagocytic activity, e.g., are capable of phagocytosis. Cells engage in phagocytosis, engulfing particles, enabling the sequestration and destruction of foreign invaders, like bacteria or dead cells.

[000642] A purified fusosome composition as produced by any one of the methods described in previous Examples comprising a fusosome from a mammalian macrophage having partial or complete nuclear inactivation was capable of phagocytosis assayed via pathogen bioparticles. This estimation was made by using a fluorescent phagocytosis assay according to the following protocol.

[000643] Macrophages (positive control) and fusosomes were plated immediately after harvest in separate confocal glass bottom dishes. The macrophages and fusosomes were incubated in DMEM+10%FBS+1%P/S for 1h to attach. Fluorescein-labeled *E. coli* K12 and non-fluorescein-labeled *Escherichia coli* K-12 (negative control) were added to the macrophages/fusosomes as indicated in the manufacturer's protocol, and were incubated for 2h, tools.thermofisher.com/content/sfs/manuals/mp06694.pdf. After 2h, free fluorescent particles were quenched by adding Trypan blue. Intracellular fluorescence emitted by engulfed particles was imaged by confocal microscopy at 488 excitation. The number of phagocytotic positive fusosome were quantified using image J software.

[000644] The average number of phagocytotic fusosomes was at least 30% 2h after bioparticle introduction, and was greater than 30% in the positive control macrophages.

Example 61: Measuring ability to cross a cell membrane or the blood brain barrier

[000645] This Example describes quantification of fusosomes crossing the blood brain barrier. In some embodiments, fusosomes will cross, e.g., enter and exit, the blood brain barrier, e.g., for delivery to the central nervous system.

[000646] Eight week old C57BL/6J mice (Jackson Laboratories) are intravenously injected with fusosomes or leukocytes (positive control) that express firefly luciferase. Fusosomes are produced from cells that stably express firefly luciferase or cells that do not express luciferase (negative control) by any one of the methods described in previous Examples. A bioluminescent imaging system (Perkin Elmer) is used to obtain whole-animal images of bioluminescence at one, two, three, four, five, six, eight, twelve, and twenty-four hours after fusosome or cell injection.

[000647] Five minutes before imaging, mice receive an intraperitoneal injection of bioluminescent substrate (Perkin Elmer) at a dose of 150mg/kg in order to visualize luciferase. The imaging system is calibrated to compensate for all device settings. The bioluminescent signal is measured, with total flux used as a measured value. The region of interest (ROI) is generated by surrounding the signal of the ROI in order to give a value in photons/second. The ROI selected is the head of the mouse around the area that includes the brain.

[000648] In some embodiments, the photons/second in the ROI will be greater in the animals injected with cells or fusosomes that express luciferase than the negative control fusosomes that do not express luciferase indicating accumulation of luciferase-expressing fusosomes in or around the brain.

Example 62: Measuring potential for protein secretion

[000649] This Example describes quantification of secretion by fusosomes. In some embodiments, fusosomes will be capable of secretion, e.g., protein secretion. Cells can dispose or discharge of material via secretion. In some embodiments, fusosomes will chemically interact and communicate in their environment via secretion.

[000650] The capacity of fusosomes to secrete a protein at a given rate is determined using the Gaussia luciferase flash assay from ThermoFisher Scientific (catalog #16158). Mouse embryonic fibroblast cells (positive control) or fusosomes as produced by any one of the methods described in previous Examples are incubated in growth media and samples of the media are collected every 15 minutes by first pelleting the fusosomes at 1600g for 5min and then collecting the supernatant. The collected samples are pipetted into a clear-bottom 96-well plate. A working solution of assay buffer is then prepared according to the manufacturer's instructions.

[000651] Briefly, colenterazine, a luciferin or light-emitting molecule, is mixed with flash assay buffer and the mixture is pipetted into each well of the 96 well plate containing samples. Negative control wells that lack cells or fusosomes include growth media or assay buffer to determine background Gaussia luciferase signal. In addition, a standard curve of purified Gaussia luciferase (Athena Enzyme Systems, catalog #0308) is prepared in order to convert the luminescence signal to molecules of Gaussia luciferase secretion per hour.

[000652] The plate is assayed for luminescence, using 500 msec integration. Background Gaussia luciferase signal is subtracted from all samples and then a linear best-fit curve is calculated for the Gaussia luciferase standard curve. If sample readings do not fit within the standard curve, they are diluted appropriately and re-assayed. Using this assay, the capacity for fusosomes to secrete Gaussia luciferase at a rate (molecules/hour) within a given range is determined.

[000653] In some embodiments, fusosomes will be capable of secreting proteins at a rate that is 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or greater than the positive control cells.

Example 63: Measuring signal transduction potential

[000654] This Example describes quantification of signal transduction in fusosomes. In some embodiments, fusosomes are capable of signal transduction. Cells can send and receive molecular signals from the extracellular environment through signaling cascades, such as phosphorylation, in a process known as signal transduction. A purified fusosome composition as produced by any one of the methods described in previous Examples comprising a fusosome from a mammalian cell having partial or complete nuclear inactivation is capable of signal transduction induced by insulin. Signal transduction induced by insulin is assessed by measuring AKT phosphorylation levels, a key pathway in the insulin receptor signaling cascade, and glucose uptake in response to insulin.

[000655] To measure AKT phosphorylation, cells, e.g., Mouse Embryonic Fibroblasts (MEFs) (positive control), and fusosomes are plated in 48-well plates and left for 2 hours in a humidified incubator at 37°C and 5% CO_2. Following cell adherence, insulin (e.g. at 10 nM), or a negative control solution without insulin, is add to the well containing cells or fusosomes for 30 min. After 30 minutes, protein lysate is made from the fusosomes or cells, and phospho-AKT

levels are measured by western blotting in insulin stimulated and control unstimulated samples.

[000656] Glucose uptake in response to insulin or negative control solution is measured as it is explained in the glucose uptake section by using labeled glucose (2-NBDG). (S. Galic et al., *Molecular Cell Biology* 25(2): 819-829, 2005).

[000657] In some embodiments, fusosomes will enhance AKT phosphorylation and glucose uptake in response to insulin over the negative controls by at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or greater.

Example 64: Measuring ability to transport glucose across cell membrane

[000658] This Example describes quantification of the levels of a 2-NBDG (2-(*N*-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)Amino)-2-Deoxyglucose) a fluorescent glucose analog that can be used to monitor glucose uptake in live cells, and thus measure active transport across the lipid bilayer. In some embodiments, this assay or an equivalent can be used to measure the level of glucose uptake and active transport across the lipid bilayer of the fusosome.

[000659] A fusosome composition is produced by any one of the methods described in previous Examples. A sufficient number of fusosomes are then incubated in DMEM with no glucose, 20% Fetal Bovine Serum and 1x Penicillin/Streptomycin for 2hr at 37°C and 5% CO_2. After a 2hr glucose starvation period, the medium is changed such that it includes DMEM with no glucose, 20% Fetal Bovine Serum, 1x Penicillin/Streptomycin and 20 μM 2-NBDG (ThermoFisher) and incubated for an additional 2hr at 37°C and 5% CO_2.

[000660] Negative control fusosomes are treated the same, except an equal amount of DMSO is added in place of 2-NBDG.

[000661] The fusosomes are then washed thrice with 1xPBS and re-suspended in an appropriate buffer, and transferred to a 96 well imaging plate. 2-NBDG fluorescence is then measured in a fluorimeter using a GFP light cube (469/35 excitation filter and a 525/39 emission filter) to quantify the amount of 2-NBDG that has been transported across the fusosome membrane and accumulated in the fusosome in the 1hr loading period.

[000662] In some embodiments, 2-NBDG fluorescence will be higher in the fusosome with 2-NBDG treatment as compared to the negative (DMSO) control. Fluorescence measure with a

525/39 emission filter will correlate with to the number of 2-NBDG molecules present.

Example 65: Lumen of fusosomes are miscible with aqueous solutions

[000663] This example assesses the miscibility of a fusosome lumen with aqueous solutions, such as water.

[000664] The fusosomes are prepared as described in previous Examples. The controls are dialysis membranes with either hypotonic solution, hyperosmotic solution or normal osmotic solutions.

[000665] Fusosomes, positive control (normal osmotic solution) and negative control (hypotonic solution) are incubated with hypotonic solution (150 mOsmol). The cell size is measured under a microscope after exposing each sample to the aqueous solution. In some embodiments, fusosome and positive control sizes in the hypotonic solution increase in comparison to a negative control.

[000666] Fusosomes, positive control (normal osmotic solution) and negative control (hyperosmotic solution) are incubated with a hyperosmotic solution (400 mOsmol). The cell size is measured under a microscope after exposing each sample to the aqueous solution. In some embodiments, fusosome and positive control sizes in a hyperosmotic solution will decrease in comparison to the negative control.

[000667] Fusosomes, positive control (hypotonic or hyperosmotic solution) and negative control (normal osmotic) are incubated with a normal osmotic solution (290 mOsmol). The cell size is measured under a microscope after exposing each sample to the aqueous solution. In some embodiments, fusosome and positive control sizes in a normal osmotic solution will remain substantially the same in comparison to the negative control.

Example 66: Measuring esterase activity in the cytosol

[000668] This Example describes quantification of esterase activity, as a surrogate for metabolic activity, in fusosomes. The cytosolic esterase activity in fusosomes is determined by quantitative assessment of calcein-AM staining (Bratosin et al., Cytometry 66(1): 78-84, 2005).

[000669] The membrane-permeable dye, calcein-AM (Molecular Probes, Eugene OR USA),

is prepared as a stock solution of 10 mM in dimethylsulfoxide and as a working solution of 100 mM in PBS buffer, pH 7.4. Fusosomes as produced by any one of the methods described in previous Examples or positive control parental Mouse Embryonic Fibroblast cells are suspended in PBS buffer and incubated for 30 minutes with calcein-AM working solution (final concentration in calcein-AM: 5 mM) at 37°C in the dark and then diluted in PBS buffer for immediate flow cytometric analysis of calcein fluorescence retention.

[000670] Fusosomes and control parental Mouse Embryonic Fibroblast cells are experimental permeabilized as a negative control for zero esterase activity with saponin as described in (Jacob et al., *Cytometry* 12(6): 550-558, 1991). Fusosomes and cells are incubated for 15 min in 1% saponin solution in PBS buffer, pH 7.4, containing 0.05% sodium azide. Due to the reversible nature of plasma membrane permeabilization, saponin is included in all buffers used for further staining and washing steps. After saponin permeabilization, fusosomes and cells are suspended in PBS buffer containing 0.1% saponin and 0.05% sodium azide and incubated (37°C in the dark for 45 min) with calcein-AM to a final concentration of 5 mM, washed three times with the same PBS buffer containing 0.1% saponin and 0.05% sodium azide, and analyzed by flow cytometry. Flow cytometric analyses are performed on a FACS cytometer (Becton Dickinson, San Jose, CA, USA) with 488nm argon laser excitation and emission is collected at 530+/-30nm. FACS software is used for acquisition and analysis. The light scatter channels are set on linear gains, and the fluorescence channels are set on a logarithmic scale, with a minimum of 10,000 cells analyzed in each condition. Relative esterase activities are calculated based on the intensity of calcein-AM in each sample. All events are captured in the forward and side scatter channels (alternatively, a gate can be applied to select only the fusosome population). The fluorescence intensity (FI) value for the fusosomes is determined by subtracting the FI value of the respective negative control saponin-treated sample. The normalized esterase activity for the fusosomes samples are normalized to the respective positive control cell samples in order to generate quantitative measurements for cytosolic esterase activities.

[000671] In some embodiments, a fusosome preparation will have within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or greater esterase activity compared to the positive control cell.

[000672] See also, Bratosin D, Mitrofan L, Palii C, Estaquier J, Montreuil J. Novel fluorescence assay using calcein-AM for the determination of human erythrocyte viability and aging. Cytometry A. 2005 Jul;66(1):78-84; and Jacob BC, Favre M, Bensa JC. Membrane cell permeabilisation with saponin and multiparametric analysis by flow cytometry. Cytometry 1991;12:550–558.

Example 67: Measuring acetylcholinesterase activity in fusosomes

[000673] Acetylcholinesterase activity is measured using a kit (MAK119, SIGMA) that follows a procedure described previously (Ellman, et al., Biochem. Pharmacol. 7, 88, 1961) and following the manufacturer's recommendations.

[000674] Briefly, fusosomes are suspended in 1.25 mM acetylthiocholine in PBS, pH 8, mixed with 0.1 mM 5,5-dithio-bis(2-nitrobenzoic acid) in PBS, pH 7. The incubation is performed at room temperature but the fusosomes and the substrate solution are pre-warmed at 37 °C for 10 min before starting the optical density readings.

[000675] Changes in absorption are monitored at 450 nm for 10 min with a plate reader spectrophotometer (ELX808, BIO-TEK instruments, Winooski, VT, USA). Separately, a sample is used for determining the protein content of the fusosomes via bicinchoninic acid assay for normalization. Using this assay, the fusosomes are determined to have <100 AChE activity units/μg of protein.

[000676] In some embodiments, AChE activity units/μg of protein values will be less than 0.001, 0.01, 0.1, 1, 10, 100, or 1000.

Example 68: Measuring metabolic activity level

[000677] This Example describes quantification of the measurement of citrate synthase activity in fusosomes.

[000678] Citrate synthase is an enzyme within the tricarboxylic acid (TCA) cycle that catalyzes the reaction between oxaloacetate (OAA) and acetyl-CoA to generate citrate. Upon hydrolysis of acetyl-CoA, there is a release of CoA with a thiol group (CoA-SH). The thiol group reacts with a chemical reagent, 5,5-Dithiobis-(2-nitrobenzoic acid) (DTNB), to form 5-thio-2-nitrobenzoic acid (TNB), which is a yellow product that can be measured spectrophotometrically

at 412 nm (Green 2008). Commercially-available kits, such as the Abcam Human Citrate Synthase Activity Assay Kit (Product #ab119692) provide all the necessary reagents to perform this measurement.

[000679] The assay is performed as per the manufacturer's recommendations. Fusosome sample lysates are prepared by collecting the fusosomes as produced by any one of the methods described in previous Examples and solubilizing them in Extraction Buffer (Abcam) for 20 minutes on ice. Supernatants are collected after centrifugation and protein content is assessed by bicinchoninic acid assay (BCA, ThermoFisher Scientific) and the preparation remains on ice until the following quantification protocol is initiated.

[000680] Briefly, fusosome lysate samples are diluted in 1X Incubation buffer (Abcam) in the provided microplate wells, with one set of wells receiving only 1X Incubation buffer. The plate is sealed and incubated for 4 hours at room temperature with shaking at 300rpm. The buffer is then aspirated from the wells and 1X Wash buffer is added. This washing step is repeated once more. Then, 1X Activity solution is added to each well, and the plate is analyzed on a microplate reader by measuring absorbance at 412nm every 20 seconds for 30 minutes, with shaking between readings.

[000681] Background values (wells with only 1X Incubation buffer) are subtracted from all wells, and the citrate synthase activity is expressed as the change in absorbance per minute per μg of fusosome lysate sample loaded (ΔmOD@412nm/min/μg protein). Only the linear portion from 100-400 seconds of the kinetic measurement is used to calculate the activity.

[000682] In some embodiments, a fusosome preparation will have within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or greater synthase activity compared to the control cell.

[000683] See, for example, Green HJ et al. Metabolic, enzymatic, and transporter response in human muscle during three consecutive days of exercise and recovery. Am J Physiol Regul Integr Comp Physiol 295: R1238-R1250, 2008.

Example 69: Measuring respiration levels

[000684] This Example describes quantification of the measurement of respiration level in

fusosomes. Respiration level in cells can be a measure of oxygen consumption, which powers metabolism. Fusosome respiration is measured for oxygen consumption rates by a Seahorse extracellular flux analyzer (Agilent) (Zhang 2012).

[000685] Fusosomes as produced by any one of the methods described in previous Examples or cells are seeded in a 96-well Seahorse microplate (Agilent). The microplate is centrifuged briefly to pellet the fusosomes and cells at the bottom of the wells. Oxygen consumption assays are initiated by removing growth medium, replacing with a low-buffered DMEM minimal medium containing 25mM glucose and 2mM glutamine (Agilent) and incubating the microplate at 37°C for 60 minutes to allow for temperature and pH equilibrium.

[000686] The microplate is then assayed in an extracellular flux analyzer (Agilent) that measures changes in extracellular oxygen and pH in the media immediately surrounding adherent fusosomes and cells. After obtaining steady state oxygen consumption (basal respiration rate) and extracellular acidification rates, oligomycin (5μM), which inhibits ATP synthase, and proton ionophore FCCP (carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone; 2μM), which uncouples mitochondria, are added to each well in the microplate to obtain values for maximal oxygen consumption rates.

[000687] Finally, 5 μM antimycin A (inhibitor of mitochondria complex III) is added to confirm that respiration changes are due mainly to mitochondrial respiration. The minimum rate of oxygen consumption after antimycin A addition is subtracted from all oxygen consumption measurements to remove the non-mitochondrial respiration component. Cell samples that do not appropriately respond to oligomycin (at least a 25% decrease in oxygen consumption rate from basal) or FCCP (at least a 50% increase in oxygen consumption rate after oligomycin) are excluded from the analysis. Fusosomes respiration level is then measured as pmol O2/min/1e4 fusosomes.

[000688] This respiration level is then normalized to the respective cell respiration level. In some embodiments, fusosomes will have at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or greater respiration level compared to the respective cell samples.

[000689] See, for example, Zhang J, Nuebel E, Wisidagama DRR, et al. Measuring energy metabolism in cultured cells, including human pluripotent stem cells and differentiated

cells. Nature protocols. 2012;7(6):10.1038/nprot.2012.048. doi:10.1038/nprot.2012.048.

Example 70: Measuring phosphatidylserine levels of fusosomes

[000690] This Example describes quantification of the level of annexin-V binding to the surface of fusosomes.

[000691] Dying cells can display phosphatidylserine on the cell surface which is a marker of apoptosis in the programmed cell death pathway. Annexin-V binds to phosphatidylserine, and thus, annexin-V binding is a proxy for viability in cells.

[000692] Fusosomes were produced as described herein. For detection of apoptosis signals, fusosomes or positive control cells were stained with 5% annexin V fluor 594 (A13203, Thermo Fisher, Waltham, MA). Each group (detailed in the table below) included an experimental arm that was treated with an apoptosis-inducer, menadione. Menadione was added at 100 μM menadione for 4 h. All samples were run on a flow cytometer (Thermo Fisher, Waltham, MA) and fluorescence intensity was measured with the YL1 laser at a wavelength of 561 nm and an emission filter of 585/16 nm. The presence of extracellular phophatidyl serine was quantified by comparing fluorescence intensity of annexin V in all groups.

[000693] The negative control unstained fusosomes were not positive for annexin V staining.

[000694] In some embodiments, fusosomes were capable of upregulating phosphatidylserine display on the cell surface in response to menadione, indicating that non-menadione stimulated fusosomes are not undergoing apoptosis. In some embodiments, positive control cells that were stimulated with menadione demonstrated higher-levels of annexin V staining than fusosomes not stimulated with menadione.

Table 22: Annexin V staining parameter

Experimental Arm	Mean Fluorescence Intensity of Annexin V Signal (and standard deviation)
Unstained Fusosomes (negative control)	941 (937)
Stained Fusosomes	11257 (15826)
Stained Fusosomes + Menadione	18733 (17146)
Stained Macrophages + Menadione (positive	14301 (18142)

control)	

Example 71: Measuring juxtacrine-signaling levels

[000695] This Example describes quantification of juxtacrine-signaling in fusosomes.

[000696] Cells can form cell-contact dependent signaling via juxtacrine signaling. In some embodiments, presence of juxtacrine signaling in fusosomes will demonstrate that fusosomes can stimulate, repress, and generally communicate with cells in their immediate vicinity.

[000697] Fusosomes produced by any one of the methods described in previous Examples from mammalian bone marrow stromal cells (BMSCs) having partial or complete nuclear inactivation trigger IL-6 secretion via juxtacrine signaling in macrophages. Primary macrophages and BMSCs are co-cultured. Bone marrow-derived macrophages are seeded first into 6-well plates, and incubated for 24h, then primary mouse BMSC-derived fusosomes or BMSC cells (positive control parental cells) are placed on the macrophages in a DMEM medium with 10% FBS. The supernatant is collected at different time points (2, 4, 6, 24 hours) and analyzed for IL-6 secretion by ELISA assay. (Chang J. et al., 2015).

[000698] In some embodiments, a level of juxtacrine signaling induced by BMSC fusosomes is measured by an increase in macrophage-secreted IL-6 levels in the media. In some embodiments, a level of juxtacrine signaling will be at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or greater than the levels induced by the positive control bone marrow stromal cells (BMSCs).

Example 72: Measuring paracrine-signaling levels

[000699] This Example describes quantification of paracrine signaling in fusosomes.

[000700] Cells can communicate with other cells in the local microenvironment via paracrine signaling. In some embodiments, fusosomes will be capable of paracrine signaling, e.g., to communicate with cells in their local environment. In asome n embodiments, ability of fusosomes to trigger Ca^{2+} signaling in endothelial cells via paracrine-derived secretion with the following protocol will measure Ca^{2+} signaling via the calcium indicator, fluo-4 AM.

[000701] To prepare the experimental plate, murine pulmonary microvascular endothelial

cells (MPMVECs) are plated on a 0.2% gelatin coated 25mm glass bottom confocal dish (80% confluence). MPMVECs are incubated at room temperature for 30 min in ECM containing 2% BSA and 0.003% pluronic acid with 5 μM fluo-4 AM (Invitrogen) final concentration to allow loading of fluo-4 AM. After loading, MPMVECs are washed with experimental imaging solution (ECM containing 0.25% BSA) containing sulfinpyrazone to minimize dye loss. After loading fluo-4, 500μl of pre-warmed experimental imaging solution is added to the plate, and the plate is imaged by a Zeiss confocal imaging system.

[000702] In a separate tube, freshly isolated murine macrophages are either treated with 1μg/mL LPS in culture media (DMEM+10% FBS) or not treated with LPS (negative control). After stimulation, fusosomes are generated from macrophages by any one of the methods described in previous Examples.

[000703] Fusosomes or parental macrophages (positive control) are then labeled with cell tracker red, CMTPX (Invitrogen), in ECM containing 2%BSA and 0.003% pluronic acid. Fusosomes and macrophages are then washed and resuspended in experimental imaging solution. Labeled fusosomes and macrophages are added onto the fluo-4 AM loaded MPMVECs in the confocal plate.

[000704] Green and red fluorescence signal is recorded every 3s for 10-20 min using Zeiss confocal imaging system with argon ion laser source with excitation at 488 and 561 nm for fluo-4 AM and cell tracker red fluorescence respectively. Fluo-4 fluorescence intensity changes are analyzed using imaging software (Mallilankaraman, K. et al., *J Vis Exp.* (58): 3511, 2011). The level of Fluo-4 intensity measured in negative control fusosome and cell groups is subtracted from LPS-stimulated fusosome and cell groups.

[000705] In some embodiments, fusosomes, e.g., activated fusosomes, will induce an increase in Fluo-4 fluorescence intensity that is at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or greater than the positive control cell groups.

Example 73: Measuring ability to polymerize actin for mobility

[000706] This Example describes quantification of cytoskeletal components, such as actin, in fusosomes. In some embodiments, fusosomes comprise cytoskeletal components such as actin,

and are capable of actin polymerization.

[000707] Cells use actin, which is a cytoskeletal component, for motility and other cytoplasmic processes. The cytoskeleton is essential to creating motility driven forces and coordinating the process of movement

[000708] C2C12 cells were enucleated as described herein. Fusosomes obtained from the 12.5% and 15% Ficoll layers were pooled and labeled 'Light', while fusosomes from the 16 – 17% layers were pooled and labeled 'Medium'. Fusosomes or cells (parental C2C12 cells, positive control) were resuspended in DMEM + Glutamax + 10% Fetal Bovine Serum (FBS), plated in 24-well ultra-low attachment plates (#3473, Corning Inc, Corning, NY) and incubated at 37 °C + 5% CO_2. Samples were taken periodically (5.25 hr, 8.75 hr, 26.5 hr) and stained with 165 μM rhodamine phalloidin (negative control was not stained) and measured on a flow cytometer (#A24858, Thermo Fisher, Waltham, MA) with a FC laser YL1 (561 nm with 585/16 filter) to measure F-actin cytoskeleton content. The fluorescence intensity of rhodamine phalloidin in fusosomes was measured along with unstained fusosomes and stained parental C2C12 cells.

[000709] Fusosome fluorescence intensity was greater (Figure 4) than the negative control at all timepoints, and fusosomes were capable of polymerizing actin at a similar rate to the parental C2C12 cells.

[000710] Additional cytoskeletal components, such as those listed in the table below, are measured via a commercially available ELISA systems (Cell Signaling Technology and MyBioSource), according to manufacturer's instructions.

Table 23: Cytoskeletal components

Cytoskeletal protein measured	Commercial Kit Type	Kit ID
Actin	Path Scan Total B-Actin Sandwich ELISA Kit	Cell Signaling, 7880
Arp2/3	Human Actin Related protein 2/3 complex subunit(APRC2)	MyBioSource, MBS7224740

	ELISA KIT	
Formin	**Formin Binding Protein 1 (FNBP1), ELISA Kit**	MyBioSource, MBS9308864
Coronin	Human Coronin 1A ELISA Kit	MyBioSource, MBS073640
Dystrophin	Human dystrophin ELISA Kit	MyBioSource MBS722223
Keratin	Human Keratin 5 ELISA Kit	MyBioSource, MBS081200
Myosin	Human Myosin IG (MYO1G) ELISA Kit	MyBioSource, MBS9312965
Tubulin	Human Tubulin Beta 3 ELISA Kit	MyBioSource, MBS097321

[000711] Then 100 µL of appropriately-diluted lysate is added to the appropriate well from the microwell strips. The microwells are sealed with tape and incubated for 2 hrs at 37°C. After incubation, the sealing tape is removed and the contents are discarded. Each microwell is washed four times with 200 µL of 1X Wash Buffer. After each individual wash, plates are struck onto an absorbent cloth so that the residual wash solution is removed from each well. However, wells are not completely dry at any time during the experiment.

[000712] Next, 100 µL of the reconstituted Detection Antibody (green) is added each individual well, except for negative control wells. Then wells are sealed and incubated for 1 hour at 37°C. The washing procedure is repeated after incubation is complete. 100 µL of reconstituted HRP-Linked secondary antibody (red) is added to each of the wells. The wells are sealed with tape and incubated for 30 minutes at 37°C. The sealing tape is then removed and the washing procedure is repeated. 100 µL of TMB Substrate is then added to each well. The wells are sealed with tape, then incubated for 10 minutes at 37°C. Once this final incubation is complete, 100 µL of STOP solution is added to each of the wells and the plate is shaken gently for several seconds.

[000713] Spectrophotometric analysis of the assay is conducted within 30 minutes of adding the STOP solution. The underside of the wells is wiped with lint-free tissue and then absorbance is read at 450nm. In some embodiments, fusosome samples that have been stained with the

detection antibody will absorb more light at 450 nm that negative control fusosome samples, and absorb less light than cell samples that have been stained with the detection antibody.

Example 74: Measuring average membrane potential

[000714] This Example describes quantification of the mitochondrial membrane potential of fusosomes. In some embodiments, fusosomes comprising a mitochondrial membrane will maintain mitochondrial membrane potential.

[000715] Mitochondrial metabolic activity can be measured by mitochondrial membrane potential. The membrane potential of the fusosome preparation is quantified using a commercially available dye, TMRE, for assessing mitochondrial membrane potential (TMRE: tetramethyl rhodamine, ethyl ester, perchlorate, Abcam, Cat# T669).

[000716] Fusosomes are generated by any one of the methods described in previous Examples. Fusosomes or parental cells are diluted in growth medium (phenol-red free DMEM with 10% fetal bovine serum) in 6 aliquots (untreated and FCCP-treated triplicates). One aliquot of the samples is incubated with FCCP, an uncoupler that eliminates mitochondrial membrane potential and prevents TMRE staining. For FCCP-treated samples, 2μM FCCP is added to the samples and incubated for 5 minutes prior to analysis. Fusosomes and parental cells are then stained with 30nM TMRE. For each sample, an unstained (no TMRE) sample is also prepared in parallel. Samples are incubated at 37°C for 30 minutes. The samples are then analyzed on a flow cytometer with 488nm argon laser, and excitation and emission is collected at 530+/-30nm.

[000717] Membrane potential values (in millivolts, mV) are calculated based on the intensity of TMRE. All events are captured in the forward and side scatter channels (alternatively, a gate can be applied to exclude small debris). The fluorescence intensity (FI) value for both the untreated and FCCP-treated samples are normalized by subtracting the geometric mean of the fluorescence intensity of the unstained sample from the geometric mean of the untreated and FCCP-treated sample. The membrane potential state for each preparation is calculated using the normalized fluorescent intensity values with a modified Nernst equation (see below) that can be used to determine mitochondrial membrane potential of the fusosomes or cells based on TMRE fluorescence (as TMRE accumulates in mitochondria in a Nernstian fashion).

[000718] Fusosome or cell membrane potential is calculated with the following formula: (mV) = -61.5 * log(FIuntreated-normalized/FIFCCP-treated-normalized). In some embodiments, using this assay or an equivalent on fusosome preparations from C2C12 mouse myoblast cells, the membrane potential state of the fusosome preparation will be within about 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or greater than the parental cells. In some embodiments, the range of membrane potential is about -20 to -150mV.

Example 75: Measuring persistence half-life in a subject

[000719] This Example describes the measurement of fusosome half-life.

[000720] Fusosomes are derived from cells that express gaussia-luciferase produced by any one of the methods described in previous Examples, and pure, 1:2, 1:5, and 1:10 dilutions in buffered solution are made. A buffered solution lacking fusosomes is used as a negative control.

[000721] Each dose is administered to three eight week old male C57BL/6J mice (Jackson Laboratories) intravenously. Blood is collected from the retro-orbital vein at 1, 2, 3, 4, 5, 6, 12, 24, 48, and 72 hours after intravenous administration of the fusosomes. The animals are sacrificed at the end of the experiment by CO_2 inhalation.

[000722] Blood is centrifuged for 20 min at room temperature. The serum samples are immediately frozen at −80°C until bioanalysis. Then, each blood sample is used to carry out a Gaussia-luciferase activity assay after mixing the samples with Gaussia-luciferase substrate (Nanolight, Pinetop, AZ). Briefly, colenterazine, a luciferin or light-emitting molecule, is mixed with flash assay buffer and the mixture is pipetted into wells containing blood samples in a 96 well plate. Negative control wells that lack blood contain assay buffer to determine background Gaussia luciferase signal.

[000723] In addition, a standard curve of positive-control purified Gaussia luciferase (Athena Enzyme Systems, catalog #0308) is prepared in order to convert the luminescence signal to molecules of Gaussia luciferase secretion per hour. The plate is assayed for luminescence, using 500 msec integration. Background Gaussia luciferase signal is subtracted from all samples and then a linear best-fit curve is calculated for the Gaussia luciferase standard curve. If sample readings do not fit within the standard curve, they are diluted appropriately and re-assayed. The

luciferase signal from samples taken at 1, 2, 3, 4, 5, 6, 12, 24, 48, and 72 hours is interpolated to the standard curve. The elimination rate constant k_e (h^{-1}) is calculated using the following equation of a one-compartment model: $C(t) = C_0 \times e^{-kext}$, in which $C(t)$ (ng/mL) is the concentration of fusosomes at time t (h) and C_0 the concentration of fusosomes at time = 0 (ng/mL). The elimination half-life $t_{1/2,e}$ (h) is calculated as $\ln(2)/k_e$.

[000724] In some embodiments, fusosomes will have a half-life of at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or greater than the negative control cells.

Example 76: Measuring retention of fusosomes in circulation

[000725] This example describes quantification of fusosome delivery into the circulation and retention at organs. In some embodiments, fusosomes are delivered into the circulation, and are not captured and retained in organ sites.

[000726] In some embodiments, fusosomes delivered into the peripheral circulation evade capture and retention by the reticulo-endothelial system (RES) in order to reach target sites with high efficiency. The RES comprises a system of cells, primarily macrophages, which reside in solid organs such as the spleen, lymph nodes and the liver. These cells are usually tasked with the removal of "old" cells, such as red blood cells.

[000727] Fusosomes are derived from cells expressing CRE recombinase (agent), or cells not expressing CRE (negative control). These fusosomes are prepared for in vivo injection as in Example 62.

[000728] The recipient mice harbor a loxp-luciferase genomic DNA locus that is modified by CRE protein made from mRNA delivered by the fusosomes to unblock the expression of luciferase (JAX#005125). Luciferase can be detected by bioluminescent imaging in a living animal. The positive control for this example are offspring of recipient mice mated to a mouse strain that expresses the same protein exclusively in macrophage and monocyte cells from its own genome (Cx3cr1-CRE JAX#025524). Offspring from this mating harbor one of each allele (loxp-luciferase, Cx3cr1-CRE).

[000729] Fusosomes are injected into the peripheral circulation via tail vein injection (IV,

Example #48) into mice that harbor a genetic locus that when acted on by the CRE protein results in the expression of luciferase. The non-specific capture mechanism of the RES is phagocytic in nature releasing a proportion of the CRE protein from the fusosome into the macrophage resulting in genomic recombination. IVIS measurements (as described in Example 62) identify where non-fusogen controls accumulate and fuse. Accumulation in the spleen, lymph nodes and liver will be indicative of non-specific RES-mediated capture of the fusosome. IVIS is carried out at 24, 48 and 96 hours post-fusosome injection.

[000730] Mice are euthanized and spleen, liver and major lymphatic chain in the gut are harvested.

[000731] Genomic DNA is isolated from these organs and subjected to quantitative polymerase chain reaction against the recombined genomic DNA remnant. An alternative genomic locus (not targeted by CRE) is also quantified to provide a measure of the number of cells in the sample.

[000732] In embodiments, low bioluminescent signals will be observed for both the agent and negative control throughout the animal and specifically at the liver and splenic sites. In embodiments, the positive control will show increased signal in the liver (over negative control and agent) and high signals in the spleen and a distribution consistent with lymph nodes.

[000733] In some embodiments, genomic PCR quantification of these tissues will indicate a high proportion of the recombination signals over the alternative locus in the positive control in all tissues examined, while for or agent and negative controls, the level of recombination will be negligible in all tissues.

[000734] In some embodiments, the result of this Example will indicate that the non-fusogen controls are not retained by the RES and will be able to achieve broad distribution and exhibit high bioavailability.

Example 77: Fusosome longevity with immunosuppression

[000735] This Example describes quantification of the immunogenicity of a fusosome composition when it is co-administered with an immunosuppressive drug.

[000736] Therapies that stimulate an immune response can sometimes reduce the therapeutic

efficacy or cause toxicity to the recipient. In some embodiments, fusosomes will be substantially non-immunogenic.

[000737] A purified composition of fusosomes as produced by any one of the methods described in previous Examples is co-administered with an immunosuppressive drug, and immunogenic properties are assayed by the longevity of the fusosome in vivo. A sufficient number of fusosomes, labeled with luciferase, are injected locally into the gastrocnemius muscle of a normal mouse with tacrolimus (TAC, 4mg/kg/day; Sigma Aldrich), or vehicle (negative control), or without any additional agent (positive control). The mice are then subjected to in vivo imaging at 1, 2, 3, 4, 5, 6, 12, 24, 48, and 72 hours post injection.

[000738] Briefly, mice are anesthetized with isoflurane and D-luciferin is administered intraperitoneally at a dose of 375 mg per kilogram of body weight. At the time of imaging, animals are placed in a light-tight chamber, and photons emitted from luciferase expressing fusosomes transplanted into the animals are collected with integration times of 5 sec to 5 min, depending on the intensity of the bioluminescence emission. The same mouse is scanned repetitively at the various timepoints set forth above. BLI signal is quantified in units of photons per second (total flux) and presented as log [photons per second]. The data is analyzed by comparing the intensity and fusosome injection with and without TAC.

[000739] In embodiments, the assay will show an increase in fusosome longevity in the TAC co-administered group relative to the fusosome alone and vehicle groups at the final timepoint. In addition to the increase in fusosome longevity, in some embodiments, an increase in BLI signal from the fusosome plus TAC arm versus the fusosome plus vehicle or fusosomes alone at each of the time points will be observed.

Example 78: Measuring pre-existing IgG and IgM antibodies reactive against fusosomes

[000740] This Example describes quantification of pre-existing anti-fusosome antibody titers measured using flow cytometry.

[000741] A measure of immunogenicity for fusosomes is antibody responses. Antibodies that recognize fusosomes can bind in manner that can limit fusosome activity or longevity. In some

embodiments, some recipients of a fusosome described herein will have pre-existing antibodies which bind to and recognize fusosomes.

[000742] In this Example, anti-fusosome antibody titers are tested using fusosomes produced using a xenogeneic source cell by any one of the methods described in a previous Example. In this Example, a fusosome naïve mouse is assessed for the presence of anti-fusosome antibodies. Notably, the methods described herein may be equally applicable to humans, rats, monkeys with optimization to the protocol.

[000743] The negative control is mouse serum which has been depleted of IgM and IgG, and the positive control is serum derived from a mouse that has received multiple injections of fusosomes generated from a xenogeneic source cell.

[000744] To assess the presence of pre-existing antibodies which bind to fusosomes, sera from fusosome-naïve mice is first decomplemented by heating to 56°C for 30 min and subsequently diluted by 33% in PBS containing 3% FCS and 0.1% NaN3. Equal amounts of sera and fusosomes ($1x10^2$ - $1x10^8$ fusosomes per mL) suspensions are incubated for 30 min at 4°C and washed with PBS through a calf-serum cushion.

[000745] IgM xenoreactive antibodies are stained by incubation of the cells with PE-conjugated goat antibodies specific for the Fc portion of mouse IgM (BD Bioscience) at 4°C for 45 min. Notably, anti-mouse IgG1 or IgG2 secondary antibodies may also be used. Cells from all groups are washed twice with PBS containing 2% FCS and then analyzed on a FACS system (BD Biosciences). Fluorescence data are collected by use of logarithmic amplification and expressed as mean fluorescent intensity. In some embodiments, the negative control serum will show negligible fluorescence comparable to the no serum or secondary alone controls. In an embodiment, the positive control will show more fluorescence than the negative control, and more than the no serum or secondary alone controls. In an embodiment, in cases where immunogenicity occurs, serum from fusosome-naïve mice will show more fluorescence than the negative control. In an embodiment, in cases where immunogenicity does not occur, serum from fusosome-naïve mice will show similar fluorescence compared to the negative control.

Example 79: Measuring IgG and IgM antibody responses after multiple administrations of fusosomes

[000746] This Example describes quantification of the humoral response of a modified fusosome following multiple administrations of the modified fusosome. In some embodiments, a modified fusosome, e.g., modified by a method described herein, will have a reduced (e.g., reduced compared to administration of an unmodified fusosome) humoral response following multiple (e.g., more than one, e.g., 2 or more), administrations of the modified fusosome.

[000747] A measure of immunogenicity for fusosomes is the antibody responses. In some embodiments, repeated injections of a fusosome can lead to the development of anti-fusosome antibodies, e.g., antibodies that recognize fusosomes. In some embodiments, antibodies that recognize fusosomes can bind in a manner that can limit fusosome activity or longevity.

[000748] In this Example, anti-fusosome antibody titers are examined after one or more administrations of fusosomes. Fusosomes are produced by any one of the previous Examples. Fusosomes are generated from: unmodified mesenchymal stem cells (hereafter MSCs), mesenchymal stem cells modified with a lentiviral-mediated expression of HLA-G (hereafter MSC-HLA-G), and mesenchymal stem cells modified with a lentiviral-mediated expression of an empty vector (hereafter MSC-empty vector). Serum is drawn from the different cohorts: mice injected systemically and/or locally with 1, 2, 3, 5, 10 injections of vehicle (Fusosome naïve group), MSC fusosomes, MSC-HLA-G fusosomes, or MSC-empty vectors fusosomes.

[000749] To assess the presence and abundance of anti-fusosomes antibodies, sera from the mice is first decomplemented by heating to 56°C for 30 min and subsequently diluted by 33% in PBS with 3% FCS and 0.1% NaN3. Equal amounts of sera and fusosomes ($1x10^2$ - $1x10^8$ fusosomes per mL) are incubated for 30 min at 4°C and washed with PBS through a calf-serum cushion.

[000750] Fusosome reactive IgM antibodies are stained by incubation of the cells with PE-conjugated goat antibodies specific for the Fc portion of mouse IgM (BD Bioscience) at 4°C for 45 min. Notably, anti-mouse IgG1 or IgG2 secondary antibodies may also be used. Cells from all groups are washed twice with PBS containing 2% FCS and then analyzed on a FACS system (BD Biosciences). Fluorescence data are collected by use of logarithmic amplification and expressed as mean fluorescent intensity.

[000751] In some embodiments, MSC-HLA-G fusosomes will have decreased anti-fusosome

IgM (or IgG1/2) antibody titers (as measured by fluorescence intensity on FACS) after injections, as compared to MSC fusosomes or MSC-empty vector fusosomes.

Example 80: Modification of fusosome source cells to express tolerogenic protein to reduce immunogenicity

[000752] This Example describes quantification of immunogenicity in fusosomes derived from a modified cell source. In some embodiments, fusosomes derived from a modified cell source have reduced immunogenicity in comparison to the fusosomes derived from an unmodified cell source.

[000753] Therapies that stimulate an immune response can sometimes reduce the therapeutic efficacy or cause toxicity to the recipient. In some embodiments, substantially non-immunogenic fusosomes are administered to a subject. In some embodiments, immunogenicity of the cell source can be assayed as a proxy for fusosome immunogenicity.

[000754] iPS cells modified using lentiviral mediated expression of HLA-G or expressing an empty vector (Negative control) are assayed for immunogenic properties as follows. A sufficient number of iPS cells, as a potential fusosome cell source, are injected into C57/B6 mice, subcutaneously in the hind flank and are given an appropriate amount of time to allow for teratomas to form.

[000755] Once teratomas are formed, tissues are harvested. Tissues prepared for fluorescent staining are frozen in OCT, and those prepared for immunohistochemistry and H&E staining are fixed in 10% buffered formalin and embedded in paraffin. The tissue sections are stained with antibodies, polyclonal rabbit anti-human CD3 anti-body (DAKO), mouse anti–human CD4 mAb (RPA-T4, BD PharMingen), mouse anti-human CD8 mAb (RPA-T8, BD PharMingen), in accordance with general immunohistochemistry protocols. These are detected by using an appropriate detection reagent, namely an anti-mouse secondary HRP (Thermofisher), or anti-rabbit secondary HRP (Thermofisher).

[000756] Detection is achieved using peroxidase-based visualization systems (Agilent). The data is analyzed by taking the average number of infiltrating CD4+ T-cells, CD8+ T-cells, CD3+ NK-cells present in 25, 50 or 100 tissue sections examined in a 20x field using a light microscope.

In embodiments, iPSCs which are not modified or iPSCs expressing an empty vector will have a higher number of infiltrating CD4+ T-cells, CD8+ T-cells, CD3+ NK-cells present in the fields examined as compared to iPSCs that express HLA-G.

[000757] In some embodiments, a fusosome's immunogenic properties will be substantially equivalent to that of the source cell. In some embodiments, fusosomes derived from iPS cells modified with HLA-G will have reduced immune cell infiltration versus their unmodified counterparts.

Example 81: Modification of fusosome source cells to knockdown immunogenic protein to reduce immunogenicity

[000758] This Example describes quantification of the generation of a fusosome composition derived from a cell source, which has been modified to reduce expression of a molecule which is immunogenic. In some embodiments, a fusosome can be derived from a cell source, which has been modified to reduce expression of a molecule which is immunogenic.

[000759] Therapies that stimulate an immune response can reduce the therapeutic efficacy or cause toxicity to the recipient. Thus, immunogenicity is an important property for a safe and effective therapeutic fusosomes. Expression of certain immune activating agents can create an immune response. MHC class I represents one example of an immune activating agent.

[000760] In this Example, fusosomes are generated by any one of the methods described in previous Examples. Fusosomes are generated from: unmodified mesenchymal stem cells (hereafter MSCs, positive control), mesenchymal stem cells modified with a lentiviral-mediated expression of an shRNA targeting MHC class I (hereafter MSC-shMHC class I), and mesenchymal stem cells modified with a lentiviral-mediated expression of a non-targeted scrambled shRNA (hereafter MSC-scrambled, negative control).

[000761] Fusosomes are assayed for expression of MHC class I using flow cytometry. An appropriate number of fusosomes are washed and resuspended in PBS, held on ice for 30 minutes with 1: 10-1: 4000 dilution of fluorescently conjugated monoclonal antibodies against MHC class I (Harlan Sera-Lab, Belton, UK). Fusosomes are washed three times in PBS and resuspended in PBS. Nonspecific fluorescence is determined, using equal aliquots of fusosomes preparation

incubated with and appropriate fluorescently conjugated isotype control antibody at equivalent dilutions. Fusosomes are assayed in a flow cytometer (FACSort, Becton-Dickinson) and the data is analyzed with flow analysis software (Becton-Dickinson).

[000762] The mean fluorescence data of the fusosomes derived from MSCs, MSCs-shMHC class I, MSC-scrambled, is compared. In some embodiments, fusosomes derived from MSCs-shMHC class I will have lower expression of MHC class I compared to MSCs and MSC-scrambled.

Example 82: Modification of fusosome source cells to evade macrophage phagocytosis

[000763] This Example describes quantification of the evasion of phagocytosis by modified fusosomes. In some embodiments, modified fusosomes will evade phagocytosis by macrophages.

[000764] Cells engage in phagocytosis, engulfing particles, enabling the sequestration and destruction of foreign invaders, like bacteria or dead cells. In some embodiments, phagocytosis of fusosomes by macrophages would reduce their activity.

[000765] Fusosomes are generated by any one of the methods described in previous Examples. Fusosomes are created from: CSFE-labelled mammalian cells which lack CD47 (hereafter NMC, positive control), CSFE-labelled cells that are engineered to express CD47 using lentiviral mediated expression of a CD47 cDNA (hereafter NMC-CD47), and CSFE-labelled cells engineered using lentiviral mediated expression of an empty vector control (hereafter NMC-empty vector, negative control).

[000766] Reduction of macrophage mediate immune clearance is determined with a phagocytosis assay according to the following protocol. Macrophages are plated immediately after harvest in confocal glass bottom dishes. Macrophages are incubated in DMEM+10%FBS+1%P/S for 1h to attach. An appropriate number of fusosomes derived from NMC, NMC-CD47, NMC-empty vector are added to the macrophages as indicated in the protocol, and are incubated for 2h, tools.thermofisher.com/content/sfs/manuals/mp06694.pdf.

[000767] After 2h, the dish is gently washed and intracellular fluorescence is examined. Intracellular fluorescence emitted by engulfed particles is imaged by confocal microscopy at 488 excitation. The number of phagocytotic positive macrophage is quantified using imaging software.

The data is expressed as the phagocytic index = (total number of engulfed cells/total number of counted macrophages) × (number of macrophages containing engulfed cells/total number of counted macrophages) × 100.

[000768] In some embodiments, a phagocytic index will be reduced when macrophages are incubated with fusosomes derived from NMC-CD47, versus those derived from NMC, or NMC-empty vector.

Example 83: Modification of fusosome source cells for decreased cytotoxicity mediated by PBMC cell lysis

[000769] This Example described the generation of fusosomes derived from cells modified to have decreased cytotoxicity due to cell lysis by PBMCs.

[000770] In some embodiments, cytotoxicity mediated cell lysis of source cells or fusosomes by PBMCs is a measure of immunogenicity for fusosomes, as lysis will reduce, e.g., inhibit or stop, the activity of a fusosome.

[000771] In this Example, fusosomes are generated by any one of the methods described in a previous Example. Fusosomes are created from: unmodified mesenchymal stem cells (hereafter MSCs, positive control), mesenchymal stem cells modified with a lentiviral-mediated expression of HLA-G (hereafter MSC-HLA-G), and mesenchymal stem cells modified with a lentiviral-mediated expression of an empty vector (hereafter MSC-empty vector, negative control).

[000772] PMBC mediated lysis of a fusosome is determined by europium release assays as described in Bouma, et al. Hum. Immunol. 35(2):85-92; 1992 & van Besouw et al. Transplantation 70(1):136-143; 2000. PBMCs (hereafter effector cells) are isolated from an appropriate donor, and stimulated with allogeneic gamma irradiated PMBCs and 200IU/mL IL-2 (proleukin, Chiron BV Amsterdam, The Netherlands) in a round bottom 96 well plate for 7 days at 37°C. The fusosomes are labeled with europium-diethylenetriaminepentaacetate (DTPA) (sigma, St. Louis, MO, USA).

[000773] At day 7 cytotoxicity-mediated lysis assays is performed by incubating ^{63}Eu-labelled fusosomes with effector cells in a 96-well plate for 1, 2, 3, 4, 5, 6, 8, 10, 15, 20, 24, 48 hours after plating at effector/target ratios ranging from 1000:1-1:1 and 1:1.25-1:1000. After incubation, the plates are centrifuged and a sample of the supernatant is transferred to 96-well

plates with low background fluorescence (fluoroimmunoplates, Nunc, Roskilde, Denmark).

[000774] Subsequently, enhancement solution (PerkinElmer, Groningen, The Netherlands) is added to each well. The released europium is measured in a time-resolved fluorometer (Victor 1420 multilabel counter, LKB-Wallac, Finland). Fluorescence is expressed in counts per second (CPS). Maximum percent release of europium by a target fusosome is determined by incubating an appropriate number (1x 10^2 -1x 10^8) of fusosomes with 1% triton (sigma-aldrich) for an appropriate amount of time. Spontaneous release of europium by target fusosomes is measured by incubation of labeled target fusosomes without effector cells. Percentage leakage is then calculated as: (spontaneous release/maximum release) x100%. Finally, the percentage of cytotoxicity mediated lysis is calculated as %lysis= [(measured lysis-spontaneous lysis- spontaneous release)/(maximum release-spontaneous release)]x100%. The data is analyzed by looking at the percentage of lysis as a function of different effector target ratios.

[000775] In some embodiments, fusosomes generated from MSC-HLA-G cells will have a decreased percentage of lysis by target cells, at specific timepoints as compared to MSCs or MSC-scrambled generated fusosomes.

Example 84: Modification of fusosome source cells for decreased NK lysis activity

[000776] This Example describes the generation of a fusosome composition derived from a cell source, which has been modified to decrease cytotoxicity mediated cell lysis by NK cells. In some embodiments cytotoxicity mediated cell lysis of source cells or fusosomes by NK cells is a measure of immunogenicity for fusosomes.

[000777] In this Example, fusosomes are generated by any one of the methods described in a previous Example. Fusosomes are created from: unmodified mesenchymal stem cells (hereafter MSCs, positive control), mesenchymal stem cells modified with a lentiviral-mediated expression of HLA-G (hereafter MSC-HLA-G), and mesenchymal stem cells modified with a lentiviral-mediated expression of an empty vector (hereafter MSC-empty vector, negative control).

[000778] NK cell mediated lysis of a fusosome is determined by europium release assays as described in Bouma, et al. Hum. Immunol. 35(2):85-92; 1992 & van Besouw et al. Transplantation 70(1):136-143; 2000. NK cells (hereafter effector cells) are isolated from an appropriate donor

according to the methods in Crop et al. Cell transplantation (20):1547-1559; 2011, and stimulated with allogeneic gamma irradiated PMBCs and 200IU/mL IL-2 (proleukin, Chiron BV Amsterdam, The Netherlands) in a round bottom 96 well plate for 7 days at 37°C. The fusosomes are labeled with europium-diethylenetriaminepentaacetate (DTPA) (sigma, St. Louis, MO, USA).

[000779] At day 7 cytotoxicity-mediated lysis assays is performed by incubating ^{63}Eu-labelled fusosomes with effector cells in a 96-well plate for 1, 2, 3, 4, 5, 6, 8, 10, 15, 20, 24, 48 hours after plating at effector/target ratios ranging from 1000:1-1:1 and 1:1.25-1:1000. After incubation, the plates are centrifuged and a sample of the supernatant is transferred to 96-well plates with low background fluorescence (fluoroimmunoplates, Nunc, Roskilde, Denmark).

[000780] Subsequently, enhancement solution (PerkinElmer, Groningen, The Netherlands) is added to each well. The released europium is measured in a time-resolved fluorometer (Victor 1420 multilabel counter, LKB-Wallac, Finland). Fluorescence is expressed in counts per second (CPS). Maximum percent release of europium by a target fusosome is determined by incubating an appropriate number (1x 10^2 -1x 10^8) of fusosomes with 1% triton (Sigma-Aldrich) for an appropriate amount of time. Spontaneous release of europium by target fusosomes is measured by incubation of labeled target fusosomes without effector cells. Percentage leakage is then calculated as: (spontaneous release/maximum release) x100%. Finally, the percentage of cytotoxicity mediated lysis is calculated as %lysis= [(measured lysis-spontaneous lysis- spontaneous release)/(maximum release-spontaneous release)]x100%. The data is analyzed by looking at the percentage of lysis as a function of different effector target ratios.

[000781] In some embodiments, fusosomes generated from MSC-HLA-G cells will have a decreased percentage of lysis at appropriate timepoints as compared to MSCs or MSC-scrambled generated fusosomes.

Example 85: Modification of fusosome source cells for decreased CD8 Killer T cell lysis

[000782] This Example describes the generation of a fusosome composition derived from a cell source, which has been modified to decrease cytotoxicity mediated cell lysis by CD8+ T-cells. In some embodiments, cytotoxicity mediated cell lysis of source cells or fusosomes by CD8+ T-cells is a measure of immunogenicity for fusosomes.

[000783] In this Example, fusosomes are generated by any one of the methods described in a previous Example. Fusosomes are created from: unmodified mesenchymal stem cells (hereafter MSCs, positive control), mesenchymal stem cells modified with a lentiviral-mediated expression of HLA-G (hereafter MSC-HLA-G), and mesenchymal stem cells modified with a lentiviral-mediated expression of an empty vector (hereafter MSC-empty vector, negative control).

[000784] CD8+ T cell mediated lysis of a fusosome is determined by europium release assays as described in Bouma, et al. Hum. Immunol. 35(2):85-92; 1992 & van Besouw et al. Transplantation 70(1):136-143; 2000. CD8+ T-cells (hereafter effector cells) are isolated from an appropriate donor according to the methods in Crop et al. Cell transplantation (20):1547-1559; 2011, and stimulated with allogeneic gamma irradiated PMBCs and 200IU/mL IL-2 (proleukin, Chiron BV Amsterdam, The Netherlands) in a round bottom 96 well plate for 7 days at 37°C. The fusosomes are labeled with europium-diethylenetriaminepentaacetate (DTPA) (sigma, St. Louis, MO, USA).

[000785] At day 7 cytotoxicity-mediated lysis assays is performed by incubating ^{63}Eu-labelled fusosomes with effector cells in a 96-well plate for 1, 2, 3, 4, 5, 6, 8, 10, 15, 20, 24, 48 hours after plating at effector/target ratios ranging from 1000:1-1:1 and 1:1.25-1:1000. After incubation, the plates are centrifuged and 20 μL of the supernatant is transferred to 96-well plates with low background fluorescence (fluoroimmunoplates, Nunc, Roskilde, Denmark).

[000786] Subsequently, enhancement solution (PerkinElmer, Groningen, The Netherlands) is added to each well. The released europium is measured in a time-resolved fluorometer (Victor 1420 multilabel counter, LKB-Wallac, Finland). Fluorescence is expressed in counts per second (CPS). Maximum percent release of europium by a target fusosome is determined by incubating an appropriate number (1x 10^2 -1x 10^8) of fusosomes with 1% triton (sigma-aldrich) for an appropriate amount of time. Spontaneous release of europium by target fusosomes is measured by incubation of labeled target fusosomes without effector cells. Percentage leakage is then calculated as: (spontaneous release/maximum release) x100%. Finally, the percentage of cytotoxicity mediated lysis is calculated as %lysis= [(measured lysis-spontaneous lysis- spontaneous release)/(maximum release-spontaneous release)]x100%. The data is analyzed by looking at the percentage of lysis as a function of different effector target ratios.

[000787] In some embodiments, fusosomes generated from MSC-HLA-G cells will have a decreased percentage of lysis at appropriate timepoints as compared to MSCs or MSC-scrambled generated fusosomes.

Example 86: Modification of fusosome source cells for decreased T-cell activation

This Example describes the generation of modified fusosomes that will have reduced T cell activation and proliferation as assessed by a mixed lymphocyte reaction (MLR).

[000788] T-cell proliferation and activation are measures of immunogenicity for fusosomes. Stimulation of T cell proliferation in an MLR reaction by a fusosome composition, could suggest a stimulation of T cell proliferation in vivo.

[000789] In some embodiments, fusosomes generated from modified source cells have reduced T cell activation and proliferation as assessed by a mixed lymphocyte reaction (MLR). In some embodiments, fusosomes generated from modified source cells do not generate an immune response in vivo, thus maintaining the efficacy of the fusosome composition.

[000790] In this Example, fusosomes are generated by any one of the methods described in a previous Example. Fusosomes are generated from: unmodified mesenchymal stem cells (hereafter MSCs, positive control), mesenchymal stem cells modified with a lentiviral-mediated expression of IL-10 (hereafter MSC-IL-10), and mesenchymal stem cells modified with a lentiviral-mediated expression of an empty vector (hereafter MSC-empty vector, negative control).

[000791] BALB/c and C57BL/6 splenocytes are used as either stimulator or responder cells. Notably, the source of these cells can be exchanged with commonly used human-derived stimulator/responder cells. Additionally, any mammalian purified allogeneic CD4+ T cell population, CD8+ T-cell population, or CD4-/CD8- may be used as responder population.

[000792] Mouse Splenocytes are isolated by mechanical dissociation using fully frosted slides followed by red blood cell lysis with lysing buffer (Sigma-Aldrich, St-Louis, MO). Prior to the experiment, stimulator cells are irradiated with 20 Gy of γray to prevent them from reacting against responder cells. A co-culture is then made by adding equal numbers of stimulator and responder cells (or alternative concentrations while maintaining a 1:1 ratio) to a round bottom 96-well plate in complete DMEM-10 media. An appropriate number of fusosomes (at several

concentrations from a range of $1x10^1$-$1x10^8$) are added to the co-culture at different time intervals, t = 0, 6, 12, 24, 36, 48h.

[000793] Proliferation is assessed by adding 1μCi of [^{3}H]- thymidine (Amersham, Buckinghamshire, UK) to allow for incorporation. [^{3}H]- thymidine is added to the MLR at t= 2, 6, 12, 24, 36, 48, 72h, and the cells are harvested onto glass fiber filters using a 96 well cell harvester (Inoteck, Bertold, Japan) after 2, 6, 12, 18, 24, 36 and 48h of extended culture. All of the T-cell proliferation experiments are done in triplicate. [^{3}H]- thymidine incorporation is measured using a microbeta lLuminescence counter (Perkin Elmer, Wellesley, MA). The results can be represented as counts per minute (cpm).

[000794] In some embodiments, MSC-IL10 fusosomes will show a decrease in T-cell proliferation as compared to the MSC-Empty vector or the MSC unmodified fusosome controls.

Example 87: Measuring targeting potential in a subject

[000795] This Example assesses the ability of a fusosome to target a specific body site. In some embodiments, a fusosome can target a specific body site. Targeting is a way to restrict activity of a therapeutic to one or more relevant therapeutic sites.

[000796] Eight week old C57BL/6J mice (Jackson Laboratories) are intravenously injected with fusosomes or cells that express firefly luciferase. Fusosomes are produced from cells that stably express firefly luciferase or cells that do not express luciferase (negative control) by any one of the methods described in previous Examples. Groups of mice are euthanized at one, two, three, four, five, six, eight, twelve, and twenty-four hours after fusosome or cell injection.

[000797] Five minutes before euthanization, mice receive an IP injection of bioluminescent substrate (Perkin Elmer) at a dose of 150mg/kg in order to visualize luciferase. The bioluminescent imaging system is calibrated to compensate for all device settings. Mice are then euthanized and liver, lungs, heart, spleen, pancreas, GI, and kidney are collected. The imaging system (Perkin Elmer) is used to obtain images of bioluminescence of these ex vivo organs. The bioluminescent signal is measured using Radiance Photons, with Total Flux used as a measured value. The region of interest (ROI) is generated by surrounding the ex vivo organ in order to give a value in photons/second. The ratio of photons/second between target organs (e.g. liver) and non-target

organs (e.g. the sum of photons/second from lungs, heart, spleen, pancreas, GI, and kidney) is calculated as a measure of targeting to the liver.

[000798] In some embodiments, in both fusosomes and cells, the ratio of photons/second between liver and the other organs will be greater than 1, which would indicate that fusosomes target the liver. In some embodiments, negative control animals will display much lower photons/second in all organs.

Example 88: Measuring delivery of an exogenous agent in a subject

[000799] This Example describes quantification of delivery of fusosomes comprising an exogenous agent in a subject. Fusosomes are prepared from cells expressing Gaussia-luciferase or from cells not expressing luciferase (negative control) by any one of the methods described in previous Examples.

[000800] Positive control cells or fusosomes are intravenously injected into mice. Fusosomes or cells are delivered within 5–8 seconds using a 26-gauge insulin syringe-needle. In vivo bioluminescent imaging is performed on mice 1, 2, or 3 days after injection using an in vivo imaging system (Xenogen Corporation, Alameda, CA).

[000801] Immediately before use, coelenterazine, a luciferin or light-emitting molecule, (5 mg/mL) is prepared in acidified methanol and injected immediately into the tail vein of the mice. Mice are under continuous anesthesia on a heated stage using the XGI-8 Gas Anesthesia System.

[000802] Bioluminescence imaging is obtained by acquiring photon counts over 5 min immediately after intravenous tail-vein injection of coelenterazine (4 μg/g body weight). Acquired data are analyzed using software (Xenogen) with the overlay on light-view image. Regions of interest (ROI) are created using an automatic signal intensity contour tool and normalized with background subtraction of the same animal. A sequential data acquisition using three filters at the wavelengths of 580, 600 and 620 nm with exposure time 3–10 min is conducted to localize bioluminescent light sources inside a mouse.

[000803] Furthermore, at each timepoint, urine samples are collected by abdominal palpation.

[000804] Blood samples (50 μL) are obtained from the tail vein of each mouse into

heparinized or EDTA tubes. For plasma isolation, blood samples are centrifuged for 25 min at 1.3×g at 4°C.

[000805] Then, 5 µL of blood, plasma or urine sample are used to carry out a Gaussia-luciferase activity assay after mixing the samples with 50 µM Gaussia-luciferase substrate (Nanolight, Pinetop, AZ).

[000806] In some embodiments, a negative control sample will be negative for luciferase, and a positive control sample will be from animals administered cells. In some embodiments, samples from animals administered fusosomes expressing Gaussia-luciferase will be positive for luciferase in each sample.

[000807] See, for example, El-Amouri SS et al., *Molecular biotechnology* 53(1): 63-73, 2013.

Example 89: Active transport across a lipid bilayer of a fusosome

[000808] This example describes quantification of the level of 2-NBDG (2-(*N*-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)Amino)-2-Deoxyglucose), a fluorescent glucose analog that can be used to monitor glucose uptake in live cells and thus active transport across the lipid bilayer. In some embodiments, this assay or an equivalent can be used to measure the level of glucose uptake and active transport across the lipid bilayer of the fusosome.

[000809] A fusosome composition as produced by any one of the methods described in previous Examples. A sufficient number of fusosomes are then incubated in DMEM containing no glucose, 20% Fetal Bovine Serum and 1x Penicillin/Streptomycin for 2hr at 37°C and 5% CO_2. After the 2hr glucose starvation period, the medium is changed such that it includes DMEM with no glucose, 20% Fetal Bovine Serum, 1x Penicillin/Streptomycin, and 20 µM 2-NBDG (ThermoFisher) and incubated for 2hr at 37°C and 5% CO_2. Negative control fusosomes are treated the same, except an equal amount of DMSO, the vehicle for 2-NBDG is added in place of 2-NBDG.

[000810] The fusosomes are then washed thrice with 1xPBS and re-suspended in an appropriate buffer, and transferred to a 96 well imaging plate. 2-NBDG fluorescence is then measured in a fluorimeter using a GFP light cube (469/35 excitation filter and a 525/39 emission

filter) to quantify the amount of 2-NBDG that has transported across the fusosome membrane and accumulated in the fusosome in the 1hr loading period.

[000811] In some embodiments, 2-NBDG fluorescence will be higher in the fusosomes with 2-NBDG treatment as compared to the negative (DMSO) control. Fluorescence measure with a 525/39 emission filter will be relatively to the number of 2-NBDG molecules present.

Example 90: Delivery of fusosomes via non-endocytic pathway

[000812] This example describes quantification of fusosome delivery of Cre to a recipient cell via a non-endocytic pathway.

[000813] In some embodiments, fusosomes will deliver agents via a fusosome-mediated, non-endocytic pathway. Without wishing to be bound by theory, delivery of an agent, e.g., Cre, which is carried within the lumen of the fusosomes, directly to the cytosol of the recipient cells without any requirement for endocytosis-mediated uptake of the fusosomes, will occur through a fusosome-mediated, non-endocytic pathway delivery.

[000814] In this example, the fusosome comprises a HEK293T cell expressing the Sendai virus H and F protein on its plasma membrane (Tanaka et al., 2015, Gene Therapy, 22(October 2014), 1–8. https://doi.org/10.1038/gt.2014.123). In addition, the fusosome expresses mTagBFP2 fluorescent protein and Cre recombinase. The target cell is a RPMI8226 cell which stably-expresses "LoxP-GFP-stop-LoxP-RFP" cassette under a CMV promoter, which upon recombination by Cre switches from GFP to RFP expression, indicating fusion and Cre, as a marker, delivery.

[000815] Fusosomes produced by the herein described methods are assayed for delivery of Cre via a non-endocytic pathway as follows. The recipient cells are plated into a black, clear-bottom 96-well plate. Next, 24 hours after plating the recipient cells, the fusosomes expressing Cre recombinase protein and possessing the particular fusogen protein are applied to the recipient cells in DMEM media. To determine the level of Cre delivery via a non-endocytic pathway, a parallel group of recipient cells receiving fusosomes is treated with an inhibitor of endosomal acidification, chloroquine (30 μg/mL). The dose of fusosomes is correlated to the number of recipient cells plated in the well. After applying the fusosomes, the cell plate is centrifuged at 400g for 5 minutes

to help initiate contact between the fusosomes and the recipient cells. The cells are then incubated for 16 hours and agent delivery, Cre, is assessed via imaging.

[000816] The cells are imaged to positively identify RFP-positive cells versus GFP-positive cells in the field or well. In this example cell plates are imaged using an automated fluorescence microscope. The total cell population in a given well is determined by first staining the cells with Hoechst 33342 in DMEM media for 10 minutes. Hoechst 33342 stains cell nuclei by intercalating into DNA and therefore is used to identify individual cells. After staining, the Hoechst media is replaced with regular DMEM media.

[000817] The Hoechst is imaged using the 405 nm LED and DAPI filter cube. GFP is imaged using the 465 nm LED and GFP filter cube, while RFP is imaged using 523 nm LED and RFP filter cube. Images of the different cell groups are acquired by first establishing the LED intensity and integration times on a positive-control well; i.e., recipient cells treated with adenovirus coding for Cre recombinase instead of fusosomes.

[000818] Acquisition settings are set so that RFP and GFP intensities are at the maximum pixel intensity values but not saturated. The wells of interest are then imaged using the established settings.

[000819] Analysis of GFP and RFP-positive wells is performed with software provided with the fluorescence microscope or other software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, 1997–2007). The images are pre-processed using a rolling ball background subtraction algorithm with a 60 μm width. The total cell mask is set on the Hoechst-positive cells. Cells with Hoechst intensity significantly above background intensities are used to set a threshold, and areas too small or large to be Hoechst-positive cells are excluded.

[000820] Within the total cell mask, GFP and RFP-positive cells are identified by again setting a threshold for cells significantly above background and extending the Hoechst (nuclei) masks for the entire cell area to include the entire GFP and RFP cellular fluorescence.

[000821] The number of RFP-positive cells identified in control wells containing recipient cells is used to subtract from the number of RFP-positive cells in the wells containing fusosomes (to subtract for non-specific Loxp recombination). The number of RFP-positive cells (recipient

cells that received Cre) is then divided by the sum of GFP-positive cells (recipient cells that have not received Cre) and RFP-positive cells to quantify the fraction of fusosome Cre delivered to the recipient cell population. The level is normalized to the given dose of fusosomes applied to the recipient cells. To calculate the value of fusosome Cre delivered via a non-endocytic pathway, the level of fusosome Cre delivery in the presence of chloroquine (FusL+CQ) is determined as well as the level of fusosome Cre delivery in the absence of chloroquine (FusL-CQ). To determine the normalized value of fusosome Cre delivered via a non-endocytic pathway, the following equation is used: [(FusL-CQ)-(FusL+CQ)]/(FusL-CQ).

[000822] In some embodiments, an average level of fusosome Cre delivered via a non-endocytic pathway for a given fusosome will be in the range of 0.1-0.95, or at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater than chloroquine treated recipient cells.

Example 91: Delivery of fusosomes via endocytic pathway

[000823] This example describes fusosome delivery of Cre to a recipient cell via an endocytic pathway.

[000824] In some embodiments, fusosomes will deliver agents via a fusosome-mediated, endocytic pathway. Without wishing to be bound by theory, delivery of an agent, e.g., a cargo, carried in the lumen of the fusosomes, to the recipient cells with the route of uptake being endocytosis-dependent will occur through a fusosome-mediated, endocytic pathway delivery.

[000825] In this example the fusosome comprises microvesicles that were produced by extruding a HEK293T cell expressing a fusogen protein on its plasma membrane through a 2 μm filter (Lin et al., 2016, Biomedical Microdevices, 18(3). doi.org/10.1007/s10544-016-0066-y)(Riedel, Kondor-Koch, & Garoff, 1984, The EMBO Journal, 3(7), 1477–83. Retrieved from www.ncbi.nlm.nih.gov/pubmed/6086326). In addition, the fusosome expresses mTagBFP2 fluorescent protein and Cre recombinase. The target cell is a PC3 cell which stably-expresses "LoxP-GFP-stop-LoxP-RFP" cassette under a CMV promoter, which upon recombination by Cre switches from GFP to RFP expression, indicating fusion and Cre, as a marker, delivery.

[000826] Fusosomes produced by the herein described methods are assayed for delivery of

Cre via an endocytic pathway as follows. The recipient cells are plated into a cell culture multi-well plate compatible with the imaging system to be used (in this example cells are plated in a black, clear-bottom 96-well plate). Next, 24 hours after plating the recipient cells, the fusosomes expressing Cre recombinase protein and possessing the particular fusogen protein are applied to the recipient cells in DMEM media. To determine the level of Cre delivery via an endocytic pathway, a parallel group of recipient cells receiving fusosomes is treated with an inhibitor of endosomal acidification, chloroquine (30 μg/mL). The dose of fusosomes is correlated to the number of recipient cells plated in the well. After applying the fusosomes, the cell plate is centrifuged at 400g for 5 minutes to help initiate contact between the fusosomes and the recipient cells. The cells are then incubated for 16 hours and agent delivery, Cre, is assessed via imaging.

[000827] The cells are imaged to positively identify RFP-positive cells versus GFP-positive cells in the field or well. In this example cell plates are imaged using an automated fluorescent microscope. The total cell population in a given well is determined by first staining the cells with Hoechst 33342 in DMEM media for 10 minutes. Hoechst 33342 stains cell nuclei by intercalating into DNA and therefore is used to identify individual cells. After staining the Hoechst media is replaced with regular DMEM media.

[000828] The Hoechst is imaged using the 405 nm LED and DAPI filter cube. GFP is imaged using the 465 nm LED and GFP filter cube, while RFP is imaged using 523 nm LED and RFP filter cube. Images of the different cell groups are acquired by first establishing the LED intensity and integration times on a positive-control well; i.e., recipient cells treated with adenovirus coding for Cre recombinase instead of fusosomes.

[000829] Acquisition settings are set so that RFP and GFP intensities are at the maximum pixel intensity values but not saturated. The wells of interest are then imaged using the established settings.

[000830] Analysis of GFP and RFP-positive wells is performed with software provided with the fluorescent microscope or other software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, 1997–2007). The images are pre-processed using a rolling ball background subtraction algorithm with a 60 μm width. The total cell mask is set on the Hoechst-positive cells. Cells with Hoechst intensity significantly above background intensities are

thresholded and areas too small or large to be Hoechst-positive cells are excluded.

[000831] Within the total cell mask, GFP and RFP-positive cells are identified by again thresholding for cells significantly above background and extending the Hoechst (nuclei) masks for the entire cell area to include the entire GFP and RFP cellular fluorescence.

[000832] The number of RFP-positive cells identified in control wells containing recipient cells is used to subtract from the number of RFP-positive cells in the wells containing fusosomes (to subtract for non-specific Loxp recombination). The number of RFP-positive cells (recipient cells that received Cre) is then divided by the sum of the GFP-positive cells (recipient cells that have not received Cre) and RFP-positive cells to quantify the fraction of fusosome Cre delivered to the recipient cell population. The level is normalized to the given dose of fusosomes applied to the recipient cells. To calculate the value of fusosome Cre delivered via an endocytic pathway, the level of fusosome Cre delivery in the presence of chloroquine (FusL+CQ) is determined as well as the level of fusosome Cre delivery in the absence of chloroquine (FusL-CQ). To determine the normalized value of fusosome Cre delivered via an endocytic pathway, the following equation is used: (FusL+CQ)/(FusL-CQ).

[000833] In some embodiments, an average level of fusosome Cre delivered via an endocytic pathway for a given fusosome will be in the range of 0.01-0.6, or at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater than chloroquine treated recipient cells.

Example 92: Delivery of fusosomes via a dynamin mediated pathway, a macropinocytosis pathway, or an actin mediated pathway

[000834] This example describes fusosome delivery of Cre to a recipient cell via a dynamin mediated pathway. A fusosome comprising a microvesicle may be produced as described in the preceding example. Fusosomes are assayed for delivery of Cre via a dynamin-mediated pathway according to the preceding example, except that a group of recipient cells receiving fusosomes is treated with an inhibitor of dynamin, Dynasore (120 μM). To calculate the value of fusosome Cre delivered via a dynamin-mediated pathway, the level of fusosome Cre delivery in the presence of Dynasore (FusL+DS) is determined as well as the level of fusosome Cre delivery in the absence of Dynasore (FusL-DS). The normalized value of fusosome Cre delivered may be calculated as

described in the preceding example.

[000835] This example also describes delivery of Cre to a recipient cell via macropinocytosis. A fusosome comprising a microvesicle may be produced as described in the preceding example. Fusosomes are assayed for delivery of Cre via macropinocytosis according to the preceding example, except that a group of recipient cells receiving fusosomes is treated with an inhibitor of macropinocytosis, 5-(*N*-ethyl-*N*-isopropyl)amiloride (EIPA) (25 μM). To calculate the value of fusosome Cre delivered via macropinocytosis, the level of fusosome Cre delivery in the presence of EIPA (FusL+EPIA) is determined as well as the level of fusosome Cre delivery in the absence of EPIA (FusL-EIPA). The normalized value of fusosome Cre delivered may be calculated as described in the preceding example.

[000836] This example also describes fusosome delivery of Cre to a recipient cell via an actin mediated pathway. A fusosome comprising a microvesicle may be produced as described in the preceding example. Fusosomes are assayed for delivery of Cre via macropinocytosis according to the preceding example, except that a group of recipient cells receiving fusosomes is treated with an inhibitor of actin polymerization, Latrunculin B (6 μM). To calculate the value of fusosome Cre delivered via an actin-mediated pathway, the level of fusosome Cre delivery in the presence of Latrunculin B (FusL+ LatB) is determined as well as the level of fusosome Cre delivery in the absence of Latrunculin B (FusL- LatB). The normalized value of fusosome Cre delivered may be calculated as described in the preceding example.

Example 93: Delivery of organelles

[000837] This example describes fusosome fusion with a cell *in vitro*. In some embodiments, fusosome fusion with a cell *in vitro* can result in delivery of fusosomal mitochondrial cargo to the recipient cell.

[000838] A fusosome produced by the methods described by the herein described methods was assayed for its ability to deliver its mitochondria to the recipient cell as follows.

[000839] In this particular example, the fusosome was a HEK293T cell expressing a fusogen protein on its membrane, as well as mitochondrial-targeted DsRED (mito-DsRED) protein to label mitochondria. The recipient cells were plated into a cell culture multi-well plate compatible with

the imaging system to be used (in this example cells were plated in a glass-bottom imaging dish). The recipient cells stably-expressed cytosolic GFP.

[000840] Next, 24 hours after plating the recipient cells, the fusosome expressing mito-DsRED and possessing the particular fusogen protein was applied to the recipient cells in DMEM media. The dose of fusosomes was correlated to the number of recipient cells plated in the well. After applying the fusosomes the cell plate was centrifuged at 400g for 5 minutes to help initiate contact between the fusosomes and the recipient cells. The cells were then incubated for 4 hours and VSVG-mediated fusion was induced by one minute exposure to pH 6.0 phosphate-buffered saline (or control cells are exposed to pH 7.4 phosphate-buffered saline). Following induction of fusion, cells were incubated an additional 16 hours and mitochondria delivery was assessed via imaging.

[000841] In this example, cells were imaged on a Zeiss LSM 710 confocal microscope with a 63x oil immersion objective while maintained at 37°C and 5% CO_2. GFP was subjected to 488nm laser excitation and emission was recorded through a band pass 495-530nm filter. DsRED was subjected to 543 nm laser excitation and emission was recorded through a band pass 560 to 610nm filter. The cells were scanned to positively identify cells positive for cytosolic GFP fluorescence and mito-DsRED fluorescence.

[000842] The presence of both cytosolic GFP and mito-DsRED mitochondria were found in the same cell indicating the cell has undergone VSVG-mediated fusion, and thus mitochondria have been delivered from the fusosome to the recipient cell.

Example 94: In vitro delivery of DNA

[000843] This example describes the delivery of DNA using fusosomes to cells *in vitro*. This example quantifies the ability of fusosomes to deliver DNA using a plasmid encoding an exogenous gene, GFP, a surrogate therapeutic cargo.

[000844] A fusosome composition, resulting from cell-derived vesicles or cell-derived cytobiologics as produced by any one of the methods described in previous Examples, except the fusosome is engineered such that the fusogen is in-frame with the open reading frame of Cre. Following production of the fusosome, it is additionally nucleofected with a plasmid having a

sequence that codes for GFP (System Biosciences, Inc.).

[000845] See, for example, Chen X, et al., Genes Dis. 2015 Mar;2(1):96-105.DOI:10.1016/j.gendis.2014.12.001.

[000846] As a negative control, fusosomes are nucleofected with a plasmid having a sequence that codes for beta-actin.

[000847] A sufficient number of fusosomes are then incubated at 37°C and 5% CO_2 together with a recipient NIH/3T3 fibroblast cell line that has a loxP-STOP-loxP-tdTomato reporter for a period of 48h in in DMEM containing 20% Fetal Bovine Serum and 1x Penicillin/Streptomycin. Following the 48 hr incubation, the tdTomato positive cells are then isolated via FACS, using a FACS cytometer (Becton Dickinson, San Jose, CA, USA) with 561nm laser excitation and emission is collected at 590+/-20nm. Total DNA is then isolated using a DNA extraction solution (Epicentre) and PCR is performed using primers specific to GFP (see Table 222) that amplify a 600bp fragment. A 600bp fragment present on a gel following gel electrophoresis would then substantiate the present of DNA delivery to the recipient cell.

Table 24. GFP Primers sequences that amplify a 500bp fragment

Primer	Sequence (SEQ ID NO)
GFP-F	ATGAGTAAAGGAGAAGAACTTTTCAC (SEQ ID NO: 600)
GFP-R	GTCCTTTTACCAGACAACCATTAC (SEQ ID NO: 601)

[000848] In some embodiments, delivery of nucleic acid cargo with fusosomes *in vitro* is higher in fusosomes with GFP plasmid as compared to the negative control. Negligible GFP fluorescence is detected in the negative control.

Example 95: In vivo delivery of DNA

[000849] This example describes the delivery of DNA to cells *in vivo* via fusosomes. Delivery of DNA to cells *in vivo* results in the expression of proteins within the recipient cell.

[000850] Fusosome DNA delivery in vivo will demonstrates the delivery of DNA and protein expression in recipient cells within an organism (mouse).

[000851] Fusosomes that express a liver directed fusogen are prepared as described herein. Following production of the fusosome, it is additionally nucleofected with a plasmid having a sequence that codes for Cre recombinase.

[000852] Fusosomes are prepared for in vivo delivery. Fusosome suspensions are subjected to centrifugation. Pellets of the fusosomes are resuspended in sterile phosphate buffered saline for injection.

[000853] Fusosomes are verified to contain DNA using a nucleic acid detection method, e.g., PCR.

[000854] The recipient mice harbor a loxp-luciferase genomic DNA locus that is modified by CRE protein made from DNA delivered by the fusosomes to unblock the expression of luciferase (JAX#005125). The positive control for this example are offspring of recipient mice mated to a mouse strain that expresses the same protein exclusively in the liver from its own genome (albumin-CRE JAX#003574). Offspring from this mating harbor one of each allele (loxp-luciferase, albumin-CRE). Negative controls are carried out by injection of recipient mice with fusosomes not expressing fusogens or fusosomes with fusogens but not containing Cre DNA.

[000855] The fusosomes are delivered into mice by intravenous (IV) tailvein administration. Mice are placed in a commercially available mouse restrainer (Harvard Apparatus). Prior to restraint, animals are warmed by placing their cage on a circulating water bath. Once inside the restrainer, the animals are allowed to acclimate. An IV catheter consisting of a 30G needle tip, a 3" length of PE-10 tubing, and a 28G needle is prepared and flushed with heparinized saline. The tail is cleaned with a 70% alcohol prep pad. Then, the catheter needle is held with forceps and slowly introduced into the lateral tail vein until blood becomes visible in the tubing. The fusosome solution (~500K-5M fusosomes) is aspirated into a 1 cc tuberculin syringe and connected to an infusion pump. The fusosome solution is delivered at a rate of 20 μL per minute for 30 seconds to 5 minutes, depending on the dose. Upon completion of infusion, the catheter is removed, and pressure is applied to the injection site until cessation of any bleeding. Mice are returned to their cages and allowed to recover.

[000856] After fusion, the DNA will be transcribed and translated into CRE protein which will then translocates to the nucleus to carry out recombination resulting in the constitutive

expression of luciferase. Intraperitoneal administration of D-luciferin (Perkin Elmer, 150 mg/kg) enables the detection of luciferase expression via the production of bioluminescence. The animal is placed into an in vivo bioluminescent imaging chamber (Perkin Elmer) which houses a cone anesthetizer (isoflurane) to prevent animal motion. Photon collection is carried out between 8-20 minutes post-injection to observe the maximum in bioluminescence due to D-luciferin pharmacokinetic clearance. A specific region of the liver is created in the software and collection exposure time set so that count rates are above 600 (in this region) to yield interpretable radiance (photons/sec/cm^2/steradians) measurements. The maximum value of bioluminescent radiance is recorded as the image of bioluminescence distribution. The liver tissue is monitored specifically for radiance measurements above background (untreated animals) and those of negative controls. Measurements are carried out at 24 hours post-injection to observe luciferase activity. Mice are then euthanized and livers are harvested.

[000857] Freshly harvested tissue is subjected to fixation and embedding via immersion in 4% paraformaldehyde/0.1M sodium phosphate buffer pH7.4 at 4°C for 1-3hrs. Tissue is then immersed in sterile 15% sucrose/1xPBS (3 hrs. to overnight) at 4°C. Tissue is then embedded in O.C.T. (Baxter No. M7148-4). Tissue is oriented in the block appropriately for sectioning (cross-section). Tissue is then frozen in liquid nitrogen using the following method: place the bottom third of the block into the liquid nitrogen, allow to freeze until all but the center of the O.C.T. is frozen, and allow freezing to conclude on dry ice. Blocks are sectioned by cryostat into 5-7 micron sections placed on slides and refrozen for staining.

[000858] In situ hybridization is carried out (using standard methods) on tissue sections using digoxygenin labeled nucleic acid probes (for CRE DNA and luciferase mRNA detection), labeled by anti-digoxygenin fluorescent antibodies, and observed by confocal microscopy.

[000859] In some embodiments, positive control animals (recombination via breeding without fusosome injection) will show bioluminescence intensity in liver as compared to untreated animals (no CRE and no fusosomes) and negative controls, while agent injected animals will show bioluminescence in liver as compared to negative controls (fusosomes without fusogen) and untreated animals.

[000860] In embodiments, detection of nucleic acid in tissue sections in agent injected

animals will reveal detection of CRE recombinase and luciferase mRNA compared to negative controls and untreated animals in cells in the tissue, while positive controls will show levels of both luciferase mRNA and CRE recombinase DNA throughout the tissue.

[000861] Evidence of DNA delivery by fusosomes will be detected by in situ hybridization-based detection of the DNA and its colocalization in the recipient tissue of the animal. Activity of the protein expressed from the DNA will be detected by bioluminescent imaging. In embodiments, fusosomes will deliver DNA that will result in protein production and activity.

Example 96: In vitro delivery of mRNA

[000862] This example describes fusosome fusion with a cell *in vitro*. In some embodiments, fusosome fusion with a cell *in vitro* results in delivery of a specified mRNA to the recipient cell.

[000863] A fusosome produced by the herein described methods was assayed for its ability to deliver a specified mRNA to the recipient cell as follows. In this particular example, the fusosome was a cytobiologic (lacking a nucleus), which was generated from a 3T3 mouse fibroblast cell expressing Cre and GFP. The cytobiologic was then treated with HVJ-E fusogen protein to produce the fusosome.

[000864] The recipient mouse macrophage cells were plated into a cell culture multi-well plate compatible with the imaging system to be used (in this example cells are plated in a glass-bottom imaging dish). The recipient cells stably-expressed "LoxP-stop-LoxP-tdTomato" cassette under CMV promoter, which upon recombination by Cre induces tdTomato expression, indicating delivery of Cre protein to the recipient cell.

[000865] Next, 24 hours after plating the recipient cells, the fusosome expressing Cre recombinase protein and possessing the particular fusogen protein was applied to the recipient cells in DMEM media. The dose of fusosomes was correlated to the number of recipient cells plated in the well. After applying the fusosomes the cell plate was centrifuged at 400g for 5 minutes to help initiate contact between the fusosomes and the recipient cells. The cells were then incubated for 16 hours and mRNA delivery was assessed via imaging.

[000866] The cells were stained with 1 μg/mL Hoechst 33342 in DMEM media for 10 minutes prior to imaging. In this example cells were imaged on a Zeiss LSM 710 confocal

microscope with a 63x oil immersion objective while maintained at 37°C and 5% CO_2. Hoechst was subjected to 405nm laser excitation and emission was recorded through a band pass 430-460nm filter. GFP was subjected to 488nm laser excitation and emission was recorded through a band pass 495-530nm filter. tdTomato was subjected to 543 nm laser excitation and emission was recorded through a band pass 560 to 610nm filter.

[000867] First, the cells were scanned to positively identify single-nucleated, tdTomato-positive cells. The presence of a tdTomato-positive cell indicated a cell that has undergone fusion, and the single nucleus indicated the fusion was by a cytobiologic fusosome donor. These identified cells were first imaged and then subsequently photo-bleached using a 488nm laser to partially quench GFP fluorescence. The cells were then imaged over-time to assess recovery of GFP fluorescence, which would demonstrate translation of new GFP protein and thus presence of GFP mRNA delivered by the donor fusosome.

[000868] Analysis of Hoechst, GFP, and tdTomato fluorescence in the cells of interest was performed using ImageJ software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, rsb.info.nih.gov/ij/, 1997–2007). First the images were pre-processed using a rolling ball background subtraction algorithm with a 60 μm width. Within a photo-bleached cell, the GFP fluorescence was thresholded to remove background. Then the GFP mean fluorescence intensity for the photo-bleached cell was analyzed at different times before and after photo-bleaching.

[000869] Within this particular Example, 3T3 mouse fibroblast cytobiologics expressing Cre and GFP and either possessing the applied fusogen HVJ-E (+fusogen) were applied to recipient mouse macrophage cells expressing "LoxP-stop-LoxP-tdTomato" cassette. Representative images and data are shown in FIG. 5. For this particular example the GFP fluorescence intensity recovered up to 25% of the original intensity 10 hours after photo-bleaching, indicating the delivery of actively-translated mRNA in the recipient cell.

Example 97: In vitro delivery of siRNA

[000870] This example describes delivery of short interfering RNA (siRNA) to cell *in vitro* via fusosomes. Delivery of siRNA to cells *in vitro* results in the suppression of the expression of proteins within the recipient cell. This can be used to inhibit the activity of a protein whose

expression is injurious to the cell, thus permitting the cell to behave normally.

[000871] A fusosome produced by the herein described methods is assayed for its ability to deliver a specified siRNA to the recipient cell as follows. Fusosomes are prepared as described herein. Following production of the fusosome, it is additionally electroporated with an siRNA having a sequence that specifically inhibits GFP. The sequence of the double stranded siRNA targeted against GFP is 5' GACGUAAACGGCCACAAGUUC 3' and its complement 3' CGCUGCAUUUGCCGGUGUUCA 5' (note that there are overhangs 2 basepairs long at 3' ends of the siRNA sequence). As a negative control fusosomes are electroporated with an siRNA having a sequence that specifically inhibits luciferase. The sequence of the double stranded siRNA targeted against luciferase is 5' CUUACGCUGAGUACUUCGATT 3' and its complement 3' TTGAAUGCGACUCAUGAAGCU 5' (note that there are overhangs 2 basepairs long at 3' ends of the siRNA sequence).

[000872] The fusosomes are then applied to the recipient cells that constitutively express GFP. The recipient cells are plated into a black, clear-bottom 96-well plate. Next, 24 hours after plating the recipient cells, the fusosomes expressing are applied to the recipient cells in DMEM media. The dose of fusosomes is correlated to the number of recipient cells plated in the well. After applying the fusosomes, the cell plate is centrifuged at 400g for 5 minutes to help initiate contact between the fusosomes and the recipient cells. The cells are then incubated for 16 hours and agent delivery, siRNA, is assessed via imaging.

[000873] The cells are imaged to positively identify GFP-positive cells in the field or well. In this example cell plates are imaged using an automated fluorescence microscope (www.biotek.com/products/imaging-microscopy-automated-cell-imagers/lionheart-fx-automated-live-cell-imager/). The total cell population in a given well is determined by first staining the cells with Hoechst 33342 in DMEM media for 10 minutes. Hoechst 33342 stains cell nuclei by intercalating into DNA and therefore is used to identify individual cells. After staining, the Hoechst media is replaced with regular DMEM media.

[000874] The Hoechst is imaged using the 405 nm LED and DAPI filter cube. GFP is imaged using the 465 nm LED and GFP filter cube. Images of the different cell groups are acquired by first establishing the LED intensity and integration times on an untreated well; i.e., recipient cells

that were not treated with any fusosomes.

[000875] Acquisition settings are set so that GFP intensities are at the maximum pixel intensity values but not saturated. The wells of interest are then imaged using the established settings.

[000876] Analysis of GFP positive wells is performed with software provided with the fluorescence microscope or other software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/, 1997–2007). The images are pre-processed using a rolling ball background subtraction algorithm with a 60 μm width. The total cell mask is set on the Hoechst-positive cells. Cells with Hoechst intensity significantly above background intensities are thresholded and areas too small or large to be Hoechst-positive cells are excluded.

[000877] Within the total cell mask, GFP - positive cells are identified by again thresholding for cells significantly above background and extending the Hoechst (nuclei) masks for the entire cell area to include the entire GFP cellular fluorescence. The percentage of GFP-positive cells out of total cells is calculated.

[000878] In embodiments, the percentage of GFP positive cells in wells treated with fusosomes containing an siRNA against GFP will be at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% less than the percentage of GFP positive cells in well treated with fusosomes containing an siRNA against luciferase.

Example 98: In vivo delivery of mRNA

[000879] This example describes the delivery of messenger RNA (mRNA) to cells *in vivo* via fusosomes. In some embodiments, delivery of mRNA to cells *in vivo* results in the expression of proteins within the recipient cell. In some embodiments, this method of delivery can be used to supplement a protein not present due to a genetic mutation, permitting the cell to behave normally, or re-direct the activity of a cell to carry out a function, e.g., a therapeutic function.

[000880] In some embodiments, fusosome mRNA delivery *in vivo* demonstrates the delivery of messenger RNA and protein expression in recipient cells within an organism (e.g., a mouse).

[000881] In some embodiments, fusosomes that express a liver directed fusogen, and produce

mRNA expressing Cre are prepared for *in vivo* delivery.

[000882] Fusosomes are prepared as described herein. Fusosome suspensions are subjected to centrifugation. Pellets of the fusosomes are resuspended in sterile phosphate buffered saline for injection.

[000883] Fusosomes are verified to express mRNA using a nucleic acid detection method, e.g., PCR.

[000884] The recipient mice harbor a loxp-luciferase genomic DNA locus that is modified by CRE protein made from mRNA delivered by the fusosomes to unblock the expression of luciferase (JAX#005125). The positive controls for this example are offspring of recipient mice mated to a mouse strain that expresses the same protein exclusively in the liver from its own genome (albumin-CRE JAX#003574). Offspring from this mating harbor one of each allele (loxp-luciferase, albumin-CRE). Negative controls are carried out by injection of recipient mice with fusosomes not expressing fusogens or fusosomes with fusogens but not expressing Cre mRNA.

[000885] The fusosomes are delivered into mice by intravenous (IV) tail vein administration. Mice are placed in a commercially available mouse restrainer (Harvard Apparatus). Prior to restraint, animals are warmed by placing their cage on a circulating water bath. Once inside the restrainer, the animals are allowed to acclimate. An IV catheter consisting of a 30G needle tip, a 3" length of PE-10 tubing, and a 28G needle is prepared and flushed with heparinized saline. The tail is cleaned with a 70% alcohol prep pad. Then, the catheter needle is held with forceps and slowly introduced into the lateral tail vein until blood becomes visible in the tubing. The fusosome solution (~500K-5M fusosomes) is aspirated into a 1 cc tuberculin syringe and connected to an infusion pump. The fusosome solution is delivered at a rate of 20 μL per minute for 30 seconds to 5 minutes, depending on the dose. Upon completion of infusion, the catheter is removed, and pressure is applied to the injection site until cessation of any bleeding. Mice are returned to their cages and allowed to recover.

[000886] After fusion, the mRNA is translated in the recipient cytoplasm into CRE protein which then translocates to the nucleus to carry out recombination resulting in the constitutive expression of luciferase. Intraperitoneal administration of D-luciferin (Perkin Elmer, 150 mg/kg) enables the detection of luciferase expression via the production of bioluminescence. The animal

is placed into an *in vivo* bioluminescent imaging chamber (Perkin Elmer) which houses a cone anesthetizer (isoflurane) to prevent animal motion. Photon collection is carried out between 8-20 minutes post-injection to observe the maximum in bioluminescence due to D-luciferin pharmacokinetic clearance. A specific region of the liver is created in the software and collection exposure time set so that count rates are above 600 (in this region) to yield interpretable radiance (photons/sec/cm^2/steradians) measurements. The maximum value of bioluminescent radiance is recorded as the image of bioluminescence distribution. The liver tissue is monitored specifically for radiance measurements above background (untreated animals) and those of negative controls. Measurements are carried out at 24 hours post-injection to observe luciferase activity. Mice are then euthanized and livers are harvested.

[000887] Freshly harvested tissue is subjected to fixation and embedding via immersion in 4% paraformaldehyde/0.1M sodium phosphate buffer pH7.4 at 4°C for 1-3hrs. Tissue is then immersed in sterile 15% sucrose/1xPBS (3 hrs. to overnight) at 4°C. Tissue is then embedded in O.C.T. (Baxter No. M7148-4). Tissue is oriented in the block appropriately for sectioning (cross-section). Tissue is then frozen in liquid nitrogen using the following method: place the bottom third of the block into the liquid nitrogen, allow to freeze until all but the center of the O.C.T. is frozen, and allow freezing to conclude on dry ice. Blocks are sectioned by cryostat into 5-7 micron sections placed on slides and refrozen for staining.

[000888] *In situ* hybridization is carried out (using standard methods) on tissue sections using digoxygenin labeled RNA probes (for CRE mRNA and luciferase mRNA detection), labeled by anti-digoxygenin fluorescent antibodies, and observed by confocal microscopy.

[000889] In some embodiments, positive control animals (e.g., recombination via breeding without fusosome injection), will show bioluminescence intensity in liver as compared to untreated animals (e.g., no CRE or fusosomes), and negative controls. In some embodiments, fusosome injected animals will show bioluminescence in liver as compared to negative controls (e.g., fusosomes without fusogen), and untreated animals.

[000890] In some embodiments, detection of mRNA in tissue sections in animals administered fusosomes will reveal detection of CRE recombinase and luciferase mRNA compared to negative controls, and untreated animals in cells in the tissue. In some embodiments,

positive controls will show levels of both luciferase mRNA and CRE recombinase mRNA throughout the tissue.

[000891] In some embodiments, evidence of mRNA delivery by fusosomes will be detected by *in situ* hybridization-based detection of the mRNA, and its colocalization in the recipient tissue of the animal. In some embodiments, activity of the protein expressed from the mRNA delivered by the fusosome is detected by bioluminescent imaging. In some embodiments, fusosomes deliver mRNA that will result in protein production and activity.

Example 99: In vitro delivery of protein

[000892] This example demonstrates fusosome fusion with a cell *in vitro*. In this example, fusosome fusion with a cell in vitro results in delivery of Cre protein to the recipient cell.

[000893] In this example, the fusosomes were generated from a 3T3 mouse fibroblast cell possessing the Sendai virus HVJ-E protein (Tanaka et al., 2015, Gene Therapy, 22(October 2014), 1–8. doi.org/10.1038/gt.2014.12). In addition, the fusosomes expressed Cre recombinase. The target cell was a primary HEK293T cell which stably-expressed "LoxP -GFP-stop-LoxP-RFP" cassette under a CMV promoter, which upon recombination by Cre switches from GFP to RFP expression, indicating fusion and Cre, as a marker, delivery.

[000894] Fusosomes produced by the herein described methods were assayed for the ability to deliver Cre protein to recipient cells as follows. The recipient cells were plated into a cell culture multi-well plate compatible with the imaging system to be used (in this example cells were plated in a black, clear-bottom 96-well plate). Next, 24 hours after plating the recipient cells, the fusosome expressing Cre recombinase protein and possessing the particular fusogen protein were applied to the recipient cells in DMEM media. The dose of fusosomes was correlated to the number of recipient cells plated in the well. After applying the fusosomes the cell plate was centrifuged at 400g for 5 minutes to help initiate contact between the fusosomes and the recipient cells. The cells were then incubated for 16 hours and protein delivery was assessed via imaging.

[000895] The cells were imaged to positively identify RFP-positive cells versus GFP-positive cells in the field or well. In this example cell plates were imaged using an automated microscope. The total cell population in a given well was determined by first staining the cells with 1 μg/mL

Hoechst 33342 in DMEM media for 10 minutes. Hoechst 33342 stains cell nuclei by intercalating into DNA and therefore is used to identify individual cells. After staining the Hoechst media was replaced with regular DMEM media. The Hoechst was imaged using the 405 nm LED and DAPI filter cube. GFP was imaged using the 465 nm LED and GFP filter cube, while RFP was imaged using 523 nm LED and RFP filter cube. Images of the different cell groups were acquired by first establishing the LED intensity and integration times on a positive-control well; i.e., cells treated with adenovirus coding for Cre recombinase. Acquisition settings were set so that RFP and GFP intensities are at the maximum pixel intensity values but not saturated. The wells of interest were then imaged using the established settings.

[000896] Analysis of Hoechst, GFP, and RFP-positive wells was performed in the Gen5 software provided with the LionHeart FX or by ImageJ software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/, 1997–2007). First the images were pre-processed using a rolling ball background subtraction algorithm with a 60 μm width. Next the total cell mask was set on the Hoechst-positive cells. Cells with Hoechst intensity significantly above background intensities were thresholded and areas too small or large to be Hoechst-positive cells were excluded. Within the total cell mask GFP and RFP-positive cells were identified by again thresholding for cells significantly above background and extending the Hoechst (nuclei) masks for the entire cell area to include the entire GFP and RFP cellular fluorescence.

[000897] The number of RFP-positive cells identified in control wells containing only recipient cells was used to subtract from the number of RFP-positive cells in the wells containing fusosome (to subtract for non-specific Loxp recombination). The number of RFP-positive cells (recipient cells that received the agent) was then divided by the sum of the GFP-positive cells (recipient cells that have not received the agent) and RFP-positive cells to quantify the fraction of fusosome agent delivery within the recipient cell population.

[000898] Within this particular example, 3T3 mouse fibroblast cells expressing Cre and either possessing the applied fusogen HVJ-E (+fusogen) or not (-fusogen) were applied to recipient 293T cells expressing "LoxP-GFP-stop-LoxP-RFP" cassette. Delivery of Cre protein is assessed by the induction of RFP expression in the recipient cells. The graph in Figure 6 shows the

quantification of the RFP-positive cells (rightmost bar of each pair) out of the total cells stained positive for Hoechst (leftmost bar of each pair). For this particular Example the fraction of fusosome delivery to recipient cells is 0.44 for 3T3 Cre cells possessing HVJ-E fusogen.

Example 100: In vivo delivery of protein

[000899] This example describes the delivery of therapeutic agents to the eye by fusosomes.

[000900] Fusosomes are derived from hematopoietic stem and progenitor cells using any of the methods described in previous Examples and are loaded with a protein that is deficient in a mouse knock-out.

[000901] Fusosomes are injected subretinally into the right eye of a mouse that is deficient for the protein and vehicle control is injected into the left eye of the mice. A subset of the mice is euthanized when they reach 2 months of age.

[000902] Histology and H&E staining of the harvested retinal tissue is conducted to count the number of cells rescued in each retina of the mice (described in Sanges et al., The Journal of Clinical Investigation, 126(8): 3104-3116, 2016).

[000903] The level of the injected protein is measured in retinas harvested from mice euthanized at 2 months of age via a western blot with an antibody specific to the PDE6B protein.

[000904] In some embodiments, the left eyes of mice, which are administered fusosomes, will have an increased number of nuclei present in the outer nuclear level of the retina compared to the right eyes of mice, which are treated with vehicle. The increased protein is suggestive of complementation of the mutated PBE6B protein.

Example 101: Delivery to edit recipient DNA

[000905] This example describes fusosomes for delivery of genome CRISPR-Cas9 editing machinery to a cell *in vitro.* In some embodiments, delivery of genome CRISPR-Cas9 editing machinery to a cell *in vitro* via a fusosome results in a loss of function of a specific protein in a recipient cell. Genome editing machinery referred to, in this example, is the *S. pyogenes* Cas9 protein complexed with a guide RNA (gRNA) specific for GFP.

[000906] In some embodiments, fusosomes are a chassis for the delivery of therapeutic agents. In some embodiments, therapeutic agents, such as genome editing machinery that can be delivered to cells with high specificity and efficiency could be used to inactivate genes, and thus subsequent gene products (e.g. proteins) that when expressed at high levels or in the wrong cell type become pathological.

[000907] A fusosome composition as produced by any one of the methods described in previous Examples, except the fusosome is engineered such that the fusosome also includes the *S. pyogenes* Cas9 protein complexed with a guide RNA (gRNA) sequence that is specific for the sequence of *A. Victoria* EGFP. This is achieved by co-nucleofecting a PiggyBac vector that has the open reading frame of the Neomycin resistance gene that is an in-frame fusion with the open reading frame of *S. pyogenes* Cas9, separated by a P2A cleavage sequence. The additional co-nucleofected PiggyBac vector also includes the gRNA sequence (GAAGTTCGAGGGCGACACCC) driven by the U6 promoter. As a negative control a fusosome is engineered such that the fusosome includes the *S. pyogenes* Cas9 protein complexed with a scrambled gRNA (GCACTACCAGAGCTAACTCA) sequence that is not-specific for any target in the mouse genome.

[000908] A sufficient number of fusosomes are incubated at 37°C and 5% CO_2 together with NIH/3T3 GFP+ cells for a period of 48h in in DMEM containing 20% Fetal Bovine Serum and 1x Penicillin/Streptomycin. Following the 48 hr incubation, genomic DNA is prepared and used as a template with primers specific for region within 500 bp of the predicted gRNA cleavage site in the GFP gene (see Table 25).

Table 25. GFP Primers sequences that amplify a 500bp fragment for TIDE analysis

Primer	Sequence (SEQ ID NO)
GFP-F	ATGAGTAAAGGAGAAGAACTTTTCAC (SEQ ID NO: 602)
GFP-R	GTCCTTTTACCAGACAACCATTAC (SEQ ID NO: 603)

[000909] The PCR amplicon is then purified, sequenced by capillary sequencing and then uploaded to Tide Calculator, a web tool that rapidly assesses genome editing by CRISPR-Cas9 of a target locus determined by a guide RNA. Based on the quantitative sequence trace data from two

standard capillary sequencing reactions, the software quantifies the editing efficacy. An indel (insert or deletion) at the predicted gRNA cleavage site with the GFP locus results in the loss of GFP expression in the cells and is quantified via FACS using a FACS analysis (Becton Dickinson, San Jose, CA, USA) with 488nm argon laser excitation and emission is collected at 530+/-30nm. FACS software is used for acquisition and analysis. The light scatter channels are set on linear gains, and the fluorescence channels on a logarithmic scale, with a minimum of 10,000 cells analyzed in each condition. The indel and subsequent loss of GFP function is calculated based on the intensity of GFP signal in each sample.

[000910] In some embodiments, an indel (insert or deletion) at the predicted gRNA cleavage site with the GFP locus and loss of GFP fluorescence in the cell, in comparison to the negative control, will indicate the ability of a fusosome to edit DNA and result in a loss of protein function in vitro. In some embodiments, fusosomes with the scrambled gRNA sequence will demonstrate no indels or subsequent loss of protein function.

Example 102: Assessment of teratoma formation after administration of fusosome

[000911] This Example describes the absence of teratoma formation with a fusosome. In some embodiments, a fusosome will not result in teratoma formation when administered to a subject.

[000912] Fusosomes are produced by any one of the methods described in a previous Example. Fusosomes, tumor cells (positive control) or vehicle (negative control) are subcutaneously injected in PBS into the left flank of mice (12-20 weeks old). Teratoma, e.g., tumor, growth is analyzed 2-3 times a week by determination of tumor volume by caliper measurements for eight weeks after fusosome, tumor cell, or vehicle injection.

[000913] In some embodiments, mice administered fusosomes or vehicle will not have a measurable tumor formation, e.g., teratoma, via caliper measurements. In some embodiments, positive control animals treated with tumor cells will demonstrate an appreciable tumor, e.g., teratoma, size as measured by calipers over the eight weeks of observation.

Example 103: Fusosomes deliver active protein to recipient cells of a subject in vivo

[000914] This Example demonstrates that fusosomes can deliver a protein to a subject

in vivo. This is exemplified by delivery of the nuclear editing protein Cre. Once inside of a cell, Cre translocates to the nucleus, where it recombines and excises DNA between two LoxP sites. Cre-mediated recombination can be measured microscopically when the DNA between the two LoxP sites is a stop codon and is upstream of a distal fluorescent protein, such as the red fluorescent protein tdTomato.

[000915] Fusosomes that contain CRE and the fusogen VSV-G, purchased from Takara (Cre Recombinase Gesicles, Takara product 631449), were injected into B6.Cg-*Gt(ROSA)26Sor*$^{tm9(CAG-tdTomato)Hze}$/J mice (Jackson Laboratories strain 007909). Animals were injected at the anatomical sites, injection volumes, and injection sites as described in Table 26. Mice that do not have tdTomato (FVB.129S6(B6)-*GT(ROSA)26Sor*$^{tm1(Luc)Kael}$/J, Jackson Laboratories strain 005125) and were injected with fusosomes and B6.Cg-*Gt(ROSA)26Sor*$^{tm14(CAG-tdTomato)Hze}$/J mice that were not injected with fusosomes were used as negative controls.

Table 26: Injection parameters

Anatomical Site	**Injection Volume**	**Injection Site(s)**
Brain	10 µL	anterior posterior axis: -2 Lateral/medial axis: 1.8 ventral: 1.5 side: right
Eye	1 µL	intravitreal
Liver	25 µL	center of frontal lobe
Spleen	10 µL	approximately in the center, both lengthwise and widthwise
Kidney	20 µL	center of left kidney
Small intestine lining	10 µL	loop of small intestine nearest the peritoneal wall was isolated outside peritoneum, and injected into lining.

Anatomical Site	Injection Volume	Injection Site(s)
Heart	5 µL	near apex
White Adipose (Epididymal fat pad)	25 µL	left, top and central
Brown adipose (intrascapular)	25 µL	left lobe, as central as possible
Lung	10 µL	inferior lobe right lung
Testis	10 µL	left testis, as central as possible
Ovary	1 µL	left ovary, as central as possible

[000916] Two days after injections, the animals were sacrificed and samples were collected. The samples were fixed for 8 hours in 2% PFA, fixed overnight in 30% sucrose, and shipped for immediate embedding in OCT and sectioning to slides. Slides were stained for nuclei with DAPI. DAPI and tdTomato fluorescence was imaged microscopically.

[000917] All anatomical sites listed in Table 26 demonstrated tdTomato fluorescence (Figure 9). In addition, delivery to muscle tissue was confirmed using fluorescence microscopy for tdTomato (Figure 11). Negative control mice did not have any tissues with tdTomato fluorescence. This result demonstrates that fusosomes are capable of turning on tdTomato fluorescence in the cells of a mouse at various anatomical sites, and that this does not occur if the mice are not treated with fusosomes or if the mice do not have tdTomato in their genome. Hence, fusosomes deliver active Cre recombinase to the nucleus of mouse cells *in vivo*.

[000918] It was also shown that different routes of administration can deliver deliver fusosomes to tissue *in vivo*. Fusosomes that contain CRE and the fusogen VSV-G, purchased from Takara (Cre Recombinase Gesicles, Takara product 631449), were injected into FVB.129S6(B6)-*GT(ROSA)26Sor*$^{tm1(Luc)Kael}$/J (Jackson Laboratories strain 005125) intramuscularly (in 50 µL to the right tibialis anterior muscle), intraperitoneally (in 50 µL to the peritoneal cavity), and

subcutaneously (in 50 µL under the dorsal skin).

[000919] The legs, ventral side, and dorsal skin was prepared for intramuscular, intraperitoneal, and subcutaneous injection, respectively, by depilating the area using a chemical hair remover for 45 seconds, followed by 3 rinses with water.

[000920] On day 3 after injection, an in vivo imaging system (Perkin Elmer) was used to obtain whole animal images of bioluminescence. Five minutes before imaging, mice received an intraperitoneal injection of bioluminescent substrate (Perkin Elmer) at a dose of 150mg/kg in order to visualize luciferase. The imaging system was calibrated to compensate for all device settings.

[000921] Administration by all three routes resulted in luminescense (Figure 10) indicating successful delivery of active Cre recombinase to mouse cells *in vivo*.

[000922] In conclusion, fusosomes are capable of delivering active protein to cells of a subject *in vivo.*

Example 104: Sonication-mediated loading of nucleic acid in fusosomes

[000923] This example describes loading of nucleic acid payloads into a fusosome via sonication. Sonication methods are disclosed e.g., in Lamichhane, TN, *et al.*, Oncogene Knockdown via Active Loading of Small RNAs into Extracellular Vesicles by Sonication. *Cell Mol Bioeng,* (2016), the entire contents of which are hereby incorporated by reference.

[000924] Fusosomes are prepared by any one of the methods described in a previous example. Approximately 10^6 fusosomes are mixed with 5-20µg nucleic acid and incubated at room temperature for 30 minutes. The fusosome/nucleic acid mixture is then sonicated for 30 seconds at room temperature using a water bath sonicator (Brason model #1510R-DTH) operated at 40kHz. The mixture is then placed on ice for one minute followed by a second round of sonication at 40kHz for 30 seconds. The mixture is then centrifuged at 16,000g for 5 minutes at 4ºC to pellet the fusosomes containing nucleic acid. The supernatant containing unincorporated nucleic acid is removed and the pellet is resuspended in phosphate-buffered saline. After DNA loading, the fusosomes are kept on ice before use.

Example 105: Sonication-mediated loading of protein in fusosomes

[000925] This example describes loading of protein payloads into a fusosome via sonication. Sonication methods are disclosed e.g., in Lamichhane, TN, *et al.*, Oncogene Knockdown via Active Loading of Small RNAs into Extracellular Vesicles by Sonication. *Cell Mol Bioeng,* (2016), the entire contents of which are hereby incorporated by reference.

[000926] Fusosomes are prepared by any one of the methods described in a previous example. Approximately 10^6 fusosomes are mixed with 5-20μg protein and incubated at room temperature for 30 minutes. The fusosome/protein mixture is then sonicated for 30 seconds at room temperature using a water bath sonicator (Brason model #1510R-DTH) operated at 40kHz. The mixture is then placed on ice for one minute followed by a second round of sonication at 40kHz for 30 seconds. The mixture is then centrifuged at 16,000g for 5 minutes at 4°C to pellet the fusosomes containing protein. The supernatant containing unincorporated protein is removed and the pellet is resuspended in phosphate-buffered saline. After protein loading, the fusosomes are kept on ice before use.

Example 106: Hydrophobic-carrier mediated loading of nucleic acid in fusosomes

[000927] This example describes loading of nucleic acid payloads into a fusosome via hydrophobic carriers. Exemplary methods of hydrophobic loading are disclosed, e.g., in Didiot *et al.*, Exosome-mediated Delivery of Hydrophobically Modified siRNA for Huntingtin mRNA Silencing, *Molecular Therapy* 24(10): 1836–1847, (2016), the entire contents of which are hereby incorporated by reference.

[000928] Fusosomes are prepared by any one of the methods described in a previous example. The 3' end of a RNA molecule is conjugated to a bioactive hydrophobic conjugate (triethylene glycol–Cholesterol). Approximately 10^6 fusosomes are mixed in 1 mL with 10 μmol/l of siRNA conjugate in PBS by incubation at 37° C for 90 minutes with shaking at 500 rpm. The hydrophobic carrier mediates association of the RNA with the membrane of the fusosome. In some embodiments, some RNA molecules are incorporated into the lumen of the fusosome, and some are present on the surface of the fusosome. Unloaded fusosomes are separated from RNA-loaded fusosomes by ultracentrifugation for 1 hour at 100,000g, 4° C in a tabletop ultracentrifuge using a

TLA-110 rotor. Unloaded fusosomes remain in the supernatant and RNA-loaded fusosomes form a pellet. The RNA-loaded fusosomes are resuspended in 1 mL PBS and kept on ice before use.

Example 107: Processing fusosomes

[000929] This example described the processing of fusosomes. Fusosomes produced via any of the described methods in the previous Examples may be further processed.

[000930] In some embodiments, fusosomes are first homogenized, e.g., by sonication. For example, the sonication protocol includes a 5 second sonication using an MSE sonicator with microprobe at an amplitude setting of 8 (Instrumentation Associates, N.Y.). In some embodiments, this short period of sonication is enough to cause the plasma membrane of the fusosomes to break up into homogenously sized fusosomes. Under these conditions, organelle membranes are not disrupted and these are removed by centrifugation (3,000 rpm, 15 min 4°C). Fusosomes are then purified by differential centrifugation as described in Example 16.

[000931] Extrusion of fusosomes through a commercially available polycarbonate membrane (e.g., from Sterlitech, Washington) or an asymmetric ceramic membrane (e.g., Membralox), commercially available from Pall Execia, France, is an effective method for reducing fusosome sizes to a relatively well defined size distribution. Typically, the suspension is cycled through the membrane one or more times until the desired fusosome size distribution is achieved. The fusosomes may be extruded through successively smaller pore membranes (e.g., 400 nm, 100 nm and/or 50 nm pore size) to achieve a gradual reduction in size and uniform distribution.

[000932] In some embodiments, at any step of fusosome production, though typically prior to the homogenization, sonication and/or extrusion steps, a pharmaceutical agent (such as a therapeutic), may be added to the reaction mixture such that the resultant fusosome encapsulates the pharmaceutical agent.

Example 108: Measuring total RNA in a fusosome and source cell

[000933] This Example describes a method to quantify the amount of RNA in a fusosome relative to a source cell. In some embodiments, a fusosome will have similar RNA levels to the source cell. In this assay, RNA levels are determined by measuring total RNA.

[000934] Fusosomes are prepared by any one of the methods described in previous Examples.

Preparations of the same mass as measured by protein of fusosomes and source cells are used to isolate total RNA (e.g., using a kit such as Qiagen RNeasy catalog #74104), followed by determination of RNA concentration using standard spectroscopic methods to assess light absorbance by RNA (e.g. with Thermo Scientific NanoDrop).

[000935] In some embodiments, the concentration of RNA in fusosomes will be 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% of that of source cells per mass of protein.

Example 109: Fusosme Fusing to T cell In Vitro

a. DNA Payload

[000936] This example describes the delivery of DNA using fusosomes to CD3+ T cells *in vitro*. This example quantifies the ability of fusosomes to deliver DNA using a plasmid encoding an exogenous gene, a chimeric antigen receptor directed against CD19, which is a therapeutic cargo.

[000937] A fusosome composition, resulting from cell-derived vesicles or cell-derived cytobiologics as produced by any one of the methods described in previous Examples, except the fusosome is engineered such that the fusogen is in-frame with the open reading frame of Cre but separated by a P2A self-cleaving peptide sequence. Following production of the fusosome, it is additionally nucleofected with a plasmid having a sequence that codes for the CAR.

[000938] See, for example, Chen X, et al., Genes Dis. 2015 Mar;2(1):96-105.DOI:10.1016/j.gendis.2014.12.001.

[000939] As a negative control, fusosomes are nucleofected with a plasmid that codes for GFP.

[000940] A sufficient number of fusosomes are then incubated at 37°C and 5% CO_2 together with recipient CD3+ T cells that have a loxP-STOP-loxP-tdTomato reporter for a period of 48h in T-cell medium comprising X-VIVO 15 medium (Lonza, Basel, CH, Switzerland) supplemented with 5% fetal bovine serum (FBS) (Gibco, LAX, CA, USA), 100 U mL– 1 penicillin, 100 µg mL – 1 streptomycin, 1.25 µg mL – 1 amphotericin B, 2 mM L-glutamine (Gibco), and 100 U mL – 1 hIL-2 (PerproTech, Rocky Hill, CT, USA). Fusosome fusion with the CD3+ T cells that have have a loxP-STOP-loxP-tdTomato reporter results in tdTomato expression due to Cre recombinase

excising from DNA the stop codon that blocks tdTomato expression. Following the 48 hr incubation, the tdTomato positive cells are then isolated via FACS, using a FACS cytometer (Becton Dickinson, San Jose, CA, USA) with 561nm laser excitation and emission is collected at 590+/-20nm. Total DNA is then isolated using a DNA extraction solution (Epicentre). Quantitative Real-time PCR is performed using a QuantStudio3 Real-time PCR System (ThermoFisher Scientific) with TaqMan® Fast Advanced Master Mix (ThermoFisher Scientific), 100ng of DNA template, a primer and probe set that is specific for the variable regions of the anti-CD19 CAR (designed using Taqman online primer and probe design program). cA standard curve is prepared for absolute quantitation of anti-CD19 CAR transgene DNA copies by making serial dilutions of the plasmid that encodes the CAR. A primer and probe set specific for beta-lactamase (AMP^r gene) was used to normalize for DNA quantity. The C_t value is used to compare the amount of CAR DNA in the CD3+ T cells treated with fusosomes with CAR plasmid or with negative control.

In some embodiments, delivery of DNA cargo with fusosomes *in vitro* is higher in fusosomes with CAR plasmid as compared to the negative control.

b. mRNA Payload

[000941] This example describes the delivery of mRNA using fusosomes to CD3+ T cells *in vitro*. This example quantifies the ability of fusosomes to deliver mRNA encoding an exogenous gene, a chimeric antigen receptor directed against CD19, which is a therapeutic cargo.

[000942] A fusosome composition, resulting from cell-derived vesicles or cell-derived cytobiologics as produced by any one of the methods described in previous Examples, except the fusosome is engineered such that the fusogen is in-frame with the open reading frame of Cre but separated by a P2A self-cleaving peptide sequence. Following production of the fusosome, it is additionally nucleofected with an mRNA having a sequence that codes for the CAR. See, for example, Chen X, et al., Genes Dis. 2015 Mar;2(1):96- 105.DOI:10.1016/j.gendis.2014.12.001.

[000943] As a negative control, fusosomes are nucleofected with an mRNA that codes for GFP.

[000944] A sufficient number of fusosomes are then incubated at 37°C and 5% CO_2 together with recipient CD3+ T cells that have a loxP-STOP-loxP-tdTomato reporter for a period of 48h in T-cell medium comprising X-VIVO 15 medium (Lonza, Basel, CH, Switzerland) supplemented

with 5% fetal bovine serum (FBS) (Gibco, LAX, CA, USA), 100 U mL– 1 penicillin, 100 μg mL – 1 streptomycin, 1.25 μg mL – 1 amphotericin B, 2 mM L-glutamine (Gibco), and 100 U mL – 1 hIL-2 (PerproTech, Rocky Hill, CT, USA). Fusosome fusion with the CD3+ T cells that have a loxP-STOP-loxP-tdTomato reporter results in tdTomato expression due to Cre recombinase excising from DNA the stop codon that blocks tdTomato expression. Following the 48 hr incubation, the tdTomato positive cells are then isolated via FACS, using a FACS cytometer (Becton Dickinson, San Jose, CA, USA) with 561nm laser excitation and emission is collected at 590+/-20nm.

[000945] Total RNA is isolated (e.g., using a kit such as Qiagen RNeasy catalog #74104), followed by determination of RNA concentration using standard spectroscopic methods to assess light absorbance by RNA (e.g. with Thermo Scientific NanoDrop). Reverse transcription is performed using the Superscript III First-Strand Synthesis supermix for RT-PCR (Thermo Fisher Scientific), and RNA (100 ng) is reverse transcribed into cDNA. Quantitative Real-time PCR is performed using a QuantStudio3 Real-time PCR System (ThermoFisher Scientific) with TaqMan® Fast Advanced Master Mix (ThermoFisher Scientific), 100ng of cDNA template, a primer and probe set that is specific for the variable regions of the anti-CD19 CAR (designed using Taqman online primer and probe design program), and primer probe set designed to amplify β–actin as an endogenous loading control. The C_t value is used to compare the amount of CAR cDNA in the qRT-PCR reaction between CD3+ T cells treated with fusosomes containing the CAR mRNA and treated with fusosomes containing negative control. The relative expression is calculated using the ΔΔCt method. A higher relative expression level of CAR is due to a higher level of CAR mRNA that is purified from the sorted CD3+ T cells.

[000946] In some embodiments, delivery of mRNA cargo with fusosomes *in vitro* is higher in fusosomes containing CAR mRNA as compared to negative control fusosomes.

c. Protein / mRNA Payload, wherein payload is expressed by donor cell

[000947] This example describes fusosome fusion with a cell *in vitro*. In some embodiments, fusosome fusion with a CD3+ T cell in vitro results in delivery of a chimeric antigen receptor protein to the membrane of the CD3+ T cell.

[000948] A fusosome composition, resulting from cell-derived vesicles or cell-derived

cytobiologics as produced by any one of the methods described in previous Examples, except the fusosome is engineered such that the fusogen is in-frame with the open reading frame of Cre and with the open reading frame of a CAR that targets CD19, separated by a P2A and T2A self-cleaving peptide sequence respectively. A negative control fusosme is engineered such that the fusogen is in-frame with the open reading frame of Cre and with the open reading frame of the blue fluorescent protein mTagBFP2, each separated by a P2A self-cleaving peptide sequence. See, for example, Chen X, et al., Genes Dis. 2015 Mar;2(1):96-105.DOI:10.1016/j.gendis.2014.12.001.

[000949] A sufficient number of fusosomes are then incubated at 37°C and 5% CO_2 together with recipient CD3+ T cells that have a loxP-STOP-loxP-tdTomato reporter for a period of 48h in T-cell medium comprising X-VIVO 15 medium (Lonza, Basel, CH, Switzerland) supplemented with 5% fetal bovine serum (FBS) (Gibco, LAX, CA, USA), 100 U mL– 1 penicillin, 100 μg mL – 1 streptomycin, 1.25 μg mL – 1 amphotericin B, 2 mM L-glutamine (Gibco), and 100 U mL – 1 hIL-2 (PerproTech, Rocky Hill, CT, USA. Fusosome fusion with the CD3+ T cells that have a loxP-STOP-loxP-tdTomato reporter results in tdTomato expression due to Cre recombinase excising from DNA the stop codon that blocks tdTomato expression. Following the 48 hr incubation, the tdTomato positive cells are then isolated via FACS, using a FACS cytometer (Becton Dickinson, San Jose, CA, USA) with 561nm laser excitation and emission is collected at 590+/-20nm.

[000950] mRNA delivery to the sorted T cells is assayed. Total RNA is isolated (e.g., using a kit such as Qiagen RNeasy catalog #74104), followed by determination of RNA concentration using standard spectroscopic methods to assess light absorbance by RNA (e.g. with Thermo Scientific NanoDrop). Reverse transcription is performed using the Superscript III First-Strand Synthesis supermix for RT-PCR (Thermo Fisher Scientific), and RNA (100 ng) is reverse transcribed into cDNA. Quantitative Real-time PCR is performed using a QuantStudio3 Real-time PCR System (ThermoFisher Scientific) with TaqMan® Fast Advanced Master Mix (ThermoFisher Scientific), 100ng of cDNA template, a primer and probe set that is specific for the variable regions of the anti-CD19 CAR (designed using Taqman online primer and probe design program), and primer probe set designed to amplify β–actin as an endogenous loading control. The C_t value is

used to compare the amount of CAR cDNA in the qRT-PCR reaction between CD3+ T cells treated with fusosomes containing the CAR mRNA and treated with fusosomes containing negative control. The relative expression is calculated using the ΔΔCt method. A higher relative expression level of CAR is due to a higher level of CAR mRNA that is purified from the sorted CD3+ T cells.

[000951] In some embodiments, delivery of the CAR mRNA cargo with fusosomes *in vitro* is higher in fusosomes derived from cells expressing the CAR as compared to the negative control fusosmes derived from cells expressing CFP.

[000952] CAR expression on the surface of the sorted cells is assayed. Sorted tdTomato+ CD3+ cells are incubated with CD19sIg1-4 conjugated to Alexa Fluor 488 (CD19sIg1-4:AF488), as described in De Oliveira et al., J Transl Med 11:23, 2013. CD19sIg1-4:AF488 labels cells that express CD19 CAR. 2×10^5 cells are incubated with 450 ng of CD19sIg1-4:AF488 at 4°C for 30 minutes in the dark, after being blocked by human serum from AB plasma (Sigma-Aldrich) for 10 minutes. After being washed two times with PBS, cells are analyzed on a LSR II (BD Biosciences, San Jose, CA.) machine running the FACSDiva™ software (BD Biosciences, San Jose, CA.). tdTomato+ CD3+ cells cells that were incubated with the negative control fusogen are used to set up the negative gate for CD19sIg1-4:AF488 signal. The gate is chosen such that % of positive events for CD19sIg1-4:AF488 is equal to 0.0% The percent of events that are positive for CD19sIg1-4:AF488 is measured in sorted cells that were treated with fusosomes derived from cells expressing the CAR.

[000953] In some embodiments, the percent of sorted cells with surface CAR expression is higher in cells treated with fusosomes derived from cells expressing the CAR as compared to the negative control fusosmes derived from cells expressing mTagBFP2.

Example 110: T-cell Specific Fusosme Fusing to T cell In Vitro

[000954] This example describes fusosome fusion that is preferential for a CD3+ T cell *in vitro*. In some embodiments, the fusosome delivers its payload to a CD3+ T cell at a greater efficiency than an alternative cell type.

[000955] A fusosome composition, resulting from cell-derived vesicles or cell-derived cytobiologics as produced by any one of the methods described in previous Examples, except the

fusosome is engineered such that the fusogen is in-frame with the open reading frame of Cre but separated by a P2A self-cleaving peptide sequence.

[000956] A sufficient number of fusosomes are then incubated at 37°C and 5% CO_2 together with recipient CD3+ T cells that have a loxP-STOP-loxP-tdTomato reporter for a period of 48h in T-cell medium comprising X-VIVO 15 medium (Lonza, Basel, CH, Switzerland) supplemented with 5% fetal bovine serum (FBS) (Gibco, LAX, CA, USA), 100 U mL– 1 penicillin, 100 µg mL – 1 streptomycin, 1.25 µg mL – 1 amphotericin B, 2 mM L-glutamine (Gibco), and 100 U mL – 1 hIL-2 (PerproTech, Rocky Hill, CT, USA. Fusosome fusion with the CD3+ T cells that have a loxP-STOP-loxP-tdTomato reporter results in tdTomato expression due to Cre recombinase excising from DNA the stop codon that blocks tdTomato expression.

[000957] In a separate experiment, the same number of fusosomes are incubated at 37°C and 5% CO_2 together with a recipient NIH/3T3 fibroblast cell line that has a loxP-STOP-loxP-tdTomato reporter for a period of 48h in in DMEM containing 20% Fetal Bovine Serum and 1x Penicillin/Streptomycin. Fusosome fusion with the NIH/3T3 fibroblasts that have a loxP-STOP-loxP-tdTomato reporter results in tdTomato expression due to Cre recombinase excising from DNA the stop codon that blocks tdTomato expression.

[000958] Following the 48 hr incubations, cells are run on a FACS cytometer (Becton Dickinson, San Jose, CA, USA) with 561nm laser excitation and emission collected at 590+/-20nm. A gate is set up to measure positive tdTomato expression. The gate is chosen such that CD3+ T cells and NIH/3T3 fibroblasts that have not been contacted with fusosmes are all negative. The percent of cells that are positive for tdTomato expression is measured in CD3+ T cells and NIH/3T3 fibroblasts that have been contacted by fusosomes.

[000959] In some embodiments, a percent of cells that are positive for tdTomato expression is higher in CD3+ cells contacted with fusosomes than in NIH/3T3 fibroblasts contacted with fusosomes, which demonstrates that fusosomes fuse preferentially with the target CD3+ cells.

Example 111: T-cell Specific Fusosme Fusing to T cell In Vivo

[000960] This example describes fusosome fusion that is preferential for a CD3+ T cell *in vivo*. In some embodiments, a fusosome delivers its payload to a CD3+ T cell at a greater efficiency

than any alternative cell type.

[000961] A fusosome composition, resulting from cell-derived vesicles or cell-derived cytobiologics as produced by any one of the methods described in previous Examples, except the fusosome is engineered such that the fusogen is in-frame with the open reading frame of Cre but separated by a P2A self-cleaving peptide sequence.

[000962] 3 x 10^{11} fusosomes or PBS are then administered slowly over 20 min through a rodent tail-vein catheter using a programmable BS-300 infusion pump (both Braintree Scientific Inc.) daily for 5 days to B6.Cg-*Gt(ROSA)26Sor*$^{tm9(CAG\text{-}tdTomato)Hze}$/J mice (Jackson Laboratories strain 007909). Three days after the final treatment, peripheral blood is collected from fusosome treated mice and mice that received PBS treatment. Blood is collected into 1ml PBS containing 5 μM EDTA and mixed immediately to prevent clotting. The tubes are kept on ice and red blood cells are removed using a buffered ammonium chloride (ACK) solution. Cells are stained with a murine CD3-FITC antibody (Thermo Fisher **Catalog #:**11-0032-82), at 4°C for 30 minutes in the dark, after being blocked with bovsine serum albumin for 10 minutes. After being washed two times with PBS, cells are analyzed on a LSR II (BD Biosciences, San Jose, CA.) with 488nm laser excitation and emission collected at 530+/-30nmrunning the FACSDiva™ software (BD Biosciences, San Jose, CA.). Unstained cells from mice that received PBS treatment are used to draw a gate for negative FITC and negative tdTomato fluorescence.

[000963] In some embodiments, a percent of cells that are positive for tdTomato fluorescence is higher in mice treated with fusosomes than in mice treated with PBS. In some embodiments, a percent of tdTomato positive cells that stain positive for FITC is greater than those that stain negative for FITC in mice treated with fusosomes. This demonstrates that fusosomes targeting CD3+ cells specifically fuse with CD3+ cells *in vivo.*

Example 112: T cells engineered *in vitro* lyse cells associated with a target antigen *in vitro*

[000964] This example demonstrates that CD3+ T cells expressing a CAR after being contacted by a fusosome as described in any one of the methods in previous Examples are capable of lysing cells associated with a target antigen, e.g., CD19, *in vitro*.

[000965] CD3+ T cells expressing a CAR targeting CD19 are incubated with CD19+ Eμ-ALL01 leukaemia cells (target) or CD19- B16F10 melanoma tumor cells (control). Prior to the incubation, the CD3+ T cells are activated using CD3- and CD28-specific magnetic beads at three beads/cell (Invitrogen Life Technologies, Carlsbad, CA, USA and theEμ-ALL01 leukaemia cells and B16F10 melanoma tumor cells are labeled with membrane dye PKH-26 (Sigma-Aldrich), washed with RPMI containing 10% foetal calf serum, and resuspended in the same medium at a concentration of $1x10^5$ tumor cells per mL. T cells are then added to the suspension at various ratios of T cell to tumor cell, ranging from 0 T cells : 1 tumor cells to 100 T cells : 1 tumor cells, in 96-well plates (final volume, 200 μl), and incubated for 3 h at 37 °C. Then, cells are transferred to V-bottom 96-well plates and stained with Annexin V-Brilliant Violet 421 (BioLegend). Following a wash in PBS, the cells are analyzed by flow cytometry on a LSR II (BD Biosciences, San Jose, CA.) machine running the FACSDiva™ software (BD Biosciences, San Jose, CA.). Cells from the incubation of 0 T cells : 1 tumor cells and a separate batch of T cells are first run on the flow cytometer. A gate is first drawn to distinguish T cells and tumor cells based upon PKH-26 fluorescence. The gate is drawn such that T cells are negative and tumor cells that were incubated with PKH-26 are positive. Next, a gate is drawn to measure Annexin V-Brilliant Violet 421 staining. The gate is drawn such that the cells from the incubation of 0 T cells : 1 tumor cells are all negative for Annexin V-Brilliatn Violet 421. Using these two gates, cells from each of the incubations of various ratios of T cells to tumor cells are run on the flow cytometer.

[000966] In some embodiments, a percent of cells that are positive for PKH-26 and Annexin V-Brilliant Violet 421 increases with increasing ratios of T cells to tumor cells for the Eμ-ALL01 leukaemia cells, and the percent of cells that are positive for PKH-26 and Annexin V-Brilliant Violet 421 does not increase with increasing ratios of T cells to tumor cells for the B16F10 melanoma tumor cells. This demonstrates that T cells that express a CAR targeting CD19 after being contacted by fusomes are capable of specifically lysing cells that are CD19-positive.

[000967] See, for example, Smith T, et al., Nature Nanotechnology. 2017. DOI: 10.1038/NNANO.2017.57

Example 113: T cells engineered *in vivo* lyse cells associated with a target antigen *in vitro*

[000968] This example demonstrates that CD3+ T cells engineered to express a CAR after being contacted by a fusosomes *in vivo* are capable of lysing cells associated with a target antigen *in vitro*.

[000969] A fusosome composition, resulting from cell-derived vesicles or cell-derived cytobiologics as produced by any one of the methods described in previous Examples, except the fusosome is engineered such that the fusogen is in-frame with the open reading frame of Cre but separated by a P2A self-cleaving peptide sequence. In addition, the fusosome is engineered to deliver a CAR targeting CD-19 to a target cell as described in previous Examples.

[000970] 3 x 10^{11} fusosomes or PBS are then administered slowly over 20 min through a rodent tail-vein catheter using a programmable BS-300 infusion pump (both Braintree Scientific Inc.) daily for 5 days to B6.Cg-*Gt(ROSA)26Sor*$^{tm9(CAG\text{-}tdTomato)Hze}$/J mice (Jackson Laboratories strain 007909). Three days after the final treatment, peripheral blood is collected from fusosome treated mice and mice received PBS treatment. Blood is collected into 1ml PBS containing 5 μM EDTA and mixed immediately to prevent clotting. The tubes are kept on ice and red blood cells are removed using a buffered ammonium chloride (ACK) solution. Cells are stained with a murine CD3-FITC antibody (Thermo Fisher **Catalog #:**11-0032-82), at 4°C for 30 minutes in the dark, after being blocked with bovsine serum albumin for 10 minutes. After being washed two times with PBS, cells are analyzed on a LSR II (BD Biosciences, San Jose, CA.) with 488nm laser excitation and emission collected at 530+/-30nmrunning the FACSDiva™ software (BD Biosciences, San Jose, CA.). Sorted cells from mice treated with fusosomes or PBS are then incubated with CD19+ Eμ-ALL01 leukaemia cells. Prior to the incubation, Eμ-ALL01 leukaemia cells are labeled with membrane dye PKH-26 (Sigma-Aldrich), washed with RPMI containing 10% foetal calf serum, and resuspended in the same medium at a concentration of 1x10^5 tumor cells per mL. T cells are then added to the suspension at various ratios of T cell to tumor cell, ranging from 0 T cells : 1 tumor cells to 100 T cells : 1 tumor cells, in 96-well plates (final volume, 200 μl), and incubated for 3 h at 37 °C. Then, cells are transferred to V-bottom 96-well plates and stained with Annexin V-Brilliant Violet 421 (BioLegend). Following a wash in PBS, the cells are

analyzed by flow cytometry on a LSR II (BD Biosciences, San Jose, CA.) machine running the FACSDiva™ software (BD Biosciences, San Jose, CA.). Cells from the incubation of 0 T cells : 1 tumor cells and a separate batch of sorted T cells are first run on the flow cytometer. A first gate is first drawn to distinguish T cells and tumor cells based upon PKH-26 fluorescence. The gate is drawn such that T cells are negative and tumor cells that were incubated with PKH-26 are positive. Next, a gate is drawn to measure Annexin V-Brilliant Violet 421 staining. The gate is drawn such cells from the incubation of 0 T cells : 1 tumor cells are all negative for Annexin V-Brilliant Violet 421. Using these two gates, cells from each of the incubations of various ratios of T cells to tumor cells are run on the flow cytometer.

[000971] In some embodiments, a percent of cells that are positive for PKH-26 and Annexin V-Brilliant Violet 421 increases with increasing ratios of T cells to Eμ-ALL01 leukaemia cells from mice treated with fusosomes, and the percent of cells that are positive for PKH-26 and Annexin V-Brilliant Violet 421 does not increase with increasing ratios of T cells to Eμ-ALL01 leukaemia cells from mice treated with PBS. This demonstrates that T cells engineered to express a CAR that targets CD19 after treatment with fusosomes are capable of lysing cells associated with CD19. See, for example, Smith T, et al., Nature Nanotechnology. 2017. DOI: 10.1038/NNANO.2017.57

Example 114: T cells engineered in vivo lyse tumor cells associated with a target antigen in vivo

[000972] This example demonstrates that CD3+ T cells engineered to express a CAR after being contacted by a fusosomes *in vivo* are capable of treating a tumor *in vivo*.

[000973] A fusosome composition, resulting from cell-derived vesicles or cell-derived cytobiologics as produced by any one of the methods described in previous Examples, except the fusosome is engineered such that the fusogen is in-frame with the open reading frame of Cre but separated by a P2A self-cleaving peptide sequence. In addition, the fusosome is engineered to deliver a CAR targeting CD-19 as described in previous Examples.

[000974] A model of leukemia is established by systemically injecting luciferase-expressing Eμ-ALL01 leukaemia cells into 4–6-week-old female albino B6 (C57BL/6J-Tyr < c-2J>) mice (Jackson Laboratories) and allowing them to develop for 1 week. Mice are then randomly assigned

to experimental cohorts. 3 x 10^{11} fusosomes or PBS are then administered slowly over 20 min through a rodent tail-vein catheter using a programmable BS-300 infusion pump (both Braintree Scientific Inc.) daily for 5 days.

[000975] Luminescence as a proxy for the number of live leukemia cells is then measured daily. D-luciferin (Xenogen) in PBS (15 mg mL–1) is used as a substrate for F-luc expressed by the leukemia cells. Bioluminescence images are collected with a Xenogen IVIS Spectrum Imaging System (Xenogen). Living Image software version 4.3.1 (Xenogen) is used to acquire (and later quantitate) the data obtained over a range of 10-35 min after intraperitoneal injection of D-luciferin into animals anesthetized with 150 mg kg–1 of 2% isoflurane (Forane, Baxter Healthcare). Acquisition times range from 10 s to 5 min. To correct for background bioluminescence, the signals acquired from tumor-free mice (injected with D-luciferin) is subtracted from the measurement region of interest (ROI).

[000976] In embodiments, the luciferase signal increases over the course of 21 days in mice treated with PBS and the luciferase signal decreases over the course of 21 days in mice treated with fusosomes.

[000977] The survival of mice that received PBS or fusosomes is also tracked. In embodiments, mice that received PBS have a median survival that is less than mice treated with fusosomes.

[000978] This demonstrates that fusosomes are capable of engineering T cells to target tumor cells *in vivo*. See, for example, Smith T, et al., Nature Nanotechnology. 2017. DOI: 10.1038/NNANO.2017.57

Example 115: Fusosomes deliver a transmembrane protein to recipient cells

[000979] This example describes fusosome fusion with a cell *in vitro*. In some embodiments, fusosome fusion results in delivery of a transmembrane protein to a recipient cell.

[000980] A fusosome composition, resulting from cell-derived vesicles or cell-derived cytobiologics as produced by any one of the methods described in previous Examples, except the fusosome is engineered such that the fusogen is in-frame with the open reading frame of Cre and with the open reading frame of the human Insulin Receptor, separated by a P2A and T2A self-

cleaving peptide sequence respectively. A negative control fusosme is engineered such that the fusogen is in-frame with the open reading frame of Cre and with the open reading frame of the blue fluorescent protein mTagBFP2, each separated by a P2A self-cleaving peptide sequence. See, for example, Chen X, et al., Genes Dis. 2015 Mar;2(1):96-105.DOI:10.1016/j.gendis.2014.12.001.

[000981] A sufficient number of fusosomes are then incubated at 37°C and 5% CO_2 together with recipient HEK293 cells that have a loxP-STOP-loxP-tdTomato reporter for a period of 48h in complete media (DMEM + 10% FBS + Pen/Strep). Fusosome fusion with the HEK293 cells that have a loxP-STOP-loxP-tdTomato reporter results in tdTomato expression due to Cre recombinase excising from DNA the stop codon that blocks tdTomato expression. Following the 48 hr incubation, the tdTomato positive cells are then isolated via FACS, using a FACS cytometer (Becton Dickinson, San Jose, CA, USA) with 561nm laser excitation and emission is collected at 590+/-20nm.

[000982] mRNA delivery to the sorted HEK293 cells is assayed. Total RNA is isolated (e.g., using a kit such as Qiagen RNeasy catalog #74104), followed by determination of RNA concentration using standard spectroscopic methods to assess light absorbance by RNA (e.g. with Thermo Scientific NanoDrop). Reverse transcription is performed using the Superscript III First-Strand Synthesis supermix for RT-PCR (Thermo Fisher Scientific), and RNA (100 ng) is reverse transcribed into cDNA. Quantitative Real-time PCR is performed using a QuantStudio3 Real-time PCR System (ThermoFisher Scientific) with TaqMan® Fast Advanced Master Mix (ThermoFisher Scientific), 100ng of cDNA template, a primer and probe set that is specific for the variable regions of the human Insulin Receptor (designed using Taqman online primer and probe design program), and primer probe set designed to amplify β–actin as an endogenous loading control. The C_t value is used to compare the amount of Insulin Receptor cDNA in the qRT-PCR reaction between HEK293 cells treated with fusosomes containing the Insulin Receptor mRNA and treated with fusosomes containing negative control. The relative expression is calculated using the ΔΔCt method. A higher relative expression level of Insulin Receptor is due to a higher level of Insulin Receptor mRNA that is purified from the sorted HEK293 T cells.

[000983] In some embodiments, delivery of the Insulin Receptor mRNA cargo with

fusosomes *in vitro* is higher in fusosomes derived from cells expressing the Insulin Receptor as compared to the negative control fusosmes derived from cells expressing CFP.

[000984] Insulin Receptor expression on the surface of the sorted cells is assayed. Sorted tdTomato+ HEK293 cells are incubated with Insulin Receptor Alpha Antibody conjugated to Alexa Fluor 488 (Bioss Antibodies, catalog number bs-0260R-A488). The antibody labels cells that express the Insulin Receptor proportional to the amount of Insulin Receptor expression. 2×10^5 cells are incubated with 450 ng of the Insulin Receptor Alpha Antibody conjugated to Alexa Fluor 488 at 4°C for 30 minutes in the dark, after being blocked by human serum from AB plasma (Sigma-Aldrich) for 10 minutes. After being washed two times with PBS, cells are analyzed on a LSR II (BD Biosciences, San Jose, CA.) machine running the FACSDiva™ software (BD Biosciences, San Jose, CA.). tdTomato+ HEK293 cells that were incubated with the negative control fusogen are used to set up the negative gate for the Insulin Receptor Alpha Antibody conjugated to Alexa Fluor 488 signal. The gate is chosen such that the percent of positive events for Insulin Receptor Alpha Antibody conjugated to Alexa Fluor 488 is equal to 0.0% The percent of events that are positive for the Insulin Receptor Alpha Antibody conjugated to Alexa Fluor 488 is measured in sorted cells that were treated with fusosomes derived from cells expressing the Insulin Receptor.

[000985] In some embodiments, the percent of sorted cells with surface Insulin Receptor expression is higher in cells treated with fusosomes derived from cells expressing the Insulin Receptor as compared to the negative control fusosmes derived from cells expressing mTagBFP2.

Example 116: Fusosomes deliver a heterologous, signal peptide-targeted transmembrane protein to recipient cells

[000986] This example describes fusosome fusion with a cell *in vitro*. In some embodiments, fusosome fusion with a recipient cell results in the delivery of a heterologous membrane protein payload to the recipient cell's plasma membrane.

[000987] A fusosome composition, resulting from cell-derived vesicles or cell-derived cytobiologics as produced by any one of the methods described in previous Examples, except the fusosome is engineered such that the fusogen is in-frame with the open reading frame of Cre and with the open reading frame of a membrane-targeted GFP, separated by a P2A and T2A self-

cleaving peptide sequence respectively. The membrane-targeted GFP is generated by fusing the N terminus of the coding sequence of GFP to the first twenty-six amino acids of LCK, a Src family tyrosine kinase containing two palmitoylation domains and a single myristoylation domain. A negative control fusosme is engineered such that the fusogen is in-frame with the open reading frame of Cre and with the open reading frame of cytosolic GFP (the coding sequence of GFP without any additional targeting peptide sequences), each separated by a P2A self-cleaving peptide sequence. See, for example, Chen X, et al., Genes Dis. 2015 Mar;2(1):96 105.DOI:10.1016/j.gendis.2014.12.001, and Benediktsson A, et al, Journal of Neuroscience Methods 2005 141, 41-53.

[000988] A sufficient number of fusosomes are then incubated at 37°C and 5% CO_2 together with recipient HEK293 cells that have a loxP-STOP-loxP-tdTomato reporter for a period of 48h in complete media (DMEM + 10% FBS + Pen/Strep). Fusosome fusion with the HEK293 cells that have a loxP-STOP-loxP-tdTomato reporter results in tdTomato expression due to Cre recombinase excising from DNA the stop codon that blocks tdTomato expression. Following the 48 hr incubation, the tdTomato positive cells are then isolated via FACS, using a FACS cytometer (Becton Dickinson, San Jose, CA, USA) with 561nm laser excitation and emission is collected at 590+/-20nm.

[000989] Membrane localization of GFP in the plasma membrane of the sorted HEK293 cells is assayed via confocal microscopy. Prior to confoncal microscopy sorted HEK293 cells are stained with a reagent that labels the plasma membrane (e.g. CellMask Deep Red Plasma Membrane Stain, Invitrogen, Catalog number C10046). Imaging experiments are performed with a Zeiss LSM 710 inverted microscope using a Plan Apochromat 63 x 1.4 numerical aperture oil objective. A 488 nm argon laser is used to excite GFP/EGFP and a 632 nm Helium-Neon laser is used to excite the plasma membrane stain. A MATLAB script is written to determine the average GFP intensity of the plasma membrane and cytosol for each cell. In the script the average intensity in the plasma membrane (defined by 6 pixels on either side of the plasma membrane as defined by the plasma membrane stain) and the cytosol (region within the plasma membrane region) are calculated. The values for plasma membrane and cytosol intensity for each cell are then used to calculate the %plasma membrane localization. The %plasma membrane localization is calculated

with the following equation: plasma membrane intensity over the total (plasma membrane + cytosol) intensity x 100%. See, for example, Johnson A, et al., Scientific Reports 6: 19125 (2015).

In some embodiments, the sorted cells treated with fusosomes containing plasma-membrane localized GFP have higher % plasma membrane localization of GFP than the sorted cells treated with fusosomes containing cytosolic GFP.

Example 117: Fusosome delivery of a nuclear-targeted protein

[000990] This example describes fusosome fusion with a cell *in vitro*. In an embodiment, fusosome fusion with a cell *in vitro* results in delivery of a protein to the recipient cell nucleus.

[000991] A fusosome composition, resulting from cell-derived vesicles or cell-derived cytobiologics as produced by any one of the methods described in previous Examples, contains mRNA encoding LRH-1 (e.g., as described in Mamrosh et al., *eLife* 3: e01694, 2014). As a negative control, a fusosome composition contains mRNA with a missense mutation that prevents translation of LRH-1.

[000992] A sufficient number of fusosomes are then incubated at 37°C and 5% CO2 together with a recipient HEK293 cell line for a period of 12 hours in in DMEM containing 20% Fetal Bovine Serum and 1x Penicillin/Streptomycin.

[000993] Following the 12 hr incubation, the cells are prepared for imaging. The cells are fixed, permeabilized, blocked, and immunostained with an anti-LRH-1 antibody conjugated FITC (for example, LSBio catalog number LC-C423401-100). Following immunostaining, the cells are counterstained with DAPI to label the nucleus.

[000994] In this example cells are imaged on a Zeiss LSM 710 confocal microscope with a 63x oil immersion objective while maintained at 37C and 5% CO2. DAPI is subjected to 405nm laser excitation and FITC is subjected to 488nm laser excitation.

[000995] A MATLAB script is written to determine the average FITC intensity of the nucleus in the field of view. In the script the average intensity in the nucleus (defined by 6 pixels on either side of the nucleus as defined by the DAPI stain). Any FITC signal outside of the nucleus is defined as non-nuclear. The values for nuclear and non-nuclear intensity in each field of view are then used to calculate the %nuclear localization. The %nuclear localization is calculated with the

following equation: nuclear localization intensity over the total (nuclear + non-nuclear) intensity x 100%.

[000996] In some embodiments, the cells treated with fusosomes containing LRH-1 mRNA will have a higher % nuclear localization of FITC signal than the cells treated with fusosomes containing missense LRH-1 mRNA. This assay can be used to show fusosomes delivering therapeutic proteins to the nucleus of recipient cells.

Example 118: Delivery of GFP that does not naturally go to the nucleus, but targets the nucleus upon addition of a nuclear localization sequence

[000997] This example describes fusosome fusion with a cell *in vitro*. In an embodiment, fusosome fusion with a cell *in vitro* results in delivery of a protein to the recipient cell nucleus.

[000998] A fusosome composition, resulting from cell-derived vesicles or cell-derived cytobiologics as produced by any one of the methods described in previous Examples, contains mRNA encoding a translational fusion between EGFP and a nuclear localization sequence (e.g. one of the nuclear localization sequences in Table 6-1). As a negative control, a fusosome composition contains mRNA encoding EGFP without a nuclear localization sequence.

[000999] A sufficient number of fusosomes are then incubated at 37°C and 5% CO2 together with a recipient HEK293 cell line for a period of 12 hours in in DMEM containing 20% Fetal Bovine Serum and 1x Penicillin/Streptomycin.

[0001000] Following the 12 hr incubation, the cells are imaged. Prior to imaging the cells are stained with 1 µg/mL Hoechst 33342 to label the nucleus and with a stain that labels the plasma membrane (e.g. CellMask Deep Red Plasma Membrane Stain, Invitrogen, Catalog number C10046). In this example cells are imaged on a Zeiss LSM 710 confocal microscope with a 63x oil immersion objective while maintained at 37C and 5% CO2. Hoechst is subjected to 405nm laser excitation, EGFP is subjected to 488nm laser excitation, and the plasma membrane stain is subjected to 632 nm Helium-Neon laser excitation.

[0001001] A MATLAB script is written to determine the average EGFP intensity of the nucleus and cytosol for each cell. In the script the average intensity in the nucleus (defined by 6 pixels on either side of the nucleus as defined by the Hoechst 33342 stain) and the cytosol (region

within the plasma membrane region and outside of the nucelus) are calculated. The values for nucleus and cytosol intensity for each cell are then used to calculate the %nuclear localization. The %nuclear localization is calculated with the following equation: nuclear localization intensity over the total (nuclear + cytosol) intensity x 100%.

[0001002] In some embodiments, the cells treated with fusosomes containing EGFP-NLS fusion protein will have higher % nuclear localization of EGFP than the cells treated with fusosomes containing EGFP. This assay can be used to show fusosomes delivering proteins to the nucleus of recipient cells.

Example 119: Delivery of a nanobody containing a nuclear localization sequence that drives localization of an endogenous cytosolic protein to the nucleus in a recipient cell

[0001003] This example describes fusosome fusion with a cell *in vitro*. In an embodiment, fusosome fusion with a cell *in vitro* results in delivery of nanobody containing a nuclear localization sequence that subsequently drives an endogenous cytosolic protein to the nucleus in the recipient cell.

[0001004] A fusosome composition, resulting from cell-derived vesicles or cell-derived cytobiologics as produced by any one of the methods described in previous Examples, contains a DNA plasmid encoding a nanobody that recognizes GFP protein, and the nanobody also contains a nuclear localization sequence (e.g. one of the nuclear localization sequences in Table 6-1). As a negative control, a fusosome composition contains the same GFP-targeted nanobody but lacking the nuclear localization sequence.

[0001005] A sufficient number of fusosomes are then incubated at 37°C and 5% CO2 together with a recipient HEK293 cell line endogenously expressing GFP for a period of 24 hours in in DMEM containing 10% Fetal Bovine Serum and 1x Penicillin/Streptomycin.

[0001006] Following the 24 hr incubation, the cells are imaged. Prior to imaging the cells are stained with 1 µg/mL Hoechst 33342 to label the nucleus and with a stain that labels the plasma membrane (e.g. CellMask Deep Red Plasma Membrane Stain, Invitrogen, Catalog number C10046). In this example cells are imaged on a Zeiss LSM 710 confocal microscope with a 63x oil immersion objective while maintained at 37C and 5% CO2. Hoechst is subjected to 405nm

laser excitation, GFP is subjected to 488nm laser excitation, and the plasma membrane stain is subjected to 632 nm Helium-Neon laser excitation.

[0001007] An ImageJ macro is written to determine the average EGFP intensity of the nucleus and cytosol for each cell. In the macro the average intensity in the nucleus (defined by 6 pixels on either side of the nucleus as defined by the Hoechst 33342 stain after thresholding and subtracting background appopriately) and the cytosol (region within the plasma membrane region and outside of the nucleus) are calculated. The values for nucleus and cytosol integrated pixel intensity for each cell are then used to calculate the % nuclear localization. The % nuclear localization is calculated with the following equation: nuclear localization integrated intensity over the total (nuclear + cytosol) intensity x 100%.

[0001008] In some embodiments, the cells treated with fusosomes containing the GFP-targeted nanobody with nuclear localization sequencen will have higher % nuclear localization of GFP than the cells treated with fusosomes containing GFP-targeted nanobody without the nuclear localization sequence. This assay can be used to show fusosomes delivering a targeted nanobody that can drive endogenous protein localization to the nucleus of recipient cells.

Example 120: Delivery of DNA in the presence of absence of a nuclear-targeting protein

[0001009] This example describes fusosome fusion with a cell *in vitro*. In an embodiment, fusosome fusion with a cell *in vitro* results in delivery of DNA to the nucleus of the cell.

[0001010] A fusosome composition, resulting from cell-derived vesicles or cell-derived cytobiologics as produced by any one of the methods described in previous Examples, contains a DNA plasmid containing a tetracycline repressor (TetR) binding site and a DNA-probe binding site along with TetR-NLS, which is a TetR protein translationally fused to a nuclear localization sequence (e.g. one of the nuclear localization sequences in Table 6-1). As a negative control, a fusosome composition contains on the DNA plasmid containing a tetracycline repressor (TetR) binding site and a DNA-probe binding site. In some embodiments, TetR-NLS will bind to the TetR binding site on the plasmid and carry the plasmid along with it into the nucleus as the protein is transported across the nuclear envelope due to its NLS.

[0001011] A sufficient number of fusosomes are then incubated at 37°C and 5% CO2 together

with a recipient HEK293 cell line for a period of 12 hours in in DMEM containing 20% Fetal Bovine Serum and 1x Penicillin/Streptomycin.

[0001012] Following the 12 hr incubation, the cells are prepared for fluorescence in situ hybridization (FISH). FISH is conducted using FISH Tag DNA Orange Kit, with Alex Fluor 555 dye (ThermoFisher catalog number F32948). Briefly, a DNA probe that binds to the DNA-probe binding site on the plasmid is generated through a procedure of nick translation, dye labeling, and purification as described in the Kit manual. The cells are then labeled with the DNA probe as described in the Kit manual. Subsequent to labeling with the DNA probe, the cells are stained with 1 μg/mL Hoechst 33342 to label the nucleus.

[0001013] Next, the cells are imaged on a Zeiss LSM 710 confocal microscope with a 63x oil immersion objective while maintained at 37C and 5% CO2. Hoechst is subjected to 405nm laser excitation, and the DNA probe is subjected to 555nm laser excitation to stimulate Alexa Flour.

[0001014] A MATLAB script is written to measure the localization of the Alex Fluor intensity of the nucleus for each cell. In the script the average intensity in the nucleus is defined by 6 pixels on either side of the nucleus as defined by the Hoechst 33342 stain.

[0001015] In some embodiments, the cells treated with fusosomes containing the plasmid and TetR-NLS will have higher fluorescence intensity in the nucleus than the cells treated with fusosomes containing the plasmid alone. This assay can be used to show fusosomes delivering a protein that localizes a DNA payload to the nucleus of recipient cells.

Example 121: Delivery of synthetic transcription factor that upregulates gene expression

[0001016] This example describes fusosome fusion with a cell *in vitro*. In an embodiment, fusosome fusion with a cell *in vitro* results in delivery of a transcription factor to the recipient cell nucleus.

[0001017] A fusosome composition, resulting from cell-derived vesicles or cell-derived cytobiologics as produced by any one of the methods described in previous Examples, contains mRNA encoding VP64-p65-Rta translationally fused to the C terminus of a nuclease null Streptococcus pyogenes cas9 (VPR-dCas9) and a guide RNA targeting a protospacer. As a negative control, a fusosome composition contains mRNA encoding VPR-dCas9 with a scrambled

guide RNA. VP64-p65-RTA is a translational fusion of three transcriptional activators (VP64, p65, and RTA) that recruit transcriptional machinery to a gene. In this example, the gene that is affected by VP64-p65-RTA is determined by the location of dCas9 binding DNA as directed by a guide RNA, e.g., as described in Chavez et al., *Nature Methods* 2015 doi: 10.1038/nmeth.3312.

[0001018] A sufficient number of fusosomes are then incubated at 37°C and 5% CO2 for a period of 48 hours in in DMEM containing 20% Fetal Bovine Serum and 1x Penicillin/Streptomycin with a recipient HEK293 cell line that has integrated into the genome a protospacer upstream of a mini-CMV promoter and tdTomato. As an additional negative control, the same HEK293 cells are not exposed to any fusosomes.

[0001019] Following the 48 hr incubation, the cells are imaged. Prior to imaging the cells are stained with 1 μg/mL Hoechst 33342 to label the nucleus. Cells are imaged on a Zeiss LSM 710 confocal microscope with a 63x oil immersion objective while maintained at 37C and 5% CO2. Hoechst is subjected to 405nm laser excitation, and tdTomato is subject to 561nm laser excitation. Cells are identified based on the presence of Hoechst and amount of tdTomato fluorescence per cell is measured.

[0001020] In some embodiments, the cells treated with fusosomes containing VPR-dCas9 and a guide RNA targeting the protospacer will have higher tdTomato fluorescence than cells treated with VPR-dCas9 and a scrambeled guide RNA or cells that were not treated with fusosomes. This assay can be used to show fusosomes delivering proteins to the nucleus of recipient cells that alter the transcription level of a target gene.

Example 122: Delivery of synthetic epigenetic factor that alters gene expression

[0001021] This example describes fusosome fusion with a cell *in vitro*. In an embodiment, fusosome fusion with a cell *in vitro* results in delivery of a epigenetic modulator to the recipient cell nucleus.

[0001022] A fusosome composition, resulting from cell-derived vesicles or cell-derived cytobiologics as produced by any one of the methods described in previous Examples, contains mRNA encoding the Tet1 enzymatic domain translationally fused to the C terminus of a nuclease null Streptococcus pyogenes cas9 (Tet1-dCas9) and a guide RNA targeting a the *Snrpn* promoter

region. As a negative control, a fusosome composition contains mRNA encoding Tet1-dCas9 with a scrambled guide RNA. Tet1 (ten-eleven translocation) dioxygenases methyl groups on 5-cytosine to form 5-hydroxymethylcytosine, which is then restored to unmodified cytosine. Methylation at 5-cytosine represses adjacent DNA sequence transcription, thus Tet1 activates transcription by removing these methyl groups. In this example, the gene that is affected by Tet1 is determined by the location of dCas9 binding DNA as directed by a guide RNA, e.g., as described in Liu et al., *Cell* 167(1): 233-247, 2016.

[0001023] A sufficient number of fusosomes are then incubated at 37°C and 5% CO2 for a period of 72 hours in in DMEM containing 20% Fetal Bovine Serum and 1x Penicillin/Streptomycin with a reporter line. The reporter line is a HEK293 cell line that has integrated into the genome at the *Dazl* promoter locus a synthetic methylation-sensing promoter (conserved sequence elements from the promoter of an imprinted gene, *Snrpn*) that controls the expression of GFP (Stelzer et al., *Cell* 163: 218-229, 2015). The *Dazl* promoter is hypermethylated, and thus the *Snrpn* promoter is not active and GFP is not expressed in these cells. As an additional negative control, the same HEK293 cells are not exposed to any fusosomes.

[0001024] Following the 72 hr incubation, mCherry fluorescence is measured with flow cytometry. The cells are run on a FACS cytometer (Becton Dickinson, San Jose, CA, USA) with 561nm laser excitation and emission collected at 590+/-20nm. A gate is set up to measure positive tdTomato expression. The gate is chosen such that the HEK293 cells not exposed to any fusosomes are negative for tdTomato expression. The percent of cells that are positive for tdTomato expression is then measured.

[0001025] In some embodiments, the cells treated with fusosomes containing Tet1-dCas9 and a guide RNA targeting the *Snrpn* promoter region will have higher tdTomato fluorescence than cells treated with Tet1-dCas9 and a scrambeled guide RNA or cells that were not treated with fusosomes. This assay can be used to show fusosomes delivering proteins to the nucleus of recipient cells that alter the epigenetic state of a target gene.

Example 123: Delivery of protein that is naturally targeted to mitochondria

[0001026] This example describes fusosome fusion with a cell *in vitro*. In an embodiment, fusosome fusion with a cell *in vitro* results in delivery of a protein to the recipient cell

mitochondria.

[0001027] A fusosome composition, resulting from cell-derived vesicles or cell-derived cytobiologics as produced by any one of the methods described in previous Examples, contains mRNA encoding Ornithine Transcarbamylase (OTC). As a negative control, a fusosome composition contains mRNA with a missense mutation that prevents translation of OTC.

[0001028] A sufficient number of fusosomes are then incubated at 37°C and 5% CO2 together with a recipient HEK293 cell line for a period of 24 hours in in DMEM containing 10% Fetal Bovine Serum and 1x Penicillin/Streptomycin.

[0001029] Following the 24 hr incubation, the cells are prepared for imaging. The cells are stained with Mitotracker Deep Red FM (Thermofisher catalog number M22426), fixed, permeabilized, blocked, and immunostained with an anti-OTC antibody (for example, abcam catalog number ab91418). Following immunostaining, the cells are counterstained with a secondary antibody conjugated to Alexa Fluor 488 (for example, abcam catalog number ab150077).

[0001030] In this example cells are imaged on a Zeiss LSM 710 confocal microscope with a 63x oil immersion objective while maintained at 37C and 5% CO2. Mitotracker Deep Red FM is subjected to 632 nm laser excitation and emission captured at 665±20nm, and Alexa Fluor is subjected to 488nm laser excitation and emission captured at 510±15nm.

[0001031] Colocalization of Alexa Fluor 488 (OTC signal) with Mitotracker Deep Red-labeled mitochondria is calculated by masking mitochondrial regions based on MitoTracker Deep Red fluorescence followed by determining the Alexa Fluor 488 average fluorescence intensity in the mitochondrial region and outside the mitochondrial region. All images are processed in ImageJ (NIH) and mitochondrial regions are thresholded using the Moments threshold algorithm to identify regions after subtracting background.

[0001032] The average mitochondrial OTC signal is defined as the average fluorescence intensity of Alexa Fluor 488 within the mitochondrial regions and the average non-mitochondrial OTC signal is defined as the average fluorescence intensity of Alexa Fluor 488 outside of the mitochondrial regions (but within the cell boundary dictated by parallel phase contrast images).

The normalized mitochondrial OTC signal for a given cell is calculated as the average mitochondrial OTC signal normalized to the non-mitochondrial OTC signal. For each group at least 30-40 cells are imaged and analyzed.

[0001033] In some embodiments, the cells treated with fusosomes containing OTC mRNA will have a higher average fluorescence intensity of Alexa Fluor 488 within the mitochondrial regions than the cells treated with fusosomes containing missense OTC mRNA. In some embodiments, the cells treated with fusosomes containing OTC mRNA will have a higher normalized mitochondrial OTC signal than the cells treated with fusosomes containing missense OTC mRNA. This assay can be used to show fusosomes delivering therapeutic proteins to the mitochondria of recipient cells.

Example 124: Delivery of protein that is not naturally targeted to the mitochondria except upon addition of a mitochondrial localization sequence

[0001034] This example describes fusosome fusion with a cell *in vitro*. In an embodiment, fusosome fusion with a cell *in vitro* results in delivery of a protein to the recipient cell mitochondria.

[0001035] A fusosome composition, resulting from cell-derived vesicles or cell-derived cytobiologics as produced by any one of the methods described in previous Examples, contains mRNA encoding a translational fusion between EGFP and a mitochondrial localization sequence (e.g. one of the mitochondrial localization sequences in Table 6-2). As a negative control, a fusosome composition contains mRNA encoding EGFP without a mitochondrial localization sequence.

[0001036] A sufficient number of fusosomes are then incubated at 37°C and 5% CO2 together with a recipient HEK293 cell line for a period of 12 hours in in DMEM containing 10% Fetal Bovine Serum and 1x Penicillin/Streptomycin.

[0001037] Following the 24 hr incubation, the cells are imaged. Prior to imaging the cells are stained with Mitotracker Deep Red FM (Thermofisher catalog number M22426) to label the mitochondria and with a stain that labels the plasma membrane (e.g. CellMask Orange Plasma Membrane Stain, Thermofisher, Catalog number C10045). In this example cells are imaged on a

Zeiss LSM 710 confocal microscope with a 63x oil immersion objective while maintained at 37C and 5% CO2. The plasma membrane stain is subjected to 554 nm laser excitation with emission captured at 580±20nm, Mitotracker Deep Red FM is subjected to 632 nm laser excitation with emission captured at 665±20nm, and EGFP is subjected to 488nm laser excitation with emission captured at 510±15nm.

[0001038] Colocalization of EGFP with Mitotracker Deep Red-labeled mitochondria is determined by masking mitochondrial regions based on MitoTracker Deep Red fluorescence followed by determining the EGFP average fluorescence intensity in the mitochondrial region and outside the mitochondrial region. All images are processed in ImageJ (NIH) and mitochondrial regions are thresholded using the Moments threshold algorithm to identify regions after subtracting background.

[0001039] The average mitochondrial EGFP signal is defined as the average fluorescence intensity of EGFP within the mitochondrial regions and the average non-mitochondrial EGFP signal is defined as the average fluorescence intensity of EGFP outside of the mitochondrial regions (but within the cell boundary dictated by the plasma membrane stain). The normalized mitochondrial EGFP signal for a given cell is calculated as the average mitochondrial EGFP signal normalized to the non-mitochondrial EGFP signal. For each group at least 30-40 cells are imaged and analyzed.

[0001040] In some embodiments, the cells treated with fusosomes containing mitochondrial-targeted EGFP mRNA will have a higher average mitochondrial EGFP signal than the cells treated with fusosomes containing non-mitochondrial-targeted EGFP mRNA. In some embodiments, the cells treated with fusosomes containing mitochondrial-targeted EGFP mRNA will have a higher normalized mitochondrial EGFP signal than the cells treated with fusosomes containing non-mitochondrial-targeted EGFP mRNA. This assay can be used to show fusosomes delivering proteins to the mitochondria of recipient cells.

Example 125: Delivery of fusosomes via a pathway that is independent of lysosome acidification

[0001041] Often, entry of complex biological cargo into target cells is accomplished by endocytosis. Endocytosis requires the cargo to enter an endosome, which matures into an

acidified lysosome. Disadvantageously, cargo that enters a cell through endocytosis may become trapped in an endosome or lysosome and be unable to reach the cytoplasm or other desired compartment of the target cell. The cargo may also be damaged by acidic conditions in the lysosome. Some viruses are capable of non-endocytic entry into target cells; however this process is incompletely understood. This example demonstrates that a viral fusogen can be isolated from the rest of the virus and confer non-endocytic entry on a fusosome that lacks other viral proteins.

[0001042] Fusosomes from HEK-293T cells expressing the Nipah virus receptor-binding G protein and fusion F protein (NivG+F) on the cell surface and expressing Cre recombinase protein were generated according by the standard procedure of ultracentrifugation through a Ficoll gradient to obtain small particle fusosomes, as described herein. To demonstrate delivery of the fusosome to a recipient cell via a non-endocytic pathway, the NivG+F fusosomes were used to treat recipient HEK-293T cells engineered to express a "Loxp-GFP-stop-Loxp-RFP" cassette under CMV promoter. NivF protein is a pH-independent envelope glycoprotein that has been shown to not require environmental acidification for activation and subsequent fusion activity (Tamin, 2002).

[0001043] The recipient cells were plated 30,000 cells/well into a black, clear-bottom 96-well plate. Four to six hours after plating the recipient cells, the NivG+F fusosomes expressing Cre recombinase protein were applied to the target or non-target recipient cells in DMEM media. The fusosome sample was first measured for total protein content by bicinchoninic acid assay (BCA, ThermoFisher, Cat #23225) according to manufacturer's instructions. Recipient cells were treated with 10 μg of fusosomes and incubated for 24 hrs at 37ºC and 5% CO_2. To demonstrate that Cre delivery via NivG+F fusosomes was through a non-endocytic pathway, a parallel wells of recipient cells receiving NivG+F fusosome treatment were co-treated with an inhibitor of endosome/lysosome acidification, bafilomycin A1 (Baf; 100 nM; Sigma, Cat #B1793).

[0001044] Cell plates were imaged using an automated microscope (www.biotek.com/products/imaging-microscopy-automated-cell-imagers/lionheart-fx-automated-live-cell-imager/). The total cell population in a given well was determined by staining the cells with Hoechst 33342 in DMEM media for 10 minutes. Hoechst 33342 stains cell nuclei by intercalating into DNA and was therefore used to identify individual cells. Hoechst staining

was imaged using the 405 nm LED and DAPI filter cube. GFP was imaged using the 465 nm LED and GFP filter cube, while RFP was imaged using the 523 nm LED and RFP filter cube. Images of target and non-target cell wells were acquired by first establishing the LED intensity and integration times on a positive control well containing recipient cells treated with adenovirus coding for Cre recombinase instead of fusosomes.

[0001045] Acquisition settings were set so that Hoescht, RFP, and GFP intensities were at the maximum pixel intensity values but not saturated. The wells of interest were then imaged using the established settings. Focus was set on each well by autofocusing on the Hoescht channel and then using the established focal plane for the GFP and RFP channels. Analysis of GFP and RFP-positive cells was performed with Gen5 software provided with automated fluorescent microscope (https://www.biotek.com/products/software-robotics-software/gen5-microplate-reader-and-imager-software/).

[0001046] The images were pre-processed using a rolling ball background subtraction algorithm with a 60 μm width. Cells with GFP intensity significantly above background intensities were thresholded and areas too small or large to be GFP-positive cells were excluded. The same analysis steps were applied to the RFP channel. The number of RFP-positive cells (recipient cells receiving Cre) was then divided by the sum of the GFP-positive cells (recipient cells that did not show delivery) and RFP-positive cells to quantify the percentage RFP conversion, which indicates the amount of fusosome fusion with the recipient cells.

[0001047] With this assay, the fusosome derived from a HEK-293T cell expressing NivG+F on its surface and containing Cre recombinase protein showed significant delivery via a lysosome-independent pathway, which is consistent with entry via a non-endocytic pathway, as evidenced by a significant delivery of Cre cargo by NivG+F fusosomes even when recipient cells were co-treated with Baf to inhibit endocytosis-mediated uptake (Table 27 below). In this case, the inhibition of cargo delivery by Baf co-treatment was 23.4%.

[0001048] **Table 27: RFP conversion in HEK-293T cells**

Group	**% RFP conversion (± SD)**
Recipient + no fusosome	0.4 ± 0.2%

Recipient + NivG+F fusosome	88.9 ± 3.4%
Recipient + NivG+F fusosome + Baf	68.1 ± 2.7%

Example 126: Measuring fusion with a target cell

[0001049] Fusosomes derived from HEK-293T cells expressing the engineered hemagglutinin glycoprotein of measles virus (MvH) and the fusion protein (F) on the cell surface and containing Cre recombinase protein were generated, as described herein. The MvH was engineered so that its natural receptor binding is ablated and target cell specificity is provided through a single-chain anbitody (scFv) that recognizes the cell surface antigen, in this case the scFv is designed to target CD8, a co-receptor for the T cell receptor. A control fusosome was used which was derived from HEK-293T cells expressing the fusogen VSV-G on its surface and containing Cre recombinase protein. The target cell was a HEK-293T cell engineered to express a "Loxp-GFP-stop-Loxp-RFP" cassette under CMV promoter, as well as engineered to over-express the co-receptors CD8a and CD8b. The non-target cell was the same HEK-293T cell expressing "Loxp-GFP-stop-Loxp-RFP" cassette but without CD8a/b over-expression. The target or non-target recipient cells were plated 30,000 cells/well into a black, clear-bottom 96-well plate and cultured in DMEM media with 10% fetal bovine serum at 37ºC and 5% CO2. Four to six hours after plating the recipient cells, the fusosomes expressing Cre recombinase protein and MvH+F were applied to the target or non-target recipient cells in DMEM media. Recipient cells were treated with 10 µg of fusosomes and incubated for 24 hours at 37°C and 5% CO_2.

[0001050] Cell plates were imaged using an automated microscope (www.biotek.com/products/imaging-microscopy-automated-cell-imagers/lionheart-fx-automated-live-cell-imager/). The total cell population in a given well was determined by staining the cells with Hoechst 33342 in DMEM media for 10 minutes. Hoechst 33342 stains cell nuclei by intercalating into DNA and therefore is used to identify individual cells. The Hoechst was imaged using the 405 nm LED and DAPI filter cube. GFP was imaged using the 465 nm LED and GFP filter cube, while RFP was imaged using 523 nm LED and RFP filter cube. Images of target and non-target cell wells were acquired by first establishing the LED intensity and integration

times on a positive-control well; i.e., recipient cells treated with adenovirus coding for Cre recombinase instead of fusosomes.

[0001051] Acquisition settings were set so that Hoescht, RFP, and GFP intensities are at the maximum pixel intensity values but not saturated. The wells of interest were then imaged using the established settings. Focus was set on each well by autofocusing on the Hoescht channel and then using the established focal plane for the GFP and RFP channels. Analysis of GFP and RFP-positive cells was performed with Gen5 software provided with automated fluorescent microscope (https://www.biotek.com/products/software-robotics-software/gen5-microplate-reader-and-imager-software/).

[0001052] The images were pre-processed using a rolling ball background subtraction algorithm with a 60 μm width. Cells with GFP intensity significantly above background intensities were thresholded and areas too small or large to be GFP-positive cells were excluded. The same analysis steps were applied to the RFP channel. The number of RFP-positive cells (recipient cells receiving Cre) was then divided by the sum of the GFP-positive cells (recipient cells that did not show delivery) and RFP-positive cells to quantify the percent RFP conversion, which describes the amount of fusosome fusion within the target and non-target recipient cell population. For amounts of targeted fusion (fusosome fusion to targeted recipient cells), the percent RFP conversion value is normalized to the percentage of recipient cells that are target recipient cells (i.e., expressing CD8), which was assessed by staining with anti-CD8 antibody conjugated to phycoerythrin (PE) and analyzed by flow cytometry. Finally, the absolute amount of targeted fusion was determined by subtracting the amount of non-target cell fusion from the target cell fusion amount (any value <0 was considered to be 0).

[0001053] With this assay, the fusosome derived from a HEK-293T cell expressing the engineered MvH(CD8)+F on its surface and containing Cre recombinase protein showed a percentage RFP conversion of 25.2 +/- 6.4% when the recipient cell was the target HEK-293T cell expressing the "Loxp-GFP-stop-Loxp-RFP" cassette, and 51.1% of these recipient cells were observed to be CD8-positive. From these results, the normalized percentage RFP conversion or amount of targeted fusion was determined to be 49.3 +/- 12.7% for targeted fusion. The same fusosome showed a percentage RFP conversion of 0.5 +/- 0.1% when the recipient was the non-

target HEK-293T cell expressing “Loxp-GFP-stop-Loxp-RFP” but with no expression of CD8. Based on the above, the absolute amount of targeted fusion for the MvH(CD8)+F fusosome determined to be 48.8% and the absolute amount of targeted fusion for the control VSV-G fusosome was determined to be 0%.

CLAIMS

What is claimed is:

1. A fusosome comprising:

(a) a lipid bilayer comprising a plurality of lipids derived from a source cell;

(b) a lumen surrounded by the lipid bilayer;

(c) a fusogen that is exogenous or overexpressed relative to the source cell, wherein the fusogen is disposed in the lipid bilayer; and

(d) a nuclear payload agent for delivery to the nucleus of a target cell.

2. The fusosome of claim 1, wherein the nuclear payload agent comprises or encodes a protein having a nuclear localization signal (NLS) which is the same as the NLS in a naturally-occurring counterpart of the protein.

3. The fusosome of claim 1, wherein the nuclear protein payload agent comprises or encodes a protein having a NLS which is not comprised by a naturally-occurring counterpart of the protein.

4. The fusosome of any of claims 1-3, wherein the nuclear protein payload agent comprises nuclear localization signal set forth in any one of SEQ ID NOS: 128-507 and 605-626.

5. The fusosome of any of claims 1-4, wherein:

i) when a target cell is contacted with a plurality of the fusosomes, the nuclear protein payload agent or a protein encoded therein becomes present in the nucleus of the target cell at a level at least 10%, 20%, 50%, 100%, 2-fold, 5-fold, 10-fold, 20-fold, 50-fold, or 100-fold higher than the level of the nuclear protein payload agent in the cytoplasm of the target cell;

ii) when a population of target cells is contacted with a plurality of the fusosomes, the nuclear protein payload agent or a protein encoded therein becomes enriched in the

nucleus of at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of cells in the population of target cells;

iii) when a population of target cells is contacted with a plurality of the fusosomes, the nuclear protein payload agent or a protein encoded therein becomes present in a sub-population, which is the recipient cell population, of the population of target cells, wherein the nuclear protein payload agent or protein encoded therein becomes enriched in the nucleus in at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of cells in the recipient cell population; and/or

iv) at least 1, 2, 5, 10, 20, 50, 100, 200, 500, or 1,000 copies of the nuclear protein payload agent, or a protein encoded by a nucleic acid nuclear protein payload agent, are present in the nucleus in a target cell.

6. The fusosome of any of claims 1-5, wherein the nuclear payload agent comprises or encodes one or more of a transcriptional activator, transcriptional repressor, epigenetic modifier, histone acetyltransferase, histone deacetylase, histone methyltransferase, DNA methyltransferase, a DNA nickase, a site-specific DNA editing enzyme, optionally a deaminase, DNA transposase, DNA integrase, an RNA editor, an RNA splicing factor, or a PIWI protein.

7. The fusosome of any of claims 1-6, wherein the nuclear payload agent comprises or encodes: a transcription factor, a nuclease, a recombinase, an epigenetic factor, a post-transcriptional RNA modification factor, a non-coding RNA or ribonucleic protein, or a structural protein.

8. The fusosome of any of claims 1-5, wherein the nuclear payload agent comprises or encodes an antibody molecule, e.g., a Fab, an scFv, an scFab, a sdAb, a duobody, a minibody, a nanobody, a diabody, a zybody, a camelid antibody, a BiTE, a quadroma, or a bsDb.

9. The fusosome of any of claims 1-6, wherein the nuclear protein payload agent comprises or encodes a fusion protein comprising an effector domain and a localization domain.

10. The fusosome of claim 9, wherein the localization domain comprises a TAL domain, a Zinc Finger domain, a Cas9 domain, or a meganuclease domain.

11. The fusosome of claim 9 or claim 10, wherein the effector domain comprises a nuclease, recombinase, integrase, base editor (deaminase), transcription factor, or epigenetic modifier.

12. The fusosome of any of claims 1-6, wherein the nuclear payload agent comprises or encodes a gene editing protein or nucleic acid, optionally Cas9 or a guide RNA.

13. The fusosome of any of claims 1-6, wherein the nuclear payload agent comprises a functional RNA.

14. The fusosome of any of claims 1-6, wherein the nuclear payload agent comprises (i) a nucleic acid, and (ii) a protein that promotes nuclear import of the nucleic acid.

15. The fusosome of claim 14, wherein the nucleic acid comprises a binding site, and the protein that promotes nuclear import binds to the binding site.

16. The fusosome of claim 15, wherein the binding site comprises a TetR binding site and the protein comprises TetR.

17. The fusosome of any of claims 1-6, wherein the nuclear payload agent comprises a synthetic transcription factorthat is a fusion protein comprising (i) a DNA binding domain, optionally wherein the DNA binding domain ia TAL domain, ZF domain, or Cas9 domain and (ii) a transcriptional activator domain or transcriptional repressor domain.

18. The fusosome of any of claims 1-6, wherein the nuclear payload agent comprises a synthetic epigenetic modifier that is a fusion protein comprising (i) a DNA binding domain and (ii) an epigenetic modifier domain.

19. The fusosome of any of claims 1-6, wherein the nuclear protein payload agent is or comprises a protein selected from fibrillarin; KRI1 homolog; nucleolar protein 11; upstream binding transcription factor; EBNA1 binding protein 2; coilin; promyelocytic leukemia; chromatin target of PRMT1; TERF1 interacting nuclear factor 2; ABL proto-oncogene 1, non-receptor tyrosine kinase; lamin B1; centromere protein B; H1 histone family member 0; kinetochore scaffold 1; RB binding protein 7, chromatin remodeling factor; signal transducer and activator of transcription 3; forkhead box P3; hepatocyte nuclear factor 4 alpha; runt related transcription factor 1; lymphoid enhancer binding factor 1; forkhead box P1; cut like homeobox 2; POU class 2 homeobox 1; SIX homeobox 3; RELA proto-oncogene, NF-kB subunit; SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1; chromodomain helicase DNA binding protein 7; chromatin accessibility complex subunit 1; APOBEC1 complementation factor; bromodomain containing 4; Cbp/p300 interacting transactivator with Glu/Asp rich carboxy-terminal domain 1; lysine acetyltransferase 2A; histone acetyltransferase 1; TATA-box binding protein associated factor 5 like; lysine acetyltransferase 5; sirtuin 1; sirtuin 2; histone deacetylase 11; histone deacetylase 3; histone deacetylase 6; ASH1 like histone lysine methyltransferase; DOT1 like histone lysine methyltransferase; nuclear receptor binding SET domain protein 1; SET nuclear proto-oncogene; lysine methyltransferase 2A; lysine demethylase 2A; lysine demethylase 1A; lysine demethylase 3A; lysine demethylase 4B; lysine demethylase 5C; DNA methyltransferase 1; DNA methyltransferase 3 alpha; adenosine deaminase 2; adenosine deaminase, RNA specific; apolipoprotein B mRNA editing enzyme catalytic subunit 1; spliceosome associated factor 3, U4/U6 recycling protein; cyclin dependent kinase 19; cyclin L1; pre-mRNA processing factor 31; splicing factor 3a subunit 3; mediator of DNA damage checkpoint 1; ATM serine/threonine kinase; poly(ADP-ribose) polymerase 1; DNA topoisomerase I; or DNA ligase 4.

20. A fusosome comprising:

(a) a lipid bilayer comprising a plurality of lipids derived from a source cell;

(b) a lumen surrounded by the lipid bilayer, wherein the lumen does not comprise an organelle;

(c) a fusogen that is exogenous or overexpressed relative to the source cell, e.g., wherein the fusogen is disposed in the lipid bilayer; and

(d) an organellar payload agent for delivery to a cytoplasmic organelle of a target cell, wherein, when a target cell is contacted with a plurality of the fusosomes, the organellar protein payload agent or polypeptide encoded therein becomes enriched in one or more of the Golgi apparatus, endoplasmic reticulum, vacuole, acrosome, autophagosome, centriole, glycosome, glyoxysome, hydrogenosome, melanosome, mitosome, cnidocyst, peroxisome, proteasome, vesicle, or stress granule of the target cell relative to the cyosol or plasma membrane of the target cell.

21. The fusosome of claim 20, wherein the organellar payload agent comprises or encodes a protein having a signal sequence which is the same as the signal sequence in a naturally-occurring counterpart of the protein.

22. The fusosome of claim 20, wherein the organellar payload agent comprises or encodes a protein having a signal sequence which is not comprised by a naturally-occurring counterpart of the protein.

23. A fusosome comprising:

(a) a lipid bilayer comprising a plurality of lipids derived from a source cell;

(b) a lumen surrounded by the lipid bilayer;

(c) a fusogen that is exogenous or overexpressed relative to the source cell, wherein the fusogen is disposed in the lipid bilayer; and

(d) an organellar payload agent that comprises or encodes a heterologous signal sequence to the organellar payload agent that is sufficient to enrich the organellar payload agent in a cytoplasmic organelle of a target cell.

24. The fusosome of claim 23, wherein the cytoplasmic organelle is chosen from a mitochondrion, Golgi apparatus, lysosome, endoplasmic reticulum, vacuole, endosome, acrosome, autophagosome, centriole, glycosome, glyoxysome, hydrogenosome, melanosome,

mitosome, cnidocyst, peroxisome, proteasome, vesicle, or stress granule.

25. The fusosome of any of claims 20-24, wherein the heterologous signal sequence is set forth in any one of SEQ ID NOS: 39-127 and 605-626.

26. The fusosome of any of claims 20-25, wherein, when a target cell is contacted with a plurality of the fusosomes, the organellar payload agent becomes enriched in one or more of the Golgi apparatus, endoplasmic reticulum, vacuole, acrosome, autophagosome, centriole, glycosome, glyoxysome, hydrogenosome, melanosome, mitosome, cnidocyst, peroxisome, proteasome, vesicle, or stress granule of the target cell relative to the cyosol or plasma membrane of the target cell.

27. The fusosome of any of claims 20-26, wherein the cytoplasmic organelle is a secretory pathway organelle and the heterologous signal sequence is set forth in any one of SEQ ID NOS: 108-120.

28. The fusosome of any of claims 20-26, wherein the cytoplasmic organelle is a endoplasmic reticulum and the heterologous signal sequence is set forth in any one of SEQ ID NOS: 72-93 and 121-127.

29. The fusosome of any of claims 20-26, wherein the cytoplasmic organelle is a Golgi apparatus and the heterologous signal sequence is set forth in any one of SEQ ID NOS: 94-107 and 609-610.

30. The fusosome of any of claims 23-25, wherein, when a target cell is contacted with a plurality of the fusosomes, the encoded polypeptide becomes enriched in the lysosome and/or endosome, relative to the cytosol or plasma membrane of the target cell.

31. The fusosome of any of claims 23-25 and 30, wherein the cytoplasmic organelle is a lysosome and the heterologous signal sequence is set forth in any one of SEQ ID NOS: 39-

71 and 605-608.

32. The fusosome of any of claims 23-25, wherein the organellar payload agent becomes enriched in mitochondria of the target cell at a level at least 10%, 20%, 50%, 100%, 2-fold, 5-fold, 10-fold, 20-fold, 50-fold, or 100-fold higher than the level of the organellar payload agent in the cytoplasm or the target cell plasma membrane.

33. The fusosome of any of claims 20-32, wherein the organellar payload agent comprises or encodes a polypeptide chosen from a mitochondrial membrane protein, a outer mitochondrial membrane protein, an inner mitochondrial membrane protein, a mitochondrial inner boundary membrane protein, a mitochondrial cristal membrane protein, a mitochondrial DNA protein, a mitochondrial intermembrane space protein, a mitochondrial matrix protein, a mitochondrial matrix granule protein, a mitochondrial cristae protein, a mitochondrial ribosome protein, a mitochondrial intracristal protein, a mitochondrial peripheral space protein, a peroxisomal membrane protein, a peroxisomal crystalloid core protein, a peroxisomal matrix protein, a peroxin.

34. The fusosome of any of claims 20-33, wherein the organellar payload agent comprises or encodes a polypeptide chosen from:

viii) a metabolic protein;
ix) a protease;
x) a chaperone;
xi) a protein transport protein;
xii) a mitochondrial ribosome protein;
xiii) a mitochondrial transcription protein; or
xiv) a mitochondrial replication protein.

35. The fusosome of any of claims 20-34, wherein the organellar payload agent comprises a protein selected from B cell receptor associated protein 31; calnexin; KDEL endoplasmic reticulum protein retention receptor 3; reticulon 4; Sec61 translocon alpha 1

subunit; Sarcoplasmic/endoplasmic reticulum calcium ATPase 1; Derlin-2; Stress-associated endoplasmic reticulum protein 2; Reticulocalbin-2; Atlastin-3; cytochrome c, somatic; translocase of inner mitochondrial membrane 44; translocase of outer mitochondrial membrane 40; voltage dependent anion channel 2; coenzyme Q8B; Acetyl-CoA acetyltransferase, mitochondrial; Citrate synthase, mitochondrial; Cytochrome c oxidase subunit 7A-related protein, mitochondrial; Fumarate hydratase, mitochondrial; NADPH:adrenodoxin oxidoreductase, mitochondrial; Induced myeloid leukemia cell differentiation protein Mcl-1; NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 13; Transcription factor A, mitochondrial; fatty acyl-CoA reductase 1; acetyl-CoA acyltransferase 1; ATP binding cassette subfamily D member 3; hydroxysteroid 17-beta dehydrogenase 4; peroxisomal biogenesis factor 14; Non-specific lipid-transfer protein; Peroxisomal biogenesis factor 19; Enoyl-CoA delta isomerase 2; Acyl-CoA-binding domain-containing protein 5; golgin A1; golgi integral membrane protein 4; golgi associated PDZ and coiled-coil motif containing; trans-golgi network vesicle protein 23 homolog C; zinc finger DHHC-type containing 7; Coatomer subunit delta; BSD domain-containing protein 1; Platelet glycoprotein 4; Conserved oligomeric Golgi complex subunit 7; Coatomer subunit epsilon; Protein FAM3C; Alpha-(1,6)-fucosyltransferase; General vesicular transport factor p115; Transmembrane protein 165; Vacuolar protein sorting-associated protein 54; Protein YIPF3; Fas associated factor family member 2; lysophosphatidylcholine acyltransferase 2; NAD(P) dependent steroid dehydrogenase-like; perilipin 3; patatin like phospholipase domain containing 2; cadherin 4; catenin beta 1; epidermal growth factor receptor; ezrin; syntaxin 4; ATP-binding cassette sub-family G member 2; Caskin-2; Junction plakoglobin; Neural cell adhesion molecule 1; Pannexin-1; Solute carrier family 2, facilitated glucose transporter member 1; Stomatin-like protein 3; FUS RNA binding protein; TAR DNA binding protein; TATA-box binding protein associated factor 15; EWS RNA binding protein 1; DEAD-box helicase 4; TAR DNA-binding protein 43; Heterogeneous nuclear ribonucleoprotein A1; Heterogeneous nuclear ribonucleoproteins A2/B1; Nucleolysin TIA-1 isoform p40; centrosomal protein 164; doublecortin domain containing 2; keratin 8; myosin heavy chain 10; actin bet; Coiled-coil domain-containing protein 14; Keratin, type I cytoskeletal 18; Pericentrin; Tubulin alpha-1A chain; or Actin, alpha skeletal muscle.

36. The fusosome of any of claims 20-34, wherein the organellar payload agent comprises or encodes an antibody molecule, e.g., a Fab, an scFV, an scFab, a sdAb, a duobody, a minibody, a nanobody, a diabody, a zybody, a camelid antibody, a BiTE, a quadroma, or a bsDb.

37. The fusosome of any of claims 20-34, wherein the organellar payload agent comprises a functional RNA.

38. The fusosome of any of claims 20-37, wherein, when a population of target cells is contacted with a plurality of the fusosomes, the organellar payload agent becomes enriched in the cytoplasmic organelle of at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of cells in the population of target cells.

39. The fusosome of any of claims 1-38, wherein the source cell is a primary cell, a cultured cell, an immortalized cell, or a cell line , optionally wherein the cell line is amyelobast cell line, optionally C2C12.

40. The fusosome of any of claims 1-39, wherein the source cell is an endothelial cell, a fibroblast, a blood cell (e.g., a macrophage, a neutrophil, a granulocyte, a leukocyte), a stem cell (e.g., a mesenchymal stem cell, an umbilical cord stem cell, bone marrow stem cell, a hematopoietic stem cell, an induced pluripotent stem cell e.g., an induced pluripotent stem cell derived from a subject's cells), an embryonic stem cell (e.g., a stem cell from embryonic yolk sac, placenta, umbilical cord, fetal skin, adolescent skin, blood, bone marrow, adipose tissue, erythropoietic tissue, hematopoietic tissue), a myoblast, a parenchymal cell (e.g., hepatocyte), an alveolar cell, a neuron (e.g., a retinal neuronal cell) a precursor cell (e.g., a retinal precursor cell, a myeloblast, myeloid precursor cells, a thymocyte, a meiocyte, a megakaryoblast, a promegakaryoblast, a melanoblast, a lymphoblast, a bone marrow precursor cell, a normoblast, or an angioblast), a progenitor cell (e.g., a cardiac progenitor cell, a satellite cell, a radial gial cell, a bone marrow stromal cell, a pancreatic progenitor cell, an endothelial progenitor cell, a blast cell), or an immortalized cell (e.g., HeLa, HEK293, HFF-1, MRC-5, WI-38, IMR 90, IMR 91, PER.C6, HT-1080, or BJ cell).

41. The fusosome of any of claims 1-40, wherein the source cell is allogeneic, e.g., obtained from a different organism of the same species as the target cell.

42. The fusosome of any of claims 1-40, wherein the source cell is autologous, e.g., obtained from the same organism as the target cell.

43. The fusosome of any of claims 1-42, wherein the source cell is selected from a white blood cell or a stem cell.

44. The fusosome of any of claims 1-43, wherein the source cell is selected from a neutrophil, a lymphocyte (e.g., a T cell, a B cell, a natural killer cell), a macrophage, a granulocyte, a mesenchymal stem cell, a bone marrow stem cell, an induced pluripotent stem cell, an embryonic stem cell, or a myeloblast.

45. The fusosome of any of claims 1-44, wherein the fusosome is from a source cell having a modified genome that exhibits reduced immunogenicity, optionally by genome editing to remove MHC complexes.

46. The fusosome of any of claims 1-45 wherein the fusosome has a diameter that is less than about 0.01% or 1%, of that of the source cell.

47. The fusosome of any of claims 1-46, wherein the fusogen is a mammalian fusogen or a viral fusogen.

48. The fusosome of any of claims 1-47, wherein the fusogen is active at a pH of 6-8.

49. The fusosome of any of claims, wherein the fusosome comprises a targeting domain which localizes the fusosome to a target cell.

50. The fusosome of claim 49, wherein the targeting domain interacts with a target cell moiety on the target cell.

51. The fusosome of any one of claims 1-50, wherein the target cell is in an organism.

52. The fusosome of any one of claims 1-50, wherein the target cell is a primary cell isolated from an organism.

53. The fusosome of any one of claims 1-52, wherein the target cell is selected from an endothelial cell, a fibroblast, a blood cell (e.g., a macrophage, a neutrophil, a granulocyte, a leukocyte), a stem cell (e.g., a mesenchymal stem cell, an umbilical cord stem cell, bone marrow stem cell, a hematopoietic stem cell, an induced pluripotent stem cell e.g., an induced pluripotent stem cell derived from a subject's cells), an embryonic stem cell (e.g., a stem cell from embryonic yolk sac, placenta, umbilical cord, fetal skin, adolescent skin, blood, bone marrow, adipose tissue, erythropoietic tissue, hematopoietic tissue), a myoblast, a parenchymal cell (e.g., hepatocyte), an alveolar cell, a neuron (e.g., a retinal neuronal cell) a precursor cell (e.g., a retinal precursor cell, a myeloblast, myeloid precursor cells, a thymocyte, a meiocyte, a megakaryoblast, a promegakaryoblast, a melanoblast, a lymphoblast, a bone marrow precursor cell, a normoblast, or an angioblast), a progenitor cell (e.g., a cardiac progenitor cell, a satellite cell, a radial gial cell, a bone marrow stromal cell, a pancreatic progenitor cell, an endothelial progenitor cell, a blast cell), or an immortalized cell (e.g., HeLa, HEK293, HFF-1, MRC-5, WI-38, IMR 90, IMR 91, PER.C6, HT-1080, or BJ cell).

54. A pharmaceutical composition comprising a fusosome of any of claims 1-53.

55. A method of manufacturing a fusosome composition, comprising:

i) providing a plurality of fusosomes according to any of claims 1-53; and

ii) formulating the plurality of fusosomes, fusosome composition, or pharmaceutical composition e.g., as a fusosome drug product suitable for administration to a subject.

56. A method of administering a fusosome composition to a subject, comprising administering to the subject a fusosome composition comprising a plurality of fusosomes according to any of claims 1-53, thereby administering the fusosome composition to the subject.

57. A method of delivering a protein membrane payload to a subject, comprising administering to the subject a fusosome composition comprising a plurality of fusosomes according to any of claims 1-53, wherein the fusosome composition is administered in an amount and/or time such that the protein payload gene is delivered.

58. A method of treating a disease or disorder in a patient comprising administering to the subject a plurality of fusosomes according to any of claims 1-53, wherein the fusosome composition is administered in an amount and/or time such that the disease or disorder is treated.

59. The method of claim 58, wherein the disease or disorder is selected from cancer, autoimmune disorder, or infectious disease.

60. The method of any of claims 56-59, wherein the subject is a human subject.