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(54) Titre : COMPOSITIONS A BASE DE FUSOSOMES POUR L'ADMINISTRATION DE SNC
(54) Title: FUSOSOME COMPOSITIONS FOR CNS DELIVERY

(57) Abrégé/Abstract:

The present disclosure provides, at least in part, methods and compositions for in vivo fusosome delivery. In some embodiments, the fusosome comprises a combination of elements that promote specificity for target cells, e.g., one or more of a fusogen, a positive target cell- specific regulatory element, and a non-target cell- specific regulatory element. In some embodiments, the fusosome comprises one or more modifications that decrease an immune response against the fusosome.



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(57) Abstract: The present disclosure provides, at least in part, methods and compositions for in vivo fusosome delivery. In some embodiments, the fusosome comprises a combination of elements that promote specificity for target cells, e.g., one or more of a fusogen, a positive target cell-specific regulatory element, and a non-target cell-specific regulatory element. In some embodiments, the fusosome comprises one or more modifications that decrease an immune response against the fusosome.



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JUMBO APPLICATIONS/PATENTS

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FUSOSOME COMPOSITIONS FOR CNS DELIVERY

Cross-Reference to Related Applications

This application claims priority to U.S. provisional applications: 62/767,358, filed November 14, 2018, entitled “FUSOSOME COMPOSITIONS FOR CNS CELL DELIVERY”;
5 and 62/900,064, filed September 13, 2019, entitled “FUSOSOME COMPOSITIONS FOR CNS CELL 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
10 format. The Sequence Listing is provided as a file entitled 186152003340SeqList.TXT, created November 14, 2019, which is 819 kilobytes in size. The information in the electronic format of the Sequence Listing is incorporated by reference in its entirety.

BACKGROUND

15 Complex biologics are promising therapeutic candidates for a variety of diseases. However, it is difficult to deliver large biologic agents into a cell because the plasma membrane acts as a barrier between the cell and the extracellular space. There is a need in the art for new methods of delivering complex biologics into cells in a subject.

SUMMARY

20 The present disclosure provides, at least in part, fusosome methods and compositions for in vivo delivery. In some embodiments, the fusosome comprises a combination of elements that promote specificity for target cells, e.g., one or more of a fusogen, a positive target cell-specific regulatory element, and a non-target cell-specific regulatory element. In some embodiments, the fusosome comprises one or more modifications that decrease an immune response against the
25 fusosome.

Enumerated embodiments

1. A fusosome comprising:
 - a) a lipid bilayer comprising a fusogen; and
 - b) a nucleic acid that comprises:

5 (i) a payload gene encoding an exogenous agent, e.g. a payload gene encoding an exogenous agent of Table 5 or Table 6, optionally wherein the exogenous agent is set forth in any one of SEQ ID NOS: 134-154, a functional fragment thereof, or a functional variant thereof comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%, identity to an amino acid sequence set forth in any one of SEQ
10 ID NOS: 134-154; and

(ii) a positive target cell-specific regulatory element (e.g., a target cell-specific promoter) operatively linked to the payload gene, wherein the positive target cell-specific regulatory element increases expression of the payload gene in a target cell relative to an otherwise similar fusosome lacking the positive target cell-specific
15 regulatory element, wherein the target cell is a CNS cell.

2. The fusosome of embodiment 1, wherein the nucleic acid further comprises a non-target cell-specific regulatory element (NTCSRE) (e.g., a non-target cell-specific miRNA recognition sequence), operatively linked to the payload gene, wherein the NTCSRE decreases
20 expression of the payload gene in a non-target cell relative to an otherwise similar fusosome lacking the NTCSRE, optionally wherein the target cell is a first type of CNS cell and the non-target cell is a second, different type of CNS cell or a non-CNS cell, optionally wherein:

the target cell is a neuron and the non-target cell is a glial cell (e.g., an oligodendrocyte, an astrocyte, or a microglia cell), or

25 the target cell is a glial cell (e.g., an oligodendrocyte, an astrocyte, or a microglia cell) and the non-target cell is a neuron.

3. A fusosome comprising:
 - a) a lipid bilayer comprising a fusogen; and
 - b) a nucleic acid that comprises:

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(i) a payload gene encoding an exogenous agent, e.g., an exogenous agent of Table 5 or Table 6, optionally wherein the exogenous agent is set forth in any one of SEQ ID NOS: 134-154, a functional fragment thereof, or a functional variant thereof comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%,
 5 identity to an amino acid sequence set forth in any one of SEQ ID NOS: 134-154; and

(ii) a promoter operatively linked to the payload gene, wherein the promoter is chosen from a SYN, NSE, CaMKII, aTubulin, PDGF, fSST, fNPY, GAD67, DLX5/6, VGLUT1, Dock10, ChAT, VACHT, Drd1a, TPH-2, GFAP, EAAT1, GS, CX3CR1, TMEM119, MBP, CNP, or CRFR2 β promoter, e.g., according to a sequence of a promoter in
 10 Table 3, or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

4. A fusosome comprising:

a) a lipid bilayer comprising a fusogen; and

15 b) a nucleic acid that comprises:

(i) a payload gene encoding an exogenous agent, e.g. a payload gene encoding an exogenous agent of Table 5 or Table 6, optionally wherein the exogenous agent is set forth in any one of SEQ ID NOS: 134-154, a functional fragment thereof, or a functional variant thereof comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%,
 20 95%, 96%, 97%, 98%, or 99%, identity to an amino acid sequence set forth in any one of SEQ ID NOS: 134-154; and

(ii) a non-target cell-specific regulatory element (NTCSRE) (e.g., a non-target cell-specific miRNA recognition sequence), operatively linked to the payload gene, wherein the NTCSRE decreases expression of the payload gene in a non-target cell or
 25 tissue relative to an otherwise similar fusosome lacking the NTCSRE.

5. A fusosome comprising:

a) a lipid bilayer comprising a fusogen; and

b) a nucleic acid that comprises:

(i) a payload gene encoding an exogenous agent, e.g. a payload gene encoding an exogenous agent of Table 5 or Table 6, optionally wherein the exogenous agent is
 30

set forth in any one of SEQ ID NOS: 134-154, a functional fragment thereof, or a functional variant thereof comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%, identity to an amino acid sequence set forth in any one of SEQ ID NOS: 134-154; and

- 5 (ii) a negative target cell-specific regulatory element (negative TCSRE) (e.g., a tissue-specific miRNA recognition sequence), operatively linked to the payload gene, wherein the negative TCSRE decreases expression of the exogenous agent in a non-target cell or tissue relative to an otherwise similar nucleic acid lacking the negative TCSRE.

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6. The fusosome of either embodiment 4 or 5, wherein the nucleic acid further comprises a positive target cell-specific regulatory element (e.g., a target cell-specific promoter) operatively linked to the payload gene, wherein the positive target cell-specific regulatory element increases expression of the payload gene in a target cell relative to an otherwise similar fusosome lacking the positive target cell-specific regulatory element, wherein the target cell is a first type of CNS cell, optionally wherein the non-target cell is a second, different type of CNS cell or a non-CNS cell, optionally wherein:

- the target cell is a neuron and the non-target cell is a glial cell (e.g., an oligodendrocyte, an astrocyte, or a microglia cell), or
- 20 the target cell is a glial cell (e.g., an oligodendrocyte, an astrocyte, or a microglia cell) and the non-target cell is a neuron.

7. A fusosome comprising:
- a) a lipid bilayer comprising a fusogen;
- 25 b) a nucleic acid that comprises a payload gene encoding an exogenous agent, e.g. a payload gene encoding an exogenous agent of Table 5 or Table 6, optionally wherein the exogenous agent is set forth in any one of SEQ ID NOS: 134-154, a functional fragment thereof, or a functional variant thereof comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%, identity to an amino acid sequence set forth in any one of SEQ ID NOS: 134-154; and
- 30 c) one or both of:

(i) a first exogenous or overexpressed immunosuppressive protein on the lipid bilayer; or

(ii) a first immunostimulatory protein that is absent or present at reduced levels (e.g., reduced by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%) compared to a fusosome generated from an otherwise similar, unmodified source cell.

8. 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%, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 20-fold, 50-fold, or 100-fold;

ii) the fusosome fuses at a higher rate with a target cell than with another fusosome, 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;

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%, of target cells after 24, 48, or 72 hours;

iv) the fusosome delivers the nucleic acid, e.g., retroviral nucleic acid, to a target cell at a higher rate than to 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;

v) the fusosome delivers the nucleic acid, e.g., retroviral nucleic acid, to a target cell at a higher rate than to another fusosome, 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; or

vi) the fusosome delivers the nucleic acid, e.g., retroviral nucleic acid, to a target cell 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.

9. The fusosome of any of the preceding embodiments, wherein one or more of (e.g., 2 or all 3 of) the following apply: the fusosome is a retroviral vector, the lipid bilayer is comprised by an envelope, e.g., a viral envelope, and the nucleic acid is a retroviral nucleic acid.

10. The fusosome of any of the preceding embodiments, wherein the nucleic acid comprises one or more of (e.g., all of) the following nucleic acid sequences: 5' LTR (e.g., comprising U5 and lacking a functional U3 domain), Psi packaging element (Psi), Central polypurine tract (cPPT) Promoter operatively linked to the payload gene, payload gene (optionally comprising an intron before the open reading frame), Poly A tail sequence, WPRE, and 3' LTR (e.g., comprising U5 and lacking a functional U3).

11. The fusosome of any of the preceding embodiments, which comprises one or more of (e.g., all of) a polymerase (e.g., a reverse transcriptase, e.g., pol or a portion thereof), an integrase (e.g., pol or a portion thereof, e.g., a functional or non-functional variant), a matrix protein (e.g., gag or a portion thereof), a capsid protein (e.g., gag or a portion thereof), a nucleocapsid protein (e.g., gag or a portion thereof), and a protease (e.g., pro).

12. The fusosome of embodiment 7, which comprises (i) and (ii).

13. The fusosome of any of embodiments 7-12, which further comprises a second exogenous or overexpressed immunosuppressive protein on the lipid bilayer.

14. The fusosome of any of embodiments 7-13, which further comprises a second immunostimulatory protein that is absent or present at reduced levels (e.g., reduced by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%) compared to a fusosome generated from an otherwise similar, unmodified source cell.

15. The fusosome of any of embodiments 7-14, wherein the nucleic acid, e.g., retroviral vector, further comprises a positive target cell-specific regulatory element (e.g., a target cell-specific promoter) operatively linked to the payload gene, wherein the positive target cell-specific regulatory element increases expression of the payload gene in a target cell relative to an otherwise similar fusosome lacking the positive target cell-specific regulatory element, wherein the target cell is a CNS cell.

16. The fusosome of any of embodiments 7-15, wherein the nucleic acid, e.g., retroviral nucleic acid, further comprises a non-target cell-specific regulatory element (NTCSRE) (e.g., a non-target cell-specific miRNA recognition sequence), operatively linked to the payload gene, wherein the NTCSRE decreases expression of the payload gene in a non-target cell or tissue relative to an otherwise similar fusosome lacking the NTCSRE, optionally wherein the target cell is a first type of CNS cell and the non-target cell is a second, different type of CNS cell or a non-CNS cell, optionally wherein:

the target cell is a neuron and the non-target cell is a glial cell (e.g., an oligodendrocyte, an astrocyte, or a microglia cell), or

the target cell is a glial cell (e.g., an oligodendrocyte, an astrocyte, or a microglia cell) and the non-target cell is a neuron.

17. The fusosome of any of embodiments 7-15, wherein the nucleic acid, e.g., retroviral nucleic acid, further comprises a negative target cell-specific regulatory element (negative TCSRE) (e.g., a tissue-specific miRNA recognition sequence), operatively linked to the payload gene, wherein the negative TCSRE decreases expression of the exogenous agent in a non-target cell or tissue relative to an otherwise similar nucleic acid, e.g., retroviral nucleic acid, lacking the negative TCSRE.

18. The fusosome of any of embodiments 7-17, wherein, when administered to a subject (e.g., a human subject or a mouse), one or more of:

i) the fusosome does not produce a detectable antibody response (e.g., after a single administration or a plurality of administrations), or antibodies against the fusosome are present at a level of less than 10%, 5%, 4%, 3%, 2%, or 1% above a background level, e.g., by a FACS antibody detection assay, e.g., an assay of Example 13 or Example 14);

ii) the fusosome does not produce a detectable cellular immune response (e.g., T cell response, NK cell response, or macrophage response), or a cellular immune response against the fusosome is present at a level of less than 10%, 5%, 4%, 3%, 2%, or 1% above a background level, e.g., by a PBMC lysis assay (e.g., an assay of Example 5), by an NK cell lysis assay (e.g., an assay of Example 6), by a CD8 killer T cell lysis assay (e.g., an assay of Example 7), or by a macrophage phagocytosis assay (e.g., an assay of Example 8);

iii) the fusosome does not produce a detectable innate immune response, e.g., complement activation (e.g., after a single administration or a plurality of administrations), or the innate immune response against the fusosome is present at a level of less than 10%, 5%, 4%, 3%, 2%, or 1% above a background level, e.g., by a complement activity assay (e.g., an assay of Example 9);

iv) less than 10%, 5%, 4%, 3%, 2%, or 1% of fusosomes are inactivated by serum, e.g., by a serum inactivation assay, e.g., an assay of Example 11 or Example 12;

v) a target cell that has received the exogenous agent from the fusosome does not produce a detectable antibody response (e.g., after a single administration or a plurality of administrations), or antibodies against the target cell are present at a level of less than 10%, 5%, 4%, 3%, 2%, or 1% above a background level, e.g., by a FACS antibody detection assay, e.g., an assay of Example 15; or

vi) a target cell that has received the exogenous agent from the fusosome does not produce a detectable cellular immune response (e.g., T cell response, NK cell response, or macrophage response), or a cellular response against the target cell is present at a level of less than 10%, 5%, 4%, 3%, 2%, or 1% above a background level, e.g., by a macrophage phagocytosis assay (e.g., an assay of Example 16), by a PBMC lysis assay (e.g., an assay of Example 17), by an NK cell lysis assay (e.g., an assay of Example 18), or by a CD8 killer T cell lysis assay (e.g., an assay of Example 19).

19. The fusosome of embodiment 18, wherein the background level is the corresponding level in the same subject prior to administration of the fusosome.

20. The fusosome of any of embodiments 7-19, wherein the immunosuppressive protein (e.g., first immunosuppressive protein or second immunosuppressive protein) is a complement regulatory protein or CD47.

21. The fusosome of any of embodiments 7-20, wherein the immunostimulatory protein (e.g., first immunostimulatory protein or second immunostimulatory protein) is an MHC I (e.g., HLA-A, HLA-B, HLA-C, HLA-E, or HLA-G) or MHC II (e.g., HLA-DP, HLA-DM, HLA-DOA, HLA-DOB, HLA-DQ, or HLA-DR) protein.

22. The fusosome of any of the preceding embodiments, wherein the exogenous agent is chosen from: SYNE1, SETX, FMR1, SLC6A8, UBE3A, SOD1, TDP43, C9orf72, FXN, MECP2, ASPA, or ALDH7A1; or the exogenous agent is chosen from: TPP1, FUCA1, GALC,
 5 HEXA, HEXB, MANBA, ARSA, GNPTAB, or MCOLN1.

23. The fusosome of any of the preceding embodiments, wherein the fusogen comprises VSV-G.

10 24. The fusosome of any embodiments 1, 2, 6, 15, 22, or 23, wherein the positive target cell-specific regulatory element comprises a CNS cell-specific promoter, a CNS cell-specific enhancer, a CNS cell-specific splice site, a CNS cell-specific site extending half-life of an RNA or protein, a CNS cell-specific mRNA nuclear export promoting site, a CNS cell-specific translational enhancing site, or a CNS cell-specific post-translational modification site.

15 25. The fusosome of any embodiments 1, 2, 6, 15, or 22-24, wherein the positive target cell-specific regulatory element comprises a CNS cell-specific promoter.

20 26. The fusosome of embodiment 25, wherein the CNS cell-specific promoter comprises a motif of Table 3.

25 27. The fusosome of embodiment 25 or 26, wherein the positive CNS cell-specific regulatory element comprises a promoter chosen from a SYN, NSE, CaMKII, aTubulin, PDGF, fSST, fNPY, GAD67, DLX5/6, VGLUT1, Dock10, ChAT, VACHT, Drd1a, TPH-2, GFAP, EAAT1, GS, CX3CR1, TMEM119, MBP, CNP, or CRFR2 β promoter.

30 28. The fusosome of any of embodiments 4-6, or 16-21, wherein the negative TCSRE or NTCSRE comprises a non-target cell-specific miRNA recognition sequence, non-target cell-specific protease recognition site, non-target cell-specific ubiquitin ligase site, non-target cell-specific transcriptional repression site, or non-target cell-specific epigenetic repression site.

29. The fusosome of any of embodiments 4-6, 16-21, or 28, wherein the negative TCSRE or NTCSRE comprises a tissue-specific miRNA recognition sequence, tissue-specific protease recognition site, tissue-specific ubiquitin ligase site, tissue-specific transcriptional repression site, or tissue-specific epigenetic repression site.

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30. The fusosome of any of embodiments 4-6, 16-21, 28, or 29, wherein the negative TCSRE or NTCSRE comprises a non-target cell-specific miRNA recognition sequence, non-target cell-specific protease recognition site, non-target cell-specific ubiquitin ligase site, non-target cell-specific transcriptional repression site, or non-target cell-specific epigenetic repression site.

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31. The fusosome of any of embodiments 4-6, 16-21, or 28-30, wherein the negative TCSRE or NTCSRE comprises a non-target cell-specific miRNA recognition sequence bound by a miRNA of Table 4, e.g., by one or more of (e.g., two or more of) miR-338-3p, miR-125b-5p, miR-342-3p, or miR-124 optionally wherein the miRNA is or comprises the sequence set forth in any one of SEQ ID NOS: 156-162.

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32. The fusosome of any of embodiments 28-31, wherein the negative TCSRE or NTCSRE is situated or encoded within a transcribed region (e.g., the transcribed region encoding the exogenous agent), e.g., such that an RNA produced by the transcribed region comprises the miRNA recognition sequence within a UTR or coding region.

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33. The fusosome of any of the preceding embodiments, wherein the nucleic acid, e.g., retroviral nucleic acid, comprises one or more insulator elements.

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34. The fusosome of embodiment 33, wherein the nucleic acid, e.g., retroviral nucleic acid, comprises two insulator elements, e.g., a first insulator element upstream of the payload gene and a second insulator element downstream of the payload gene, e.g., wherein the first insulator element and second insulator element comprise the same or different sequences.

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35. The fusosome of any of the preceding embodiments, which is not genotoxic or does not increase the rate of tumor formation in target cells.

36. The fusosome of any of the preceding embodiments, wherein the nucleic acid, e.g., retroviral nucleic acid, is capable of integrating into the genome of a target cell.

37. The fusosome of embodiment 36, wherein the nucleic acid, e.g., retroviral nucleic acid, is an integration-competent lentivirus or an integration-deficient lentivirus.

38. The fusosome of any of the preceding embodiments, wherein the target cell is chosen from a CNS cell, a pan-neuronal cell, a GABAergic neuron, a Glutamatergic neuron, a Cholinergic neuron, a Dopaminergic neuron, a Serotonergic neuron, an astrocyte, a microglia, an oligodendrocytes, or a choroid plexus cell.

39. The fusosome of any of embodiments 4-6 and 9-38, wherein one or more of:

i) less than 10%, 5%, 4%, 3%, 2%, or 1% of the exogenous agent detectably present in the subject is in non-target cells;

ii) at least 90%, 95%, 96%, 97%, 98%, or 99% of the cells of the subject that detectably comprise the exogenous agent, are target cells (e.g., cells of a single cell type);

iii) less than 1,000,000, 500,000, 200,000, 100,000, 50,000, 20,000, or 10,000 cells of the cells of the subject that detectably comprise the exogenous agent are non-target cells;

iv) average levels of the exogenous agent in all target cells in the subject are at least 100-fold, 200-fold, 500-fold, or 1,000-fold higher than average levels of the exogenous agent in all non-target cells in the subject; or

v) the exogenous agent is not detectable in any non-target cell in the subject.

40. The fusosome of any of the preceding embodiments, wherein the nucleic acid, e.g., retroviral nucleic acid, encodes a positive TCSRE and/or a NTCSRE or negative TCSRE.

41. The fusosome of any of the preceding embodiments, wherein the nucleic acid, e.g., retroviral nucleic acid, comprises the complement of a positive TCSRE and/or a NTCSRE or negative TCSRE.

42. The fusosome of either embodiment 40 or 41, wherein the positive TCSRE comprises a target cell-specific promoter that is at least 10%, 25%, 50%, 75%, 100%, 150%, 200%, 250%, 300%, 400%, 500%, 750%, 1000% or more active in a target cell than a non-target cell.

43. The fusosome of any of embodiments 40-42, wherein the negative TCSRE or NTCSRE comprises a miRNA recognition sequence that decreases gene expression by at least 10%, 25%, 50%, 75%, or 100% in a non-target cell compared to a target cell.

44. The fusosome of any of the preceding embodiments, which does not deliver nucleic acid, e.g., retroviral nucleic acid, to a non-target cell, e.g., a neuron, a glial cell, an antigen presenting cell, an MHC class II+ cell, a professional antigen presenting cell, an atypical antigen presenting cell, a macrophage, a dendritic cell, a myeloid dendritic cell, a plasmacyteoid dendritic cell, a CD11c+ cell, a CD11b+ cell, a splenocyte, a B cell, a hepatocyte, an endothelial cell, or a non-cancerous cell.

45. The fusosome of any of the preceding embodiments, wherein less than 10%, 5%, 2.5%, 1%, 0.5%, 0.1%, 0.01%, 0.001%, 0.0001%, 0.00001%, or 0.000001% of a non-target cell type (e.g., one or more of a neuron, a glial cell, an antigen presenting cell, an MHC class II+ cell, a professional antigen presenting cell, an atypical antigen presenting cell, a macrophage, a dendritic cell, a myeloid dendritic cell, a plasmacyteoid dendritic cell, a CD11c+ cell, a CD11b+ cell, a splenocyte, a B cell, a hepatocyte, an endothelial cell, or a non-cancerous cell) comprise the nucleic acid, e.g., retroviral nucleic acid, e.g., using quantitative PCR, e.g., using an assay of Example 1.

46. The fusosome of any of the preceding embodiments, wherein the target cells comprise 0.00001-10, .0001-10, .001-10, .01-10, .1-10, .5 – 5, 1-4, 1-3, or 1-2 copies of the nucleic acid, e.g., retroviral nucleic acid, or a portion thereof, per host cell genome, e.g., wherein

copy number of the nucleic acid, e.g., retroviral nucleic acid, is assessed after administration in vivo.

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

less than 10%, 5%, 2.5%, 1%, 0.5%, 0.1%, 0.01% of the non-target cells (e.g., a neuron, a glial cell, an antigen presenting cell, an MHC class II+ cell, a professional antigen presenting cell, an atypical antigen presenting cell, a macrophage, a dendritic cell, a myeloid dendritic cell, a plasmacyteoid dendritic cell, a CD11c+ cell, a CD11b+ cell, a splenocyte, a B cell, a hepatocyte, an endothelial cell, or a non-cancerous cell) comprise the exogenous agent; or

the exogenous agent (e.g., protein) is not detectably present in a non-target cell, e.g., a neuron, a glial cell, an antigen presenting cell, an MHC class II+ cell, a professional antigen presenting cell, an atypical antigen presenting cell, a macrophage, a dendritic cell, a myeloid dendritic cell, a plasmacyteoid dendritic cell, a CD11c+ cell, a CD11b+ cell, a splenocyte, a B cell, a hepatocyte, an endothelial cell, or a non-cancerous cell.

48. The fusosome of any of the preceding embodiments, wherein the fusosome delivers the nucleic acid, e.g., retroviral nucleic acid to a target cell, e.g., a CNS cell, a pan-neuronal cell, a GABAergic neuron, a Glutamatergic neuron, a Cholinergic neuron, a Dopaminergic neuron, a Serotonergic neuron, a glial cell an astrocyte, a microglial cell, an oligodendrocyte, or a choroid plexus cell.

49. The fusosome of any of the preceding embodiments, wherein at least 0.00001%, 0.0001%, 0.001%, 0.001%, 0.01%, 0.1%, 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of target cells (e.g., one or more of a CNS cell, a pan-neuronal cell, a GABAergic neuron, a Glutamatergic neuron, a Cholinergic neuron, a Dopaminergic neuron, a Serotonergic neuron, a glial cell, an astrocyte, a microglial cell, an oligodendrocyte, or a choroid plexus cell) comprise the nucleic acid, e.g., retroviral nucleic acid, e.g., using quantitative PCR, e.g., using an assay of Example 3.

50. The fusosome of any of the preceding embodiments, wherein at least 0.00001%, 0.0001%, 0.001%, 0.001%, 0.01%, 0.1%, 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%,

80%, or 90% of target cells (e.g., a CNS cell, a pan-neuronal cell, a GABAergic neuron, a Glutamatergic neuron, a Cholinergic neuron, a Dopaminergic neuron, a Serotonergic neuron, a glial cell, an astrocyte, a microglial cell, an oligodendrocyte, or a choroid plexus cell) comprise the exogenous agent.

5

51. The fusosome of any of the preceding embodiments, wherein, upon administration, the ratio of target cells comprising the nucleic acid, e.g., retroviral nucleic acid, to non-target cells comprising the nucleic acid, e.g., retroviral nucleic acid, is at least 1.5, 2, 3, 4, 5, 10, 25, 50, 100, 500, 1000, 5000, 10,000, e.g., according to a quantitative PCR assay, e.g.,
10 using assays of Example 1 and Example 3.

52. The fusosome of any of the preceding embodiments, wherein the ratio of the average copy number of nucleic acid, e.g., retroviral nucleic acid, or a portion thereof in target cells to the average copy number of nucleic acid, e.g., retroviral nucleic acid, or a portion thereof
15 in non-target cells is at least 1.5, 2, 3, 4, 5, 10, 25, 50, 100, 500, 1000, 5000, 10,000, e.g., according to a quantitative PCR assay, e.g., using assays of Example 1 and Example 3.

53. The fusosome of any of the preceding embodiments, wherein the ratio of the median copy number of of nucleic acid, e.g., retroviral nucleic acid, or a portion thereof in target
20 cells to the median copy number of nucleic acid, e.g., retroviral nucleic acid, or a portion thereof in non-target cells is at least 1.5, 2, 3, 4, 5, 10, 25, 50, 100, 500, 1000, 5000, 10,000, e.g., according to a quantitative PCR assay, e.g., using assays of Example 1 and Example 3.

54. The fusosome of any of the preceding embodiments, wherein the ratio of target
25 cells comprising the exogenous RNA agent to non-target cells comprising the exogenous RNA agent is at least 1.5, 2, 3, 4, 5, 10, 25, 50, 100, 500, 1000, 5000, 10,000, e.g., according to a reverse transcription quantitative PCR assay.

55. The fusosome of any of the preceding embodiments, wherein the ratio of the
30 average exogenous RNA agent level of target cells to the average exogenous RNA agent level of

non-target cells is at least 1.5, 2, 3, 4, 5, 10, 25, 50, 100, 500, 1000, 5000, 10,000, e.g., according to a reverse transcription quantitative PCR assay.

56. The fusosome of any of the preceding embodiments, wherein the ratio of the median exogenous RNA agent level of target cells to the median exogenous RNA agent level of non-target cells is at least 1.5, 2, 3, 4, 5, 10, 25, 50, 100, 500, 1000, 5000, 10,000, e.g., according to a reverse transcription quantitative PCR assay.

57. The fusosome of any of the preceding embodiments, wherein the ratio of target cells comprising the exogenous protein agent to non-target cells comprising the exogenous protein agent is at least 1.5, 2, 3, 4, 5, 10, 25, 50, 100, 500, 1000, 5000, 10,000, e.g., according to a FACS assay, e.g., using assays of Example 2 and Example 4.

58. The fusosome of any of the preceding embodiments, wherein the ratio of the average exogenous protein agent level of target cells to the average exogenous protein agent level of non-target cells is at least 1.5, 2, 3, 4, 5, 10, 25, 50, 100, 500, 1000, 5000, 10,000, e.g., according to a FACS assay, e.g., using assays of Example 2 and Example 4.

59. The fusosome of any of the preceding embodiments, wherein the ratio of the median exogenous protein agent level of target cells to the median exogenous protein agent level of non-target cells is at least 1.5, 2, 3, 4, 5, 10, 25, 50, 100, 500, 1000, 5000, 10,000, e.g., according to a FACS assay, e.g., using assays of Example 2 and Example 4.

60. The fusosome of any of the preceding embodiments, which comprises one or both of:

i) an exogenous or overexpressed immunosuppressive protein on the lipid bilayer, e.g., envelope; and

ii) an immunostimulatory protein that is absent or present at reduced levels (e.g., reduced by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%) compared to a fusosome generated from an otherwise similar, unmodified source cell.

61. The fusosome of any of the preceding embodiments, which comprises one or more of:

i) a first exogenous or overexpressed immunosuppressive protein on the lipid bilayer, e.g., envelope, and a second exogenous or overexpressed immunosuppressive protein on the lipid bilayer, e.g., envelope;

ii) a first exogenous or overexpressed immunosuppressive protein on the lipid bilayer, e.g., envelope, and a second immunostimulatory protein that is absent or present at reduced levels (e.g., reduced by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%) compared to a fusosome generated from an otherwise similar, unmodified source cell; or

iii) a first immunostimulatory protein that is absent or present at reduced levels (e.g., reduced by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%) compared to a fusosome generated from an otherwise similar, unmodified source cell and a second immunostimulatory protein that is absent or present at reduced levels (e.g., reduced by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%) compared to a fusosome generated from an otherwise similar, unmodified source cell.

62. The fusosome of any of the preceding embodiments, wherein the fusosome is in circulation at least 0.5, 1, 2, 3, 4, 6, 12, 18, 24, 36, or 48 hours after administration to the subject.

63. The fusosome of any of the preceding embodiments, wherein at least 0.001%, 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of fusosomes are in circulation 30 minutes after administration.

64. The fusosome of any of the preceding embodiments, wherein at least 0.001%, 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of fusosomes are in circulation 1 hour after administration.

65. The fusosome of any of the preceding embodiments, wherein at least 0.001%, 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of fusosomes are in circulation 2 hours after administration.

66. The fusosome of any of the preceding embodiments, wherein at least 0.001%, 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of fusosomes are in circulation 4 hours after administration.

5 67. The fusosome of any of the preceding embodiments, wherein at least 0.001%, 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of fusosomes are in circulation 8 hours after administration.

10 68. The fusosome of any of the preceding embodiments, wherein at least 0.001%, 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of fusosomes are in circulation 12 hours after administration.

15 69. The fusosome of any of the preceding embodiments, wherein at least 0.001%, 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of fusosomes are in circulation 18 hours after administration.

20 70. The fusosome of any of the preceding embodiments, wherein at least 0.001%, 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of fusosomes are in circulation 24 hours after administration.

71. The fusosome of any of the preceding embodiments, wherein at least 0.001%, 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of fusosomes are in circulation 36 hours after administration.

25 72. The fusosome of any of the preceding embodiments, wherein at least 0.001%, 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of fusosomes are in circulation 48 hours after administration.

30 73. The fusosome of any of the preceding embodiments, which has a reduction in immunogenicity as measured by a reduction in humoral response following one or more administration of the fusosome to an appropriate animal model, e.g., an animal model described

herein, compared to reference fusosome, e.g., an unmodified fusosome otherwise similar to the fusosome.

74. The fusosome of embodiment 73, wherein the reduction in humoral response is
5 measured in a serum sample by an anti-cell antibody titre, e.g., anti-retroviral antibody titre, e.g., by ELISA.

75. The fusosome of any of the preceding embodiments, wherein a serum sample
from animals administered the fusosome has a reduction of 1%, 5%, 10%, 20%, 30%, 40%, 50%,
10 60%, 70%, 80%, 90%, or more of an anti-fusosome antibody titer compared to the serum sample from a subject administered an unmodified cell.

76. The fusosome of any of the preceding embodiments, wherein a serum sample
from a subject administered the fusosome has an increased anti-cell antibody titre, e.g., increased
15 by 1%, 2%, 5%, 10%, 20%, 30%, or 40% from baseline, e.g., wherein baseline refers to serum sample from the same subject before administration of the fusosome.

77. The fusosome of any of the preceding embodiments, wherein:
the subject to be administered the fusosome or a pharmaceutical composition comprising
20 the fusosome has, or is known to have, or is tested for, a pre-existing antibody (e.g., IgG or IgM) reactive with the fusosome;

the subject to be administered the fusosome does not have detectable levels of a pre-existing antibody reactive with the fusosome;

a subject that has received the fusosome or a pharmaceutical composition comprising the
25 fusosome has, or is known to have, or is tested for, an antibody (e.g., IgG or IgM) reactive with the fusosome;

the subject that received the fusosome or a pharmaceutical composition comprising the fusosome (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; or

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.

5 78. The fusosome of any of the preceding embodiments, wherein the fusosome is produced by the methods of Example 5, 6, or 7, e.g., from cells transfected with HLA-G or HLA-E cDNA.

10 79. The fusosome of any of the preceding embodiments, wherein fusosomes generated from NMC-HLA-G cells have a decreased percentage of lysis, e.g., PBMC mediated lysis, NK cell mediated lysis, and/or CD8+ T cell mediated lysis, at specific timepoints as compared to fusosomes generated from NMCs or NMC-empty vector.

15 80. The fusosome of any of the preceding embodiments, wherein the modified fusosome evades phagocytosis by macrophages.

 81. The fusosome of any of the preceding embodiments, wherein the fusosome is produced by the methods of Example 8, e.g., from cells transfected with CD47 cDNA.

20 82. The fusosome of any of the preceding embodiments, wherein the phagocytic index is reduced when macrophages are incubated with fusosomes derived from NMC-CD47, versus those derived from NMC, or NMC-empty vector.

25 83. The fusosome of any of the preceding embodiments, which 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 fusosome, e.g., an unmodified fusosome otherwise similar to the fusosome, wherein the reduction in macrophage phagocytosis is determined by assaying the phagocytosis index *in vitro*, e.g., as described in Example 8.

30

84. The fusosome of any of the preceding embodiments, wherein the fusosome composition has a phagocytosis index of 0, 1, 10, 100, or more, e.g., as measured by an assay of Example 8, when incubated with macrophages in an *in vitro* assay of macrophage phagocytosis.

5 85. The fusosome of any of the preceding embodiments, which is modified and has reduced complement activity compared to an unmodified fusosome.

86. The fusosome of any of the preceding embodiments, which is produced by the methods of Example 9, e.g., from cells transfected with a cDNA coding for a complement
10 regulatory protein, e.g., DAF.

87. The fusosome of any of the preceding embodiments, wherein the dose of fusosome at which 200 pg/ml of C3a is present is greater for the modified fusosome (e.g., HEK293-DAF) incubated with corresponding mouse sera (e.g., HEK-293 DAF mouse sera) than
15 for the reference fusosome (e.g., HEK293 retroviral vector) incubated with corresponding mouse sera (e.g., HEK293 mouse sera).

88. The fusosome of any of the preceding embodiments, wherein the dose of fusosome at which 200 pg/ml of C3a is present is greater for for the modified fusosome (e.g.,
20 HEK293-DAF) incubated with naive mouse sera than for the reference fusosome (e.g., HEK293 retroviral vector) incubated with naive mouse sera.

89. The fusosome of any of the preceding embodiments, wherein the fusosome is resistant to complement mediated inactivation in patient serum 30 minutes after administration
25 according to an assay of Example 9.

90. The fusosome of any of the preceding embodiments, wherein at least 0.001%, 0.01%, 0.1%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of fusosomes are resistant to complement mediated inactivation.

91. The fusosome of any of embodiments 86-90, wherein the complement regulatory protein comprises one or more of proteins that bind decay-accelerating factor (DAF, CD55), e.g. factor H (FH)-like protein-1 (FHL-1), e.g. C4b-binding protein (C4BP), e.g. complement receptor 1 (CD35), e.g. Membrane cofactor protein (MCP, CD46), eg. Protectin (CD59), e.g. proteins that inhibit the classical and alternative complement pathway CD/C5 convertase enzymes, e.g. proteins that regulate MAC assembly.

92. The fusosome of any of the preceding embodiments, which is produced by the methods of Example 10, e.g., from cells transfected with a DNA coding for an shRNA targeting MHC class I, e.g., wherein retroviral vectors derived from NMC- shMHC class I has lower expression of MHC class I compared to NMCs and NMC-vector control.

93. The fusosome of any of the preceding embodiments, wherein a measure of immunogenicity for fusosomes is serum inactivation, e.g., serum inactivation measured as described herein, e.g., as described in Example 11.

94. The fusosome of any of the preceding embodiments, wherein the percent of cells which receive the exogenous agent is not different between fusosome samples that have been incubated with serum and heat-inactivated serum from fusosome naïve mice.

95. The fusosome of any of the preceding embodiments, wherein the percent of cells which receive the exogenous agent is not different between fusosome samples that have been incubated with serum from fusosome naïve mice and no-serum control incubations.

96. fusosome of any of the preceding embodiments, wherein the percent of cells which receive the exogenous agent is less in fusosome samples that have been incubated with positive control serum than in fusosome samples that have been incubated with serum from fusosome naïve mice.

97. The fusosome of any of the preceding embodiments, wherein a modified fusosome, e.g., modified by a method described herein, has a reduced (e.g., reduced compared to

administration of an unmodified fusosome) serum inactivation following multiple (e.g., more than one, e.g., 2 or more), administrations of the modified fusosome.

98. The fusosome of any of the preceding embodiments, wherein a fusosome
5 described herein is not inactivated by serum following multiple administrations.

99. The fusosome of any of the preceding embodiments, wherein a measure of immunogenicity for the fusosome is serum inactivation, e.g., after multiple administrations, e.g., serum inactivation after multiple administrations measured as described herein, e.g., as described
10 in Example 12.

100. The fusosome of any of the preceding embodiments, wherein the percent of cells which receive the exogenous agent is not different between fusosome samples that have been incubated with serum and heat-inactivated serum from mice treated with modified (e.g.,
15 HEK293-HLA-G) fusosomes.

101. The fusosome of any of the preceding embodiments, wherein the percent of cells which receive the exogenous agent is not different between fusosome samples that have been incubated from mice treated 1, 2, 3, 5 or 10 times with modified (e.g., HEK293-HLA-G)
20 fusosomes.

102. The fusosome of any of the preceding embodiments, wherein the percent of cells which receive the exogenous agent is not different between fusosome samples that have been incubated with serum from mice treated with vehicle and from mice treated with modified (e.g.,
25 HEK293-HLA-G) fusosomes.

103. The fusosome of any of the preceding embodiments, wherein the percent of cells which receive the exogenous agent is less for fusosomes derived from a reference cell (e.g., HEK293) than for modified (e.g., HEK293-HLA-G) fusosomes.
30

104. The fusosome of any of the preceding embodiments, wherein a measure of immunogenicity for a fusosome is antibody response.

105. The fusosome of any of the preceding embodiments, wherein a subject that
5 receives a fusosome described herein has pre-existing antibodies which bind to and recognize fusosome, e.g., measured as described herein, e.g., as described in Example 13.

106. The fusosome of any of the preceding embodiments, wherein serum from fusosome -naïve mice shows more signal (e.g., fluorescence) than the negative control, e.g.,
10 serum from a mouse depleted of IgM and IgG, e.g., indicating that immunogenicity has occurred.

107. The fusosome of any of the preceding embodiments, wherein serum from fusosome -naïve mice shows similar signal (e.g., fluorescence) compared to the negative control,
15 e.g., indicating that immunogenicity did not detectably occur.

108. The fusosome of any of the preceding embodiments, which is a modified fusosome, e.g., modified by a method described herein, and which has a reduced (e.g., reduced compared to administration of an unmodified fusosome) humoral response following multiple
20 (e.g., more than one, e.g., 2 or more), administrations of the modified fusosome, e.g., measured as described herein, e.g., as described in Example 14.

109. The fusosome of any of the preceding embodiments, wherein the fusosome is produced by the methods of Example 5, 6, 7, or 14, e.g., from cells transfected with HLA-G or
25 HLA-E cDNA.

110. The fusosome of any of the preceding embodiments, wherein humoral response is assessed by determining a value for the level of anti-fusosome antibodies (e.g., IgM, IgG1, and/or IgG2 antibodies).

111. The fusosome of any of the preceding embodiments, wherein modified (e.g., NMC-HLA-G) fusosomes have decreased anti-viral IgM or IgG1/2 antibody titers (e.g., as measured by fluorescence intensity on FACS) after injections, as compared to a control, e.g., NMC fusosomes or NMC-empty fusosomes.

5

112. The fusosome of any of the preceding embodiments, wherein recipient cells are not targeted by an antibody response, or an antibody response will be below a reference level, e.g., measured as described herein, e.g., as described in Example 15.

10

113. The fusosome of any of the preceding embodiments, signal (e.g., mean fluorescence intensity) is similar for recipient cells from mice treated with fusosomes and mice treated with PBS.

15

114. The fusosome of any of the preceding embodiments, wherein a measure of the immunogenicity of recipient cells is the macrophage response.

115. The fusosome of any of the preceding embodiments, wherein recipient cells are not targeted by macrophages, or are targeted below a reference level.

20

116. The fusosome of any of the preceding embodiments, wherein the phagocytic index, e.g., measured as described herein, e.g., as described in Example 16, is similar for recipient cells derived from mice treated with fusosomes and mice treated with PBS.

25

117. The fusosome of any of the preceding embodiments, wherein a measure of the immunogenicity of recipient cells is the PBMC response.

118. The fusosome of any of the preceding embodiments, wherein recipient cells do not elicit a PBMC response.

119. The fusosome of any of the preceding embodiments, wherein the percent of CD3+/CMG+ cells is similar for recipient cells derived from mice treated with fusosome and mice treated with PBS, e.g., as measured as described herein, e.g., as described in Example 17.

5 120. The fusosome of any of the preceding embodiments, wherein a measure of the immunogenicity of recipient cells is the natural killer cell response.

121. The fusosome of any of the preceding embodiments, wherein recipient cells do not elicit a natural killer cell response or elicit a lower natural killer cell response, e.g., lower
10 than a reference value.

122. The fusosome of any of the preceding embodiments, wherein the percent of CD3+/CMG+ cells is similar for recipient cells derived from mice treated with fusosome and mice treated with PBS, e.g., as measured as described herein, e.g., as described in Example 18.

15 123. The fusosome of any of the preceding embodiments, wherein a measure of the immunogenicity of recipient cells is the CD8+ T cell response.

124. The fusosome of any of the preceding embodiments, wherein recipient cells do not elicit a CD8+ T cell response or elicit a lower CD8+ T cell response, e.g., lower than a
20 reference value.

125. The fusosome of any of the preceding embodiments, wherein the percent of CD3+/CMG+ cells is similar for recipient cells derived from mice treated with fusosome and
25 mice treated with PBS, e.g., as measured as described herein, e.g., as described in Example 19.

126. The fusosome of any of the preceding embodiments, wherein the fusogen is a re-targeted fusogen.

30 127. The fusosome of any of the preceding embodiments, which comprises a nucleic acid, e.g., retroviral nucleic acid, that encodes one or both of: (i) a positive target cell-specific

regulatory element operatively linked to a nucleic acid encoding an exogenous agent, or (ii) a non-target cell-specific regulatory element or negative TCSRE operatively linked to the nucleic acid encoding the exogenous agent.

5 128. A pharmaceutical composition comprising the fusosome of any of the preceding embodiments, and a pharmaceutically acceptable carrier, diluent, or excipient.

 129. A method of delivering an exogenous agent to a subject (e.g., a human subject) comprising administering to the subject a fusosome of any of embodiments 1-127 or
10 pharmaceutical composition of embodiment 128, thereby delivering the exogenous agent to the subject.

 130. A method of modulating a function, in a subject (e.g., a human subject), target tissue or target cell (e.g., a CNS cell, e.g., a neuron or a glial cell), comprising contacting, e.g.,
15 administering to, the subject, the target tissue or the target cell a fusosome of any of embodiments 1-127, or the pharmaceutical composition of embodiment 128.

 131. The method of embodiment 130, wherein the target tissue or the target cell is present in a subject.

20

 132. A method of treating a genetic deficiency in a subject (e.g., a human subject) comprising administering to the subject a fusosome of any of embodiments 1-127 or the pharmaceutical composition of claim 128.

25 133. The method of embodiment 132, wherein the genetic deficiency is a genetic deficiency of Table 5 or Table 6.

 134. The method of embodiment 132 or 133, wherein the genetic deficiency is a genetic deficiency able to be treated by the payload gene encoding the exogenous agent.

30

135. The method of any of embodiments 132-134, wherein the genetic deficiency is associated with a CNS disease or disorder or a lysosomal disease or disorder, wherein the method treats the CNS disease or disorder or a lysosomal disease or disorder.

5 136. The method of embodiment 135, wherein the CNS disease or disorder or a lysosomal disease or disorder Spinocerebellar Ataxia; Autosomal Recessive, Type 1; Ataxia with Oculomotor Apraxia, Type 2; Fragile X Syndrome; Cerebral Creatine Deficiency Syndrome 1; Angelman Syndrome; Amyotrophic Lateral Sclerosis; Friedreich's Ataxia; Rett Syndrome; Canavan Disease; Pyridoxine-Dependent Epilepsy; Batten Disease, Fucosidosis; Krabbe
10 Disease; Tay Sachs Disease; Sandhoff Disease; Beta-mannosidosis; Metachromatic Leukodystrophy; Mucopolysaccharidosis Type IIIa; Mucopolysaccharidosis Type IIIb; or Mucopolysaccharidosis Type IV.

137. A fusosome of any of embodiments 1-127 or pharmaceutical composition of embodiment 128 for use in treating a subject (e.g. a human subject) with a genetic deficiency.

15

138. Use of a fusosome of any of embodiments 1-127 or pharmaceutical composition of embodiment 128 for manufacture of a medicament for use in treating a subject (e.g. a human subject) with a genetic deficiency.

20 139. The fusosome or pharmaceutical composition for use of embodiment 137 or the use of embodiment 138, wherein the fusosome comprises a payload gene encoding an exogenous agent for treating the genetic deficiency.

25 140. The fusosome or pharmaceutical composition for use of embodiment 137 or 139 or the use of embodiment 138 or 139, wherein the genetic deficiency is associated with a CNS disease or disorder or a lysosomal disease or disorder, wherein the method treats the CNS disease or disorder or a lysosomal disease or disorder.

30 141. The fusosome or pharmaceutical composition for use of embodiment 137, 139 or 140, or the use of embodiment 138, 139 or 140, wherein the CNS disease or disorder or a lysosomal disease or disorder Spinocerebellar Ataxia; Autosomal Recessive, Type 1; Ataxia with

Oculomotor Apraxia, Type 2; Fragile X Syndrome; Cerebral Creatine Deficiency Syndrome 1; Angelman Syndrome; Amyotrophic Lateral Sclerosis; Friedreich's Ataxia; Rett Syndrome; Canavan Disease; Pyridoxine-Dependent Epilepsy; Batten Disease, Fucosidosis; Krabbe Disease; Tay Sachs Disease; Sandhoff Disease; Beta-mannosidosis; Metachromatic

5 Leukodystrophy; Mucopolysaccharidosis Type IIIa; Mucopolysaccharidosis Type IIIb; or Mucopolysaccharidosis Type IV.

142. A method of making a fusosome of any of embodiments 1-127, comprising:

- 10 a) providing a cell that comprises the nucleic acid, e.g., retroviral nucleic acid, and the fusogen;
- b) culturing the cell under conditions that allow for production of the fusosome, and
- c) separating, enriching, or purifying the fusosome from the cell, thereby making the fusosome.

15 Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

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

20 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 May 15, 2018. When one gene or protein

25 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

The following detailed description of the invention will be better understood when read

30 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.

FIG. 1 quantifies staining of fusosomes with a dye for F-actin.

FIG. 2 is a graph showing the capacity for fusosomes and parent cells to polymerase actin
5 over a period of 3, 5, and 24 hours.

FIG. 3 is a table showing size distribution statistics of fusosomes and parental cells as measured by NTA and microscopy.

FIG. 4 is a table showing the average size and volume of fusosomes and parental cells.

FIG. 5 is a series of diagrams showing the soluble:insoluble ratio observed for fusosomes
10 or a cell preparation.

FIG. 6 is a series of diagrams showing MvH(CD8)+F fusosome fusion to target or non-target cells and absolute amount of targeted fusion.

FIG. 7 is a diagram showing 2-NBDG mean fluorescence intensity in VSV-G fusosomes.

FIG. 8 is a diagram showing esterase activity in the cytosol of VSV-G fusosomes.

FIGS. 9A-9B are a series of diagrams showing Cre recombinase delivery by fusosomes
15 as detected by bioluminescent imaging in mice. (A) Ventral image and luminescent signal overlay of exposed liver and spleen of IV fusosome treated mice (1x and 3x concentration). Lower portion is luminescent signal alone. (B) Total flux signal of fusosome targeted spleen and liver; y-scale is on log10 scale. Mice treated with a concentration of 3x fusosome treatment had a
20 significantly greater signal in the spleen ($p=0.0004$) than background 72 hours post-treatment.

FIGS. 10A-10B are a series of diagrams showing Cre recombinase to murine liver and spleen by fusosomes as detected by bioluminescent imaging. (A) From left to right; dorsal
25 image and luminescent signal overlay of excised liver, heart, lungs, kidney, small intestines, pancreas, and spleen collected and imaged within 5 minutes of euthanasia. Lower portion is luminescent signal alone. (B) Total flux signal of fusosome targeted spleen and liver and other tissues; y-scale is on log10 scale. Mice treated with a concentration of 3x fusosome treatment had a significantly greater signal in the spleen ($p<0.0001$) as compared to the tissue with the lowest signal (heart).

FIG. 11 is a table showing delivery of Cre cargo by NivG+F fusosomes via a non-
30 endocytic pathway.

FIG. 12 is a graph showing GAPDH: Total protein ratios measured by bicinchoninic acid assay in fusosomes and parental cells.

FIG. 13 is a graph showing lipid: protein ratios measured by bicinchoninic acid assay in fusosomes and parental cells.

5 FIG. 14 is a graph showing protein: DNA ratios measured by bicinchoninic acid assay in fusosomes and parental cells.

FIG. 15 is a graph showing lipids: DNA ratios measured by bicinchoninic acid assay in fusosomes and parental cells.

10 FIG. 16 is a graph showing protein levels of the exosome marker CD63 in exosomes and fusosomes.

FIG. 17 is a graph showing the intensity of calnexin signal detected in fusosomes and parental cells.

FIG. 18 is a graph showing lipid:DNA ratios determined for fusosomes and parental cells.

15 FIGS. 19A-19B are a series of graphs showing the proportion of lipid species as a percentage of total lipids in parental cells, exosomes, and fusosomes.

FIG. 20 is a series of graphs showing the protein content of parental cells, exosomes, and fusosomes with respect to proteins associated with specific compartments, as indicated.

20 FIG. 21 is a series of graphs showing the level of ARRDC1 (left panel) or TSG101 (right panel) as a percentage of total protein content in parental cells, exosomes, and fusosomes.

DETAILED DESCRIPTION

The present disclosure provides, at least in part, fusosome methods and compositions for in vivo delivery. In some embodiments, the fusosome comprises a combination of elements that
25 promote specificity for target cells, e.g., one or more of a re-targeted fusogen, a positive target cell-specific regulatory element, and a non-target cell-specific regulatory element. In some embodiments, the fusosome comprises one or more modifications that decrease an immune response against the fusosome.

I. Definitions

Terms used in the claims and specification are defined as set forth below unless otherwise specified.

As used herein, “detectably present”, when used in the context of an exogenous agent
5 being detectably present, means that the exogenous agent itself is detectably present. For instance, if the exogenous agent is a protein, the exogenous protein agent can be detectably present regardless of whether a nucleic acid that encodes it is detectably present or not.

As used herein, “fusosome” refers to a bilayer of amphipathic lipids enclosing a lumen or cavity and a fusogen that interacts with the amphipathic lipid bilayer. In embodiments, the
10 fusosome comprises a nucleic acid. In some embodiments, the fusosome is a membrane enclosed preparation. In some embodiments, the fusosome is derived from a source cell.

As used herein, “fusosome composition” refers to a composition comprising one or more fusosomes.

As used herein, “fusogen” refers to an agent or molecule that creates an interaction
15 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., a lumen of a retroviral vector 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. In some embodiments, the fusogen comprises a targeting
20 domain.

As used herein, an “insulator element” refers to a nucleotide sequence that blocks enhancers or prevents heterochromatin spreading. An insulator element can be wild-type or mutant.

The term “effective amount” as used herein means an amount of a pharmaceutical
25 composition which is sufficient enough to significantly and positively modify the symptoms and/or conditions to be treated (e.g., provide a positive clinical response). The effective amount of an active ingredient for use in a pharmaceutical composition will vary with the particular condition being treated, the severity of the condition, the duration of treatment, the nature of concurrent therapy, the particular active ingredient(s) being employed, the particular
30 pharmaceutically-acceptable excipient(s) and/or carrier(s) utilized, and like factors with the knowledge and expertise of the attending physician.

An “exogenous agent” as used herein with reference to a virus, VLP or fusosome, refers to an agent that is neither comprised by nor encoded in the corresponding wild-type virus or fusogen made from a corresponding wild-type source cell. In some embodiments, the exogenous agent does not naturally exist, such as a protein or nucleic acid that has a sequence that is altered (e.g., by insertion, deletion, or substitution) relative to a naturally occurring protein. In some
5 embodiments, the exogenous agent does not naturally exist in the source cell. In some embodiments, the exogenous agent exists naturally in the source cell but is exogenous to the virus. In some embodiments, the exogenous agent does not naturally exist in the recipient cell. In some embodiments, the exogenous agent exists naturally in the recipient cell, but is not
10 present at a desired level or at a desired time. In some embodiments, the exogenous agent comprises RNA or protein.

The term “pharmaceutically acceptable” as used herein, refers to excipients, compositions and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation,
15 allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

As used herein, a “promoter” refers to a cis- regulatory DNA sequence that, when operably linked to a gene coding sequence, drives transcription of the gene. The promoter may comprise a transcription factor binding sites. In some embodiments, a promoter works in concert
20 with one or more enhancers which are distal to the gene.

As used herein, a “positive target cell-specific regulatory element” (or positive TCSRE) refers to a nucleic acid sequence that increases the level of an exogenous agent in a target cell compared to in a non-target cell, wherein the nucleic acid encoding the exogenous agent is operably linked to the positive TCSRE. In some embodiments, the positive TCSRE is a
25 functional nucleic acid sequence, e.g., the positive TCSRE can comprise a promoter or enhancer. In some embodiments, the positive TCSRE encodes a functional RNA sequence, e.g., the positive TCSRE can encode a splice site that promotes correct splicing of the RNA in the target cell. In some embodiments, the positive TCSRE encodes a functional protein sequence, or the positive TCSRE can encode a protein sequence that promotes correct post-translational
30 modification of the protein. In some embodiments, the positive TCSRE decreases the level or activity of a downregulator or inhibitor of the exogenous agent.

As used herein, a “negative target cell-specific regulatory element” (or negative TCSRE) refers to a nucleic acid sequence that decreases the level of an exogenous agent in a non-target cell compared to in a target cell, wherein the nucleic acid encoding the exogenous agent is operably linked to the negative TCSRE. In some embodiments, the negative TCSRE is a functional nucleic acid sequence, e.g., a miRNA recognition site that causes degradation or inhibition of the retroviral nucleic acid in a non-target cell. In some embodiments, the nucleic acid sequence encodes a functional RNA sequence, e.g., the nucleic acid encodes an miRNA sequence present in an mRNA encoding an exogenous protein agent, such that the mRNA is degraded or inhibited in a non-target cell. In some embodiments, the negative TCSRE increases the level or activity of a downregulator or inhibitor of the exogenous agent.

As used herein, a “non-target cell-specific regulatory element” (or NTCSRE) refers to a nucleic acid sequence that decreases the level of an exogenous agent in a non-target cell compared to in a target cell, wherein the nucleic acid encoding the exogenous agent is operably linked to the NTCSRE. In some embodiments, the NTCSRE is a functional nucleic acid sequence, e.g., a miRNA recognition site that causes degradation or inhibition of the retroviral nucleic acid in a non-target cell. In some embodiments, the nucleic acid sequence encodes a functional RNA sequence, e.g., the nucleic acid encodes an miRNA sequence present in an mRNA encoding an exogenous protein agent, such that the mRNA is degraded or inhibited in a non-target cell. In some embodiments, the NTCSRE increases the level or activity of a downregulator or inhibitor of the exogenous agent. The terms “negative TCSRE” and “NTCSRE” are used interchangeably herein.

As used herein, a “non-CNS cell specific regulatory element” refers to a non-target cell-specific regulatory element (NTCSRE), wherein the target cell is a CNS cell. Thus, a non-CNS cell specific regulatory element refers to a nucleic acid sequence that decreases the level of an exogenous agent in a non-CNS cell compared to in a CNS cell, wherein the nucleic acid encoding the exogenous agent is operably linked to the non-CNS cell-specific regulatory element.

As used herein, a “re-targeted fusogen” refers to a fusogen that comprises a targeting moiety having a sequence that is not part of the naturally-occurring form of the fusogen. In embodiments, the fusogen comprises a different targeting moiety relative to the targeting moiety in the naturally-occurring form of the fusogen. In embodiments, the naturally-occurring form of

the fusogen lacks a targeting domain, and the re-targeted fusogen comprises a targeting moiety that is absent from the naturally-occurring form of the fusogen. In embodiments, the fusogen is modified to comprise a targeting moiety. In embodiments, the fusogen comprises one or more sequence alterations outside of the targeting moiety relative to the naturally-occurring form of the fusogen, e.g., in a transmembrane domain, fusogenically active domain, or cytoplasmic domain.

As used herein, a “retroviral nucleic acid” refers to a nucleic acid containing at least the minimal sequence requirements for packaging into a retrovirus or retroviral vector, alone or in combination with a helper cell, helper virus, or helper plasmid. In some embodiments, the retroviral nucleic acid further comprises or encodes an exogenous agent, a positive target cell-specific regulatory element, a non-target cell-specific regulatory element, or a negative TCSRE. In some embodiments, the retroviral nucleic acid comprises one or more of (e.g., all of) a 5’ LTR (e.g., to promote integration), U3 (e.g., to activate viral genomic RNA transcription), R (e.g., a Tat-binding region), U5, a 3’ LTR (e.g., to promote integration), a packaging site (e.g., psi (Ψ)), RRE (e.g., to bind to Rev and promote nuclear export). The retroviral nucleic acid can comprise RNA (e.g., when part of a virion) or DNA (e.g., when being introduced into a source cell or after reverse transcription in a recipient cell). In some embodiments, the retroviral nucleic acid is packaged using a helper cell, helper virus, or helper plasmid which comprises one or more of (e.g., all of) gag, pol, and env.

As used herein, a “target cell” refers to a cell of a type to which it is desired that a fusosome (e.g., lentiviral vector) deliver an exogenous agent. In embodiments, a target cell is a cell of a specific tissue type or class, e.g., a CNS cell, e.g., a neuron or a glial cell. In some embodiments, a target cell is a diseased cell, e.g., a cancer cell. In some embodiments, the fusogen, e.g., re-targeted fusogen (alone or in combination with the positive TCSRE, NTCSRE, negative TCSRE, or any combination thereof) leads to preferential delivery of the exogenous agent to a target cell compared to a non-target cell.

As used herein a “non-target cell” refers to a cell of a type to which it is not desired that a lentiviral vector delivers an exogenous agent. In some embodiments, a non-target cell is a cell of a specific tissue type or class. In some embodiments, a non-target cell is a non-diseased cell, e.g., a non-cancerous cell. In some embodiments, the fusogen, e.g., re-targeted fusogen (alone or in combination with the positive TCSRE, NTCSRE, negative TCSRE or any combination

thereof) leads to lower delivery of the exogenous agent to a non-target cell compared to a target cell.

As used herein, the terms “treat,” “treating,” or “treatment” refer to ameliorating a disease or disorder, e.g., slowing or arresting or reducing the development of the disease or disorder, e.g., a root cause of the disorder or at least one of the clinical symptoms thereof.

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.

II. Fusosomes, e.g., cell-derived fusosomes

Fusosomes can take various forms. For example, in some embodiments, a fusosome described herein is derived from a source cell. A fusosome may be or comprise, e.g., an extracellular vesicle, a microvesicle, a nanovesicle, an exosome, an apoptotic body (from apoptotic cells), a microparticle (which may be derived from, e.g., platelets), an ectosome (derivable from, e.g., neutrophils and monocytes in serum), a prostatosome (obtainable from prostate cancer cells), a cardiosome (derivable from cardiac cells), or any combination thereof. In some embodiments, a fusosome is released naturally from a source cell, and in some embodiments, the source cell is treated to enhance formation of fusosomes. In some embodiments, the fusosome is between about 10-10,000 nm in diameter, e.g., about 30-100 nm in diameter. In some embodiments, the fusosome comprises one or more synthetic lipids.

In some embodiments, the fusosome is or comprises a virus, e.g., a retrovirus, e.g., a lentivirus. For instance, in some embodiments, the fusosome’s bilayer of amphipathic lipids is or comprises the viral envelope. The viral envelope may comprise a fusogen, e.g., a fusogen that is endogenous to the virus or a pseudotyped fusogen. In some embodiments, the fusosome’s lumen or cavity comprises a viral nucleic acid, e.g., a retroviral nucleic acid, e.g., a lentiviral nucleic acid. The viral nucleic acid may be a viral genome. In some embodiments, the fusosome further comprises one or more viral non-structural proteins, e.g., in its cavity or lumen.

Fusosomes may have various properties that facilitate delivery of a payload to a target cell. For instance, in some embodiments, the fusosome and the source cell together comprise nucleic acid(s) sufficient to make a particle that can fuse with a target cell. In embodiments,

these nucleic acid(s) encode proteins having one or more of (e.g., all of) the following activities: gag polyprotein activity, polymerase activity, integrase activity, protease activity, and fusogen activity.

Fusosomes may also comprise various structures that facilitate delivery of a payload to a target cell. For instance, in some embodiments, the fusosome and the source cell together comprise nucleic acid(s) sufficient to make a particle that can fuse with a target cell. In embodiments, these nucleic acid(s) encode proteins having one or more of (e.g., all of) the following activities: gag polyprotein activity, polymerase activity, integrase activity, protease activity, and fusogen activity.

Fusosomes may also comprise various structures that facilitate delivery of a payload to a target cell. For instance, in some embodiments, the fusosome (e.g., virus, e.g., retrovirus, e.g., lentivirus) comprises one or more of (e.g., all of) the following proteins: gag polyprotein, polymerase (e.g., pol), integrase (e.g., a functional or non-functional variant), protease, and a fusogen. In some embodiments, the fusosome further comprises rev. In some embodiments, one or more of the aforesaid proteins are encoded in the retroviral genome, and in some embodiments, one or more of the aforesaid proteins are provided in trans, e.g., by a helper cell, helper virus, or helper plasmid. In some embodiments, the fusosome nucleic acid (e.g., retroviral nucleic acid) comprises one or more of (e.g., all of) the following nucleic acid sequences: 5' LTR (e.g., comprising U5 and lacking a functional U3 domain), Psi packaging element (Psi), Central polypurine tract (cPPT) Promoter operatively linked to the payload gene, payload gene (optionally comprising an intron before the open reading frame), Poly A tail sequence, WPRE, and 3' LTR (e.g., comprising U5 and lacking a functional U3). In some embodiments the fusosome nucleic acid (e.g., retroviral nucleic acid) further comprises one or more insulator element. In some embodiments the fusosome nucleic acid (e.g., retroviral nucleic acid) further comprises one or more miRNA recognition sites. In some embodiments, one or more of the miRNA recognition sites are situated downstream of the poly A tail sequence, e.g., between the poly A tail sequence and the WPRE.

In some embodiments, a fusosome provided herein is 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 a genetic

deficiency, such as any listed in Table 5 or Table 6. In some embodiments, the fusosome contains nucleic acid sequences encoding an exogenous agent for treating the disease or condition, such as for treating the genetic deficiency.

A. Fusosomes generated from viruses.

5 For instance, in some embodiments, the fusosome (e.g., virus, e.g., retrovirus, e.g., lentivirus) comprises one or more of (e.g., all of) the following proteins: gag polyprotein, polymerase (e.g., pol), integrase (e.g., a functional or non-functional variant), protease, and a fusogen. In some embodiments, the fusosome further comprises rev. In some embodiments, one or more of the aforesaid proteins are encoded in the retroviral genome, and in some
10 embodiments, one or more of the aforesaid proteins are provided in trans, e.g., by a helper cell, helper virus, or helper plasmid. In some embodiments, the fusosome nucleic acid (e.g., retroviral nucleic acid) comprises one or more of (e.g., all of) the following nucleic acid sequences: 5' LTR (e.g., comprising U5 and lacking a functional U3 domain), Psi packaging element (Psi), Central polypurine tract (cPPT) Promoter operatively linked to the payload gene, payload gene
15 (optionally comprising an intron before the open reading frame), Poly A tail sequence, WPRE, and 3' LTR (e.g., comprising U5 and lacking a functional U3). In some embodiments the fusosome nucleic acid (e.g., retroviral nucleic acid) further comprises one or more insulator element. In some embodiments the fusosome nucleic acid (e.g., retroviral nucleic acid) further comprises one or more miRNA recognition sites. In some embodiments, one or more of the
20 miRNA recognition sites are situated downstream of the poly A tail sequence, e.g., between the poly A tail sequence and the WPRE.

i) Lentiviral components and helper cells

In some embodiments, the retroviral nucleic acid comprises one or more of (e.g., all of): a 5' promoter (e.g., to control expression of the entire packaged RNA), a 5' LTR (e.g., that
25 includes R (polyadenylation tail signal) and/or U5 which includes a primer activation signal), a primer binding site, a psi packaging signal, a RRE element for nuclear export, a promoter directly upstream of the transgene to control transgene expression, a transgene (or other exogenous agent element), a polypurine tract, and a 3' LTR (e.g., that includes a mutated U3, a R, and U5). In some embodiments, the retroviral nucleic acid further comprises one or more of a
30 cPPT, a WPRE, and/or an insulator element.

A retrovirus typically replicates by reverse transcription of its genomic RNA into a linear double-stranded DNA copy and subsequently covalently integrates its genomic DNA into a host genome. Illustrative retroviruses suitable for use in particular embodiments, include, but are not limited to: Moloney murine leukemia virus (M-MuLV), Moloney murine sarcoma virus (MoMSV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), gibbon ape leukemia virus (GaLV), feline leukemia virus (FLV), spumavirus, Friend murine leukemia virus, Murine Stem Cell Virus (MSCV) and Rous Sarcoma Virus (RSV) and lentivirus.

In some embodiments the retrovirus is a Gammaretrovirus. In some embodiments the retrovirus is an Epsilonretrovirus. In some embodiments the retrovirus is an Alpharetrovirus. In some embodiments the retrovirus is a Betaretrovirus. In some embodiments the retrovirus is a Deltaretrovirus. In some embodiments the retrovirus is a Lentivirus. In some embodiments the retrovirus is a Spumaretrovirus. In some embodiments the retrovirus is an endogenous retrovirus.

Illustrative lentiviruses include, but are not limited to: HIV (human immunodeficiency virus; including HIV type 1, and HIV type 2); visna-maedi virus (VMV) virus; the caprine arthritis-encephalitis virus (CAEV); equine infectious anemia virus (EIAV); feline immunodeficiency virus (FIV); bovine immune deficiency virus (BIV); and simian immunodeficiency virus (SIV). In some embodiments, HIV based vector backbones (i.e., HIV cis-acting sequence elements) are used.

In some embodiments, a vector herein is a nucleic acid molecule capable transferring or transporting another nucleic acid molecule. The transferred nucleic acid is generally linked to, e.g., inserted into, the vector nucleic acid molecule. A vector may include sequences that direct autonomous replication in a cell, or may include sequences sufficient to allow integration into host cell DNA. Useful vectors include, for example, plasmids (e.g., DNA plasmids or RNA plasmids), transposons, cosmids, bacterial artificial chromosomes, and viral vectors. Useful viral vectors include, e.g., replication defective retroviruses and lentiviruses.

A viral vector can comprise, e.g., a nucleic acid molecule (e.g., a transfer plasmid) that includes virus-derived nucleic acid elements that typically facilitate transfer of the nucleic acid molecule or integration into the genome of a cell or to a viral particle that mediates nucleic acid transfer. Viral particles will typically include various viral components and sometimes also host cell components in addition to nucleic acid(s). A viral vector can comprise, e.g., a virus or viral

particle capable of transferring a nucleic acid into a cell, or to the transferred nucleic acid (e.g., as naked DNA). Viral vectors and transfer plasmids can comprise structural and/or functional genetic elements that are primarily derived from a virus. A retroviral vector can comprise a viral vector or plasmid containing structural and functional genetic elements, or portions thereof, that are primarily derived from a retrovirus. A lentiviral vector can comprise a viral vector or plasmid containing structural and functional genetic elements, or portions thereof, including LTRs that are primarily derived from a lentivirus.

In embodiments, a lentiviral vector (e.g., lentiviral expression vector) may comprise a lentiviral transfer plasmid (e.g., as naked DNA) or an infectious lentiviral particle. With respect to elements such as cloning sites, promoters, regulatory elements, heterologous nucleic acids, etc., it is to be understood that the sequences of these elements can be present in RNA form in lentiviral particles and can be present in DNA form in DNA plasmids.

In some vectors described herein, at least part of one or more protein coding regions that contribute to or are essential for replication may be absent compared to the corresponding wild-type virus. This makes the viral vector replication-defective. In some embodiments, the vector is capable of transducing a target non-dividing host cell and/or integrating its genome into a host genome.

The structure of a wild-type retrovirus genome often comprises a 5' long terminal repeat (LTR) and a 3' LTR, between or within which are located a packaging signal to enable the genome to be packaged, a primer binding site, integration sites to enable integration into a host cell genome and gag, pol and env genes encoding the packaging components which promote the assembly of viral particles. More complex retroviruses have additional features, such as rev and RRE sequences in HIV, which enable the efficient export of RNA transcripts of the integrated provirus from the nucleus to the cytoplasm of an infected target cell. In the provirus, the viral genes are flanked at both ends by regions called long terminal repeats (LTRs). The LTRs are involved in proviral integration and transcription. LTRs also serve as enhancer-promoter sequences and can control the expression of the viral genes. Encapsidation of the retroviral RNAs occurs by virtue of a psi sequence located at the 5' end of the viral genome.

The LTRs themselves are typically similar (e.g., identical) sequences that can be divided into three elements, which are called U3, R and U5. U3 is derived from the sequence unique to the 3' end of the RNA. R is derived from a sequence repeated at both ends of the RNA and U5 is

derived from the sequence unique to the 5' end of the RNA. The sizes of the three elements can vary considerably among different retroviruses.

For the viral genome, the site of transcription initiation is typically at the boundary between U3 and R in one LTR and the site of poly (A) addition (termination) is at the boundary between R and U5 in the other LTR. U3 contains most of the transcriptional control elements of the provirus, which include the promoter and multiple enhancer sequences responsive to cellular and in some cases, viral transcriptional activator proteins. Some retroviruses comprise any one or more of the following genes that code for proteins that are involved in the regulation of gene expression: *tot*, *rev*, *tax* and *rex*.

With regard to the structural genes *gag*, *pol* and *env* themselves, *gag* encodes the internal structural protein of the virus. *Gag* protein is proteolytically processed into the mature proteins MA (matrix), CA (capsid) and NC (nucleocapsid). The *pol* gene encodes the reverse transcriptase (RT), which contains DNA polymerase, associated RNase H and integrase (IN), which mediate replication of the genome. The *env* gene encodes the surface (SU) glycoprotein and the transmembrane (TM) protein of the virion, which form a complex that interacts specifically with cellular receptor proteins. This interaction promotes infection, e.g., by fusion of the viral membrane with the cell membrane.

In a replication-defective retroviral vector genome *gag*, *pol* and *env* may be absent or not functional. The R regions at both ends of the RNA are typically repeated sequences. U5 and U3 represent unique sequences at the 5' and 3' ends of the RNA genome respectively.

Retroviruses may also contain additional genes which code for proteins other than *gag*, *pol* and *env*. Examples of additional genes include (in HIV), one or more of *vif*, *vpr*, *vpx*, *vpu*, *tat*, *rev* and *nef*. EIAV has (amongst others) the additional gene S2. Proteins encoded by additional genes serve various functions, some of which may be duplicative of a function provided by a cellular protein. In EIAV, for example, *tat* acts as a transcriptional activator of the viral LTR (Derse and Newbold 1993 *Virology* 194:530-6; Maury et al. 1994 *Virology* 200:632-42). It binds to a stable, stem-loop RNA secondary structure referred to as TAR. *Rev* regulates and co-ordinates the expression of viral genes through *rev*-response elements (RRE) (Martarano et al. 1994 *J. Virol.* 68:3102-11). The mechanisms of action of these two proteins are thought to be broadly similar to the analogous mechanisms in the primate viruses. In addition, an EIAV

protein, Ttm, has been identified that is encoded by the first exon of tat spliced to the env coding sequence at the start of the transmembrane protein.

In addition to protease, reverse transcriptase and integrase, non-primate lentiviruses contain a fourth pol gene product which codes for a dUTPase. This may play a role in the ability of these lentiviruses to infect certain non-dividing or slowly dividing cell types.

In embodiments, a recombinant lentiviral vector (RLV) is a vector with sufficient retroviral genetic information to allow packaging of an RNA genome, in the presence of packaging components, into a viral particle capable of infecting a target cell. Infection of the target cell can comprise reverse transcription and integration into the target cell genome. The RLV typically carries non-viral coding sequences which are to be delivered by the vector to the target cell. In embodiments, an RLV is incapable of independent replication to produce infectious retroviral particles within the target cell. Usually the RLV lacks a functional gag-pol and/or env gene and/or other genes involved in replication. The vector may be configured as a split-intron vector, e.g., as described in PCT patent application WO 99/15683, which is herein incorporated by reference in its entirety.

In some embodiments, the lentiviral vector comprises a minimal viral genome, e.g., the viral vector has been manipulated so as to remove the non-essential elements and to retain the essential elements in order to provide the required functionality to infect, transduce and deliver a nucleotide sequence of interest to a target host cell, e.g., as described in WO 98/17815, which is herein incorporated by reference in its entirety.

A minimal lentiviral genome may comprise, e.g., (5')R-U5-one or more first nucleotide sequences-U3-R(3'). However, the plasmid vector used to produce the lentiviral genome within a source cell can also include transcriptional regulatory control sequences operably linked to the lentiviral genome to direct transcription of the genome in a source cell. These regulatory sequences may comprise the natural sequences associated with the transcribed retroviral sequence, e.g., the 5' U3 region, or they may comprise a heterologous promoter such as another viral promoter, for example the CMV promoter. Some lentiviral genomes comprise additional sequences to promote efficient virus production. For example, in the case of HIV, rev and RRE sequences may be included. Alternatively or combination, codon optimization may be used, e.g., the gene encoding the exogenous agent may be codon optimized, e.g., as described in WO 01/79518, which is herein incorporated by reference in its entirety. Alternative sequences which

perform a similar or the same function as the rev/RRE system may also be used. For example, a functional analogue of the rev/RRE system is found in the Mason Pfizer monkey virus. This is known as CTE and comprises an RRE-type sequence in the genome which is believed to interact with a factor in the infected cell. The cellular factor can be thought of as a rev analogue. Thus, CTE may be used as an alternative to the rev/RRE system. In addition, the Rex protein of HTLV-I can functionally replace the Rev protein of HIV-I. Rev and Rex have similar effects to IRE-BP.

In some embodiments, a retroviral nucleic acid (e.g., a lentiviral nucleic acid, e.g., a primate or non-primate lentiviral nucleic acid) (1) comprises a deleted gag gene wherein the deletion in gag removes one or more nucleotides downstream of about nucleotide 350 or 354 of the gag coding sequence; (2) has one or more accessory genes absent from the retroviral nucleic acid; (3) lacks the tat gene but includes the leader sequence between the end of the 5' LTR and the ATG of gag; and (4) combinations of (1), (2) and (3). In an embodiment the lentiviral vector comprises all of features (1) and (2) and (3). This strategy is described in more detail in WO 99/32646, which is herein incorporated by reference in its entirety.

In some embodiments, a primate lentivirus minimal system requires none of the HIV/SIV additional genes vif, vpr, vpx, vpu, tat, rev and nef for either vector production or for transduction of dividing and non-dividing cells. In some embodiments, an EIAV minimal vector system does not require S2 for either vector production or for transduction of dividing and non-dividing cells.

The deletion of additional genes may permit vectors to be produced without the genes associated with disease in lentiviral (e.g. HIV) infections. In particular, tat is associated with disease. Secondly, the deletion of additional genes permits the vector to package more heterologous DNA. Thirdly, genes whose function is unknown, such as S2, may be omitted, thus reducing the risk of causing undesired effects. Examples of minimal lentiviral vectors are disclosed in WO 99/32646 and in WO 98/17815.

In some embodiments, the retroviral nucleic acid is devoid of at least tat and S2 (if it is an EIAV vector system), and possibly also vif, vpr, vpx, vpu and nef. In some embodiments, the retroviral nucleic acid is also devoid of rev, RRE, or both.

In some embodiments the retroviral nucleic acid comprises vpx. The Vpx polypeptide binds to and induces the degradation of the SAMHD1 restriction factor, which degrades free

dNTPs in the cytoplasm. Thus, the concentration of free dNTPs in the cytoplasm increases as Vpx degrades SAMHD1 and reverse transcription activity is increased, thus facilitating reverse transcription of the retroviral genome and integration into the target cell genome.

Different cells differ in their usage of particular codons. This codon bias corresponds to a bias in the relative abundance of particular tRNAs in the cell type. By altering the codons in the sequence so that they are tailored to match with the relative abundance of corresponding tRNAs, it is possible to increase expression. By the same token, it is possible to decrease expression by deliberately choosing codons for which the corresponding tRNAs are known to be rare in the particular cell type. Thus, an additional degree of translational control is available. An additional description of codon optimization is found, e.g., in WO 99/41397, which is herein incorporated by reference in its entirety.

Many viruses, including HIV and other lentiviruses, use a large number of rare codons and by changing these to correspond to commonly used mammalian codons, increased expression of the packaging components in mammalian producer cells can be achieved.

Codon optimization has a number of other advantages. By virtue of alterations in their sequences, the nucleotide sequences encoding the packaging components may have RNA instability sequences (INS) reduced or eliminated from them. At the same time, the amino acid sequence coding sequence for the packaging components is retained so that the viral components encoded by the sequences remain the same, or at least sufficiently similar that the function of the packaging components is not compromised. In some embodiments, codon optimization also overcomes the Rev/RRE requirement for export, rendering optimized sequences Rev independent. In some embodiments, codon optimization also reduces homologous recombination between different constructs within the vector system (for example between the regions of overlap in the gag-pol and env open reading frames). In some embodiments, codon optimization leads to an increase in viral titer and/or improved safety.

In some embodiments, only codons relating to INS are codon optimized. In other embodiments, the sequences are codon optimized in their entirety, with the exception of the sequence encompassing the frameshift site of gag-pol.

The gag-pol gene comprises two overlapping reading frames encoding the gag-pol proteins. The expression of both proteins depends on a frameshift during translation. This frameshift occurs as a result of ribosome "slippage" during translation. This slippage is thought

to be caused at least in part by ribosome-stalling RNA secondary structures. Such secondary structures exist downstream of the frameshift site in the gag-pol gene. For HIV, the region of overlap extends from nucleotide 1222 downstream of the beginning of gag (wherein nucleotide 1 is the A of the gag ATG) to the end of gag (nt 1503). Consequently, a 281 bp fragment spanning the frameshift site and the overlapping region of the two reading frames is preferably not codon optimized. In some embodiments, retaining this fragment will enable more efficient expression of the gag-pol proteins. For EIAV, the beginning of the overlap is at nt 1262 (where nucleotide 1 is the A of the gag ATG). The end of the overlap is at nt 1461. In order to ensure that the frameshift site and the gag-pol overlap are preserved, the wild type sequence may be retained from nt 1156 to 1465.

Derivations from optimal codon usage may be made, for example, in order to accommodate convenient restriction sites, and conservative amino acid changes may be introduced into the gag-pol proteins.

In some embodiments, codon optimization is based on codons with poor codon usage in mammalian systems. The third and sometimes the second and third base may be changed.

Due to the degenerate nature of the genetic code, it will be appreciated that numerous gag-pol sequences can be achieved by a skilled worker. Also, there are many retroviral variants described which can be used as a starting point for generating a codon optimized gag-pol sequence. Lentiviral genomes can be quite variable. For example there are many quasi-species of HIV-I which are still functional. This is also the case for EIAV. These variants may be used to enhance particular parts of the transduction process. Examples of HIV-I variants may be found in the HIV databases maintained by Los Alamos National Laboratory. Details of EIAV clones may be found at the NCBI database maintained by the National Institutes of Health.

The strategy for codon optimized gag-pol sequences can be used in relation to any retrovirus, e.g., EIAV, FIV, BIV, CAEV, VMR, SIV, HIV-I and HIV -2. In addition this method could be used to increase expression of genes from HTLV-I, HTLV-2, HFV, HSRV and human endogenous retroviruses (HERV), MLV and other retroviruses.

As described above, the packaging components for a retroviral vector can include expression products of gag, pol and env genes. In addition, packaging can utilize a short sequence of 4 stem loops followed by a partial sequence from gag and env as a packaging signal. Thus, inclusion of a deleted gag sequence in the retroviral vector genome (in addition to the full

gag sequence on the packaging construct) can be used. In embodiments, the retroviral vector comprises a packaging signal that comprises from 255 to 360 nucleotides of gag in vectors that still retain env sequences, or about 40 nucleotides of gag in a particular combination of splice donor mutation, gag and env deletions. In some embodiments, the retroviral vector includes a gag sequence which comprises one or more deletions, e.g., the gag sequence comprises about 360 nucleotides derivable from the N-terminus.

The retroviral vector, helper cell, helper virus, or helper plasmid may comprise retroviral structural and accessory proteins, for example gag, pol, env, tat, rev, vif, vpr, vpu, vpx, or nef proteins or other retroviral proteins. In some embodiments the retroviral proteins are derived from the same retrovirus. In some embodiments the retroviral proteins are derived from more than one retrovirus, e.g. 2, 3, 4, or more retroviruses.

The gag and pol coding sequences are generally organized as the Gag-Pol Precursor in native lentivirus. The gag sequence codes for a 55-kD Gag precursor protein, also called p55. The p55 is cleaved by the virally encoded protease (a product of the pol gene) during the process of maturation into four smaller proteins designated MA (matrix [p17]), CA (capsid [p24]), NC (nucleocapsid [p9]), and p6. The pol precursor protein is cleaved away from Gag by a virally encoded protease, and further digested to separate the protease (p10), RT (p50), RNase H (p15), and integrase (p31) activities.

Native Gag-Pol sequences can be utilized in a helper vector (e.g., helper plasmid or helper virus), or modifications can be made. These modifications include, chimeric Gag-Pol, where the Gag and Pol sequences are obtained from different viruses (e.g., different species, subspecies, strains, clades, etc.), and/or where the sequences have been modified to improve transcription and/or translation, and/or reduce recombination.

In various examples, the retroviral nucleic acid includes a polynucleotide encoding a 150-250 (e.g., 168) nucleotide portion of a gag protein that (i) includes a mutated INS1 inhibitory sequence that reduces restriction of nuclear export of RNA relative to wild-type INS1, (ii) contains two nucleotide insertion that results in frame shift and premature termination, and/or (iii) does not include INS2, INS3, and INS4 inhibitory sequences of gag.

In some embodiments, a vector described herein is a hybrid vector that comprises both retroviral (e.g., lentiviral) sequences and non-lentiviral viral sequences. In some embodiments, a

hybrid vector comprises retroviral e.g., lentiviral, sequences for reverse transcription, replication, integration and/or packaging.

According to certain specific embodiments, most or all of the viral vector backbone sequences are derived from a lentivirus, e.g., HIV-1. However, it is to be understood that many different sources of retroviral and/or lentiviral sequences can be used, or combined and numerous substitutions and alterations in certain of the lentiviral sequences may be accommodated without impairing the ability of a transfer vector to perform the functions described herein. A variety of lentiviral vectors are described in Naldini et al., (1996a, 1996b, and 1998); Zufferey et al., (1997); Dull et al., 1998, U.S. Pat. Nos. 6,013,516; and 5,994,136, many of which may be adapted to produce a retroviral nucleic acid.

At each end of the provirus, long terminal repeats (LTRs) are typically found. An LTR typically comprises a domain located at the ends of retroviral nucleic acid which, in their natural sequence context, are direct repeats and contain U3, R and U5 regions. LTRs generally promote the expression of retroviral genes (e.g., promotion, initiation and polyadenylation of gene transcripts) and viral replication. The LTR can comprise numerous regulatory signals including transcriptional control elements, polyadenylation signals and sequences for replication and integration of the viral genome. The viral LTR is typically divided into three regions called U3, R and U5. The U3 region typically contains the enhancer and promoter elements. The U5 region is typically the sequence between the primer binding site and the R region and can contain the polyadenylation sequence. The R (repeat) region can be flanked by the U3 and U5 regions. The LTR is typically composed of U3, R and U5 regions and can appear at both the 5' and 3' ends of the viral genome. In some embodiments, adjacent to the 5' LTR are sequences for reverse transcription of the genome (the tRNA primer binding site) and for efficient packaging of viral RNA into particles (the Psi site).

A packaging signal can comprise a sequence located within the retroviral genome which mediate insertion of the viral RNA into the viral capsid or particle, see e.g., Clever et al., 1995. J. of Virology, Vol. 69, No. 4; pp. 2101-2109. Several retroviral vectors use a minimal packaging signal (a psi [Ψ] sequence) for encapsidation of the viral genome.

In various embodiments, retroviral nucleic acids comprise modified 5' LTR and/or 3' LTRs. Either or both of the LTR may comprise one or more modifications including, but not limited to, one or more deletions, insertions, or substitutions. Modifications of the 3' LTR are

often made to improve the safety of lentiviral or retroviral systems by rendering viruses replication-defective, e.g., virus that is not capable of complete, effective replication such that infective virions are not produced (e.g., replication-defective lentiviral progeny).

In some embodiments, a vector is a self-inactivating (SIN) vector, e.g., replication-defective vector, e.g., retroviral or lentiviral vector, in which the right (3') LTR enhancer-promoter region, known as the U3 region, has been modified (e.g., by deletion or substitution) to prevent viral transcription beyond the first round of viral replication. This is because the right (3') LTR U3 region can be used as a template for the left (5') LTR U3 region during viral replication and, thus, absence of the U3 enhancer-promoter inhibits viral replication. In
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embodiments, the 3' LTR is modified such that the U5 region is removed, altered, or replaced, for example, with an exogenous poly(A) sequence. The 3' LTR, the 5' LTR, or both 3' and 5' LTRs, may be modified LTRs.

In some embodiments, the U3 region of the 5' LTR is replaced with a heterologous promoter to drive transcription of the viral genome during production of viral particles.
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Examples of heterologous promoters which can be used include, for example, viral simian virus 40 (SV40) (e.g., early or late), cytomegalovirus (CMV) (e.g., immediate early), Moloney murine leukemia virus (MoMLV), Rous sarcoma virus (RSV), and herpes simplex virus (HSV) (thymidine kinase) promoters. In some embodiments, promoters are able to drive high levels of transcription in a Tat-independent manner. In certain embodiments, the heterologous promoter
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has additional advantages in controlling the manner in which the viral genome is transcribed. For example, the heterologous promoter can be inducible, such that transcription of all or part of the viral genome will occur only when the induction factors are present. Induction factors include, but are not limited to, one or more chemical compounds or the physiological conditions such as temperature or pH, in which the host cells are cultured.

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In some embodiments, viral vectors comprise a TAR (trans-activation response) element, e.g., located in the R region of lentiviral (e.g., HIV) LTRs. This element interacts with the lentiviral trans-activator (tat) genetic element to enhance viral replication. However, this element is not required, e.g., in embodiments wherein the U3 region of the 5' LTR is replaced by a heterologous promoter.

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The R region, e.g., the region within retroviral LTRs beginning at the start of the capping group (i.e., the start of transcription) and ending immediately prior to the start of the poly A tract

can be flanked by the U3 and U5 regions. The R region plays a role during reverse transcription in the transfer of nascent DNA from one end of the genome to the other.

The retroviral nucleic acid can also comprise a FLAP element, e.g., a nucleic acid whose sequence includes the central polypurine tract and central termination sequences (cPPT and CTS) of a retrovirus, e.g., HIV-1 or HIV-2. Suitable FLAP elements are described in U.S. Pat. No.

6,682,907 and in Zennou, et al., 2000, *Cell*, 101:173, which are herein incorporated by reference in their entireties. During HIV-1 reverse transcription, central initiation of the plus-strand DNA at the central polypurine tract (cPPT) and central termination at the central termination sequence (CTS) can lead to the formation of a three-stranded DNA structure: the HIV-1 central DNA flap.

In some embodiments, the retroviral or lentiviral vector backbones comprise one or more FLAP elements upstream or downstream of the gene encoding the exogenous agent. For example, in some embodiments a transfer plasmid includes a FLAP element, e.g., a FLAP element derived or isolated from HIV-1.

In embodiments, a retroviral or lentiviral nucleic acid comprises one or more export elements, e.g., a cis-acting post-transcriptional regulatory element which regulates the transport of an RNA transcript from the nucleus to the cytoplasm of a cell. Examples of RNA export elements include, but are not limited to, the human immunodeficiency virus (HIV) rev response element (RRE) (see e.g., Cullen et al., 1991. *J. Virol.* 65: 1053; and Cullen et al., 1991. *Cell* 58: 423), and the hepatitis B virus post-transcriptional regulatory element (HPRE), which are herein incorporated by reference in their entireties. Generally, the RNA export element is placed within the 3' UTR of a gene, and can be inserted as one or multiple copies.

In some embodiments, expression of heterologous sequences in viral vectors is increased by incorporating one or more of, e.g., all of, posttranscriptional regulatory elements, polyadenylation sites, and transcription termination signals into the vectors. A variety of posttranscriptional regulatory elements can increase expression of a heterologous nucleic acid at the protein, e.g., woodchuck hepatitis virus posttranscriptional regulatory element (WPRE; Zufferey et al., 1999, *J. Virol.*, 73:2886); the posttranscriptional regulatory element present in hepatitis B virus (HPRE) (Huang et al., *Mol. Cell. Biol.*, 5:3864); and the like (Liu et al., 1995, *Genes Dev.*, 9:1766), each of which is herein incorporated by reference in its entirety. In some embodiments, a retroviral nucleic acid described herein comprises a posttranscriptional regulatory element such as a WPRE or HPRE

In some embodiments, a retroviral nucleic acid described herein lacks or does not comprise a posttranscriptional regulatory element such as a WPRE or HPRE.

Elements directing the termination and polyadenylation of the heterologous nucleic acid transcripts may be included, e.g., to increase expression of the exogenous agent. Transcription
5 termination signals may be found downstream of the polyadenylation signal. In some embodiments, vectors comprise a polyadenylation sequence 3' of a polynucleotide encoding the exogenous agent. A polyA site may comprise a DNA sequence which directs both the termination and polyadenylation of the nascent RNA transcript by RNA polymerase II. Polyadenylation sequences can promote mRNA stability by addition of a polyA tail to the 3' end
10 of the coding sequence and thus, contribute to increased translational efficiency. Illustrative examples of polyA signals that can be used in a retroviral nucleic acid, include AATAAA, ATATAA, AGTAAA, a bovine growth hormone polyA sequence (BGHpA), a rabbit β -globin polyA sequence (r β gpA), or another suitable heterologous or endogenous polyA sequence.

In some embodiments, a retroviral or lentiviral vector further comprises one or more
15 insulator elements, e.g., an insulator element described herein.

In various embodiments, the vectors comprise a promoter operably linked to a polynucleotide encoding an exogenous agent. The vectors may have one or more LTRs, wherein either LTR comprises one or more modifications, such as one or more nucleotide substitutions, additions, or deletions. The vectors may further comprise one or more accessory elements to
20 increase transduction efficiency (e.g., a cPPT/FLAP), viral packaging (e.g., a Psi (Ψ) packaging signal, RRE), and/or other elements that increase exogenous gene expression (e.g., poly (A) sequences), and may optionally comprise a WPRE or HPRE.

In some embodiments, a lentiviral nucleic acid comprises one or more of, e.g., all of, e.g., from 5' to 3', a promoter (e.g., CMV), an R sequence (e.g., comprising TAR), a U5
25 sequence (e.g., for integration), a PBS sequence (e.g., for reverse transcription), a DIS sequence (e.g., for genome dimerization), a psi packaging signal, a partial gag sequence, an RRE sequence (e.g., for nuclear export), a cPPT sequence (e.g., for nuclear import), a promoter to drive expression of the exogenous agent, a gene encoding the exogenous agent, a WPRE sequence (e.g., for efficient transgene expression), a PPT sequence (e.g., for reverse transcription), an R
30 sequence (e.g., for polyadenylation and termination), and a U5 signal (e.g., for integration).

ii) Vectors engineered to remove splice sites

Some lentiviral vectors integrate inside active genes and possess strong splicing and polyadenylation signals that could lead to the formation of aberrant and possibly truncated transcripts.

5 Mechanisms of proto-oncogene activation may involve the generation of chimeric transcripts originating from the interaction of promoter elements or splice sites contained in the genome of the insertional mutagen with the cellular transcriptional unit targeted by integration (Gabriel et al. 2009. Nat Med 15: 1431 -1436; Bokhoven, et al. J Virol 83:283-29). Chimeric fusion transcripts comprising vector sequences and cellular mRNAs can be generated either by
10 read- through transcription starting from vector sequences and proceeding into the flanking cellular genes, or vice versa.

In some embodiments, a lentiviral nucleic acid described herein comprises a lentiviral backbone in which at least two of the splice sites have been eliminated, e.g., to improve the safety profile of the lentiviral vector. Species of such splice sites and methods of identification
15 are described in WO2012156839A2, all of which is included by reference.

iii) Retroviral production methods

Large scale viral particle production is often useful to achieve a desired viral titer. Viral particles can be produced by transfecting a transfer vector into a packaging cell line that
20 comprises viral structural and/or accessory genes, e.g., gag, pol, env, tat, rev, vif, vpr, vpu, vpx, or nef genes or other retroviral genes.

In embodiments, the packaging vector is an expression vector or viral vector that lacks a packaging signal and comprises a polynucleotide encoding one, two, three, four or more viral structural and/or accessory genes. Typically, the packaging vectors are included in a packaging
25 cell, and are introduced into the cell via transfection, transduction or infection. A retroviral, e.g., lentiviral, transfer vector can be introduced into a packaging cell line, via transfection, transduction or infection, to generate a source cell or cell line. The packaging vectors can be introduced into human cells or cell lines by standard methods including, e.g., calcium phosphate transfection, lipofection or electroporation. In some embodiments, the packaging vectors are
30 introduced into the cells together with a dominant selectable marker, such as neomycin, hygromycin, puromycin, blastocidin, zeocin, thymidine kinase, DHFR, Gln synthetase or ADA,

followed by selection in the presence of the appropriate drug and isolation of clones. A selectable marker gene can be linked physically to genes encoding by the packaging vector, e.g., by IRES or self cleaving viral peptides.

Packaging cell lines include cell lines that do not contain a packaging signal, but do stably or transiently express viral structural proteins and replication enzymes (e.g., gag, pol and env) which can package viral particles. Any suitable cell line can be employed, e.g., mammalian cells, e.g., human cells. Suitable cell lines which can be used include, for example, CHO cells, BHK cells, MDCK cells, C3H 10T1/2 cells, FLY cells, Psi-2 cells, BOSC 23 cells, PA317 cells, WEHI cells, COS cells, BSC 1 cells, BSC 40 cells, BMT 10 cells, VERO cells, W138 cells, MRC5 cells, A549 cells, HT1080 cells, 293 cells, 293T cells, B-50 cells, 3T3 cells, NIH3T3 cells, HepG2 cells, Saos-2 cells, Huh7 cells, HeLa cells, W163 cells, 211 cells, and 211A cells. In embodiments, the packaging cells are 293 cells, 293T cells, or A549 cells.

A source cell line includes a cell line which is capable of producing recombinant retroviral particles, comprising a packaging cell line and a transfer vector construct comprising a packaging signal. Methods of preparing viral stock solutions are illustrated by, e.g., Y. Soneoka et al. (1995) *Nucl. Acids Res.* 23:628-633, and N. R. Landau et al. (1992) *J. Virol.* 66:5110-5113, which are incorporated herein by reference. Infectious virus particles may be collected from the packaging cells, e.g., by cell lysis, or collection of the supernatant of the cell culture. Optionally, the collected virus particles may be enriched or purified.

iv) Packaging plasmids and cell lines

In some embodiments, the source cell comprises one or more plasmids coding for viral structural proteins and replication enzymes (e.g., gag, pol and env) which can package viral particles. In some embodiments, the sequences coding for at least two of the gag, pol, and env precursors are on the same plasmid. In some embodiments, the sequences coding for the gag, pol, and env precursors are on different plasmids. In some embodiments, the sequences coding for the gag, pol, and env precursors have the same expression signal, e.g., promoter. In some embodiments, the sequences coding for the gag, pol, and env precursors have a different expression signal, e.g., different promoters. In some embodiments, expression of the gag, pol, and env precursors is inducible. In some embodiments, the plasmids coding for viral structural proteins and replication enzymes are transfected at the same time or at different times. In some

embodiments, the plasmids coding for viral structural proteins and replication enzymes are transfected at the same time or at a different time from the packaging vector.

In some embodiments, the source cell line comprises one or more stably integrated viral structural genes. In some embodiments expression of the stably integrated viral structural genes is inducible.

In some embodiments, expression of the viral structural genes is regulated at the transcriptional level. In some embodiments, expression of the viral structural genes is regulated at the translational level. In some embodiments, expression of the viral structural genes is regulated at the post-translational level.

In some embodiments, expression of the viral structural genes is regulated by a tetracycline (Tet)-dependent system, in which a Tet-regulated transcriptional repressor (Tet-R) binds to DNA sequences included in a promoter and represses transcription by steric hindrance (Yao et al, 1998; Jones et al, 2005). Upon addition of doxycycline (dox), Tet-R is released, allowing transcription. Multiple other suitable transcriptional regulatory promoters, transcription factors, and small molecule inducers are suitable to regulate transcription of viral structural genes.

In some embodiments, the third-generation lentivirus components, human immunodeficiency virus type 1 (HIV) Rev, Gag/Pol, and an envelope under the control of Tet-regulated promoters and coupled with antibiotic resistance cassettes are separately integrated into the source cell genome. In some embodiments the source cell only has one copy of each of Rev, Gag/Pol, and an envelope protein integrated into the genome.

In some embodiments a nucleic acid encoding the exogenous agent (e.g., a retroviral nucleic acid encoding the exogenous agent) is also integrated into the source cell genome. In some embodiments a nucleic acid encoding the exogenous agent is maintained episomally. In some embodiments a nucleic acid encoding the exogenous agent is transfected into the source cell that has stably integrated Rev, Gag/Pol, and an envelope protein in the genome. See, e.g., Milani et al. *EMBO Molecular Medicine*, 2017, which is herein incorporated by reference in its entirety.

In some embodiments, a retroviral nucleic acid described herein is unable to undergo reverse transcription. Such a nucleic acid, in embodiments, is able to transiently express an exogenous agent. The retrovirus or VLP, may comprise a disabled reverse transcriptase protein,

or may not comprise a reverse transcriptase protein. In embodiments, the retroviral nucleic acid comprises a disabled primer binding site (PBS) and/or att site. In embodiments, one or more viral accessory genes, including rev, tat, vif, nef, vpr, vpu, vpx and S2 or functional equivalents thereof, are disabled or absent from the retroviral nucleic acid. In embodiments, one or more
5 accessory genes selected from S2, rev and tat are disabled or absent from the retroviral nucleic acid.

v) Strategies for packaging a retroviral nucleic acid

Typically, modern retroviral vector systems consist of viral genomes bearing cis-acting
10 vector sequences for transcription, reverse-transcription, integration, translation and packaging of viral RNA into the viral particles, and (2) producer cells lines which express the trans-acting retroviral gene sequences (e.g., gag, pol and env) needed for production of virus particles. By separating the cis-and trans-acting vector sequences completely, the virus is unable to maintain replication for more than one cycle of infection. Generation of live virus can be avoided by a
15 number of strategies, e.g., by minimizing the overlap between the cis-and trans-acting sequences to avoid recombination.

A viral vector particle which comprises a sequence that is devoid of or lacking viral RNA may be the result of removing or eliminating the viral RNA from the sequence. In one embodiment this may be achieved by using an endogenous packaging signal binding site on gag.
20 Alternatively, the endogenous packaging signal binding site is on pol. In this embodiment, the RNA which is to be delivered will contain a cognate packaging signal. In another embodiment, a heterologous binding domain (which is heterologous to gag) located on the RNA to be delivered, and a cognate binding site located on gag or pol, can be used to ensure packaging of the RNA to be delivered. The heterologous sequence could be non-viral or it could be viral, in which case it
25 may be derived from a different virus. The vector particles could be used to deliver therapeutic RNA, in which case functional integrase and/or reverse transcriptase is not required. These vector particles could also be used to deliver a therapeutic gene of interest, in which case pol is typically included.

In an embodiment, gag-pol are altered, and the packaging signal is replaced with a
30 corresponding packaging signal. In this embodiment, the particle can package the RNA with the

new packaging signal. The advantage of this approach is that it is possible to package an RNA sequence which is devoid of viral sequence for example, RNAi.

An alternative approach is to rely on over-expression of the RNA to be packaged. In one embodiment the RNA to be packaged is over-expressed in the absence of any RNA containing a packaging signal. This may result in a significant level of therapeutic RNA being packaged, and that this amount is sufficient to transduce a cell and have a biological effect.

In some embodiments, a polynucleotide comprises a nucleotide sequence encoding a viral gag protein or retroviral gag and pol proteins, wherein the gag protein or pol protein comprises a heterologous RNA binding domain capable of recognising a corresponding sequence in an RNA sequence to facilitate packaging of the RNA sequence into a viral vector particle.

In some embodiments, the heterologous RNA binding domain comprises an RNA binding domain derived from a bacteriophage coat protein, a Rev protein, a protein of the U1 small nuclear ribonucleoprotein particle, a Nova protein, a TF111A protein, a TIS11 protein, a trp RNA-binding attenuation protein (TRAP) or a pseudouridine synthase.

In some embodiments, a method herein comprises detecting or confirming the absence of replication competent retrovirus. The methods may include assessing RNA levels of one or more target genes, such as viral genes, e.g. structural or packaging genes, from which gene products are expressed in certain cells infected with a replication-competent retrovirus, such as a gammaretrovirus or lentivirus, but not present in a viral vector used to transduce cells with a heterologous nucleic acid and not, or not expected to be, present and/or expressed in cells not containing replication-competent retrovirus. Replication competent retrovirus may be determined to be present if RNA levels of the one or more target genes is higher than a reference value, which can be measured directly or indirectly, e.g. from a positive control sample containing the target gene. For further disclosure, see WO2018023094A1.

vi) Repression of a gene encoding an exogenous agent in a source cell

(Over-)expressed protein in the source cell may have an indirect or direct effect on vector virion assembly and/or infectivity. Incorporation of the exogenous agent into vector virions may also impact downstream processing of vector particles.

In some embodiments, a tissue-specific promoter is used to limit expression of the exogenous agent in source cells. In some embodiments, a heterologous translation control system

is used in eukaryotic cell cultures to repress the translation of the exogenous agent in source cells. More specifically, the retroviral nucleic acid may comprise a binding site operably linked to the gene encoding the exogenous agent, wherein the binding site is capable of interacting with an RNA-binding protein such that translation of the exogenous agent is repressed or prevented in the source cell.

In some embodiments, the RNA-binding protein is tryptophan RNA-binding attenuation protein (TRAP), for example bacterial tryptophan RNA-binding attenuation protein. The use of an RNA-binding protein (e.g. the bacterial trp operon regulator protein, tryptophan RNA-binding attenuation protein, TRAP), and RNA targets to which it binds, will repress or prevent transgene translation within a source cell. This system is referred to as the Transgene Repression In vector Production cell system or TRIP system.

In embodiments, the placement of a binding site for an RNA binding protein (e.g., a TRAP-binding sequence, tbs) upstream of the NOI translation initiation codon allows specific repression of translation of mRNA derived from the internal expression cassette, while having no detrimental effect on production or stability of vector RNA. The number of nucleotides between the tbs and translation initiation codon of the gene encoding the exogenous agent may be varied from 0 to 12 nucleotides. The tbs may be placed downstream of an internal ribosome entry site (IRES) to repress translation of the gene encoding the exogenous agent in a multicistronic mRNA.

vii) Kill switch systems and amplification

In some embodiments, a polynucleotide or cell harboring the gene encoding the exogenous agent utilizes a suicide gene, e.g., an inducible suicide gene, to reduce the risk of direct toxicity and/or uncontrolled proliferation. In specific aspects, the suicide gene is not immunogenic to the host cell harboring the exogenous agent. Examples of suicide genes include caspase-9, caspase-8, or cytosine deaminase. Caspase-9 can be activated using a specific chemical inducer of dimerization (CID).

In certain embodiments, vectors comprise gene segments that cause target cells, e.g., immune effector cells, e.g., T cells, to be susceptible to negative selection in vivo. For instance, the transduced cell can be eliminated as a result of a change in the in vivo condition of the individual. The negative selectable phenotype may result from the insertion of a gene that

confers sensitivity to an administered agent, for example, a compound. Negative selectable genes are known in the art, and include, inter alia the following: the Herpes simplex virus type I thymidine kinase (HSV-I TK) gene (Wigler et al., Cell 11:223, 1977) which confers ganciclovir sensitivity; the cellular hypoxanthine phosphoribosyltransferase (HPRT) gene, the cellular adenine phosphoribosyltransferase (APRT) gene, and bacterial cytosine deaminase, (Mullen et al., Proc. Natl. Acad. Sci. USA. 89:33 (1992)).

In some embodiments, transduced cells, e.g., immune effector cells, such as T cells, comprise a polynucleotide further comprising a positive marker that enables the selection of cells of the negative selectable phenotype in vitro. The positive selectable marker may be a gene which, upon being introduced into the target cell, expresses a dominant phenotype permitting positive selection of cells carrying the gene. Genes of this type include, inter alia, hygromycin-B phosphotransferase gene (hph) which confers resistance to hygromycin B, the amino glycoside phosphotransferase gene (neo or aph) from Tn5 which codes for resistance to the antibiotic G418, the dihydrofolate reductase (DHFR) gene, the adenosine deaminase gene (ADA), and the multi-drug resistance (MDR) gene.

In some embodiments, the positive selectable marker and the negative selectable element are linked such that loss of the negative selectable element necessarily also is accompanied by loss of the positive selectable marker. For instance, the positive and negative selectable markers can be fused so that loss of one obligatorily leads to loss of the other. An example of a fused polynucleotide that yields as an expression product a polypeptide that confers both the desired positive and negative selection features described above is a hygromycin phosphotransferase thymidine kinase fusion gene (HyTK). Expression of this gene yields a polypeptide that confers hygromycin B resistance for positive selection in vitro, and ganciclovir sensitivity for negative selection in vivo. See Lupton S. D., et al, Mol. and Cell. Biology 11:3374-3378, 1991. In addition, in embodiments, the polynucleotides encoding the chimeric receptors are in retroviral vectors containing the fused gene, particularly those that confer hygromycin B resistance for positive selection in vitro, and ganciclovir sensitivity for negative selection in vivo, for example the HyTK retroviral vector described in Lupton, S. D. et al. (1991), supra. See also the publications of PCT U591/08442 and PCT/U594/05601, describing the use of bifunctional selectable fusion genes derived from fusing dominant positive selectable markers with negative selectable markers.

Suitable positive selectable markers can be derived from genes selected from the group consisting of hph, nco, and gpt, and suitable negative selectable markers can be derived from genes selected from the group consisting of cytosine deaminase, HSV-I TK, VZV TK, HPRT, APRT and gpt. Other suitable markers are bifunctional selectable fusion genes wherein the positive selectable marker is derived from hph or neo, and the negative selectable marker is derived from cytosine deaminase or a TK gene or selectable marker.

viii) Strategies for regulating lentiviral integration

Retroviral and lentiviral nucleic acids are disclosed which are lacking or disabled in key proteins/sequences so as to prevent integration of the retroviral or lentiviral genome into the target cell genome. For instance, viral nucleic acids lacking each of the amino acids making up the highly conserved DDE motif (Engelman and Craigie (1992) *J. Virol.* 66:6361-6369; Johnson et al. (1986) *Proc. Natl. Acad. Sci. USA* 83:7648-7652; Khan et al. (1991) *Nucleic Acids Res.* 19:851-860) of retroviral integrase enables the production of integration defective retroviral nucleic acids.

For instance, in some embodiments, a retroviral nucleic acid herein comprises a lentiviral integrase comprising a mutation that causes said integrase to be unable to catalyze the integration of the viral genome into a cell genome. In some embodiments, said mutations are type I mutations which affect directly the integration, or type II mutations which trigger pleiotropic defects affecting virion morphogenesis and/or reverse transcription. Illustrative non-limitative examples of type I mutations are those mutations affecting any of the three residues that participate in the catalytic core domain of the integrase: DX₃₉₋₅₈DX₃₅E (D64, D116 and E152 residues of the integrase of the HIV-1). In a particular embodiment, the mutation that causes said integrase to be unable to catalyze the integration of the viral genome into a cell genome is the substitution of one or more amino acid residues of the DDE motif of the catalytic core domain of the integrase, preferably the substitution of the first aspartic residue of said DEE motif by an asparagine residue. In some embodiment the retroviral vector does not comprise an integrase protein.

In some embodiments the retrovirus integrates into active transcription units. In some embodiments the retrovirus does not integrate near transcriptional start sites, the 5' end of genes, or DNaseI cleavage sites. In some embodiments the retrovirus integration does not active proto-

oncogenes or inactive tumor suppressor genes. In some embodiments the retrovirus is not genotoxic. In some embodiments the lentivirus integrates into introns.

In some embodiments, the retroviral nucleic acid integrates into the genome of a target cell with a particular copy number. The average copy number may be determined from single
5 cells, a population of cells, or individual cell colonies. Exemplary methods for determining copy number include polymerase chain reaction (PCR) and flow cytometry.

In some embodiments DNA encoding the exogenous agent is integrated into the genome. In some embodiments DNA encoding the exogenous agent is maintained episomally. In some
10 embodiments the ratio of integrated to episomal DNA encoding the exogenous agent is at least 0.01, 0.1, 0.5, 1.0, 2, 5, 10, 100.

In some embodiments DNA encoding the exogenous agent is linear. In some
embodiments DNA encoding the exogenous agent is circular. In some embodiments the ratio of linear to circular copies of DNA encoding the exogenous agent is at least 0.01, 0.1, 0.5, 1.0, 2, 5,
10, 100.

15 In embodiments the DNA encoding the exogenous agent is circular with 1 LTR. In some
embodiments the DNA encoding the exogenous agent is circular with 2 LTRs. In some
embodiments the ratio of circular, 1 LTR-comprising DNA encoding the exogenous agent to
circular, 2 LTR-comprising DNA encoding the exogenous agent is at least 0.1, 0.5, 1.0, 2, 5, 10,
20, 50, 100.

ix) Maintenance of an episomal virus

In retroviruses deficient in integration, circular cDNA off-products of the
retrotranscription (e.g., 1-LTR and 2-LTR) can accumulate in the cell nucleus without
integrating into the host genome (see Yáñez-Muñoz R J et al., Nat. Med. 2006, 12: 348-353).

25 Like other exogenous DNA those intermediates can then integrate in the cellular DNA at equal
frequencies (e.g., 10^3 to 10^5 /cell).

In some embodiments, episomal retroviral nucleic acid does not replicate. Episomal
virus DNA can be modified to be maintained in replicating cells through the inclusion of
eukaryotic origin of replication and a scaffold/matrix attachment region (S/MAR) for association
30 with the nuclear matrix.

Thus, in some embodiments, a retroviral nucleic acid described herein comprises a eukaryotic origin of replication or a variant thereof. Examples of eukaryotic origins of replication of interest are the origin of replication of the β -globin gene as have been described by Aladjem et al (Science, 1995, 270: 815-819), a consensus sequence from autonomously replicating
5 sequences associated with alpha-satellite sequences isolated previously from monkey CV-1 cells and human skin fibroblasts as has been described by Price et al Journal of Biological Chemistry, 2003, 278 (22): 19649-59, the origin of replication of the human c-myc promoter region has have been described by McWinney and Leffak (McWinney C. and Leffak M., Nucleic Acid Research 1990, 18(5): 1233-42). In embodiments, the variant substantially maintains the ability to initiate
10 the replication in eukaryotes. The ability of a particular sequence of initiating replication can be determined by any suitable method, for example, the autonomous replication assay based on bromodeoxyuridine incorporation and density shift (Araujo F. D. et al., supra; Frappier L. et al., supra).

In some embodiments, the retroviral nucleic acid comprises a scaffold/matrix attachment
15 region (S/MAR) or variant thereof, e.g., a non-consensus-like AT-rich DNA element several hundred base pairs in length, which organizes the nuclear DNA of the eukaryotic genome into chromatin domains, by periodic attachment to the protein scaffold or matrix of the cell nucleus. They are typically found in non-coding regions such as flanking regions, chromatin border regions, and introns. Examples of S/MAR regions are 1.8 kbp S/MAR of the human IFN- γ gene
20 (hIFN- γ^{large}) as described by Bode et al (Bode J. et al., Science, 1992, 255: 195-7), the 0.7 Kbp minimal region of the S/MAR of the human IFN- γ gene (hIFN- γ^{short}) as has have been described by Ramezani (Ramezani A. et al., Blood 2003, 101: 4717-24), the 0.2 Kbp minimal region of the S/MAR of the human dehydrofolate reductase gene (hDHFR) as has been described by Mesner
25 L. D. et al., Proc Natl Acad Sci USA, 2003, 100: 3281-86). In embodiments, the functionally equivalent variant of the S/MAR is a sequence selected based on the set six rules that together or alone have been suggested to contribute to S/MAR function (Kramer et al (1996) *Genomics* 33, 305; Singh et al (1997) *Nucl. Acids Res* 25, 1419). These rules have been merged into the MAR-Wiz computer program freely available at genomecluster.secs.oakland.edu/MAR-Wiz. In
30 embodiments, the variant substantially maintains the same functions of the S/MAR from which it derives, in particular, the ability to specifically bind to the nuclear the matrix. The skilled person can determine if a particular variant is able to specifically bind to the nuclear matrix, for example

by the in vitro or in vivo MAR assays described by Mesner et al. (Mesner L. D. et al, supra). In some embodiments, a specific sequence is a variant of a S/MAR if the particular variant shows propensity for DNA strand separation. This property can be determined using a specific program based on methods from equilibrium statistical mechanics. The stress-induced duplex destabilization (SIDD) analysis technique “[. . .] calculates the extent to which the imposed level of superhelical stress decreases the free energy needed to open the duplex at each position along a DNA sequence. The results are displayed as an SIDD profile, in which sites of strong destabilization appear as deep minima [. . .]” as defined in Bode et al (2005) *J. Mol. Biol.* 358,597. The SIDD algorithm and the mathematical basis (Bi and Benham (2004) *Bioinformatics* 20, 1477) and the analysis of the SIDD profile can be performed using the freely available internet resource at WebSIDD (www.genomecenter.ucdavis.edu/benham). Accordingly, in some embodiment, the polynucleotide is considered a variant of the S/MAR sequence if it shows a similar SIDD profile as the S/MAR.

B. Cell-derived fusosomes

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.

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
 5 progenitor cell (e.g., a cardiac progenitor cell, a satellite cell, a radial glial 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).

The cultured cells may be from epithelial, connective, muscular, or nervous tissue or
 10 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);
 15 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,
 20 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).

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
 25 some embodiments, the cells are from fetal tissue.

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).

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
 30 nucleotides in length, between 6,000-10,000 nucleotides in length).

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).

5 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
10 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.

 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
15 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.

 In some embodiments, fusosomes are generated by inducing budding of an exosome, microvesicle, membrane vesicle, extracellular membrane vesicle, plasma membrane vesicle,
20 giant plasma membrane vesicle, apoptotic body, mitoparticle, pyrenocyte, lysosome, or other membrane enclosed vesicle.

 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
25 DNA to ρ^0 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
30 typical location such that the cell contains the nucleus but it is non-functional.

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.

5 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.

10 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;

15 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;

20 b) a mechanical method, e.g., centrifugation (e.g., ultracentrifugation or density centrifugation); freeze thaw; or sonication.

i) Modifications to Cells Prior to Fusosome Generation

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.

25

a) Physical Modifications

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.

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
5 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.

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
10 molecules or lipids on the cell surface that enhance targeting of the fusosome to an organ, tissues, or cell-type.

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.
15 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.

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
20 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
25 modifier that increases expression of the endogenous fusogen.

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.

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.

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,
5 endoplasmic reticulum, intracellular vesicles (such as lysosomes, autophagosomes).

b) Genetic Modifications

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
10 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
15 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.

In some embodiments, a cell is genetically modified prior to generating the fusosome to
20 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
25 construct. In some embodiments, the nucleic acid encodes an inhibitor of a fusogen repressor.

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.
30 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.

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.

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.

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 an 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.

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.

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.

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
5 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
10 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
15 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.

20 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
25 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.

One example of a suitable promoter is the immediate early cytomegalovirus (CMV) promoter sequence. This promoter sequence is a strong constitutive promoter sequence capable
30 of driving high levels of expression of any polynucleotide sequence operatively linked thereto. Another example of a suitable promoter is Elongation Growth Factor-1 α (EF-1 α). 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.

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.

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.

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.

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.

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.

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.

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-
5 thawing, mechanical disruption, and filtration.

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
10 described herein.

The present disclosure provides, in some aspects, a fusosome comprising: (a) a lipid bilayer, (b) a lumen (e.g., comprising cytosol) surrounded by the lipid bilayer; (c) an exogenous or overexpressed fusogen, e.g., wherein the fusogen is disposed in the lipid bilayer, wherein the fusosome is derived from a source cell; and wherein the fusosome has partial or complete
15 nuclear inactivation (e.g., nuclear removal).

The present disclosure provides, in some aspects, a fusosome composition comprising a plurality of fusosomes derived from a source cell, wherein the fusosomes of the plurality comprise: (a) a lipid bilayer, (b) a lumen comprising cytosol, wherein the lumen is surrounded by the lipid bilayer; (c) an exogenous or overexpressed fusogen disposed in the lipid bilayer, (d) a
20 nucleic acid, e.g., a nucleic acid comprising a payload gene; and wherein the fusosome does not comprise a nucleus; wherein the amount of viral capsid protein in the fusosome composition is less than 1% of total protein; 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 10-fold more target cells than non-target cells or reference cells, or (ii) the fusosomes of the plurality
25 fuse at a higher rate with a target cell than with a non-target cell or reference cell by at least at least 50%; wherein the target cell is chosen from a pan-neuronal cell, a GABAergic neuron, a Glutamatergic neuron, a Cholinergic neuron, a Dopaminergic neuron, a Serotonergic neuron, a glial cell, an astrocyte, a microglial cell, an oligodendrocyte, or a choroid plexus cell.

The present disclosure provides, in some aspects, a fusosome composition comprising a
30 plurality of fusosomes derived from a source cell, wherein the fusosomes of the plurality comprise: (a) a lipid bilayer, (b) a lumen comprising cytosol, wherein the lumen is surrounded by

the lipid bilayer; (c) an exogenous or overexpressed fusogen disposed in the lipid bilayer, (d) a nucleic acid comprising a payload gene encoding an exogenous agent of Table 5 or Table 6, wherein the fusosome does not comprise a nucleus; and wherein the amount of viral capsid protein in the fusosome composition is less than 1% of total protein.

5 The present disclosure provides, in some aspects, a fusosome composition comprising a plurality of fusosomes derived from a source cell, wherein the fusosomes of the plurality comprise: (a) a lipid bilayer, (b) a lumen comprising cytosol, wherein the lumen is surrounded by the lipid bilayer; (c) an exogenous or overexpressed fusogen disposed in the lipid bilayer, (d) a nucleic acid comprising a payload gene, wherein the nucleic acid comprises a NTCSRE operably
10 linked to the payload gene, wherein the NTCSRE comprises a non- target cell-specific miRNA recognition sequence, e.g., a non-target cell-specific miRNA recognition sequence bound by a miRNA present in a non-target cell at a higher level than in a target cell, e.g., a non-target cell-specific miRNA recognition sequence bound by a miRNA of Table 4, wherein the target cell is a first type of CNS cell, optionally wherein the non-target cell is a second, different type of CNS
15 cell or a non-CNS cell; and wherein the fusosome does not comprise a nucleus; and wherein the amount of viral capsid protein in the fusosome composition is less than 1% of total protein.

In some embodiments, the miRNA is present in a non-target cell (e.g., a non-target cell described herein) at a level at least 10, 100, 1,000, or 10,000 times higher than the level of the miRNA present in the target cell (e.g., a CNS cell). In some embodiments, the miRNA is not
20 detectably present in a target cell (e.g., a CNS cell, e.g., a CNS cell described herein). In some embodiments, the miRNA is not present in the target cell (e.g., a CNS cell, e.g., a CNS cell described herein).

The present disclosure provides, in some aspects, a fusosome composition comprising a plurality of fusosomes derived from a source cell, wherein the fusosomes of the plurality
25 comprise: (a) a lipid bilayer, (b) a lumen comprising cytosol, wherein the lumen is surrounded by the lipid bilayer; (c) an exogenous or overexpressed fusogen disposed in the lipid bilayer, (d) a nucleic acid comprising a payload gene, wherein the nucleic acid comprises a promoter operably linked to the payload gene, wherein the promoter is a CNS cell-specific promoter, e.g., is a promoter specific for a CNS cell, a pan-neuronal cell, a GABAergic neuron, a Glutamatergic
30 neuron, a Cholinergic neuron, a Dopaminergic neuron, a Serotonergic neuron, a glial cell, an astrocyte, a microglial cell, an oligodendrocyte, or a choroid plexus cell; wherein the fusosome

does not comprise a nucleus; and wherein the amount of viral capsid protein in the fusosome composition is less than 1% of total protein.

The present disclosure provides, in some aspects, a fusosome composition comprising a plurality of fusosomes derived from a source cell, wherein the fusosomes of the plurality
 5 comprise: (a) a lipid bilayer, (b) a lumen comprising cytosol, wherein the lumen is surrounded by the lipid bilayer; (c) an exogenous or overexpressed fusogen disposed in the lipid bilayer, (d) a nucleic acid comprising a payload gene, wherein the nucleic acid comprises a promoter having sequence of a promoter in Table 3, or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; wherein the fusosome does not comprise a
 10 nucleus; and wherein the amount of viral capsid protein in the fusosome composition is less than 1% of total protein;

The present disclosure provides, in some aspects, a fusosome composition comprising a plurality of fusosomes derived from a source cell, wherein the fusosomes of the plurality
 15 comprise: (a) a lipid bilayer, (b) a lumen comprising cytosol, wherein the lumen is surrounded by the lipid bilayer; (c) an exogenous or overexpressed fusogen disposed in the lipid bilayer, (d) a nucleic acid comprising: (i) a payload gene; (ii) a NTCSRE operably linked to the payload gene, e.g., wherein the NTCSRE comprises a non-target cell-specific miRNA recognition sequence, e.g., a non-target cell-specific miRNA recognition sequence bound by a miRNA of Table 4, and

(iii) optionally, a positive target cell-specific regulatory element, e.g., a positive target
 20 cell-specific regulatory element (e.g., a target cell-specific promoter) operatively linked to the payload gene, wherein the positive target cell-specific regulatory element increases expression of the payload gene in a target cell relative to an otherwise similar fusosome lacking the positive target cell-specific regulatory element, wherein the target cell is a first type of CNS cell; optionally wherein the non-target cell is a second, different type of CNS cell or a non-CNS cell,
 25 optionally wherein: the target cell is a neuron and the non-target cell is a glial cell (e.g., an oligodendrocyte, an astrocyte, or a microglial cell), or the target cell is a glial cell (e.g., an oligodendrocyte, an astrocyte, or a microglial cell) and the non-target cell is a neuron; wherein the fusosome does not comprise a nucleus; and wherein the amount of viral capsid protein in the fusosome composition is less than 1% of total protein.

30 In some embodiments, one or more of the following is present: 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 42; v) the fusosome fuses at a higher rate with a target cell
 5 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 42; 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 42; vii) the fusogen is present at a copy number
 10 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 26; 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,
 15 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 88; 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
 20 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
 25 the source cell; xi) the fusosome comprises a proteomic composition similar to that of the source cell, e.g., using an assay of Example 87; 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 40; 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
 30 in the source cell, e.g., as measured using an assay of Example 41; 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 91; 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 60; 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 50; 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 51; 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 53; 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 54; 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 55, 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 55, 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 55, 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 33; 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 38; 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 39; 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 49; 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 64; 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 56; 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 57; 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 58; 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 59, 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 44, 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 48; 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; xxxiv) 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.

The present disclosure also provides, in some aspects, a fusosome comprising: a) a lipid bilayer and a lumen that is miscible with an aqueous solution, e.g., water, wherein the fusosome is derived from a source cell, b) an exogenous or overexpressed fusogen disposed in the lipid bilayer, and c) an organelle, e.g., a therapeutically effective number of organelles, disposed in the lumen.

In some embodiments, one or more of the following is present: i) 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 organelle is selected from a Golgi apparatus, lysosome, endoplasmic reticulum, mitochondria, vacuole, endosome, acrosome, autophagosome, centriole, glycosome, glyoxysome, hydrogenosome, melanosome, mitosome, cnidocyst, peroxisome, proteasome, vesicle, and stress granule; iii) the fusosome has a size of greater than 5 μm , 10 μm , 20 μm , 50 μm , or 100 μm ; iv) the fusosome, or a composition or preparation comprising a plurality of the fusosomes, has a density of other than between 1.08 g/ml and 1.12 g/ml, e.g., the fusosome has a density of 1.25 g/ml $\pm$ 0.05, e.g., as measured by an assay of Example 30; v) the fusosome is not captured by the scavenger system in circulation or by Kupffer cells in the sinus of the liver; vi) the source cell is other than a 293 cell; vii) the source cell is not transformed or immortalized; viii) the source cell is transformed, or immortalized using a method other than adenovirus-

mediated immortalization, e.g., immortalized by spontaneous mutation, or telomerase expression; ix) the fusogen is other than VSVG, a SNARE protein, or a secretory granule protein; x) the fusosome does not comprise Cre or GFP, e.g., EGFP; xi) the fusosome further comprises an exogenous protein other than Cre or GFP, e.g., EGFP xii) the fusosome further
 5 comprises an exogenous nucleic acid (e.g., RNA, e.g., mRNA, miRNA, or siRNA) or an exogenous protein (e.g., an antibody, e.g., an antibody), e.g., in the lumen; or xiii) the fusosome does not comprise mitochondria.

The present disclosure also provides, in some aspects, a fusosome comprising: (a) a lipid bilayer, (b) a lumen (e.g., comprising cytosol) surrounded by the lipid bilayer, (c) an exogenous
 10 or overexpressed fusogen, e.g., wherein the fusogen is disposed in the lipid bilayer, and (d) a functional nucleus, wherein the fusosome is derived from a source cell.

In some embodiments, one or more of the following is present: 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,
 15 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) the fusosome was
 20 made according to good manufacturing practices (GMP); vii) the fusosome has a pathogen level below a predetermined reference value, e.g., is substantially free of pathogens; or viii) the fusosome has a contaminant level below a predetermined reference value, e.g., is substantially free of contaminants.

The present disclosure also provides, in some aspects, a fusosome composition
 25 comprising a plurality of fusosomes derived from a source cell, wherein the fusosomes of the plurality comprise: (a) a lipid bilayer, (b) a lumen comprising cytosol, wherein the lumen is surrounded by the lipid bilayer; (c) an exogenous or overexpressed fusogen disposed in the lipid bilayer, (d) a cargo; and wherein the fusosome does not comprise a nucleus; wherein the amount of viral capsid protein in the fusosome composition is less than 1% of total protein; wherein the
 30 plurality of 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% of the number of cells in the target cell population compared to the reference target cell population.

The present disclosure also provides, in some aspects, a fusosome composition comprising a plurality of fusosomes derived from a source cell, and wherein the fusosomes of the plurality comprise: (a) a lipid bilayer; (b) a lumen comprising cytosol, wherein the lumen is surrounded by the lipid bilayer; (c) an exogenous or overexpressed re-targeted fusogen disposed in the lipid bilayer; (d) a cargo; and wherein the fusosome does not comprise a nucleus; wherein the amount of viral capsid protein in the fusosome composition is less than 1% of total protein; 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%.

The present disclosure also provides, in some aspects, a fusosome composition comprising a plurality of fusosomes derived from a source cell, and wherein the fusosomes of the plurality comprise: (a) a lipid bilayer, (b) a lumen surrounded by the lipid bilayer; (c) an exogenous or overexpressed fusogen, wherein the fusogen is disposed in the lipid bilayer; and (d) a cargo; wherein the fusosome does not comprise a nucleus; and wherein one or more of (e.g., at least 2, 3, 4, or 5 of): i) the fusogen is present at a copy number of at least 1,000 copies; ii) the fusosome comprises a therapeutic agent at a copy number of at least 1,000 copies; iii) 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; iv) the fusosome comprises a proteomic composition similar to that of the source cell; v) 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; vi) 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; or 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.

5 In embodiments, one or more of: 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 EGFP; vi) the therapeutic agent is a nucleic acid (e.g., RNA, e.g., mRNA, miRNA, or siRNA) or an exogenous protein (e.g., an antibody, e.g., an antibody), e.g., in the lumen; or vii) the fusosome does not comprise mitochondria.

10 In embodiments, one or more of: 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 size of other than between 40 and 150 nm, e.g., greater than 150 nm, 200 nm, 300 nm, 400 nm, or 500 nm.

15 In embodiments, one or more of: i) the therapeutic agent is a soluble protein 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 an exogenous therapeutic transmembrane protein; or vi) the fusosome does not comprise CD63 or GLUT4, or the fusosome comprises less than or equal to 0.05%, 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, or 10% CD63 (e.g., about 0.048% or less), e.g., as determined according to the method described in Example 89.

25 In embodiments, the fusosome: i) does not comprise a virus, is not infectious, or does not propagate in a host cell; ii) is not a viral vector iii) is not a VLP (virus like particle); iv) does not comprise a viral structural protein, e.g., a protein derived from gag, e.g. a viral capsid protein, e.g. a viral capsule 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 mass spectrometry, e.g. using an assay of Example 93; v) does not comprise a viral matrix protein; vi) does not comprise a viral non-structural protein; e.g. pol or a fragment or variant thereof, a viral reverse transcriptase protein, a viral integrase protein, or a viral protease protein.

5 vii) does not comprise viral nucleic acid; e.g. viral RNA or viral DNA; viii) 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, or 1,000,000,000 copies per vesicle of a viral structural protein; or ix) the fusosome is not a virosome.

10 In some embodiments, the fusosome comprises (or is identified as comprising) less than about 0.01%, 0.05%, 0.1%, 0.5%, 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% viral capsid protein (e.g., about 0.05% viral capsid protein). In embodiments, the viral capsid protein is Complex of Rabbit Endogenous Lentivirus (RELK) Capsid with Cyclophilin A. In embodiments, the viral capsid protein: total protein ratio is (or is identified as being) about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09,

15 or 0.1.

In some embodiments, the fusosome does not comprise (or is identified as not comprising) a gag protein or a fragment or variant thereof, or the amount of gag protein or fragment or variant thereof 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 93.

20 In embodiments, 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, 20:1, 10:1, 5:1, or 1:1; or is between 100:1 and 50:1, 50:1 and 20:1, 20:1 and 10:1, 10:1 and 5:1 or 1:1. In embodiments, 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,

25 100:1, 50:1, 20:1, 10:1, 5:1, or 1:1.

In embodiments, one or more of: 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; or iv) the organelle is selected from a mitochondrion, Golgi apparatus, lysosome, endoplasmic reticulum, vacuole, endosome, acrosome, autophagosome, centriole,

30 glycosome, glyoxysome, hydrogenosome, melanosome, mitosome, cnidocyst, peroxisome, proteasome, vesicle, and stress granule.

In embodiments, one or more of: 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
 5 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; or v) the fusosome further comprises an exogenous therapeutic agent having one or more post-translational modifications, e.g., glycosylation.

In embodiments, the fusosome is unilamellar or multilamellar.

10 In embodiments, one or more of: 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 an exogenous fusogen; vi) the fusosome has a size of at least 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 size of at least 80 nm, 100 nm, 200 nm,
 15 500 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,
 20 dystrophin, keratin, myosin, or tubulin; ix) the fusosome, or a composition or 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
 25 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
 30 ligand or both of PS and CD40 ligand, e.g., when measured in an assay of Example 92; xii) the fusosome is enriched for PS compared to the source cell, e.g., in a population of fusosomes at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% are positive for PS, 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; 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/ug of protein, e.g., by an assay of Example 52; 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 89; 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/ug 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/ug in the source cell, e.g., using an assay of Example 36; 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 / ug total protein, or wherein the fusosome comprises less Calnexin per total protein in ng/ug 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 37 or 90, or wherein the average fractional content of Calnexin in the fusosome is less than about 1×10^{-4} , 1.5×10^{-4} , 2×10^{-4} , 2.1×10^{-4} , 2.2×10^{-4} , 2.3×10^{-4} , 2.4×10^{-4} , 2.43×10^{-4} , 2.5×10^{-4} , 2.6×10^{-4} , 2.7×10^{-4} , 2.8×10^{-4} , 2.9×10^{-4} , 3×10^{-4} , 3.5×10^{-4} , or 4×10^{-4} , or wherein the fusosome comprises an amount of Calnexin per total protein that is lower than that of the parental cell by about 70%, 75%, 80%, 85%, 88%, 90%, 95%, 99%, or more; xvii) the fusosome comprises an exogenous agent (e.g., an exogenous protein, mRNA, or siRNA) e.g., as measured using an assay of Example 34; 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.

In embodiments, one or more of: i) the fusosome is an exosome; ii) the fusosome is not a microvesicle; iii) the fusosome has a size 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 size of less than 80 nm, 100 nm, 200 nm, 500 nm, 1000 nm, 1200 nm, 1400 nm, or 1500 nm; iv) the fusosome does not comprise an organelle; 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) the fusosome, or a composition or preparation comprising a plurality of the fusosomes, has 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 92; 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, 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; 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/ug of protein, e.g., by an assay of Example 52; 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 89; 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/ug 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/ug in the source cell, e.g., using an assay of Example 36; 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 / ug total protein, or wherein the fusosome comprises less Calnexin per total protein in ng/ug 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 90, or wherein the average fractional content of Calnexin in the fusosome is less than about 1×10^{-4} , 1.5×10^{-4} , 2×10^{-4} , 2.1×10^{-4} , 2.2×10^{-4} , 2.3×10^{-4} , 2.4×10^{-4} , 2.43×10^{-4} , 2.5×10^{-4} , 2.6×10^{-4} , 2.7×10^{-4} , 2.8×10^{-4} , 2.9×10^{-4} , 3×10^{-4} , 3.5×10^{-4} , or 4×10^{-4} , or wherein the fusosome comprises an amount of Calnexin per total protein that is lower than that of the parental cell by about 70%, 75%, 80%, 85%, 88%, 90%, 95%, 99%, or more; 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.

In embodiments, one or more of: 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.

In embodiments, the fusosome comprises cytosol.

In embodiments, one or more of: i) the fusosome or the source cell does not form a teratoma when implanted into subject, e.g., by an assay of Example 65; ii) the fusosome is 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 45; iii) the fusosome is capable of homing, e.g., at the site of an injury, wherein the

fusosome or cytobiologic is from a human cell, e.g., using an assay of Example 46, e.g., wherein the source cell is a neutrophil; or iv) the fusosome is capable of phagocytosis, e.g., wherein phagocytosis by the fusosome is detectable within 0.5, 1, 2, 3, 4, 5, or 6 hours in using an assay of Example 47, e.g., wherein the source cell is a macrophage.

5 In embodiments, the fusosome or fusosome composition 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.

In embodiments, 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
10 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
15 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.

In embodiments, 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,
20 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 24 and DNA replication is measured by an assay of Example 25; b) the fusosome is not capable of transcription or has transcriptional activity of less than 1%, 2.5% 5%, 10%, 20%,
25 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 24; 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 25; d) the fusosome lacks chromatin or has a
30 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 32; 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 31; 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 31, 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 32; 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.

In embodiments, the fusosome comprises mtDNA or vector DNA. In embodiments, the fusosome does not comprise DNA.

In embodiments, the source cell is a primary cell, immortalized cell or a cell line (e.g., myeloblast cell line, e.g., C2C12). In embodiments, 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 or MHC complexes). In embodiments, the source cell is from a cell culture treated with an anti-inflammatory signal. In embodiments, the source cell is from a cell culture treated with an immunosuppressive agent. In embodiments, the source cell is substantially non-immunogenic, e.g., using an assay described herein. In embodiments, the source cell comprises an exogenous agent, e.g., a therapeutic agent. In embodiments, the source cell is a recombinant cell.

In embodiments, the fusosome further comprises an exogenous agent, e.g., a therapeutic 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). In embodiments, 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, e.g., 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. In embodiments, the fusosome has an altered, e.g., increased or decreased level of one or more endogenous molecules, e.g., protein or nucleic acid, e.g., due to treatment of the mammalian cell with a siRNA or gene editing enzyme. In embodiments, the endogenous molecule is present at, e.g. 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, 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., 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. In embodiments, 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 , 5.0×10^3 , 10^4 , 5.0×10^4 , 10^5 , 5.0×10^5 , 10^6 , 5.0×10^6 , 1.0×10^7 , 5.0×10^7 , or 1.0×10^8 , greater than its concentration in the source cell.

In embodiments, the active 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. In embodiments, the exogenous agent comprises a site-specific nuclease, e.g., Cas9 molecule, TALEN, or ZFN.

In embodiments, the fusogen is a viral fusogen, e.g., HA, HIV-1 ENV, HHV-4, gp120, or VSV-G. In embodiments, the fusogen is a mammalian fusogen, e.g., a SNARE, a Syncytin, myomaker, myomixer, myomerger, or FGFR1. In embodiments, the fusogen is active at a pH of 4-5, 5-6, 6-7, 7-8, 8-9, or 9-10. In embodiments, the fusogen is not active at a pH of 4-5, 5-6, 6-7, 7-8, 8-9, or 9-10. In embodiments, the fusosome fuses to a target cell at the surface of the target cell. In embodiments, the fusogen promotes fusion in a lysosome-independent manner. In embodiments, the fusogen is a protein fusogen. In embodiments, the fusogen is a lipid fusogen, e.g., oleic acid, glycerol mono-oleate, a glyceride, diacylglycerol, or a modified unsaturated fatty acid. In embodiments, the fusogen is a chemical fusogen, e.g., PEG. In embodiments, the fusogen is a small molecule fusogen, e.g., halothane, an NSAID such as meloxicam, piroxicam, tenoxicam, and chlorpromazine. In embodiments, the fusogen is recombinant. In embodiments, the fusogen 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. In embodiments, the fusogen is biosynthetically incorporated, e.g. expressed in a source cell under conditions that allow the fusogen to associate with the lipid bilayer.

In embodiments, the fusosome binds a target cell. In embodiments, the target cell is other than a HeLa cell, or the target cell is not transformed or immortalized.

5 In some embodiments involving fusosome compositions, the plurality of fusosomes are the same. In some embodiments, the plurality of fusosomes are different. In some embodiments the plurality of fusosomes are from one or more source cells. In some embodiments 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
10 composition. In some embodiments 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. In some embodiments, the fusosome composition has less than about 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, 5%, variability in size distribution within 10%, 50%, or 90% of the source cell population variability
15 in size distribution, e.g., based on Example 28. In some embodiments, 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. In some embodiments, at least 50%, 60%, 70%, 80%, 90%, 95%, or 99% of fusosomes in the plurality have a copy number of the therapeutic agent
20 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. In some embodiments, the fusosome composition comprises at least 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} , 10^{11} , 10^{12} , 10^{13} , 10^{14} , or 10^{15} or more fusosomes. In some embodiments, the fusosome composition is in a volume of at least 1 ul, 2 ul, 5 ul, 10 ul, 20 ul, 50 ul, 100 ul, 200 ul, 500 ul, 1 ml, 2 ml, 5 ml, or 10 ml.

25 In some embodiments, the fusosome composition delivers the cargo to at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of the number of cells in the target cell population compared to the reference 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% of the cargo to the target
30 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.

In some embodiments, less than 10% of cargo enters the cell by endocytosis.

5 In some embodiments, the inhibitor of endocytosis is an inhibitor of lysosomal acidification, e.g., bafilomycin A1. In some embodiments, the inhibitor of endocytosis is a dynamin inhibitor, e.g., Dynasore.

In some embodiments, the target cell population is at a physiological pH (e.g., between 7.3-7.5, e.g., between 7.38-7.42).

10 In some embodiments, the cargo delivered is determined using an endocytosis inhibition assay, e.g., an assay of Example 80.

In some embodiments, cargo enters the 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-
15 isopropyl)amiloride (EIPA), e.g., at a concentration of 25 μ M), 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 μ M).

In some embodiments, the fusosomes of the plurality further comprise a targeting moiety. In embodiments, the targeting moiety is comprised by the fusogen or is comprised by a separate
20 molecule.

In some embodiments, 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 10-fold more target cells than non-target cells.

In some embodiments, when the plurality of fusosomes are contacted with a cell
25 population comprising target cells and non-target cells, the cargo is present at least 2-fold, 5-fold, 10-fold, 20-fold, or 50-fold higher in target cells than non-target cells and/or the cargo is present at least 2-fold, 5-fold, 10-fold, 20-fold, or 50-fold higher in target cells than reference cells.

In some embodiments, the fusosomes of the plurality fuse at a higher rate with a target cell than with a non-target cell by at least 50%.

30 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.

In embodiments, less 50%, 40%, 30%, 20%, or 10% of the cargo is delivered to an endosome or lysosome.

In some embodiments, the fusosomes of the plurality comprise exosomes, microvesicles, or a combination thereof.

5 In some embodiments, the plurality of fusosomes has an average size of at least 50 nm, 100 nm, 200 nm, 500 nm, 1000 nm, 1200 nm, 1400 nm, or 1500 nm. In other embodiments, the plurality of fusosomes has an average size of less than 100 nm, 80 nm, 60 nm, 40 nm, or 30 nm.

In some embodiments, the fusogen (e.g., re-targeted fusogen) comprises a mammalian fusogen. In some embodiments, the fusogen (e.g., re-targeted fusogen) comprises a viral
10 fusogen. In some embodiments, the fusogen (e.g., re-targeted fusogen) is a protein fusogen. In some embodiments, the fusogen (e.g., re-targeted fusogen) comprises a sequence chosen from a Nipah virus protein F, a measles virus F protein, a tupaia paramyxovirus F protein, a paramyxovirus F protein, a Hendra virus F protein, a Henipavirus F protein, a Morbilivirus F protein, a respirovirus F protein, a Sendai virus F protein, a rubulavirus F protein, or an
15 avulavirus F protein, or a derivative thereof.

In some embodiments, the fusogen (e.g., re-targeted fusogen) is active at a pH of 4-5, 5-6, 6-7, 7-8, 8-9, or 9-10. In some embodiments, the fusogen (e.g., re-targeted fusogen) is not active at a pH of 4-5, 5-6, 6-7, 7-8, 8-9, or 9-10.

In some embodiments, the fusogen is present at a copy number of at least 1, 2, 5, or 10
20 copies per fusosome.

In some embodiments, the fusogen (e.g., re-targeted fusogen) comprises a Nipah virus protein G, a measles protein H, a tupaia paramyxovirus H protein, a paramyxovirus G protein, a paramyxovirus H protein, a paramyxovirus HN protein, a Morbilivirus H protein, a respirovirus HN protein, a sendai HN protein, a rubulavirus HN protein, an avulavirus HN protein, or a
25 derivative thereof. In some embodiments, the fusogen (e.g., re-targeted fusogen) comprises a sequence chosen from Nipah virus F and G proteins, measles virus F and H proteins, tupaia paramyxovirus F and H proteins, paramyxovirus F and G proteins or F and H proteins or F and HN proteins, Hendra virus F and G proteins, Henipavirus F and G proteins, Morbilivirus F and H proteins, respirovirus F and HN protein, a Sendai virus F and HN protein, rubulavirus F and HN
30 proteins, or avulavirus F and HN proteins, or a derivative thereof, or any combination thereof.

In some embodiments, the cargo comprises an exogenous protein or an exogenous nucleic acid. In some embodiments, the cargo comprises or encodes a cytosolic protein. In some embodiments the cargo comprises or encodes a membrane protein. In some embodiments, the cargo comprises a therapeutic agent. In some embodiments, the cargo is present at a copy number of at least 1, 2, 5, 10, 20, 50, 100, or 200 copies per fusosome (e.g., up to about 1,000 copies per fusosome). In some embodiments, the ratio of the copy number of the fusogen (e.g., re-targeted fusogen) to the copy number of the cargo is between 1000:1 and 1:1, or between 500:1 and 1:1 or between 250:1 and 1:1, or between 150:1 and 1:1, or between 100:1 and 1:1, or between 75:1 and 1:1 or between 50:1 and 1:1 or between 25:1 and 1:1 or between 20:1 and 1:1 or between 15:1 and 1:1 or between 10:1 and 1:1 or between 5:1 and 1:1 or between 2:1 and 1:1 or between 1:1 and 1:2.

In some embodiments, the fusosome composition comprises a viral capsid protein or a DNA integration polypeptide. In some embodiments, the cargo comprises a viral genome.

In some embodiments, the fusosome composition is capable of delivering a nucleic acid to a target cell, e.g., to stably modify the genome of the target cell, e.g., for gene therapy.

In some embodiments, the fusosome composition does not comprise a viral nucleocapsid protein, or the amount of viral nucleocapsid protein is less than 10%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.2%, or 0.1% of total protein, e.g., by mass spectrometry, e.g. using an assay of Example 93.

In embodiments, the fusosome composition comprises at least 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} , 10^{11} , 10^{12} , 10^{13} , 10^{14} , or 10^{15} fusosomes. In embodiments, the fusosome composition comprises 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.

In embodiments, 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 or MHC complexes). In embodiments, the source cell is from a cell culture treated with an anti-inflammatory signal. In embodiments, the method further comprises contacting the source cell of step a) with an immunosuppressive agent or anti-inflammatory signal, e.g., before or after inactivating the nucleus, e.g., enucleating the cell.

In one aspect, provided herein is a fusosome composition comprising a plurality of fusosomes derived from a source cell, wherein the fusosomes of the plurality comprise: (a) a

lipid bilayer, (b) a lumen comprising cytosol, wherein the lumen is surrounded by the lipid bilayer; (c) an exogenous or overexpressed fusogen disposed in the lipid bilayer, (d) a cargo; and wherein the fusosome does not comprise a nucleus; wherein the amount of viral capsid protein in the fusosome composition is less than 1% of total protein; wherein the plurality of 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% of the number of cells in the target cell population compared to the reference target cell population.

In embodiments, the fusosome composition delivers the cargo to at least 40%, 50%, 60%, 70%, or 80% of the number of cells in the target cell population compared to the reference target cell population or to a non-target cell population; or delivers the cargo, e.g., at least 40%, 50%, 60%, 70%, or 80% of the cargo, to the target cell population compared to the reference target cell population or to a non-target cell population. In embodiments, less than 10% of cargo enters the cell by endocytosis. In embodiments, the inhibitor of endocytosis is an inhibitor of lysosomal acidification, e.g., bafilomycin A1. In embodiments, cargo delivered is determined using an endocytosis inhibition assay, e.g., an assay of Example 80. In embodiments, cargo enters the 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 μ M), 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 μ M).

C. Fusogens and pseudotyping

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.

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., CNS

cell). In some embodiments, the specific cell type targeted by the one or more fusogens is a CNS cell, a pan-neuronal cell, a GABAergic neuron, a Glutamatergic neuron, a Cholinergic neuron, a Dopaminergic neuron, a Serotonergic neuron, a glial cell, an astrocyte, a microglial cell, an oligodendrocyte, or a choroid plexus cell. 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 particular embodiments, a fusogen is a fusogen or a re-targeted fusogen that binds to a target cell from among a CNS cell a pan-neuronal cell, a GABAergic neuron, a Glutamatergic neuron, a Cholinergic neuron, a Dopaminergic neuron, a Serotonergic neuron, a glial cell, an astrocyte, a microglial cell, an oligodendrocyte, or a choroid plexus cell. In some embodiments, the fusosome comprising the fusogen will integrate the membrane into a lipid bilayer of a target cell. In some embodiments, one or more of the fusogens described herein may be included in the fusosome.

The fusosomes (e.g., retroviral vectors) described herein can comprise a fusogen, e.g., an endogenous fusogen or a pseudotyped fusogen.

i) Protein fusogens

In some embodiments, the fusogen comprises a protein (e.g., glycoprotein), lipid, or small molecule. A fusogen can be, for instance, a mammalian fusogen or a viral fusogen. 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. In some embodiments, a viral fusogen is a Class I viral membrane fusion protein, a Class II viral membrane 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.

In embodiments, the fusogen is a viral fusogen, e.g., HA, HIV-1 ENV, HHV-4, gp120, or VSV-G. In embodiments, the fusogen is a mammalian fusogen, e.g., a SNARE, a Syncytin,

myomaker, myomixer, myomerger, or FGFR1. In embodiments, the fusogen is active at a pH of 4-5, 5-6, 6-7, 7-8, 8-9, or 9-10. In embodiments, the fusogen is not active at a pH of 4-5, 5-6, 6-7, 7-8, 8-9, or 9-10. In embodiments, the fusosome fuses to a target cell at the surface of the target cell. In embodiments, the fusogen promotes fusion in a lysosome-independent manner. In
 5 embodiments, the fusogen is a protein fusogen. In embodiments, the fusogen is a lipid fusogen, e.g., oleic acid, glycerol mono-oleate, a glyceride, diacylglycerol, or a modified unsaturated fatty acid. In embodiments, the fusogen is a chemical fusogen, e.g., PEG. In embodiments, the fusogen is a small molecule fusogen, e.g., halothane, an NSAID such as meloxicam, piroxicam, tenoxicam, and chlorpromazine. In embodiments, the fusogen is recombinant. In embodiments,
 10 the fusogen is biochemically incorporated, e.g., the fusogen is provided as a purified protein and contacted with a lipid bilayer under conditions that allow for association of the fusogen with the lipid bilayer. In embodiments, the fusogen is biosynthetically incorporated, e.g. expressed in a source cell under conditions that allow the fusogen to associate with the lipid bilayer.

In some embodiments, the fusogen (e.g., re-targeted fusogen) comprises a mammalian
 15 fusogen. In some embodiments, the fusogen (e.g., re-targeted fusogen) comprises a viral fusogen. In some embodiments, the fusogen (e.g., re-targeted fusogen) is a protein fusogen. In some embodiments, the fusogen (e.g., re-targeted fusogen) comprises a sequence chosen from a Nipah virus protein F, a measles virus F protein, a tupaia paramyxovirus F protein, a paramyxovirus F protein, a Hendra virus F protein, a Henipavirus F protein, a Morbillivirus F
 20 protein, a respirovirus F protein, a Sendai virus F protein, a rubulavirus F protein, or an avulavirus F protein, or a derivative thereof.

In some embodiments, the fusogen (e.g., re-targeted fusogen) is active at a pH of 4-5, 5-6, 6-7, 7-8, 8-9, or 9-10. In some embodiments, the fusogen (e.g., re-targeted fusogen) is not active at a pH of 4-5, 5-6, 6-7, 7-8, 8-9, or 9-10.

25 In some embodiments, the fusogen is present at a copy number of at least 1, 2, 5, or 10 copies per fusosome.

In some embodiments, the fusogen (e.g., re-targeted fusogen) comprises a Nipah virus protein G, a measles protein H, a tupaia paramyxovirus H protein, a paramyxovirus G protein, a paramyxovirus H protein, a paramyxovirus HN protein, a Morbillivirus H protein, a respirovirus
 30 HN protein, a sendai HN protein, a rubulavirus HN protein, an avulavirus HN protein, or a derivative thereof. In some embodiments, the fusogen (e.g., re-targeted fusogen) comprises a

sequence chosen from Nipah virus F and G proteins, measles virus F and H proteins, tupaia paramyxovirus F and H proteins, paramyxovirus F and G proteins or F and H proteins or F and HN proteins, Hendra virus F and G proteins, Henipavirus F and G proteins, Morbilivirus F and H proteins, respirovirus F and HN protein, a Sendai virus F and HN protein, rubulavirus F and HN proteins, or avulavirus F and HN proteins, or a derivative thereof, or any combination thereof.

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).

Fusogens, which include viral envelope proteins (env), generally determine the range of host cells which can be infected and transformed by fusosomes. In the case of lentiviruses, such as HIV-1, HIV-2, SIV, FIV and EIV, the native env proteins include gp41 and gp120. In some embodiments, the viral env proteins expressed by source cells described herein are encoded on a
 5 separate vector from the viral gag and pol genes, as has been previously described.

Illustrative examples of retroviral-derived env genes which can be employed include, but are not limited to: MLV envelopes, 10A1 envelope, BAEV, FeLV-B, RD114, SSAV, Ebola, Sendai, FPV (Fowl plague virus), and influenza virus envelopes. Similarly, genes encoding envelopes from RNA viruses (e.g., RNA virus families of Picornaviridae, Calciviridae,
 10 Astroviridae, Togaviridae, Flaviviridae, Coronaviridae, Paramyxoviridae, Rhabdoviridae, Filoviridae, Orthomyxoviridae, Bunyaviridae, Arenaviridae, Reoviridae, Birnaviridae, Retroviridae) as well as from the DNA viruses (families of Hepadnaviridae, Circoviridae, Parvoviridae, Papovaviridae, Adenoviridae, Herpesviridae, Poxviridae, and Iridoviridae) may be utilized. Representative examples include, FeLV, VEE, HFVW, WDSV, SFV, Rabies, ALV,
 15 BIV, BLV, EBV, CAEV, SNV, ChTLV, STLV, MPMV, SMRV, RAV, FuSV, MH2, AEV, AMV, CT10, and EIAV.

In some embodiments, envelope proteins for display on a fusosome include, but are not limited to any of the following sources: Influenza A such as H1N1, H1N2, H3N2 and H5N1 (bird flu), Influenza B, Influenza C virus, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus,
 20 Hepatitis D virus, Hepatitis E virus, Rotavirus, any virus of the Norwalk virus group, enteric adenoviruses, parvovirus, Dengue fever virus, Monkey pox, Mononegavirales, Lyssavirus such as rabies virus, Lagos bat virus, Mokola virus, Duvenhage virus, European bat virus 1 & 2 and Australian bat virus, Ephemerovirus, Vesiculovirus, Vesicular Stomatitis Virus (VSV), Herpesviruses such as Herpes simplex virus types 1 and 2, varicella zoster, cytomegalovirus,
 25 Epstein-Bar virus (EBV), human herpesviruses (HHV), human herpesvirus type 6 and 8, Human immunodeficiency virus (HIV), papilloma virus, murine gammaherpesvirus, Arenaviruses such as Argentine hemorrhagic fever virus, Bolivian hemorrhagic fever virus, Sabia-associated hemorrhagic fever virus, Venezuelan hemorrhagic fever virus, Lassa fever virus, Machupo virus, Lymphocytic choriomeningitis virus (LCMV), Bunyaviridae such as Crimean-Congo
 30 hemorrhagic fever virus, Hantavirus, hemorrhagic fever with renal syndrome causing virus, Rift Valley fever virus, Filoviridae (filovirus) including Ebola hemorrhagic fever and Marburg

hemorrhagic fever, Flaviviridae including Kaysanur Forest disease virus, Omsk hemorrhagic fever virus, Tick-borne encephalitis causing virus and Paramyxoviridae such as Hendra virus and Nipah virus, variola major and variola minor (smallpox), alphaviruses such as Venezuelan equine encephalitis virus, eastern equine encephalitis virus, western equine encephalitis virus, SARS-associated coronavirus (SARS-CoV), West Nile virus, any encephalitis causing virus.

In some embodiments, a source cell described herein produces a fusosome, e.g., recombinant retrovirus, e.g., lentivirus, pseudotyped with the VSV-G glycoprotein.

A fusosome or pseudotyped virus generally has a modification to one or more of its envelope proteins, e.g., an envelope protein is substituted with an envelope protein from another virus. For example, HIV can be pseudotyped with a fusion protein from rhabdovirus, e.g., vesicular stomatitis virus G-protein (VSV-G) envelope proteins, which allows HIV to infect a wider range of cells because HIV envelope proteins (encoded by the env gene) normally target the virus to CD4+ presenting cells. In some embodiments, lentiviral envelope proteins are pseudotyped with VSV-G. In one embodiment, source cells produce recombinant retrovirus, e.g., lentivirus, pseudotyped with the VSV-G envelope glycoprotein.

Furthermore, a fusogen or viral envelope protein can be modified or engineered to contain polypeptide sequences that allow the transduction vector to target and infect host cells outside its normal range or more specifically limit transduction to a cell or tissue type. For example, the fusogen or envelope protein can be joined in-frame with targeting sequences, such as receptor ligands, antibodies (using an antigen-binding portion of an antibody or a recombinant antibody-type molecule, such as a single chain antibody), and polypeptide moieties or modifications thereof (e.g., where a glycosylation site is present in the targeting sequence) that, when displayed on the transduction vector coat, facilitate directed delivery of the virion particle to a target cell of interest. Furthermore, envelope proteins can further comprise sequences that modulate cell function. Modulating cell function with a transducing vector may increase or decrease transduction efficiency for certain cell types in a mixed population of cells. For example, stem cells could be transduced more specifically with envelope sequences containing ligands or binding partners that bind specifically to stem cells, rather than other cell types that are found in the blood or bone marrow. Non-limiting examples are stem cell factor (SCF) and Flt-3 ligand. Other examples include, e.g., antibodies (e.g., single-chain antibodies that are

specific for a cell-type), and essentially any antigen (including receptors) that binds tissues as lung, liver, pancreas, heart, endothelial, smooth, breast, prostate, epithelial, vascular cancer, etc.

Protein fusogens or viral envelope protein may be re-targeted by mutating amino acid residues in a fusion protein or a targeting protein (e.g. the hemagglutinin protein). In some
 5 embodiments the fusogen is randomly mutated. In some embodiments the fusogen is rationally mutated. In some embodiments the fusogen is subjected to directed evolution. In some
 embodiments the fusogen is truncated and only a subset of the peptide is used in the retroviral vector or VLP. For example, amino acid residues in the measles hemagglutinin protein may be
 mutated to alter the binding properties of the protein, redirecting fusion (doi:10.1038/nbt942,
 10 Molecular Therapy vol. 16 no. 8, 1427–1436 Aug. 2008, doi:10.1038/nbt1060, DOI:
 10.1128/JVI.76.7.3558–3563.2002, DOI: 10.1128/JVI.75.17.8016–8020.2001, doi:
 10.1073pnas.0604993103).

In some embodiments, the protein fusogen or viral envelope protein is re-targeted by i)
 mutating amino acid residues in the natural fusogen protein sequence or viral envelope protein
 15 sequence and/or ii) engineering the fusogen protein or viral envelope protein to contain
 polypeptide sequences that allow the fusogen or viral envelope protein to target and fuse or
 infect host cells outside its normal range.

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. Fusogens
 20 include without limitation protein based, lipid based, and chemical based fusogens. The fusogen
 may bind a partner on a target cells' surface. In some embodiments, the fusosome comprising
 the fusogen will integrate the membrane into a lipid bilayer of a target cell.

In some embodiments the fusogen is a paramyxovirus fusogen. In some embodiments the
 fusogen is a Nipah virus protein F, a measles virus F protein, a tupaia paramyxovirus F protein, a
 25 paramyxovirus F protein, a Hendra virus F protein, a Henipavirus F protein, a Morbilivirus F
 protein, a respirovirus F protein, a Sendai virus F protein, a rubulavirus F protein, or an
 avulavirus F protein.

In some embodiments, the fusogen is a poxviridae fusogen.

Additional exemplary fusogens are disclosed in US 9,695,446, US 2004/0028687, US
 30 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.

In some embodiments, a fusogen described herein comprises an amino acid sequence of Table 1, 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 1. In some embodiments, a nucleic acid sequence described herein encodes an amino acid sequence of Table 1, 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.

In some embodiments, a fusogen described herein comprises an amino acid sequence set forth in any one of SEQ ID NOS: 1-57, 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: 1-57. In some embodiments, a nucleic acid sequence described herein encodes an amino acid sequence set forth in any one of SEQ ID NOS: 1-57, 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 1. 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	Nucleotide s of CDS	Full Gene Name	Sequence	#Sequences /Cluster	SEQ ID NO

KP31792 7	5630-7399	gb:KP317 927:5630- 7399 Orga nism:Hum an respiratory syncytial virus Strai n Name:Kili fi_9465_7 _RSVB_2 011 Protei n Name:fusi on glycoprote in Gene Symbol:F	MIPQARTELN LGQITMELLIHRSSAIFL TLAINALYLTSSQNITEEFYQSTCSAVS RGYLSALRTGWYTSVITIELSNIKETK CNGTDTKVLIKQELDKYKNAVTELQ LLMQNTPAANNRARRREAPQYMNYTI NTTGSLNVSISKRRKRFLGFLGVS AIASGIAVSKVLHLEGEVNIKINALLS TNKAVVSLSNGVSVLT SKVLDLKNYI NNQLLPIVNQQSCRISNIETVIEFQQKN SRLLEITREFSVNAGVTTPLSTYMLTN SELLSLINDMPITNDQKKLMSSNVQIV RQQSYSIMSIIKEEV LAYVVQLPIYGV DTPCWKLHTSPLCTTNIKEGSNICLTR TDRGWYCDNAGSVSFFPQADTCKVQ SNRVFCDTMNSLTLPSEVSLCNTDIFN SKYDCKIMTSKTDISSVITSLGAIVSC YGKTKCTASNKNRGIKTF SNGCDYV SNKGVDTVSVGNTLYYV NKLEGKNL YVKGEPIINYYDPLVFP SDEFDASISQV NEKINQSLAFIRRSDELLHNVNTGKST TNIMITAIIIVIVVLLSLIAIGLLLYCKA KNTPVTL SKDQLSGINNIAFSK	993	1
AB52440 5	4556-6217	gb:AB524 405:4556- 6217 Orga nism:New castle disease virus Strai n Name:Go ose/Alask a/415/91 P rotein Name:fusi on protein Ge ne Symbol:F	MDPKPSTSYLHAFPLIFVAISLVFMAG RASALDGRPLAAAGIVVTGDKAVNIY TSSQTGTIIKLLPNMPKDKEQCAKSPL DAYNRTLTTLLAPLGDSIRRIQESVTT SGGERQERLVGAIIGGVALGVATAAQ ITAASALI QANQNAANILKLKESIAAT NEAVHEVTSGLSQLAVAVGKMQQFV NDQFNKTAQEIDCIKITQQVGVELNLY LTELTTVF GPQITSPALTQ LTIQALYNL AGGNMDYMLTKLGVGNNQLSSLISS GLISGNPILYDSQTQLLGIQVTLPSVGN LNNMRATYLET LSVSTNKG FASALVP KVVTVQVGSVIEELDTSYCIETDL DLYC TRIVTFPMSPGIF SCLGGNTSACMYSK TEGALTTPYMTLKG SVIANCKMTTCR CADPPGIISQNYGEAVSLIDKKVCNILT LDGITLRLSGEFDATYQKNISIQDSQV VITGNLDISTELGNVNNSISNALDKLE ESNSKLDKVNVR LTSTSALITYIVLTTI ALICGIVSLVLACYIMYKQKAQQKTL LWLGNN TLDQMRATTKM	418	2
AF26628 6	4875-7247	gb:AF266 286:4875- 7247 Orga nism:Mea sles virus strain AIK- C Strain Name:Me asles virus strain Edmonsto n (AIK-C	MSIMGLKVNVS AIFMAVLLTLQTPTG QIHWGNLSKIGVV GIGSASYKVMTRS SHQSLVIKLPNITLLNNCTRVEIAEY RRLRLTVLEPIRDALNMTQNI RPVQS VASSRRHKRFAGVVLAGAALGVATA AQITAGIALHQ SMLNSQAIDNLRASLE TTNQAIEAIRQAGQEMILAVQGVQDY INNELIPSMNQLSCDLIGQKLGLKLLR YYTEILSLFGPSLRDPISAEISIQALS YA LGGDINKVLEKLGYSGGDLLGILES RG IKARITHVDTESYFIVLSIAYPTLSEIKG VIVHRLEGVSYNIGSQEWYTTVPKYV ATQGYLISNFDESSCTFMPEGTVCSQN	128	3

		vaccine)IP rotein Name:fusi on proteinlGe ne Symbol:F	ALYPMSPLLQECLRGYTKSCARTLVS GSFGNRFILSQGNLIANCASILCKCYTT GTIINQDPDKILTYIAADNCPVVEVNG VTIQVGSRRYPDAVYLHRIDLGPILL ERLDVGTNLGNIAKLEDAKELLESS DQILRSMKGLSSTCIVYILIAVCLGGLI GIPALICCCRGRGNKKGEQVGMSPRG LKPDLTGTSKSYVRSL		
AB50385 7	3068-4687	gb:AB503 857:3068- 4687 Orga nism:Hum an metapneu moviruslSt rain Name:Jpn 03- 11 Protein Name:fusi on glycoprote in precursorl Gene Symbol:F	MSWKVVIIIFSLITPQHGLKESYLEES CSTITEGYLSVLRTGWYTNVFTLEV DVENLTCSDGPSLIKTELDLTKSALRE LKTVSADQLAREEQIEKPRQSRFVLG AIALGVATAAAVTAGVAIAKTIRLESE VTAIKNALKTTNEAVSTLGNNGVRVLA TAVRELKDFVSKNLTRAINKNKCDID DLKMAVSFSQFNRRFLNVVRQFSDNA GITPAISLDLMTDAELARAVSNMPTSA GQIKLMLENRAMVRRKGFILIGVYG SSVIYMVQLPIFGVIDTPCWIVKAAPS CSEKKGNYACLLREDQGWYCNAGS TVYYPNEKDCETRGRDHVFCDTAAGIN VAEQSKECNINISTTNYPCKVSTGRHP ISMVALSPLGALVACYKGVSCSIGSNR VGIKQLNKGCSYITNQDADTVTIDNT VYQLSKVEGEQHVIGRPVSSSFDPK FPEDQFNVALDQVFENIENSQALVDQ SNRILSSAEKGNTGFIIIVILIAVLGSSM ILVSIFIIKKTKKPTGAPPELSGVTNNG FIPHS	125	4
EU27765 8	5078-6700	gb:EU277 658:5078- 6700 Orga nism:Bovi ne parainflue nza virus 3 Strain Name:Q5 592 Protei n Name:fusi on proteinlGe ne Symbol:F	MIIIVITMILSLTPSSLCQIDITKLQSVG VLVNSPKGKISQNFETRYLILSLIPKIE DSHSCGNQQIDQYKKLLDRLIPLYDG LKLQKDVIVVNHESHNTNLRTKRFF GEIIGTIAIGIATSAQITAAVALVEAKQ ARSDIDKLKEAIKDTNKAVQSIQSSVG NLIVAVKSVQDYVNNEIVPSITRLGCE AAGLQLGIALTQHYSLETNIFGDNIGT LGEKGVKLQGIASLYRTNITEVFTTST VDQYDIYDLLFTESIKMRVIDVDLSY SITLQVRLPLLTKVSNTQIYKVDSISYN IQGKEWYIPLPHHIMTKGAFLGGADIK ECIESFSNYICPSDPGFILNHEMENCLS GNITQCPKTIVTSDIVPRYAFVDGGVI ANCIPTTCTCNGIDNRINQSPDQGIKIT YKECQIVGINGMLFKTNQEGTLAKYT FDNIKLNNVALNPIDISLELNKAKSD LEESKRWIEKSNQKLD SIGSWHQSSVT IIIIVMIVVLLIINAIIMIMIRYLDRNR HLNNKDSEPYVLTNRQ	93	5
AB04087 4	4546-6162	gb:AB040 874:4546- 6162 Orga nism:Mu mps viruslStrai n	MKVFLVTCLGFAVFSSVCVNINILQQ IGYIKQVRQLSYYSQSSSYIVVKLL PNIQPTDNSCEFKSVTQYNKTLNLLL PIAENINNIASPSSGSRHRKRFAGIAIGI AALGVATAAQVTAASVSLVQAQTNAR AIAAMKNSIQATNRAVFEVKEGTQRL AIAVQAIQDHINTIMNTQLNNMSCQIL	89	6

		Name:MiyaharaProtein Name:fusion proteinGene Symbol:F	DNQLATSLGLYLTELTTVFQPQLINPALSPISIQALRSLLGSMTPAVVQATLSTSISAAEILSAGLMEGQIVSVLLDEMQMI VKINIPTIVTQSNALVIDFYSISSFINNQ ESIIQLPDRILEIGNEQWSYPANKCKLTRHHIFCQYNEAERLSLESKLCLAGNIS ACVFSPIAGSYMRRFVALDGTIVANC RSLTCLCKSPSYPIYQPDHHA VTTIDL TACQTLSDGLDFSI VLSNITYAENLT ISLSQTINTQPIDISTELSKVNASLQNA VKYIKESNHQLQSVNVNSKIGAIIVAA LVLSILSIISLLFCCWAYVATKEIRIN FKTNHINTISSSVDDLIRY		
AB475097	4908-6923	gb:AB475097:4908-6923 Organism:Canine distemper virus Strain Name:M25CR Protein Name:fusion proteinGene Symbol:F	MNPHEQTIPMHEKIPKRSKTQHTTQQ DLPQQHSTKSAESKTSRARHSITSAQR STHYDPRTADWPDYIMKRTRSCQ ASYRSDNIPAHGDHGDGIIHHTPESVSQ GAKSRLKMGQSNVKSQSCTWLVL WCIGVASLFLCSKAQIHWNNLSTIGII GTDSVHYKIMTRPSHQYLVIKLMPNV SLIDNCTKAELDEYEKLLSSILEPINQA LTLMTKNVKPLQSVGSGRRQRRFAG VVLAGAALGVATAAQITAGIALHQS LNAQAIQSLRTSLEQSNKAIEEIREATQ ETVIAVQGVQDYVNNELVPAMQHMS CELVGQRLGLKLLRYYTELLSIFGPSL RDPISAEISIQALSALGGEIHKILEKL GYSGNDMIAILESRIKTKITHVDLPG KFIILSVSYPTLSEVKGIVVHRLEAVSY NIGSQEWYTTVPYVATNGYLISNFD ESSCVFVSESAICSQNSLYPMSPLLQQ CIRGDTSSCARTLVSGTMGNKFILSKG NIVANCASILCKCYSTSTINQSPDKLL TFIASDTCPLVEIDGVTIQVGSRQYPD MVYESKVALGPAISLERLDVGTNLGN ALKKLDDAKVLIDSSNQILETVRRSSF NFGSLLSVPILSCTALALLLICCKRR YQQTHKQNTKVDPTFKPDLTGTSRSY VRSL	46	7
AJ849636	5526-7166	gb:AJ849636:5526-7166 Organism:Peste-des-petits-ruminants virus Strain Name:Turkey 2000 Protein Name:fusion proteinGene	MTRVAITLFLFPNAVACQIHWGNL SKIGIVGTGSASYKVMTRPSHQTLVIK LMPNITAI DNCTKSEIAEYKRLITVLK PVEDALSVITKNVRPIQTLTPGRRTRR FAGAVLAGVALGVATAAQITAGVAL HQSLMNSQAIESLKTSLKSNQAIEEIR LANKETILAVQGVQDYINNELVPSVH RMSCELVGHKLGLKLLRYYTEILSIFG PSLRDPIAAEISIQALSALGGDINRIL DKLGYSGGDFLAILESKGIKARVTYV DTRDYFIILSIAYPTLSEIKGVIVHKIEA ITYNIGAQEWYTTIPKYVATQGYLISN FDETS CVFTP DGTVC SQNALYPMSPLL QECFQGSTKSCARTLVSGTISNRFILSK GNLIANCASVLCKCYTTETVISQDPDK LLTVVASDKCPVVEVDGVTIQVGSRE YPDSVYLHKIDLGAISLEKLDVGTNL	34	8

		ne Symbol:F	GNAVTRLENAKELLDASDQILKTVKG VPFGGNMYIALAACIGVSLGLVTLICC CKGRCKNKEVPISKINPGLKPDLTGTS KSYVRSL		
AF01714 9	6618-8258	gb:AF017 149 Organ ism:Hendr a virus Strai n Name:UN KNOWN- AF017149 Protein Name:fusi on Gene Symbol:F	MATQEVRLKCLLCGIIVLVLSLEGLGI LHYEKLKSKIGLVKGITRKYKIKSNPLT KDIVIKMIPNVSNVSKCTGTVMENYK SRLTGILSPIKGAIELYNNNTHDLVGD VKLAGVVMAGIAIGIATAAQITAGVA LYEAMKNADNINKLKSSIESTNEAVV KLQETAECTVYVLTALQDYINTNLVP TIDQISCKQTEALDLALSKYLSDLLF VFGPNLQDPVSNSTIQAISQAFGGN YETLLRTLGYATEDFDDLLESDSIAGQ IVYVDLSSYYIIVRVYFPILTEIQQAYV QELLPVSFNNDNSEWISIVPNFVLIRNT LISNIEVKYCLITKKSVCINQDYATPM TASVRECLTGSTDKCPRELVSSSHVPR FALSGGVLFANCISVTCQCQTGRAIS QSGEQTLLMIDNTTCTTVVLGNIIISLG KYLGSINYNSESIAGVPPVYTDKVDIS SQISSMNQSLQQSKDYIKEAQKILDTV NPSLISMLSMILYVLSIAALCIGLITFIS FVIVEKKRGNYSLDDRQVRPVSNGD LYYIGT	29	9
AB00579 5	4866-6563	gb:AB005 795:4866- 6563 Orga nism:Send ai virus Strai n Name:Ohi tal Protein Name:fusi on protein Ge ne Symbol:F	MATYIQRVQCISALLSVVLTTLVSCQI PRDRLSNIGVIVDEGKSLKIAGSHESR YIVLSLVPGIDLENGCGTAQVIQYKSL LNRLLIPLRDALDLQEALITVTNDTMT GADVPSRFFGAVIGTIALGVATSAQI TAGIALAEAREAKRDIALIKESMTKTH KSIELLQNAVGEQILALKTLQDFVNDE IKPAISELGCETAALRLGIKLTQHYSSEL LTAFGSNFGTIGEKSLTLQALSSLYSA NITEIMTTIRTGQSNIDYVYIEQIKGT VIDVDLERYMVTLSVKIPILSEVPGVLI HKASSISYNIDGEEWYVTVPSHILSRA SFLGGANIADCVESRLTYICPRDPAQLI PDSQQKCILGDTTRCPVTKVVDNIIPK FAFVNGGVVANCIASTCTCGTGRRPIS QDRSKGVVFLTHDNCGLIGVNGIELY ANRKGHDATWGVQNLTVGPAIAIRPV DISLNLAAATDFLQDSRAELEKARKIL SEVGRWYNSGATLTIIVVMIVVLVVII VIVIVLYRLRRSMLMSNPAGRISRDTY TLEPKIRHMYTNGGFDAMTEKR	23	10
AF45710 2	5088-6755	gb:AF457 102 Organ ism:Huma n parainflue nza virus 1 strain Washingto n/1964 Str ain	MQKSEILFLVYSSLLSSSLCQIPVEKL SNVGVIINEGKLLKIAGSYESRYIVLSL VPSIDLQDGC GTTQIIQYKNLLNRLIP LKDALDLQESLITITNDTTVTNDNPQT RFFGAVIGTIALGVATAAQITAGIALA EAREARKDIALIKDSIVKTHNSVELIQ RGIGEQUIALKTLQDFVNDEIRPAIGEL RCETTALKLGIKLTQHYSSELATAFSSN LGTIGEKSLTLQALSSLYSANITEILST TKKDKSDIYDIYTEQVKGTVIDVDLE	21	11

		Name: Washington 1964 Protein Name: F glycoprotein Gene Symbol: F	KYMTVLLVKIPILSEIPGVLIYRASSISY NIEGEEWHVAIPNYIINKASSLGGADV TNCIESKLAYICPRDPTQLIPDNQQKCI LGDVSKCPVTKVINNLVPKFAFINGG VVANCIASCTCTCGTNRIPVNQDRSRG VTFLTYTNCGLIGINGIELYANKRGRD TTWGNQIIKVGPAVSIRPVDISLNLAS ATNFLEESKTELMKARAIISAVGGWH NTESTQIIMIIIVCIIIIICGILYYLYRVR RLLVMINSTHNSPVNAYTLESRMNP YMGNNNSN		
AB910309	4951-6582	gb:AB910309:4951-6582 Organism: Felin e morbillivir us Strain Name: SS1 Protein Name: fusi on protein Ge ne Symbol: F	MGKIRVIIISSLLLSNITTAQVGWDNLT SIGVISTKQYDYKITTLNQLMVIKM VPNISSINCKPELMKYRELVLGVIRP INESLELMNSYINMRAGSERFIGAVIA GVALGVATAAQITSGIALHNSIMNKR QIQELRKALSTTNKAIDEIRIAGERTLI AVQGVQDYINNIIPMQDKLQCDILSS QLAIALLRYYTNILTVFGPSIRDPVTSII SIQALSQAFNGNLQALLDGLGYTGRD LRDLLESRSITGQIIHADMTDLFLVLRI NYPSITEMQGVTIYELNSITYHIGPEE WYTIMPNFIAVQGFLTSNFDERKCSIT KSSILCQQNSIYPMSTEMQRCIKGEIRF CPRSKAVGTLVNRFILTKGNLMANCL GVICRCYSSGQIITQDPSKLITISQEEC KEVGVDGIRIMVGPRKLPDVIFNARLE VGVPISLSKLDVGTDLAIASAKLNNSK ALLEQSDKILDSMSKLDINSRITGLIL AIMAIFITVTIIWIIYKRCRNKDNKFST SIEPLYIPPSYNPSHSVVKSI	12	12
KT071755	4310-6070	gb:KT071755:4310-6070 Organism: Avian paramyxovirus 2 Strain Name: AP MV-2 Procard uelis nipalensis/ China/Suiling/53/2013 Protein Name: fusi on protein Ge ne Symbol: F	MIAALFISLFATCGALDNSVLAPVGIA SAQEWQLAAYTNLTSGTIAVRFPVL PGNLSTCAQATLAEYNKTVTNILGPL KENLETLLSEPTKTAARFVGAIIGTVA LGVATSAQITAVALNQAQENARNIW RLKESIRKTNEAVLELKDGLASTAIAL DKVQKFINEDIIPQIKEIDCQVVANKL GVYLSLYLTELTTFGAQITNPALTPLS YQALYNLCGGDMGKLTTELIGVKAKDI NSLYEANLITGQVIGYDSESQIILIQVS YPSVSEVTGVRATELVTVSVTPKGE GRAIAPKYVAQSRVVTEELDTSTCRFS KTLTYCRSIIITRPLPLIANCLNGLYQD CQYTTEIGALSSRFITVNGGIIANCRAT ICKCVNPPKIIVQSDASSLTVIDSAICK DVVLDNVQLRLEGKLSAQYFTNITIDL SQITTSGLDISSEIGSINNTVNKVEELI AESNAWLQAVNPHLVNNTSIIIVLCVL AAIFVWLVALTGCLAYYIKKSSATR MVGIGSSPAGNPYVAQSATKM	12	13
AY029299	4598-6265	gb:AY029299 Organism: Avian paramyxovirus	MGARLGPLAMAPGRYVIIFNILLHRV VSLDNSRLLQQGIMSATEREIKVYTNS ITGSIAVRLIPNLPQEVLKCSAGQIKSY NDTLNRIFTPIKANLERLLATPSMLED	11	14

		virus 6 Strain Name:AP MV- 6/duck/Tai wan/Y1/9 8 Protein Name:fusi on protein Ge ne Symbol:F	NQNPAPEPRLIGAIIGTAALGLATAAQ VTAALALNQAQDNAKAILNLKESITK TNEAVLELKDATGQIAIALDKTQRFIN DNILPAINNLTCEVAGAKVGVELSLYL TELSTVFGSQITNPALSTLSIQALMSLC GNDFNYLLNLMGAKHSDLGALYEAN LINGRIIYDQASQIMVIQSVSPSSIS GLRLTELFTLSIETPVGEGKAVVPQFV VESGQLLEEIDTQACTLTDTTAYCTIV RTKPLPELVAQCLRGDESRCQYTTGIG MLESRFGVFDGLVIANCKATICRCLAP EMIITQNKGLPLTVISQETCKRILIDGV TLQIEAQVSGSYSRNITVGNSQIAPSGP LDISSELGKVNQSLSNVEDLIDQSNQL LNRVNPNIWNNTAHVTIVLLVLLVLW CLALTISILYVSKHAVRMIKTVPNPYV MQAKSPGSATQF		
AY14176 0	5028-6665	gb:AY141 760 Organ ism:Fer- de-Lance paramyxo virus Strai n Name:AT CC VR- 895 Protei n Name:fusi on protein Gene Symbol:F	MTRITILQIILTLTLPVMCQVSFDNLEQ VGVMFDPKPKFLKITGPASTATMIKLIP TLGTMESCGTSAVNEYKKTLDITLVP LRDTINKLSTDITVVEGTSNISNKREK RFVGIAIAVGAVALATSAQITAGIALS NTIKNAEAIESIKSSIQASNQAIQKVID AQGRTVTVINGIQDHINSVINPALNQL GCDVAKNLTLAISLTQYFSKLSLLFGPN LRNPVEQPLSVQAIAGLMDGDINAVV SQLGYTQSDLLDLLSTESIVGTVTAID MVNYMIQIEMSFPQYITIPDTKVLEGH KITFNDKGSEWQTQVPSTIAVRDILIA GVDPDGCSITSTSYICKNDPTYAMSEV LTNCFRGNTQECPRARITSTFATRFAT ARSTVIANCVA AVCLCGDPGIPVVQK AEVTLTAMTLDQCSLITVDGLQIKPSK SIANVTANFGNITLGPVVSVGDLDLA ELTKVQSDLKEAQDKLDESNAIQLGI NNKILTAPTSIALIVVSVVILLIIGMIS WLVLWTKAVRRSNTSRERVTPSAYN NLGFIK	8	15
EU87797 6	4330-6410	gb:EU877 976:4330- 6410 Orga nism:Avia n paramyxo virus 4 Strain Name:AP MV- 4/KR/YJ/0 6 Protein Name:fusi on protein Ge ne Symbol:F	MRLSRTILTLILGTLTGYLMGAHSTNV NEGPKSEGIRGDLIPGAGIFVTQVRQL QIYQQSGYHDLVIRLLPLLPAELNDCQ REVVT EYNNTVS QLLQPIKTNLDTLL ADGGTRDADIQPRFIGAIIATGALAVA TVAEVTAAQALSQSKTNAQNILKLRD SIQATNQAVFEISQGLEATATVLSKLQ TELNENIIPSLNNLSAAMGNRLGVSL SLYLTLMTTLFGDQITNPVLTPISTL SAMAGGHIGPVMKILAGSVTSQ LGA EQLIASGLIQSQVVGYSQYQLLVIRV NLVRIQEVQNTRVVSRLTAVNRDGG LYRAQVPPEVVERS G I A E R F Y A D D C V LTTTDYICSSIRSSRLNPELVKCLSGAL DSCTFERESALLSTPFFVYNKAVVAN CKAATCRCNKPPSIIAQYSASALVTITT DTCADLEIEGYRFNIQTESNSWVAPNF TVSTSQIVSVDPIDISSDIKINSSIEAA	8	16

			REQLELSNQILSRINPRIVNDESLIAIIV TIVVLSLLVIGLIVVLGVMYKNLKKV QRAQAAMMMQMQSSSQPVTTKLGTP F		
AB17653 1	4793-6448	gb:AB176 531:4793- 6448 Orga nism:Hum an parainflue nza virus 2 Strain Name:Nis hiolProtein Name:fusi on protein Ge ne Symbol:F	MHHLHPMIVCIFVMYTGIVGSDAIG DQLLNIGVIOQSKIRSLMYTDDGGASFI VVKLLPNLPPSNGTCNITSLDAYNVTL FKLLTPLIENLSKISTVTDTKTRQKRFA GVVVGLAALGVATAAQITAATAVAIVK ANANAAAINNLASSIQSTNKAVSDVID ASRTIATAVQAIQDRINGAIVNGITSAS CRAHDALIGSILNLYLTELTTFIFHNQIT NPALTPLSIQALRILLGSTLPVIESKLN TNFNTAELLSSGLLTGQIISISPMYMQ MLIQINVPTFIMQPGAKVIDLIAISANH KLQEVVVQVPRILEYANELQNYPAN DCVVTNPNSVFCRYNEGSPICESQYQCL RGNLNSCTFTPIIGNFLKRFAFANGVL YANCKSLLCRCADPPHVVSQDDTQGI SIIDIKRCSEMMLDTFSFRITSTFNATY VTDFSMINANIVHLSPLDLSNQINSINK SLKSAEDWIADSNFFANQARTAKTLY SLSAIALILSVITLVVVGLLIAIYIKLVS QIHQFRSLAATTMFHRENPAFFSKNN HGNIYGIS	7	17
BK00591 8	4677-6302	gb:BK005 918 Organ ism:Porcin e rubulaviru s Strain Name:UN KNOWN- BK00591 8 Protein Name:fusi on protein Ge ne Symbol:F	MPQQQVAHTCVMLWGIISTVSGINTE ALSQYGVVVVTNVRQLTYTQAGSTY LAVRLLPSLASPDQSCALHSIINYNAT LQAILSPIAENLNLISTALREQHRKKRF AGVAIGLTALGVATAAQATAAVALV RANKNAEKVEQLSQALGETNAAISDL IDATKNLGFVQAIQNQINTAILPQIH NLSCQVIDAQLGNILSLYTELTTFVFQ PQLTNPALSPLTIQALRAVLGTTLPAL LSEKLKSNIPGLDLMSSGLLKQQLVGL NLQNMLMIIELYIPTLSTHSTAKVLDL VTISSHVNGREVEIQVPRNVLELGSEV LGYGGSECALTMSHILCPFNDARVLS TDMKYCLQGNITHCIFSPVVGSLRRF ALVNGVVIANCADMSCVCDFPQEIHY QNFQEPTTVIDIKKCGKVQLDTLTFTIS TFANRTYGPPAYVPPDNIIQSEPLDISG NLIAVNNSLSSALNHLATSEILRNEQI WTSSLGISTIVALVIIGILIICLVVTWAA LWALLKEVRGLNSAVNSQLSSYVMG DKFIRY	7	18
KC23706 3	4530-6185	gb:KC237 063:4530- 6185 Orga nism:Parai nfluenza virus 5 Strain Name:08- 1990 Prote in	MGTRIQFLMVSCLLAGTGSLDPAALM QIGVIPTNVRQLMYYTEASSAFIVVKL MPTIDSPISGCNITSISSYNATMTKLLQ PIGENLETIRYQLIPTRRRRRFVGVVIG LAALGVATAAQVTAVALVKANKN AAAILNLKNAIQKTNAAVADVQAT QSLGTAVQAVQDHINSVVSAPITAN CKAQDAIIGSILNLYLTELTTFIFHNQIT NPALSPITIQALRILLGSTLPVVRKSF NTQISAAELLSSGLLTGQIVGLDLTYM	7	19

		Name:fusion protein Gene Symbol:Fl Segment:4	QMVIKIELPTLTVQPATQIIDLVTISAFI NNREVMAQLPTRIIVTGSLIQAYPASQ CTITPNTVYCRYNDAQVLSDDTMACL QGNLTRCTFSPVVGSLTRFVLFDGIV YANCRSMLCKCMQPAAVILQPSSSPV TVIDMHKCVSLQLDNLRFTITQLANIT YNSTIKLETSQILPIDPLDISQNLA AVN KSLSDALQH LAQSDTYLSAITSATTTS VLSIIAICLGSGLILILISVVVWKLTI VAANRRNMENFVYHNSAFHHSRSDL 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 VRNTNEAVVSLTNGMSVLAKVDDDL KNFISKELLPKINRVSCDVHDITAVIRF QQLNKRLLLEVSRFSSNAGLTHTVSSF MLTDRELTSIVGGMAVSAGQKEIMLS SKAIMRRNGLAILSSVNADTLVYVIQL PLFGVMDTDCWVIRSSIDCHNIADKY ACLARADNGWYCHNAGSLSYFPSPT DCEIHNGYAFCDTLKSLTVPVTSRECN SNMYTTNYDCKISTSKTYVSTAVLTT MGCLVSCYGHNSCTVINNDKGIIRTL DGCHYISNKGVD RVQVGNTVYYLSK EVGKSIVVRGEPLVLKYDPLSFPDDKF DVAIRDVEHSINQTRTFLKASDQLDL SENRENKNLNKSYILTTLLFVVMILHIM AVIGFILYKVLKMIRDNKLKSKSTPGL TVLS	6	20
AB543336	5174-6805	gb:AB543336:5174-6805 Organism:Human parainfluenza virus 4a Strain Name:M-25 Protein Name:fusion protein Gene Symbol:F	MGVKGLSLIMIGLLISPITNLDITHLMN LGTVPPTAIRSLVYYTYTKPSYLTVDLI PNLKNLDQNCNYSSLNYYNKTALSLI QPIADNINRLTKPITSSEIQSRFFGAVIG TIALGVATAAQVTAAIGLAKAQENAK LILTLKKAATETNEAVRDLANSNKIV VKMISAIQNQINTIIQPAIDQINCQIKDL QVANILNLYLTEITTVFHNQLTNPAL SISIQALKSLLGPTLPEVLSKLDLNNIS AASVMASGLIKGQIIAVDIPTMTLVLM VQIPSISPLRQAKIIDLTSITIHTNSQEV QAVVPARFLEIGSEILGFDGSVCQITK DTIFCPYNDA YELPIQKRCLOQGQTRD CVFTPVAGTFPRRFLT TYGTIVANCRD LVCSCLRPPQIIYQPDENPVTIIDKDLC TTLTLD SITIEIQKSINSTFRREVVEST QVRSLTPLDLSTD LNQYNQLLKS AED HIQRSTDY LNSINPSIVNNNAIILILCI LLILTVTICHIWLKYLTK EVKNVARNQ RLNRDADLFYKIPSQIPVPR	5	21
AF298895	4834-6450	gb:AF298895 Organism:Tioman	MRIALTAVIVSIHFDLAFPMNKNLSLS VGLVHKS VKNLYFY SQGSPSYIVVKL VPTLGNVPGNCTLN SLVRYKSTVSSL LSPLAENLEYLQKTLTVSRGGRRRRF	5	22

		virus Strain Name:UNKNOWN- AF298895 Protein Name:fusion protein Gene Symbol:F	AGVAIGLAALGVAAAAQATAAVALV EARQNAAQIQSLSEAIQNTNLAVNEL KTAIGASATAIQAIQTQINEVINPAINR LSCEILDAQLASMLNLYLIHLTTVFQN QLTNPALTPLSIQSLQSLQGTSSVLTN ITSSSKLALNDALVTGLITGQVVGLN MTSLQIVIAAYVPSVAKLSNAVVHNF RITTSVNGTEVIIQSPTIMEQNEVMYD LKTGHCTESDLNIYCPYVDAQLLSPG MTNCINGRLNDCTFSKVVGSPTRFA AVEGAILANCKYLQCNCLTPPYIITPL NGEMISMIDLSKCQRLDLGTIVFDINN PVNVTFNGNYRADVGQMIVTNPLDIS AELNQINTSLSNAQGFLSKSDAWLHV SQWVTNSGTIFILHGLIVGIVYMIINT YVVVQIIEINRMRTSDRAHLLKGSIS SIST		
FJ215863	4499-6130	gb:FJ215863:4499-6130 Organism:Avian paramyxovirus 8 Strain Name:goose/Delaware/1053/76 Protein Name:fusion protein Gene Symbol:F	MGQISVYLINSVLLLLVYPVNSIDNTLI APIGVASANEWQLAAYTTSLSGTIAV RFLPVLDPDNMTTCLRETITTYNNTVN NILGPLKSNLDALLSSETYPQTRLIGA VIGSIALGVATSAQITA AVALKQAQD NARNILALKEALSKTNEAVKELSSGL QQTALALGKIQSFVNEEILPSINQLSCE VTANKLGVYLSLYLTELTTIFGAQLTN PALTSLSYQALYNLCGGNMAMLTQKI GIKQQDVNSLYEAGLITGQVIGYDSQ YQLLVIQVNYPSISEVTGVRATELVTV SVTTDKGEGKAIVPQFVAESRVTIEEL DVASCKFSSTTLYCRQVNTRALPLV ASCLRGNYDDCQYTTEIGALSSRYITL DGGVLVNCKSIVCRCLNPSKIISQNTN AAVTYVDATICKTIQLDDIQLQLEGSL SSVYARNISIEISQVTTSGSLDISSEIGNI NNTVNRVEDLIHQSEEWLAKVNPHIV NNTTLIVLCVLSALAVIWLAVLTAIHYY LRTKLKTISALAVTNTIQSNPYVNQTK RESKF	5	23
JN689227	4689-6521	gb:JN689227:4689-6521 Organism:Tailor virus Strain Name:TL8K Protein Name:fusion protein Gene Symbol:F	MKLSVVYTTLLVSTFYSDLARSQALAL SELTKIGVIPGRSYDLKISTQASYQYM VVKLIPNLTLGNCTNGTIEAYKKML NRLLSPIDAALRKMMDAVNDKPPEV GNVKFWGAVIGGVALGVATSAQITA GVALHNSIQNANAILALKDSIRQSNKA IQELQTAMSTTVVVLNALQDQINNQL VPAINSLGCQVVANTLGLKLNQYFSEI SLVFGPNLRDPTSETLSIQALSRAFNG DFDSMLSKLKYDDSDFLDLLESDSIRG RIIDVSLSDYLITIQIEYPALLSIKDAVI QTFNLISYNTRGTEWISIFPKQLLVRG T YISNIDISQCVAATSIICKSDTSTPISSA TWSCATGNITNCARTRVVNAHVPRFA LYGGVVFANCAPVVCKCQDPLY SINQ EPKVTNVMVDVDACKEMYLDGLYIT LGKTQISRAMYAEDVSLGGPISVDPID LGNEINSINSAINRSEEHLNHANELLD	5	24

			KVNPRIVNVKTFGVMIGLLVLVVLWC VITLVWLICLTQKLARTAYAGSMGSR ASTVNSLSGFVG		
JX85740 9	4831-6615	gb:JX857 409:4831- 6615 Orga nism:Porci ne parainflue nza virus 1 Strain Name:S20 6N Protein Name:fusi on protein Ge ne Symbol:F	MQVTTLRPAIILSIALLVGTGQVPRDKL ANLGIHKDSKALKIAGSYENRYIVLSL VPTIDNVNGCGSIQIAKYKEMLERLLI PIKDALDLQESLIVIDNETVNNNYSQP YRFVGAIIGTIALGVATAAQVTAGVA LMEAREAKRDISMLKEAIEKTQNSIEK LQNSAGEQILALKMLQDYVNGEIKPA IEELGCETAALKLGIALTQHYTELNTA FGSNLGSIGESLTLQALSSLYKTNTN ILTATNLGKTDIYDIIYAEQVKGRVID VDLKRYMVTISVKIPILSEIPGVLIYEV SSISYNIDGAEWYAAVPDHILSKSAYI GGADISDCIESRLTYICPDPAQIIADN QQQCFFGHLDKCPITKVIDNLVPKFAF INGGVVANCIASTCTCGEERIQVSQDR NKGVTFLTHNCCGLIGINGIEFHANKK GSDATWNVSPIGVGPVSLRPVDSLQ IVAATNFLNSSRKDLMAKEILNQVG NLKDLTTTIIINIVIIIILICVIGLGILYH QLRSALGMRDKMSVLNNSYSLEPRT AQVQVIKPTSFMG	5	25
AY64031 7	2932-4571	gb:AY640 317:2932- 4571 Orga nism:Avia n metapneu movirus St rain Name:LA H A Protein Name:FlG ene Symbol:F	MDVRICTLLFLISNPSSCIQETYNNEESC STVTRGYKSVLRTGWYTNVFNLEIGN VENTCNDGPSLIDTELVLTKNALREL KTVSADQVAKESRLSSPRRRRFVLGAI ALGVATAAAVTAGVALAKTIRLEGEV KAIKNALRNTNEAVSTLGNGVRVLAT AVNDLKEFISKKLTPAINQNKCNADI KMAISFGQNNRRFLNVVRQFSDSAGI TSAVSLDLMTDDELVRRAINRMPTSSG QISLMLNNRAMVRRKGFGILIGVYDG TVVYMVQLPIFGVIETPCWRVVAAPL CRKRRGNYACILREDQGWYCTNAGS TAYYPNKDDCEVRDDYVFCDTAAGI NVALEVDQCNYNISTSKYPCKVSTGR HPVSMVALTPLGGLVSCYESVSCSIGS NKVGIIKQLGKGCTHIPNNEADTITID NTVYQLSKVVGEQRTIKGAPVVNNFN PILFPVDQFNVALDQVFESIDRSQDLID KNDLLGADAKSKAGIAIAIVVLVILG IFFLLAVIYYCSRVRKTKPKHDYPATT GHSSMAYVS	4	26
KU64651 3	4641-6498	gb:KU646 513:4641- 6498 Orga nism:Avia n paramyxo virus 13 goose/Kaz akhstan/57 51/2013 St rain	MARFSWEIFRLSTILLIAQTCQGSIDGR LTLAAGIVPVGDRPISITSSQTGIIVV KLIPNLPDNKKDCAKQSLQSYNETLS RILTPLATAMSAIRGNSTTQVRENRLV GAIIGSV ALGVATAAQITAATALIQAN QNAANIARLANSIKTNEAVTDLTEG LGTLAGVGKLQDYVNEQFNNTAVAI DCLTLESRLGIQLSLYLTELMGVFGNQ LTSPALTPITIQUALYNLAGGNLALLS RLGASETQLGSLINSGLIKGMPIMYDD ANKLLAVQVELPSIGKLNARSTLLET	4	27

		Name:AP MV- 13/white fronted goose/Nor thern Kazakhsta n/5751/20 13 Protein Name:fusi on protein Ge ne Symbol:F	LAVDTTRGPSSPIIPSAVIEIGGAMEEL DLSPCITTDLDMFCTKIISYPLSQSTLS CLNGNLSDCVFSRSEGLSTPYMTIKG KIVANCKQVICRCMDPPQILSQNYGE ALLLIDENTCRSLELSGVILKLAGTYE SEYTRNLTVDP SQVIITGPLDISAELSK VNQSIDSAKENIAESNKFLSQVNVKLL SSSAMITYIVATVVCLIIAITGCVIGIYT LTKLKSQQKTLLWLGNN AEMHGSR KTSF		
AF326114	4818-6482	gb:AF326114 Organism:Meningle virus Strain Name:UNKNOWN-AF326114 Protein Name:fusi on protein Ge ne Symbol:F	MMPRVLGMIVLYLTHSQILCINRNTL YQIGLIHRSVKKNFYSSQGSYSYIVVK LVPTLAAIPPNCISLQRYKETVTSLV QPISDN LGYLQDKLVTGQSRRRRFA GVAIGLAALGVAAAAQATAAVALVE TRENAGKIQALSESIQNTNQAVHSLKT ALGFSATAIQAIQNQVNEVINPAINKL SCEVLDSQLASMLNLYLIHLTTVFQTO LTNPALTPLSIQALTSVLQGTSGVLMN STNSTLTQPIDLLATGLITGQIISVNMT SLQLIIATFMPSIAELPNAVLHSHFRITT SVNLTEVMIQSPEFIMEQNGVFYDFNT AHCQLGDN NVYCPYIDAARLSSMMT NCINGNLGECVFSRVIGSFPSRFVSLN GAILANCKFMRCNCL SPEKIITPLDGE MISLIDLRVCQKLT LGTITFEISQPVNV SFQGGFVANAGQIIVTNPFDISAELGQI NNSLND AQGFLDQSNNWLK VSGWIN NSGSLFIAGIVVIGLIVLCIVIIIYINVQII REVNRLRSFIYRDYVLDHDKAPYSPES SSPHRKS LKTVS	3	28
GU206351	5441-7468	gb:GU206351:5441-7468 Organism:Avian paramyxovirus 5 Strain Name:budgerigar/Kunitachi/74 Protein Name:fusi on protein Ge ne Symbol:F	MLQLPLTILLSILSAHQSLCLDNSKLIH AGIMSTTEREVNVYAQSITGSIVVRLIP NIPSNHKSCATSQIKLYNDTLTRLLTPI KANLEGLISAVSQDQSQNSGKRKRKF VGAVIGAAALGLATAAQVTATVALN QAQENARNILRLKNSIQKTNEAVMEL KDAVGQTAVAIKDTQAFINNQILPAIS NLSCEVLGNKIGVQLSLYLTELTTVFG NQLTNPALTTL SLQALYNLCGDDFN LINLLNAKNRNLASLYEANLIQGRITQ YDSMNQLLIQVQIPSISTVSGMRVTEL FTLSVDTPIGEGKALVPKYVLSSGRIM EEVDLSSCAITSTS VFCSSHSRPLPLETI NCLNGNV TQCQFTANTGTLESRYAVI GGLVIANCKAIVCRCLNPPGVIAQNLG LPITHSSNTCQRINLEQITLSLGN SILST YSANLSQVEMNLAPSNPLDISVELNR VNTSLSKVESLIKESNSILDSVNPQILN VKTVIILAVIIIGLIVVWC FILTCLIVRGF MLLVKQQKFKGLSVQNNPYVSNNSH	3	29

JQ00177 6	6129-8166	gb:JQ001776:6129-8166 Organism:Cedar virus Strain Name:CG1a Protein Name:fusion glycoprotein Gene Symbol:F	MSNKRTTVLIISYTLFYLNNAIVGFD FDKLNKIGVVQGRVLNYKIKGDPMTK DLVLKFIPNIVNITECVREPLSRYNETV RRLLLPIHNMLGLYLNNNTNAKMTGL MIAGVIMGGIAIGIATAAQITAGFALY EAKKNTENIQKLTDSIMKTQDSIDKLT DSVGTSLILNKLQTYINNQLVPLELL SCRQNKIEFDLMLTKYLVDLMTVIGP NINNPVNKDMTIQSLSLFDGNYDIM MSELGYTPQDFLDLIESKSITGQIIYVD MENLYVVIRTYLPTLIEVPDAQIYEFN KITMSSNGGEYLSTIPNFILIRGNYSN IDVATCYMTKASVICNQDYSLPMSQN LRSCYQGETEYCPVEAVIASHSPRFAL TNGVIFANCINTICRCQDNGKTITQIN QFVSMIDNSTCNDVMVDKFTIKVGKY MGRKDINNINIQIGPQIIIDKVDLSNEIN KMNQSLKDSIFYLREAKRILDSVNISLI SPSVQLFLIISVLSFIILLIIVYLYCKSK HSYKYNKFIDDPDYNDYKRERINGK ASKSNNIYYVGD	3	30
LC16874 9	4869-7235	gb:LC168749:4869-7235 Organism:Rinderpest morbillivirus Strain Name:Lv1 Protein Name:F protein Gene Symbol:F	MGILFAALLAMTNPHLATGQIHWGNL SKIGVVGTSASYKVMTQSSHQSLVI KLMPNVTAIDNCTKTEIMEYKRLGT VLKPIREALNAITKNIKPIQSSTTSRRH KRFAGVVLGAALGVATAAQITAGIA LHQSMMSQAIESLKASLETTNQAIEE IRQAGQEMVLAVQGVQDYINNELVP AMGQLSCEIVGQKLGLKLLRYYTEILS LFGPSLRDPVSAELSIQALSALGGDI NKILEKLGYSGSDLLAILESGKIKAKIT YVDIESYFIVLSIAYPSLSEIKGVIVHRL ESVSYNIGSQEWYTTVPRYVATQGYL ISNFDDTPCAFTPEGTICSQNALYPMS LLQECFRGSTRSCARTLVSGSIGNRFIL SKGNLIANCASILCKCYTTGSIISQDPD KILTYIAADQCPVVEVGGVTIQVGSRE YSDAVYLHEIDLGPPISEKLDVGTNL WNAVTKLEKAKDLLDSSDLILENIK VSVTNTGYILVGVGLIAVVGILIITCCC KKRRSDNKVSTMVLNPGLRPDLTGTS KSYVRSL	2	31
LC18731 0	6250-7860	gb:LC187310:6250-7860 Organism:Avian paramyxovirus 10 Strain Name:rAPMV-10-FI324/Ym HA Protein Name:fusi	MTRTRLLFLLTCYIPGAVSLDNSILAP AGIISASERQIAIYTQTLQGTLALRFIPV LPQNLSSCAKDTLESYNSTVSNLLLPI AENLNALLKDADKPSQRIIGAIIGSVA LGVATTAQVTAALAMTQAQQNARNI WKLKESIKNTNQAVLELKDGLQQSAI ALDKVQSFINSEILPQINQLGCEVAAN KLGIFLSLYLTEITTVFKNQITNPALST LSYQALYNLCGGNMAALTQKIGIKDT EINSLYEAELITGQVIGYDSADQILLIQ VSYPVSRVQGVRAVELLTVSVATPK GEGKAIAPSFIAQSNIIEELDTQPCKF SKTTLYCRQVNTRTLPVRVANCLKGK YNDQCQYTTEIGALASRYVTITNGVVA	2	32

		on protein Gene ne Symbol:F	NCRSIIICRCLDPEGIVAQNSDAAITVID RSTCKLIQLGDITLRLEGKLSYSKNI TIDISQVTTSGSLDISSELGSINNTITKV EDLISKSNWLSKVNPTLISNDTIIALC VIAGIVVIWLVIITILSYIILIKLKNVAL LSTMPKKDLNPNYVNTKF		
NC_0052 83	5277-6935	gb:NC_00 5283:5277 - 6935 Orga nism:Dolp hin morbillivir us Strain Name:UN KNOWN- NC_0052 83 Protein Name:fusi on protein Gene ne Symbol:F	MAASNGGVMYQSFLTIIILVIMTEGQI HWGNLSKIGIVGTGSASYKVMTRPNH QYLVIKLMPNVTMIDNCTRTEVTEYR KLLKTVLEPVKNALTVITKNIKPIQSLT TSRRSKRFAGVVLAVAGVATAAQI TAGVALHQSIMNSQSIDNLRSTSLEKSN QAIEEIRQASQETVLAVQGVQDFINNE LIPSMHQLSCEMLGQKLGLKLLRYYT EILSIFGPSLRDPVSAEISIQALSALGG DINKILEKLGYSADLLAILESRIKA KVTHVDLEGYFIVLSIAYPTLSEVKGV IVHKLEAVSYNLGSQEWYTTLPKYVA TNGYLISNFDSESCAFMSEVTICSQNA LYPMSPLLQQCLRGSTASCARSLVSG TIGNRFILSKGNLIANCASVLCKCYST GTIISQDPDKLLTFVAADKCPLEVDG ITIQVGSREYPDSVYVSRIDLGPASLE KLDVGTNLGSALTCLDNADLLDSSN QILENVRRSSFSGAMYIGILVCAGALV ILCVLVYCCRRHCRKRVQTPPKATPG LKPDLTGTTKSYVRS	2	33
NC_0053 39	5374-7602	gb:NC_00 5339:5374 - 7602 Orga nism:Mos sman virus Strai n Name:UN KNOWN- NC_0053 39 Protein Name:fusi on protein Gene ne Symbol:F	MSNYFPARVHIVSLITAVSCQISFQNL TIGVFKFKEYDYRVSGDYNEQFLAIK MVPNVTGVENCTASLIDEYRHVIYNL LQPINTTLTASTSNVDPYAGNKKFFGA VIAGVALGVATAAQVTAGVALYEAR QNAAAIAEIKESLHYTHKAIESLQISQ KQTVVAIQGIQDQINTNIIPQINALTCEI ANQRLRLMLLQYYTEMLSSFGPIIQDP LSGHITVQALSQAAGGNITGLMREL YSSKDLRYILSVNGISANIADPEIGSI ILRIRYPSMIKIPDVAVMELSYLAYHA AGGDWLTVGPRFILKRGYSLSNLDITS CTIGEDFLLCSKDVSSPMSLATQSCLR GDTQMCSRTAVQDREAPRFLLLQGNL IVNCMSVNCKCEDPEETITQDPAYPL MVLGSDTCKIHIDGIRIKLGKVQLPPI TVLNTLSLGPVVLNPIDVSNQLSLVE TTVKESEDHLKNAIGALRSQSRVGGV GIVAIVGLIIATVSLVVLVISGCCLVKY FSRTATLESSLTIEHGPTLAPKSGPIIP TYINPVYRHD	2	34
NC_0074 54	4635-6384	gb:NC_00 7454:4635 - 6384 Orga nism:J- virus Strai n Name:UN	MKPVALIYLTILAFVTKVRSQALSDL TKIGIIPAKSYELKISTQAAQQLMVIKL IPNVNGLTNCTIPVMDSYKKMLDRIL KPIDDALNHVKNAIQDKQGDGVP RFGWAIIGGVALGVATSAQITAGVAL HNSIQNANAILQLKESIRNSNKAIEELQ AGLQSTVLVINALQDQINSQVPAINT LGCSVIANTLGLRLNQYFSEISLVFGP	2	35

		KNOWN-NC_007454 Protein Name:fusion protein Gene Symbol:F	NLRDPTSQTLISQAIKAFNGDFDSM MKKMHYTDSDFLDLLESDSIRGRIISV SLEDYLIIQIDYPGLTTIPNSVVQTFNL ITYNYKGTEWESIFPRELLIRGSYISNI DISQCVGTSKSMICKSDTSTTISPATW ACATGNLTSCARTRVVNSHSTRFALS GGVLFANCAPIACRCQDPQYSINQEPK TTNVMVTSEDCKELYIDGFYLTGKK MLDRAMYAEDVALGGSVSVDPIDIGN ELNSINESINKSHEYLDKANELLEQVN PNIVNVSSFSFILVISILLIWFIVTLVWL IYLTKHMNFIVGKVMGSRSSSTVNSL 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 QTDSSHCSYHSIENYNLTTLKLLPLQ ENLHQITDSLSSRRRKKRFAGVAVGL AALGVATAAQVTAIAIVVKAKENSA KIAQLTSAISETNRAVQDLIEGSKQLA VAVQAIQDQINNVIQPLTNLSCQVA DAQVGTILNMYLTELTTFVHPQITNSA LTPITIQUALRSLGSLPQVVTSTIKTD VPLQDLLTSGLLKGQIVYLDLQSMIM VVSVSVP TIALHSMKVYTLKAISAH VNNAEVQM QVPSRVMELGSEIMGYD IDQCEETSRYLFCPYNGGSILSATMKM CLNGNISQCVFTPIYGSFLQRFLVDG VIVANCRDMTCACKSPSKIITQPDSLP VTIIDSTSCSNLVLDLTELPIISINNATY RPVQYVGPNQIIFSQPLDLLSQLGKINS SLSDAIEHLAKSDEILEQIQWDSPPQGY TLIALTSVLAFFVVAIVGLLISTRYLIF EIRRINTTLTQQLSSYVLSNKIIQY	2	36
NC_017937	4534-6330	gb:NC_017937:4534-6330 Organism:Nariva virus Strain Name:UN KNOWN-NC_017937 Protein Name:fusion protein Gene Symbol:F	MAEQEKTPLRYKILLIIVINHYNITNV FGQIHLANLSSIGVFVTKTLDYRTTSD PTEQLLVINMLPNISNIQDCAQGVVNE YKHLISSLLTPINDTLDLITSNINPYSGR NKLFGELIAGAALT VATSAQITAGVAL YEARQNAKDIAAIKESLGYAYKAIDK LTTATREITVVINELQDQINNRLIPRIN DLACEVWATRLQAMLLQYYAEIFSVI GPNLQDPLSGKISIQALARAAGGNIKL MVDELNYSQGDL SRLVKVGAIKGQII DADPSLGVVIKMRYPNIIKIPNVAISE LSYVSYSSDGQDWITTPNYIVTRGYS IANIQTSSCSVGDDFVLCDRDMTYPM SQVTQDCLRGNIALCSRMVVRDREAP RYLILQGNMVANCM SITCRCEEPESI YQSPDQPLTLLTRDTCDTHVVDGIRIR LGVRLPTISVINNITLGPIITDPIDVS NQLNAVVS TIDQSAELLHQAQRVLSE RARGARDHILATAAIVICVVLAVLILV LLIGLVYLYRTQNEILVKTTMLEQVPT FAPKSFPMESQIYSGKTNKGYDPAE	2	37
NC_025256	6865-8853	gb:NC_025256:6865	MKKKTDNPTISKRGHNHSRGIKSRL LRETDNYSNGLIVENLVRNCHHPSKN	2	38

		- 8853 Orga nism:Bat Paramyxo virus Eid_hel/G H- M74a/GH A/2009 Str ain Name:Bat PV/Eid_h el/GH- M74a/GH A/2009 Pr otein Name:fusi on protein Ge ne Symbol:F	NLNYTKTQKRDSTIPYRVEERKGHYP KIKHLIDKSYKHIKRGKRRNGHNGNII TIILLILILKTQMSEGAHYETLSKIGLI KGITREYKVKGTPSSKDIVIKLIPNVTG LNKCTNISMENYKEQLDKILIPINNIIE LYANSTKSAPGNARFAGVIIAGVALG VAAAAQITAGIALHEARQNAERINLL KDSISATNNAVAELQEATGGIVNVITG MQDYINTNLVPQIDKLQCSQIKTALDI SLSQYYSEILTVFGPNLQNPVTTSMIS QAISQSFGGNIDLLNLLGYTANDLLD LLESKSITGQITYINLEHYFMVIRVYYP IMTTISNAYVQELIKISFNVDGSEWVS LVPSYILIRNSYLSNIDISECLITKNSVI CRHDFAMPMSYTLKECLTGDTEKCP EAVVTSYVPRFAISGGVIYANCLSTTC QCYQTGKVIAQDGSQTLMMIDNQTC IVRIIEILISTGKYLGSQEYNTMHVSV GNPVFTDKLDITSQISNINQSIEQSKFY LDKSKAILDKINLNLIGSVPIILFIAIL SLILSIITFVIVMIIVRRYNYKTPLINSDP SSRRSTIQDVYIIPNPGEHSIRSAARSID RDRD		
NC_0253 47	4471-6386	gb:NC_02 5347:4471 - 6386 Orga nism:Avia n paramyxo virus 7 Strain Name:AP MV- 7 dove/Te nessee/4/ 75 Protein Name:fusi on protein Ge ne Symbol:F	MRVRPLIHLVLLVLLWLNILPVIGLDN SKIAQAGHISAQEYAVNVYSQSNEAYI ALRTVPYIPPHNLSCFQDLINTYNTTIQ NIFSPIQDQITSITSASTLPSSRFAGLVV GAIALGVATSAQITAAVALTKAQQNA QEIIRLRDSIQNTINAVNDITVGLSSIGV ALSKVQNYLNDVINPALQNLSCQVSA LNLGIQLNLYLTEITTIFGPQITNPSLTP LSIQALYTLAGDNLQMFLTRYGYGET SVSSILESGLISAQIVSFDKQTGAILYV TLPSIATLSGSRVTKLMSVSVQTVGVE GSAIVPSYVIQQTVEEFIPDSCIFTRS DVCYCTQLYSKLLPDSILQCLQGSMAD CQFTRSLGSFANRFMTVAGGVIANCQ TVLCRCYNPVMII PQNNGIAVTLIDGS LCKELELEGIRLT MADPVFASYSRDLII NGNQFAPSDALDISSELGQLNNSISSA TDNLQKAQESLNKSIIPATSSWLIILL FVLVSISLVIGCISYFIYKHSTTNRSRN LSSDIISNPYIQKAN	2	39
NC_0253 48	4790-6570	gb:NC_02 5348:4790 - 6570 Orga nism:Tuh ko virus 2 Strain Name:UN KNOWN- NC_0253 48 Protein Name:fusi on	MAPCVLFLSSLLLISTISPSHGINQPAL RRIGAIVSSVKQLKFYSKTKPNYIIVKL LPTINLSKSNCLTSINRYKESVIEIIP LADNIDNLNQKLLPKNRRKRMAGVAI GLAALGVAAAAQATAAVALVEARKN TQMIQSLADSIQDTNAAVQAVNIGLQ NSAVAIQAIQNQINN VINPALDRLNCE VLDAQIASILNLYLIKSVTIFQNQLTNP ALQQLSIQMLSIVMQDTAKILGNFTIG DKFDQHDLLGSLITGQVVGVNLTNL QLIIAAFIPSIAPLPQAYIIDLISITISVND TEAVIQIPERIMEHGSSYQFGGKQCV YGFSAAYCPFSDAVLMTQDLQLCMK	2	40

		proteinGene Symbol:F	GNIEHCIFSSVLGSFPNRFASVDGVFY ANCKYMSCACSDPLQVIHQDDSVNL MVIDSSVCRSLTLGHVTFPIIAFSNVSY QMKTNISIEQMIVTSPLDLSTELKQINN SVNIANTFLDSSNRALKTSIFGTSSQIIL IVLLIFTCLLILYVIFLTYIHKILIKEVKR LRDGNSTGSKLSFINPDV		
NC_025350	4663-6428	gb:NC_025350:4663-6428 Organism:Tuhoko virus 3 Strain Name:UNKNOWN-NC_025350 Protein Name:fusion proteinGene Symbol:F	MLWLTILIALVGNHESTCMNINFLQSL GQINSQKRFLNFYTQQPPSYMVIRLVP TLQLSANNCTLGSIYRYRNAIKELIQP MDENLRWLSSNLIPQRRGKRFAGVAV GLAALGVAVAAQATAAVALVEARAN AEKIASMSQSIQETNKAVTSLSQAVSA SGIAIQAIQNEINNVIHPILNQVQCDVL DARVGNILNLYLIKVTTFQNLTPA LQRLSTQALSMLMQSTSSYLRLNLSSE SAINADLSMTNLIEAQIVGINMTNLQL VLAVFIPSIARLNGALLYDFISITISSNQ TEVMLQIPHRVLEIGNSLYTFEGTQCE MTKLNAYCLYSDAIPVTESLRDCMNG LFSQCGFVRIIGSFANRFASVNGVIYA NCKHLTCSCLOPDEIITQDTNVPLTIID TKRCTKISLGHLTFTIREYANVTYSLR TEIANSQITVVSPDLSSQLTTINNSLA DATNHIMNSDRILDRLNSGLYSKWVII FLICASIVSLIGLVFLGFLIRGLILELRS KHRSNLNKASTYSIDSSIGLT	2	41
NC_025352	5950-8712	gb:NC_025352:5950-8712 Organism:Mojjang virus Strain Name:Tongguan Protein Name:fusion proteinGene Symbol:F	MALNKNMFSSFLGYLLVYATTVQSS IHYDSLKVGVIKGLTYNYKIKGSPST KLMVVKLIPNIDSVKNCTQKQYDEYK NLVRKALEPVKMAIDTMLNNVKSNG NKYRFAGAIMAGVALGVATAATVTA GIALHRSENAQAIAANMKSAIQNTNE AVKQLQLANKQTLAVIDTIRGEINNNI IPVINQLSCDTIGLSVGIRLTQYYSEIIT AFGPALQNPVNTRITQAISSVFNGNF DELLKIMGYTSGDLYEILHSELIRGNII DVDVDAGYIALEIEFPNLTLVNAV QELMPISYNIDGDEWVTLVPRFVLTRT TLLSNIDTSRCTITDSSVICDNDYALPM SHELIGCLQGDTSKCAREKVVSSYVP KFALSDGLVYANCLNTICRCMDTDTP ISQSLGATVSLLDNKRCSVYQVGDVLI SVGSYLGDGEYNADNVELGPPIVIDKI DIGNQLAGINQTLQEAEDYIEKSEEF KGVNPSIITLGSMMVLYIFMILIAIVSVI ALVLSIKLTVKGNVVRQQFTYTQHVP SMENINYVSH	2	42
NC_025363	4622-6262	gb:NC_025363:4622-6262 Organism:Avian paramyxovirus	MAIPVPSSTALMIFNILVSLAPASALD GRLLLGAGIVPTGDRQNVVYTSSQTGI IALKLLPNLPKDKENCAEVSIRSNET LTRILTPLAQSMIAIRGNSTVSTRGRE PRLVGAIIGGVALGVATAAQITAATAL IQANQNAENIARLAKGLAATNEAVTD LTKGVGSLAIGVGKLQDYVNEQFNRT GEAIECLTIESRVGVQLSLYLTEVIGVF	2	43

		12 Strain Name:Wi geon/Italy/ 3920_1/20 05 Protein Name:fusi on protein Ge ne Symbol:F	GDQITSPALSDISIQALYNLAGGNLNV LLQKMGIEGTQLGSLINSLIKGRPIM YDDGNKILGIQVTLPSVGRINGARATL LEAIAVATPKGNASPLIPRAVISVGS EELDMTPCVLTPTDIFCTRILSYPLSDS LTTCLKGNLSSCVFSRTEGALSTPYVS VHGKIVANCKSVVCRCVEPQQIISQN YGEALSLIDESLCRILELNGVILKMDG QFTSEYTKNITIDPVQVIISGPIDISSELS QVNQSLDSALENIKESNSYLSKVN LISSAMITYIVITVICLILTFVALV LSYTKIRSQQKTLIWMGNNIARSKEG NRF		
NC_0253 73	4617-6582	gb:NC_02 5373:4617 - 6582 Orga nism:Avia n paramyxo virus 3 Strain Name:turk ey/Wiscon sin/68 Prot ein Name:fusi on protein Ge ne Symbol:F	MASPMVPLLIITVVPALISSQSANIDKL IQAGIIMGSGKELHIYQESGSLDLYLR LLPVIPSNLSHCQSEVITQYNSTVTRLL SPIAKNLNHLQPRPSGRLFGAVIGSIA LGVATSAQISAAIALVRAQQNANDIL ALKAAIQSSNEAIKQLTYGQEKQLLAI SKIQKAVNEQVIPALTALDCAVLGNK LAAQLNLYLIEMTTIFGDQINNPVLTPI PLSYLLRLTGSELNDVLLQQTRSSLSLI HLVSKGLLSGQIIGYDPSVQGIIRIGLI RTQRIDRSLVFXPYVLPITISSNIATPIIP DCVVKKGVIIEGMLKSNCIELERDIIC KTINTYQITKETRACLQGNITMCKYQ QSRTQLSTPFITYNGVVIANCDLVSCR CIRPPMIITQVKGYPITINRNLCNELS VDNLILNIETNHNFSNPTIIDSQSRLIA TSPLEIDALIQDAQHHAAAALLKVEES NAHLLRVTGLGSSSWHILILTLVCTI AWLIGLSIYVCRIKNDDSTDKEPTTQS SNRGIGVGSIQYMT	2	44
NC_0253 86	5548-7206	gb:NC_02 5386:5548 - 7206 Orga nism:Sale m virus Strai n Name:UN KNOWN- NC_0253 86 Protein Name:fusi on protein Ge ne Symbol:F	MNPLNQTLIAKVLGFLLSSTFTVGQI GFENLTRIGVHVQVKQYGYKLAHYN HQLLLIRMIPTVNGTHNCTHQTTRYR EMVREIITPIKGALDIMKKA VSPDLVG ARIFGAIVAGAALGIATSAQITAGVAL HRTKLNGQEISKLKEAVSLTNEAVEQ LQYSQGKSILAIQGIQDFINFNVVPLLE EHTCGIAKLHLEMALMEYFQKLILVF GPNLRDPIGSTIGIQALATLQNNMFE VSLRLGYAGDDLEDVLQSN SIRANIE AEPDSGFIVLAIRYPTLTLVEDQVITEL AHITFNDGPQEWVATIPQFVITYRGLV LANIDVSTCTFTERNVICARDQTYPMII DLQLCMRGNIACGRTRVTGSTASRF LLKDGNMYANCIATMCRCMSSSSIIN QEPSHLTTLIVKETCSEVMIDTIRITLG ERKHPPIDYQTTITLGQPIALAPLDVGT ELANAVSYLNKSKVLEHSNEVLSSV STAHTSLTATIVLGIVVGGAILIVVMF LFLEAQVIKVQRAMMLCPITNHGYLP NEDLLTRGHSIPTIG	2	45
NC_0253 90	4805-6460	gb:NC_02 5390:4805	MGYFHLLILTAIAISAHLCYTTTLDG RKLLGAGIVITEEKQVRVYTAAQSGTI	2	46

		- 6460 Organism: Avian paramyxovirus 9 Strain Name: duck/New York/22/1978 Protein Name: fusion protein Gene Symbol: F	VLRSFRVVS LDRYSCMESTIESYNKTV YNILAPLGDAIRRIQASGVSVIREGR IFGAILGGVALGVATAAQITAAIALIQ ANENAKNILRIKDSITKTNEAVRDVTN GVSQTLIAVGKLQDFVNKEFNKTTEAI NCVQAAQQLGVELSLYLTEITTVFGP QITSPALSKLTIQALYNLAGVSLDVLL GRLGADNSQLSSLVSSGLITGQPILYD SESQLALQVSLPSISDLRGVVRATYLDT LAVNTAAGLASAMIPKVVIQSNNIVEE LDTTACIAAEADLYCTRITTFPIASAVS ACILGDVSQCLYSKTNGVLTTPYVAV KGKIVANCKHVTCTRCVDPTSIIISQNYG EAATLIDDQLCKVINLDGVSQISLSTF ESTYVRNVSSISANKVIVSSSIDISNELE NVNSSLSSALEKLDESDAALS KVN VH LTSTSAMATYIVLTIVIALILGFVGLGL GCFAMIKVKSQAKTLLWLGAHADRS YILQSKPAQSST		
NC_025403	4826-6649	gb:NC_025403:4826-6649 Organism: Achimota virus 11 Strain Name: UNKNOWN-NC_025403 Protein Name: fusion protein Gene Symbol: F	MWIMILSLFQIIPGVTPINSKVLTLQLG VITKHTRQLKFYSHSTPSYLVVKLVPT INTESTVCNFTSLSRKDSVRELITPLA KNIDNLNSILTIPKRRKRMAGVVIGLA ALGVAAAAQATAAVALIEAKKNTAQI QALSESIQNTNKA VSSIEKGLSSAAIA VQAIQNQINN VINPAL TALDCGVTD A QLGNILNLYLIKTLTVFQKQITNPALQ PLSIQALNIIMQETSSVLRNFTKTDEIE HTDLLTSGSLITGQVVG VNLTNLQLIIA AFIPSIAPLNQAYILDFIRITVNINNS SES MIQIPERIMEHGISLYQFGGDQCTFSD WSAYCPYSDATLMAPGLQNCFRGQA ADCVFSTVMGSFPNRFVS VQGVFYVN CKFIRCACTQPQRLITQDDSLSLTQIDA KTCRMLTLGFVQFSINEYANVTYSFK NNVTAGQLIMTNPIDLSTEIKQMND S VDEAARYIEKSNAALNKL MYGGRSDI VTTVLLVGFILLV VYVIFVTYILKILM KEVARLRNSNHPDLIKPYNYPM	2	47
NC_025404	4772-6647	gb:NC_025404:4772-6647 Organism: Achimota virus 21 Strain Name: UNKNOWN-NC_025404 Protein Name: fusion protein Gene Symbol: F	MLNSFYQIICLAVCLTTYTVISIDQHNL LKAGVIVKSIKGLNFYSRGQANYIIVK LIPNVNVTDTDCDIGSIKRYNETVYSLI KPLADNIDYLRTQFAPTKRKRKFAGV AIGLTALGVATAAQVTA AVALVKAQ ENARKLDALADSIQATNEAVQDLSTG LQAGAIQAIQSEINHVINPALERLSC EIIDTRVASILNLYLIRLTTVFHRQLVN PALTPLSIQALNHLLQGETEGLVKNES KMTDSKIDLLMSGLITGQVVG VNIKH MQLMIAVFPVPTTAQLPNAYVINLLTIT ANINNSEVLVQLPNQILERSGIIYQFRG KDCVSSPNHMYCPYSDASILSPELQLC LQGRLEMCLFTQVVG SFPTRFASDKGI VYANCRHLQCACSEPEGIIYQDDTSAI TQIDASKCSTLKLDM LTFKLSTYANK TFDASFVSGKDQMLVTNLLDLSAELK	2	48

			TMNASVAHANKLIDKSNLLIQSNALIG HSNTIFIVVIVILAVMVLVLIIVTYIIKVI MVEVSRLKRMNIYSIDK		
NC_0254 10	4958-6751	gb:NC_02 5410:4958 - 6751 Orga nism:Tuho ko virus 1 Strain Name:UN KNOWN- NC_0254 10 Protein Name:fusi on protein Ge ne Symbol:F	MVTHIKPLILLVTVILQISGHIDTTALTS IGAVIASSKEIMYYAQSTPNYIVIKLIP NLPNIPSQC�NFSSIAYYNKTLLDLFTPI SDNINMLHQRLSNTGRNRRFAGVAIG LAALGVATAAQVTAAAFALVEAKSNT AKIAQIGQAIQNTNAAINSLNAGIGGA VTAIQAIQTQINGIITDQINAATCTALD AQIGTLLNMYLLQLTTTFQPQIQNPAL QPLSIQALHRIMQGTSTIVLSNLTDSSK YGLNDALSAGLITGQIVSVDLRLMQIT IAANVPTLSRLENAIAHDIMRITTNVN NTEVIVQLPETIMEHAGRLYQFNKDH CLSSTQRRFCPYSDAKLLTSKISSCLSG IRGDCIFSPVVGNFATRFISVKGVIAN CKFIRCTCLQPEGHISQLDDHTLTVIDL KLCNKLDLGLIQFDLQVLSNISYEMTL NTSQNLILTDPDLSELQTMNQSN NAANFIEKSNSLLNSSTYEFNRSVALL VALILLSLTILYVIVLTCVVKLLVHEVS KNRRHIQDLESHHK	2	49
NC_0282 49	4850-7055	gb:NC_02 8249:4850 - 7055 Orga nism:Phoc ine distemper virus Strai n Name:PD V/Wadden _Sea.NLD /1988 Prot ein Name:fusi on protein Ge ne Symbol:F	MTRVKKLPVPTNPPMHSLDSPFLNP EHATGKISITDDTSSQLTNFLYHKYHK TTINHLSTISGTDPPSAKLNKFGSPILS TYQIRSALWWIAMVILVHCVMGQIH WTNLSTIGIIGTDSSHYKIMTRSSHQY LVLKMPNVSIIDNCTKAELDEYEKLL NSVLEPINQALTLMTKNVKSLSLGS GRRQRRFAGVVIAGAALGVATAAQIT AGVALYQSNLNAQAIQSLRASLEQSN KAIDEVRQASQNIIAVQGVQDYVNN EIVPALQHMSCELIGQRLGLKLLRYYT ELLSVFGPSLRDPVSAEISIQALSYALG GEIHKILEKLGYSNDMV AILETKGIR AKITHVDLSGKFIVLSISYPTLSEVKGV VVHRLEAVSYNIGSQEWYTTVPRYVA TNGYLISNFEDESSCVFVSESAICSQNSL YPMSPILQQCLRGETASCARTLVSGTL GNKFILSKGNIIANCASILCKCHSTSKII NQSPDKLLTFIASDTCSLVEIDGVTIQV GSRQYPDVVYASKVILGPAISLERLDV GTNLGSALKKLNDAKVLISSDQILD VKNSYLSLGTIALPVSIGLGLILLLLIC CCKKRYQHLFSQSTKVAPVFKPDLTG TSKSYVRSL	2	50
NC_0283 62	5217-6842	gb:NC_02 8362:5217 - 6842 Orga nism:Capr ine parainflue nza virus 3 Strain	MIKKIICFSMPILLSFCQVDIILQRVG ILVSKPKSIKISQNFETRYLVNLIPNIE NAQSCGDQKIKYKLLDRLIPLYDG LRLQQDIIVVDNNLKNNTNHRKRFF GEIIGTIALGVATSAQITA AVALVEAK QARSDIERVKNAVRDTNKAVQSIQGS VGNLIVAVKSVQDYVNNEIVPSIKRLG CEAAGLQLGIALTQHYSELTNIFGDNI GTLKEKGIKLQGIASLYHTNITEIFTS	2	51

		Name:JS2013Protein Name:fusion proteinGene Symbol:F	TVDQYDIYDLLFTESIKMRVIDVDLND YSITLQVRLPLLTKISDAQIYNVDSVS YNIGGTEWYIPLPRNIMTKGAFLGGA NLQDCIESFSDYICPSDPGFILNRDIEN CLSGNITQCPKTLVISDIVPRYAFVDG GVIANCLSTTCTCNGIDNRINQAPDQG IKIITYKDCQTIGINGMLFKTNQEGTLA AYTPVDITLNNSVNLDPIDLSIELNRA RSDLAESKEWIKRSEAKLDSVGSWYQ SSTTEIIQIVMIIVLFIINIIVLIVLIKYSRS QNQSMNNHMEPYILTNNKVQ		
AF079780	5919-7580	gb:AF079780 Organism:Tupaia paramyxovirus Strain Name:UNKNOWN-AF079780 Protein Name:fusion proteinGene Symbol:F	MASLLKTICYYLITYAKLEPTPKSQL DLDSLASIGVVDAGKYNYKLMTTGSE KLMVIKLVPNITYATNCNLTAHTAYT KMIERLLTPINQSLYEMRSVITERDGG TIFWGAIAGAAALGVATAAAITAGVAL HRAEQNARNIAALKDALRNSNEAIQH LKDAQGHTVLAIQGLQEQINNNIIPKL KESHCLGVNNQLGLLLNQYYSEILTV FGPNLQNPVSASLTQAIKAFNGDFN SLMTNLNYDPTDLLDILESNSINGRIID VNLNEKYIALSIEIPNFITLDAKIQTFN RITYGYGSNEWLTLPDNILEYGNLISN VDLTSCVKTKSSYICNQDTSYPISSELT RCLRGDTSSCPRTPVVNSRAPTFALSG GHIYANCAKAACRCEKPPMAIVQPAT STLTFLTEKECQEVVIDQINIQLAPNRL NKTIITDGDILGPEVIINPIDVSAELGNI ELEMCKTQKALDRSNKILDSMITEVT PDKLLIAMIVVFGILLWLFGVSYAF KIWSKLHFLDSYVYSLRNPSHRSNG HQNHFSFSTDISG	1	52
EU403085	4664-6585	gb:EU403085:4664-6585 Organism:Avian paramyxovirus 3 Strain Name:APMV3/PKT/Netherlands/449/75 Protein Name:fusion proteinGene Symbol:F	MQPGSALHLPPLYIIIALVSDGTLGQT AKIDRLIQAGIVLGSGKELHISQDSGTL DLFVRLLPVLPNSLSHCQLEAITQYNK TVTRLLAPIGKNLEQVLQARPRGRLF GPIIGSIALGVATSAQITAAIALVRAQQ NANDILALKNALQSSNEAIRQLTYGQ DKQLLAISKIQKAVNEQILPALDQLDC AVLGTKLAVQLNLYLIEMTTIFGEQIN NPVLATIPLSYILRLTGAELENNVLMKQ ARSSSLVQLVSKGLLSGQVIGYDPSV QGLIIRVNL MRTQKIDRALVYQPYVLP ITLNSNIVTPIAPECVIQKGTIEGMSRK DCTELEQDIICRTVTTYTLARDTRLCL QGNISSCRYQSGTQLHTPFITYNGAV IANCDLVSCRCLRPPMIITQVKGYPLTI ITRSVCQELSVDNLVLNIETHHNFSLN PTIIDPLTRVIATTPLEIDSLIQAQDHA NAALAKVEESDKYLRAVTGGNYSNW YIVLVIVLLFGNLGWSLLLTVLLCRSR KQRRYQQDDSVGSEKGVGVGTIQYMS	1	53
KX258200	4443-6068	gb:KX258200:4443-6068 Organism:	MEKGTVLFLAALTLYNVKALDNTKL LGAGIASGKEHELKIQSSVNGYIAVK LIPFLPSTKRECYNEQLKKNYNATINRL	1	54

		nism:Avia n paramyxo virus 14 Strain Name:AP MV14/duc k/Japan/11 OG0352/2 011 Protei n Name:fusi on protein Ge ne Symbol:F	MGPINDNIKLVLSGVKTRTREGKLIGA IIGTAALGLATAAQVTAIALEQAQD NARAILTLKESIRNTNNAVSELKTGLS EVSIALSKTQDYINTQIMPALSNLSCEI VGLKIGIQLSQYLTEVTAVFGNQITNP ALQPLSMQALYQLCGGDFSLLLDKIG ADRNELESLEYEANLVTGRIVQYDTAD QLVIIQVSIPSVSTLSGYRVTELQSI V DMDHGEKGKAVIPRYIVTSGRVIEEMDI SPCVLTATAVYCNRLTTSPLPESVLKC LDGDHSSCTYTSNSGVLETRYIAFDG MLIANCRSIVCKCLDPPYIIPQNKKGKPL TIISKEVCKKVTLDGITLLIDAEFTGEY GLNITIGPDQFAPSGALDISTELGKLNN SINKAEDYIDKSNELLNRVNVNDIVNDT AVIVLCVMSALVVWCIGLTVGLIYV SKNTLRAVAIKGTSIENPYVSSGKHAK NSS		
KY51104 4	4592-6247	gb:KY511 044:4592- 6247 Orga nism:Avia n paramyxo virus UPO216 S train Name:AP MV- 15/WB/Kr /UPO216/ 2014 Prote in Name:fusi on protein Ge ne Symbol:F	MIFTMYHVTVLLLLSLTLPLGIQLAR ASIDGRQLAAAGIVVTGEKAINLYTSS QTGTIVVKLLPNVPQGREACMRDPLT SYNKTLTSLLSPLGEAIRRIHESTTETA GLVQARLVGAIIGSVLGVATSAQITA AAALIQANKNAENILKLKQSIAATNE AVHEVTDGLSQLAVAVGKMQDFINT QFNNTAQEIDCIRISQQLGVNELNLYLT ELTTVFGPQITSPALSPLSIQALYNLAG GNLDVLLSKIGVGNNQLSALISSGLIS GSPILYDSQTQLLGIQVTLPSVSSLNN MRAIFLETLSVSTDKGFAAALIPKVVT TVGTVTEELDTSYCIETDIDLFCRIVT FPMSPGIYACLNGNTSECMYSKTQGA LTPPYMSVKGSIVANCKMTTCRCADP ASIISQNYGEAVSLIDSSVCRVITLDGV TLRLSGSFDSTYQKNITIRDSQVIITGS LDISTELGNVNNNSINNALDKIEESNQIL ESVNVSLTSTNALIVYIICTALALICGIT GLILSCYIMYKMRSQQKTLMWLGNN TLDQMRAQTKM	1	55
NC_0253 60	6104-8123	gb:NC_02 5360:6104 - 8123 Orga nism:Atla ntic salmon paramyxo virus Strai n Name:AS PV/Yrkje3 71/95 Prot ein Name:fusi on protein Ge ne	MDGPKFRFVLLILLTAPARGQVDYDK LLKVGIFEKGTANLKISVSSQQRVMVI KMMPNLGPMNQCGIKEVNLYKESILR LITPISTTLNLIKSEIQVEREVALQPNG TIVRFFGLIVAAGALTLATSAQITAGIA LHNSLENAKAIKGLTDAIKESNLAIQK IQDATAGTVIALNALQDQVNTNIIPAI NTLGCTAAGNTLGIALTRYSELIMIF GPSLGNPVEAPLTIQALAGAFNGDLH GMIREYGYTPSDIEDILRTNSVTGRVI DVDLVGMNIVLEINLPTLYTLRDTKIV NLGKITYNVDGSEWQTLVPEWLAINR TLMGGVDLRCVVSRRDLICKQDPVF SLDTSIISCLNGNTESCPRNRVNSVA PRYAVIRGNILANCISTTCLCGDPGVPI IQKGDNTLTAMSINDCKLVGVDGYVF RPGPKAVNVTFNLPHLNLGPEVNVNP	1	56

		ne Symbol:F	VDISGALGKVEQDLASSRDHLAKSEKI LSGINPNIINTEMVLVAVILSLVCAMV VIGIVCWLSILTKWVRSCRADCRRPN KGPDLGPIMSSQDNLSF		
UniProt ID: Q9IH63		FUS_NIP AV Fusion glycoprote in F0 OS=Nipah virus	MVVILDKRCYCNLLILMISECSVGIL HYEKLKIGLVKGVTRKYKIKSNPLT KDIVIKMIPNVSNMSQCTGSVMENYK TRLNGILTPIKGALEIYKNNTHDLVGD VRLAGVIMAGVAIGIATAAQITAGVA LYEAMKNADNINKLKSSIESTNEAVV KLQETAECTVYVLTALQDYINTNLVP TIDKISCKQTELSLDLALSKYLSDLLFV FGPNLQDPVSNMTIQAISQAFGGNYE TLLRTLGYATEDFDDLLESDSITGQIY VDLSSYYIIVRVYFPILTEIQQAYIQEL LPVSFNNDNSEWISIVPNFILVRNTLIS NIEIGFCLITKRSVICNQDYATPMTNN MRECLTGSTEKCPRELVVSSHVPRFA LSNGVLFANCISVTCQCQTTGRAISQS GEQTLLMIDNTTCPTAVLGNVILSLGK YLGSVNYNSEGIAIGPPVFTDKVDISS QISSMNQSLQSKDYIKEAQRLLDTV NPSLISMLSMILYVLSIASLCIGLITFIS FIIVEKKRNTYSRLEDRRVRPTSSGDL YYIGT		57

In some embodiments, a fusogen described herein comprises an amino acid sequence of Table 2, 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 2. In some embodiments, a nucleic acid sequence described herein encodes an amino acid sequence of Table 2, 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.

In some embodiments, a fusogen described herein comprises an amino acid sequence set forth in any one of SEQ ID NOS: 58-133, 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: 58-133. In some embodiments, a nucleic acid sequence described herein encodes an amino acid sequence set forth in any one of SEQ ID NOS: 58-133, 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.

- 10 Table 2. 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/Homo sapiens/USA/Texas/10506/2014 Protein Name:attachment glycoprotein Gene Symbol:G	MSKTKDQRTAKTLERTWDTLNHLLFISSC LYKLNLSIAQITLSILAMIISTSLIIAIIIFIA SANHKVTLTTAIIQDATNQIKNTTPTYLTQ NPQLGISFSNLSGTTLQSTTILASTTPSAEST PQSTTVKIINTTTTQILPSKPTTKQRQNKPKQ NKPNNDFHFEVENFVPCSICSNPTCWAIC KRIPNKKPGKKTTPKPTKPTLKTTKKDP KPQTTKPKEALTTPKPTGKPTINTTKTNIRTT LLTSNTKGNPEHTSQEETLHSTTSEGYLSP SQVYTTSGQEETLHSTTSEGYLSPSQVYTT SEYLSQSLSSSNTTK	706	58
AB524405	6424-8274	gb:AB524405:6424-8274 Organism:Newcastle disease virus Strain Name:Goose/Alaska/415/91 Protein Name:hemaggl	MERGVSVQVALENDEREAKNTWRLVFRVT VLFLTIVTLAISAAALAFSMNASTPQDLEGI PVAISKVEDKITSALGASQDVMMDRIYKQV ALESPLALLNTESTIMNALTSLSYQINGAA NASGCGAPVPDPDYIGGIGKELIVDDTSDV TSFYPSAFQEHLNFIPAPTTGSGCTRIPSFD MSATHYCYTHNVILSGCRDHSQSHQYLAL GVLRTSATGRVFFSTLRSINLDDTQNRKSC SVSATPLGCDMLCSKVTETEEEDYQSTDP TLMVHGRLGFDGQYHERDLVDVHTLFGDW	418	59

		utin- neuraminidase proteinlGene Symbol:HN	VANYPGVGGGSFINNRVWFPVYGGGLKPGS PTDKRQEGQYAIYKRYNDTCPDDQEYQV RMAKSAYKPNRFGGKRVQQAILSIGVSTT LADDPVLTVTSNTITLMGAEGRVMTVGTS HYLYQRGSSYYSPAILYPLTIANKTATLQD PYKFNAFTRPGSVPCQASARCPNSCVTGV YTDPIVIVFHKNHTRLRGVFGTMLDDEQAR LNPVSAVFDSIARSRVTRVSSSSTKAAYTT STCFKVVKTGKVYCLSIAEISNTLFGEFRIV PLLVEILRDEGRSEARSALTTQGHGPGWND EVVDPIFCAVTNQTDHRQKLEEY AQSWP		
JQ5 828 44	4686- 5636	gb:JQ582844:4 686- 5636lOrganism :Human respiratory syncytial viruslStrain Name:NH1067l Protein Name:receptor- binding glycoproteinlG ene Symbol:G	MSKNKNQRTARTLEKTWDTLNLHIVISSC LYKLNLSIAQIALSVLAMIISTSLIIAIIIFI SANHKVTLTTVTVTQTIKNTHEKNITTYLTQ VSPERVSPSKQPTTTPPIHTNSATISPNTKSE THHTTAQTKGRTTTPQNNKPSTKPRPKN PPKKPKDDYHFEVFNFPVPCICGNNQLCKS ICKTIPNNKPKKPTTKPTNKPPTKTNKR DPKTPAKTLKKETTINPTTKKPTPKTTERD TSTPQSTVLDTTTTSKHTERDSTPQSTVLD TTTSKHTIQQQLHSITPENPTNSTQTPTAS EPSTSNSTQKL	278	60
AB2 544 56	7271- 9136	gb:AB254456: 7271- 9136lOrganism :Measles viruslStrain Name:SSPE- Kobe-1lProtein Name:Hemaggl utinilGene Symbol:H	MSPHRDRINAFYRDNPHPKGSRIVINREHL MIDRPYVLLAVLFVMFLSLIGLLAIAGIRL HRAAIYTAEIHKSLSTNLDVTNSIEHQVKD VLTPLFKIIGDEVGLRTPQRFTDLVKFISDK IKFLNPDREYDFRDLTWCINPPERIKLDYD QYCADVA AEELMNALVNSTLLEARATNQ FLAVSKGNCSGPTTIRGQFSNMSLSLLDLY LSRGYNVSSIVTMTSQGMYGGTYLVGKPN LSSKGSELSQLSMHRVFEVGVIRNPGLGAP VFHMTNYFEQPVSNDFSNCMVALGELRFA ALCHREDSVTVPYQSGSGKGVSFQLVKLG WKSPTDMQSWVPLSTDDPVIDRLYLSSH GVIADNQAKWAVPTTRTDDKLRMETCFQ QACKGKNQALCENPEWAPLKDNRIPSYG VLSVNLSLTVELKIKIASGFGPLITHGSGM DLYKTNHDNVYWLTIPTPMKNLALGVINTL EWIPRFKVSPNLFTVPIKEAGEDCHAPTYL PAEVDGDVVLSSNLVILPGQDLQYVLATY DTSRVEHAVVYVYVSPSRFSYFYFPFRLPI KGVPIELQVECFTWDQKLWCRHFCVLADS ESGGHITHSGMVGVMGVSCVTVTREDGTNR RQGCQ	128	61
AB0 408 74	6614- 8362	gb:AB040874: 6614- 8362lOrganism :Mumps viruslStrain Name:Miyahar alProtein Name:hemaggl utin- neuraminidase	MEPSKLFTMSDNATFAPGPVINAADKKTF RTCFRILVLSVQAVTLILVIVTLGELVRMIN DQGLSNQLSSIADKIRESATMIASAVGVM NQVIHGVTVSLPLQIEGNQNQLLSTLATIC TGKKQVSNCSNIPLVNDLRFINGINKFIE DYATHDFSIGHPLNMPFIPTATSPNGCTRI PSFSLGKTHWCYTHNVINANCKDHTSSNQ YISMGILVQTASGYPMFKTLKIQYLSDGLN RKSCSIATVPDGCAMYCYVSTQLETDDYA GSSPPTQKLTLIFYNDTVTERTISPTGLEGN	87	62

		Gene Symbol:HN	WATLVPGVSGSIYFENKLIFPAYGGVLPNS SLGVKSAREFFRPVNPYNPCSGPQQDLDQ RALRSYFSPSYFSNRRVQSAFLVCAWNQIL VTNCELVVPSNNQTLMGAEGRVLLINNRL LYYQRSTSWWPYELLYEISFTFTNSGQSSV NMSWIPIYSFTRPGSGNCSGENVCPTACVS GVYLDPWPLTPYSHQSGINRNFYFTGALL NSSTTRVNPTLYVSALNNLKVLPYGNQG LFASYTTTTCFQDTGDASVYCVYIMELAS NIVGEFQILPVLTRLTIT		
AB7 361 66	6709- 8427	gb:AB736166: 6709- 8427 Organism :Human respirovirus 3 Strain Name:ZMLS/2 011 Protein Name:hemaggl utinin- neuraminidase Gene Symbol:HN	MEYWKHTNHGKDAGNELETATATHGNR LTNKITYILWTITLVLLSIVFIIVLINSIKSEK AHESLLQDINNEFMVTEKIQVASDNTND LIQSGVNTRLTLIQSHVQNYIPISLTQQISDL RKFISETIRNDNQEVPPQRITHDVGKPLNP DDFWRCTSGLPSLMRTPKIRLMPGPGLLA MPTTVDGCVRTPSLVINDLIYAYTSNLITR GCQDIGKSYQVLQIGIITVNSDLVPDLNPRI SHTFNINDNRKSCSLALLNTDVYQLCSTPK VDERSDYASSGIEDIVLDIVNYDGSISTTRF KNNNISFDQPYAALYPSVGPYIYKKGKIIFL GYGGLEHPINENAICNTTGCPGKTQRDCN QASHSPWFSDRRMVNSIIVVDKGLNSVPK LKVWTISMRQNYWGSEGRLLLLGNKIYY TRSTSWHSLQLGIIDITDYSIRIKWTWH NVLSRPGNNECPWGHSCPDGCITGVYTDA YPLNPTGSIVSSVILDSQKSRVNPVITYSTA TERVNELAIRNKTLASGYTTTSCITHYKNG YCFHIVEINHKSLLNTFQPMFLFKTEIPKSCS	78	63
KJ6 273 96	6166- 6885	gb:KJ627396:6 166- 6885 Organism :Human metapneumovir us Strain Name:HMPV/ Homo sapiens/PER/F LI1305/2010/A Protein Name:attachme nt glycoprotein GI Gene Symbol:G	MEVKVENIRAIDMLKARVKNRVARSKCF KNASLILIGITTLIALNIYLIINYTIQKTTSE SEHHTSSPPTESNKETSTIPIDNPDITPNSQH PTQQSTESLTLYPASSMSPSETEPASTPGIT NRLSLADRSTTQPSESRTKTNSTVHKKNK KNISSTISRTQSPRRTAKAVSRRTALRMSS TGERPTTTSVQSDSSTTAQNHEETGPANPQ ASVSTM	71	64
AB4 750 97	7079- 8902	gb:AB475097: 7079- 8902 Organism :Canine distemper virus Strain Name:M25CR Protein Name:hemaggl utinin Gene Symbol:H	MLSYQDKVGAFYKDNARANSSKLSLVTE EQGRRPPYLLFVLLILLVGILALLAIAAGVR FRQVSTSNVEFGRLKDDLEKSEAVHHQV MDVLTPLFKIIGDEIGLRLPQKLNEIKQFIL QKTNFFNPNNREFDFRDLHWCINPPSKIKVN FTNYCDAIGVRKSIASAANPILLSALSGGR GDIFPPYRCSGATTSGRVFPLSVLSMSLI SKTSEIISMLTAISDGVYGYKTYLLVPDYIER EFDTKIRVFEIGFIKRWLNDMPLLQTTNY MVLPENSKAKVCTIAVGELTLASLCVDES TVLLYHDSNGSQDSILVVTLGIFGATPMNQ VEEVIPVAHPSVERIHITNHRGFIKDSVAT	45	65

			WMVPALVSEQQEGQKNCLESACQRKSYP MCNQTSWEPFGGVQLPSYGRLTLPLDASI DLQLNISFTYGPVILNGDGMYYENPLDLS GWLTIIPKNGTILGLINKASRGDQFTVTPH VLTFAPRESSGNCYLPQTSQIMDKDVLTE SNLVVLPTQNFYVAVATYDISRENHAIVY 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:haemaggl utinin Gene Symbol:H	MSAQRERINAFYKDNPHKNHRVILDRER LVIERPYILLGVLLVMFLSLIGLLAIAGIRL HRATVGTSEIQSRLNTNIELTESIDHQTkd VLTPLFKIIGDEVGIRIPQKFSDLVKFISDKI KFLNPDREYDFRDLRWCMNPPERVKINFD QFCEYKAAVKSIEHIFESPLNKSKKLQSLT LGPGTGCLGRTVTRAHFSELTTLMDLDL EMKHNVSSVFTVVEEGLFGRTYTVWRSD ARDPSTD LGIGHFLRVFEIGLVRDLGLGPP VFHMTNYLTVNMSDDYRRCLLAVGELKL TALCSSSETVTLGERGVPKREPLVVILNL AGPTLGGELYSVLPTSDLMVEKLYLSSHR GIIKDDEANWVVPSTDVVDLQNKGECLVE ACKTRPPSFCNGTGSGPWSEGRIPAYGVIR VSLDLASDPGVVITSVFGPLPHLSGMDLY NNPFSRAVWLAVPPYEQSFLGMINTIGFPN RAEVMPHILTTEIRGPRGRCHVPIELSRRV DDDIKIGSNMVILPTIDLRYITATYDVSRS HAIVYYIYDTGRSSSYFYPVRLNFKGNPLS LRIECFPWRHKVWCYHDCLIYNTITDEEV HTRGLTGIEVTCNPV	34	66
AB0 057 95	6693- 8420	gb:AB005795: 6693- 8420 Organism :Sendai virus Strain Name:Ohita Pr oteins Name:hemaggl utinin- neuraminidase protein Gene Symbol:HN	MDGDRSKRDSYWSTSPGGSTTKLVSDSER SGKVDTWLLILAFTQWALSIAIVICIVIAA RQGYSMERYSMTEALNTSNKEVKESLTS LIRQEVITRAANIQSSVQTGIPVLLNKNSRD VIRLIEKSCNRQELTQLCDSTIAVHHAEGIA PLEPHSFWRCPAGEPYLSSDPEVSLLPGPSL LSGSTTISGCVRLPSLSIGEAIYAYSSNLITQ GCADIGKSYQVLQLGYISLNSDMFPDLNP VVSHTYDINDNRKSCSVVATGTRGYQLCS MPIVDERTDYSSDGEDLVLDILDKGRTK SHRYSNSEIDLHPFSALYPSVSGSIATEGS LIFLGYGGLTTPQLQGDTKCRIQGCQVVSQD TCNEALKITWLGKQVVSVLIQVNDYLSE RPRIRVTTPITQNYLGAEGRLKLGDQVYI YTRSSGWHSQLQIGVLDVSHPLTISWTPHE ALSRPGNEDCNWYNTCPKECISGVYTDAY PLSPDAANVATVTLYANTSRVNPTIMYSN TTNIINMLRIKDVQLEAAYTTTSCITHFGK GYCFHIIENQKSLNTLQPMFLKTSIPKLCK AES	23	67
AF4 571 02	6903- 8630	gb:AF457102 Organism:Hum an parainfluenza virus 1 strain Washington/19	MAEKGKTNSYWSYSTRNDNSTVNTHINTP AGRTHIWLLIATTMHTVLSFIIMILCIDLIK QDTCMKTNIMTVSSMNESAKIIEKETITELIR QEVISRTINIQSSVQSGIPILLNKQSRDLTQL IEKSCNRQELAQICENTIAIHHADGISPLDP HDFWRCPVGEPLLSNNPNISLLPGPSLLSG	21	68

		64 Strain Name:Washing ton 1964 Protein Name:HN glycoprotein G ene Symbol:HN	STTISGCVRLPSLSIGDAIYAYSSNLITQGC ADIGKSYQVLQLGYISLNSDMYPDLNPVIS HTYDINDNRKSCSVIAAGTRGYQLCSLPTV NETTDYSSEGIEDLVFDILDLDLKGKTKSHRY KNEDITFDHPFSAMYPVSGSGIKIENTLIFL GYGGLTTPLOQDTCVINRCTNVNQSVCN DALKITWLKKRQVVNVLRINNYLSDRPKI VVETIPTQNYLGAEGRLCLKGKKIYIYTRS SGWHSNLQIGSLDINNPMTIKWAPHEVLS RPGNQDCNWNRCPRECISGVYTDAYPLS PDVNVVATTTLYANTSRVNPTIMYSNTSEI INMLRLKNVQLEAAAYTTTSCITHFGKGYC FHIVEINQASLNTLQPMFLKTSIPKICKITS		
KJ6 273 97	6146- 6888	gb:KJ627397:6 146- 6888 Organism :Human metapneumovir us Strain Name:HMPV/ Homo sapiens/PER/F PP00098/2010/ B Protein Name:attachme nt glycoprotein G Gene Symbol:G	MEVRVENIRAIDMFKAKMKNRIRSSKCYR NATLILIGLTALSMALNIFLIIDYATLKNMT KVEHCVMPPVEPSKSKSPMTSAADLNTKL NPQQATQLTTEDSTSLAATSENHLHTETTP TSDATISQQATDEHTTLRLPINRQTQTTE KKPTGATTKKDKKEKETTTRTTSTAATQTL NTTNQTSNGREATTSARSRNGATTQNSD QTIQAADPSSKPYHTQNTTTTAHNTDTSSL SS	21	69
AF0 171 49	8913- 1072 7	gb:AF017149 Organism:Hend ra virus Strain Name:UNKNO WN- AF017149 Prot ein Name:glycopro tein Gene Symbol:G	MMADSKLVSLNNNLGKIKDQGGKVIKYN YGTMDIKKINDGLLDSKILGAFNTVIALLG SIIIVMNIMIIQNYTRTTDNQALIKESLQSV QQQIKALTDKIGTEIGPKVSLIDTSSTITIPA NIGLLGSKISQSTSSINENVNDKCKFTLPPL KIHECNISCPNPLPFREYRPISQGVSDLVGL PNQICLQKTTSTILKPRILISYTLPI NTREGVC ITDPLLAVDNGFFAYSHLEKIGSCTRGIK QRIIGVGEVLDRGDKVPSMFMTNVWTPPN PSTIIHCSSTYHEDFYTLCAVSHVGDPI NSTSWTESLSLRLAVRPKSDSGDYNQKYI AITKVERGKYDKVMPYGPSGIKQGDLYF PAVGFLPRTEFQYNDSCPIIHCYKSKAEN CRLSMGVNSKSHYILRSGLLKYNLSLGGDI ILQFIEIADNRLTIGSPSKIYNLSLQPVFYQA SYSWDTMIKLGVDVTDVPLRVQWRNNSV ISRPGQSQCPRFNVCEVCWEGTYNDAFLI DRLNWVSAGVYLSNQTAE NPVFAVFKD NEILYQVPLAEDDTNAQKTITDCFLE NVI WCISLVEIYDTGDSVIRPKLFAVKIPAQCSE S	14	70
AF2 123 02	8943- 1075 1	gb:AF212302 Organism:Nipa h virus Strain Name:UNKNO WN- AF212302 Prot ein	MPAENKKVRFENTTSDKGKIPSKVIKSY GTMDIKKINEGLLDSKILSAFNTVIALLGSI VIIVMNIMIIQNYTRSTDNQA VIKDALQGIQ QQIKGLADKIGTEIGPKVSLIDTSSTITIPAN IGLLGSKISQSTASINENVNEKCKFTLPPLKI HECNISCPNPLPFREYRPQTEGVSNLVGLP NNICLQKTSNQLKPKLISYTL PVVGQSGT	14	71

		Name:attachment glycoproteinGene Symbol:G	CITDPLLAMDEGYFAYSHLERIGSCSRGVS KQRIIGVGEVLDRGDEVPSLFMTNVWTPP NPNTVYHCSAVYNNEFYVLCVSTVGD PILNSTYWSGSLMMTRLAVKPKSNGGGY NQHQLALRSIEKGRYDKVMPYGPSGIKQG DTLYFPAVGFLVRTEFKYNDSPITKCQY SKPENCRLSMGIRPNHYILRSGLLKYNLS DGENPKVVFIEISDQRLSIGSPSKIYDSLQ PVFYQASFSWDTMIKFGDVLTVNPLVVN WRNNTVISRPGQSQCPRFNTCPEICWEGV YNDAFLIDRINWISAGVFLDSNQTAEVNF TVFKDNEILYRAQLASEDTNAQKTITNCF LKNKIWCISLVEIYDTGDNVIRPKLFAVKIP EQCT		
EU4 394 28	6751- 8638	gb:EU439428:6751-8638 Organism:Swine parainfluenza virus 3 Strain Name:92-7783_ISU-92 Protein Name:hemagglutinin-neuraminidase HNI Gene Symbol:HN	MEYWKHTNSTKDTNNELGTTDRHSSKA TNIIMYIFWTTTSTILSVIFIMILINLIQENNH NKLMLQEIKKEFAVIDTKIQKTSDDISTSQ SGINTRLLTIQSHVQNYIPLSLTQQMSDLR KFINDLTTKREHGEVPIQRMTHDSGIEPLN PDKFWRCTSGNPSLTSSPKIRLIPGPGLLAT STTVNGCIRIPSLAINNLIYAYTSNLITQGC QDIGKSYQVLQIGIITNSDLVPLNPRVTH TFNIDNDRKSCSLALLNTDVYQLCSTPKV DERSDYASTGIEDIVLDIVTSNGLIITTRFTN NNITFDKPYAALYPSVGPYIYKDKVIFLG YGGLEHEENGDVICNTTGCPGKTQRDCNQ ASYSPWFSNRRMVNSIIVVDKSIDTTFSLR VWTIPMRQNYWGSEGRLLLLGDRIYIYTR STSWHSLQLGLVIDISDYNINRINWTWHN VLSRPGNDECPWGHSCPDGCITGVYTDAY PLNPSGSVVSSVILDSQKSRENPIITYSTAT NRVNELAIYNRTLPAAYTTTNCITHYDKG YCFHIVEINHRSLNTFQPMFLKTEVPKNCS	14	72
KF5 301 64	6157- 6906	gb:KF530164:6157-6906 Organism:Human metapneumovirus Strain Name:HMPV/AUS/17283278/8/2004/B Protein Name:attachment glycoprotein G Gene Symbol:G	MEVRVENIRAIDMFKAKIKNRIRSSRCYRN ATLILIGLTALSMALNIFLIIDHATLRNMIK TENCANMPSAEPSKKTTPMTSTAGPSTKPN PQQATQWTTENSTSPAATLEGHPYTGTQT TPDTPAPQQTDDKHTALPKSTNEQITQTTT EKKTTTRATTQKREKRKENTNQTSTAATQ TTNTTNQTRNASETITTSBGPRIDTTQSSE QTARATEPGSSPYHARRGAGPR	14	73
AB9 103 09	6960- 8747	gb:AB910309:6960-8747 Organism:Feline morbillivirus Strain Name:SS1 Protein Name:hemaggl	MKNINIKYYKDSNRYLGKILDEHKIVNSQL YLSIKVITHAIIVSLIATIMTIINATSGRTTL NSNTDILLNQRDEIHSIHEMIFDRVYPLITA MSTELGLHIPTLLDELTKAIDQKIKIMNPPV DTVTSDLSCWIKPPNGIIDPKGYCESMELS KTYKLLLDQLDVSRRKSLTINRKNINQCQ LVDDSEIIFATVNIQSTPRFLNFGHTVSNQR ITFGQGTYSSTYILTIQEDGITDVQYRVFEI GYISDQFGVFPSSLIVSRVLPIRMVLMESC	12	74

		utinin protein Gene Symbol:H	TLTSRQGGYFLCMNTLTRSIYDYVNIRDL KSLYITLPHYGKVNITYFNFNGKIRSPHEID KLWLTSDRGQIISGYFAAFVTTIRNYNNY PYKCLNNPCFDNSENCRGWYKNITGTDD VPILAYLLVEMYDEEGPLITLVAIPPYNYT APSHNSLYYDDKINKLIMTTSHIGYIQINEV HEVIVGDNLKAILLNRLSDEHPNLTACRLN QGIKEQYKSDGMIISNSALIDIQERMYITVK AIPVGNYNFTVELHSRNTSYILLPKQFN AKYDKLHLECFNWDKSWWCALIPQFSLS WNESLSVDTAIFNLINCK		
AB7 591 18	7116- 8957	gb:AB759118: 7116- 8957 Organism :Avian paramyxovirus 6 Strain Name:red- necked stint/Japan/8KS 0813/2008 Prot ein Name:hemaggl utinin- neuraminidase Gene Symbol:HN	MASPSELNRSQATLYEGDPNSKRTWRTVY RASTLILDLAILCVSIVAIVRMSTLTPSDVT DSISSITSLSDTYQSVWSDTHQKVNIFKE VGISIPVTLDKMQVEMGTAVNIITDAVRQL QGVNGSAGFSITNSPEYSGGIDALIYPQKSL NGKSLAISDLLEHPSFIPAPTTSHGCTRIPTF HLGYPHWCYSHNTIESGCHDAGESIMYLS MGAVGVGHQGKPVFTTSAAVILDDGKNR KSCSVVANPNGCDVLCSLVKQTEDQDYA DPTPTPMIHGRLHFNGTYTESMLDQSLFTG HWVAQYPAVSGSVSHGRLFFPLYGGISK SSSLFPKLRAHAYFTHNEELECKNLTSKQR EDLFNAYMPGKIAGSLWAQGIVICNLTTL ADCKIAVANTSTMMAAEGRQLVQDK VVLYQRSSSWWPVLIYYDILVSELVNARH LDIVNWVPYPQSKFPRPTWTKGLCEKPSIC PAVCVTGVYQDVWVSVGDFSNETVVIG GYLEAASERKDPWIAAANQYNWLTRRQL FTAQTEAAYSSTTCFRNTHQDKVFCLTIME VTDNLLGDWRIAPLLYEVTVVDRQQSSRK AVAMSEAHRTFRKYYSPENKFTPPQH	11	75
AY1 417 60	6791- 8485	gb:AY141760 Organism:Fer- de-Lance paramyxovirus Strain Name:ATCC VR-895 Protein Name:hemaggl utinin- neuraminidase protein HN Gene Symbol:HN	MDPKSYCNEDLRSDGGEKSPGGDLYKGI ILVSTVISLIIAISLAFIIDNKINIQLDPLRG LEDSYLVPIKDKSEISQDIQEGIFPRLNLIT AATTTTIPRSIAIQTCDLSDLMNRCYPSVV NNDTSCDVLGAIHSNLSQLDPSTYWTG SSGTPTMNQTVKLLPDNSQIPGSTYSTGCV RIPTFSLGSMIYSYSHNVIYEGCNDHSSQ YWQLGYISTSKTGEPLQQVSRTLTLNGL NRKSCSTVAQGRGAYLLCTNVVEDERTD YSTEGIQDLTLDYIDIFGAERSYRYTNNEV DLDRPYAALYPSVSGTVYNDRLFLGYG GLMTPYGDQAMCQAPECTSATQEGCNSN QLIGYFSGRQIVNCIIEITVGTEKPIIRVRTI PNSQVWLGAEGRIQTLGGVLYLYIRSSGW HALAQTGIILTLDPIRISWIVNTGYSRPGNG PCSASSRCPAQCTGVYTDIFPLSQNYGYL ATVTLLSGVDRVNPVISYGTSTGRVADSQ LTSSSQVAAYTTTTCTFNQKGYCYHIEL SPATLGIFQPVLVVTEIPKICS	8	76
EU8 779 76	6248- 8161	gb:EU877976:6 248- 8161 Organism :Avian paramyxovirus	MQGNMEGSRDNLTVDELKTTWRLAYR VVSLLLMVSALIHIVILTRDNSQSIITAINQS SDADSKWQTGIEGKITSIMTDLTDTRNAAL LHIPLQLNTLEANLLSALGGNTGIGPGDLE HCRYPVHDTAYLHGVNRLINQTAADYTAE	8	77

		4 Strain Name: APMV-4/KR/YJ/06 Protein Name: hemagglutinin-neuraminidase Gene Symbol: HN	GPLDHVNFIPAPVTTTGTCTRIPSFVSSSIW CYTHNVIETGCNDHSGSNQYISMGVKRA GNGLPYFSTVVSKYLT DGLNRKSCSVAAG SGHCYLLCSLVSEPEPDDYVSPDPTMRLG VLTWDGSYTEQAVPERIFKNIWSANYPGV GSGAIVGNKVLFPFYGGVRNGSTPEVMNR GRYYYIQDPNDYCPDPLQDQILRAEQSY PTRFGRRMVMQGV LACPVSNNSTIASQCQ SYYFNNSLGFIGAESRIYYLNGNIYLYQRS SSWWPHPQIYLLDSRIASPGTQNIDSGVNL KMLNVTVITRPSSGFCNSQSRCPNDCFLGV YSDIWPLSLTSDSIFAFTMYLQGKTTRIDPA WALFSNHAIGHEARLFNKEVSAAYSTTTC FSDTIQNQVYCLSILEVRSELLGAFKIVPFL YRVL		
AB1 765 31	6821- 8536	gb:AB176531: 6821- 8536 Organism :Human parainfluenza virus 2 Strain Name: NishiolP rotein Name: hemagglutinin- neuraminidase protein Gene Symbol: HN	MEDYSNLSLKSIKRTCRIFRTATILGICTL IVLCSSILHEIHLDVSSGLMDSDDSQGGIIQ PIESLKSILALANQILYNVAIIPLKIDSIEIV IFSALKDMHTGMSNTNCTPGNLLLHDAA YINGINKFLVLKSYNGTPKYGPLLNIPSFIP SATSPNGCTRIPSFSLIKTHWCYTHNVM LG DCLDFTTSNQYLAMGIIQSSAAAFPIFRTM KTIYLSGGINRKSCSVTAIPGGCVLYCYVA TRSEKEDYATTDLAELRLAFYYYNDTFIER VISLPNTTGQWATINPAVSGGIYHLGFILFP VYGGGLISGTPSYNKQSSRYFIPKHPNITCAG NSSEQAAAARSSYVIRYHSNRLIQSAVLIC PLSDMHTARC�LVMFNNSQVMMGAEGR LYVIDNNLYYYQRSSSWWSASLFYRINTD FSKGIPPIEAQWVPSYQVPRPGVMPCNAT SFCPANCITGVYADVWPLNDPEPTSQNAL NPNYRFAGAF LRNESNRTNPTFYTASASA LLNTTGFNNTNHKAAYSSTCFKNTGTQK IYCLIIHEMGSSLLGEFQIIPFLRELIP	7	78
AF0 527 55	6584- 8281	gb:AF052755 Organism: Parai nfluenza virus 5 Strain Name: W3A Pro tein Name: hemagglutinin- neuraminidase protein Gene Symbol: HN	MVAEDAPVRATCRVLFRTTTLIFLCTLLAL SISILYESLITQKQIMSQAGSTGSNSGLGSIT DLLNNILSVANQIIYNSAVALPLQLDTLEST LLTAIKSLQTS DKLEQNCSWSAALINDNRY INGINQFYFSIAEGRNLTLGPLLNMPSTFIPT ATTPEGCTRIPSFSLTKTHWCYTHNVILNG CQDHVSSNQFVSMGIIPTSAGFPFFRTLKT LYLSDGVNRKSCSISTVPGGCM MYCFVST QPERDDYFSAAPPEQRHIMYYNDTIVERII NPPGVLDVWATLNP GTGSGVYYLGWVLF PIYGGVIKGTSLWNNQANKYFIPQMVAAL CSQNQATQVQNAKSSYYSSWFGNRM IQS GILACPLRQDLTNECLVLPFSNDQVLMGA EGRLYMYGDSVYYYQRSNSWWPMTMLY KVTITFTNGQPSAISAQNVPTQQVPRPGTG DCSATNRCPGFCLTGVYADAWLLTNPSST STFGSEATFTG SYLNTATQRINPTMYIANN TQISSQQFGSSGQEAAYGHTTCFRDTGSV MVYCIYIHELSSSLLGQFQIVPFIRQVTL S	7	79
BK0 059 18	6560- 8290	gb:BK005918 Organism: Porci ne	MSQLGTDQIMHLAQPAIARRTWRLCFRIF ALFILIAIVITQIFMLTFDHTLLTTTQFLTSIG NLQSTITSWTPDVQAMLSISNQLIYTTSITL	7	80

		rubulavirus Strain Name:UNKNO WN- BK005918 Protein Name:attachment protein Gene Symbol:HN	PLKISTTEMSILT AIRDHCHCPDCSSACPTR QMLLNDPRYMSGV NQFIGAPTESINITFGP LFGIPSF IPTSTTTQ GCTRIPSFALGPSHCY THNFITAGCADGGHSNQYLAMGTIQSASD GSPLLITARSYYLSDGVNRKSCSIAVVP GG CAMYCYVATRSETDYYAGNSPPQQLLTL VFSNDTIERTIHPTGLANGWVMLVPGVGS GTLYNEYLLFPAYGGMQQILANQSGEINQ FFTPYNATVRCAMAQPQFSQRAAASYYPR YFSNRWIRSAIVACPYRAIYQTQCTLIPLN RMVMMGSEGRIFTLGDRLFYYQRSSSWW PYPLLYQVGLNFLTTPPSVSSMTQVPLEHL ARPGKGGCPGNSHCPATCVTGVYADVWP LTDPRSGVGGTSLVAAGGLDSTSERMAPV NYLAIGESLLSKTYLLSKTQPAAYTTTTCF RDTDTGKIYCITIAELGKVLLGEFQIVPFLR EIKIQSRY		
EU3 384 14	6015- 7913	gb:EU338414:6 015- 7913 Organism :Avian paramyxovirus 2 Strain Name:APMV- 2/Chicken/Calif ornia/Yucaipa/ 56 Protein Name:hemaggl utinin- neuraminidase Gene Symbol:HN	MDFPSRENLAAGDISGRKTWRLFRILTL IGVVCLAINIATIAKLDHLDNMASTWTTT EADRVISSITPLKVPVNQINDMFRIVALDL PLQMTSLQKEITSQVGFLAESINNVLSKNG SAGLVLVNDPEYAGGIAVSLYQGDASAGL NFQPISLIEHPSFVPGPTTAKGCIRIPTFHM PSHCYSHNIIASGCQDASHSSMYISLGV KASQTGSPIFLTASHLVDDNINRKSCSIVA SKYGCILCSIVETENEDYRSDPATSMIIG RLFFNGSYTESKINTGSIFSLFSANYPAVGS GIVVGDEAAFPYGGVKQNTWLFNQLKDF GYFTHNDVYKCNRTDIQQTILDAYRPPKIS GRLWVQGILLCPVSLRPDPGCRLKVFNTS NVMMGAEARLIQVGSTVYLYQRSSSWWV VGLTYKLDVSEITSQTGNLNVDPPIAHTK FPRPSFRRDACARPNICPAVCVSGVYQDIW PISTATNNSNIVWVGQYLEAFYSRKDPRIG IATQYEWKVTNQLFNSNTEGGYSTTTTCFR NTKRDKAYCVVISEYADGVFGSYRIVPQLI EIRTTTGKSE	7	81
KC4 039 73	6234- 6964	gb:KC403973: 6234- 6964 Organism :Human metapneumovirus Strain Name:HMPV/ USA/TN-82- 518/1982/A Pro tein Name:attachment glycoprotein G Gene Symbol:G Seg ment: 8	MEVKVENIRTIDMLKARVKNRVARSKCFK NASLILIGITLSIALNIYLIINYTMQENTSES EHHTSSSPMESSRETPTVPIDNSDTNPSSQY PTQQSTEGSTLYFAASASSPETEPTSTPDTT SRPPFVDTHTPPSASRTKTSPAVHTKNNP RISSRTHSPPWAMTRTVRRTTTLRTSSIRK RSSTASVQPDSSATTHKHEEASPVSPQTS STTRPQRKSMEASTSTTYNQTS	6	82
KF0 152 81	4511- 5844	gb:KF015281:4 511- 5844 Organism :Canine	MRPAEQLIQENYKLTSLSMGRNFEVSGST TNLNFERTQYPDTFRAVVKVNQMCKLIAG VLTSAAVAVCVGVIMYSVFTSNHKANS QNATIRNSTSAPPQPTAGPPTTEQGTTPKFT	6	83

		pneumovirus Strain Name:dog/Bari/100-12/ITA/2012 Protein Name:attachment protein Gene Symbol:G	KPPTKTTHHEITEPAKMVTPSEDPYQCSS NGYLDRLPEDFKLVLDVICKPPGPEHHS TNCYEKREINLGSVCPDLVTMKANMGLN NGGGEEAAPYIEVITLSTYSNKRAMCVHN GCDQGFCCFLSGLSTDQKRAVLELGGQQA IMELHYDSYWKHYWSNSNCVVPRTNCNL TDQTVILFSPFNNKNQSQCTTCADSAGLD NKFYLTCDGLSRNLPLVGLPSLSPQAHKA ALKQSTGTTTAPTETRNPTPAPRRSKPLS RKKRALCGVDSSREPKPTMPYWCPMLQL FPRRSNS		
KF97339	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	MSKTKDQRAAKTLEKTWDTLNHLLFISSC LYKSNLKSIAQITLSILAMTIPTSLIIVATTFI ASANNKVTPTTAIIQDATSQIKNTTPHLLT QNPQPGISFFNLSGTISQTTAILAPTPSVEP ILQSTTVKTKNTTTTQIQPSKLTTKQRQNK PPNKPNDDFHFEVFNFPVCSICSNNPTCWA ICKRIPSKKPGKKTTKPTKKQTIKTTKKD LKPQTTKPKEAPTT	6	84
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	MSNIASSLENIVEQDSRKTWRAIFRWSVL LITGCLALSIVSIVQIGNLKIPSVGDLADE VVTPLKTTLSDTLRNPINQINDIFRIVALDIP LQVTSIQKDLASQFSLMLIDSLNAIKLGNGT NLIPTSDKEYAGGIGNPVFTVDAGGSIGFK QFSLIEHPSFIAGPTTTRGCTRIPTFHMSESH WCYSHNIIAAGCQDASASSMYISMVGLHV SSSGTPIFLTASELIDDGVNRKSCSIVATQ FGCDILCSIVIEKEGDDYWSDTPTPMRHGR FSFNGSFVETELPVSSMFSSFSANYPAVGS GEIVKDRILFPIYGGIKQTSPEFTLVKYGL FVSTPTTVCQSSWTYDQVKAAYRPDYISG RFWAQVILSCALDAVDLSSCIVKIMNSSTV MMAAEGRIKIGIDYFYQYQRSSSWWPLAF VTKLDPQELADTNSIWLNTSIPIQSKFPRP SYSENYCTKPAVCPATCVTGVYSDIWPLT SSSSLPSIIWIGQYLDAPVGRTYPRFGIANQ SHWYLQEDILPTSTASAYSTTTCFKNTARN RVFCVTIAEFADGLFGEYRITPQLYELVRN N	5	85
JX857409	6619-8542	gb:JX857409:6619-8542 Organism:Porcine parainfluenza virus 1 Strain Name:S206N Protein Name:haemagglutinin	MEETKVKTSEYWARSPQIHATNHPNVQN REKIKEILTILSFISSLSLVLVIAVLIMQSLH NGTILRCKDVGLSINKSTYSISNAILDVIK QELITRIINTQSSVQVALPILINKKIQDLSLII EKSSKVHQNSPTCSGVAALTHVEGIKPLDP DDYWRCPSGEPYLEDELTLSPGPSMLAG TSTIDGCVRLPSLAIGKSLYAYSSNLITKGC QDIGKSYQVLQLGIITLNSDLHPDLNPIISH TYDINDNRKSCSVAVSETKGYQLCSMPRV NEKTDYTSDGIEDIVFDVLDLKGSSRSFKF SNNDINFDHPFSALYPSVSGSIIWKNELYF	5	86

		protein Gene Symbol:H	LGYGALTTALQGNTKCNLMGCPGATQDN CNKFISSSWLYSKQMVNVLQVKGYLSSK PSIIVRTIPITENYVGAEGKLVGTRERIYYT RSTGWHNTLQIGVLNINHPITITWTDHRVL SRPGRSPCAWNNKCPRNCTTGVYTDAYPI SPDANYVATVTLLSNSTRNNPTIMYSSSDR VYNMLRLRNTELEAAYTTTSCIVHFDRGY CFHIIENQKELNTLQPMFLKTAIPKACRIS NL		
KF9 082 38	7510- 9249	gb:KF908238:7 510- 9249 Organism :Human parainfluenza virus 4b Strain Name:QLD- 01 Protein Name:hemaggl utinin- neuraminidase protein Gene Symbol:HN	MQDSRGNTQIFSQANS MVKRTWRLLFRIV TLILLISIFVLSLIIVLQSTPGNLQSDVDIIRK ELDELMENFETTSKSLLSVANQITYDVSVL TPIRQEATETNIIAKIKDHCKDRVVKGEST CTLGHKPLHDVSFLNGFNKFYFTYRDNVQ IRLNPLLDYPNFIPTATTPHGCIRIPSFSLSQ THWCYTHNTILRGCEDTASSKQYVSLGTL QTLENGDPYFKVEYSHYLNDRKNRKS CSV VAVLDGCLLYCVIMTKNETENFKDPQLAT QLLTYISYNGTIKERIINPPGSSRDWVHISP GVGSGILYSNYIIFPLYGGLMENS MIYNNQ SGKYFFPNSTKLPCSNKTSEKITGAKDSYTI TYFSKRLIQSAFLICDLRQFLSEDCEILIPSN DHMLVGAEGRLYNIENNIFYYQRGSSWW PYPSLYRIKLNSNKKYPRIIEIKFTKIEIAPRP GNKDCPGNKACPKECITGVYQDIWPLSYP NTAFPHKKRAYYTGFYLNNSLARRNPTFY TADNLDYHQERLGKFNLTAGYSTTTCFK QTTTARLYCLYILEVGDSVIGDFQIFPFLRS IDQAIT	5	87
KT0 717 57	6066- 7962	gb:KT071757:6 066- 7962 Organism :Avian paramyxovirus 2 Strain Name:APMV- 2/Emberiza spodocephala/C hina/Daxing'anl ing/974/2013 P rotein Name:hemaggl utinin- neuraminidase protein Gene Symbol:HN	MDALSRENLTESISQGGRRRTWRMLFRILTL VLTLVCLAINIATIAKLDSIDTSKVQTWTTT ESDRVIGSLTDTLKIPIQVNDMFRIVALDL PLQMTTLQKEIASQVGFLAESINNFLSKNG SAGSVLVNDPEYAGGIGTSLFHGDSASGL DFEAPSLIEHPSFIPGPTTAKGCIRIPTFHMS ASHWCYSHNIIASGCQDAGHSSMYISM GV LKATQAGSPSFLT TASQLVDDKLN RKSCSI ISTTYGCDILCSLVVENEDADYRSDPPTDM ILGRLFFNGTYESKLN TSAIFQLFSANYPA VGSGIVLGDEIAFPVYGGVKQNTWLFNQL KDYGYFAHNNVYKCNNSNIHQTVLNAYR PPKISGRLWSQVVLICPMRLFINTDCRIKVF NTSTVMMGAEARLIQVGSDIYLYQRSSSW WVVGLTYKLDFQELSSKTGNILNNVSPIA HAKFPRPSYSRDACARPNICPAVCVSGVY QDIWPISTAHNLSQVWVGQYLEAFYARK DPWIGIATQYDWKKNVRLFNANTEGGYS TTTCFRNTKRDKAFCVIIEYADGVFGSYR IVPQLIEIRTTSSKKGLPS	5	88
LC0 411 32	6605- 8437	gb:LC041132:6 605- 8437 Organism :Avian paramyxovirus goose/Shimane/ 67/2000 Strain	MQPGISEVSFVNDERSESGTWRLFRILTI VLCLTSIGIGIPALIYSKEAATSGDIDKSLEA VKTGMSTLSSKIDESINTEQKIYRQVILEAP VSQNLMESNILSAITSLSYQIDGTSNSSGCG SPMHDQDFVGGINKEIWTTDNVNLGEITL TPFLEHLNFIPAPTTGNGCTRIPSF DLGLTH WCYTHNVILSGCQDYSSSFQYIALGV LKIS	4	89

		Name:goose/Sh imane/67/2000 Protein Name:hemaggl utinin- neuraminidase Gene Symbol:HN	ATGHVFLSTMRSINLDDERNRKSCSISATSI GCDIICSLVTEREVDYNSPAATPMIHGRL DFSGKYNEVDLNVGQLFGDWSANYPGVG GGSFLNGRVWFPIYGGVKEGTPTFKENDG RYAIYTRYNDTCPDSESEQVSRKSSYRPS YFGGKLVQQAVLSIKIDDTLGLDPVLTISN NSITLMGAESRVLQIEEKLYFYQRGTSWFP SLIMYPLTVDDKMVRFEPTIFDQFTRPGN HPCSADSRCPNACVTGVYTDGYPIVFHNN HSIAAVYGMQLNDVTNRLNPRSAVWYGV SMSNVIRVSSSTTKAAYTTSTCFKVKKTQR VYCLSIGEIGNTLFGEFRIVPLLLEVYSEKG KSLKSSFDGWEDISINNPLRPLDNHRVDPIL ISNYTSSWP		
AF0 929 42	4705- 5478	gb:AF092942 Organism:Bovi ne respiratory syncytial virus Strain Name:ATue51 908 Protein Name:attachme nt glycoprotein G ene Symbol:G	MSNHTHHLKFKTLKRAWKASKYFIVGLSC LYKFNLKSLVQTALTTLAMITLTSLVITAI YISVGNAKAKPTSKPTIQQTQQPQNHTSPF FTEHNYKSTHTSIQSTTLSQLPNTDTRTET YSHSINETQNRKIKSQSTLPATRKPPINPSG SNPPENHQDHNSQTLPLYVPCSTCEGNLA CLSLCQIGPERAPSRAPTITLKKTPKPKTTK KPTKTTHHRTSPEAKLQPKNNTAAPQQGI LSSPEHHTNQSTTQI	3	90
AF3 261 14	6691- 847	gb:AF326114 Organism:Men angle virus Strain Name:UNKNO WN- AF326114 Prot ein Name:attachme nt protein Gene Symbol:HN	MWNSIPQLVSDHEEAKGKFTDIPLQDDTD SQHPSGSKSTCRTLFRTVSIILSLVILVLGVT STMFSKYSGGCATNSQLLGVSNLINQIQK SIDSLISEVNQVSITTAVTLPKIMDFGKSVT DQVTQMIRQCNTVCKGPGQKPGSQNVRI MPSNNLSTFQINMSARGIAYQDVPLTFV RPIKNPQSCSRFSPYSVSFGVHCFANAVTD QTCELNQNTFYRVVLSVSKGNISDPSSLET KAETRTPKGTTPVRTCSIISSVYGCYLLCSK ATVPESEEMKTIGFSQMFILYLSMDSKRIIY DNIVSSTSIAIWGSLYPGEGAGIWHMGQLF FPLWGGIPFLTPLGQKILNSTLDIPEVGSKC KSDLTSNPAKTKDMLFSPYYGENVMVFGF LTCYLLSNVPTNCHADYLNSTVLGFGSKA QFYDYRGIVYMYIQSAGWYPFTQIFRITLQ LKQNRQLAKSIKRIEVTSTTRPGNRECSVL RNCPIYCATGLFQVPWIVNSDAITSKEVDN MVFVQAWAADTFEVRKILSLCSQVSCPI NDLLSKDNSYMRDTTTTYCFPQTPVNILSCT SFVEWGGDSGNPINILEIHYEVIFVAS	3	91
GU2 063 51	7500- 9714	gb:GU206351: 7500- 9714 Organism :Avian paramyxovirus 5 Strain Name:budgerig ar/Kunitachi/74 Protein Name:hemaggl utinin	MDKSYYTEPEDQRGNSRTWRLLFRLIVLT LLCLIACTSVSQLFYPWLPQVLSTLISLNSSI ITSSNGLKKEILNQNIKEDLIYREVAINIPLT LDRVTVEVGTAVNQITDALRQLQSVNGSA AFALSNPDYSGGIEHLVFQRNTLINRSVS VSDLIEHPSFIPTTQHGCTRIPTFHLGTRH WCYSHNIIGQGCADSGASMMYISMALG VSSLGTPTFTTSATSILSDSLNRKSCSIVATT EGCDVLCISIVTQTEDQDYADHTPTPMIHG RLWFNGTYTERSLSQSLFLGTWAAQYPAV GSGIMTPGRVIFPFYGGVIPNSPLFLDLERF	3	92

		neuraminidase protein Gene Symbol:HN	ALFTHNGDLECRNLTQYQKEAIYSAYKPP KIRGSLWAQGFIVCSVGDGMGNC SLKVINT STVMMGAEGRLQLVGDSVMYYQRSSSW WPVGILYRLSLVDIIARDIQVINSEPLPLS KFPRPTWTPGVCQKPNVCPAVCVTGVYQ DLWAISAGETLSEMTFFGGYLEASTQRKD PWIGVANQYSWFMRRRLFKTSTEAAYSSS TCFRNTRLDRNFCLLIFELTDNLLGDWRIV PLLFELTIV		
JQ00176	8170-10275	gb:JQ001776:8170-10275 Organism:Cedar virus Strain Name:CG1alProtein Name:attachment glycoprotein Gene Symbol:G	MLSQ LQKNYLDNSNQGD KMNNPDKKLS VNFNPLELDKGQKDLNKSYYVKNKNYNV SNLLNESLHDIKFCIYCIFSLIIITIIITISIV ITRLKVHEENNGMESPNLQSIQDSLSSLTN MINTETPRIGILVTATSVTLSSSINYVGTKT NQLVNELKDYITKSCGFKVPELKLHECNIS CADPKISKSAMYSTNAY AELAGPPKIFCKS VSKDPDFRLKQIDYVIPVQQDRSICMNNPL LDISDGGFTYIHYEGINSCKKSDSFKVLLSH GEIVDRGDYRPSLYLLSSHYHPYSMQVINC VPVTCNQSSFVFC HISSNNTKTLDNSDYSSD EYYITYFNGIDRPKTKKIPINMTADNRYI HFTFSGGGGVCLGEEFIIPVTTVINTDVFTH DYCESFNCSVQTGKSLKEICESLRSPTNSS RYNLNGIMIISQNNMTDFKIQLNGITYNKL SFGSPGRLSKTLGQVLYYQSSMSWDTYLK AGFVEKWKPFPTPNWMNNTVISRPNQGNC PRYHKCPEICYGGTYNDIAPLDLGKDMYV SVILDSQDLAENPEITVFNSTTILYKERVSK DELNTRSTTTSCFLFLDEPWCISVLETNRF NGKSIRPEIYSYKIPKYC	3	93
KP271123	6644-8431	gb:KP271123:6644-8431 Organism:Teviot virus Strain Name:Geelong Protein Name:attachment protein Gene Symbol:HN	MWSTQASKHPAMVNSATNLVDIPLDHPSS AQFPINRKRTGRLIYRLFSLCNLILISILISL VVIWSRSSRDCAKSDGLSSVDNQLSSLRS INSLITEVNQISVTTAINLPIKLEFGKSVVD QVTQMIRQCNAACKGPGEKPGIQNVRINIP NNFSTYSELNRTANSLNFQSRTALFARNP YPKTCSRFPSSVYFGIHCFSHAVTDSSCE LSDSTYYRLVIGVADKNLSDPADVKYIGE TTTPVRVQTRGCSVSSYIGCYLLCSKSNQ DYQDDFREQGFHQMFI LFLSRELKTTFFDD MVSSTTVTWNGLYPGEGSGIWHMGHLVF PLWGGIRFGTHASEGILNSTLELPPVGPSC KRSLADNGLINKDVLFSFYFGDSVMVFAY LSCYMLSNVPTHQCQVETMNSSVLGFGSRA QFYDLKGIVYLYIQSAGWFSYTLFRLSLQ SKGYKLSVKQIKRIPISSTRPGTEPCDIIHN CPYTCATGLFQAPWIVNGDSIRDRDVRNM AFVQAWSGAINTFQRPFMSICSQYSCPLSE LLDSESSIMRSTTTYCFPSL TESILQCVSFIE WGGPVGNPISINEVYSSISFRPD	3	94
AY28609	7644-9542	gb:AY286409 Organism:Moss man virus Strain Name:UNKNO WN-	MVDPPAVSYTYGTGRNDRVKVVTQTSTN PYWAHNPQNQLRRLIDMVVNVIMVTGVIF ALINIILGIVIISQSAGSRQDTSKSLDIIQHVD SSVAITKQIVMENLEPKIRSILDSVSFQIPKL LSSLLGPGKTDPPIALPTKASTPVIPTIYPSL NTTCLRIEESVTQNAALFNISFDLKTVM	2	95

		AY286409 Protein Name:attachme nt glycoprotein Gene Symbol:G	YELVTRTGGCVTLPSYSELYTRVRTFSTAI RNPKTCQRAGQETDLNLIPAFIGTDTGILIN SCVRQPVIATGDGIYALTYLTMRGTCQDH RHAVRHFEIGLVRRDAWVDPVLTPIHHFT EPGTPVFDGCSLTVQNQTALALCTLTTDG PETDIHNGASLGLALVHFNIRGEFSKHKVD PRNIDTQNQGLHLVTTAGKSAVKKGILYS FGYMVTRSPEPGDSKCVTEECNQNNQEK NAYSKTTLDPKPRSMIIFQIDVGAEYFTV DKVVVVVPRTQYYQLTSGDLFYTGEEENDLL YQLHNKGWYNKPIRGRVTFDQGVTLHEH SRTYDSLNSQRACNPRLGCPSTCELTSMAS YFPLDKDFKAAVGVIALRNGMTPITTYSTD DWRNHWKYIKNADLEFSESSLSCYSPNPP LDDYVLCTAVITAKVMSNTNPQLLATSW YQYDKCHT		
AY900001	7809-9938	gb:AY900001 Organism:J-virus Strain Name:UNKNO WN-AY900001 Protein Name:attachme nt glycoprotein Gene Symbol:G	MNPVAMSNFYGINQADHLREKGDQPEKG PSVLTYVSLITGLLSLFTIALNVTNIIYLTG SGGTMATIKDNQQSMSGSMRDISGMLVE DLKPKTDLINSMVSYTIPSQISAMSAMIKN EVLROCTPSFMFNNTICPIAEHPVHTSYFEE VGIEAISMCTGTNRKLVVNQGINFVEYPSF IPGSTKPGGCVRRLPSFSLGLEVFAYAHAIT QDDCTSSSTPDYYFSVGRIADHGTDPVFE TLAEWFLDDKMNRSCSVTAAGKGGWL GCSILVGSFTDELTSPEVNRISLSYMDTFGK KKDWLYTGSEVRADQSWALFFSVGSGV VIGDTVYFLVWGGLNHPINVDAMCRAPG CQSPTQSLCNYAIKPQEWGGNQIVNGILHF KHDTNEKPTLHVRTLSPDNNWMGAEGRL FHFHNSGKTFIYTRSSTWHTLPQVGILTLG WPLSVQWVDITSISRPQGSPCEYDNRCPHQ CVTG VYTDLFPLGVSYEYSVTAYLDQVQS RMNPKIALVGAQEKIYEKTITTTNTQHADY TTTSCFAYKLRVWCVSIVEMSPGVITTRQP VPFLYHLNLGCQDTSTGSLTPLDAHGGTY LNTDPVGNKVDCYFVLHEGQIYFGMSVGP INYTYSIVGRSREIGANMNVSLNQLCHSVY TEFLKEKEHPGTRNNIDVEGWLLKRIETLN GTKIFGLDDLEGSGPGHQSGPEDPSIPIGH N	2	96
EF199772	6150-6944	gb:EF199772:6150-6944 Organism :Avian metapneumovirus Strain Name:PL-2 Protein Name:attachme nt glycoprotein Gene Symbol:G	MEVKVENVGKSQELKVKVKNFIKRSDCK KKLFALILGLVSFELTMNIMLSVMYVESNE ALSLCRIQGTAPPRDNKTNTENATKETTLH TTTTTRDPEVRETKTTPQANEGATNPSR NLTTKGDKHQTTTRATTEAELEKQSKQTTE PGTSTQKHTPARPSSKSPTTTQATAQPTTP TAPKASTAPKNRQATTCKKTETDTTTASRA RNTNNPTETATTTPKATTETGKGKEGPTQ HTTKEQPETTARETTTPQPRRTAGASPRAS	2	97
JF424833	5981-7156	gb:JF424833:5981-7156 Organism	MGSKLYMVQGTSA YQTAVGFWLDIGRRY ILAIVLSAFGLTCTVTIALTVSVIVEQSVLE ECRNYNGGDRDWSTTQEQTTPAPSATP	2	98

		:Avian metapneumovirus Strain Name:IT/Ty/A/ 259- 01/03 Protein Name:attachme nt protein Gene Symbol:G	AGNYGGLQTARTRKSESLHVQISYGDM YSRSDTVLGGFDCMGLLVLCCKSGPICQRD NQVDPTALCHCRVDLSSVDCCKVNKISTN SSTTSEPQKTNPAPWPSQDNTDSDPNPQGIT TSTATLLSTSLGLMLTSKTGTHKSGPPQAL PGSNTNGKTTTDRRELGSTNQPNSTTNGQH NKHTQRM TLPPSYDNTRTILQHTTPWEKT FSTYKPTHSPNESDQSLPTTQNSINCEHFD PQGKEKICYRVGSYNSNITKQCRIDVPLCS TYNTVCMKTYYTEPFNCWRRIRWRLCDD GVGLVEWCCTS		
JN6 892 27	7918- 1244 4	gb:JN689227:7 918- 12444 Organism: Tailam virus Strain Name:TL8K Protein Name:attachme nt glycoprotein Gene Symbol:G	MSQLAAHN LAMS NFYGIHQGGQSTSQKE EEQPVGQVIRYASMI VGLLSLFTIHALNVTN IYMTESGGTMQSIKNAQGSIDGSMKDL SG TIMEDIKPKTDLINS MVSYNIP AQLSMIHQI IKNDVLKQCTPSFMFNNTICPLAENP THSR YFEEVNLD SISECSG NEMSLELGTEPEFIEY PSFAPGSTKPGSCVRLPSFSLSSSTVFAYTHT IMGHGCSELDVGDHYLAIGRIADAGHEIPQ FETISSWFINDKINRRSCTVAAGVMETWM GCVIMTETFYDDLDLSDTGKITISYLDVFG RKKEWIYTRSEILYDYTYTSVYFSIGSGVV VGDTVYFLLWGSLSPIEETAYCYAPGCSN YNQRM CNEAQRPAKFGHRQMANAILRFK TNSMGKPSISVRTLSPTVIPFGTEGRLIYSD FTKIIYLYLRSTSWYVLPLTG LLLGPPVSIS WVTQEAVSRPGEYPCGASNRCPKDCITGV YTDLFPLGARYEYAVTVYLNAETYRVNPT LALIDRSKIIARKKITTESQKAGYTTTTCFV FKLRIWCMSVVELAPATMTAFEPVPFLYQ LDLTCKRNGTTAMQFSGQDGM YKSGRY KSPRNECF FEKVS NKYYFVVSTPEGIQPYE VRDLTPERVSHVIMYISDVCAPALSAFKKL IPAMRPITTLTIGNWQFRPV DISGGLRVNIY RNLTRYGDL SMSAPEDPGTDTFFPGTHAPS KGHEEVGHYTL PNEKLSEVTTAAVKTKES LNLIPDTKDTRGEEENGSG LNEITGHTTPG HIKTHPAETKVTKHTVIIPQIEEDGSGATT S TELQDETGYHTEDYNTTNTNGSLTAPNER NNYTSGDHTVSGEDITHITVSDRTKTTQT LPTDNTFNQTPTKIQEGSPKSESTPKDYTAI ESED SHFTDPTLIRSTPEGTIVQVIGDQFHS AVTQLGESNAIGNSEPIDQGNLPIPTDRG TMDNTSSQSHSSTTSTQGS HSAGHGSQSN MNLTA LADTDSVTDQSTSTQ EIDHEHENV SSILNPLSRHTRVMRDTVQEALTGA WGFI RMIP	2	99
KC5 622 42	6178- 6926	gb:KC562242: 6178- 6926 Organism: Human metapneumovirus Strain Name:HMPV/ USA/C1- 334/2004/B Pro	MEVRVENIRAIDMFKAKIKNRIRNSRCYR NATLILIGLTALS MALNIFLIIDHATLRNMI KTENCANMPSAEPSKKT PMTSIAGPSTKPN PQQATQWTTENSTSPAATLEGHPYTGT TQ TPD TTAPQQTTDKHTALPKSTNEQITQTTT EKKTTTRATTQKRKKEKKTQT K PQVQLQP KQPTPPTKSEMQRVRSQHPTDPELTPLPKA VNRQPGQQNQAPHHIMHGEVQDPGERNT QVSHPS	2	100

		tein Name:attachme nt glycoprotein GI Gene Symbol:G			
KC9 150 36	6154- 7911	gb:KC915036: 6154- 7911 Organism :Avian metapneumovir us type C Strain Name:GDY Pro tein Name:attachme nt glycoprotein G ene Symbol:G	MEVKIENVGKSQELRVKVKNFIRSDCKK KLFALILGLISFDITMNIMLSVMYVESNEA LSSCRVQGTPAPRDNRTNTENTAKETTLH TMTTTRNTEAGGTTKTPQADERATSPSK NPTIGADKHKTTRATTEAEQEKQSKQTTE PGTSTPKHIPARPSSKSPATTKTTTQPTTPT VAKGGTAPKNRQTTTKKTEADTPTTSRAK QTNKPTGTETTPPRATTETDKDKEGPTQH TTKEQPETTAGGTTTPQPRRTTSRPAPTTN TKEGAETTGTTRTTKSTQTSASPPRPTSTPS KTATGTNKRATTTKGPNTASTDRRQQTRT TPKQDQQTQTKAKTTTNAHAKAATTPE HNTDTTDSMKENSKEDKTTRDPSSKATTK QENTSKGTTATNLGNNTTEAGARTPPTTTP TRHTTEPATSTAGGHTKARTTRWKSTAAR QPTRNNTTADTKTAQSKQTTTPAQLGNNTT PENTTPPDNKSNSQTNVAPTEEIEIGSSLWR RRYVYGPCRENALEHPMNPCLKDNTTWI YLDNGRNLPAGYYSKTDKIICYGIYRGN SYCYGRIECTCKNGTGLLSYCCNSYNWS	2	101
LC1 687 49	7239- 9196	gb:LC168749:7 239- 9196 Organism :Rinderpest morbillivirus St rain Name:Lvl Protei n Name:H protein Gene Symbol:H	MSSPRDRVNAFYKDNLQFKNTRVVLNKE QLLIERPYMLLAVLFVFMFLSLVGLLAIA RLHRAAVNTAEINSGLTTSIDITKSIEYQVK DVLTPLFKIIGDEVGLRTPQRFTDLTKFISD KIKFLNPDKEYDFRDINWCISPPERIKINYD QYCAHTAAEELITMLVNSSLAGTAVLRSTS LVNLGRSCTGSTTTKGQFSNMSLALSGIYS GRGYNISSMITTEKGMYGSTYLVGKHQ GARRPSTAWQRDYRVFEVGIIRLGVGTP VFHMTNYLELPRQPELEICMLALGEFKLA ALCLADNSVALHYGGLRDDHKIRFVKLG VWSPADSDTLATLSAVDPTLDGLYITTHR GIIAAGKAVWAVPVTRTDDQRKMGQCRR EACREKPPFCNSTDWEPLEAGRIPAYGIL TIRLGLADKPEIDIIESEFGLITHDSGMDLYT PLDGNEYWLTIPPLQNSALGTVNTLVLEPS LKISPNIITLPIRSGGDCYTPTYLSDLADD DVKLSSNLVILPSRNLQYVSATYDTSRVEH AIVYYIYSTGRLSSYYYPVKLPIKGDVSL QIGCFPWGLKLWCHHFCVIDSGTGKQVT HTGAVGIEITCNSR	2	102
LC1 873 10	8144- 9871	gb:LC187310:8 144- 9871 Organism :Avian paramyxovirus 10 Strain Name:rAPMV- 10- FI324/YmHA P rotein	MDSSQMNILDAMDRESSKRTWRGVFRVT TIIMVVTVCVLSAITLSKVAHPQGFDTNEL GNGIVDRVSDKITEALTPVNNQIGEIFKIVA LDLHLVSSSQAIAGQIGMLAESINSILSQ NGSASTILSSSPEYAGGIGVPLFSNKLNTGT VIKPTLIEHPSFIPGPTTIGGCTRIPTFHMAS SHWCYSHNIIKKGCKDSGISSMYISLGVLQ VLKKGTPVFLVTASAVLSDDRNRKSCSIIS SRFGCEILCSLVTEAESDDYKSDTPTGMVH GRLYFNGTYREGLVDTETIFRDFSANYPG	2	103

		Name:hemagglutinin-neuraminidase Gene Symbol:HN	VGSGEIVEGHIHFPIYGGVKQNTGLYNSLT PYWLDANKYDYCKLPYTNQTIQNSYKPP FIHGRFWAQGILSCELDLFLNLCNLCIIRS DKVMMGAESRLMLVGSKLLMYQRASSW WPLGITQEIDIAELHSSNTTILREVKPISSK FPRPSYQPNYCTKPSVCPAVCVTVGVYTD WPISITGNISDYAWISHYLDAPTSRQQPRIG IANQYFWIHQTTIFPTNTQSSYSTTTCFRNQ VRSRMFCLSIAEFADGVFGEFRIVPLLYEL RV		
NC_004074	6590-8563	gb:NC_004074:6590-8563 Organism:Tioman virus Strain Name:UNKNO WN-NC_004074 Protein Name:attachement protein Gene Symbol:HN	MWATSESKAPIPANSTLNLVDVPLDEPQTI TKHRKQKRTGRLVFRLLSLVLSMTVILV LVILASWSQKINACATKEGFNSLDLQISGL VKSINSLITEVNQISITTAINLPIKLSDFGKSI VDQVTQMIRQCNVCKGPGEKPGIQNIRI NIPNNFSTYLELNNTVKSIELQRRPALLARP NPIPKSCSRFPSYSVNFQIHCFHAITDQSC ELSDKTYRLAIGISDKNLSDPDVKYIGE AFTPMGLQARGCSVISSYGYLLCSKSNQ GYEADFQTQGFHQMYILFLSRDLKTTLFN DMISSTTVVWNGLYPGEAGIWHMGYLIF PLWGGIKIGTPASTSILNSTLDLPLVGPSC STLEENNLINKDVLFSYFGEVSMVFGFLS CYMLSNVPTHQVEVLNSSLVGFGRSRL MDLKGVYLYIQSAGWYSYTLFRLSLQS RGYKLTVKQIRRIPISTTRPGTAPCDVVH NCPYTCATGLFQAPWIVNGDSILDRDVRN LVFVQAWSGNFNTFQKGLISICNQYTCPLT TLLDNDNSIMRSTTTYCYPSLSEYNLQCQS FIEWGGPVGNPIGILEVHYIIFK	2	104
NC_005283	7091-8905	gb:NC_005283:7091-8905 Organism:Dolphin morbillivirus Strain Name:UNKNO WN-NC_005283 Protein Name:haemagglutinin Gene Symbol:H	MSSPRDKVDAFYKDIPRPRNNRVLLDNER VIERPLILVGVLA VMFLSLVGLLAIAGVRL QKATTNSIEVNRKLSTNLETTVSIHHVKD VLTPLFKIIGDEVGLRMPQKLTEIMQFISNK IKFLNPDREYDFNDLHWCVNPPDQVKIDY AQYCNHIAAEELIVTKFKELMNHSLDMSK GRIFPPKNCSGSVITRGQTIKPGTLVNIYT TRNFEVSFMTVISGGMYGKTYFLKPEP DDPFEFQAFRIFEVLVRDVGSREPVLMQ TFMVIDEDEGLNFCLLSVGELRLAAVCVR GRPVTTKDIGGYKDEPFKVVTLGIIIGGLS NQKTEIYPTIDSSIEKLYITSHRGIIRNSKAR WSVPAIRSDDKDKMEKCTQALCKSRPPPS CNSSDWEPLTSNRIPAYAYIALEIKEDSGLE LDITSNYGPLIIHGAGMDIYEGPSSNQDWL AIPPLSQSVLGVINVDFTAGFDIKPHTLT TAVDYESGKCYVPVELSGAKDQDLKLESNL VVLPTKDFGYVTATYDTSRSEHAIVYVY DTARSSSYFFPRIKARGEPIYLRIECFPWS RQLWCHHYCMINSTVSNEIVVDNLVSIN MSCSR	2	105
NC_007803	7978-12504	gb:NC_007803:7978-12504 Organism:Beilong virus Strain	MSQLAAHNLAMSNFYGTHQGDLSGSQKG EEQQVQGVIRYVSMIVSLLSLFTIILNVTN IYMTESGGTMQSIKTAQGSIDGSMREISG VIMEDVKPKTDLINSMVSYNIPALSMIHQ IKNNDVPKQCTPSFMFNNTICPLAENPHTSR	2	106

		Name:LilProtein Name:attachme nt glycoproteinlG ene Symbol:G	YFEEVNLDSECSGPDMLHLGLGVNPEFIE FPSFAPGSTKPGSCVRLPSFSLSTTVFAYTH TIMGHGCSELDVGDHYFSVGRIADAGHEIP QFETISSWFINDKINRRSCTVAAGAMEAW MGCVIMTETFYDDRNSLDTGKLTISYLDV FGRKKEWIYTRSEILYDYTYTSVYFSVGS VVVGDTVYFLIWGSLSSPIEETAYCFAPDC SNYNQRMCMNEAQRPSKFGHRQMVNGILK FKTTSTGKPLLSVGTLSPSVVPFGSEGRML YSEITKIIYLYLRSTSWHALPLTGLFVLGPP TSISWIVQRAVSRPGEFPCGASNRCPKDCV TGVYTDLFPLGSRYEYAATVYLNSETYRV NPTLALINQTNIIASKKVTTESQRAGYTTTT CFVFKLRVWCISVVELAPSTMAYEPIPF YQLDLTCKGKNGSLAMRFAGKEGTYKSG RYKSPRNECFEKEVSNKYFIVSTPEGIQ YEIRDLTPDRMPHIIMYISDVCAPALSAFK KLLPAMRPITTLTIGNWQFRPVEVSGGLRV NIGRNLTKEGDLTMSAPEDPGSNTFPGNHI PGNGILDAGYYTVEYPKE		
NC_ 009 489	6559- 8512	gb:NC_009489 :6559- 8512 Organism :Mapuera virus Strain Name:BeAnn 370284 Protein Name:attachme nt protein Gene Symbol:HN	MASLQSEPGSQKPHYQSDDQLVKRTWRSF FRFSVLVVTITSLALSITLIGVNRISTAKQIS NAFAAIQANILSSIPDIRPINSLLNQLVYTSS VTLPLRISSLESNVLAIIQEAQTYRDSQSSC SATMSVMNDQRYIEGIQVYSGSFLDLQKH TLSPPIAFPSFIPTSTTTVGCTRIPSFLTKTH WCYTHNYIKTGCRDATQSNQYIALGTIYT DPDGTGPGFSTSRSQYLNDGVNRKSCSISAV PMGCALYCFISVKEEVDYYKGTVPQAQTLI LFFFNQTVHEHRIVPSSMNSEWVWMLSPGV GSGVFYNNYIIFPLYGGMTKDKAERKRGEL TRFFTPKNSRSLCKMNDVFSNAAQSAYY PPYFSSRWIRSGLLACNWNQIITNCEILTF SNQVMMMGAEGRLLILINDDLFYQQRSTS WWPRPLVYKLDIELNYPDSHIQRVDQVEV TFPTRPGWGGCVGNFPCPMICVSGVYQD VWPVTNPVNTTDSRTLWVGGLLSNTTRE NPASVVTSGGSISQTVSWFNQTVPGAYSTT TCFNDQVQGRIFCLIFEVGGGLLGEYQIVP FLKELKYQGAVHA	2	107
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	MAPINYPASYTNNNAERPVVITTKSTESKG QRPLPLGNARFWEYFGHVCGLTFCMSLI GIIVGIIALANYSSDKDWKGRIGGDIQVTR MATEKTVKLILEDTPKLRNILDSVLFQLP KMLASIASKINTQTPPPPTTSGHSTALATQ CSSNCENRPEIGYDYLQVEQSLQRITNISI QLLEASEIHSMAGAYPNALYKIRTQDSWS VTAKECPLQAFQPNLNLIPAMIGTATGALI RNCVRQPVIVDDGVYMLTYLAMRGSCQ DHQKSVRHFEMGVITSDPFGDPVPTPLRH WTKRALPAYDGCALAVKGHAGFALCTET SVGPLRDRTAKRKPNIVLFKASLVGELSER VIPPQSWLSGFSFVSVYTVAGKGYAYHSKF HAFGNVVRVGQSEYQAKCRGTGCPTANQ DDCNTAQRVSQEDNTYLHQAILSVDIDSVI DPEDVVYVIERDQYYQASAGDLYRVPETG	2	108

			EILYNLHNGGWSNEVQVGRIQPSDRFYMR EIQLTSTRVPAPNGCNRVKGCPGGCVAVIS PAFTPMHPEFNVGVGIFPMNQPHNPSIMH VQQQTELFWKPIVGGNITLHESSIACYSTV PPNPSYDLCIGVMTLLHQQQLPQFQALS WYQPTMCNGNAPQNRRLIPVIVEDSKA MSVSSDAPRTP		
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 id_hel/GH- M74a/GHA/20 09 Protein Name:glycopro tein Gene Symbol:G	MPQKTVEFINMNSPLERGVSTLSDKKT QSKITKQGYFGLGSHSERNWKKQKNQND HYMTVSTMILEILVVLGIMFNLIVLTMVYY QNDNINQRMAELTSNITVLNLNLNQLTNKI QREIIPRITLIDTATTITIPSAITYILATLTTRI SELLPSINQKCEFKTPTLVNDCRINCTPPL NPSDGVKMSSLATNLVAHGSPCRNFSSV PTIYYRIPGLYNRTALDERCILNPRLTISST KFAYVHSEYDKNCTRGFKYYELMTFGEIL EGPEKEPRMFSRSFYSPTNVNYHSCTPIV TVNEG YFLCLECTSSDPLYKANLSNSTFHL VILRHNDKDEKIVMSPFNLDQEYVQIIPA EGGGTAESGNLYFPCIGRLLHKRVTHPLC KKSNC SRTDDESCLKSYYNQGSPQHQQV CLIRIRNAQRDNPTWDVITVDLTNTYPGSR SRIFGSFSKPMLYQSSVSWHTLLQVAEITD LDKYQLDWLDTPIYISRPGGSECPFGNYCP TVCWEGTYNDVYSLTPNNDLFVTVYLKSE QVAENPYFAIFSRDQILKEFPLDAWISSART TTISCFMFNNEIWCAALEITRLNDDIIRPIY YSFWLPTDCRTPYPHTGKMTRVPLRSTYN Y	2	109
NC_ 025 347	6398- 8418	gb:NC_025347 :6398- 8418 Organism :Avian paramyxovirus 7 Strain Name:APMV- 7/dove/Tenness ee/4/75 Protein Name:hemaggl utinin- neuraminidase Gene Symbol:HN	MESIGKGTWRTVYRVLTILLDVVVIILSVIA LISLGLKPGERIINEVNGSIHNQLVPLSGITS DIQAKVSSIYRSNLLSIPLQLDQINQAISSA RQIADTINSFLALNGSGTFIYTN SFEFANGF NRAMFPTLNQSLNMLTPGNLIEFTNFIPPT TKSGCIRIPSFMSSSHWCYTHNIIASGCQD HSTSSEYISMGVVEVTDQAYPNFRTTLSIT LADNLNRKSCSIAATGFGCDILCSVVTETE NDDYQSPEPTQMIYGRLLFFNGTYSEMSLN VNQMFADWVANYPVAVGSGVELADVFIFP LYGGVKITSTLGASLSQYYYIPKVPTVNCS ETDAQQIEKAKASYSPPKVAPNIWAQAVV RCNKS VNLANSCIELTFNTSTMMMGAEGR LLMIGKNVYFYQRSSSYWPVGIIYKLDLQE LTTFSSNQLLSTIPIPFKFRPASTAGVCSK PNVCPAVCQTGVYQDLWVLYDLGKLENT TAVGLYLNSAVGRMNPFIGIANTLSWYNT TRLFAQGT PASYSTTTCFKNTKIDTAYCLSI LELSDSLLGSWRITPLLYNITLSIMS	2	110
NC_ 025 348	6590- 8548	gb:NC_025348 :6590- 8548 Organism :Tuhoko virus 2 Strain Name:UNKNO WN- NC_025348 Pr	MPPVPTVSQSIDEGSFTDIPLSPDDIKHPLS KKTCKRKLFRIVTLIGVGLISILTIISLAQQTGI LRKVDSSDFQSYVQESFKQVLNLMKQFSS NLNSLIEITSVTLPRIDQFGTDIKTQVAQL VRQCNVAVCRGPIKGPTTQNVYPALYETSL NKTLETKNVRIQEVQRQEVDPVPGPLSNG CTRNPFSVYHGVWCYTHATSGNCSGL GTSQ LFRIGNVLEGDGGAPYHKS LATHLL	2	111

		otein Name:hemaggl utinin- neuraminidase Gene Symbol:HN	TTRNVSQRQCSATASYGFCSEPVLT DDYETPGIEPITIFRLDPDGNWVFPNINRF TEYSLKALYPGIGSGVLFQGLIFPMYGGI DKERLSALGLGNIGLIERRMADTCNHTEK ELGRSFPFAGFSSPYHDAVMLNFLICEMI ENLPGDCDLQILNPTNMSMGSESQSLVLD NELFLYQRSASWWPYTLIYRLNMRYTGK YLKPKSIIPMVIKSNTRPGYEGCNHERVCP KVCVTGVFQAPWILSIGRDHKERVSNVTY MVAWSMDKSDRTYPAVSVCGSDTCKLTV PLGDSKVHSAYSVTRCYLSRDHMSAYCLV IFELDARPAEMRIQSFLYKLILT		
NC_ 025 350	6451- 8341	gb:NC_025350 :6451- 8341 Organism :Tuhoko virus 3 Strain Name:UNKNO WN- NC_025350 Pr otein Name:hemaggl utinin- neuraminidase Gene Symbol:HN	MHNRTQSVSSIDTSSDVYLPRRKKAVTKF TFKKIFRVLILTLLLSIIIIAVIFPKIDHIRETC DNSQILETITNQNSEIKNLINSAITNLNVLLT STTVDLPIKLNNFGKSIVDQVTMMVRQCN AVCRGPGDRPTQNIELFKGLYHTSPSNTS TKLSMITEASNPDIVPRPGKLLGCTRFPSF SVHYGLWCYGHMASTGNCSSGSSPSVQIIRI GSIGTNKDGTGPKYVIIASASLPETTRLHYCS VTMTSIGCYILCTTPSVSETDDYSTMGIEK MSISFLSLDGYLTQLGQPTGLDNQNLAL YPGPGSGVIFRDFLIFPMMGGIRLMDAQK MLNRNITYRGFPSETCTESELKLEKQEVAN MLTSPYYGEVLVLNFLYVCSLLDNIPGDCS VQLIPPDNMTLGAESRLYVLNGSLIMYKR GSSWWPYTELYQINRVNNRAFRVRESVR INTTSTSRPGVQGCNLEKVCVKCVSGIYQ SPGIISAPVNPTRQEEGLLYFLVWTSMSR TGPLSSLCDHSTCRITYPIGDDTIFIGYTDSS CFMSSIKEGIYCIAFLELDNQPYSMMAIRSL SYIIN	2	112
NC_ 025 352	8716- 1125 7	gb:NC_025352 :8716- 11257 Organis m:Mojiang virus Strain Name:Tonggua n1 Protein Name:attachme nt glycoprotein G ene Symbol:G	MATNRDNTITSAEVSQEDKVKKYYGVET AEKVADSISGNKVFILMNTLLITGAIITITL NITNLTAASKSQNMLKIIQDDVNKLEMF VNLDQLVKGEIKPKVSLINTAVSVSIPGQIS NLQTKFLQKYVYLEESITKQCTCNPLSGIF PTSGPTYPTDKPDDDTDDDDKVDTTIKPI EYPKPDGCNRTGDHFTMEPGANFYTPVNL GPASSNSDECYTNPSFSIGSSIMFSQEIRK TDCTAGEILSIQIVLGRIVDKGQGPQASPL LVWAVPNPKIINSCAVAAGDEMGWVLC VTLTAASGEPIPHMFDGFWLYKLEPDTEV VSYRITGYAYLLDKQYDSVFIGKGGGIQK GNDLYFQMYGLSRNRQSFKALCEHGSCL GTGGGGYQVLCRAVMSFGSEESLITNAY LKVNDLASGKPVIIQTFPPSDSYKGSNGR MYTIGDKYGLYLAPSSWNRYLRFGITPDIS VRSTTWLKSQDPIMKILSTCTNTDRDMCP EICNTRGYQDIFLSESEYYTYIGITPNN GTKNFVAVRSDGHIASIDILQNYYSITSA TISCFMYKDEIWCIAITEGKKQKDNQPRIY AHSYKIRQMCYNMKSATVTVGNAKNITIR RY	2	113

NC_025363	6503-8347	gb:NC_025363:6503-8347 Organism:Avian paramyxovirus 12 Strain Name:Wigeon/Italy/3920_1/2005 Protein Name:hemagglutinin-neuraminidase Gene Symbol:HN	MESATSQVSFENDKTSRRTWRAVFRVL MIILALSSLCVTVAALIYSAKAAIPGNIDAS EQRILSSVEAVQVPVSRLEDTSQKIYRQVIL EAPVTQLNMETNILNAITSLSYQIDASANS SGCGAPVHDSDFGGVGRELLQEAENVLT IIRPSKFLEHLNFIPAPTTGNGCTRIPSFDLG QTHWCYTHNVVLNGCRDRGHSFQYVALG ILRTSATGSVFLSTLRSVNLDDDRNRKSCS VSATPIGCEMLCSLVTETEEGDYDSIDPTP MVHGRGLGFDGKYREVDLSEKEIFADWRA NYPVGGGAFFGNRVWFPVYGGGLKEGTQ SERDAEKGYAIYKRFNNTCPDDNTTQIAN AKASYRPSRFGGRFIQQGILSFKVEGNLGS DPILSLTDNSITLMGAEARVMNIENKLYLY QRGTSWFPSALVYPLDVANTAVKVRAPYI FDKFTRPGGHPSCASSRCPNVCVTGVYTD AYPLVFSRSHDIVAVYGMQLAAGTARLDP QAAIWYGNEMSTPTKVSSSTTKAAYTTST CFKVTKTKRIYCISIAEIGNTLFGEFRIVPLL IEVQKTPLTRRSELRRQQMPQPPIDLVIDNPF CAPSGNLSRKNAIDEYANSWP	2	114
NC_025373	6619-8605	gb:NC_025373:6619-8605 Organism:Avian paramyxovirus 3 Strain Name:turkey/Wisconsin/68 Protein Name:hemagglutinin Gene Symbol:HN	MEPTGSKVDIVPSQGTKRCTFYRLILIL NLIIILTHISYVSISTDQHKLNCNEADSLH SIVEPITVPLGTDSDVEDELREIRRDGTINIP IQIDNTENIILTTLASINSNIARLHNATDESP TCLSPVNDPRFIAGINKITKGSIMYRNFNL IEHVNFIPTTSLSGCTRIPSFSLSKTHWCYS HNVISTGCQDHAASSQYISIGIVDTGLNNE PYLRTMSSRLNDGLNRKSCSVTAGAGVC WLLCSVVTESESADYRSRAPTAMILGRFN FYGDYTESPVASLFSGRFTANYPGVSGST QLNGTLYFPIYGGVVNDSDIELSNRGKSFR PRNPTNPCPDPEVTQSQRAQASYYPTRFGR LLIQQAILACRISDTTCTDYLLYFDNNQV MMGAEARIIYLLNNQMYLYQRSSSWWPH PLFYRFSPLPHCEPMSVCMITDTHLILTYATS RPGTSICTGASRCPNNCVDGVYTDVWPLT EGTTQDPDSYYTVFLNSPNRRISPTISIYSY NQKISSRLAVGSEIGAAAYTTSTCFSTRDTG ALYCITIEAVNTIFGQYRIVPILVQLISD	2	115
NC_025386	7541-9403	gb:NC_025386:7541-9403 Organism:Salem virus Strain Name:UNKNOWN-NC_025386 Protein Name:attachment glycoprotein Gene Symbol:G	MKAMHYKNDFAADPGTNDNSSDLTTNPFI SNQIKSNLSPPVLAEGHLSPPIPKFRKILLT ISFVSTIVVLTVILLVLTIRILTIEASAGDEK DIHTILSSLNTFMNEYIPVFKNLVSIISLQIP QMLIDLKTSSTQMMQSLKTFPRDLETLS VTQSVAVLLEKAKSTIPDINKFYKNVGKV TFNDPNIKVLTLEVPAPWLPIVRQCLKQDFR QVISNSTGFALIGALPSQLFNEFEGYPSLAI VSEVYAITYLKGVMFENQENFLYQYFEIG TISPDGYNKPYFLRHTSVMLSTFKLSGKCT AAVDYRGGIFLCTPSPKIPKILQNPPDLPTL TVVSIPFDGRYTIRNISLMLTDEADIIYDLD TLQGRGVLQAMRFYALVRVISSSSPRHFPF CKNSWCPTADDKICDQSRRLGADGNYPV MYGLISIPAHSSYQGNVSLKLIDPKYYAYT RDASLFYNSMTDTYHYSFGTRGWVSRPII	2	116

			GELLGDDIVLTRYTVRSVSRATAGDCTT VSMCPQACSGGMNSIFYPLNFDKPQVTGV AIRQYERQQEGIIIVTMNDHYYYSVPIKN GTLLISSVTDCFWLMGDLWCMSLMEKNN LPLGVRSLAHLTWNHWS		
NC_ 025 390	6647- 8386	gb:NC_025390 :6647- 8386 Organism :Avian paramyxovirus 9 Strain Name:duck/New York/22/1978 P rotein Name:hemaggl utinin- neuraminidase Gene Symbol:HN	MESGISQASLVNDNIELRNTWRTAFRVVS LLLGFTSLVLTACALHFALNAATPADLSSI PVAVDQSHHEILQTLMSDIGNKIYKQVA LDSPVALLNTESTLMSAITSLSYQINNAAN NSGCGAPVHDKDFINGVAKELFVGSQYNA SNYRPSRFLEHLNFIPAPTTGKGCTRIPSFD LAATHWCYTHNVILNGCNDHAQSYQYISL GILKVSATGNVFLSTLRSINLDDDENRKSC SISATPLGCDLLCAKVERTEREADYNSDAAT RLVHGRLGFDGVYHEQALPVESLFSWDV ANYPSVGGGSYFDNRVWFGVYGGIRPGS QTDLLQSEKYAIYRRYNNTCPDNNPTQIER AKSSYRPQRFGQRLVQQAILSIRVEPSLGN DPKLSVLDNTVVLMGAEARIMTFGHVAL MYQRGSSYFPSALLYPLSLTNGSAAASKPF IFEQYTRPGSPPCQATARCPNSCVTGVYTD AYPLFWSEDHKVNNGVYGMMLDDITSRLN PVAAIFDRYGRSRVTRVSSSSTKAAYTTNT CFKVVKTKRVYCLSAEIENTLFGFRITPL LSEIFDPNLEPSDTSRN	2	117
NC_ 025 403	6692- 8645	gb:NC_025403 :6692- 8645 Organism :Achimota virus 1 Strain Name:UNKNO WN- NC_025403 P rotein Name:attachme nt protein Gene Symbol:HN	MATNLSTITNGKFSQNSDEGSLTELPFFE NRKVATTKRTCRFVFRSVITLCNLTLIVTV VVLFFQQAGFIKRTESNQVCETLQNDMHGV VTMSKGVITTLNLIETSVNLPFQMKQFG QGIVTQVTQMVRQCNAVCKGPTIGPDIQNI VYPASYESMIKHPVNNSNILLSEIRQPLNFV PNTGKLNCTRTPSFSVYNGFWCYTHAES DWNCGSSPYMQVFRVGVVTSYDYNVI HKTLLHTKTSRLANVTYQCSTISTGYECYFL CSTPNVDEITDYKTPGIESLQIYKIDNRGTF AKFPITDQLNKELLTALYPGPGNGVLYQG RLLFPMHGGMQSSELNKNVNLNNTVLSQFN DNKGCNATEIKLESEFFGTFTSPYYSNQVM LNYILICEMIENLPGNCDLQIVAPKNMSMG SESQLYSINNKLILYQRSSSRWPYPLIYEV GTRLTNRQFRLRAINRFLIKSTTRPGSEGC NIYRVCPKVCVTGVYQAPWILHVS KAGSQ SIKVLVYAVAWSKDHMSRKGPLFSICDND TCFLTKSLASEHVHSGYSITRCYLENSERHI ICVVIMELDASPWAEMRIQSVIYNITLPS	2	118
NC_ 025 404	6655- 8586	gb:NC_025404 :6655- 8586 Organism :Achimota virus 2 Strain Name:UNKNO WN- NC_025404 P rotein Name:attachme	MDNSMSISTISLDAQPRIWSRHESRRTWRN IFRITSLVLLGVTVIICIWLCCEVARESELEL LASPLGALIMAINTIKSSVVKMTTELNQVT FTTSIILPNKVDQFGQNVVSQVAQLVKQC NAVCRGHQDTPELEQFINQKNPTWILQPN YTTKLTLNLHEIDSIPLVDYPGFSKSTRFPS FSEGSKFWCFTYAVVKEPCSDISSIQVVK YGAIKANHSDGNPYLVLGTKVLDDGKFR RGCSITSSLYGCYLLCSTANVSEVNDY AHT PAYPLTLELISKDGITTDLSPTYTVQLDKW SALYPGIGSGVIFKGYLMFPVYGGPLPKSP	2	119

		nt protein Gene Symbol:HN	LISASWVGPGNKWPVDFSCSEDQYSTFNF SNPYSALYSPHFSNNIVVSALFVCPLNENL PYSCEVQVLPQGNLTIGAEGRLYVIDQDL YYYQRSTSWWPYLQLYKLNIRITNRVFRV RSLSLPIKSTTRPGYGNCITYFKLCPHICVT GVYQSPWLISIRDKRPHEEKNILYFIGWSP DEQIRQNPLVSLCHETACFINRSLATNKTH AGYSESHCVQSFERNKLTCTVFYELTAKP WAEMRVQSLLFQVDFL		
NC_ 025 410	6799- 8869	gb:NC_025410 :6799- 8869 Organism :Tuhoko virus Strain Name:UNKNO WN- NC_025410 Pr otein Name:hemaggl utinin- neuraminidase Gene Symbol:HN	MDSRSDSFTDIPLDNRIERTVTSKKTWRSIF RVTAILLIICVVVSSISLNQHNDAPLNGAG NQATSGFMDAIAKSLEKLMSQTINELNQVV MTTSVQLPNRITKFGQDILDQVTQMVRQC NAVCRGPGVGPSIQNYVIQGHAPTVSFDPI SAEYQKFVFGITEKTLITAYHNPWECLRFP SQHLFDTTWCVSQILTQNCSDHGPRTVI QLGEIMIANNLSTVFRDPVIKYIRHHIWLRS CSVVAYYSQCTIFCTSTNKSEPSDYADTGY EQLFLATLQSDGTFTEHSMHGVNIVHQWN AIYGGVGNGVIIGRNMLIPLYGGINYYDHN TTIVQTVDLRPYPIDSCSQTNDNYQTNLYP SMFTNSYYGTNLVVSGLSCRLMAGTPTS CSIRVIPIENMTMGSEGQFYLINNQLYYYK RSSNWIRDTQVYLLSYSDKGNIEITSAERY IFKSVTSPDEGDCVTNHGCPSPNCIGGLFQA PWILNDFKLCGSNITCPKIVTVWADQDPDK RSNPMLSIAETDKLLLHKSININYHTAVGYS TVLCFDSPKLNLKTCVVLQELMSDDKLLI RISYSIVSIMVE	2	120
NC_ 028 249	7059- 9010	gb:NC_028249 :7059- 9010 Organism :Phocine distemper virus Strain Name:PDV/Wa dden_Sea.NLD /1988 Protein Name:hemaggl utinin protein Gene Symbol:H	MFSHQDKVGAFYKNNARANSKLSLVT EVEERRSPWFLSILLILLVIGILILLAITGIRFH QVVKSNLEFNKLLIEDMEKTKAVHHQVK DVLTPLFKIIGDEVGLRLPQKLNEIKQFIVQ KTNFFNPNNREFDFRELHWCINPPSKVKVNF TQYCEITEFKEATRSVANSILLTLYRGRD DIFPPYKCRGATTSMGNVFPLAVSLSMSLI SKPSEVINMLTAISEGIYKTYLLVTDDTE ENFETPEIRVFEIGFINRWLGDMPLFQTTN YRIISNNSNTKICTIAVGELALASLCTKESTI LLNLGDEESQNSVLVILGLFGATHMDQL EEVIPVAHPSIEKIHITNHRGFIKDSVATWM VPALALSEQGEQINCLRSACKRRTYPMCN QTSWEPFGDKRLPSYGRLLSLDVSTDLSI NVSVAQGPPIIFNGDGM DY YEGTLLNSGWL TIPKNGTILGLINQASKGDQFIVTPHILTF PRESSTDCHLPQTYQIQDDDDVLLESNLVV LPTQSFEYVVA TYDVSRS DHAIVYYVYDP ARTVSYTYPFRLRTKGRPDILRIECFVWDG HLWCHQFYRFQLDATNSTSVVENLIRIRFS CDRLDP	2	121
NC_ 028 362	6951- 8675	gb:NC_028362 :6951- 8675 Organism :Caprine parainfluenza virus 3 Strain	MEYWGHTNPNPDKINRKVGVDQVRDRSKT LKIITFIISMMSIMSTVALILILIMFIQNNNN NRIILQELRDETD AIEARIQKASNDIGVSIQS GINTRLLTIQNHVQNYIPLALTQQVSSLRES INDVITKREETQSKMPIQRMTHDDGIEPLIP DNFWKCPSGIPTISASPKIRLIPGPGLLATST	2	122

		Name:JS2013IP rotein Name:hemaggl utinin- neuraminidase Gene Symbol:HN	TINGCIRLPSLVINNLIYAYTSNLITQGCQDI GKSYQVLQIGIITINSDLVDPDLNPRITHTFDI DDNRKSCSLALRNADVYQLCSTPKVDE DYSSIGIEDIVLDIVTSEGTVSTTRFTNNIT FDKPYAALYPSVGPYIYDNKIIFLGYGGL EHEENGDVICNITGCPGKTQHDCNQASYS PWFSNRRMVNAILVNKGLNKVPSLQVWT IPMRQNYWGSEGRLLLLGNKIYIYTRSTS WHSKLQLGTLDISNYNDIRIRWTHHDVLS RPGSEECPWGNTCPRGCITGVYNDAYPLN PSGSVVSSVILDSRTSRENPIITYSTDTSRVN ELAIRNNTLSAAYTTTNCVTHYKGKGYCFH IIEINHKSNTLQPMFLFKTEIPKSCN		
AB5 484 28	5999- 7261	gb:AB548428: 5999- 7261Organism :Avian metapneumovir uslStrain Name:VCO3/6 0616lProtein Name:attachme nt glycoproteinlG ene Symbol:G	MGSELYHIEGVSSSEIVLKQVLRSSKILLG LVLSALGLTLTSTIVISICISVEQVKLRQCV DTYWAENGLHPGQSTENTSTRGKTTTKD PRRLQATGAGKFESCGLYVQVVDGDMHDR SYAVLGGVDCGLLLALCESGPICQGDWTS EDGNFCRCTFSSHGVSCKKPKSKATTAQ RNSKPANSKSTPPVHSDRASKEHNPSQGE QPRRGPTSSKTTIASTPSTEDTAKPTISKPK LTIRPSQRGPSGSTKAASSTPSHKTNTRGTS KTDDQRPRTGPTPERPRQTHSTATPPPTTPI HKGRAPTPKPTTDLKVNPREGSTSPTAIQK NPTTQSNLVDCTLSDPDEPQRICYQVGTY NPSQSGTCNIEVPKCTYGHACMATLYDT PFNCWRRTRRCICDSGGELIEWCCTSQ	1	123
AF0 797 80	8118- 1011 5	gb:AF079780l Organism:Tupa ia paramyxovirusl Strain Name:UNKNO WN- AF079780lProt ein Name:hemaggl utininlGene Symbol:H	MDYHSHTTQTGSNETLYQDPLQSQSGSRD TLDGPPSTLQHYSNPPPYSEEDQGIDGPQR SQPLSTPHQYDRYYGVNIQHTRVYNHLGT IYKGLKLAFQILGWVSVIITMIITVTTLKKM SDGNSQDSAMKSLDENFDAIQEVANLLD NEVRPKLGVTMTQTTFQLPKELSEIKRYLL RLERNCPVCGTEATPQGSKNASGDTAFC PPCLTRQCSSEDSTHDQGPVGEVTSRHNKG KINFPHILQSDDCGRSDNLIVYSINLVPGLS FIQLPSGTHKCIIDVSYTFSDTLAGYLIVGG VDGCQLHNKAIHLYSLGYKTKMIYPPDYI AIATYTYDLVPLNRDCSIAVNQTSALAAICT SKKTKENQDFSTSGVHPFYIFLTNDGIFT VTVIEQSQLKLDYQYAALYPATGPGIFIGD HLVFLMWGGLMTKAEGDAYCQASGCND AHRTSCNIAQMPSAYGHRQLVNGLLMLPI KELGSHLIQPSLETISPKNWAGGHGRLYY NWEINTTYIYIEGKTWRSRPNLGIISWSKPL SIRWIDHSVARRPGARPCDSANDCPEDCL VGGYYDMFPMSSDYKTAITHPTHHQWPSS PALKLFNTNREVRVVMILRPPNNVKTTIS CIRIMQTNWCLGFIIFKEGNNAWGQIYSYI YQVESTCPNTK	1	124
AY5 906 88	6138- 7935	gb:AY590688: 6138- 7935Organism :Avian metapneumovir uslStrain	MEVKVENVGKSQELKVKVKNFIKRSDCK KKLFALILGLVSFELTMNIMLSVMYVESNE ALSLCRIQGTAPRDNKNTENATKETTLH TTTTTRDPEVRETKTKPQANEGATNPSR NLTTKGDKHQTTTRATTEAELEKQSKQTTE PGTSTQKHTPTRSSKSPTTTQAIAQLTTPT	1	125

		Name:Colorado lProtein Name:attachme nt glycoproteinlG ene Symbol:G	TPKASTAPKNRQATTKKKTETDTTASRAR NTNNPTETATTTTPKATTETGKSKEGPTQHT TKEQPETTAGETTTTQPRRTASRPAPTTKIE EEAETTKTRTTKSTQTSTGPPRPTGGAPSG AATEGSGRAAAAGGPPSAASAGGRRRTEA AAERDRRTRAGAGPTAGGARARTAAASE RGADTAGSAGGGPGDGATGGLSGGAPA EREDASGGTAAAGPGDGTEADGRAPPAA ALAGRTTESAAGAAGDSGRAGTAGWGSA ADGRSTGGNAAAEAGAAQSGRAAPRQPS GGTAPESTAPPNSGGSGRADAAPTEEVGV GSLWRGRYVCGPCGESVPEHPMNPFCG DGTAWICSDDGGSLPAGCYDGGTDGVVC CGVCGGNSCCGRVECTCGGGAGLLSCC CGSYSWS		
EU4 030 85	6620- 8593	gb:EU403085:6 620- 8593lOrganism :Avian paramyxovirus 3lStrain Name:APMV3/ PKT/Netherlan d/449/75lProtei n Name:hemaggl utinin- neuraminidase proteinlGene Symbol:HN	MESPPSGKDAPAFREPKRTRCLCYRATTL LNLTIIVLSHSIYVSTQTGANNSCVNPTIVT PDYLTGSTTGSVEDLADLESQLEIRRDGT INLPVQIDNTENLILTTLASINSNLRFLQNA TTESQTCCLSPVNDPRFVAGINRIPAGSMAY NDFSNLIEHVNFIPTTLTGCTRIPSFSLSK THWCYTHNVISNGCLDHAASSQYISIGIVD TGLNNEPYFRTMSSKSLNDGLNRKSCSVT AAANACWLLCSVVTEYEAADYRSRTPTA MVLGRFDFNGEYTEIAVPSSSLFDGRFASNY PGVGSQTQVNGTLYFPLYGGVLNGSDIET ANKGKSFRPQNPKNRCPDSEAIQSFRAQDS YYPTRFGKVLIIQQAIIACRISNKSCTDFYLL YFDNNRVMGAEARLYLNNQLYLYQR SSSWWPHPLFYISLPSQCALAVCQITEAH LTLTYATSRPGMSICTGASRCPNNCVDGV YTDVWPLTKNDAQDPNLFYTVYLNSTR RISPTISLYTYDRRIKSKLAVGSDIGAAAYT STCFGRSDTGAVYCLTIMETVNTIFGQYRI VPILLRVTSR	1	126
FJ97 756 8	6139- 7936	gb:FJ977568:6 139- 7936lOrganism :Avian metapneumovir uslStrain Name:aMPV/ MN/turkey/2a/ 97lProtein Name:attachme nt glycoproteinlG ene Symbol:G	MEVKVENVGKSQELKVKVKNFIKRSACK KKLFALILGLVSFELTMNIMLSVMYVESNE ALSLCRIQGTAPPRDNKTNTENATKETTLH TTTTTRDPEVRETKTKPKQANEGATNPSR NLTTKGDKHQTTTRATTEAELEKQSKQTTE PGTSTQKHTPARPSSKSPTTTQATAQPTTP TAPKASTAPKNRQATTKKKTETDTTASRA RNTNNPTETATTTTPKATTETGKGKEGPTQ HTTKEQPETTARETTTPQPRRTASRPAPTT KIEEEAETTKTRTTKNTQTSTGPPRPTRSTP SKTATENNKRTTTTKRPNTASTDSRQQTR TTAEQDQQTQTRAKPTTNGAHPQTTTTPE HNTDTTNSTKGSPKEDKTTRDPSSKTPTEQ EDASKGTAAANPGGSAEADRRAPPATTPT GRTTESAAGTTGDDSGAETTRRRSAADRR PTGGSTAAEAGTAQSGRATPKQPSGGTAA GNTAPPNNESSGRADAAPAEAEAGVGPSIR RGRHACGPRRESAEPHTNPCPGDGTAWT RSDGGGNLPAGRHDGADGAARRGARGG NPRRRGRAERTRGGGAGPPSCRCGSHNRS	1	127

HG934339	5997-7166	gb:HG934339:5997-7166 Organism:Avian metapneumovirus type D Strain Name:Turkey/1985/Fr85.1 Protein Name:attachment glycoprotein Gene Symbol:G	MGAKLYAISGASDAQLMKKTCAKLLEKV VPIILAVLGITGTTIALSISISIERAVLSDCT TQLRNGTTSGLSNPTRSTTSTA VTTTRDIR GLQTTRTRELKSCSNVQIAYGYLHDSSNP VLDSIGCLGLLALCESGPFQCRNYNPRDRP KCRCTLRGKDISCKEPPTAVTTSKTPPWG TEVHPTYPTQVTPQSQPATMAHQATATANQ RSSTTEPVGSQGNTTSSNPEQQTEPPSPQH PPTTTSQDQSTETADGQEHPTPKTPTATS NRRSPTPKRQETGRATPRNTATTQSGSSPP HSSPPGVDANMEGQCKELQAPKPNVCK GLDIYREALPRGCDKVLPLCKTSTIMCVD AYYSKPPICFGYNQRCFCMETFGPIEFCK S	1	128
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	MSKNKNQRTARTLEKTWDTLNHLIVISSC LYKLNLSIAQIALSVLAMIHSTSLIAAIIIFI SANHKVTLTTVTVQTIKNHTEKNITTYLTQ VSPERVSPSKQPTTTPPIHTNSATISPNTKSE IHHTTAQTKGRTSTPTQNNKPNTKPRPKNP PKKDDYHFEVFNFPVCSICGNNQLCKSICK TIPSNKPRKNQP	1	129
KX258200	6254-7996	gb:KX258200:6254-7996 Organism:Avian paramyxovirus 14 Strain Name:APMV14/duck/Japan/11OG0352/2011 Protein Name:hemagglutinin-neuraminidase protein Gene Symbol:HN	MEGSRTVIYQGDPNEKNTWRLVFRTLTLI LNLAILSVTIASIIHITSKITLSEVTTLKTEGVE EVITPLMATLSDSVQOEKMIYKEVAISIPLV LDKIQTVDVGTSAQITDALRQIQGVNGTQ AFALSNAPESYSGGIEVPLFQIDSFVNKSMIS SGLLEHASFIPSPTTLHGCTRIPSFHLGPRH WCYTHNIIGSRRCRDEGFSSMYISGAITVNR DGNPLFITASTILADDNNRKSCHASSYG CDLLCSIVTESENDDYANPNPTKMVHGRF LYNGSYVEQALPNSLFDQKWVAQYPGVG SGITTHGKVLFPPIYGGIKNTQLFYELSKY GFFAHNKELECKNMTEEQIRDIKAAAYLPS KTSGNLFAGQHHCNISKLGDCNVAVLNTS TTMMGAEGRLQMMGEYVYYYQRSSSWW PVGIVYKKSALAEMLNGINMEVLSFEPIPLS KFPRPTWTAGLCQKPSICPDVCVTGVYTD LFSVTIGSTTDKDTYFGVYLDATERKDP WVAAADQYEWNRNRVRLFESTTEAAYTTS TCFKNTVNNRVFCVSIVELRENLLGDWKI VPLLFQIGVSQGPPIPK	1	130
KX940961	7978-12504	gb:KX940961:7978-12504 Organism:Beilong virus Strain Name:ERN081008_1 Protein Name:attachment	MSQLAAHNLAMSNFYGTHQGDLSGSQKG EEQQVQGVIRYVSMIVGLLSLFTIHALNVT NIIYMTESGGTMQSIKTAQGSIDGSMREIS GVIMEDVKPKTDLINSMVSYNIPALQSMIH QIIKNDVLKQCTPSFMFNNTICPLAENPHS RYFEEVNLDSECSGPDMLGLGVNPEFI EFPSFAPGSTKPGSCVRLPSFSLSTTVFAYT HTIMGHGCSELDVGDHYFSVGRIADAGHE IPQFETISSWFINDKINRRSCTVAAGAMEA	1	131

		glycoprotein ene Symbol:G	WMGCVIMTETFYDDLNSLDTGKLTISYLD VFGRKKEWIYTRSEILYDYTYTSVYFSVGS GVVVGDTVYFLIWGSLSSPIEETAYCFAPD CSNYNQRMCEAQRPSKFGHRQMVNGIL KFKTTSTGKPLLSVGTLSPSVVPFGSEGR MYSEITKIIYLYLRSTSWHALPLTGLFVLGP PTSISWIVQRAVSRPGEFPCGASNRCPKDC VTGVYTDLFPLGSRYEYAATVYLNSETYR VNPTLALINQTNIIASKKVTTESQRAGYTT TTCFVFKLRVWCISVVELAPSTMAYEPI FLYQLDLTCKGKNGSLAMRFTGKEGTYS GRYKSPRNECFEKKVSNKYFIVSTPEGIQ PYEIRDLTPDRMPHIIMYISDVCAPALSAFK KLLPAMRPITTLTIGNWQFRPVEVSGGLRV SIRNLTKEGDLTMSAPEDPGSNTFPGGHI PGNGLFDAGYYTVEYPKEWKQTPPKPSEG GNIIDKNKTPVIPSRDNPTSDSSIPHRESIEP VRPTREVLKSSDYVTIVSTDGSGSGDFAT GVPWTGVSPKAPQNGINLPGTELPHPTVL DRINTPAPSDPKVSADSDHTRDTIDPTALS KPLNHDTTGDTRINTGTATYGFTPGRE ATSSGKLANDLTNSTSVSEAHPSASTSEA SKPEKNTDNRVTDQPTSGTAERPTTNAPV DGKHSTQLTDARPNTADPERTSQHSSSTTR DEVKPSLPSTTEASTHQRTEAATPELVNN 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 VVSVITLGLTAASLVYSMNAAPADFDGII PAVQQVGTSLTNSIGGMQDVLDRITYKQV ALESPLTLLNMESTIMNAITSLSYKINNGG NSSGCGAPIHDPEYIGGIGKELLIDNDVDV TSFYPSAFKEHLNFIPAPTTGAGCTRIPSFD LSATHYCYTHNVILSGCQDHS SHQYIAL GVLKLSDTGNVFFSTLRSINLDDTANRKSC SISATPLGCDILCSKVTELEEDYKSEETP MVHGRLSFDGTYSEKDLVDNNLFSWTA NYPVSGGGSYIGNRVWYAVYGGGLKPGSN TDQSQRDKYVIYKRYNNTCPDPEDYQINK AKSSYTPSYFGSKRVQQAILSIASPTLGSD PVLTPLSNDVVLMAEGRVMHIGGYTYL YQRGTSYYSPALLYPLNIQDKSATASSPYK FADFTRPGSVPCQADARCPQSCVTGVYTD PYPLIFAKDHSIRGVYGMMLNDVTARLNPI AAVFSNISRSQITRVSSSSTKAAYTTSTCFK VIKTNRIYCMSIAEISNTLFGEFRIVPLLVEI LSNGGNTARSAGGTPVKESPKGWSDAIAE PLFCTPTNVTRYNADIRRYAYSWP	1	132
NC_ 025 360	8127- 1015 8	gb:NC_025360: 8127- 10158 Organis m:Atlantic salmon paramyxovirus Strain Name:ASPV/Y	MPPAPSPVHDPSSFYGGSLFNEDTASRKGT SEEIHLGIRWNTVLIVLGLILAIIGIGIGASS FSASGITGNTTKEIRLIVEEMSYGLVRISDS VRQEISPKVTLLQNAVLSSIPALVTTETNTII NAVKNHCNSPPTPPPPTEAPLKKHETGMA PLDPTTYWTCTSGTFRFYSSPNATFIPGPSP LPHTATPGGCVRIPSMHIGSEIYAYTSNLIA SGCQDIGKSYQNVQIGVLDRTPEGNPEMS	1	133

	rkje371/95lProtein Name:hemagglutinin- neuraminidase proteinlGene Symbol:HN	PMLSHTFPINDNRKSCSIVTLKRAAYIYCS QPKVTEFVDYQTPGIEPMSLDHINANGTTK TWIYSPTEVVTDVPYASMYPSVSGSVVID GKLVFLVYGGLNGIQVPAMCLSPECPGID QAACNASQYNQYLSGRQVVNGIATVDLM NGQKPHISVETISPSKNWFGAEGRLVYMG GRLYIYIRSTGWHSPIQIGVIYTMNPLAITW VTNTVLSRPGSAGCDWNNRCPKACLSGV YTDAYPISPDYNHLATMILHSTSTRSNPVM VYSSPTNMVNYAQLTTTAQIAGYTTTSCF TDNEVGYCATALELTPGTLSSVQPILVMT KIPKECV		

ii) Lipid Fusogens

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
5 embodiments, the saturated fatty acids have longer-chain carboxylic acids. In some embodiments, the saturated fatty acids are mono-esters.

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,
10 glycerides, diacylglycerol, modified unsaturated fatty acids, and any combination thereof.

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
15 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).

Without wishing to be bound by theory, in some embodiments positive curvature lipids
20 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).

iii) Chemical fusogens

5 In some embodiments, the fusosome may be treated with fusogenic chemicals. In some embodiments, the fusogenic chemical is polyethylene glycol (PEG) or derivatives thereof.

10 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.

In some embodiments, the chemical fusogen is a positive cation. Some nonlimiting examples of positive cations include Ca^{2+} , Mg^{2+} , Mn^{2+} , Zn^{2+} , La^{3+} , Sr^{3+} , and H^{+} .

In some embodiments, the chemical fusogen binds to the target membrane by modifying surface polarity, which alters the hydration-dependent intermembrane repulsion.

15 In some embodiments, the chemical fusogen is a soluble lipid soluble. Some nonlimiting examples include oleoylglycerol, dioleoylglycerol, trioleoylglycerol, and variants and derivatives thereof.

In some embodiments, the chemical fusogen is a water-soluble chemical. Some nonlimiting examples include polyethylene glycol, dimethyl sulphoxide, and variants and derivatives thereof.

20 In some embodiments, the chemical fusogen is a small organic molecule. A nonlimiting example includes n-hexyl bromide.

In some embodiments, the chemical fusogen does not alter the constitution, cell viability, or the ion transport properties of the fusogen or target membrane.

25 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.

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
5 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.

iv) Small molecule fusogens

In some embodiments, the fusosome may be treated with fusogenic small molecules. Some
10 nonlimiting examples include halothane, nonsteroidal anti-inflammatory drugs (NSAIDs) such as meloxicam, piroxicam, tenoxicam, and chlorpromazine.

In some embodiments, the small molecule fusogen may be present in micelle-like aggregates or free of aggregates.

15 v) Fusogen modifications

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
20 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).

In some embodiments the fusogen is linked to an affinity tag. In some embodiments the affinity tag aids in fusosome separation and isolation. In some embodiments the affinity tag is
25 cleavable. In some embodiments the affinity tag is non-covalently linked to the fusogen. In some embodiments the affinity tag is present on the fusosome and separate from the fusogen.

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-I- (SEQ ID NO: 155)) 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.

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.

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; Vlieghe, 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.

III. Positive target cell-specific regulatory element

In some embodiments, a fusosome described herein, e.g. a virus, e.g., a retrovirus, contains a nucleic acid (e.g., the gene encoding the exogenous agent), e.g. a retroviral nucleic

acid, that comprises a positive target cell-specific regulatory element such as a tissue-specific promoter, a tissue-specific enhancer, a tissue-specific splice site, a tissue-specific site extending half-life of an RNA or protein, a tissue-specific mRNA nuclear export promoting site, a tissue-specific translational enhancing site, or a tissue-specific post-translational modification site.

5 In some embodiments, a fusosome, e.g. virus, e.g. retrovirus, described herein contains a nucleic acid, e.g. a retroviral nucleic acid, that can comprise regions, e.g., non-translated regions such as origins of replication, selection cassettes, promoters, enhancers, translation initiation signals (Shine Dalgarno sequence or Kozak sequence), introns, a polyadenylation sequence, 5' and 3' untranslated regions—which interact with host cellular proteins to carry out transcription
10 and translation, and which are capable of directing, increasing, regulating, or controlling the transcription or expression of an operatively linked polynucleotide. Such elements may vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including ubiquitous promoters and inducible promoters may be used.

15 In particular embodiments, control elements are capable of directing, increasing, regulating, or controlling the transcription or expression of an operatively linked polynucleotide in a cell-specific manner. In particular embodiments, a nucleic acid, e.g. retroviral nucleic acids, comprise one or more expression control sequences that are specific to particular cells, cell types, or cell lineages e.g., target cells; that is, expression of polynucleotides operatively linked to an
20 expression control sequence specific to particular cells, cell types, or cell lineages is expressed in target cells and not (or at a lower level) in non-target cells.

In particular embodiments, a nucleic acid, e.g. a retroviral nucleic acid, can include exogenous, endogenous, or heterologous control sequences such as promoters and/or enhancers.

In embodiments, the promoter comprises a recognition site to which an RNA polymerase
25 binds. An RNA polymerase initiates and transcribes polynucleotides operably linked to the promoter. In particular embodiments, promoters operative in mammalian cells comprise an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated and/or another sequence found 70 to 80 bases upstream from the start of transcription, a CNCAAT region where N may be any nucleotide.

30 In embodiments, an enhancer comprises a segment of DNA which contains sequences capable of providing enhanced transcription and in some instances can function independent of

orientation relative to another control sequence. An enhancer can function cooperatively or additively with promoters and/or other enhancer elements. In some embodiments, a promoter/enhancer segment of DNA contains sequences capable of providing both promoter and enhancer functions.

5 Illustrative ubiquitous expression control sequences include, but are not limited to, a cytomegalovirus (CMV) immediate early promoter, a viral simian virus 40 (SV40) (e.g., early or late), a Moloney murine leukemia virus (MoMLV) LTR promoter, a Rous sarcoma virus (RSV) LTR, a herpes simplex virus (HSV) (thymidine kinase) promoter, H5, P7.5, and P11 promoters from vaccinia virus, an elongation factor 1-alpha (EF1a) promoter, early growth response 1
10 (EGR1), ferritin H (FerH), ferritin L (FerL), Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), eukaryotic translation initiation factor 4A1 (EIF4A1), heat shock 70 kDa protein 5 (HSPA5), heat shock protein 90 kDa beta, member 1 (HSP90B1), heat shock protein 70 kDa (HSP70), β -kinesin (β -KIN), the human ROSA 26 locus Orions et al., *Nature Biotechnology* 25, 1477-1482 (2007)), a Ubiquitin C promoter (UBC), a phosphoglycerate kinase-1 (PGK)
15 promoter, a cytomegalovirus enhancer/chicken β -actin (CAG) promoter, a β -actin promoter and a myeloproliferative sarcoma virus enhancer, negative control region deleted, d1587rev primer-binding site substituted (MND) promoter (Challita et al., *J Virol.* 69(2):748-55 (1995)).

In some embodiments, a promoter may be paired with a heterologous gene to impart the regulatory functions of that promoter on the heterologous gene. In some embodiments, the cis-
20 regulatory elements from a first gene's promoter may be linked to segments of a different gene's promoter to create chimeric promoters that have properties of both promoters.

In some embodiments, the promoter is a tissue-specific promoter, e.g., a promoter that drives expression in CNS cells, e.g., pan-neuronal cells, GABAergic neurons, Glutamatergic neurons, Cholinergic neurons, Dopaminergic neurons, Serotonergic neurons, astrocytes,
25 microglia, oligodendrocytes, or choroid plexus cells. Various suitable CNS cell-specific promoters are described in Table 3 below. In some embodiments, a fusosome (e.g., viral vector) described herein comprises, in its nucleic acid, a promoter having a sequence of a promoter in Table 3, or transcriptionally active fragment thereof, or a variant having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto. In some embodiments, a fusosome
30 (e.g., viral vector) described herein comprises, in its nucleic acid, a promoter having transcription factor binding sites from the region within 3 kb of the transcriptional start site for the genes listed

in Table 3. In some embodiments, a fusosome (e.g., viral vector) described herein comprises, in its nucleic acid, a region within 2.5 kb, 2 kb, 1.5 kb, 1 kb, or 0.5 kb immediately upstream of the transcriptional start site of a gene listed in Table 3, or a transcriptionally active fragment thereof, or a variant having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

Table 3. Exemplary promoters, e.g., CNS cell-specific promoters

Target cell type	Exemplary promoters
Pan-neuronal	SYN, NSE, CaMKII, aTubulin, PDGF
GABAergic neurons	fSST, fNPY, GAD67, DLX5/6
Glutamatergic neurons	VGLUT1, Dock10
Cholinergic neurons	ChAT, VACHT
Dopaminergic neurons	Drd1a
Serotonergic neurons	TPH-2
Astrocytes	GFAP, EAAT1, GS
Microglia	CX3CR1, TMEM119
Oligodendrocytes	MBP, CNP
Choroid plexus	CRFR2 β

In some embodiments, the CNS cell-specific promoter is a promoter described in Hioki et al., Ther. 2007 Jun;14(11):872-82, herein incorporated by reference in its entirety, e.g., the CNS cell-specific promoter is a SYN, NSE, CaMKII, aTubulin, or PDGF promoter. In some embodiments, the CNS cell-specific promoter is a promoter described in Nathanson et al., Front. Neural Circuits, 2009, 3:19. doi: 10.3389/neuro.04.019.2009, herein incorporated by reference in its entirety, e.g., the CNS cell-specific promoter is a fSST or fNPY promoter. In some embodiments, the CNS cell-specific promoter is a promoter described in Delzor et al., Hum Gene Ther Methods. 2012 Aug;23(4):242-54, herein incorporated by reference in its entirety, e.g., the CNS cell-specific promoter is a GAD67 or DLX5/6 promoter. In some embodiments, the CNS cell-specific promoter is a promoter described in Egashira et al., Sci Rep. 2018 Oct 11;8(1):15156, herein incorporated by reference in its entirety, e.g., the CNS cell-specific promoter is a VGLUT1 or Dock10 promoter. In some embodiments, the CNS cell-specific promoter is a promoter described in Naciff et al., J. Neurochem., 1999 Jan;72(1):17-28, herein incorporated by reference in its entirety, e.g., the CNS cell-specific promoter is a ChAT

promoter. In some embodiments, the CNS cell-specific promoter is a VACHT promoter. In some embodiments, the CNS cell-specific promoter is a promoter described in Delzor et al., Hum Gene Ther Methods. 2012 Aug; 23(4): 242–254, herein incorporated by reference in its entirety, e.g., the CNS cell-specific promoter is a Drd1a promoter. In some embodiments, the CNS cell-specific promoter is a promoter described in Benzekroufa et al., Gene Ther. 2009 May;16(5):681-8, herein incorporated by reference in its entirety, e.g., the CNS cell-specific promoter is a TPH-2 promoter. In some embodiments, the CNS cell-specific promoter is a promoter described in Merienne et al., Gene Ther. 2015 Oct;22(10):830-9, herein incorporated by reference in its entirety, e.g., the CNS cell-specific promoter is a GFAP, EAAT1, or GS promoter. In some embodiments, the CNS cell-specific promoter is a promoter described in Immgen consortium, herein incorporated by reference in its entirety, e.g., the CNS cell-specific promoter is a CX3CR1 promoter. In some embodiments, the CNS cell-specific promoter is a TMEM119 promoter. In some embodiments, the CNS cell-specific promoter is a promoter described in McIver et al., J Neurosci Res. 2005 Nov 1;82(3):397-403, herein incorporated by reference in its entirety, e.g., the CNS cell-specific promoter is a MBP promoter. In some embodiments, the CNS cell-specific promoter is a promoter described in Kagiava et al., J Gene Med. 2014 Nov-Dec;16(11-12):364-73, herein incorporated by reference in its entirety, e.g., the CNS cell-specific promoter is a MBP or CNP promoter. In some embodiments, the CNS cell-specific promoter is a promoter described in Regev et al., Proc Natl Acad Sci U S A. 2010 Mar 2;107(9):4424-9, herein incorporated by reference in its entirety, e.g., the CNS cell-specific promoter is a CRFR2 β promoter. In some embodiments, the CNS cell-specific promoter is a transcriptionally active fragment of any of the foregoing. In some embodiments, the CNS-cell specific promoter is a variant having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to any of the foregoing.

An internal ribosome entry site (IRES) typically promotes direct internal ribosome entry to the initiation codon, such as ATG, of a cistron (a protein encoding region), thereby leading to the cap-independent translation of the gene. See, e.g., Jackson et al, (1990) Trends Biochem Sci 15(12):477-83) and Jackson and Kaminski. (1995) RNA 1 (10):985-1000. In particular embodiments, a vector includes one or more exogenous genes encoding one or more exogenous agents. In particular embodiments, to achieve efficient translation of each of the plurality of

exogenous protein agents, the polynucleotide sequences can be separated by one or more IRES sequences or polynucleotide sequences encoding self-cleaving polypeptides.

The nucleic acid, e.g. retroviral nucleic acids herein, can also comprise one or more Kozak sequences, e.g., a short nucleotide sequence that facilitates the initial binding of mRNA to the small subunit of the ribosome and increases translation. The consensus Kozak sequence is (GCC)RCCATGG, where R is a purine (A or G) (Kozak, (1986) Cell. 44(2):283-92, and Kozak, (1987) Nucleic Acids Res. 15(20): 8125-48).

Promoters responsive to a heterologous transcription factor and inducer

In some embodiments, a nucleic acid, retroviral nucleic acid, comprises an element allowing for conditional expression of the exogenous agent, e.g., any type of conditional expression including, but not limited to, inducible expression; repressible expression; cell type-specific expression, or tissue-specific expression. In some embodiments, to achieve conditional expression of the exogenous agent, expression is controlled by subjecting a cell, tissue, or organism to a treatment or condition that causes the exogenous agent to be expressed or that causes an increase or decrease in expression of the exogenous agent.

Illustrative examples of inducible promoters/systems include, but are not limited to, steroid-inducible promoters such as promoters for genes encoding glucocorticoid or estrogen receptors (inducible by treatment with the corresponding hormone), metallothionine promoter (inducible by treatment with various heavy metals), MX-1 promoter (inducible by interferon), the “GeneSwitch” mifepristone-regulatable system (Sirin et al., 2003, *Gene*, 323:67), the cumate inducible gene switch (WO 2002/088346), tetracycline-dependent regulatory systems, etc.

Transgene expression may be activated or repressed by the presence or absence of an inducer molecule. In some cases the inducer molecule activates or represses gene expression in a graded manner, and in some cases the inducer molecules activates or represses gene expression in an all-or-nothing manner.

A commonly used inducible promoter/system is tetracycline (Tet)-regulated system. The Tet system is based on the coexpression of two elements in the respective target cell: (i) the tetracycline response element containing repeats of the Tet-operator sequences (TetO) fused to a minimal promoter and connected to a gene of interest (e.g., a gene encoding the exogenous agent) and (ii) the transcriptional transactivator (tTA), a fusion protein of the Tet-repressor

(TetR) and the transactivation domain of the herpes simplex virus derived VP16 protein.

Whereas in the originally described version, transgene expression was active in the absence of tetracycline or its potent analogue doxycycline (Dox), referred to as Tet-OFF system, modification of four amino acids within the transactivator protein resulted in a reverse tTA (rtTA), which only
 5 binds to TetO in the presence of Dox (Tet-ON system). In some embodiments, in the transactivator, the VP16 domain has been replaced by minimal activation domains, potential splice-donor and splice acceptor sites have been removed, and the protein has been codon optimization, resulting in the improved Transactivator variant rtTA2S-M2 with higher sensitivity to Dox and lower baseline activity. Furthermore, different Tet-responsive promoter elements
 10 have been generated, including modification in the TetO with 36-nucleotide spacing from neighboring operators to enhance regulation. Additional modifications may be useful to further reduce basal activity and increase the expression dynamic range. As an example, the pTet-T11 (short: T11) variant displays a high dynamic range and low background activity.

Conditional expression can also be achieved by using a site specific DNA recombinase.
 15 According to certain embodiments, the nucleic acid, e.g. retroviral nucleic acid, comprises at least one (typically two) site(s) for recombination mediated by a site specific recombinase, e.g., an excisive or integrative protein, enzyme, cofactor or associated protein that is involved in recombination reactions involving one or more recombination sites (e.g., two, three, four, five, seven, ten, twelve, fifteen, twenty, thirty, fifty, etc.), which may be wild-type proteins (see
 20 Landy, Current Opinion in Biotechnology 3:699-707 (1993)), or mutants, derivatives (e.g., fusion proteins containing the recombination protein sequences or fragments thereof), fragments, and variants thereof. Illustrative examples of recombinases include, but are not limited to: Cre, Int, IHF, Xis, Flp, Fis, Hin, Gin, Φ C31, Cin, Tn3 resolvase, TndX, XerC, XerD, TnpX, Hjc, Gin, SpCCE1, and ParA.

Riboswitches to regulate exogenous agent expression

Some of the compositions and methods provided herein include one or more riboswitches or polynucleotides that include one or more riboswitch. Riboswitches are a common feature in bacteria to regulate gene expression and are a means to achieve RNA control of biological
 30 functions. Riboswitches can be present in the 5'-untranslated region of mRNAs and can allow for regulatory control over gene expression through binding of a small molecule ligand that induces

or suppresses a riboswitch activity. In some embodiments, the riboswitch controls a gene product involved in the generation of the small molecule ligand. Riboswitches typically act in a cis-fashion, although riboswitches have been identified that act in a trans-fashion. Natural riboswitches consist of two domains: an aptamer domain that binds the ligand through a three-dimensional folded RNA structure and a function switching domain that induces or suppresses an activity in the riboswitch based on the absence or presence of the ligand. Thus, there are two ligand sensitive conformations achieved by the riboswitch, representing on and off states (Garst et al., 2011). The function switching domain can affect the expression of a polynucleotide by regulating: an internal ribosome entry site, pre-mRNA splice donor accessibility in the retroviral gene construct, translation, termination of transcription, transcript degradation, miRNA expression, or shRNA expression (Dambach and Winkler 2009). The aptamer and function switching domains can be used as modular components allowing for synthetic RNA devices to control gene expression either as native aptamers, mutated/evolved native aptamers, or totally synthetic aptamers that are identified from screening random RNA libraries (McKeague et al 2016).

The purine riboswitch family represents one of the largest families with over 500 sequences found (Mandal et al 2003; US20080269258; and WO2006055351). The purine riboswitches share a similar structure consisting of three conserved helical elements/stem structures (P1, P2, P3) with intervening loop/junction elements (J1-2, L2, J2-3, L3, J3-1). The aptamer domains of the purine family of riboswitches naturally vary in their affinity/regulation by various purine compounds such as adenine, guanine, adenosine, guanosine, deoxyadenosine, deoxyguanosine, etc. due to sequence variation (Kim et al. 2007)

In some embodiments, a nucleic acid, e.g. retroviral nucleic acid, described herein comprises a polynucleotide encoding the exogenous agent operably linked to a promoter and a riboswitch. The riboswitch include one or more of, e.g., all of: a.) an aptamer domain, e.g., an aptamer domain capable of binding a nucleoside analogue antiviral drug and having reduced binding to guanine or 2'-deoxyguanosine relative to the nucleoside analogue antiviral drug; and b.) a function switching domain, e.g., a function switching domain capable of regulating expression of the exogenous agent, wherein binding of the nucleoside analogue by the aptamer domain induces or suppresses the expression regulating activity of the function switching domain, thereby regulating expression of the exogenous agent. In some embodiments, the

exogenous agent can be a polypeptide, an miRNA, or an shRNA. For example, in an embodiment, the riboswitch is operably linked to a nucleic acid encoding a chimeric antigen receptor (CAR). In non-limiting illustrative examples provided herein, the exogenous gene encodes one or more engineered signaling polypeptides. For instance, the riboswitch and the target polynucleotide encoding one or more engineered signaling polypeptides can be found in the genome of a source cell, in a replication incompetent recombinant retroviral particle, in a T cell and/or in an NK cell.

The aptamer domains can be used, e.g., as modular components and combined with any of the function switching domains to affect the RNA transcript. In any of the embodiments disclosed herein, the riboswitch can affect the RNA transcript by regulating any of the following activities: internal ribosomal entry site (IRES), pre-mRNA splice donor accessibility, translation, termination of transcription, transcript degradation, miRNA expression, or shRNA expression. In some embodiments, the function switching domain can control binding of an anti-IRES to an IRES (see, e.g. Ogawa, RNA (2011), 17:478- 488, the disclosure of which is incorporated by reference herein in its entirety). In any of the embodiments disclosed herein, the presence or absence of the small molecule ligand can cause the riboswitch to affect the RNA transcript. In some embodiments, the riboswitch can include a ribozyme. Riboswitches with ribozymes can inhibit or enhance transcript degradation of target polynucleotides in the presence of the small molecule ligand. In some embodiments, the ribozyme can be a pistol class of ribozyme, a hammerhead class of ribozyme, a twisted class of ribozyme, a hatchet class of ribozyme, or the HDV (hepatitis delta virus).

IV. Non-target cell-specific regulatory element

In some embodiments, the non-target cell specific regulatory element or negative TCSRE comprises a tissue-specific miRNA recognition sequence, tissue-specific protease recognition site, tissue-specific ubiquitin ligase site, tissue-specific transcriptional repression site, or tissue-specific epigenetic repression site.

In some embodiments, a non-target cell comprises an endogenous miRNA. In some embodiments, a fusosome described herein, e.g. a virus, e.g., a retrovirus, contains a nucleic acid, e.g. retroviral nucleic acid (e.g., the gene encoding the exogenous agent) that may comprise a recognition sequence for that miRNA. Thus, if the nucleic acid, retroviral nucleic acid, enters

the non-target cell, the miRNA can downregulate expression of the exogenous agent. This helps achieve additional specificity for the target cell versus non-target cells.

In some embodiments, the miRNA is a small non-coding RNAs of 20-22 nucleotides, typically excised from ~70 nucleotide foldback RNA precursor structures known as pre-miRNAs.

5 In general, miRNAs negatively regulate their targets in one of two ways depending on the degree of complementarity between the miRNA and the target. First, miRNAs that bind with perfect or nearly perfect complementarity to protein-coding mRNA sequences typically induce the RNA-mediated interference (RNAi) pathway. miRNAs that exert their regulatory effects by binding to imperfect complementary sites within the 3' untranslated regions (UTRs) of their mRNA targets, 10 typically repress target-gene expression post-transcriptionally, apparently at the level of translation, through a RISC complex that is similar to, or possibly identical with, the one that is used for the RNAi pathway. Consistent with translational control, miRNAs that use this mechanism reduce the protein levels of their target genes, but the mRNA levels of these genes are only minimally affected. miRNAs (e.g., naturally occurring miRNAs or artificially designed 15 miRNAs) can specifically target any mRNA sequence. For example, in one embodiment, the skilled artisan can design short hairpin RNA constructs expressed as human miRNA (e.g., miR-30 or miR-21) primary transcripts. This design adds a Drosha processing site to the hairpin construct and has been shown to greatly increase knockdown efficiency (Pusch et al., 2004). The hairpin stem consists of 22-nt of dsRNA (e.g., antisense has perfect complementarity to desired 20 target) and a 15-19-nt loop from a human miR. Adding the miR loop and miR30 flanking sequences on either or both sides of the hairpin results in greater than 10-fold increase in Drosha and Dicer processing of the expressed hairpins when compared with conventional shRNA designs without microRNA. Increased Drosha and Dicer processing translates into greater siRNA/miRNA production and greater potency for expressed hairpins.

25 Hundreds of distinct miRNA genes are differentially expressed during development and across tissue types. Several studies have suggested important regulatory roles for miRNAs in a broad range of biological processes including developmental timing, cellular differentiation, proliferation, apoptosis, oncogenesis, insulin secretion, and cholesterol biosynthesis. (See Bartel 2004 Cell 116:281-97; Ambros 2004 Nature 431 :350-55; Du et al. 2005 Development 30 132:4645-52; Chen 2005 N. Engl. J. Med. 353:1768-71; Krutzfeldt et al. 2005 Nature 438:685-89.) Molecular analysis has shown that miRNAs have distinct expression profiles in different

tissues. Computational methods have been used to analyze the expression of approximately 7,000 predicted human miRNA targets. The data suggest that miRNA expression broadly contributes to tissue specificity of mRNA expression in many human tissues. (See Sood et al. 2006 PNAS USA 103(8):2746-51.)

5 Thus, an miRNA-based approach may be used for restricting expression of the exogenous agent to a target cell population by silencing exogenous agent expression in non-target cell types by using endogenous microRNA species. MicroRNA induces sequence-specific post-transcriptional gene silencing in many organisms, either by inhibiting translation of messenger RNA (mRNA) or by causing degradation of the mRNA. See, e.g., Brown et al. 2006 Nature
10 Med. 12(5):585-91., and WO2007/000668, each of which is herein incorporated by reference in its entirety. In some embodiments, the nucleic acid, e.g. retroviral nucleic acid, comprises one or more of (e.g., a plurality of) tissue-specific miRNA recognition sequences. In some
15 embodiments, the tissue-specific miRNA recognition sequence is about 20-25, 21-24, or 23 nucleotides in length. In embodiments, the tissue-specific miRNA recognition sequence has perfect complementarity to an miRNA present in a non-target cell. In some embodiments, the
exogenous agent does not comprise GFP, e.g., does not comprise a fluorescent protein, e.g., does not comprise a reporter protein. In some embodiments, the off-target cells are not hematopoietic cell and/or the miRNA is not present in hematopoietic cells.

 In some embodiments, a method herein comprises tissue-specific expression of an
20 exogenous agent in a target cell comprising contacting a plurality of fusosomes, e.g. a virus, e.g. retroviral vectors, comprising a nucleotide encoding the exogenous agent and at least one tissue-specific microRNA (miRNA) target sequence with a plurality of cells comprising target cells and non-target cells, wherein the exogenous agent is preferentially expressed in, e.g., restricted, to the target cell.

25 For example, the nucleic acid, e.g. retroviral nucleic acid, can comprise at least one miRNA recognition sequence operably linked to a nucleotide sequence having a corresponding miRNA in a non-target cell, e.g., a hematopoietic progenitor cell (HSPC), hematopoietic stem cell (HSC), which prevents or reduces expression of the nucleotide sequence in the non-target cell but not in a target cell, e.g., differentiated cell. In some embodiments, the nucleic acid, e.g.
30 retroviral nucleic acid, comprises at least one miRNA sequence target for a miRNA which is present in an effective amount (e.g., concentration of the endogenous miRNA is sufficient to

reduce or prevent expression of a transgene) in the non-target cell, and comprises a transgene. In
embodiments, the miRNA used in this system is strongly expressed in non-target cells, such as
HSPC and HSC, but not in differentiated progeny of e.g. the myeloid and lymphoid lineage,
preventing or reducing expression of a transgene in sensitive stem cell populations, while
5 maintaining expression and therapeutic efficacy in the target cells.

In some embodiments, the negative TSCRE or NTSCRE comprises an miRNA
recognition site. Exemplary miRNAs are provided in Table 4 below. In some embodiments, the
nucleic acid (e.g., fusosome nucleic acid or retroviral nucleic acid) comprises a sequence that is
complementary to a miRNA of Table 4, or has at least 50%, 60%, 70%, 75%, 80%, 85%, 90%,
10 95%, 96%, 97%, 98%, or 99% complementarity thereto. In some embodiments, the nucleic acid
(e.g., fusosome nucleic acid or retroviral nucleic acid) comprises a sequence that is perfectly
complementary to a seed sequence within an endogenous miRNA, e.g., miRNA of Table 4. In
some embodiments, the miRNA comprises the sequence set forth in any one of SEQ ID NOS:
156-162. In embodiments, the seed sequence is at least 6, 7, 8, 9, or 10 nucleotides in length.

15

Table 4. Exemplary miRNAs.

Target cell type	miRNA name and silenced cell type (non-target cell type)	SEQUENCE	SEQ ID NO
Pan-neuronal	miR-338-3p (for de-targeting from oligodendrocytes); miR-9 and miR-125b-5p (for de-targeting from astrocytes); miR-342-3p (for de-targeting from microglia)	miR-338-3p: uccagcaucagauuuuguug	156
GABAergic neurons		miR-9-5p: ucuuugguuauacuagcuguauga	157
Glutamatergic neurons		miR-9-3p: auaaagcuagauaaccgaaagu	158
Cholinergic neurons		miR-125b-5p: ucccugagaccuaacuuguga	159
Dopaminergic neurons		miR-342-3p: ucucacacagaaaucgcacccgu	160
Serotonergic neurons			
Astrocytes	miR-124 (for de-targeting from neuronal lineage)	miR-124-3p: uaaggcacgcggugaauGCCAA	161
Microglia		miR-124-5p: cguguucacagcggaccuugau	162
Oligodendrocytes			
Choroid plexus			

In some embodiments, the negative TSCRE or NTSCRE comprises an miRNA recognition site for an miRNA described herein. Exemplary miRNAs include those found in Butovsky et al., Nat Neurosci. 2014 Jan;17(1):131-43, herein incorporated by reference in its entirety, e.g., miR-338-3p, miR-9, miR-125b-5p, or miR-342-3p. Additional exemplary miRNAs can be found in Delzor et al., Curr. Drug Targets, 2013 Oct;14(11):1336-46, herein incorporated by reference in its entirety, e.g., miR-124.

In some embodiments, a fusosome described herein comprises a nucleic acid comprising a payload gene and a positive target cell-specific regulatory element, e.g., wherein the target cell is a neuron, e.g., a pan-neuronal cell, a GABAergic neuron, a Glutamatergic neuron, a Cholinergic neuron, a Dopaminergic neuron, or a Serotonergic neuron. In some embodiments, the nucleic acid further comprises a non-target cell-specific regulatory element (NTCSRE), e.g.,

wherein the NTSCRE comprises an miRNA recognition site for an miRNA expressed in a glial cell, e.g., an astrocyte, a microglial cell, or an oligodendrocyte.

In some embodiments, a fusosome described herein comprises a nucleic acid comprising a payload gene and a positive target cell-specific regulatory element, e.g., wherein the target cell is a glial cell, e.g., an astrocyte, a microglial cell, or an oligodendrocyte. In some embodiments, the nucleic acid further comprises a non-target cell-specific regulatory element (NTCSRE), e.g., wherein the NTSCRE comprises an miRNA recognition site for an miRNA expressed in a neuron, e.g., a pan-neuronal cell, a GABAergic neuron, a Glutamatergic neuron, a Cholinergic neuron, a Dopaminergic neuron, or a Serotonergic neuron.

In some embodiments, the negative TSCRE or NTSCRE comprises an miRNA recognition site for an miRNA described herein. Exemplary miRNAs include those found in Griffiths-Jones et al. *Nucleic Acids Res.* 2006 Jan 1, 34; Chen and Lodish, *Semin Immunol.* 2005 Apr;17(2):155-65; Chen et al. *Science.* 2004 Jan 2;303(5654):83-6; Barad et al. *Genome Res.* 2004 Dec; 14(12): 2486–2494; Krichevsky et al., *RNA.* 2003 Oct;9(10):1274-81; Kasashima et al. *Biochem Biophys Res Commun.* 2004 Sep 17;322(2):403-10; Houbaviy et al., *Dev Cell.* 2003 Aug;5(2):351-8; Lagos-Quintana et al., *Curr Biol.* 2002 Apr 30;12(9):735-9; Calin et al., *Proc Natl Acad Sci U S A.* 2004 Mar 2;101(9):2999-3004; Sempere et al. *Genome Biol.* 2004; 5(3): R13; Metzler et al., *Genes Chromosomes Cancer.* 2004 Feb;39(2):167-9; Calin et al., *Proc Natl Acad Sci U S A.* 2002 Nov 26;99(24):15524-9; Mansfield et al. *Nat Genet.* 2004 Oct;36(10):1079-83; Michael et al. *Mol Cancer Res.* 2003 Oct;1(12):882-91; and at www.miRNA.org.

In some embodiments, the negative TSCRE or NTSCRE comprises an miRNA recognition site for an miRNA selected from miR-1b, miR-189b, miR-93, miR-125b, miR-130, miR-32, miR-128, miR-22, miR124a, miR-296, miR-143, miR-15, miR-141, miR-143, miR-16, miR-127, miR99a, miR-183, miR-19b, miR-92, miR-9, miR-130b, miR-21, miR-30b, miR-16, miR-99a, miR-212, miR-30c, miR-213, miR-20, miR-155, miR-152, miR-139, miR-30b, miR-7, miR-30c, miR-18, miR-137, miR-219, miR-1d, miR-178, miR-24, miR-122a, miR-215, miR-124a, miR-190, miR-149, miR-193, let-7a, miR-132, miR-27a, miR-9*, miR-200b, miR-266, miR-153, miR-135, miR-206, miR-24, miR-19a, miR-199, miR-26a, miR-194, miR-125a, miR-15a, miR-145, miR-133, miR-96, miR-131, miR-124b, miR-151, miR-7b, miR-103, and miR-208.

In some embodiments, the nucleic acid (e.g., retroviral nucleic acid) comprises two or more miRNA recognition sites. In some embodiments, the first miRNA recognition site and second miRNA recognition site are recognized by the same miRNA, and in some embodiments, the first miRNA recognition site and second miRNA recognition site are recognized by different
5 miRNAs. In some embodiments, the first miRNA recognition site and second miRNA recognition site are recognized by miRNAs present in the same non-target cell, and in some embodiments, the first miRNA recognition site and second miRNA recognition site are recognized by miRNAs present in different non-target cells. In some embodiments, one or both of the first miRNA recognition site and second miRNA recognition site are recognized by
10 miRNAs of Table 4. In some embodiments, one or more of the miRNA recognition sites on the fusosome nucleic acid (e.g. retroviral nucleic acid) are transcribed in *cis* with the exogenous agent. In some embodiments, one or more of the miRNA recognition sites on the fusosome nucleic acid (e.g., retroviral nucleic acid) are situated downstream of the poly A tail sequence, e.g., between the poly A tail sequence and the WPRE. In some embodiments, one or more of the
15 miRNA recognition sites on the fusosome nucleic acid (e.g., retroviral nucleic acid) are situated downstream of the WPRE.

V. Immune modulation

In some embodiments, a fusosome, e.g. retroviral vector, or VLP, described herein comprises elevated CD47. See, e.g., US Pat. 9,050,269, which is herein incorporated by
20 reference in its entirety. In some embodiments, a fusosome, e.g. a retroviral vector or VLP, described herein comprises elevated Complement Regulatory protein. See, e.g., ES2627445T3 and US6790641, each of which is incorporated herein by reference in its entirety. In some embodiments, a fusosome, e.g. a retroviral vector, or VLP, described herein lacks or comprises reduced levels of an MHC protein, e.g., an MHC-1 class 1 or class II. See, e.g.,
25 US20170165348, which is herein incorporated by reference in its entirety.

Sometimes fusosomes, e.g. retroviral vectors, or VLPs, are recognized by the subject's immune system. In the case of enveloped viral vector particles (e.g., retroviral vector particles), membrane-bound proteins that are displayed on the surface of the viral envelope may be recognized and the viral particle itself may be neutralised. Furthermore, on infecting a target cell,
30 the viral envelope becomes integrated with the cell membrane and as a result viral envelope proteins may become displayed on or remain in close association with the surface of the cell. The

immune system may therefore also target the cells which the viral vector particles have infected. Both effects may lead to a reduction in the efficacy of exogenous agent delivery by viral vectors.

A viral particle envelope typically originates in a membrane of the source cell. Therefore, membrane proteins that are expressed on the cell membrane from which the viral particle buds
5 may be incorporated into the viral envelope.

The immune modulating protein CD47

The internalization of extracellular material into cells is commonly performed by a process called endocytosis (Rabinovitch, 1995, Trends Cell Biol. 5(3):85-7; Silverstein, 1995,
10 Trends Cell Biol. 5(3):141-2; Swanson et al., 1995, Trends Cell Biol. 5(3):89-93; Allen et al., 1996, J. Exp. Med. 184(2):627-37). Endocytosis may fall into two general categories: phagocytosis, which involves the uptake of particles, and pinocytosis, which involves the uptake of fluid and solutes.

Professional phagocytes have been shown to differentiate from non-self and self, based
15 on studies with knockout mice lacking the membrane receptor CD47 (Oldenborg et al., 2000, Science 288(5473):2051-4). CD47 is a ubiquitous member of the Ig superfamily that interacts with the immune inhibitory receptor SIRP α (signal regulatory protein) found on macrophages (Fujioka et al., 1996, Mol. Cell. Biol. 16(12):6887-99; Veillette et al., 1998, J. Biol. Chem. 273(35):22719-28; Jiang et al., 1999, J. Biol. Chem. 274(2):559-62). Although CD47-SIRP α
20 interactions appear to deactivate autologous macrophages in mouse, severe reductions of CD47 (perhaps 90%) are found on human blood cells from some Rh genotypes that show little to no evidence of anemia (Mouro-Chanteloup et al., 2003, Blood 101(1):338-344) and also little to no evidence of enhanced cell interactions with phagocytic monocytes (Arndt et al., 2004, Br. J. Haematol. 125(3):412-4).

In some embodiments, a fusosome, e.g. a retroviral vector, or VLP (e.g., a viral particle having a radius of less than about 1 μm , 400 nm, or 150 nm), comprises at least a biologically active portion of CD47, e.g., on an exposed surface of the fusosome, e.g. retroviral vector, or VLP. In some embodiments, the fusosome, e.g. retroviral vector (e.g., lentivirus), or VLP, includes a lipid coat. In embodiments, the amount of the biologically active CD47 in the
30 fusosome, e.g. retroviral vector, or VLP, is between about 20-250, 20-50, 50-100, 100-150, 150-200, or 200-250 molecules/ μm^2 . In some embodiments, the CD47 is human CD47.

A method described herein can comprise evading phagocytosis of a particle by a phagocytic cell. The method may include expressing at least one peptide including at least a biologically active portion of CD47 in a fusosome, e.g. a retroviral vector, or VLP, so that, when the fusosome, e.g. retroviral vector, or VLP, comprising the CD47 is exposed to a phagocytic cell, the fusosome, e.g. viral particle, evades phagocytosis by the phagocytic cell, or shows decreased phagocytosis compared to an otherwise similar unmodified fusosome, e.g. retroviral vector, or VLP. In some embodiments, the half-life of the fusosome, e.g. retroviral vector, or VLP, in a subject is extended compared to an otherwise similar unmodified fusosome, e.g. retroviral vector, or VLP.

MHC deletion

The major histocompatibility complex class I (MHC-I) is a host cell membrane protein that can be incorporated into viral envelopes and, because it is highly polymorphic in nature, it is a major target of the body's immune response (McDevitt H. O. (2000) *Annu. Rev. Immunol.* 18: 1-17). MHC-I molecules exposed on the plasma membrane of source cells can be incorporated in the viral particle envelope during the process of vector budding. These MHC-I molecules derived from the source cells and incorporated in the viral particles can in turn be transferred to the plasma membrane of target cells. Alternatively, the MHC-I molecules may remain in close association with the target cell membrane as a result of the tendency of viral particles to absorb and remain bound to the target cell membrane.

The presence of exogenous MHC-I molecules on or close to the plasma membrane of transduced cells may elicit an alloreactive immune response in subjects. This may lead to immune-mediated killing or phagocytosis of transduced cells either upon ex vivo gene transfer followed by administration of the transduced cells to the subject, or upon direct in vivo administration of the viral particles. Furthermore, in the case of in vivo administration of MHC-I bearing viral particles into the bloodstream, the viral particles may be neutralised by pre-existing MHC-I specific antibodies before reaching their target cells.

Accordingly, in some embodiments, a source cell is modified (e.g., genetically engineered) to decrease expression of MHC-I on the surface of the cell. In embodiments, the source comprises a genetically engineered disruption of a gene encoding β 2-microglobulin (β 2M). In embodiments, the source cell comprises a genetically engineered disruption of one or

more genes encoding an MHC-I α chain. The cell may comprise genetically engineered disruptions in all copies of the gene encoding β 2-microglobulin. The cell may comprise genetically engineered disruptions in all copies of the genes encoding an MHC-I α chain. The cell may comprise both genetically engineered disruptions of genes encoding β 2-microglobulin and genetically engineered disruptions of genes encoding an MHC-I α chain. In some embodiments, the retroviral vector or VLP comprises a decreased number of surface-exposed MHC-I molecules. The number of surface-exposed MHC-I molecules may be decreased such that the immune response to the MHC-I is decreased to a therapeutically relevant degree. In some embodiments, the enveloped viral vector particle is substantially devoid of surface-exposed MHC-I molecules.

HLA-G/E overexpression

In some embodiments, a retroviral vector or VLP displays on its envelope a tolerogenic protein, e.g., an ILT-2 or ILT-4 agonist, e.g., HLA-E or HLA-G or any other ILT-2 or ILT-4 agonist. In some embodiments, a retroviral vector or VLP has increased expression of HLA-E, HLA-G, ILT-2 or ILT-4 compared to a reference retrovirus, e.g., an unmodified retrovirus otherwise similar to the retrovirus.

In some embodiments, a retrovirus composition has decreased MHC Class I compared to an unmodified retrovirus and increased HLA-G compared to an unmodified retrovirus.

In some embodiments, the retroviral vector or VLP 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 retrovirus, e.g., an unmodified retrovirus otherwise similar to the retrovirus, wherein expression of HLA-G or HLA-E is assayed *in vitro* using flow cytometry, e.g., FACS.

In some embodiments, the retrovirus with increased HLA-G expression demonstrates reduced immunogenicity, e.g., as measured by reduced immune cell infiltration, in a teratoma formation assay.

Complement regulatory proteins

Complement activity is normally controlled by a number of complement regulatory proteins (CRPs). These proteins prevent spurious inflammation and host tissue damage. One

group of proteins, including CD55/ decay accelerating factor (DAF) and CD46/membrane cofactor protein (MCP), inhibits the classical and alternative pathway C3/C5 convertase enzymes. Another set of proteins including CD59 regulates MAC assembly. CRPs have been used to prevent rejection of xenotransplanted tissues and have also been shown to protect viruses and viral vectors from complement inactivation.

Membrane resident complement control factors include, e.g., decay-accelerating factor (DAF) or CD55, factor H (FH)-like protein-1 (FHL-1), C4b-binding protein (C4BP), Complement receptor 1 (CD35), membrane cofactor protein (MCP) or CD46, and CD59 (protectin) (e.g., to prevent the formation of membrane attack complex (MAC) and protect cells from lysis).

Albumin binding protein

In some embodiments the lentivirus binds albumin. In some embodiments the lentivirus comprises on its surface a protein that binds albumin. In some embodiments the lentivirus comprises on its surface an albumin binding protein. In some embodiments the albumin binding protein is streptococcal Albumin Binding protein. In some embodiments the albumin binding protein is streptococcal Albumin Binding Domain.

Expression of non-fusogen proteins on the lentiviral envelope

In some embodiments the lentivirus is engineered to comprise one or more proteins on its surface. In some embodiments the proteins affect immune interactions with a subject. In some embodiments the proteins affect the pharmacology of the lentivirus in the subject. In some embodiments the protein is a receptor. In some embodiments the protein is an agonist. In some embodiments the protein is a signaling molecule. In some embodiments, the protein on the lentiviral surface comprises OKT3 or IL7.

In some embodiments, comprises a mitogenic transmembrane protein and/or a cytokine-based transmembrane protein is present in the source cell, which can be incorporated into the retrovirus when it buds from the source cell membrane. The mitogenic transmembrane protein and/or a cytokine-based transmembrane protein can be expressed as a separate cell surface molecule on the source cell rather than being part of the viral envelope glycoprotein.

Exemplary Features

In some embodiments of any of the aspects described herein, the fusosome, e.g. retroviral vector, VLP, or pharmaceutical composition is substantially non-immunogenic. Immunogenicity can be quantified, e.g., as described herein.

5 In some embodiments, a retroviral vector or VLP fuses with a target cell to produce a recipient cell. In some embodiments, a recipient cell that has fused to one or more retroviral vectors or VLPs is assessed for immunogenicity. In embodiments, a recipient cell is analyzed for the presence of antibodies on the cell surface, e.g., by staining with an anti-IgM antibody. In other embodiments, immunogenicity is assessed by a PBMC cell lysis assay. In embodiments, a recipient cell is incubated with peripheral blood mononuclear cells (PBMCs) and then assessed for lysis of the cells by the PBMCs. In other embodiments, immunogenicity is assessed by a natural killer (NK) cell lysis assay. In embodiments, a recipient cell is incubated with NK cells and then assessed for lysis of the cells by the NK cells. In other embodiments, immunogenicity is assessed by a CD8+ T-cell lysis assay. In embodiments, a recipient cell is incubated with
10 CD8+ T-cells and then assessed for lysis of the cells by the CD8+ T-cells.
15

In some embodiments, the retroviral vector or VLP comprises elevated levels of an immunosuppressive agent (e.g., immunosuppressive protein) as compared to a reference retroviral vector or VLP, e.g., one produced from an unmodified source cell otherwise similar to the source cell, or a HEK293 cell. In some embodiments, the elevated level is at least 5%, 10%,
20 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 retroviral vector or VLP comprises an immunosuppressive agent that is absent from the reference cell. In some embodiments, the retroviral vector or VLP comprises reduced levels of an immunostimulatory agent (e.g., immunostimulatory protein) as compared to a reference retroviral vector or VLP, e.g., one produced from an unmodified source cell otherwise similar to the source cell, or a HEK293 cell. In some embodiments, the reduced
25 level is at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% compared to the reference retroviral vector or VLP. In some embodiments, the immunostimulatory agent is substantially absent from the retroviral vector or VLP.

In some embodiments, the retroviral vector or VLP, or the source cell from which the
30 retroviral vector or VLP is derived, 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 retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a source cell otherwise similar to the source cell, or a HeLa cell, or a HEK293 cell;

5 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, O_x40L, OX40, CD28, B7, CD30, CD30L 4-1BB, 4-1BBL, SLAM, CD27, CD70, HVEM, LIGHT, B7-H3, or B7-H4, compared to a reference retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a cell otherwise similar to the source cell, or a HEK cell, or a reference cell described herein;

10 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 retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a cell otherwise similar to the source cell, a Jurkat cell, or a HEK293 cell;

15 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 retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a cell otherwise similar to the source cell, or a HEK293 cell;

20 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 retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a cell otherwise similar to the source cell, or a HEK293 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 retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a cell otherwise similar to the source cell, or a HEK293 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 retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a cell otherwise similar to the source cell, or a HEK293 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 retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a cell otherwise similar to the source cell, a HEK293 cell, or a 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 TCR α/β , compared to a reference retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a cell otherwise similar to the source cell, a HEK293 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 retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a cell otherwise similar to the source cell, a HEK293 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 retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a cell otherwise similar to the source cell, a HEK293 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 retroviral vector or VLP e.g., an

unmodified retroviral vector or VLP from a cell otherwise similar to the source cell, a HEK293 cell, or a Jurkat cell, or has no detectable mitochondrial MHAs.

In embodiments, the co-stimulatory protein is 4-1BB, B7, SLAM, LAG3, HVEM, or LIGHT, and the reference cell is HDLM-2. In some embodiments, the co-stimulatory protein is
5 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.

10 In some embodiments, the retroviral vector, VLP, or pharmaceutical 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, an immunogenic response by the innate immune system comprises a response by innate immune cells including, but not limited to NK cells, macrophages,
15 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.

In some embodiments, the retroviral vector, VLP, or pharmaceutical composition does
20 not substantially elicit an immunogenic response by the immune system, e.g., adaptive immune system. 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
25 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).

In some embodiments, the retroviral vector, VLP, or pharmaceutical composition is modified to have reduced immunogenicity. In some embodiments, the retroviral vector, VLP, or
30 pharmaceutical composition has an immunogenicity less than 5%, 10%, 20%, 30%, 40%, or 50% lesser than the immunogenicity of a reference retroviral vector or VLP, e.g., an unmodified

retroviral vector or VLP from a cell otherwise similar to the source cell, a HEK293 cell, or a Jurkat cell.

In some embodiments of any of the aspects described herein, the retroviral vector, VLP, or pharmaceutical 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.

In some embodiments, the retroviral vector, VLP, or pharmaceutical 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- α ;
- 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.

In some embodiments, the retroviral vector or VLP 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 retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a cell otherwise similar to the source cell, a HEK293 cell, or a Jurkat cell;

b. soluble immunosuppressive cytokines, e.g., IL-10, e.g., increased expression of IL-10 compared to a reference retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a cell otherwise similar to the source cell, a HEK293 cell, or a Jurkat cell;

5 c. soluble immunosuppressive proteins, e.g., PD-1, PD-L1, CTLA4, or BTLA; e.g., increased expression of immunosuppressive proteins compared to a reference retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a cell otherwise similar to the cell source, a HEK293 cell, or a Jurkat cell;

10 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 retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a cell otherwise similar to the source cell, a HEK293 cell, or a Jurkat cell; or

15 e. surface proteins which suppress complement activity, e.g., complement regulatory proteins, e.g. proteins that bind decay-accelerating factor (DAF, CD55), e.g. factor H (FH)-like protein-1 (FHL-1), e.g. C4b-binding protein (C4BP), e.g. complement receptor 1 (CD35), e.g. Membrane cofactor protein (MCP, CD46), eg. Profectin (CD59), e.g. proteins that inhibit the classical and alternative complement pathway CD/C5 convertase enzymes, e.g. proteins that regulate MAC assembly; e.g.
20 increased expression of a complement regulatory protein compared to a reference retroviral vector or VLP, e.g. an unmodified retroviral vector or VLP from a cell otherwise similar to the source cell, a HEK293 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
25 higher as compared to a reference retroviral vector or VLP.

In some embodiments, the retroviral vector or VLP is derived from a source cell modified to have decreased expression of an immunostimulatory agent, e.g., one, two, three, four, five, six, seven, eight or more of the following:

30 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 retroviral vector or VLP, e.g.,

an unmodified retroviral vector or VLP from a cell otherwise similar to the source cell, a HEK293 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 retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a cell otherwise similar to the source cell, a HEK293 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 retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a cell otherwise similar to the source cell, a HEK293 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 retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a cell otherwise similar to the source cell, a HEK293 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 retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a cell otherwise similar to the source cell, a HEK293 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 retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a cell otherwise similar to the source cell, a HEK293 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 retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a cell otherwise similar to the source cell, a HEK293 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 retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a cell otherwise similar to the source cell, a HEK293 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 retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a cell otherwise similar to the source cell, a HEK293 cell, or a HeLa cell.

In some embodiments, a retroviral vector, VLP, or pharmaceutical composition derived from a mammalian cell, e.g., a HEK293, modified using shRNA expressing lentivirus to decrease MHC Class I expression, has lesser expression of MHC Class I compared to an unmodified retroviral vector or VLP, e.g., a retroviral vector or VLP from a cell (e.g., mesenchymal stem cell) that has not been modified. In some embodiments, a retroviral vector or VLP derived from a mammalian cell, e.g., a HEK293, modified using lentivirus expressing HLA-G to increase expression of HLA-G, has increased expression of HLA-G compared to an unmodified retroviral vector or VLP, e.g., from a cell (e.g., a HEK293) that has not been modified.

In some embodiments, the retroviral vector, VLP, or pharmaceutical 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 *in vitro*, by IFN-gamma ELISPOT assay.

In some embodiments, the retroviral vector, VLP, or pharmaceutical 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., Cyclosporin or rapamycin).

In some embodiments, the retroviral vector, VLP, or pharmaceutical 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.

In some embodiments, the retroviral vector, VLP, or pharmaceutical composition is derived from a source cell, e.g., a mammalian cell, wherein the mammalian cell is a recombinant cell.

5 In some embodiments, the retroviral vector, VLP, or pharmaceutical is derived from a mammalian cell genetically modified to express viral immunoevasins, e.g., hCMV US2, or US11.

In some embodiments, the surface of the retroviral vector or VLP, or the surface of the source cell, is covalently or non-covalently modified with a polymer, e.g., a biocompatible polymer that reduces immunogenicity and immune-mediated clearance, e.g., PEG.

10 In some embodiments, the surface of the retroviral vector or VLP, or the surface of the source cell is covalently or non-covalently modified with a sialic acid, e.g., a sialic acid comprising glycopolymers, which contain NK-suppressive glycan epitopes.

In some embodiments, the surface of the retroviral vector or VLP, or the surface of the source cell is enzymatically treated, e.g., with glycosidase enzymes, e.g., α -N-acetylgalactosaminidases, to remove ABO blood groups

15 In some embodiments, the surface of the retroviral vector or VLP, or the surface of the source cell is enzymatically treated, to give rise to, e.g., induce expression of, ABO blood groups which match the recipient's blood type.

20 *Parameters for assessing immunogenicity*

In some embodiments, the retroviral vector or VLP 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 retroviral vector or VLP can be determined by any of the assays described herein.

25 In some embodiments, the retroviral vector or VLP 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 retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a cell otherwise similar to the source cell.

In some embodiments, the retroviral vector or VLP has a reduction in immunogenicity as measured by a reduction in humoral response following one or more implantation of the retroviral vector or VLP 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 retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a 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
5 by an anti-cell antibody titre, e.g., anti-retroviral or anti-VLP antibody titre, e.g., by ELISA. In some embodiments, the serum sample from animals administered the retroviral vector or VLP has a reduction of 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more of an anti-retroviral or anti-VLP antibody titer compared to the serum sample from animals administered an unmodified retroviral vector or VLP. In some embodiments, the serum sample
10 from animals administered the retroviral vector or VLP has an increased anti- retroviral or anti-VLP 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 retroviral vector or VLP.

In some embodiments, the retroviral vector or VLP has a reduction in macrophage
15 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 retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a 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 8. In some embodiments, the retroviral vector or VLP has a
20 phagocytosis index of 0, 1, 10, 100, or more, e.g., as measured by an assay of Example 8, when incubated with macrophages in an *in vitro* assay of macrophage phagocytosis.

In some embodiments, the source cell or recipient 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
25 otherwise similar to the source cell, or a recipient cell that received an unmodified retroviral vector or VLP, or a mesenchymal stem cells, e.g., using an assay of Example 17. In embodiments, the source cell expresses exogenous HLA-G.

In some embodiments, the source cell or recipient cell 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
30 more in NK-mediated cell lysis compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, or a recipient cell that received an unmodified retroviral vector or VLP,

wherein NK-mediated cell lysis is assayed *in vitro*, by a chromium release assay or europium release assay, e.g., using an assay of Example 18.

In some embodiments, the source cell or recipient cell 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%,
5 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, or a recipient cell that received an unmodified retroviral vector or VLP, wherein CD8 T cell mediated cell lysis is assayed *in vitro*, by an assay of Example 19.

In some embodiments, the source cell or recipient cell has a reduction in CD4+ T-cell proliferation and/or activation, e.g., a reduction of 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%,
10 70%, 80%, 90%, or more compared to a reference cell, e.g., an unmodified cell otherwise similar to the source cell, or a recipient cell that received an unmodified retroviral vector or VLP, wherein CD4 T cell proliferation is assayed *in vitro* (e.g. co-culture assay of modified or unmodified mammalian source cell, and CD4+T-cells with CD3/CD28 Dynabeads).

In some embodiments, the retroviral vector or VLP causes a reduction in T-cell IFN-gamma secretion, e.g., a reduction of 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%,
15 90%, or more in T-cell IFN-gamma secretion compared to a reference retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a cell otherwise similar to the source cell, wherein T-cell IFN-gamma secretion is assayed *in vitro*, e.g., by IFN-gamma ELISPOT.

In some embodiments, the retroviral vector or VLP causes a reduction in secretion of immunogenic cytokines, e.g., a reduction of 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%,
20 80%, 90%, or more in secretion of immunogenic cytokines compared to a reference retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a cell otherwise similar to the source cell, wherein secretion of immunogenic cytokines is assayed *in vitro* using ELISA or
25 ELISPOT.

In some embodiments, the retroviral vector or VLP 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 retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a cell
30 otherwise similar to the source cell, wherein secretion of the immunosuppressive cytokine is assayed *in vitro* using ELISA or ELISPOT.

In some embodiments, the retroviral vector or VLP 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 retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a cell otherwise similar to the source cell, wherein expression of HLA-G or HLA-E is assayed *in vitro* using flow cytometry, e.g., FACS. In some embodiments, the retroviral vector or VLP 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 retroviral vector or VLP derived from a modified cell with increased HLA-G expression demonstrates reduced immunogenicity.

In some embodiments, the retroviral vector or VLP has or causes 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 retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a cell otherwise similar to the source cell, wherein expression of T cell inhibitor ligands is assayed *in vitro* using flow cytometry, e.g., FACS.

In some embodiments, the retroviral vector or VLP 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 retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a cell otherwise similar to the source cell, wherein expression of co-stimulatory ligands is assayed *in vitro* using flow cytometry, e.g., FACS.

In some embodiments, the retroviral vector or VLP 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 retroviral vector or VLP, e.g., an unmodified retroviral vector or VLP from a 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.

In some embodiments, the retroviral vector or VLP 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:

- 5 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 retroviral vector or VLP;
- 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 retroviral vector or VLP;
- 10 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
15 (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.

20 In some embodiments, the subject to be administered the retroviral vector or VLP has, or is known to have, or is tested for, a pre-existing antibody (e.g., IgG or IgM) reactive with a retroviral vector or VLP. In some embodiments, the subject to be administered the retroviral vector or VLP does not have detectable levels of a pre-existing antibody reactive with the retroviral vector or VLP. Tests for the antibody are described, e.g., in Example 13.

25 In some embodiments, a subject that has received the retroviral vector or VLP has, or is known to have, or is tested for, an antibody (e.g., IgG or IgM) reactive with a retroviral vector or VLP. In some embodiments, the subject that received the retroviral vector or VLP (e.g., at least once, twice, three times, four times, five times, or more) does not have detectable levels of antibody reactive with the retroviral vector or VLP. In embodiments, levels of antibody do not
30 rise more than 1%, 2%, 5%, 10%, 20%, or 50% between two timepoints, the first timepoint being before the first administration of the retroviral vector or VLP, and the second timepoint being

after one or more administrations of the retroviral vector or VLP. Tests for the antibody are described, e.g., in Example 14.

VI. Exogenous agents

In some embodiments, fusosome, e.g. a retroviral vector, VLP, or pharmaceutical composition described herein contains an exogenous agent. In some embodiments, the fusosome, e.g. a retroviral vector, VLP, or pharmaceutical composition described herein contains a nucleic acid that encodes an exogenous agent.

A. Exogenous protein agents

In some embodiments, the exogenous agent comprises a cytosolic protein, e.g., a protein that is produced in the recipient cell and localizes to the recipient cell cytoplasm. In some embodiments, the exogenous agent comprises a secreted protein, e.g., a protein that is produced and secreted by the recipient cell. In some embodiments, the exogenous agent comprises a nuclear protein, e.g., a protein that is produced in the recipient cell and is imported to the nucleus of the recipient cell. In some embodiments, the exogenous agent comprises an organellar protein (e.g., a mitochondrial protein), e.g., a protein that is produced in the recipient cell and is imported into an organelle (e.g., a mitochondrial) of the recipient cell. In some embodiments, the protein is a wild-type protein or a mutant protein. In some embodiments the protein is a fusion or chimeric protein.

In some embodiments, the exogenous agent is encoded by a gene from among SYNE1, SETX, FMR1, SLC6A8, UBE3A, SOD1, TDP43, C9orf72, FXN, MECP2, ASPA, ALDH7A1, TPP1, FUCA1, GALC, HEXA, HEXB, MANBA, ARSA, GNPTAB, or MCOLN1. In some embodiments, the exogenous agent is encoded by a gene from among In some embodiments, the exogenous agent is encoded by a gene from among SYNE1, SETX, FMR1, SLC6A8, UBE3A, SOD1, TDP43, C9orf72, FXN, MECP2, ASPA, or ALDH7A1. In some embodiments, the exogenous agent is encoded by a gene from among TPP1, FUCA1, GALC, HEXA, HEXB, MANBA, ARSA, GNPTAB, or MCOLN1.

In some embodiments, the exogenous agent comprises a protein of Table 5 below. In some embodiments, the exogenous agent comprises the wild-type human sequence of any of the proteins of Table 5, a functional fragment thereof (e.g., an enzymatically active fragment

thereof), or a functional variant thereof. In some embodiments, the exogenous agent comprises an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%, identity to an amino acid sequence of Table 5, e.g., a Uniprot Protein Accession Number sequence of column 2 of Table 5 or an amino acid sequence of column 3 of Table 5. In some

5 embodiments, the payload gene encodes an an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%, identity to an amino acid sequence of Table 5.

Table 5. *CNS diseases or disorders. The first column lists exogenous agents that can be delivered to treat the indications in the fourth column, according to the methods and uses herein.*

10 Each Uniprot accession number of Table 5 is herein incorporated by reference in its entirety.

Gene	Uniprot Protein(s) Accession Number	Amino Acid Sequence (first Uniprot Accession Number)	Disease / Disorder	SEQ ID NO
SYN E1	Q8NF91	MATSRGASRCPRDIANVMQRLQDEQEIVQKRT FTKWINSHLAKRKPPMVVDDLFDMDKDGVKL LALLEVLSGQKLPCQGRMKRIHAVANIGTA LKFLEGRKIKLVNINSTDIADGRPSIVLGLMWTI ILYFQIEELTSNLPQLQSLSSASSVDSIVSSETPS PPSKRKVTTKIQGNACKALLKWVQYTAGKQT GIEVKDFGKSWRSGVAFHSHVIAIRPELVLET VKGRSNRENLEDAFTIAETELGIPRLDPEDVD VDKPDKESIMTYVAQFLKHYPDIHNASTDGQE DDEILPGFSPFANSVQNFKREDRVIFKEMKVWI EQFERDLTRAQMVESNLQDKYQSFKHFRVQY EMKRKQIEHLIQPLHRDGLSLDQALVKQSWD RVTSRLFDWHIQDLKSLPAPLGTIGAWLYRAE VALREEITVQQVHEETANTIQRKLEQHKDLLQ NTDAHKKRAFHEIYRTRSVNGIPVPPDQLEDMA ERFHFVSSSTSELHLMKMEFLELKYRLSLLVLA ESKLKSWIIKYGRRESVEQLLQNYVFSIENSKFF EQYEVITYQILKQTAEMYVKADGSVEEAENV KFMNETTAQWRNLSVEVRSVRSMLEEVISNW DRYGNTVASLQAWLEDAEKMLNQSENAKKD FFRNLPHWIQHTAMNDAGNFIETCDEMVS DLKQQLLLLNGRWRELFMEVKQYQAQADEMD RMKKEYTDCVVTLSAFATEAHKKLSEPLEVSF MNVKLLIQDLEDIEQRVPVMDAQYKIITKTAH LITKESPQEEGKEMFATMSKLKEQLTKVKECY SPLLYESQQLLIPLEELEKQMTSFYDSLKINEII TVLEREAQSSALFKQKHQELLACQENCKKTLT LIEKGSQSVQKFVTLNVLKHFDQTRLQRQIAD IHVAFQSMVKKTDGWKKHVETNSRLMKKFEE SRAELEKVLRIAQEGLEEKGDPEELLRRHTEFF SQLDQRVLNAFLKACDELTDILPEQEQQGLQE AVRKLHKQWKDLQGEAPYHLLHLKIDVEKNR FLASVEECRTELDTRETKLMPQEGSEKIIKEHRV FFSDKGPHHLCEKRLQLIEELCVKLPVRDPVRD	Spinocerebellar Ataxia, Autosomal Recessive, Type 1	134

Gene	Uniprot Protein(s) Accession Number	Amino Acid Sequence (first Uniprot Accession Number)	Disease / Disorder	SEQ ID NO
		TPGTCHVTCLKELRAAIDSTYRKLMEDPDKWK DYTSRFSEFSSWISTNETQLKGIGEAIDTANH GEVKRAVEEIRNGVTKRGETLSWLKSRLKVLT EVSSSENAQKQGDELAKLSSSFKALVTLLSEVE KMLSNFGDCVQYKEIVKNSLEELISGSKEVQE QAEKILD TENLFEAQQLLHHQQKTKRISAKK RDVQQQIAQAQQGEGLPDRGHEELRKLESTL DGLERSRERQERRIQVTLRKWERFETNKETVV RYLFQTGSSHERFLSFSSLESLSSELEQTKEFSK RTESIAVQAENLVKEASEIPLGPQNKQLLQQQA KSIKEQVKKLED TLEEDIKTMEMVKTWKDHF GSNFETLSVWITEKEKELNALETSSSAMDMQIS QIKVTIQEIESKLSSIVGLEEEAQSFAQFVTTGES ARIKAKLTQIRRYGEELREHAQCLEGTILGHLS QQQKFEENLRKIQQSVSEFEDKLAVPIKICSSAT ETYKVLQEHMDLCQALESLSSAITAFSASARK VVNRDSCVQEAALQQQYEDILRRAKERQTA LENLLAHWQRLEKELSSFLTWLERGEAKASSP EMDISADRVKVEGELQLIQALQNEVVSQASFY SKLLQLKESLFSVASKDDVKMMKLHLEQLDE RWRDL PQIINKRINFLQSVVAEHQQFDELLLSF SVWIKLFLSELQTTSEISIMDHQVALTRHKDHA AEVESKKGELQSLQGHLAKLGS LGRAEDLHLL QGKAEDCFQLFEEASQVVERRQLALSHLAEFL QSHASLSGILRQLRQTVEATNSMNKNESDLIEK DLNDALQNAKALESAAVSLDGILSKAQYHLKI GSSEQRTSCRATADQLCGEVERIQNLLGTKQSE ADALAVLKKAQDQKEELLKSIEDIEERTDKER LKEPTRQALQQRLRVFNQLEDELNSHEHELCW LKDKAKQIAQKDVAFAPEVDREINRLEVTWD DTKRLIHENQGQCCGLIDLMREYQNLKSAVSK VLENASSVIVTRTTIKDQEDLKWAFSKHETAK NKMNYKQKDLNFTSKGKHLSELKKIHSSDF SLVKTDMESTVDKWLDVSEKLEENMDRLRVS LSIWDDVLSTRDEIEGWSNNCVPQMAENISNL DNHLRAEELLKEFESEVKNKALRLEELHSHV DLKELTKNLETPPDLQFIEADLMQKLEHAKET EVAKGTLKDFTAQSTQVEKFINDITTWFTKVEE SLMNCAQNETCEALKKV KDIQKELQSQQSNIS STQENLNSLCRKYHSAELES LGRAMTGLIKKH EAVSQLCSKTQASLQESLEKHFSESMQEFQEW FLGAKAAAKESSDRTGDSKVLEAKLHDLQNIL DSVSDGQSKLDAVTQEGQTLYAHLSKQIVSSIQ EQITKANEEFQAFLKQCLKDKQALQDCASELG SFEDQHRKLN LWIHEMEERFNTENLGESKQHIP EKKNEVHKVEMFLGELLAARES LDKLSQRGQ LLSEEGHGAGQEGRLCSQLLTSHQNLLRMTKE KLRSCQVALQEHEALEEALQSMWFWVKAIQD RLACAESTLGSKDTLEKRLS IQIDILLMKGEGE VKLNMAIGKGEQALRSSNKEGQRVIQTQLETL KEVWADIMSSSVHAQSTLESVISQWNDYVERK NQLEQWMESVDQKIEHPLQPQPGLKEKFVLLD		

Gene	Uniprot Protein(s) Accession Number	Amino Acid Sequence (first Uniprot Accession Number)	Disease / Disorder	SEQ ID NO
		HLQSILSEAEDHTRALHRLIAKSRELYEKTED SFKDTAQEELKTQFNDIMTVAKEKMRKVVEIV KDHLMYLDAVHEFTDWLHSAKEELHRWSDM SGDSSATQKKLSKIKELIDSREIGASRLSRVESL APEVKQNTTASGCELMHTEMQALRADWKQW EDSVFQTSQSCLENLVSQMALSEQEFGQVAQL EQALEQFSALLKTWAQQLTLLEGKNTDEEIVE CWHKGQEILDALQKAEPRTEDLKSQNLNLCRF SRDLSTYSGKVSGLIKEYNCLCLQASKGCQNK EQILQQRFRKAFRDFQQLVNAKITTAKCFDIP QNISEVSTSLQKIQEFLSESENGQHKLNMMLSK GELLSTLLTKEKAKGIQAKVTAAKEDWKNFHS NLHQKESALENLKIQMKDFEVSAPIQDWLSK TEKMHVHESSNRLYDLPAKRREQQKLQSVLEEI HCYPQLNRLKEKAQQLWEGQAASKSFRHRV SQLSSQYLALSNLTKEKVSRLDRIVAEHNQFSL GIKELQDWMTDIAHMLDSYCHPTSDKSVLDSR TLKLEALLSVKQEKEIQMKMIVTRGESVLQNT SPEGIPTIQQQLQSVKDMWASLLSAGIRCKSQL EGALSKWTSYQDGVRFQSGWMDSMEANLNE SERQHAELRDKTTMLGKAKLLNEEVLSYSSLL ETIEVKGAGMTEHYVTQLELQDLQERYRAIQE RAKEAVTKSEKLVRLHQEYQRDLKAFEVWLG QEKEKLDQYSVLEGDAHTHETTLRDLQELQV HCAEGQALLNSVLHTREDVIPSGIPQAEDRALE SLRQDWQAYQHRLSETRTQFNNVVKLRLME QKFQQVDEWLKTAEEKVSPRTRRQSNRATKEI QLHQMKKWHEEVTAYRDEVEEVGARAQEILD ESHVNSRMGCQATQLTSRYQALLQVLEQIKF LEEEIQSLEESLSSYSYSDWYGSTHKNFKNVA TKIDKVDTVMMGKKLKTLEVLLKDMEEKGHS LKSAREKGERAVKYLEEGEAERLRKEIHDHME QLKELTSTVRKEHMTLEKGLHLAKEFSDKCKA LTQWIAEYQEILHVPEEPMELYEKKAQLSKY KSLQQTVLSSHEPSVKS SVREKGEALLELVQDVT LKDKIDQLQSDYQDLCSIGKEHVFSLEAKVKD HEDYNSELQEVEKWLLQMSGRLVAPDLLETSS LETITQQLAHHKAMMEEIAGFEDRLNNLQMK GDTLIGQCADHLQAKLKQNVHAHLQGTKDSY SAICSTAQRMYQSLEHELQKHVSRQDTLQQCQ AWLSAVQPDLEPSPQPPLSRAEAIKQVKHFRAL QEQARTYLDLLCSMCDLSNASVKTAKDIQQT EQTIEQKLVQAQNLQGWEEIKHLKSELWIYL QDADQQLQNMKRRHSELELNIAQNMVSQVKD FVKKLQSKQASVNTIIEKVNKLTKEESPEHKE INHLNDQWLDLCRQSNLCLQREEDLQRTDR YHDCMNVVEVFLEKFTTEWDNLARSDAESTA VHLEALKKLALALQERKYAIEDLKDQKQKMIE HLNLDKELVKEQTSHELRWFQLEDLIKRI QVSVTNLEELNVVQSRFQELMEWAEEQPNIA EALKQSPPDMAQNLLMDHLAICSELEAKQML LKSLIKDADRVMADLGLNERQVIQKALSDAQS		

Gene	Uniprot Protein(s) Accession Number	Amino Acid Sequence (first Uniprot Accession Number)	Disease / Disorder	SEQ ID NO
		HVNCLSDLVGQRRKYLNKALSEKTQFLMAVF QATSQIQQHERKIMFREHICLLPDDVSKQVKTC KSAQASLKTYQNEVTGLWAQGRELKKEVTEQ EKSEVLGKLQELQSVYDSVLQKCSHRLQELEK NLVSRKHFKEFDKACHWLKQADIVTFPEINL MNESSELHTQLAKYQNILEQSPEYENLLTLQR TGQTILPSLNEVDHSYLSEKLNALPRQFNVIVA LAKDKFYKVQEAILARKEYASLIELTTQSLSEL EAQFLRMSKVPTDLAVEEALSLQDGCRAILDE VAGLGEAVDELNQQKEGFRSTGQPWQPDKML HLVTLYHRLKRQTEQRVSLLEDTTSAQEHEK MCQQLERQLKSVKEEQSKVNEETLPAAEEKLK MYHSLAGSLQDSGIVLKRVTIHLEDLAPHLDP AYEKARHQIQSWQELKLLTSAIGETVTECESR MVQSIDFQTEMSRSLDWLRRVKAELSGPVYLD LNLQDIQEEIRKIQIHQEEVQSSLRIMNALSHKE KEKFTKAKELISADLEHSLAELSELGDGDIQEAL RTRQATLTEIYSQCQRYQVQFAANDWLEDA QELLQLAGNGLDVEAEENLKSHMEFFSTEDQ FHSNLEELHSLVATLDPLIKPTGKEDLEQKVAS LELRSQRMSRDSGAQVDLLQRCTAQWHDYQK AREEVIELMNDTEKKLSEFSLLKTSSSHEAEK LSEHKALVSVVNSFHEKIVALEEKASQLEKTG NDASKATLSRSMTTVWQRWTRLRAVAQDQE KILEDVDEWTGFNNKVKKATEMIDQLQDKL PGSSAEKASKAELLTLLEYHDTFVLELEQQQSA LGMLRQQTLMLQDGAAPTGPGEPPMLMQEITA MQDRCLNMQEKVKTNGLVKQELKDREMVE TQINSVKCWVQETKEYLGNPTIEIDAQLEELQI LLTEATNHRQNIKMAEEQKEKYLGLYTILPSE LSLQLAEVALDLKIRDQIQDKIKEVEQSKATSQ ELSRQIQKLAKDLTTILTKLAKTDNVVQAKT DQKVLGEELDGCNSKLMELDAAVQKFLEQNG QLKGKPLAKKIGKLTTELHQQTIRQAENRLSKLN QAASHLEEYNEMLLELILKWIEKAKVLAHGTA WNSASQLREQYILHQTLLAESKEIDSELEAMTE KLQYLTSVYCTEKMSQQVAELGRETEELRQMI KIRLQNLQDAAKDMKKFEAELKKLQAALQA QATLTSPEVGRSLSLKEQLSHRQHLLSEMESLKP KVQAVQLCQSALRIPEDVASLPLCHAALRLQ EEASRLQHTAIQQCNIMQEAVVQYEQYEQEM KHLQQLIEGAHREIEDKPVATSNIELQAQISR HEELAQQIKGYQEQIASLNSKCKMLTMKAKH ATMLLTVEVEGLAEGTEDLDGELLPTPSAHP VMMTAGRCHTLLSPVTEESGEEGTNSEISSPP ACRSPSPVANTDASVNQDIAYYQALSAERLQT DAAKIHPSTSASQEFYEPGLEPSATAKLGLDQR SWETLKNVISEKQRTLYEALERQQKYQDSLQSI STKMEAIELKLSPEPGRSPESQMAEHQALM DEILMLQDEINELQSSLAELVSESCEADPAEQ LALQSTLTVLAERMSTIRMKASGKRQLLEEK NDQLEEQRQEALQRYRCEADELDSWLLSTK		

Gene	Uniprot Protein(s) Accession Number	Amino Acid Sequence (first Uniprot Accession Number)	Disease / Disorder	SEQ ID NO
		ATLDTALSPPKEPMDMEAQLMDCQNMLVEIE QKVVALSELVHNENLLLEGKAHTKDEAEQL AGKLRRRLKGSLELQRALHDKQLNMQGTAQE KEESDVDLTATQSPGVQEWLAQARTTWTQQR QSSLQQQKELEQELAEQKSLLRSVASRGEEILI QHSAAETSGDAGEKPDVLSQELGMEGEKSSAE DQMRMKWESLHQEFSTKQKLLQNVLEQEQQ VLYSRPNRLLSGVPLYKGDVPTQDKSAVTSLL DGLNQAFEEVSSQSGGAKRQSIHLEQKLYDGV SATSTWLDDVEERLFVATALLPEETETCLFNQE ILAKDIKEMSEEMDKNKNLFSQAFPENGDNRD VIEDTLGCLLGRLLSDSVVNQRCHQMKERLQ QILNFQNDLKVLFTSLADNKYIILQKLANVFEQ PVAEQIEAIQQAEDGLKEFDAGIHELKRRGDKL QVEQPSMQELSKLQDMYDELMMIIGSRSSGLN QNLTLSQYERALQDLADLLETGQEKMAGDQ KIIVSSKEEIQQLLDKHKEYFQGLESHMILTETL FRKIISFAVQKETQFHTELMAQASAVLKRAHK RGVELEYILETWSHLEDEDQELSRQLEVVESSI PSVGLVEENEDRLIDRITLYQHLKSSLNEYQPK LYQVLDDGKRLLISISCSDESQNLQLGECWLS NTKMSKELHRLETILKHWTRYQSESADLIHW LQSAKDRLEFWTQQSVTPQELEMVRDHLNA FLEFSKEVDAQSSLKSSVLSTGNQLRLKVDVT ATLRSELSRIDSQWTDLLTNIPAVQEKHLHQLQ MDKLPKRHAISEVMSWISLMENVIQKDEDNIK NSIGYKAIHEYLQKYKGFKIDINCKQLTVDFVN QSVLQISSQDVESKRSDKTDFAEQLGAMNKS WQILQGLVTEKIQLLEGLLESWSEYENNVQCLKT WFETQEKRLKQHRIGDQASVQNALKDCQDL EDLIKAKEKEVEKIEQNGLALIQNKKEDVSSIV MSTLRELGTWANLDHMGVQLKILLKSVLDQ WSSHKVAFDKINSYLMEARYSLSRFLLTGSL EAVQVQVDNLQNLQDDLEKQERSLQKFGSITN QLLKECHPPVTETLTNTLKEVNMRWNNLLEEI AEQLQSSKALLQLWQRYKDYSKQCASTVQQQ EDRTNELLKAATNKDIADDEVATWIQDCNDLL KGLGTVKDSLFFLHELGEQLKQQVDASAASAI QSDQLSLQHLCALEQALCKQQTSLQAGVLDY ETFAKSLEALEAWIVEAEELQGDPSHSSDLS TIQERMEELKGQMLKFSSMAPDLDRNLGYR LPLNDKEIKRMQNLRHWSLISSQTTERFSKLQ SFLQHQTFLEKCETWMEFLVQTEQKLAVEIS GNYQHLLLEQQRAHELFQAEMFSRQQILHSIID GQRLLLEQQVDDRDEFNLKLTLLSNQWQGV RRAQQRRGIIDSQIRWQRYREMAEKLKRWL VEVSYLPMSGSGSVPIPLQQARTLFDEVQFKEK VFLRQQGSYILTVEAGKQLLLSADSGAEALQ AELAEIQEKWKSASMRLEEQKKKLAFLKDW EKCEKGIADSLEKLRTFKKKLSQSLPDHHEELH AEQMRCKELENAVGSWTDDLTQLSLLKDTLS AYISADDISILNERVELLQRQWEELCHQLSLRR		

Gene	Uniprot Protein(s) Accession Number	Amino Acid Sequence (first Uniprot Accession Number)	Disease / Disorder	SEQ ID NO
		QQIGERLNEWAVFSEKNKELCEWLTQMESKV SQNGDILIEEMIEKLKKDYQEEIAIAQENKIQLQ QMGERLAKASHESKASEIEYKLGKVNDRWQH LLDLIAARVKKLKETLVAVQQLDKNMSSLRT WLAHIESELAKPIVYDSCNSEEIQRKLNEQQEL QRDIEKHSTGVASVLNLCVLLHDCDACATDA ECDSIQQATRNLDRRWRNICAMSMERRLKIEE TWRLWQKFLDDYSRFEDWLKSSERTAAFSSS GVIYTVAKEELKKFEAFQRQVHECLTQLELINK QYRRLARENRTDSACSLKQMVHEGNQRWDN LQKRVTSLRRLKHFIGQREEFETARDSILVWL TEMDLQLTNIEHFSECDVQAKIKQLKAFQQEIS LNHNKIEQIIAQGEQLIEKSEPLDAAHEEELDEL RRYCQEVFGRVERYHKKLIRLPLPDDEHDLSD RELELEDSAALSDLHWHDRSADSLLSPQPSSNL SLSLAQPLRSERSGRDTPASVDSIPLEWDHDYD LSRDLESAMSRALPSEDEEGQDDKDFYLRGAV GLSGDHSALQSIRQLGKALDDSRFQIQQTENII RSKTPTGPELDTSYKGYMKLLGECSSSIDSVKR LEHKLKEEEESLPGFVNLHSTETQTAGVIDRWE LLQAQALSKELRMKQNLQKWQQFNSDLNSIW AWLGDTEEELEQLQRLELSTDIQTIELQIKKLK ELQKAVDHRKAIL SINLCSPEFTQADSKESRDL QDRLSQMNGRWDRVCSLLEEWGRLQDALM QCQGFHEMSHGLLLMLENIDRRKNEIVPIDSNL DAEILQDHHKQLMQIKHELLESQLRVASLQDM SCQLLVNAEGTDCLEAKEKVHVIGNRLKLLK EVSRHIKELEKLLDVSSSQDLSSWSSADELDT SGVSPTSGRSTPNRQKTPRGKCSLSQPGPSVSS PHSRSTKGGSDSSLSEPGPGRSGRGFLFRVLRA ALPLQLLLLLLIGLACLVPMSSEEDYSCALSNNF ARSFHPMLRYTNGPPPL		
SETX	Q7Z333	MSTCCWCTPGGASTIDFLKRYASNTPSGEFQT ADEDLCYCLECVAEYHKARDEL PFLHEVLWE LETLRLINHFESMKAEIGDDDELYIVDNNGE MPLFDITGQDFENKLRVPLLEILKYPYLLHER VNELCVEALCRMEQANCSFQVFDKHPGIYFL VHPNEMVRRWAILTARNLGKVDRDDYYDLQE VLLCLFKVIELGLLESPDIYTSSVLEKGLILLPS HMYDTTNYKSYWLGICMLLTILEEQAMDSLLL GSDKQNDFMQSILHTMEREADDDSVDPFWPA LHC FMVILDRLGSKVWGQLMDPIVAFQTIINN ASYNREIRHIRNSSVRTKLEPESYLDDMVTCSQ IVYNYNPEKTKKDSGWRTAICPDYCPNMYEE METLASVLQSDIGQDMRVHNSTFLWFIPFVQS LMDLKD LGVAYIAQVVNHL YSEVKEVLNQT AVCDKVTEFFLLILVSVIELHRNKKCLHLLWVS SQQWVEAVVKCAKLPTTAFTRSSEKSSGNCSK GTAMISSLSLHSMPSNSVQLAYVQLIRSLLKEG YQLGQQLCKRFWDKLNFLRGNLSL GWQLT SQETHELQSCLKQIIRNIKFKAPPCNTFVDL TSA CKISPASYNKEESEQMGKTSRKDMHCLEASSP	Ataxia with Oculomotor Apraxia, Type 2	135

Gene	Uniprot Protein(s) Accession Number	Amino Acid Sequence (first Uniprot Accession Number)	Disease / Disorder	SEQ ID NO
		TFSKEPMKVQDSVLIKADNTIEGDNNEQNYIK DVKLEDHLLAGSCLKQSSKNIFTERAEDQIKIS TRKQKSVKEISSYTPKDCSTRNGPERGCDRGII VSTRLLTDSSTDALEKVSTSNEDFSLKDDALAK TSKRKTKVQKDEICAKLSHVKKQHRKSTLVD NTINLDENLTVSNIESFYSRKDTGVQKGDGFH NLSLDPSGVLDDKNGEQKSQNNVLPKEKQLK NEELVIFSFHENNCIKIEFHVDGKELIPFTEMTN ASEKKSSPFKDLMTVPESRDEEMSNTSVIYSN LTREQAPDISPKSDTLTDSQIDRDLHKLSELLAQ ASVITFPSDSPQNSSLQQRKVKEDKRCFTANQN NVGDTSRGQVHIISDSDDDDDERILSLEKLTQK DKICLEREHPEQHVSTVNSKEEKNPVKEEKTET LFQFEESDSQCFEFESSSEVFSVWQDHPDDNNS VQDGEKKCLAPIANTTNGQGCTDYVSEVVKK GAEGIEEHTRPRSISVEEFCEIEVKKPKRKRSEK PMAEDPVRPSSSVRNEGQSDTNKRDLVGNDFK SIDRRTSTPNSRIQRATTVSQKKSSKLCTCTEPI RKVPVSKTPKKTHSDAKKGQNRSSNYLSCRTT PAIVPPKKFRQCPEPTSTAEKLGKKGPRKAYE LSQRSLDYVAQLRDHGKTVGVVDTRKKTCLIS PQNLSVRNNKKLLTSQELQMQRQIRPKSQKNR RRLSDCESTDVKRAGSHTAQNSDIFVPESDRSD YNCTGGTEVLANSNRKQLIKCMPSEPETIKAK HGSPATDDACPLNQCDSVVLNGTVPTNEVIVS TSEDPLGGGDPTARHIEMAALKEGEPDSSSDAE EDNLFLTQNDPEDMDLCSQMENDNYKLIELIH GKDTVEVEEDSVSRPQLESLSGKCKYKDCLE TTKNQGEYCPKHSEVKADEDVFRKPGLPPPA SKPLRPTTKIFSSKSTSRIAGLSKSLETSSALSPS LKNKSKGIQSILKVPQPVPLIAQKPVGEMKNSC NVLHPQSPNNSNRQGCKVPFGESKYFPSSSPVN ILLSSQSVSDTFVKEVLKWKYEMFLNFGQCGP PASLCQSISRVPVRFHNYGDYFNVFFPLMVLN TFETVAQEWLNSPNRENFYQLQVRKFPADYIK YWEFAVYLEECELAKQLYPKENDLVFLAPERI NEEKKDTERNDIQDLHEYHSGYVHKFRRTSV MRNGKTECYLSIQTQENFPANLNLVNCIVISS LVTTQRKLKAMSLGSRNQLARAVLNPMPMD FCTKDLLTTTSEIIAYLRDFNEDQKKAIETAY AMVKHSPSVAKICLIHGPPGTGKSKTIVGLLYR LLTENQRKGHSDENSNAKIKQNRVLVCAPSNA AVDELMKKIILEFKEKCKDKKNPLGNCGDINL VRLGPEKSINSEVLKFSLDSQVNHMRMKKELPSH VQAMHKRKEFLDYQLDELSRQRALCRGGREI QRQELDENISKVSKERQELASKIKEVQGRPQKT QSIHLESHIICCTLTSTSGLLLESFRGQGGVPFS CVIVDEAGQSCEIETLTPLIHRCNKLILVGDPKQ LPPTVISMKAQEYGYDQSMARFCRLLEENVE HNMISRLPILQLTVQYRMHPDICLFPSNYVYNR NLKTNRQTEAIRCSSDWPFQPYLVFDVGDGSE RRDNDYINVQEIKLVMEIKLIKDKRKDVSR		

Gene	Uniprot Protein(s) Accession Number	Amino Acid Sequence (first Uniprot Accession Number)	Disease / Disorder	SEQ ID NO
		NIGIITHYKAQKTMIQKDLDFEEDRKGPAAEVDT VDAFQGRQKDCVIVTCVRANSIQGSIGFLASLQ RLNVTITRAKYSLFILGHLRTLMEHQHWNQLI QDAQKRGAIKTCDKNYRHDAVKILKLKPVLPQ RSLTHPPTIAPEGSRPQGGLPSSKLDSGFAKTSV AASLYHTPSDSKEITLTVTSKDPERPPVHDQLQ DPRLLKRMGIEVKGGIFLWDPQPSSPQHPGATP PTGEPGFPVHVDLSHIQQPAAVVAALSSHKPP VRGEPPAASPEASTCQSKCDDPEEELCHRRER AFSEGEQEKCGSETHHTRRNSRWDRKRTLEQED SSSKKRKLL		
FMR1	Q06787	MEELVVEVRGSNGAFYKAFVKDVHEDSITVAF ENNWQPDRIQPFHDFRPPVGYNDKINESDE VEVYSRANEKEPCCWWLAKVRMIKGEFYVIE YAACDATYNEIVTIERLRNVNPNKPATKDTFH KIKLDVPEDLRQMCAGEAAHKDFKAVGAFS VTYDPENYQLVILSINEVTSKRAHMLIDMHFRS LRTKLSLIMRNEEASKQLESSRQLASRFHEQFI VREDLMGLAIGTHGANIQQARKVPGVTAIDL EDTCTFHIYGEDQDAVKKARSFLEFAEDVIQVP RNLVGKVGKNGKLIQEIVDKSGVVRVRIEAE EKNVPQEEEIMPPNSLPSNNSRVGNAPPEKKH LDIKENSTHFSQPNSTKVQRVLVASSVVAGESQ KPELKAWQGMVPFVFGTKDSIANATVLLDY HLNYLKEVDQLRLRLQIDEQLRQIGASSRPPP NRDKEKSYVTDDGQGMGRGSRPYRNRGHGR RGPYTSGTNSEASNASETESDHRDELSWDL APTEEERESFLRRGDGRRRGGGGRGQGGGRGR GGGFKGNDDHSRTDNRPRNPREAKGRITDGS LQIRVDCNNERSVHTKTLQNTSSEGSRLRTGK DRNQKKEKPDSDVDGQQPLVNGVP	Fragile X Syndrome	136
SLC6A8	P48029	MAKKSAENGIYSVSGDEKKGPLIAPGPDGAPA KGDGPVGLGTPGGRLAVPPRETWTRQMDFIM SCVGFVGLGNVWRFPYLCYKNGGGVFLIPY VLIALVGGIPIFFLEISLGQFMKAGSINWNICP LFKGLGYASMVIVFYCNTYYIMVLAWGFYYL VKSFTTTLPWATCGHTWNTPDCEIFRHEDCA NASLANLTCDQLADRRSPVIEFWENKVLRLSG GLEVPGALNWEVTLCLLACWVLVYFCVWKG VKSTGKIVYFTATFPYVVLVLLVRGVLLPGA LDGIIYYLKPDSKLGSPQVWIDAGTQIFFSYA IGLGALTALGSYNRFNNNCYKDAILALINSGT SFFAGFVVSILGFMAAEQGVHISKVAESGPGL AFIAYPRAVTLMPVAPLWAAALFFFMILLGLD SQFVGVEGFITGLLDLLPASYYFRFQREISVALC CALCFVIDLSMVTGGMYVFQLFDYYSASGTT LLWQAFWECVVVAWVYGADRFMDDIACMIG YRPCPWMKWCWSFFTPLVCMGIFNVVYYEP LVYNNTYVYPWWGEAMGWAFALSSMLCVPL HLLGCLLRAKGTMAERWQHLTQPIWGLHHLE YRAQDADVRGLTTLTPVSESSKVVVVESVM	Cerebral Creatine Deficiency Syndrome 1	137

Gene	Uniprot Protein(s) Accession Number	Amino Acid Sequence (first Uniprot Accession Number)	Disease / Disorder	SEQ ID NO
UBE3A	Q05086	MEKLHQCYWKSQEPQSDIEASRMKRAAAKH LIERYYHQLTEGCGNEACTNEFCASCPTFLRM DNNAAAIKALELYKINAKLCDPHPSKKGASSA YLENSKGAPNNSCSEIKMNKKGARIDFKDVTY LTEEKVYEILELCREREDYSPLIRVIGRVFSSAE ALVQSFRKVKQHTKEELKSLQAKDEDEKDEDE KEKAACSAAMEEDSEASSSRIGDSSQGDNNL QKLGPDVSVSDIDAIRRVYTRLLSNEKIETAFL NALVYLSPNVECDLTYHNVYSRDPNYLNLFIIV MENRNLHSPEYLEMALPLFCKAMSKLPLAAQ GKLIRLWSKYNADQIRRMETFQQLITYKVIS NEFNSRNLVNDDDAIVAASKCLKMVYYANVV GGEVDTNHNEEDDEEPIPESELTLQELLGEER RNKKGPRVDPLETELGVKTLDCRKPLIPFEFI NEPLNEVLEMDKDYTFKVFETENKFSFMTCPFI LNAVTKNLGLYYDNRIRMYSEIRITVLYSLVQ GQQLNPYLRLKVRDDHIIDALVRLEMIAMEN PADLKKQLYVEFEQEGGVDEGGVSKEFFQLVV EEIFNPDIGMFTYDESTKLFWFNPSSFETEGQFT LIGIVLGLAIYNNCILDVHFPVYRKLGMGKK GTFRDLGDSHPVLYQSLKDLLEYEGNVEDDM MITFQISQTDLFGNPMYDLKENGDKIPITNEN RKEFVNLYSDYILNKSVEKQKFAFRRGFHMVT NESPLKYLFRPEEIELLICGSRNLDFALEETTE YDGGYTRDSVLIREFWEIVHSFTDEQKRLFLQF TTGTDRAPVGGLGKLKMIKNGPDTERLPTS HTCFNVLLLPEYSSKEKLERLLKAITYAKGFG ML	Angelman Syndrome	138
SOD1	P00441	MATKAVCVLKGDGPVQGIINFEQKESNGPVKV WGSIKGLTEGLHGFHVHEFGDNTAGCTSAGPH FNPLSRKHGGPKDEERHVGDLGNVTADKDG ADVSIEDSVISLSGDHCHIGRTLTVVHEKADDLG KGGNEESTKTGNAGSRLACGVIGIAQ	Amyotrophic Lateral Sclerosis	139
TDP43	Q13148	MSEYIRVTEDENDIEPIESDDGTVLLSTVTAQ FPGACGLRYRNPVSQCMRGVRLVEGILHAPDA GWGNLVYVVNYPKDNKRKMDTDASSAVKV KRAVQKTSIDLIVLGLPWKTTEQDLKEYFSTFG EVLNVQVKKDLKTGHSKGFGFVRFTETQV KVMQSRHMIDGRWCDCKLPNSKQSQDEPLRS RKVFVGRCTEDMTEDELREFFSQYGDVMDVFI PKPFRAFAFVTFADDQIAQSLCGEDLIKGISVH ISNAEPKHNSNRQLERSGRFGGNPGGFGNQGG FGNSRGGGAGLGNNQGSNMGGGMNFGAFSIN PAMMAAAQAALQSSWGMMLASQQNQSG PSGNNQNQGNMQREPNAFGSGNNSYSGSNS GAAIGWGSASNAGSGSGFNGGFGSSMDSKSSG WGM	Amyotrophic Lateral Sclerosis	140
C9orf72	Q96LT7	MSTLCPPSPAVAKTEIALSGKSPLLAATFAYW DNILGPRVRHIWAPKTEQVLLSDGEITFLANHT LNGEILRNAESGAIDVKFFVLSEKGVIIIVSLIFD GNWNGDRSTYGLSIILPQTELSFYLPVLRVCVD RLTHIRKGRIMHMERQENVQKIILEGTERME	Amyotrophic Lateral Sclerosis	141

Gene	Uniprot Protein(s) Accession Number	Amino Acid Sequence (first Uniprot Accession Number)	Disease / Disorder	SEQ ID NO
		DQGQSIIPMLTGEVIPVMELLSSMKSHSVPEEID IADTVLNDDDIGDSCHEGFLNNAISSHLQTCGC SVVVGSSAEKVNKIVRTLCLFLTPAERKCSRLC EAESSFKYESGLFVQGLLKDSTGSFVLPFRQVM YAPYPTTHIDVDVNTVKQMPPCHEHIYNQRRY MRSELTAFWRATSEEDMAQDTIYTDESFTPD NIFQDVLHRDTLVKAFLDQVFQKPLSLRSTF LAQFLLVLHRKALTLIKIEDDTQKGGKPKFSK RNLKIDLDLTAEGDLNIMALAEEKIPGLHSFIF GRPFYTSVQERDVLMTF		
FXN	Q16595	MWTLGRRRAVAGLLASPSPAQAQTLTRVPRPAE LAPLCGRRLRTDIDATCTPRRASSNQRLNQI WNVKKQSVYLMNLRKSGTLGHPGSLDETTYE RLAETLDSLAEFFEDLADKPYTFEDYDVSGS GVLTVKLGGDLGTYVINKQTPNKQIWLSSPS GPKRYDWTGKNWVYSHDGVSLHELLAAELTK ALKTKLDLSSLAYSGKDA	Friedreich's Ataxia	142
MEC P2	P51608	MVAGMLGLREEKSEDQDLQGLKDKPLKFKKV KKDKKEEKEGKHEPVQPSAHHSAEPAEAGKA ETSEGSGSAPAVPEASASPKQRRSIIRDRGPMY DDPTLPEGWTRKLKQRKSGRSAGKYDVYLINP QGKAFRSKVELIAYFEKVGDTSLDPNDFDFTV TGRGSPSRREQKPPKKPKSPKAPGTGRGRGRP KSGGTTRPKAATSEGVQVKRVLEKSPGKLLVK MPFQTSPPGKAEGGGATTSTQVMVIKRPGRKR KAEADPQAIPKKRGRKPGSVVAAAAAEAKKK AVKESSIRSVQETVLPKKRKTRETVSIEVKEVV KPLLVLSTLGEKSGKGLKTCKSPGRKSKESSPKG RSSASSPPKKEHHHHHHHSESPKAPVLLPPL PPPPPEPESSEDPTSPPEPQDLSSSVCKEEKMPR GGSLESDGCPKEPAKTQPAVATAATAAEKYK HRGGERKDIVSSSMRPNREEPVDSRTPVTER VS	Rett Syndrome	143
ASPA	P45381	MTSCHIAEEHIQKVAIFGGTHGNELTGVFLVKH WLENGAEIQRGTGLEVKPFITNPRAVKKCTRYID CDLNRIFDLENLGKKMSEDLPEYVRRRAQEINH LFGPKDSEDSYDIIFDLHNTTSNMGCTLILED SRNNFLIQMFHYIKTSLAPLPCYVYLIEHPSLKYA TTRSIKYPVGIEVGPQPQGVLRADILDQMRK MIKHALDFIHHFNEGKEFPPCAIEVYKIEKVDY PRDENGIEAIIHPNLQDQDWKPLHPGDPMFLT LDGKTIPLGGDCTVYPVFNAAAYYEKKEAFA KTKLTLNAKSIRCLH	Canavan Disease	144
ALD H7A1	P49419	MWRLPRALCVHAAKTSKLSGPWSRPAAFMST LLINQPQYAWLKEGLREENEGVYNGSWGGR GEVITTYCPANNEPIARVRQASVADYEETVKK AREAWKIWADIPAPKRGEIVRQIGDALREKIQV LGSLVSLEMGKILVEGVGEVQEYVDICDYAVG LSRMIGGPILPSERSGHALIEQWNPVGLVGITA FNFPVAVYGWNNAIAMICGNVCLWKGAPTTS LISVAVTKIIAKVLEDNKLPGAICSLTCGGADIG TAMAKDERVNLLSFTGSTQVGKQVGLMVQER	Pyridoxine-Dependent Epilepsy	145

Gene	Uniprot Protein(s) Accession Number	Amino Acid Sequence (first Uniprot Accession Number)	Disease / Disorder	SEQ ID NO
		FGRSLLELGGNNAIIFEDADLSLVVPSALFAA VGTAGQRCTTARRLFIHESIHDDEVNRLKKAY AQIRVGNPWDPNVLYGPLHTKQAVSMFLGAV EEAKKEGGTVVYGGKVMDRPGNYVEPTIVTG LGHDASIAHTETFAPILYVFKFKNEEEVFAWNN EVKQGLSSSIFTKDLGRIFRWLGPKGSDCGIVN VNIPTSGAEIGGAFGGKEKHTGGGRESGSDAWK QYMRRSTCTINYSKDLPLAQGIKFQ		

In some embodiments, the target cell is a cell in the cerebellum, e.g., for the treatment of Spinocerebellar Ataxia, Autosomal Recessive, Type 1, e.g., when the exogenous agent is SYNE1. In some embodiments, the target cell is a cell in the brain, spinal cord, and/or muscles, e.g., for the treatment of Ataxia with Oculomotor Apraxia, Type 2, e.g., when the exogenous agent is SETX. In some embodiments, the target cell is a neuron or an astrocyte, e.g., for the treatment of Fragile X Syndrome, e.g., when the exogenous agent is FMR1. In some embodiments, the target cell is a motor neuron, e.g., for the treatment of Amyotrophic Lateral Sclerosis, e.g., when the exogenous agent is SOD1, TDP43, or C9orf72. In some embodiments, the target cell is a cell in the nervous system, heart, liver, and/or skeletal muscle, e.g., for the treatment of Friedreich's Ataxia, e.g., when the exogenous agent is FXN. In some embodiments, the target cell is a neuron in the brain, e.g., for the treatment of Rett Syndrome, e.g., when the exogenous agent is MECP2. In some embodiments, the target cell is an oligodendrocyte or a neuron, e.g., for the treatment of Canavan Disease, e.g., when the exogenous agent is ASPA.

In some embodiments, the exogenous agent comprises a protein of Table 6 below. In some embodiments, the exogenous agent comprises the wild-type human sequence of any of the proteins of Table 6, a functional fragment thereof (e.g., an enzymatically active fragment thereof), or a functional variant thereof. In some embodiments, the exogenous agent comprises an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%, identity to an amino acid sequence of Table 6, e.g., a Uniprot Protein Accession Number sequence of column 2 of Table 6 or an amino acid sequence of column 3 of Table 6. In some embodiments, the payload gene encodes an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%, identity to an amino acid sequence of Table 6.

Table 6. Lysosomal storage diseases or disorders and/or CNS diseases or disorders. The first column lists exogenous agents that can be delivered to treat the indications in the fourth column, according to the methods and uses herein. Each Uniprot accession number of Table 6 is herein incorporated by reference in its entirety.

Gene	Uniprot Protein(s) Accession Number	Amino Acid Sequence (first Uniprot Accession Number)	Disease / Disorder	SEQ IS NO
TPP1	O14773	MGLQACLLGLFALILSGKCSYSPEPDQRRTLPGWVSLGRADPEEELSLTFALRQQNVERLSELVQAVSDPSSPQYGKYLLENVADLVRPSPLTLHTVQKWLLAAGAQQKCHSVITQDFLTCWLSIRQAELLPGAEFHYYVGGPTETHVVRSPHPYQLPQALAPHVDFVGGGLHRFPPTSSLRQRPEPQVTGTVGLHLGVTPSVIRKRYNLTSQDVSGGTSNNSQACAQFLEQYFHDSDLAQFMRLFSGNFAHQASVARVVGQQGRGRAGIEASLDVQYLMSAGANISTWVYSSPGRHEGQEPFLQWLMLLSNESALPHVHTVSYGDDEDSLSSAYIQRVNTLMKAAARGLTLLFASGDSGAGCWSVSGRHQFRPTFPASSPYVTTVGGTSFQEPFLITNEIVDYISGGGFSNVFPRPSYQEEAVTKFLSSSPHLPPSSYFNASGRAYPDVAALSDGYWVVSNRVPIPWVSGTSASTPVFGGILSLINEHRILSGRPPLGFLNPRLYQQHGAGLFDVTRGCHESCLDEEVEGQGFCSGPGWDPVTGWGTPNFPALLKTLLNP	Batten Disease (CLN2)	146
FUC A1	P04066	MRAPGMRSRPAGPALLLLLLFLGAAESVRRAQPPRRYTPDWPSLDSRPLPAWFDEAKFGVFIHWGVFSVPAWGSEWFWWHWQGEGRPQYQRFMRDNYPPGFSYADFGPQFTARFFHPEEWADLFQAAGAKYVVLTTKHHEGFTNWSPVSWNWNSKDVGPHRDLVGELGTALRKRNIRYGLYHSLLEWFHPLYLLDKKNGFKTQHFVSAKTMPELYDLVNSYKPDLIWSDGEWECPTYWNSTNFLSWLYNDSPVKDEVVVNDRWGQNCSCHHGGYYNCEDKFKPQSLPDHKWEMCTSIDKFSWGYRRDMALSDVTEESEIISLVQTVSLGGNYLLNIGPTKDGLIVPIFQERLLAVGKWLSINGEAIYASKPWRVQWEKNTTSVWYTSKGSVYAIFLHWPENGLNLESPITTSSTKITMLGIQGDWKVSTDTPDKGLFISLPQLPPSAVPAEFAWTIKLTGVK	Fucosidosis	147
GAL C	P54803	MAEWLLSASWQRRAKAMTAAAGSAGRAAVPLLLCALLAPGGAYVLDDSDGLGREFDGIGAVSGGGATSRLLVNYPEPYRSQILDYLFKPNFGASLHILKVEIGGDGQTTDGTPEPSHMHYALDENYFRGYEWWMKEAKRNPNTLIGLPWSFGWLKGFDWPYVNLQLTAYYVVTWIVGAKRYHDLDDIDYIGIWNERSYNANYIKILRKMLNYQGLQRVKIIASDNLWESISASMLLDAELFKVV DVIGAHYPGTHSAKDAKLTKKKLWSSDFSTLNSDMGAGCWGRILNQNYINGYMTSTIAWN	Krabbe Disease	148

Gene	Uniprot Protein(s) Accession Number	Amino Acid Sequence (first Uniprot Accession Number)	Disease / Disorder	SEQ IS NO
		LVASYEEQLPYGRCGLMTAQEPWSGHYVVE SPVWVSAHTTQFTQPGWYYLKTGVHLEKGG SYVALTDGLGNLTIIETMSHKHSCIRPFLPY FNVSQQFATFVLKGSFSEIPELQVWYTKLGKT SERFLFKQLDSLWLLSDSGSFTLSLHEDELFT LTTLTTGRKGSYPLPPKSQFPSTYKDDFNVD YFFFSEAPNFADQTGVFEYFTNIEDPGEHHFT LRQVLNQRPIITWAADASNTISIIGDYNWTNLT IKCDVYIETPDTGGVFIAGRVNKGILIRSARG IFFWIFANGSYRVTDGLAGWIIYALGRVEVTA KKWYTLTLTIKGHFTSGMLNDKSLWTDIPVN FPKNGWAAIGTHSFEFAQFDNFLVEATR		
HEX A	P06865	MTSSRLWFSLLAAAFAGRATALWPWPQNF QTSDQRYVLYPNNFQFQYDVSSAAQPGCSVL DEAFQRYRDLLFGSGSWPRPYLTGKRHTLEK NVLVVSVVTPGCNQLPTLESVENYTLTINDD QCLLSETVWGALRGLETFSQLVWKS AEGTF FINKTEIEDFPRFPHRGLLDTSRHYLP LSSILD TLDVMAYNKLNVFHWHLVDDPSFPYESFTFP ELMRKGSYNPVTHIYTAQDVKEVIEYARLRGI RVLA EFDTPGHTLSWGP GIPGLLTPCYSGSEP SGTFGPVNPSLNNTYEFMSTFFLEVSSVFPDF YLHLGGDEVDFTCWKS NPEIQDFMRKKGFGE DFKQLESFYIQTL LDIVSSYGKGYVWQEVF DNKVKIQPDTHIQVWREDIPVNYMKELELVTK AGFRALLSAPWYLNRI SYGPDWKDFYIVEPL AFEGTPEQKALVIGGEACMWGEYVDNTNLV PRLWPRAGAVAERLWSNKLTS DLTFA YERLS HFRCELLRRGVQAQPLNVGFCEQEFEQT	Tay Sachs Disease	149
HEX B	P07686	MELCGLGLPRPPMLLALLLATLLAAMLALLT QVALVVQVAEAA RAPS VSAKGPALWPLPLS VKMTPNLLHLAPENFYISHSPNSTAGPSC TLL EEAFRRYHGYIFGFYKWHHEPAEFQAKTQVQ QLLV SITLQSECD AFPNISSDES YTL LVKEPVA VLKANRVWGALRGLETFSQLVYQDSYGTFTI NESTIIDSPRFSHRGILIDTSRHYLPVKIILKTL D AMAFNKFNVLHWHIVDDQSFPYQSITFPELSN KGSYSLSHVYTPNDVRMVIEYARLRGIRVLPE FDTPGHTLSWGKGQKDLLTPCYSRQNKLD SF GPINPTLNTTYSFLTTFK EISEVFPDQFIHLGG DEVEFKCWESNPKIQDFMRQKGFGTDFKKLE SFYIQKVLDIIATINKGSIVWQEVFDDKAKLAP GTIVEVWKDSAYPEELSRVTASGFPVILSAPW YLDLISYGQDWRKYKVEPLDFGGTQKQKQ LFIGGEACLWGEYVDATNLTPRLWPRASAVG ERLWSSKDVRDMDDAYDRLTRHRCRMVER GIAAQPLYAGYCNHENM	Sandhoff Disease	150
MAN BA	O00462	MRLHLLLLLALCGAGTTAAELSYSLRGNWSI CNGNGSLELPGAVPGCVHSALFQQGLIQDSY YRFNDLNYRWVSLDNWTYSKEFKIPFEISKW QKVNLI EGVDTVSKILFNEVTIGETDNMFNR YSFDITNVVRDVNSIELRFQSAVLYAAQQSKA	Beta-mannosidosis	151

Gene	Uniprot Protein(s) Accession Number	Amino Acid Sequence (first Uniprot Accession Number)	Disease / Disorder	SEQ IS NO
		HTRYQVPPDCPPLVQKGECHVNFVRKEQCSF SWDWGPSFPTQGIWKDVRIEAYNICHNLNYFT FSPIYDKSAQEWNLIESTFDVVSSKPVGGQVI VAIPKLQTQQTYSIELQPGKRIVELFVNISKNIT VETWWPHGHGNQTGYNMTVLFELDGGGLNIE KSAKVYFRTVELIEEPIKGGSPGLSFYFKINGFPI FLKGSNWIPADSFQDRVTSELLRLLLQSVVDA NMNTRLRVWGGGIYEQDEFYELCDELGIMVW QDFMFACALYPTDQGF LDSVTAEVAYQIKRL KSHPSIIIWSGNNEEALMMNWHISFTDRP IYIKDYVTLYVKNIRELVLAGDKSRPFITSSPT NGAETVAEAWVSQNPNSNYFGDVHFDYIS DCWNWKVFPKARFASEYGYQSWPSFSTLEK VSSTEDWSFNSKFSLHRQHHEGGNKQMLYQ AGLHFKLPQSTDPLRTFKDTIYLTQVMQAQC VKTETEFYRRSRSEIVDQQGHTMGALYWQLN DIWQAPSWASLEYGGKWMLHYFAQNFFAP LLPVGFENENTFYIYGVSDDLHSDYSMTLSVRV HTWSSLEPVCSRVTERTFVMKGGAEVCLYEPP VSELLRRCGNCTRESCVVSFYLSADHELLSPT NYHFLSSPKEAVGLCKAQITAIISQQGDIFVFD LETSAPFVWLDVGSIPGRFSDNGFLMTEKT RTILFYWPWEPTSKNELEQSFHVTSLTDIY		
ARS A	P15289	MGAPRSLLLALAAGLAVARPPNIVLIFADDLG YDGLGCYGHPSSTTPNLDQLAAGGLRFTDFY VPVSLCTPSRAALLTGRLPVRMGMYPGVLVP SSRGGLPLEEVTVAEVLAAARGYLTGMAGKW HLGVGPEGAFLLPHQGFHRFLGIPYSHDQGPC QNLTCFPPATPCDGGCDQGLVPIPLLANSVE AQPPWLPGLEARYMAFAHDLMAADAQRQDRP FFLYYASHHHTYPQFSGQSFAERSGRGPFGDS LMELDAAVGTLMTAIGDLGLLEETLVIFTAD NGPETMRMSRGGCSGLLRGKGTTYEGGVR EPALAFWPBGHIAPGVTHELASSDLLPTLAAL AGAPLPNVTLDDGFDLSPLLLGTGKSPRQSLFF YPSYPDEVRGVFAVRTGKYKAHFFTQGSAS DTTADPACHASSSLTAHEPPLLYDLSKDPGEN YNLLGGVAGATPEVLQALKQLQLLKAQLDA AVTFGPSQVARGEDPALQICCHPGCTPRPACC HCPDPA	Metachromatic Leukodystrophy	152
GNP TAB	Q3T906	MLFKLLQRQTYTCLSHRYGLYVCFLGVVVTI VSAFQFGEVVLEWSRDQYHVLFDSDYRDNIAG KSFQNRCLCPMPIDVVYTWVNGTDLELLKEL QQVREQMEEEQKAMREILGKNTTEPTKKSEK QLECLLTHCIKVPMLVLDPALPANITLKDLP LYPSFHSASDIFNVAKPKNPSTNVSVVVFST KDVEDAHSGLLKGNSRQTVWRGYLTDDKEV PGLVLMQDLAFLSGFPPTFKETNQLKTKLPEN LSSKVLLQLYSEASVALLKLNNPKDFQELN KQTKKNMTIDGKELTISPAYLLWDLAISQSK QDEDISASRFEDNEELRYSLSIERHAPWVRNI FIVTNGQIPSWLNLNPNRVTIVTHQDVFRNLS	Mucopolidosis Type IIIa; Mucopolidosis Type IIIb	153

Gene	Uniprot Protein(s) Accession Number	Amino Acid Sequence (first Uniprot Accession Number)	Disease / Disorder	SEQ IS NO
		HLPTFSSPAIESHIHRIEGLSQKFIYLNDDVMF GKDVWPDDFYSHSKGQKVYLTWPVPNCAEG CPGSWIKDGYCDKACNNSACDWDGGDCSGN SGGSRYIAGGGGTGSIGVGQPWQFGGGINSVS YCNQGCANSWLADKFCDQACNVLSGFDAG DCGQDHFHELYKVILLPNQTHYIIPKGECLPY FSFAEVAKRGVGAYSDNPIIRHASIANKWKT IHLIMHSGMNATTIHFNLTFQNTNDEEFKMQI TVEVDTREGPKNLSTAQKGYENLVSPITLLPE AEILFEDIPKEKRFPKFRHVDVNSTRRAQEEV KIPLVNISLLPKDAQLSLNTLDLQLEHGDITLK GYNLSKSALLRSFLMNSQHAKIKNQAIITDET NDSLVAPEKQVHKSILPNSLGVSERLQRLTF PAVSVKVNNGHDQGGNPPLDLETTARFRVETH TQKTIGGNVTKEKPPSLIVPLESQMTKEKKIT GKEKENSMEENAENHIGVTEVLLGRKLQHY TDSYLGFLPWEEKKYYFQDLLDEEESLKTQLA YFTDSKNTGRQLKDTFADSLRYVNKILNSKF GFTSRKVPAHMPHMIDRIVMQELQDMFPEEF DKTSFHKVRHSEDMQFAFSYFYLLMSAVQPL NISQVFDEVDTDQSGVLSREIRTLATRIHELP LSLQDLTGLEHMLINCSKMLPADITQLNNIPP TQESYYDPNLPPVTKSLVTNCKPVTDKIHKA YKDKNKYRFEIMGEEIEAFKMIRTNVSHVVG QLDDIRKNPRKFVCLNDNIDHNHKDAQTVKA VLRDFYESMFPIPSQFELPREYRNRFLMHMEL QEWRAIRDKLFKFWTHCVLATLIMFTIFSFFA EQLIALKRKIFPRRRHKEASPNRIRV		
MCO LN1	Q9GZU1	MTAPAGPRGSETERLLTPNPGYGTQAGPSPAP PTPPEEEDLRRRLKYFFMSPCDKFRAKGRKPC KLMLQVVKILVVTVQLILFGLSNQLAVTFREE NTIAFRHLFLLGYSDGADDTFAAYTREQLYQ AIFHAVDQYLALPDVSLGRYAYVRGGGDPW TNGSGLALCQRYYHRGHVDPANDTFDIDPM VVTDCIQVDPPERPPPPSDDLTLLESSSYKN LTLKFHKLNVNTHFRKLTINLQSLINNEIPDC YTFSLITFDNKAHSGRIPISLETQAHIQECKH PSVFQHGDNFRLFDVVILTCSLSFLLCARS LLRGFLLQNEFVGFMWRQRGRVISLWERLEF VNGWYILLVTSVLTISGTIMKIGIEAKNLAS YDVCSILLGTSTLLVWVG VIRYLTFFHNYNLI ATRLVALPSVMRFCCCVAVIYLYGYCFGWIV LGPYHVKFRSLSMVSECLFSLINGDDMFVTFA AMQAQQGRSSLVWLFSQLYLYSFISLFIYMLV SLFIALITGAYDTIKHPGGAGAESEELQAYIAQ CQDSPTSGKFRRGSGSACSLCCCGRDPSEEH SLLVN	Mucopolidosis Type IV	154

In some embodiments, the target cell is an oligodendrocyte, e.g., for the treatment of Krabbe Disease, e.g., when the exogenous agent is GALC. In some embodiments, the target cell

is a neuron, e.g., for the treatment of Tay Sachs Disease, e.g., when the exogenous agent is HEXA. In some embodiments, the target cell is a neuron, e.g., for the treatment of Sandhoff Disease, e.g., when the exogenous agent is HEXB.

In some embodiments, the protein agent is other than a clotting factor, e.g., other than Factor VII or Factor IX. In some embodiments, the protein agent is other than a reporter protein, e.g., fluorescent protein, e.g., GFP or luciferase. In some the protein agent is other than a cell surface receptor, an NGF receptor, galactocerebrosidase, gp91 phox, IFN-alpha, TK, GCV, and autoimmune antigen, cytokine, angiogenesis inhibitor, or anti-cancer agent, or a fragment or variant thereof.

VII. Insulator elements

In some embodiments, a fusosome, retroviral or lentiviral vector, or VLP further comprises one or more insulator elements, e.g., an insulator element described herein. Insulators elements may contribute to protecting lentivirus- expressed sequences, e.g., therapeutic polypeptides, from integration site effects, which may be mediated by cis-acting elements present in genomic DNA and lead to deregulated expression of transferred sequences (e.g., position effect; see, e.g., Burgess- Beusse et al, 2002, Proc. Natl. Acad. Sci., USA, 99: 16433; and Zhan et al, 2001, Hum. Genet., 109:471) or deregulated expression of endogenous sequences adjacent to the transferred sequences. In some embodiments, transfer vectors comprise one or more insulator element the 3' LTR and upon integration of the provirus into the host genome, the provirus comprises the one or more insulators at the 5' LTR and/or 3' LTR, by virtue of duplicating the 3' LTR. Suitable insulators include, but are not limited to, the chicken β -globin insulator (see Chung et al, 1993. Cell 74:505; Chung et al, 1997. N4S 94:575; and Bell et al., 1999. Cell 98:387, incorporated by reference herein) or an insulator from a human β -globin locus, such as chicken HS4. In some embodiments the insulator binds CCCTC binding factor (CTCF). In some embodiments the insulator is a barrier insulator. In some embodiments the insulator is an enhancer-blocking insulator. See, e.g., Emery et al., *Human Gene Therapy*, 2011, and in Browning and Trobridge, *Biomedicines*, 2016, both of which are included in their entirety by reference.

In some embodiments, insulators in the retroviral nucleic acid reduce genotoxicity in recipient cells. Genotoxicity can be measured, e.g., as described in Cesana et al, "Uncovering

and dissecting the genotoxicity of self-inactivating lentiviral vectors in vivo” Mol Ther. 2014 Apr;22(4):774-85. doi: 10.1038/mt.2014.3. Epub 2014 Jan 20.

VIII. Assessing fusosome content of target cell

The present disclosure also provides, in some aspects, a method of assessing fusosome content of a target cell (e.g., fusosome fusion to a target cell) in a subject, comprising providing a biological sample from a subject that has received a fusosome composition (e.g., a fusosome composition described herein), and performing an assay to determine one or more properties of the biological sample resulting from fusion of a target cell in the biological sample with a fusosome as described herein. In some aspects, the disclosure provides a method of measuring fusion with a target cell, e.g., as described in Example 71. In some embodiments, determining one or more properties of the biological sample comprises determining: the presence of a fusogen, the level of a cargo or payload, or an activity relating to a cargo or payload.

In some aspects, the present disclosure provides a method of assessing fusosome content of a target cell (e.g., fusosome fusion to a target cell) in a subject, comprising providing a biological sample from a subject that has received a fusosome composition, e.g., as described herein, and testing the biological sample for the presence of a fusogen, e.g., a fusogen described herein. In some instances, the level of the fusogen detected is greater (e.g., at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1000%, 2000%, 3000%, 4000%, 5000%, 10,000%, 50,000%, or 100,000% greater) than that observed in a corresponding biological sample from a subject that has not received a fusosome composition. In some embodiments, the subject is the same subject prior to administration of the fusosome composition, and in some embodiments, the subject is a different subject.

In some aspects, the present disclosure provides a method of assessing fusosome content of a target cell (e.g., fusosome fusion to a target cell) in a subject, comprising providing a biological sample from a subject that has received a fusosome composition, e.g., as described herein, and testing the biological sample for the presence of a cargo or payload, e.g., delivered by a fusosome as described herein. In some instances, the level of the cargo or payload detected is greater (e.g., at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1000%, 2000%, 3000%, 4000%,

5000%, 10,000%, 50,000%, or 100,000% greater) than that observed in a corresponding biological sample from a subject that has not received a fusosome composition. In some embodiments, the subject is the same subject prior to administration of the fusosome composition, and in some embodiments, the subject is a different subject.

5 In some aspects, the present disclosure provides a method of assessing fusosome content of a target cell (e.g., fusosome fusion to a target cell in a subject), comprising providing a biological sample from a subject that has received a fusosome composition, e.g., as described herein, and testing the biological sample for alteration of an activity relating to the fusosome composition, e.g., an activity relating to a cargo or payload delivered by the fusosome
10 composition. In some instances, the level of the activity detected is increased, e.g., by at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1000%, 2000%, 3000%, 4000%, 5000%, 10,000%, 50,000%, or 100,000%, relative to that of a corresponding biological sample from a subject that has not received a fusosome composition (e.g., the same subject prior to administration of the fusosome
15 composition). In some instances, the level of the activity detected is decreased, e.g., by at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1000%, 2000%, 3000%, 4000%, 5000%, 10,000%, 50,000%, or 100,000%, relative to that of a corresponding biological sample from a subject that has not received a fusosome composition. In some embodiments, the subject is the same subject prior to
20 administration of the fusosome composition, and in some embodiments, the subject is a different subject.

In one aspect, the present disclosure provides a method of assessing fusosome fusion to a target cell in a subject, comprising providing a biological sample from a subject that has received a fusosome composition, e.g., as described herein, and assessing a level of unfused fusosomes in
25 the biological sample.

In some embodiments of the methods of assessing fusosome content of a target cell (e.g., fusosome fusion to a target cell), resulting in formation of a recipient cell, in the subject, the method further comprises collecting the biological sample from the subject. In embodiments, the biological sample includes one or more recipient cells.

30 In some embodiments of the methods of assessing fusosome content of a target cell (e.g., fusosome fusion to a target cell) in the subject, the method further comprises separating recipient

cells in the biological sample from unfused fusosomes in the biological sample, e.g., by centrifugation. In some embodiments, the method further comprises enriching recipient cells relative to unfused fusosomes in the biological sample, e.g., by centrifugation. In some embodiments, the method further comprises enriching target cells relative to non-target cells in the biological sample, e.g., by FACS.

In some embodiments of the methods of assessing fusosome content of a target cell (e.g., fusosome fusion to a target cell) in a subject, the activity relating to the fusosome composition is chosen from the presence or level of a metabolite, the presence or level of a biomarker (e.g., a protein level or post-translational modification, e.g., phosphorylation or cleavage).

In some embodiments of the methods of assessing fusosome content of a target cell (e.g., fusosome fusion to a target cell) in a subject, the activity relating to the fusosome composition is immunogenicity. In embodiments, the target cell is a CD3+ cell and the biological sample is a blood sample collected from the subject. In embodiments, blood cells are enriched from the blood sample, e.g., using a buffered ammonium chloride solution. In embodiments, enriched blood cells are incubated with an anti-CD3 antibody (e.g., a murine anti-CD3-FITC antibody) and CD3+ cells are selected, e.g., by fluorescence activated cell sorting. In embodiments, cells, e.g., sorted cells, e.g., CD3+ cells are analyzed for the presence of antibodies on the cell surface, e.g., by staining with an anti-IgM antibody. In some embodiments, if antibodies are present at a level above a reference level, the subject is identified as having an immune response against recipient cells.

In embodiments, immunogenicity is assayed by a cell lysis assay. In embodiments, recipient cells from the biological sample are co-incubated with immune effector cells capable of lysing other cells. In embodiments, the immune effector cells are from the subject or from a subject not administered the fusosome composition. For instance, in embodiments, immunogenicity is assessed by a PBMC cell lysis assay. In embodiments, recipient cells from the biological sample are co-incubated with peripheral blood mononuclear cells (PBMCs) from the subject or control PBMCs from a subject not administered the fusosome composition and then assessed for lysis of the recipient cells by PBMCs. In embodiments, immunogenicity is assessed by a natural killer (NK) cell lysis assay. In embodiments, recipient cells are co-incubated with NK cells from the subject or control NK cells from a subject not administered the fusosome composition and then assessed for lysis of the recipient cells by the NK cells. In

embodiments, immunogenicity is assessed by a CD8+ T-cell lysis assay. In embodiments, recipient cells are co-incubated with CD8+ T-cells from the subject or control CD8+ T-cells from a subject not administered the fusosome composition and then assessed for lysis of the target cells by the CD8+ T-cells. In some embodiments, if cell lysis occurs at a level above a reference level, the subject is identified as having an immune response against recipient cells.

In some embodiments, immunogenicity is assayed by phagocytosis of recipient cells, e.g., by macrophages. In embodiments, recipient cells are not targeted by macrophages for phagocytosis. In embodiments, the biological sample is a blood sample collected from the subject. In embodiments, blood cells are enriched from the blood sample, e.g., using a buffered ammonium chloride solution. In embodiments, enriched blood cells are incubated with an anti-CD3 antibody (e.g., a murine anti-CD3-FITC antibody) and CD3+ cells are selected, e.g., by fluorescence activated cell sorting. In embodiments, fluorescently-labeled CD3+ cells are incubated with macrophages and then tested for intracellular fluorescence within the macrophages, e.g., by flow cytometry. In some embodiments, if macrophage phagocytosis occurs at a level above a reference level, the subject is identified as having an immune response against recipient cells.

IX. Physical and functional characteristics of fusosomes

In some embodiments, the fusosome is capable of delivering (e.g., delivers) an agent, e.g., a protein, nucleic acid (e.g., mRNA), organelle, or metabolite to the cytosol of a target cell. Similarly, in some embodiments, a method herein comprises delivering an agent to the cytosol of a target cell. In some embodiments, the agent is a protein (or a nucleic acid encoding the protein, e.g., an mRNA encoding the protein) which is absent, mutant, or at a lower level than wild-type in the target cell. In some embodiments, the target cell is from a subject having a genetic disease, e.g., a monogenic disease, e.g., a monogenic intracellular protein disease. In some embodiments, the agent comprises a transcription factor, e.g., an exogenous transcription factor or an endogenous transcription factor. In some embodiments, the fusosome further comprises, or the method further comprises delivering, one or more (e.g., at least 2, 3, 4, 5, 10, 20, or 50) additional transcription factors, e.g., exogenous transcription factors, endogenous transcription factors, or a combination thereof.

In some embodiments, the fusosome 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 each agent of the plurality acts on a step of a pathway in the target cell, e.g., wherein the pathway is a biosynthetic pathway, a catabolic pathway, or a signal transduction cascade. In embodiments, each agent in the plurality upregulates the pathway or downregulates the pathway. In some embodiments, the fusosome further comprises, or the method further comprises delivering, one more additional agents (e.g., comprises a second plurality of agents) that do not act on a step of the pathway, e.g., that act on a step of a second pathway. In some embodiments, the fusosome comprises (e.g., is capable of delivering to the target cell), or the method further comprises delivering, a plurality of agents (e.g., at least 2, 3, 4, 5, 10, 20, or 50 agents), wherein each agent of the plurality is part of a single pathway, e.g., wherein the pathway is a biosynthetic pathway, a catabolic pathway, or a signal transduction cascade. In some embodiments, the fusosome further comprises, or the method further comprises delivering, one more additional agents (e.g., comprises a second plurality of agents) that are not part of the single pathway, e.g., are part of a second pathway.

In some embodiments, the target cell comprises an aggregated or misfolded protein. In some embodiments, the fusosome is 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.

In some embodiments, the agent is selected from a transcription factor, enzyme (e.g., nuclear enzyme or cytosolic enzyme), reagent that mediates a sequence specific modification to DNA (e.g., Cas9, ZFN, or TALEN), mRNA (e.g., mRNA encoding an intracellular protein), organelle, or metabolite.

In some embodiments, the fusosome is capable of delivering (e.g., delivers) an agent, e.g., a protein, to the cell membrane of a target cell. Similarly, in some embodiments, a method herein comprises delivering an agent to the cell membrane of a target cell. In some embodiments, delivering the protein comprises delivering a nucleic acid (e.g., mRNA) encoding the protein to the target cell such that the target cell produces the protein and localizes it to the membrane. In some embodiments, 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. In some embodiments, the agent comprises a cell surface ligand or an antibody that binds a cell surface receptor. In some embodiments, 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). In some embodiments, the first agent and the second agent form a complex, wherein optionally the complex further comprises one or more additional cell surface ligands. In some embodiments, the agent comprises or encodes a cell surface receptor, e.g., an exogenous cell surface receptor. In some embodiments, the fusosome further comprises, 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).

In some embodiments, the first agent and the second agent form a complex, wherein optionally the complex further comprises one or more additional cell surface receptors. In some embodiments, the agent comprises or encodes an antigen or an antigen presenting protein.

In some embodiments, the fusosome is capable of delivering (e.g., delivers) 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., mRNA) encoding the protein to the target cell under conditions that allow the target cell to produce and secrete the protein. Similarly, in some embodiments, a method herein comprises delivering a secreted agent as described herein. In embodiments, the secreted protein is endogenous or exogenous. In embodiments, the secreted protein comprises a protein therapeutic, e.g., an antibody molecule, a cytokine, or an enzyme. In embodiments, the secreted protein comprises an autocrine signalling molecule or a paracrine signalling molecule. In embodiments, the secreted agent comprises a secretory granule.

In some embodiments, the fusosome is capable of reprogramming (e.g., reprograms) a target cell (e.g., an immune cell), e.g., by delivering an agent selected from a transcription factor or mRNA, or a plurality of said agents. Similarly, in some embodiments, a method herein comprises reprogramming a target cell. In embodiments, reprogramming comprises inducing a pancreatic endocrine cell to take on one or more characteristics of a pancreatic beta cell, by inducing a non-dopaminergic neuron to take on one or more characteristics of a dopaminergic neuron, or by inducing an exhausted T cell to take on one or more characteristics of a non-exhausted T cell, e.g., a killer T cell. In some embodiments, the agent comprises an antigen. In

some embodiments, the fusosome comprises a first agent comprising an antigen and a second agent comprising an antigen presenting protein.

In some embodiments, the fusosome is capable of donating (e.g., donates) one or more cell surface receptors to a target cell (e.g., an immune cell). Similarly, in some embodiments, a method herein comprises donating one or more cell surface receptors.

In some embodiments, a fusosome is capable of modifying, e.g., modifies, a target tumor cell. Similarly, in some embodiments, a method herein comprises modifying a target tumor cell. In embodiments, the fusosome comprises an mRNA encoding an immunostimulatory ligand, an antigen presenting protein, a tumor suppressor protein, or a pro-apoptotic protein. In some embodiments, the fusosome comprises an miRNA capable of reducing levels in a target cell of an immunosuppressive ligand, a mitogenic signal, or a growth factor.

In some embodiments, a fusosome comprises an agent that is immunomodulatory, e.g., immunostimulatory.

In some embodiments, a fusosome is capable of causing (e.g., causes) the target cell to present an antigen. Similarly, in some embodiments, a method herein comprises presenting an antigen on a target cell.

In some embodiments, the fusosome promotes regeneration in a target tissue. Similarly, in some embodiments, a method herein comprises promoting regeneration in a target tissue. In embodiments, the target cell is a cardiac cell, e.g., a cardiomyocyte (e.g., a quiescent cardiomyocyte), a hepatoblast (e.g., a bile duct hepatoblast), an epithelial cell, a naïve T cell, a macrophage (e.g., a tumor infiltrating macrophage), or a fibroblast (e.g., a cardiac fibroblast). In embodiments, the source cell is a T cell (e.g., a Treg), a macrophage, or a cardiac myocyte.

In some embodiments, the fusosome is capable of delivering (e.g., delivers) a nucleic acid to a target cell, e.g., to stably modify the genome of the target cell, e.g., for gene therapy. Similarly, in some embodiments, a method herein comprises delivering a nucleic acid to a target cell. In some embodiments, the target cell has an enzyme deficiency, e.g., comprises a mutation in an enzyme leading to reduced activity (e.g., no activity) of the enzyme.

In some embodiments, the fusosome is capable of delivering (e.g., delivers) a reagent that mediates a sequence specific modification to DNA (e.g., Cas9, ZFN, or TALEN) in the target cell. Similarly, in some embodiments, a method herein comprises delivering the reagent to the target cell. In embodiments, the target cell is a CNS cell.

In some embodiments, the fusosome is capable of delivering (e.g., delivers) a nucleic acid to a target cell, e.g., to transiently modify gene expression in the target cell.

In some embodiments, the fusosome is capable of delivering (e.g., delivers) a protein to a target cell, e.g., to transiently rescue a protein deficiency. Similarly, in some embodiments, a method herein comprises delivering a protein to a target cell. In embodiments, the protein is a membrane protein (e.g., a membrane transporter protein), a cytoplasmic protein (e.g., an enzyme), or a secreted protein (e.g., an immunosuppressive protein).

In some embodiments, the fusosome is capable of delivering (e.g., delivers) an organelle to a target cell, e.g., wherein the target cell has a defective organelle network. Similarly, in some embodiments, a method herein comprises delivering an organelle to a target cell. In embodiments, the source cell is a hepatocyte, skeletal muscle cell, or neuron.

In some embodiments, the fusosome is capable of delivering (e.g., delivers) a nucleus to a target cell, e.g., wherein the target cell has a genetic mutation. Similarly, in some embodiments, a method herein comprises delivering a nucleus to a target cell. In some embodiments, the nucleus is autologous and comprises one or more genetic changes relative to the target cell, e.g., it comprises a sequence specific modification to DNA (e.g., Cas9, ZFN, or TALEN), or an artificial chromosome, an additional genetic sequence integrated into the genome, a deletion, or any combination thereof. In embodiments, the source of the autologous nucleus is a stem cell, e.g., a hematopoietic stem cell. In embodiments, the target cell is a muscle cell (e.g., a skeletal muscle cell or cardiomyocyte), a hepatocyte, or a neuron.

In some embodiments, the fusosome is capable of intracellular molecular delivery, e.g., delivers a protein agent to a target cell. Similarly, in some embodiments, a method herein comprises delivering a molecule to an intracellular region of a target cell. In embodiments, the protein agent is an inhibitor. In embodiments, the protein agent comprises a nanobody, scFv, camelid antibody, peptide, macrocycle, or small molecule.

In some embodiments, the fusosome is capable of causing (e.g., causes) a target cell to secrete a protein, e.g., a therapeutic protein. Similarly, in some embodiments, a method herein comprises causing a target cell to secrete a protein.

In some embodiments, the fusosome is capable of secreting (e.g., secretes) an agent, e.g., a protein. In some embodiments, the agent, e.g., secreted agent, is delivered to a target site in a subject. In some embodiments, the agent is a protein that can not be made recombinantly or is

difficult to make recombinantly. In some embodiments, the fusosome that secretes a protein is from a source cell selected from an MSC or a chondrocyte.

In some embodiments, the fusosome comprises on its membrane one or more cell surface ligands (e.g., 1, 2, 3, 4, 5, 10, 20, 50, or more cell surface ligands). Similarly, in some
 5 embodiments, a method herein comprises presenting one or more cell surface ligands to a target cell. In some embodiments, 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). In some embodiments the fusosome comprises a membrane complex, e.g., a
 10 complex comprising at least 2, 3, 4, or 5 proteins, e.g., a homodimer, heterodimer, homotrimer, heterotrimer, homotetramer, or heterotetramer. In some embodiments, the fusosome comprises an antibody, e.g., a toxic antibody, e.g., the fusosome is capable of delivering the antibody to the target site, e.g., by homing to a target site. In some embodiments, the source cell is an NK cell or neutrophil.

15 In some embodiments, a method herein comprises causing secretion of a protein from a target cell or ligand presentation on the surface of a target cell. In some embodiments, the fusosome is capable of causing cell death of the target cell. In some embodiments, the fusosome is from a NK source cell.

In some embodiments, a fusosome or target cell is capable of phagocytosis (e.g., of a
 20 pathogen). Similarly, in some embodiments, a method herein comprises causing phagocytosis.

In some embodiments, a fusosome senses and responds to its local environment. In some embodiments, the fusosome is capable of sensing level of a metabolite, interleukin, or antigen.

In embodiments, a fusosome is capable of chemotaxis, extravasation, or one or more metabolic activities. In embodiments, the metabolic activity is selected from kyneurinine,
 25 gluconeogenesis, prostaglandin fatty acid oxidation, adenosine metabolism, urea cycle, and thermogenic respiration. In some embodiments, the source cell is a neutrophil and the fusosome is capable of homing to a site of injury. In some embodiments, the source cell is a macrophage and the fusosome is capable of phagocytosis. In some embodiments, the source cell is a brown adipose tissue cell and the fusosome is capable of lipolysis.

In some embodiments, the fusosome 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). In embodiments, the fusosome comprises an inhibitory nucleic acid (e.g., siRNA or miRNA) and an mRNA.

5 In some embodiments, the fusosome comprises (e.g., is capable of delivering to the target cell) a membrane protein or a nucleic acid encoding the membrane protein. In embodiments, the fusosome is capable of reprogramming or transdifferentiating a target cell, e.g., the fusosome comprises one or more agents that induce reprogramming or transdifferentiation of a target cell.

10 In some embodiments, the subject is in need of regeneration. In some embodiments, the subject suffers from cancer, an autoimmune disease, an infectious disease, a metabolic disease, a neurodegenerative disease, or a genetic disease (e.g., enzyme deficiency).

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
15 bafilomycin A1, (b) culturing the target 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% 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
20 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 80.

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
25 lysosomal acidification. In some embodiments, an inhibitor of endocytosis (e.g., Dynasore) inhibits dynamin. In some embodiments, cargo delivery is independent of dynamin activity.

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
30 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% 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 71.

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 42. 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 42. 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 42. 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 42. 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 43.

In some embodiments, 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 26. In some embodiments, 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. In embodiments, the fusosome also comprises fusogen internally, e.g., in the cytoplasm or an organelle. In some embodiments, the fusogen comprises (or is identified as comprising) about 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 5%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, or more, or about 1-30%, 5-20%, 10-15%, 12-15%, 13-14%, or 13.6% of the total protein in a fusosome, e.g., as determined according to the method described in Example 94 and/or by a mass spectrometry assay. In embodiments, the fusogen comprises (or is identified as comprising) about 13.6% of the total

protein in the fusosome. In some embodiments, the fusogen is (or is identified as being) more or less abundant than one or more additional proteins of interest, e.g., as determined according to the method described in Example 94. In an embodiment, the fusogen has (or is identified as having) a ratio to EGFP of about 140, 145, 150, 151, 152, 153, 154, 155, 156, 157 (e.g., 156.9), 158, 159, 160, 165, or 170. In another embodiment, the fusogen has (or is identified as having) a ratio to CD63 of about 2700, 2800, 2900, 2910 (e.g., 2912), 2920, 2930, 2940, 2950, 2960, 2970, 2980, 2990, or 3000, or about 1000-5000, 2000-4000, 2500-3500, 2900-2930, 2910-2915, or 2912.0, e.g., by a mass spectrometry assay. In an embodiment, the fusogen has (or is identified as having) a ratio to ARRDC1 of about 600, 610, 620, 630, 640, 650, 660 (e.g., 664.9), 670, 680, 690, or 700. In another embodiment, the fusogen has (or is identified as having) a ratio to GAPDH of about 50, 55, 60, 65, 70 (e.g., 69), 75, 80, or 85, or about 1-30%, 5-20%, 10-15%, 12-15%, 13-14%, or 13.6%. In another embodiment, the fusogen has (or is identified as having) a ratio to CNX of about 500, 510, 520, 530, 540, 550, 560 (e.g., 558.4), 570, 580, 590, or 600, or about 300-800, 400-700, 500-600, 520-590, 530-580, 540-570, 550-560, or 558.4, e.g., by a mass spectrometry assay.

In some embodiments, 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 88. In some embodiments, the fusosome comprises 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 88. In some embodiments, the fusosome comprises 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. In some embodiments, the fusosome comprises 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. In some embodiments, the fusosome comprises 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. In some
embodiments, the fusosome comprises an exogenous 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
5 copies. In some embodiments, the fusosome comprises an exogenous 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. In some embodiments, the fusosome comprises an
exogenous nucleic acid (e.g., DNA or RNA) therapeutic agent at a copy number of at least 10,
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. In some
embodiments, 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
15 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.

In some embodiments, the fusosome delivers to a target cell 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
20 agent. In some embodiments, the fusosome delivers to a target cell 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. In some embodiments, the fusosome delivers to a target cell 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,
25 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. In some embodiments, the fusosome delivers to a target cell 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. In some embodiments, the fusosome delivers to a target
30 cell 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.

In some embodiments, 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 the cargo (e.g., a therapeutic agent, e.g., an endogenous therapeutic agent or an exogenous therapeutic agent) comprised by the fusosome. In some embodiments, 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 cargo (e.g., a therapeutic agent, e.g., an endogenous therapeutic agent or an exogenous therapeutic agent) comprised by the fusosomes that fuse with the target cell(s). In some embodiments, 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 cargo (e.g., a therapeutic agent, e.g., an endogenous therapeutic agent or an exogenous therapeutic agent) comprised by the fusosome composition.

In some embodiments, the fusosome comprises 0.00000001 mg fusogen to 1 mg fusogen per mg of total protein in fusosome, e.g., 0.00000001 - 0.00000001, 0.00000001 - 0.0000001, 0.0000001 - 0.000001, 0.000001 - 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. In some embodiments, the fusosome comprises 0.00000001 mg fusogen to 5 mg fusogen per mg of lipid in fusosome, e.g., 0.00000001 - 0.00000001, 0.00000001 - 0.0000001, 0.0000001 - 0.000001, 0.000001 - 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.

In some embodiments, the cargo is a protein cargo. In embodiments, the cargo is an endogenous or synthetic protein cargo. In some embodiments, the fusosomes have (or are identified as having) at least 1, 2, 3, 4, 5, 10, 20, 50, 100, or more protein cargo molecules per fusosome. In an embodiment, the fusosomes have (or are identified as having) about 100, 110, 120, 130, 140, 150, 160, 166, 170, 180, 190, or 200 protein agent molecules per fusosome, e.g., as quantified according to the method described in Example 88. In some embodiments, the endogenous or synthetic protein cargo comprises (or is identified as comprising) about 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 1%, 5%, 10%, 15%, 20%, 25% or more of the total protein in a fusosome. In an embodiment, the synthetic protein cargo comprises (or is identified as comprising) about 13.6% of the total protein in a fusosome. In some embodiments, the synthetic protein cargo has (or is identified as having) a ratio to VSV-G of about 4×10^{-3} , 5×10^{-3} , 6×10^{-3}

(e.g., 6.37×10^{-3}), 7×10^{-3} , or 8×10^{-3} . In embodiments, the synthetic protein cargo has (or is identified as having) a ratio to CD63 of about 10, 15, 16, 17, 18 (e.g., 18.6), 19, 20, 25, or 30, or about 10-30, 15-25, 16-19, 18-19, or 18.6. In embodiments, the synthetic protein cargo has (or is identified as having) a ratio to ARRDC1 of about 2, 3, 4 (e.g., 4.24), 5, 6, or 7. In embodiments, the synthetic protein cargo has (or is identified as having) a ratio to GAPDH of about 0.1, 0.2, 0.3, 0.4 (e.g., 0.44), 0.5, 0.6, or 0.7. In embodiments, the synthetic protein cargo has (or is identified as having) a ratio to CNX of about 1, 2, 3 (e.g., 3.56), 4, 5, or 6. In embodiments, the synthetic protein cargo has (or is identified as having) a ratio to TSG101 of about 10, 15, 16, 17, 18, 19 (e.g., 19.52), 20, 21, 22, 23, 24, 25, or 30.

In some embodiments, the fusogen comprises (or is identified as comprising) at least 0.5%, 1%, 5%, 10%, or more of the total protein in a fusosome, e.g., by a mass spectrometry assay. In an embodiment, the fusogen comprises (or is identified as comprising) about 1-30%, 5-20%, 10-15%, 12-15%, 13-14%, or 13.6% of the total protein in a fusosome, e.g., by a mass spectrometry assay. In some embodiments, the fusogen is more abundant than other proteins of interest. In embodiments, the fusogen has (or is identified as having) a ratio to a payload protein, e.g., EGFP, of about 145-170, 150-165, 155-160, 156.9, e.g., by a mass spectrometry assay. In embodiments, the fusogen has (or is identified as having) a ratio to CD63 of about 1000-5000, 2000-4000, 2500-3500, 2900-2930, 2910-2915, or 2912.0, e.g., by a mass spectrometry assay. In embodiments, the fusogen has a ratio to ARRDC1 of about 300-1000, 400-900, 500-800, 600-700, 640-690, 650-680, 660-670, or 664.9, e.g., by a mass spectrometry assay. In embodiments, the fusogen has (or is identified as having) a ratio to GAPDH of about 20-120, 40-100, 50-90, 60-80, 65-75, 68-70, or 69.0, e.g., by a mass spectrometry assay. In embodiments, the fusogen has a ratio to CNX of about 200-900, 300-800, 400-700, 500-600, 520-590, 530-580, 540-570, 550-560, or 558.4, e.g., by a mass spectrometry assay. In embodiments, the mass spectrometry assay is an assay of Example 94.

In some embodiments, the number of lipid species present in both of (e.g., shared between) the fusosomes and source cells is (or is identified as being) at least 300, 400, 500, 550, 560, or 569, or is between 500-700, 550-600, or 560-580, e.g., using a mass spectrometry assay. In embodiments, the number of lipid species present in fusosomes at a level at least 25% of the corresponding lipid level in the source cells (both normalized to total lipid levels within a sample) is (or is identified as being) at least 300, 400, 500, 530, 540, or 548, or is between 400-

700, 500-600, 520-570, 530-560, or 540-550, e.g., using a mass spectrometry assay. In some embodiments, the fraction of lipid species present in both of (e.g., shared between) the fusosomes and source cells to total lipid species in the source cell is (or is identified as being) about 0.4-1.0, 0.5-0.9, 0.6-0.8, or 0.7, or at least 0.4, 0.5, 0.6, or 0.7, e.g., using a mass spectrometry assay. In some embodiments, the mass spectrometry assay is an assay of Example 86.

In some embodiments, the number of protein species present in both of (e.g., shared between) the fusosomes and source cells is (or is identified as being) at least 500, 1000, 1100, 1200, 1300, 1400, 1487, 1500, or 1600, or is (or is identified as being) between 1200-1700, 1300-1600, 1400-1500, 1450-1500, or 1480-1490, e.g., using a mass spectrometry assay. In some embodiments, the number of protein species present in fusosomes at a level at least 25% of the corresponding protein level in the source cells (both normalized to total protein levels within a sample) is (or is identified as being) at least 500, 600, 700, 800, 900, 950, 957, 1000, or 1200, e.g., using a mass spectrometry assay. In some embodiments, the fraction of protein species present in both of (e.g., shared between) the fusosomes and source cells to total protein species in the source cell is (or is identified as being) about 0.1-0.6, 0.2-0.5, 0.3-0.4, or 0.333, or at least about 0.1, 0.2, 0.3, 0.333, or 0.4, e.g., using a mass spectrometry assay. In some embodiments, the mass spectrometry assay is an assay of Example 87.

In some embodiments, CD63 is (or is identified as being) present at less than 0.048%, 0.05%, 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, or 10% the amount of total protein in fusosomes, e.g., by a mass spectrometry assay, e.g., an assay of Example 89.

In some embodiments, the fusosomes are produced by extrusion through a filter, e.g., a filter of about 1-10, 2-8, 3-7, 4-6, or 5 μm . In some embodiments, the fusosomes have (or is identified as having) an average diameter of about 1-5, 2-5, 3-5, 4-5, or 5 μm . In some embodiments, the fusosomes have (or is identified as having) an average diameter of at least 1, 2, 3, 4, or 5 μm .

In some embodiments, the fusosomes are enriched for (or are identified as being enriched for) one or more of (e.g., at least 2, 3, 4, 5, or all of) the following lipids compared to the source cells: cholesteryl ester, free cholesterol, ether-linked lyso-phosphatidylethanolamine, lyso-phosphatidylserine, phosphatidate, ether-linked phosphatidylethanolamine, phosphatidylserine, and sphingomyelin. In some embodiments, the fusosomes are depleted for (or are identified as

being depleted for) one or more of (e.g., at least 2, 3, 4, 5, or all of) the following lipids compared to the source cells: ceramide, cardiolipin, lyso-phosphatidylcholine, lyso-phosphatidylethanolamine, lyso-phosphatidylglycerol, lyso-phosphatidylinositol, ether-linked phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, and triacylglycerol. In some embodiments, the fusosomes are enriched for (or are identified as being enriched for) one or more of the aforementioned enriched lipids and depleted for one or more of the aforementioned depleted lipids. In some embodiments, the fusosomes comprise (or are identified as comprising) the enriched lipid as a percentage of total lipid that is at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 2-fold, 5-fold, or 10-fold greater than the corresponding level in source cells. In some embodiments, the fusosome comprise (or are identified as comprising) the depleted lipid as a percentage of total lipid at a level that is less than 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, or 10% of the corresponding level in the source cells. In embodiments, lipid enrichment is measured by a mass spectrometry assay, e.g., an assay of Example 96.

In some embodiments, CE lipid levels are (or are identified as being) about 2-fold greater in fusosomes than in exosomes and/or about 5, 6, 7, 8, 9, or 10-fold higher in fusosomes than in parental cells (relative to total lipid in a sample). In some embodiments, ceramide lipid levels are (or are identified as being) about 2, 3, 4, or 5-fold greater in parental cells than in fusosomes (relative to total lipid in a sample). In some embodiments, cholesterol levels are (or are identified as being) about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, or 2-fold greater in exosomes than in fusosomes and/or about 2-fold higher in fusosomes than in parental cells (relative to total lipid in a sample). In some embodiments, CL lipid levels are (or are identified as being) at least about 5, 10, 20, 30, or 40-fold greater in parental cells than in fusosomes (relative to total lipid in a sample). In some embodiments, DAG lipid levels are (or are identified as being) about 2 or 3-fold greater in exosomes than in fusosomes and/or about 1.5 or 2-fold higher in parental cells than in fusosomes (relative to total lipid in a sample). In some embodiments, PC lipid levels are (or are identified as being) about equal between exosomes and fusosomes and/or about 1.3, 1.4, 1.5, 1.6, 1.7, or 1.8-fold higher in parental cells than in fusosomes (relative to total lipid in a sample). In some embodiments, PC O- lipid levels are (or are identified as being) about equal between exosomes and fusosomes and/or about 2-fold higher in parental cells than in fusosomes (relative to total lipid in a sample). In some embodiments, PE lipid levels are (or are identified

as being) about 1.3, 1.4, 1.5, 1.6, 1.7, or 1.8-fold higher in fusosomes than in exosomes and/or about 1.3, 1.4, 1.5, 1.6, 1.7, or 1.8-fold higher in parental cells than in fusosomes (relative to total lipid in a sample). In some embodiments, PE O- lipid levels are (or are identified as being) about equal between exosomes and fusosomes and/or about 1.5, 1.6, 1.7, 1.8, 1.9, or 2-fold higher in parental cells than in fusosomes (relative to total lipid in a sample). In some embodiments, PG lipid levels are (or are identified as being) about equal between exosomes and fusosomes and/or about 2, 3, 4, 5, 6, 7, 8, 9, or 10-fold higher in parental cells than in fusosomes (relative to total lipid in a sample). In some embodiments, PI lipid levels are (or are identified as being) about equal between exosomes and fusosomes and/or about 3, 4, 5, 6, or 7-fold higher in parental cells than in fusosomes (relative to total lipid in a sample). In some embodiments, PS lipid levels are (or are identified as being) (or are identified as being) about equal between exosomes and fusosomes and/or about 2-fold higher in fusosomes than in parental cells (relative to total lipid in a sample). In some embodiments, SM lipid levels are (or are identified as being) about equal between exosomes and fusosomes and/or about 2, 2.5, or 3-fold higher in fusosomes than in parental cells (relative to total lipid in a sample). In some embodiments, TAG lipid levels are (or are identified as being) about equal between exosomes and fusosomes and/or about 10, 20, 30, 40, 50, 60, 70 80, 90, 100-fold, or more higher in parental cells than in fusosomes (relative to total lipid in a sample).

In some embodiments, the fusosomes are (or are identified as being) enriched for one or more of (e.g., at least 2, 3, 4, 5, or all of) the following lipids compared to exosomes: cholesteryl ester, ceramide, diacylglycerol, lyso-phosphatidate, and phosphatidylethanolamine, and triacylglycerol. In some embodiments, the fusosomes are (or are identified as being) depleted for one or more of (e.g., at least 2, 3, 4, 5, or all of) the following lipids compared to exosomes (relative to total lipid in a sample): free cholesterol, hexosyl ceramide, lyso-phosphatidylcholine, ether-linked lyso-phosphatidylcholine, lyso-phosphatidylethanolamine, ether-linked lyso-phosphatidylethanolamine, and lyso-phosphatidylserine. In some embodiments, the fusosomes are (or are identified as being) enriched for one or more of the aforementioned enriched lipids and depleted for one or more of the aforementioned depleted lipids. In some embodiments, the fusosomes comprise (or are identified as comprising) the enriched lipid as a percentage of total lipid that is at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 2-fold, 5-fold, or 10-fold greater than the corresponding level in exosomes. In some embodiments, the fusosome comprise

(or are identified as comprising) the depleted lipid as a percentage of total lipid at a level that is less than 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, or 10% of the corresponding level in exosomes. In embodiments, lipid enrichment is measured by a mass spectrometry assay, e.g., an assay of Example 96.

- 5 In some embodiments, ceramide lipid levels are (or are identified as being) about 2-fold higher in fusosomes than in exosomes and/or about 2-fold higher in parental cells than in fusosomes (relative to total lipid in a sample). In some embodiments, HexCer lipid levels are (or are identified as being) about 1.5, 1.6, 1.7, 1.8, 1.9, or 2-fold higher in exosomes than in fusosomes and/or about equal in parental cells and fusosomes (relative to total lipid in a sample).
- 10 In some embodiments, LPA lipid levels are (or are identified as being) about 3 or 4-fold higher in fusosomes than in exosomes and/or about 1.3, 1.4, 1.5, 1.6, 1.7, or 1.8-fold higher in fusosomes than in parental cells (relative to total lipid in a sample). In some embodiments, LPC lipid levels are (or are identified as being) about 2-fold higher in exosomes than in fusosomes and/or about 1.5, 1.6, 1.7, 1.8, 1.9, or 2-fold higher in parental cells than in fusosomes (relative
- 15 to total lipid in a sample). In some embodiments, LPC O- lipid levels are (or are identified as being) about 3 or 4-fold higher in exosomes than in fusosomes and/or about equal between parental cells and fusosomes (relative to total lipid in a sample). In some embodiments, LPE lipid levels are (or are identified as being) about 1.5, 1.6, 1.7, 1.8, 1.9, or 2-fold higher in exosomes than in fusosomes and/or about 1.5, 1.6, 1.7, 1.8, 1.9, or 2-fold higher in parental cells
- 20 than in fusosomes (relative to total lipid in a sample). In some embodiments, LPE O- lipid levels are (or are identified as being) about 2 or 3-fold higher in exosomes than in fusosomes and/or about equal between parental cells and fusosomes (relative to total lipid in a sample). In some embodiments, LPS lipid levels are (or are identified as being) about 3-fold higher in exosomes than in fusosomes (relative to total lipid in a sample). In some embodiments, PA lipid levels are
- 25 (or are identified as being) about 1.5, 1.6, 1.7, 1.8, 1.9, or 2-fold higher in fusosomes than in exosomes and/or about 2-fold higher in fusosomes than in parental cells (relative to total lipid in a sample). In some embodiments, PG lipid levels are (or are identified as being) about equal between fusosomes and exosomes and/or about 10, 11, 12, 13, 14, or 15-fold higher in parental cells than in fusosomes (relative to total lipid in a sample).
- 30 In some embodiments, 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%, or 50% of the corresponding lipid level in the source cell. In embodiments, the lipid composition of fusosomes is similar to the cells from which they are derived. In embodiments, fusosomes and parental cells have (or are identified as having) a similar lipid composition if greater than or equal to about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% of the lipid species identified in any replicate sample of the parental cells are present (or are identified as being present) in any replicate sample of the fusosomes, e.g., as determined according to Example 86. In embodiments, of identified lipids, the average level in the fusosome is greater than about 10%, 15%, 20%, 25%, 30%, 35%, or 40% of the corresponding average lipid species level in the parental cell (relative to total lipid in a sample). In an embodiment, the lipid composition of the fusosome is enriched and/or depleted for specific lipids relative to the parental cell (relative to total lipid in a sample).

In some embodiments, the lipid composition of the fusosome is (or is identified as being) enriched and/or depleted for specific lipids relative to the parental cell, e.g., as determined according to the method described in Example 96.

In some embodiments, the fusosome has (or is identified as having) a ratio of phosphatidylserine to total lipids that is greater than that of the parental cell. In embodiments, the fusosome has (or is identified as having) a ratio of phosphatidylserine to total lipids of about 110%, 115%, 120%, 121%, 122%, 123%, 124%, 125%, 130%, 135%, 140%, or more relative to that of the parental cell. In some embodiments, the fusosome is (or is identified as being) enriched for cholesteryl ester, free cholesterol, ether-linked lyso-phosphatidylethanolamine, lyso-phosphatidylserine, phosphatidate, ether-linked phosphatidylethanolamine, phosphatidylserine, and/or sphingomyelin relative to the parental cell. In some embodiments, the fusosomes is (or is identified as being) depleted for ceramide, cardiolipin, lyso-phosphatidylcholine, lyso-phosphatidylethanolamine, lyso-phosphatidylglycerol, lyso-phosphatidylinositol, ether-linked phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, and/or triacylglycerol relative to the parental cell. In some embodiments, the fusosome is (or is identified as being) enriched for cholesteryl ester, ceramide, diacylglycerol, lyso-phosphatidate, phosphatidylethanolamine, and/or triacylglycerol relative to an exosome. In some embodiments, the fusosome is (or is identified as being) depleted for free cholesterol, hexosyl ceramide, lyso-phosphatidylcholine, ether-linked lyso-phosphatidylcholine, lyso-phosphatidylethanolamine,

ether-linked lyso-phosphatidylethanolamine, and/or lyso-phosphatidylserine relative to an exosome.

In some embodiments, the fusosome has 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 has a
 5 ratio of cardiolipin: diacylglycerol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cardiolipin: diacylglycerol in the source cell; or has 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 has a ratio of cardiolipin:lysophosphatidate that is within 10%, 20%, 30%, 40%, or 50%
 10 of the ratio of cardiolipin: lysophosphatidate in the source cell; or has 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 has 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 has a ratio of cardiolipin: lyso-phosphatidylglycerol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cardiolipin :
 15 lyso-phosphatidylglycerol in the source cell; or has 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 has 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 has a ratio of cardiolipin: phosphatidate that is within 10%, 20%, 30%,
 20 40%, or 50% of the ratio of cardiolipin : phosphatidate in the source cell; or has 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 has 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 has a ratio of cardiolipin: phosphatidylglycerol
 25 that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cardiolipin : phosphatidylglycerol in the source cell; or has 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 has 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 has a ratio of cardiolipin: cholesterol ester
 30 that is within 10%, 20%, 30%, 40%, or 50% of the ratio of cardiolipin : cholesterol ester in the source cell; or has 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 has 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 has 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

5 has 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 has 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 has a ratio of phosphatidylcholine:lysophosphatidate that is within 10%, 20%, 30%, 40%, or 50% of the ratio

10 of phosphatidylcholine: lysophosphatidate in the source cell; or has 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 has 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 has

15 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 has 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 has a ratio of phosphatidylcholine: lyso-phosphatidylserine that is within 10%, 20%, 30%, 40%, or

20 50% of the ratio of phosphatidylcholine : lyso-phosphatidylserine in the source cell; or has 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 has 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 has a ratio of cardiolipin: phosphatidylglycerol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of

25 phosphatidylcholine : phosphatidylglycerol in the source cell; or has 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 has a ratio of phosphatidylcholine: phosphatidylserine that is within 10%, 20%, 30%, 40%, or 50% of the ratio

30 of phosphatidylcholine : phosphatidylserine in the source cell; or has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has a ratio of

5 phosphatidylethanolamine: triacylglycerol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylethanolamine : triacylglycerol in the source cell; or has 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 has a ratio of phosphatidylserine: diacylglycerol that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylserine:

10 diacylglycerol in the source cell; or has 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 has 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 has a ratio of phosphatidylserine: lyso-phosphatidylcholine that is within 10%, 20%, 30%, 40%, or

15 50% of the ratio of phosphatidylserine: lyso-phosphatidylcholine in the source cell; or has 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 has 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 has a

20 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 has 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 has a ratio of phosphatidylserine: phosphatidate that is within 10%, 20%, 30%, 40%, or 50% of the ratio of

25 phosphatidylserine : phosphatidate in the source cell; or has 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 has 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 has a ratio of phosphatidylserine:

30 cholesterol ester that is within 10%, 20%, 30%, 40%, or 50% of the ratio of phosphatidylserine : cholesterol ester in the source cell; or has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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 has 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.

In some embodiments, the fusosome comprises a proteomic composition similar to that of the source cell, e.g., using an assay of Example 87. In some embodiments, the protein composition of fusosomes are similar to the parental cells from which they are derived. In some embodiments, the fractional content of each of a plurality of categories of proteins is determined as the sum of intensity signals from each category divided by the sum of the intensity signals of all identified proteins in the sample, e.g., as described in Example 87. In some embodiments, the fusosome comprises (or is identified as comprising) varying amounts of compartment-specific proteins relative to parental cells and/or exosomes, e.g., as determined according to the method described in Example 97. In some embodiments, fusosomes are (or are identified as being) depleted with endoplasmic reticulum protein compared to parental cells and exosomes. In

some embodiments, fusosomes are (or are identified as being) depleted for exosomal protein compared to exosomes. In some embodiments, fusosomes have (or are identified as having) less than 15%, 20%, or 25% of the protein in the fusosome as being exosomal protein. In some embodiments, fusosomes are (or are identified as being) depleted for mitochondrial protein compared to parental cells. In some embodiments, fusosomes are (or are identified as being) enriched for nuclear protein compared to parental cells. In some embodiments, fusosomes are (or are identified as being) enriched for ribosomal proteins compared to parental cells and exosomes. In some embodiments, at least 0.025%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% or 10% of the protein in the fusosome is ribosomal protein, or about 0.025-0.2%, 0.05-0.15%, 0.06-1.4%, 0.07%-1.3%, 0.08%-1.2%, 0.09%-1.1%, 1%-20%, 3%-15%, 5%-12.5%, 7.5%-11%, or 8.5%-10.5%, or 9%-10% of the protein in the fusosome is ribosomal protein.

In some embodiments, 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 40. In embodiments, the fusosome comprises (or is identified as comprising) a ratio of lipid mass to proteins approximately equal to the lipid mass to protein ratio for nucleated cells. In embodiments, the fusosome comprises (or is identified as comprising) a greater lipid:protein ratio than the parental cell. In embodiments, the fusosome comprises (or is identified as comprising) a lipid:protein ratio of about 110%, 115%, 120%, 125%, 130%, 131%, 132%, 132.5%, 133%, 134%, 135%, 140%, 145%, or 150% of the lipid:protein ratio of the parental cell. In some embodiments, the fusosome or fusosome composition has (or is identified as having) a phospholipid:protein ratio of about 100-180, 110-170, 120-160, 130-150, 135-145, 140-142, or 141 $\mu\text{mol/g}$, e.g., in an assay of Example 83. In some embodiments, the fusosome or fusosome composition has (or is identified as having) a phospholipid:protein ratio that is about 60-90%, 70-80%, or 75% of the corresponding ratio in the source cells, e.g., in an assay of Example 83.

In some embodiments, the fusosome comprises 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 41. In embodiments, the fusosome comprises (or is identified as comprising) a ratio of protein mass to DNA mass similar to that of a parental cell. In embodiments, the fusosome comprises (or is identified as comprising) a ratio

of protein:DNA that is about about 85%, 90%, 95%, 96%, 97%, 98%, 98.2%, 99%, 100%, 101%, 102%, 103%, 104%, 105%, or 110% of the parental cell. In some embodiments, the fusosome comprises 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 41. In some embodiments, the fusosome or fusosome composition comprises (or is identified as comprising) a ratio of protein:DNA that is about 20-35, 25-30, 26-29, 27-28, or 27.8 g/g, e.g., by an assay of Example 84. In some embodiments, the fusosome or fusosome composition comprises (or is identified as comprising) a ratio of protein:DNA that is within about 1%, 2%, 5%, 10%, or 20% of the corresponding ratio in the source cells, e.g., by an assay of Exmple 84.

In some embodiments, 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 91. In some embodiments, the fusosome or fusosome composition comprises (or is identified as comprising) a ratio of lipids:DNA that is about 2.0-6.0, 3.0-5.0, 3.5-4.5, 3.8-4.0, or 3.92 $\mu\text{mol/mg}$, e.g., by an assay of Example 85. In some embodiments, the fusosome comprises 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 91. In some embodiments, the fusosome comprises (or is identified as comprising) a greater lipid:DNA ratio than the parental cell. In some embodiments, the fusosome comprises about a 105%, 110%, 115%, 120%, 125%, 130%, 135%, 140%, 145%, 150%, or greater lipid:DNA ratio compared to the parental cell.

In some embodiments, the fusosome composition 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 composition, e.g., the source cell, e.g., by an assay of Example 60. In some embodiments, the fusosome composition has 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 60. In some embodiments, the fusosome composition has a half-life of at least 1, 2, 4, 6, 12, or 24 hours in a subject, e.g., in an assay of Example 79. In some embodiments, the therapeutic agent has a half-life in a subject that is longer than the half-life of the fusosome composition, e.g., by at least 10%, 20%, 50%, 2-

fold, 5-fold, or 10-fold. For instance, the fusosome may deliver the therapeutic agent to the target cell, and the therapeutic agent may be present after the fusosome is no longer present or detectable.

In some embodiments, 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 50. In some embodiments, the fusosome transports (or is identified as transporting) glucose (e.g., labeled glucose, e.g., 2-NBDG) across a membrane at a greater level than otherwise similar fusosomes treated with phloretin, e.g., in an assay of Example 72. In embodiments, a fusosome not treated with phloretin transports (or is identified as not transporting) glucose at a level at least 1%, 2%, 3%, 5%, or 10% higher (and optionally up to 15% higher) than an otherwise similar fusosome treated with phloretin, e.g., in an assay of Example 72. In some embodiments, 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 51. In some embodiments, the fusosome comprises (or is identified as comprising) esterase activity in the lumen that is at least 10-fold, 20-fold, 50-fold, 100-fold, 200-fold, 500-fold, 1000-fold, 2000-fold, or 5000-fold higher than an unstained control, e.g., by an assay of Example 73. In some embodiments, the fusosome comprises (or is identified as comprising) esterase activity in the lumen that is about 10-100-fold lower than that of the source cells, e.g., by an assay of Example 73. In some embodiments, the fusosome comprises (or is identified as comprising) an acetylcholinesterase activity of about $1E5$ - $1E6$, $6E5$ - $8E5$, $6.5E5$ - $7E5$, or $6.83E5$ exosome equivalents, e.g., by an assay of Example 74. In some embodiments, the fusosome comprises 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 53. In some embodiments, the fusosome comprises 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 53. In some embodiments, the fusosome comprises (or is identified as comprising) a citrate synthase activity

that is about $1\text{E-}2$ - $2\text{E-}2$, $1.3\text{E-}2$ - $1.8\text{E-}2$, $1.4\text{E-}2$ - $1.7\text{E-}2$, $1.5\text{E-}2$ - $1.6\text{E-}2$, or $1.57\text{E-}2$ $\mu\text{mol}/\mu\text{g}$ fusosome/min, e.g., by an assay of Example 75. In some embodiments, the fusosome comprises a respiration level (e.g., oxygen consumption rate), e.g., basal, uncoupled, or maximal respiration level, 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 54. In some embodiments, the fusosome comprises a respiration level (e.g., oxygen consumption rate), e.g., basal, uncoupled, or maximal respiration level, 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 54. In embodiments, the fusosome comprises (or is identified as comprising) a basal respiration rate of about 8-15, 9-14, 10-13, 11-12, or 11.3 $\text{pmol}/\text{min}/20\mu\text{g}$ fusosome, e.g., by an assay of Example 76. In embodiments, the fusosome comprises (or is identified as comprising) an uncoupled respiration rate of about 8-13, 9-12, 10-11, 10-10.2, or 10.1 $\text{pmol}/\text{min}/20\mu\text{g}$ fusosome, e.g., by an assay of Example 76. In embodiments, the fusosome comprises (or is identified as comprising) a maximal respiration rate of about 15-25, 16-24, 17-23, 18-22, 19-21, or 20 $\text{pmol}/\text{min}/20\mu\text{g}$ fusosome, e.g., by an assay of Example 76. In embodiments, the fusosome has (or is identified as having) a higher basal respiration rate than uncoupled respiration rate, e.g., by about 1%, 2%, 5%, or 10%, e.g., up to about 15%, e.g., by an assay of Example 76. In embodiments, the fusosome has (or is identified as having) a higher maximal respiration rate than basal respiration rate, e.g., by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, e.g., by an assay of Example 76. In some embodiments, 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 55, 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 an otherwise similar fusosome treated with menadione in the assay of Example 55, 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 a macrophage treated with menadione in the assay of Example 55. In embodiments, the fusosome comprises (or is identified as comprising) an Annexin V-staining level that is at least about 1%, 2%, 5%, or 10% lower than the Annexin V-staining level of an otherwise similar fusosome treated with antimycin A, e.g., in an assay of Example 77. In embodiments, the fusosome

comprises (or is identified as comprising) an Annexin V-staining level that is within about 1%, 2%, 5%, or 10% of the Annexin V-staining level of an otherwise similar fusosome treated with antimycin A, e.g., in an assay of Example 77.

In some embodiments, the fusosome has a miRNA content level of at least at least 1%,
 5 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 33. In some embodiments, the fusosome has 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 33. In some embodiments, the
 10 fusosome has 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 66.

In some embodiments, the fusosome has a soluble : non-soluble protein ratio is within
 15 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 38. In embodiments, the fusosome has a soluble: non-soluble protein ratio of about 0.3-0.8, 0.4-0.7, or 0.5-0.6, e.g., about 0.563, e.g., by an assay of Example
 20 38. In some embodiments, the population of fusosomes has (or is identified as having) a soluble:insoluble protein mass ratio of about 0.3-0.8, 0.4-0.7, 0.5-0.6, or 0.563, or greater than about 0.1, 0.2, 0.3, 0.4, or 0.5. In some embodiments, the population of fusosomes has (or is identified as having) a soluble:insoluble protein mass ratio that is greater than that of the source cells, e.g., at least 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, or 20-fold higher. In embodiments, the
 25 soluble:insoluble protein mass ratio is determined by an assay of Example 70. In embodiments, the soluble: insoluble protein mass ratio is (or is identified as being) lower in the fusosome population than in the parental cells. In embodiments, when the ratio of fusosomes to parental cells is (or is identified as being) about 3%, 4%, 5%, 6%, 7%, or 8%, the soluble: insoluble ratio of the population of fusosomes is (or is identified as being) about equal to the soluble: insoluble
 30 ratio of the parental cells.

In some embodiments, 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 39. In some embodiments, the fusosome is capable of signal transduction, e.g., transmitting an extracellular signal, e.g., AKT

5 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 49. In some embodiments, the fusosome targets a tissue, e.g., liver, lungs, heart, spleen, pancreas, gastrointestinal tract, kidney, testes, 10 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 15 64. In some embodiments, the fusosome has 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 56. In some embodiments, the fusosome has a juxtacrine-signaling level of at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 20 70%, 80%, or 90% (e.g., up to 100%) 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 56. In some embodiments, the fusosome has 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 25 Example 57. In some embodiments, the fusosome has 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 to 100%) 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 57. In some embodiments, the fusosome polymerizes actin at a level within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% 30 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 58. In some embodiments, the fusosome polymerizes actin (or

is identified as polymerizing actin) at a level that is constant over time, e.g., over at least 3, 5, or 24 hours, e.g., by an assay of Example 81. In embodiments, the level of actin polymerization changes by less than 1%, 2%, 5%, 10%, or 20% over a 5-hour period, e.g. by the assay of Example 81. In some embodiments, the fusosome has a membrane potential within about 1%,
 5 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 59, or wherein the fusosome has 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.

10 In some embodiments, the fusosome has (or is identified as having) a membrane potential of about -25 to -35, -27 to -32, -28 to -31, -29 to -30, or -29.6 millivolts, e.g., in an assay of Example 78. In some embodiments, 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, e.g., using an assay of Example 44, e.g., wherein the source
 15 cell is a neutrophil, lymphocyte, B cell, macrophage, or NK cell. In some embodiments, the fusosome is 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 45. In some embodiments, the fusosome is capable of phagocytosis, e.g., at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%,
 20 80%, or 90% (e.g., up to 100%) compared to a reference cell, e.g., a macrophage, e.g., using an assay of Example 47. In some embodiments, the fusosome is capable of crossing a cell membrane, e.g., an endothelial cell membrane or the blood brain barrier. In some embodiments, 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
 25 mouse embryonic fibroblast, e.g., using an assay of Example 48. In some embodiments, 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%, 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 48.

In some embodiments, the fusosome is not capable of transcription or has transcriptional
 30 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 24. In some embodiments, 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 25. In some embodiments, the fusosome lacks chromatin or has a
5 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 32.

In some embodiments, a characteristic of a fusosome is described by comparison to a reference cell. In embodiments, the reference cell is the source cell. In embodiments, the
10 reference cell is a HeLa, HEK293, HFF-1, MRC-5, WI-38, IMR 90, IMR 91, PER.C6, HT-1080, or BJ cell. In some embodiments, a characteristic of a population of fusosomes is described by comparison to a population of reference cells, e.g., a population of source cells, or a population of HeLa, HEK293, HFF-1, MRC-5, WI-38, IMR 90, IMR 91, PER.C6, HT-1080, or BJ cells.

In some embodiments, the fusosome meets a pharmaceutical or good manufacturing
15 practices (GMP) standard. In some embodiments, the fusosome was made according to good manufacturing practices (GMP). In some embodiments, the fusosome has a pathogen level below a predetermined reference value, e.g., is substantially free of pathogens. In some embodiments, the fusosome has a contaminant level below a predetermined reference value, e.g., is substantially free of contaminants. In some embodiments, the fusosome has low
20 immunogenicity, e.g., as described herein.

In some embodiments, immunogenicity of a fusosome composition is assayed by a serum inactivation assay (e.g., an assay that detects antibody-mediated neutralization or complement mediated degradation). In some embodiments, fusosomes are not inactivated by serum, or are inactivated at a level below a predetermined value. In some embodiments, serum of a fusosome-
25 naïve subject (e.g., human or mouse) is contacted with a test fusosome composition. In some embodiments, the serum of a subject that has received one or more administrations of fusosomes, e.g., has received at least two administrations of fusosomes, is contacted with the test fusosome composition. In embodiments, serum-exposed fusosomes are then tested for ability to deliver a cargo to target cells. In some embodiments, the percent of cells that detectably comprise the
30 cargo after treatment with serum-incubated fusosomes is at least 50%, 60%, 70%, 80%, 90%, or 95% the percent of cells that detectably comprise the cargo after treatment with positive control

fusosomes not contacted with serum. In some embodiments, serum inactivation is measured using an assay of Example 99.

In some embodiments, immunogenicity of a fusosome composition is assayed by detecting complement activation in response to the fusosomes. In some embodiments, the fusosomes do not activate complement, or activate complement at a level below a predetermined value. In some embodiments, serum of a fusosome-naïve subject (e.g., human or mouse) is contacted with a test fusosome composition. In some embodiments, the serum of a subject that has received one or more administrations of fusosomes, e.g., has received at least two administrations of fusosomes, is contacted with the test fusosome composition. In embodiments, the composition comprising serum and fusosome

is then tested for an activated complement factor (e.g., C3a), e.g., by ELISA. In some embodiments, a fusosome comprising a modification described herein (e.g., elevated levels of a complement regulatory protein compared to a reference cell) undergoes reduced complement activation compared to an otherwise similar fusosome that lacks the modification, e.g., reduced by at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99%. In some embodiments, complement activation is measured using an assay of Example 100.

In some embodiments, a fusosome or population of fusosomes will not be substantially inactivated by serum. In some embodiments, a fusosome or population of fusosomes is resistant to serum inactivation, e.g., as quantified according to the method described in Example 99. In some embodiments, the fusosome or population of fusosomes is not substantially inactivated by serum or is resistant to serum inactivation following multiple administrations of the fusosome or population of fusosomes to a subject, e.g., according to the methods described herein. In some embodiments, a fusosome is modified to have a reduced serum inactivation, e.g., compared to a corresponding unmodified fusosome, e.g., following multiple administrations of the modified fusosome, e.g., as quantified according to the method described in Example 99.

In some embodiments, a fusosome does not substantially induce complement activity, e.g., as measured according to the method described in Example 100. In some embodiments, a fusosome is modified to induce reduced complement activity compared to a corresponding unmodified fusosome. In embodiments, complement activity is measured by determining expression or activity of a complement protein (e.g., DAF, proteins that bind decay-accelerating factor (DAF, CD55), e.g., factor H (FH)-like protein-1 (FHL-1), C4b-binding protein (C4BP),

complement receptor 1 (CD35), Membrane cofactor protein (MCP, CD46), Profectin (CD59), proteins that inhibit the classical and alternative complement pathway CD/C5 convertase enzymes, or proteins that regulate MAC assembly) in a cell

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 glial 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). In some embodiments, 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.

In some embodiments, the source cell expresses (e.g., overexpresses) ARRDC1 or an active fragment or variant thereof. In some embodiments, the fusosome or fusosome composition has a ratio of fusogen to ARRDC1 of about 1-3, 1-10, 1-100, 3-10, 4-9, 5-8, 6-7, 15-100, 60-200, 80-180, 100-160, 120-140, 3-100, 4-100, 5-100, 6-100, 15-100, 80-100, 3-200, 4-200, 5-200, 6-200, 15-200, 80-200, 100-200, 120-200, 300-1000, 400-900, 500-800, 600-700, 640-690, 650-680, 660-670, 100-10,000, or about 664.9, e.g., by a mass spectrometry assay. In some embodiments, the level of ARRDC1 as a percentage of total protein content is at least about 0.01%, 0.02%, 0.03%, 0.04%, 0.05%; 0.1%, 0.15%, 0.2%, 0.25%; 0.5%, 1%, 2%, 3%, 4%, 5%; or the level of ARRDC1 as a percentage of total protein content is about 0.05-1.5%, 0.1%-0.3%, 0.05-0.2%, 0.1-0.2%, 0.25-7.5%, 0.5%-1.5%, 0.25-1%, 0.5-1%, 0.05-1.5%, 10%-30%, 5-20%, or 10-20%, e.g., by mass spectrometry, e.g., as measured according to the method described in Example 98. In some embodiments, the fusosome or fusosome composition has a

ratio of fusogen to TSG101 of about 100-1,000, 100-400, 100-500, 200-400, 200-500, 200-1,000, 300-400, 1,000-10,000, 2,000-5,000, 3,000-4,000, 3,050-3,100, 3,060-3,070, or about 3,064, 10,000-100,000, 10,000-200,000, 10,000-500,000, 20,000-500,000, 30,000-400,000, e.g., using a mass spectrometry assay, e.g., an assay of Example 94. In some embodiments, the fusosome or fusosome composition has a ratio of cargo to tsg101 of about 1-3, 1-30, 1-20, 1-25, 1.5-30, 10-30, 15-25, 18-21, 19-20, 10-300, 10-200, 15-300, 15-200, 100-300, 100-200, 150-300, or about 19.5, e.g., using a mass spectrometry assay, e.g., an assay of Example 95. In some embodiments, the level of TSG101 as a percentage of total protein content is at least about 0.0001%, 0.0002%, 0.0003%, 0.0004%, 0.0005%, 0.0006%, 0.0007%, 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%; 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%; or the level of TSG101 as a percentage of total protein content is about 0.0001-0.001, 0.0001-0.002, 0.0001-0.01, 0.0001-0.1, 0.001-0.01, 0.002-0.006, 0.003-0.005, 0.001-0.1, 0.01-0.1, 0.02-0.06, 0.03-0.05, or 0.004, e.g., by mass spectrometry, e.g., as measured according to the method described in Example 98.

In some embodiments, the fusosome comprises a cargo, e.g., a therapeutic agent, e.g., an endogenous therapeutic agent or an exogenous therapeutic agent. 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, the therapeutic agent is an organelle other than a mitochondrion, e.g., an organelle selected from: nucleus, Golgi apparatus, lysosome, endoplasmic reticulum, vacuole, endosome, acrosome, autophagosome, centriole, glycosome, glyoxysome, hydrogenosome, melanosome, mitosome, cnidocyst, peroxisome, proteasome, vesicle, and stress granule. In some embodiments, the organelle is a mitochondrion.

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 62. 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-

5 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 61. 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
10 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.,
15 using an assay of Example 62. 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 63. In an
20 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 63. In an embodiment, a fusosome delivers an agent to a target cell via an actin-
25 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 63.

In some embodiments, the fusosome has a density of <1, 1-1.1, 1.05-1.15, 1.1-1.2, 1.15-
30 1.25, 1.2-1.3, 1.25-1.35, or >1.35 g/ml, e.g., by an assay of Example 30.

In some embodiments, the fusosome composition 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. In some embodiments, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%,
 5 95%, or 99% of fusosomes in the fusosome composition comprise an organelle, e.g., a mitochondrion.

In some embodiments, the fusosome further comprises an exogenous therapeutic agent. In some embodiments, the exogenous 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
 10 chromosome (e.g. a human artificial chromosome), RNA, mRNA, siRNA, miRNA, or a small molecule.

In embodiments, the fusosome enters the cell by endocytosis or a non-endocytic pathway.

In embodiments, the fusosome composition is stable 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
 15 1, 2, 3, 4, or 5 years. In embodiments, the fusosome composition is stable 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. In embodiments, the fusosome composition is stable 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.

In embodiments, the fusosome has a size, or the population of fusosomes has an average size, 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 27. In embodiments, the fusosome has a size, or the population of fusosomes has an average size, that is less than about 0.01%, 0.05%, 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%,
 20 40%, 50%, 60%, 70%, 80%, 90%, of that of the source cell, e.g., as measured by an assay of Example 27. In embodiments, the fusosomes have (or are identified as having) a size less than parental cells. In embodiments, the fusosomes have (or are identified as having) a size within about 50%, 60%, 65%, 70%, 71%, 72%, 73%, 74%, 75%, 80%, or 90% of parental cells. In
 25 embodiments, the fusosomes have (or are identified as having) less than about 70%, 60%, 50%, 40%, 30%, 20%, 10%, 5%, 1%, or less of the parental cell's variability in size distribution, e.g., within about 90% of the sample. In embodiments, the fusosomes have (or are identified as
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having) about 40%, 45%, 50%, 55%, 56%, 57%, 58%, 59%, 60%, 65%, or 70% less of the parental cell's variability in size distribution, e.g., within about 90% of the sample. In some embodiments, fusosomes have (or are identified as having) an average size of greater than 30, 35, 40, 45, 50, 55, 60, 65, or 70 nm in diameter. In embodiments, fusosomes have an average
 5 size of about 100, 110, 120, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 140, or 150 nm in diameter. In embodiments, the fusosome has a size, or the population of fusosomes has an average size, 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 size of the source cell, e.g., as measured by an assay of Example 27.

10 In embodiments, the fusosome has a size, or the population of fusosomes has an average size, 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 size of the source cell, e.g., as measured by an assay of Example 27. In
 15 embodiments, the fusosome has a diameter, or the population of fusosomes has an average diameter, of less than about 500 nm (e.g., less than about 10, 50, 100, 150, 200, 250, 300, 350, 400, or 450 nm), e.g., as measured by an assay of Example 69. In embodiments, the fusosome has a diameter, or the population of fusosomes has an average diameter, of about 80-180, 90-170, 100-160, 110-150, 120-140, or 130 nm, e.g., as measured by an assay of Example 69. In
 20 embodiments, the fusosome has a diameter, or the population of fusosomes has an average diameter, of between about 11,000 nm and 21,000 nm, e.g., as measured by an assay of Example 69. In embodiments, the fusosome has a diameter, or the population of fusosomes has an average diameter, between about 10-22,000, 12-20,000, 14-18,720 nm, 20-16,000 nm, e.g., as measured by an assay of Example 69. In embodiments, the fusosome has a volume, or the
 25 population of fusosomes has an average volume, of about 0.01-0.1 μm^3 , 0.02-1 μm^3 , 0.03-1 μm^3 , 0.04-1 μm^3 , 0.05-0.09 μm^3 , 0.06-0.08 μm^3 , 0.07 μm^3 , e.g., as measured by an assay of Example 69. In embodiments, the fusosome has a diameter, or the population of fusosomes has an average 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, 200 nm, or 250 nm e.g., as measured by an assay of Example 29. In
 30 embodiments, the fusosome has a diameter, or the population of fusosomes has an average diameter, of 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., $\pm 20\%$) e.g., as measured by an assay of Example 29. In

embodiments, the fusosome has a diameter, or the population of fusosomes has an average diameter, of 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 29. In embodiments, the fusosome has a diameter, or the population of fusosomes has an average diameter, of 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., $\pm 20\%$), e.g., as measured by an assay of Example 29. In embodiments, the population of fusosomes has (or is identified as having) one or more of: a 10% quantile diameter of about 40-90 nm, 45-60 nm, 50-55 nm or 53 nm; a 25% quantile diameter of about 70-100 nm, 80-95 nm, 85-90 nm, or 88 nm; a 75% quantile diameter of about 200-250 nm, 210-240 nm, 220-230 nm, or 226 nm; or a 90% quantile of about 4000-5000 nm, 4300-4600 nm, 4400-4500 nm, 4450 nm, e.g., by an assay of Example 68.

In embodiments, the fusosome composition comprises (or is identified as comprising) a GAPDH concentration of about 35-40, 36-39, 37-38, or 37.2 ng/mL, e.g., in an assay of Example 82. In embodiments, the GAPDH concentration of the fusosome composition is (or is identified as being) within about 1%, 2%, 5%, 10%, or 20% of the GAPDH concentration of the source cells, e.g., in an assay of Example 82. In embodiments, the GAPDH concentration of the fusosome composition is (or is identified as being) at least 1%, 2%, 5%, 10%, or 20% lower than the the GAPDH concentration of the source cells, e.g., in an assay of Example 82. In embodiments, the the fusosome composition comprises (or is identified as comprising) less than about 30, 35, 40, 45, 46, 47, 48, 49, 50, 55, 60, 65, or 70 μg GAPDH per gram total protein. In embodiments, the fusosome composition comprises (or is identified as comprising) less than about 500, 250, 100, or 50 μg GAPDH per gram total protein. In embodiments, the parental cell comprises (or is identified as comprising) at least 1%, 2.5%, 5%, 10%, 15%, 20%, 30%, 30%, 50%, or more GAPDH per total protein than the fusosome composition.

In embodiments, the average fractional content of calnexin in the fusosome is (or is identified as being) less than about 1×10^{-4} , 1.5×10^{-4} , 2×10^{-4} , 2.1×10^{-4} , 2.2×10^{-4} , 2.3×10^{-4} ; 2.4×10^{-4} , 2.43×10^{-4} , 2.5×10^{-4} , 2.6×10^{-4} , 2.7×10^{-4} , 2.8×10^{-4} , 2.9×10^{-4} , 3×10^{-4} , 3.5×10^{-4} , or 4×10^{-4} . In embodiments, the fusosome comprises an amount of calnexin per total protein that is lower than that of the parental cell by about 70%, 75%, 80%, 85%, 88%, 90%, 95%, 99%, or more.

In some embodiments, fusosomes comprise or are enriched for lipids that affect membrane curvature (see, e.g., Thiam et al., Nature Reviews Molecular Cell Biology, 14(12):

775-785, 2013). Some lipids have a small hydrophilic head group and large hydrophobic tails, which facilitate the formation of a fusion pore by concentrating in a local region. In some embodiments, fusosomes comprise or are enriched for negative-curvature lipids, such as cholesterol, phosphatidylethanolamine (PE), diglyceride (DAG), phosphatidic acid (PA), fatty acid (FA). In some embodiments, fusosomes do not comprise, are depleted of, or have few positive-curvature lipids, such as lysophosphatidylcholine (LPC), phosphatidylinositol (PtdIns), lysophosphatidic acid (LPA), lysophosphatidylethanolamine (LPE), monoacylglycerol (MAG).

In some embodiments, the lipids are added to a fusosome. In some embodiments, the lipids are added to source cells in culture which incorporate the lipids into their membranes prior to or during the formation of a fusosome. In some embodiments, the lipids are added to the cells or fusosomes in the form of a liposome. In some embodiments methyl-beta-cyclodextrane (m β -CD) is used to enrich or deplete lipids (see, e.g., Kainu et al, Journal of Lipid Research, 51(12): 3533-3541, 2010).

X. Pharmaceutical compositions and methods of making them

The present disclosure also provides, in some aspects, a pharmaceutical composition comprising the fusosome composition described herein and pharmaceutically acceptable carrier. The pharmaceutical compositions can include any of the described fusosomes, e.g. retroviral vectors, or VLPs.

In some embodiments, one or more transducing units of retroviral vector are administered to the subject. In some embodiments, at least 1, 10, 100, 1000, 10^4 , 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} , 10^{11} , 10^{12} , 10^{13} , or 10^{14} , transducing units per kg are administered to the subject. In some embodiments at least 1, 10, 100, 1000, 10^4 , 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} , 10^{11} , 10^{12} , 10^{13} , or 10^{14} , transducing units per target cell per ml of blood are administered to the subject.

Concentration and purification of retroviral virus, e.g. lentivirus

In some embodiments, a retroviral vector formulation described herein can be produced by a process comprising one or more of, e.g., all of, the following steps (i) to (vi), e.g., in chronological order:

- (i) culturing cells that produce retroviral vector;
- (ii) harvesting the retroviral vector containing supernatant;

- (iii) optionally clarifying the supernatant;
- (iv) purifying the retroviral vector to give a retroviral vector preparation;
- (v) optionally filter-sterilization of the retroviral vector preparation; and
- (vi) concentrating the retroviral vector preparation to produce the final bulk product.

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In some embodiments the process does not comprise the clarifying step (iii). In other embodiments the process does include the clarifying step (iii). In some embodiments, step (vi) is performed using ultrafiltration, or tangential flow filtration, more preferably hollow fiber ultrafiltration. In some embodiments, the purification method in step (iv) is ion exchange
10 chromatography, more preferably anion exchange chromatography. In some embodiments, the filter-sterilisation in step (v) is performed using a 0.22 μm or a 0.2 μm sterilising filter. In some embodiments, step (iii) is performed by filter clarification. In some embodiments, step (iv) is performed using a method or a combination of methods selected from chromatography, ultrafiltration/diafiltration, or centrifugation. In some embodiments, the chromatography method
15 or a combination of methods is selected from ion exchange chromatography, hydrophobic interaction chromatography, size exclusion chromatography, affinity chromatography, reversed phase chromatography, and immobilized metal ion affinity chromatography. In some embodiments, the centrifugation method is selected from zonal centrifugation, isopycnic centrifugation and pelleting centrifugation. In some embodiments, the ultrafiltration/diafiltration
20 method is selected from tangential flow diafiltration, stirred cell diafiltration and dialysis. In some embodiments, at least one step is included into the process to degrade nucleic acid to improve purification. In some embodiments, said step is nuclease treatment.

In some embodiments, concentration of the vectors is done before filtration. In some
embodiments, concentration of the vectors is done after filtration. In some embodiments,
25 concentration and filtrations steps are repeated.

In some embodiments, the final concentration step is performed after the filter-sterilisation step. In some embodiments, the process is a large scale-process for producing clinical grade formulations that are suitable for administration to humans as therapeutics. In some embodiments, the filter-sterilisation step occurs prior to a concentration step. In some
30 embodiments, the concentration step is the final step in the process and the filter-sterilisation step is the penultimate step in the process. In some embodiments, the concentration step is performed

using ultrafiltration, preferably tangential flow filtration, more preferably hollow fiber ultrafiltration. In some embodiments, the filter-sterilisation step is performed using a sterilising filter with a maximum pore size of about 0.22 μm . In another preferred embodiment the maximum pore size is 0.2 μm

5 In some embodiments, the vector concentration is less than or equal to about 4.6×10^{11} RNA genome copies per ml of preparation prior to filter-sterilisation. The appropriate concentration level can be achieved through controlling the vector concentration using, e.g. a dilution step, if appropriate. Thus, in some embodiments, a retroviral vector preparation is diluted prior to filter sterilisation.

10 Clarification may be done by a filtration step, removing cell debris and other impurities. Suitable filters may utilize cellulose filters, regenerated cellulose fibers, cellulose fibers combined with inorganic filter aids (e.g. diatomaceous earth, perlite, fumed silica), cellulose filters combined with inorganic filter aids and organic resins, or any combination thereof, and polymeric filters (examples include but are not limited to nylon, polypropylene,
15 polyethersulfone) to achieve effective removal and acceptable recoveries. A multiple stage process may be used. An exemplary two or three-stage process would consist of a coarse filter(s) to remove large precipitate and cell debris followed by polishing second stage filter(s) with nominal pore sizes greater than 0.2 micron but less than 1 micron. The optimal combination may be a function of the precipitate size distribution as well as other variables. In addition, single
20 stage operations employing a relatively small pore size filter or centrifugation may also be used for clarification. More generally, any clarification approach including but not limited to dead-end filtration, microfiltration, centrifugation, or body feed of filter aids (e.g. diatomaceous earth) in combination with dead-end or depth filtration, which provides a filtrate of suitable clarity to not foul the membrane and/or resins in the subsequent steps, will be acceptable to use in the
25 clarification step of the present invention.

In some embodiments, depth filtration and membrane filtration is used. Commercially available products useful in this regard are for instance mentioned in WO 03/097797, p. 20-21. Membranes that can be used may be composed of different materials, may differ in pore size, and may be used in combinations. They can be commercially obtained from several vendors. In
30 some embodiments, the filter used for clarification is in the range of 1.2 to 0.22 μm . In some

embodiments, the filter used for clarification is either a 1.2/0.45 µm filter or an asymmetric filter with a minimum nominal pore size of 0.22 µm

In some embodiments, the method employs nuclease to degrade contaminating DNA/RNA, i.e. mostly host cell nucleic acids. Exemplary nucleases suitable for use in the present invention include Benzonase® Nuclease (EP 0229866) which attacks and degrades all forms of DNA and RNA (single stranded, double stranded linear or circular) or any other DNase and/or RNase commonly used within the art for the purpose of eliminating unwanted or contaminating DNA and/or RNA from a preparation. In preferred embodiments, the nuclease is Benzonase® Nuclease, which rapidly hydrolyzes nucleic acids by hydrolyzing internal phosphodiester bonds between specific nucleotides, thereby reducing the size of the polynucleotides in the vector containing supernatant. Benzonase® Nuclease can be commercially obtained from Merck KGaA (code W214950). The concentration in which the nuclease is employed is preferably within the range of 1-100 units/ml.

In some embodiments, the vector suspension is subjected to ultrafiltration (sometimes referred to as diafiltration when used for buffer exchange) at least once during the process, e.g. for concentrating the vector and/or buffer exchange. The process used to concentrate the vector can include any filtration process (e.g., ultrafiltration (UF)) where the concentration of vector is increased by forcing diluent to be passed through a filter in such a manner that the diluent is removed from the vector preparation whereas the vector is unable to pass through the filter and thereby remains, in concentrated form, in the vector preparation. UF is described in detail in, e.g., Microfiltration and Ultrafiltration: Principles and Applications, L. Zeman and A. Zydney (Marcel Dekker, Inc., New York, N.Y., 1996); and in: Ultrafiltration Handbook, Munir Cheryan (Technomic Publishing, 1986; ISBN No. 87762-456-9). A suitable filtration process is Tangential Flow Filtration (“TFF”) as described in, e.g., MILLIPORE catalogue entitled “Pharmaceutical Process Filtration Catalogue” pp. 177-202 (Bedford, Mass., 1995/96). TFF is widely used in the bioprocessing industry for cell harvesting, clarification, purification and concentration of products including viruses. The system is composed of three distinct process streams: the feed solution, the permeate and the retentate. Depending on application, filters with different pore sizes may be used. In some embodiments, the retentate contains the product (lentiviral vector). The particular ultrafiltration membrane selected may have a pore size sufficiently small to retain vector but large enough to effectively clear impurities. Depending on

the manufacturer and membrane type, for retroviral vectors nominal molecular weight cutoffs (NMWC) between 100 and 1000 kDa may be appropriate, for instance membranes with 300 kDa or 500 kDa NMWC. The membrane composition may be, but is not limited to, regenerated cellulose, polyethersulfone, polysulfone, or derivatives thereof. The membranes can be flat sheets (also called flat screens) or hollow fibers. A suitable UF is hollow fibre UF, e.g., filtration using filters with a pore size of smaller than 0.1 μm . Products are generally retained, while volume can be reduced through permeation (or be kept constant during diafiltration by adding buffer with the same speed as the speed with which the permeate, containing buffer and impurities, is removed at the permeate side).

The two most widely used geometries for TFF in the biopharmaceutical industry are plate & frame (flat screens) and hollow fiber modules. Hollow fiber units for ultrafiltration and microfiltration were developed by Amicon and Ramicon in the early 1970s (Cheryan, M. Ultrafiltration Handbook), even though now there are multiple vendors including Spectrum and GE Healthcare. The hollow fiber modules consist of an array of self-supporting fibers with a dense skin layer. Fiber diameters range from 0.5 mm-3 mm. In certain embodiments, hollow fibers are used for TFF. In certain embodiments, hollow fibers of 500 kDa (0.05 μm) pore size are used. Ultrafiltration may comprise diafiltration (DF). Microsolutes can be removed by adding solvent to the solution being ultrafiltered at a rate equal to the UF rate. This washes microspecies from the solution at a constant volume, purifying the retained vector.

UF/DF can be used to concentrate and/or buffer exchange the vector suspensions in different stages of the purification process. The method can utilize a DF step to exchange the buffer of the supernatant after chromatography or other purification steps, but may also be used prior to chromatography.

In some embodiments, the eluate from the chromatography step is concentrated and further purified by ultrafiltration-diafiltration. During this process the vector is exchanged into formulation buffer. Concentration to the final desired concentration can take place after the filter-sterilisation step. After said sterile filtration, the filter sterilised substance is concentrated by aseptic UF to produce the bulk vector product.

In embodiments, the ultrafiltration/diafiltration may be tangential flow diafiltration, stirred cell diafiltration and dialysis.

Purification techniques tend to involve the separation of the vector particles from the cellular milieu and, if necessary, the further purification of the vector particles. One or more of a variety of chromatographic methods may be used for this purification. Ion exchange, and more particularly anion exchange, chromatography is a suitable method, and other methods could be used. A description of some chromatographic techniques is given below.

Ion-exchange chromatography utilises the fact that charged species, such as biomolecules and viral vectors, can bind reversibly to a stationary phase (such as a membrane, or else the packing in a column) that has, fixed on its surface, groups that have an opposite charge. There are two types of ion exchangers. Anion exchangers are stationary phases that bear groups having a positive charge and hence can bind species with a negative charge. Cation exchangers bear groups with a negative charge and hence can bind species with positive charge. The pH of the medium has an influence on this, as it can alter the charge on a species. Thus, for a species such as a protein, if the pH is above the pI, the net charge will be negative, whereas below the pI, the net charge will be positive.

Displacement (elution) of the bound species can be effected by the use of suitable buffers. Thus commonly the ionic concentration of the buffer is increased until the species is displaced through competition of buffer ions for the ionic sites on the stationary phase. An alternative method of elution entails changing the pH of the buffer until the net charge of the species no longer favours binding to the stationary phase. An example would be reducing the pH until the species assumes a net positive charge and will no longer bind to an anion exchanger.

Some purification can be achieved if impurities are uncharged, or else if they bear a charge of opposite sign to that of the desired species, but the same sign to that on the ion exchanger. This is because uncharged species and those having a charge of the same sign to that on an ion exchanger, will not normally bind. For different bound species, the strength of the binding varies with factors such as the charge density and the distribution of charges on the various species. Thus by applying an ionic or pH gradient (as a continuous gradient, or as a series of steps), the desired species might be eluted separately from impurities.

Size exclusion chromatography is a technique that separates species according to their size. Typically it is performed by the use of a column packed with particles having pores of a well-defined size. For the chromatographic separation, particles are chosen that have pore sizes that are appropriate with regard to the sizes of the species in the mixture to be separated. When

the mixture is applied, as a solution (or suspension, in the case of a virus), to the column and then eluted with buffer, the largest particles will elute first as they have limited (or no) access to the pores. Smaller particles will elute later as they can enter the pores and hence take a longer path through the column. Thus in considering the use of size exclusion chromatography for the purification of viral vectors, it would be expected that the vector would be eluted before smaller impurities such as proteins.

Species, such as proteins, have on their surfaces, hydrophobic regions that can bind reversibly to weakly hydrophobic sites on a stationary phase. In media having a relatively high salt concentration, this binding is promoted. Typically in HIC the sample to be purified is bound to the stationary phase in a high salt environment. Elution is then achieved by the application of a gradient (continuous, or as a series of steps) of decreasing salt concentration. A salt that is commonly used is ammonium sulphate. Species having differing levels of hydrophobicity will tend to be eluted at different salt concentrations and so the target species can be purified from impurities. Other factors, such as pH, temperature and additives to the elution medium such as detergents, chaotropic salts and organics can also influence the strength of binding of species to HIC stationary phases. One, or more, of these factors can be adjusted or utilised to optimise the elution and purification of product.

Viral vectors have on their surface, hydrophobic moieties such as proteins, and thus HIC could potentially be employed as a means of purification.

Like HIC, RPC separates species according to differences in their hydrophobicities. A stationary phase of higher hydrophobicity than that employed in HIC is used. The stationary phase often consists of a material, typically silica, to which are bound hydrophobic moieties such as alkyl groups or phenyl groups. Alternatively the stationary phase might be an organic polymer, with no attached groups. The sample-containing the mixture of species to be resolved is applied to the stationary phase in an aqueous medium of relatively high polarity which promotes binding. Elution is then achieved by reducing the polarity of the aqueous medium by the addition of an organic solvent such as isopropanol or acetonitrile. Commonly a gradient (continuous, or as a series of steps) of increasing organic solvent concentration is used and the species are eluted in order of their respective hydrophobicities.

Other factors, such as the pH of the elution medium, and the use of additives, can also influence the strength of binding of species to RPC stationary phases. One, or more, of these

factors can be adjusted or utilised to optimise the elution and purification of product. A common additive is trifluoroacetic acid (TFA). This suppresses the ionisation of acidic groups such as carboxyl moieties in the sample. It also reduces the pH in the eluting medium and this suppresses the ionisation of free silanol groups that may be present on the surface of stationary phases
5 having a silica matrix. TFA is one of a class of additives known as ion pairing agents. These interact with ionic groups, present on species in the sample, that bear an opposite charge. The interaction tends to mask the charge, increasing the hydrophobicity of the species. Anionic ion pairing agents, such as TFA and pentafluoropropionic acid interact with positively charged groups on a species. Cationic ion pairing agents such, as triethylamine, interact with negatively
10 charged groups.

Viral vectors have on their surface, hydrophobic moieties such as proteins, and thus RPC, potentially, could be employed as a means of purification.

Affinity chromatography utilises the fact that certain ligands that bind specifically with biomolecules such as proteins or nucleotides, can be immobilised on a stationary phase. The
15 modified stationary phase can then be used to separate the relevant biomolecule from a mixture. Examples of highly specific ligands are antibodies, for the purification of target antigens and enzyme inhibitors for the purification of enzymes. More general interactions can also be utilised such as the use of the protein A ligand for the isolation of a wide range of antibodies.

Typically, affinity chromatography is performed by application of a mixture, containing
20 the species of interest, to the stationary phase that has the relevant ligand attached. Under appropriate conditions this will lead to the binding of the species to the stationary phase. Unbound components are then washed away before an eluting medium is applied. The eluting medium is chosen to disrupt the binding of the ligand to the target species. This is commonly achieved by choice of an appropriate ionic strength, pH or by the use of substances that will
25 compete with the target species for ligand sites. For some bound species, a chaotropic agent such as urea is used to effect displacement from the ligand. This, however, can result in irreversible denaturation of the species.

Viral vectors have on their surface, moieties such as proteins, that might be capable of binding specifically to appropriate ligands. This means that, potentially, affinity chromatography
30 could be used in their isolation.

Biomolecules, such as proteins, can have on their surface, electron donating moieties that can form coordinate bonds with metal ions. This can facilitate their binding to stationary phases carrying immobilised metal ions such as Ni^{2+} , Cu^{2+} , Zn^{2+} or Fe^{3+} . The stationary phases used in IMAC have chelating agents, typically nitriloacetic acid or iminodiacetic acid covalently
5 attached to their surface and it is the chelating agent that holds the metal ion. It is necessary for the chelated metal ion to have at least one coordination site left available to form a coordinate bond to a biomolecule. Potentially there are several moieties on the surface of biomolecules that might be capable of bonding to the immobilised metal ion. These include histidine, tryptophan and cysteine residues as well as phosphate groups. For proteins, however, the predominant donor
10 appears to be the imidazole group of the histidine residue. Native proteins can be separated using IMAC if they exhibit suitable donor moieties on their surface. Otherwise IMAC can be used for the separation of recombinant proteins bearing a chain of several linked histidine residues.

Typically, IMAC is performed by application of a mixture, containing the species of interest, to the stationary phase. Under appropriate conditions this will lead to the coordinate
15 bonding of the species to the stationary phase. Unbound components are then washed away before an eluting medium is applied. For elution, gradients (continuous, or as a series of steps) of increasing salt concentration or decreasing pH may be used. Also a commonly used procedure is the application of a gradient of increasing imidazole concentration. Biomolecules having different donor properties, for example having histidine residues in differing environments, can
20 be separated by the use of gradient elution.

Viral vectors have on their surface, moieties such as proteins, that might be capable of binding to IMAC stationary phases. This means that, potentially, IMAC could be used in their isolation.

Suitable centrifugation techniques include zonal centrifugation, isopycnic ultra and
25 pelleting centrifugation.

Filter-sterilisation is suitable for processes for pharmaceutical grade materials. Filter-sterilisation renders the resulting formulation substantially free of contaminants. The level of contaminants following filter-sterilisation is such that the formulation is suitable for clinical use. Further concentration (e.g. by ultrafiltration) following the filter-sterilisation step may be
30 performed in aseptic conditions. In some embodiments, the sterilising filter has a maximum pore size of 0.22 μm .

The retroviral vectors herein can also be subjected to methods to concentrate and purify a lentiviral vector using flow-through ultracentrifugation and high-speed centrifugation, and tangential flow filtration. Flow through ultracentrifugation can be used for the purification of RNA tumor viruses (Toplin et al, Applied Microbiology 15:582-589, 1967; Burger et al., Journal of the National Cancer Institute 45: 499-503, 1970). Flow-through ultracentrifugation can be used for the purification of Lentiviral vectors. This method can comprise one or more of the following steps. For example, a lentiviral vector can be produced from cells using a cell factory or bioreactor system. A transient transfection system can be used or packaging or producer cell lines can also similarly be used. A pre-clarification step prior to loading the material into the ultracentrifuge could be used if desired. Flow-through ultracentrifugation can be performed using continuous flow or batch sedimentation. The materials used for sedimentation are, e.g.: Cesium chloride, potassium tartrate and potassium bromide, which create high densities with low viscosity although they are all corrosive. CsCl is frequently used for process development as a high degree of purity can be achieved due to the wide density gradient that can be created (1.0 to 1.9 g/cm³). Potassium bromide can be used at high densities, e.g., at elevated temperatures, such as 25° C., which may be incompatible with stability of some proteins. Sucrose is widely used due to being inexpensive, non-toxic and can form a gradient suitable for separation of most proteins, sub-cellular fractions and whole cells. Typically the maximum density is about 1.3 g/cm³. The osmotic potential of sucrose can be toxic to cells in which case a complex gradient material can be used, e.g. Nycodenz. A gradient can be used with 1 or more steps in the gradient. An embodiment is to use a step sucrose gradient. The volume of material can be from 0.5 liters to over 200 liters per run. The flow rate speed can be from 5 to over 25 liters per hour. A suitable operating speed is between 25,000 and 40,500 rpm producing a force of up to 122,000×g. The rotor can be unloaded statically in desired volume fractions. An embodiment is to unload the centrifuged material in 100 ml fractions. The isolated fraction containing the purified and concentrated Lentiviral vector can then be exchanged in a desired buffer using gel filtration or size exclusion chromatography. Anionic or cationic exchange chromatography could also be used as an alternate or additional method for buffer exchange or further purification. In addition, Tangential Flow Filtration can also be used for buffer exchange and final formulation if required. Tangential Flow Filtration (TFF) can also be used as an alternative step to ultra or high speed centrifugation, where a two step TFF procedure would be implemented. The first step would

reduce the volume of the vector supernatant, while the second step would be used for buffer exchange, final formulation and some further concentration of the material. The TFF membrane can have a membrane size of between 100 and 500 kilodaltons, where the first TFF step can have a membrane size of 500 kilodaltons, while the second TFF can have a membrane size of between 300 to 500 kilodaltons. The final buffer should contain materials that allow the vector to be stored for long term storage.

In embodiments, the method uses either cell factories that contains adherent cells, or a bioreactor that contains suspension cells that are either transfected or transduced with the vector and helper constructs to produce lentiviral vector. Non limiting examples or bioreactors, include the Wave bioreactor system and the Xcellerex bioreactors. Both are disposable systems.

However non-disposable systems can also be used. The constructs can be those described herein, as well as other lentiviral transduction vectors. Alternatively the cell line can be engineered to produce Lentiviral vector without the need for transduction or transfection. After transfection, the lentiviral vector can be harvested and filtered to remove particulates and then is centrifuged

using continuous flow high speed or ultra centrifugation. A preferred embodiment is to use a high speed continuous flow device like the JCF-A zonal and continuous flow rotor with a high speed centrifuge. Also preferably is the use of Contifuge Stratus centrifuge for medium scale Lentiviral vector production. Also suitable is any continuous flow centrifuge where the speed of centrifugation is greater than 5,000×g RCF and less than 26,000×g RCF. Preferably, the

continuous flow centrifugal force is about 10,500×g to 23,500×g RCF with a spin time of between 20 hours and 4 hours, with longer centrifugal times being used with slower centrifugal force. The lentiviral vector can be centrifuged on a cushion of more dense material (a non limiting example is sucrose but other reagents can be used to form the cushion and these are well known in the art) so that the Lentiviral vector does not form aggregates that are not filterable, as sometimes occurs with straight centrifugation of the vector that results in a viral vector pellet.

Continuous flow centrifugation onto a cushion allows the vector to avoid large aggregate formation, yet allows the vector to be concentrated to high levels from large volumes of transfected material that produces the Lentiviral vector. In addition, a second less-dense layer of sucrose can be used to band the Lentiviral vector preparation. The flow rate for the continuous flow centrifuge can be between 1 and 100 ml per minute, but higher and lower flow rates can also be used. The flow rate is adjusted to provide ample time for the vector to enter the core of

the centrifuge without significant amounts of vector being lost due to the high flow rate. If a higher flow rate is desired, then the material flowing out of the continuous flow centrifuge can be re-circulated and passed through the centrifuge a second time. After the virus is concentrated using continuous flow centrifugation, the vector can be further concentrated using Tangential

5 Flow Filtration (TFF), or the TFF system can be simply used for buffer exchange. A non-limiting example of a TFF system is the Xampler cartridge system that is produced by GB-Healthcare. Preferred cartridges are those with a MW cut-off of 500,000 MW or less. Preferably a cartridge is used with a MW cut-off of 300,000 MW. A cartridge of 100,000 MW cut-off can also be used. For larger volumes, larger cartridges can be used and it will be easy for those in the art to find

10 the right TFF system for this final buffer exchange and/or concentration step prior to final fill of the vector preparation. The final fill preparation may contain factors that stabilize the vector—sugars are generally used and are known in the art.

Protein content

15 In some embodiments the retroviral particle includes various source cell genome-derived proteins, exogenous proteins, and viral-genome derived proteins. In some embodiments the retroviral particle contains various ratios of source cell genome-derived proteins to viral-genome-derived proteins, source cell genome-derived proteins to exogenous proteins, and exogenous proteins to viral-genome derived proteins.

20 In some embodiments, the viral-genome derived proteins are GAG polyprotein precursor, HIV-1 Integrase, POL polyprotein precursor, Capsid, Nucleocapsid, p17 matrix, p6, p2, VPR, Vif.

In some embodiments, the source cell-derived proteins are Cyclophilin A, Heat Shock 70kD, Human Elongation Factor-1 Alpha (EF-1R), Histones H1, H2A, H3, H4, beta-globin,

25 Trypsin Precursor, Parvulin, Glyceraldehyde-3-phosphate dehydrogenase, Lck, Ubiquitin, SUMO-1, CD48, Syntenin-1, Nucleophosmin, Heterogeneous nuclear ribonucleoproteins C1/C2, Nucleolin, Probable ATP-dependent helicase DDX48, Matrin-3, Transitional ER ATPase, GTP-binding nuclear protein Ran, Heterogeneous nuclear ribonucleoprotein U, Interleukin enhancer binding factor 2, Non-POU domain containing octamer binding protein, RuvB like 2, HSP 90-b,

30 HSP 90-a, Elongation factor 2, D-3-phosphoglycerate dehydrogenase, a-enolase, C-1-tetrahydrofolate synthase, cytoplasmic, Pyruvate kinase, isozymes M1/M2, Ubiquitin activating

enzyme E1, 26S protease regulatory subunit S10B, 60S acidic ribosomal protein P2, 60S acidic ribosomal protein P0, 40S ribosomal protein SA, 40S ribosomal protein S2, 40S ribosomal protein S3, 60S ribosomal protein L4, 60S ribosomal protein L3, 40S ribosomal protein S3a, 40S ribosomal protein S7, 60S ribosomal protein L7a, 60S acidic ribosomal protein L31, 60S ribosomal protein L10a, 60S ribosomal protein L6, 26S proteasome non-ATPase regulatory subunit 1, Tubulin b-2 chain, Actin, cytoplasmic 1, Actin, aortic smooth muscle, Tubulin a-ubiquitous chain, Clathrin heavy chain 1, Histone H2B.b, Histone H4, Histone H3.1, Histone H3.3, Histone H2A type 8, 26S protease regulatory subunit 6A, Ubiquitin-4, RuvB like 1, 26S protease regulatory subunit 7, Leucyl-tRNA synthetase, cytoplasmic, 60S ribosomal protein L19, 26S proteasome non-ATPase regulatory subunit 13, Histone H2B.F, U5 small nuclear ribonucleoprotein 200 kDa helicase, Poly[ADP-ribose]polymerase-1, ATP-dependent DNA helicase II, DNA replication licensing factor MCM5, Nuclease sensitive element binding protein 1, ATP-dependent RNA helicase A, Interleukin enhancer binding factor 3, Transcription elongation factor B polypeptide 1, Pre-mRNA processing splicing factor 8, Staphylococcal nuclease domain containing protein 1, Programmed cell death 6-interacting protein, Mediator of RNA polymerase II transcription subunit 8 homolog, Nucleolar RNA helicase II, Endoplasmic, DnaJ homolog subfamily A member 1, Heat shock 70 kDa protein 1L, T-complex protein 1 e subunit, GCN1-like protein 1, Serotransferrin, Fructose biphosphate aldolase A, Inosine-5' monophosphate dehydrogenase 2, 26S protease regulatory subunit 6B, Fatty acid synthase, DNA-dependent protein kinase catalytic subunit, 40S ribosomal protein S17, 60S ribosomal protein L7, 60S ribosomal protein L12, 60S ribosomal protein L9, 40S ribosomal protein S8, 40S ribosomal protein S4 X isoform, 60S ribosomal protein L11, 26S proteasome non-ATPase regulatory subunit 2, Coatamer a subunit, Histone H2A.z, Histone H1.2, Dynein heavy chain cytosolic. See: Saphire et al., *Journal of Proteome Research*, 2005, and Wheeler et al., *Proteomics Clinical Applications*, 2007.

In some embodiments the retroviral vector is pegylated.

Particle size

In some embodiments the median retroviral vector diameter is between 10 and 1000 nM, 25 and 500 nm 40 and 300 nm, 50 and 250 nm, 60 and 225 nm, 70 and 200 nm, 80 and 175 nm, or 90 and 150 nm.

5 In some embodiments, 90% of the retroviral vectors fall within 50% of the median diameter of the retrovirus. In some embodiments, 90% of the retroviral vectors fall within 25% of the median diameter of the retrovirus. In some embodiments, 90% of the retroviral vectors fall within 20% of the median diameter of the retrovirus. In some embodiments, 90% of the retroviral vectors fall within 15% of the median diameter of the retrovirus. In some
10 embodiments, 90% of the retroviral vectors fall within 10% of the median diameter of the retrovirus.

XI. Indications and uses

The fusosomes, retroviral vectors, VLPs, or pharmaceutical compositions described herein can be administered to a subject, e.g., a mammal, e.g., a human. In such embodiments,
15 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 some embodiments, the disease is a genetic deficiency, e.g., a genetic deficiency listed in Table 5 or Table 6.

This disclosure also provides, in certain aspects, a method of administering a fusosome
20 composition to a subject (e.g., a human subject), a target tissue, or a cell, comprising administering to the 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.

25 This disclosure also provides, in certain aspects, a method of delivering a therapeutic agent (e.g., a polypeptide, a nucleic acid, a metabolite, an organelle, or a subcellular structure) to a subject, a target tissue, or a cell, comprising administering to the subject, or contacting the target tissue or the cell with, a plurality of fusosomes described herein, a fusosome composition comprising a plurality of fusosomes described herein, a fusosome composition described herein,
30 or a pharmaceutical composition described herein, wherein the fusosome composition is administered in an amount and/or time such that the therapeutic agent is delivered.

This disclosure also provides, in certain aspects, a method of delivering a function to a subject, a target tissue, or a cell, comprising administering to the subject, or contacting the target tissue or the cell with, a plurality of fusosomes described herein, a fusosome composition comprising a plurality of fusosomes described herein, a fusosome composition described herein, or a pharmaceutical composition described herein, wherein the fusosome composition is administered in an amount and/or time such that the function is delivered.

Target cells from mammalian (e.g., human) tissue include cells from epithelial, connective, muscular, or nervous tissue or cells, and combinations thereof. Target mammalian (e.g., human) cells and organ systems include 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 some embodiments, a non-target cells or organ system is chosen 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.

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.

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.

5 In embodiments, the fusosome composition described herein is delivered ex-vivo to a cell or tissue, e.g., a human cell or tissue.

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
10 (e.g., a disease or condition described herein).

In some embodiments, the source of fusosomes are 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
15 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.

In some embodiments, the fusosome is co-administered with an inhibitor of a protein that
20 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 suppressyn, e.g., a siRNA or inhibitory antibody.

Compositions described herein may also be used to similarly modulate the cell or tissue
25 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
30 non-human target cell or tissue or subject.

Fusosome compositions can be autologous, allogeneic or xenogeneic to the target.

XII. Additional therapeutic agents

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 some 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.

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.

In some embodiments, the immunosuppressive agent is a small molecule such as ibuprofen, acetaminophen, cyclosporine, tacrolimus, rapamycin, mycophenolate, cyclophosphamide, glucocorticoids, sirolimus, azathioprine, or methotrexate.

In some embodiments, the immunosuppressive agent is an antibody molecule, including but not limited to: muronomab (anti-CD3), Daclizumab (anti-IL12), Basiliximab, Infliximab (Anti-TNF α), or rituximab (Anti-CD20).

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.

EXAMPLES

The Examples below are set forth to aid in the understanding of the inventions, but are not intended to, and should not be construed to, limit its scope in any way.

5 **Example 1. Assaying off-target cells to detect specificity of retroviral nucleic acid delivery**

This Example describes quantification of a nucleic acid in off-target recipient cells by measuring vector copy number in single cells.

In an embodiment, treated mice have a similar vector copy number in off-target cells as those from untreated mice, e.g., no vector or a vector number similar to negative control
10 levels. In an embodiment, treated mice have a similar percent of off-target cells that contain the vector as those from untreated mice, e.g., no cells or a cell number similar to negative control levels.

In this example, the off-target recipient cell is a CD11c+ cell. However, this protocol may be adapted to any cell type for which suitable surface markers exist and which can be
15 isolated from the subject. Notably, the methods described herein may be equally applicable to humans, rats, monkeys with optimization to the protocol.

Mice are treated with retroviral vector produced as described herein or with PBS (negative control). 28 days following treatment, peripheral blood is collected from mice that received retroviral vector and mice that received PBS treatment. Blood is collected into 1ml PBS
20 containing 5 μ M 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 CD11c:APC-Cy7 antibody (Biolegend Catalog #: 117323) or an isotype control APC-Cy7 antibody (Biolegend Catalog #: 400230) at 4°C for 30 minutes in the dark, after being Fc blocked (Biolegend Catalog #: 101319) in cell staining buffer (Biolegend Catalog
25 #: 420201) for 10 minutes. After being washed two times with PBS, cells are analyzed on a FACS Aria (BD Biosciences, San Jose, CA.) with 640nm laser excitation and emission collected at 780 +/- 60 nm running the FACSDiva™ software (BD Biosciences, San Jose, CA) to set negative gates using the isotype control APC-Cy7 antibody labeled cells. APC-Cy7 positive cells are sorted into single wells of plate for vector copy number analysis.

30 Vector copy number is assessed using single-cell nested PCR. PCR is performed with qPCR using primers and probes specific to the vector and an endogenous control gene. Vector

copy number is determined by dividing the amount of vector qPCR signal by the amount of the endogenous control gene qPCR signal. A cell that received the vector will have a vector copy number of at least 1.0. Vector copy number is assessed across the population by averaging the vector copy number of the plurality of cells

5 In some embodiments, mice treated with retroviral vectors have a similar average vector copy number in off-target cells as those from mice treated with vehicle. In some embodiments, mice treated with treated with retroviral vectors have a similar percent of off-target cells that received the vector as those from mice treated with vehicle.

10 **Example 2. Assaying off-target cells to detect specificity of delivery of an exogenous protein agent**

This Example describes quantification of the expression of an exogenous agent in off-target recipient cells by exogenous agent expression in single cells.

15 In an embodiment, treated mice have similar exogenous agent expression in off-target cells as those from untreated mice. In an embodiment, treated mice have a similar percent of off-target cells that express the exogenous agent as those from untreated mice.

20 In this example, the off-target recipient cell is a CD11c+ cell. However, this protocol may be adapted to any cell type for which suitable surface markers exist and which can be isolated from the subject. Notably, the methods described herein may be equally applicable to humans, rats, monkeys with optimization to the protocol. In this example the exogenous agent is a fluorescent protein and expression is measured via flow cytometry. In other embodiments, the expression of an exogenous protein agent may be measured with immunostaining for the protein. In other embodiments expression of the exogenous protein agent may be measured via microscopy or western blot.

25 Mice are treated with retroviral vector with a tdTomato fluorescent protein agent produced via any of the methods described in this application or with PBS (negative control). 28 days following treatment, peripheral blood is collected from mice that received retroviral vector and mice that received PBS treatment. Blood is collected into 1ml PBS containing 5 μ M EDTA and mixed immediately to prevent clotting. The tubes are kept on ice and red blood cells are
30 removed using a buffered ammonium chloride (ACK) solution. Cells are stained with a murine CD11c:APC-Cy7 antibody (Biolgend Catalog #: 117323) or isotype controls APC-Cy7 antibody

(Biolegend Catalog #: 400230) at 4°C for 30 minutes in the dark, after being Fc blocked (Biolegend Catalog #: 101319) in cell staining buffer (Biolegend Catalog #: 420201) for 10 minutes. After being washed two times with PBS, cells are analyzed on a FACS Aria (BD Biosciences, San Jose, CA.) running the FACSDiva™ software (BD Biosciences, San Jose, CA).

5 A negative gate for CD11c is set using the isotype control APC-Cy7 antibody labeled cells and with a 640nm laser excitation and emission collected at 780 +/- 60. A negative gate for tdTomato expression is set with cells isolated from mice treated with vehicle and with a 552 nm laser excitation and an emission collected at 585 +/- 42 nm.

10 The percent of CD11c+ cells that are tdTomato positive is measured. In some embodiments, the percent of CD11c+ cells that are tdTomato positive is similar in cells from treated and untreated mice. The median tdTomato fluorescence level is measured in CD11c+ cells. In some embodiments, the median tdTomato fluorescence level in CD11c+ cells is similar in cells from treated and untreated mice.

15 **Example 3. Assaying target cells to detect specificity of retroviral nucleic acid delivery**

This Example describes quantification of a nucleic acid in target recipient cells by measuring vector copy number in single cells.

20 In an embodiment, treated mice have a greater vector copy number in target cells than those from untreated mice. In an embodiment, treated mice have a greater percent of target cells that contain the vector than those from untreated mice.

In this example, the target recipient cell is a CD3+ cell. However, this protocol may be adapted to any cell type for which suitable surface markers exist and which can be isolated from the subject. Notably, the methods described herein may be equally applicable to humans, rats, monkeys with optimization to the protocol.

25 Mice are treated with retroviral vector and a blood sample is collected as described above in Example 1. Cells are stained with a murine CD3:APC-Cy7 antibody (Biolegend Catalog #: 100330) or an isotype control using the protocol described above in Example 1. Vector copy number is assessed using single-cell nested PCR as described in Example 1.

30 In some embodiments, mice treated with retroviral vectors have a greater average vector copy number in target cells than those from mice treated with vehicle. In some

embodiments, mice treated with treated with retroviral vectors have a greater percent of target cells that received the vector than those from mice treated with vehicle.

Example 4. Assaying target cells to detect specificity of delivery of an exogenous protein agent

This Example describes quantification of the expression of an exogenous protein agent in target recipient cells by exogenous protein agent expression in single cells.

In an embodiment, treated mice have greater exogenous protein agent expression in target cells than those from untreated mice. In an embodiment, treated mice have a greater percent of target cells that express the exogenous protein agent than those from untreated mice.

In this example, the target recipient cell is a CD3+ cell. However, this protocol may be adapted to any cell type for which suitable surface markers exist and which can be isolated from the subject. Notably, the methods described herein may be equally applicable to humans, rats, monkeys with optimization to the protocol. In this example the exogenous protein agent is a fluorescent protein and expression is measured via flow cytometry. In other embodiments, the expression of an exogenous protein agent may be measured with immunostaining for the protein. In other embodiments expression of the exogenous protein agent may be measured via microscopy or western blot.

Mice are treated with retroviral vector and a blood sample is collected as described above in Example 2. Cells are stained with a murine CD3:APC-Cy7 antibody (Biolegend Catalog #: 100330) or isotype controls and analyzed by flow cytometry using the protocol described in Example 2.

The percent of CD3+ cells that are tdtomato positive is measured. In some embodiments, the percent of CD3+ cells that are tdtomato positive is greater in cells from treated than untreated mice. The median tdtomato fluorescence level is measured in CD3+ cells. In some embodiments, the median tdtomato fluorescence level in CD3+ cells is greater in cells from treated than untreated mice.

Example 5. Modification of retroviral vector with HLA-G or HLA-E for decreased cytotoxicity mediated by PBMC cell lysis

This Example describes retroviral vectors derived from cells modified to have decreased cytotoxicity due to cell lysis by peripheral blood mononuclear cells (PBMCs).

5 In an embodiment, cytotoxicity mediated cell lysis of retroviral vectors by PBMCs is a measure of immunogenicity of retroviral vectors, as lysis will reduce, e.g., inhibit or stop, the activity of a retroviral vector.

Retroviral vectors are created from: unmodified cells (hereinafter NMCs, positive control), cells that are transfected with HLA-G or HLA-E cDNA (hereinafter NMC-HLA-G),
10 and cells transfected with an empty vector control (hereinafter NMC-empty vector, negative control).

PBMC mediated lysis of a retroviral vector 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 (hereinafter effector cells) are isolated from an
15 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 retroviral vectors are labeled with europium-diethylenetriaminepentaacetate (DTPA) (sigma, St. Louis, MO, USA).

At day 7 cytotoxicity-mediated lysis assays is performed by incubating ⁶³Eu-labelled
20 retroviral vector with effector cells in a 96-well plate for 1, 2, 3, 4, 5, 6, 8, 10, 15, 20, 24, or 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).

Subsequently, enhancement solution (PerkinElmer, Groningen, The Netherlands) is
25 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 retroviral vector is determined by incubating an appropriate number (1×10^2 - 1×10^8) of retroviral vectors with 1% triton (sigma-aldrich) for an appropriate amount of time. Spontaneous release of europium by target retroviral
30 vector is measured by incubation of labeled target retroviral vector without effector cells. Percentage leakage is then calculated as: (spontaneous release/maximum release) x 100%. 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.

In an embodiment, retroviral vectors generated from NMC-HLA-G cells will have a decreased percentage of lysis by target cells at specific timepoints as compared to retroviral vectors generated from NMCs or NMC-empty vector.

Example 6. Modification of retroviral vector with HLA-G or HLA-E for decreased NK lysis activity

This Example describes the generation of a retroviral vector composition derived from a cell source which has been modified to decrease cytotoxicity mediated cell lysis by NK cells. In an embodiment cytotoxicity mediated cell lysis of retroviral vectors by NK cells is a measure of immunogenicity for retroviral vectors.

Retroviral vectors are created from: unmodified cells (hereinafter NMCs, positive control), cells that are transfected with HLA-G or HLA-E cDNA (hereinafter NMC-HLA-G), and cells transfected with an empty vector control (hereinafter NMC-empty vector, negative control).

NK cell mediated lysis of a retroviral vector 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 (hereinafter 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 retroviral vectors are labeled with europium-diethylenetriaminepentaacetate (DTPA) (sigma, St. Louis, MO, USA). Cytotoxicity-mediated lysis assays and data analysis are performed as described above in Example 5.

In an embodiment, retroviral vectors generated from NMC-HLA-G cells will have a decreased percentage of lysis by target cells at specific timepoints as compared to retroviral vectors generated from NMCs or NMC-empty vector.

Example 7. Modification of retroviral vector with HLA-G or HLA-E for decreased CD8 Killer T cell lysis

This Example describes the generation of a retroviral vector composition derived from a cell source which has been modified to decrease cytotoxicity mediated cell lysis by CD8+ T-cells. In an embodiment, cytotoxicity mediated cell lysis of retroviral vector by CD8+ T-cells is a measure of immunogenicity for retroviral vectors.

Retroviral vectors are created from: unmodified cells (hereinafter NMCs, positive control), cells that are transfected with HLA-G or HLA-E cDNA (hereinafter NMC-HLA-G), and cells transfected with an empty vector control (hereinafter NMC-empty vector, negative control).

CD8+ T cell mediated lysis of a retroviral vector 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 (hereinafter 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 retroviral vectors are labeled with europium-diethylenetriaminepentaacetate (DTPA) (sigma, St. Louis, MO, USA). Cytotoxicity-mediated lysis assays and data analysis are performed as described above in Example 5.

In an embodiment, retroviral vectors generated from NMC-HLA-G cells will have a decreased percentage of lysis by target cells at specific timepoints as compared to retroviral vectors generated from NMCs or NMC-empty vector.

Example 8: Modification of retroviral vector with CD47 to evade macrophage phagocytosis

This Example describes quantification of the evasion of phagocytosis by modified retroviral vector. In an embodiment, modified retroviral vector will evade phagocytosis by macrophages.

Cells engage in phagocytosis, engulfing particles, enabling the sequestration and destruction of foreign invaders, like bacteria or dead cells. In some embodiments, phagocytosis of lentiviral vectors by macrophages would reduce their activity. In some embodiments, phagocytosis of lentiviral vectors is a measure of immunogenicity of retroviral vectors.

Retroviral vectors are produced from cells which lack CD47 (hereinafter NMC, positive control), cells that are transfected with CD47 cDNA (hereinafter NMC-CD47), and cells transfected with an empty vector control (hereinafter NMC-empty vector, negative control). Prior to retroviral vector production, the cells are labeled with CFSE.

Reduction of macrophage mediated 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 retroviral vectors produced 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.

After 2h, the dish is gently washed and intracellular fluorescence is examined. Intracellular fluorescence emitted by engulfed retroviral 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.

In an embodiment, the phagocytic index will be reduced when macrophages are incubated with retroviral vectors derived from NMC-CD47, versus those derived from NMC, or NMC-empty vector.

Example 9: Modification of retroviral vector with complement regulatory proteins to evade complement

This Example describes quantification of complement activity against a retroviral vector using an in vitro assay. In some embodiments a modified retroviral vector described herein will have reduced complement activity compared to an unmodified retroviral vector.

In this Example, serum from a mouse is assessed for complement activity against a retroviral vector. The example measures the level of complement C3a, which is a central node in all complement pathways. The methods described herein may be equally applicable to humans, rats, monkeys with optimization to the protocol.

In this example, retroviral vectors are generated from HEK293 cells transfected with a cDNA coding for complement regulatory protein DAF (HEK293-DAF retroviral vector) or HEK

293 cells not expressing a complementary regulatory protein (HEK293 retroviral vector). In other embodiments, other complement regulatory proteins may be used, such as proteins that bind decay-accelerating factor (DAF, CD55), e.g. factor H (FH)-like protein-1 (FHL-1), e.g. C4b-binding protein (C4BP), e.g. complement receptor 1 (CD35), e.g. Membrane cofactor protein (MCP, CD46), eg. Prolectin (CD59), e.g. proteins that inhibit the classical and alternative complement pathway CD/C5 convertase enzymes, e.g. proteins that regulate MAC assembly.

Serum is recovered from naïve mice, mice that are administered HEK293-DAF retroviral vector, or mice that are administered HEK293 retroviral vector. Sera are collected from mice by collecting fresh whole blood and allowing it to clot completely for several hours. Clots are pelleted by centrifugation and the serum supernatants are removed. A negative control is heat inactivated mouse serum. Negative control samples are heated at 56 degrees Celsius for 1 hour. Serum may be frozen in aliquots.

The different retroviral vectors are tested for the dose at which 50% of cells in a target cell population receive the exogenous agent in the retroviral vector. The retroviral vector may contain any of the exogenous agents described herein. Many methods for assaying retroviral delivery of an exogenous agent to recipient cells are also described herein. In this particular example, the exogenous agent is Cre protein (encoded by the retroviral nucleic acid) and the target cells are RPMI8226 cells which stably-express a “LoxP-GFP-stop-LoxP-RFP” cassette under a CMV promoter, which upon recombination by Cre switches from GFP to RFP expression, as a marker of delivery. The identified dose at which 50% of the recipient cells are RFP positive is used for further experiments. In some embodiments, the identified dose at which 50% of recipient cells receive the exogenous agent will be similar across retroviral vectors.

Two-fold dilutions in phosphate-buffered saline (PBS, pH 7.4) of the retroviral vectors, starting at the dose of retroviral vectors at which 50% of the target cells receive the exogenous agent, are mixed with a 1:10 dilution of the sera from mice treated with the same retroviral vectors or naïve mice (assay volume, 20 µl) and incubated for 1 h at 37°C. The samples are further diluted 1:500 and used in an enzyme-linked immunosorbent assay (ELISA) specific for C3a. The ELISA is mouse complement C3a ELISA Kit product LS-F4210 sold by LifeSpan BioSciences Inc, which measures the concentration of C3a in a sample. The dose of retroviral vector at which 200 pg/ml of C3a is present is compared across sera isolated from mice.

In some embodiments, the dose of retroviral vector at which 200 pg/ml of C3a is present will be greater for HEK293-DAF retroviral vector incubated with HEK-293 DAF mouse sera than for HEK293 retroviral vector incubated with HEK293 mouse sera, indicating that complement activity targeting retroviral vector is greater in mice treated with HEK293 retroviral vector than HEK293-DAF retroviral vector. In some embodiments, the dose of retroviral vector at which 200 pg/ml of C3a is present will be greater for HEK293-DAF retroviral vector incubated with naive mouse sera than for HEK293 retroviral vector incubated with naive mouse sera, indicating that complement activity targeting retroviral vector is greater in mice treated with HEK293 retroviral vector than HEK293-DAF retroviral vector.

Example 10: Modification of retroviral vector to knockdown immunogenic protein to reduce immunogenicity

This Example describes the generation of a retroviral vector composition derived from a cell source which has been modified to reduce expression of a molecule which is immunogenic, and quantification of the reduced expression. In an embodiment, a retroviral vector can be derived from a cell source, which has been modified to reduce expression of a molecule which is immunogenic.

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 retroviral vectors. Expression of certain immune activating agents can create an immune response. MHC class I represents one example of an immune activating agent.

Retroviral vectors are produced from unmodified cells which normally express MHC-1 (hereinafter NMC, positive control), cells that are transfected with a DNA coding for a shRNA targeting MHC class I (hereinafter NMC- shMHC class I), and cells transfected with a DNA coding for non-targeted scrambled shRNA vector control (hereinafter NMC-vector control, negative control). Prior to retroviral production, the cells are labeled with CFSE.

Retroviral vectors are assayed for expression of MHC class I using flow cytometry. An appropriate number of retroviral vectors 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). Retroviral vectors are washed three times in PBS and resuspended in PBS. Nonspecific fluorescence is determined, using equal aliquots of

retroviral vector preparation incubated with and appropriate fluorescently conjugated isotype control antibody at equivalent dilutions. Retroviral vectors are assayed in a flow cytometer (FACSort, Becton-Dickinson) and the data is analyzed with flow analysis software (Becton-Dickinson).

5 The mean fluorescence data of the retroviral vectors derived from NMCs, NMC- shMHC class I, and NMC-vector control, is compared. In an embodiment, retroviral vectors derived from NMC- shMHC class I will have lower expression of MHC class I compared to NMCs and NMC-vector control.

10 **Example 11: Measuring pre-existing serum inactivation of retroviral vectors**

 This Example describes quantification of pre-existing serum inactivation of retroviral vectors using an *in vitro* delivery assay.

 In some embodiments, a measure of immunogenicity for retroviral vectors is serum inactivation. Serum inactivation of retroviral vectors may be due to antibody-mediated
15 neutralization or complement mediated degradation. In an embodiment, some recipients of a retroviral vectors described herein will have factors in their serum which bind to and inactivate retroviral vectors.

 In this Example, a retroviral vector naïve mouse is assessed for the presence of factors that inactivate retroviral vectors in serum. Notably, the methods described herein may be equally
20 applicable to humans, rats, monkeys with optimization to the protocol.

 The negative control is heat inactivated mouse serum and the positive control is serum derived from a mouse that has received multiple injections of retroviral vector generated from a xenogeneic source cell. Sera are collected from mice by collecting fresh whole blood and allowing it to clot completely for several hours. Clots are pelleted by centrifugation and the
25 serum supernatants are removed. Negative control samples are heated at 56 degrees Celsius for 1 hour. Serum may be frozen in aliquots.

 The retroviral vectors are tested for the dose at which 50% of cells in a target cell population receive the exogenous agent in the retroviral vector, as described above in Example 9.

 To assess serum inactivation of retroviral vectors, retroviral vectors are diluted 1:5 into
30 normal or heat-inactivated serum (or medium containing 10% heat-inactivated FBS as the no-serum control) and the mixture is incubated at 37° C for 1 h. Following the incubation, medium

is added to the reaction for an additional 1:5 dilution and then serially diluted twice at a 1:10 ratio. Following this step, the retroviral vectors should be present at the previously identified dose at which 50% of the recipient cells have received the exogenous agent (e.g. are RFP positive).

5 Retroviral vectors that have been exposed to serum are then incubated with target cells. The percent of cells which receive the exogenous agent, and thus are RFP positive, is calculated. In some embodiments, the percent of cells which receive the exogenous agent will not be different between retroviral vector samples that have been incubated with serum and heat-inactivated serum from retroviral vector naïve mice, indicating that there is not serum
10 inactivation of retroviral vector. In some embodiments, the percent of cells which receive the exogenous agent will not be different between retroviral vector samples that have been incubated with serum from retroviral vector naïve mice and no-serum control incubations, indicating that there is not serum inactivation of retroviral vectors. In some embodiments, the percent of cells which receive the exogenous agent will be less in retroviral vector samples that have been
15 incubated with positive control serum than in retroviral vector samples that have been incubated with serum from retroviral vector naïve mice, indicating that there is not serum inactivation of retroviral vectors.

20 **Example 12: Measuring serum inactivation of retroviral vectors after multiple administrations**

 This Example describes quantification of serum inactivation of retroviral vectors using an *in vitro* delivery assay following multiple administrations of the retroviral vectors. In an embodiment, a modified retroviral vector, e.g., modified by a method described herein, will have a reduced (e.g., reduced compared to administration of an unmodified retroviral vector) serum
25 inactivation following multiple (e.g., more than one, e.g., 2 or more) administrations of the modified retroviral vector. In an embodiment, a retroviral vector described herein will not be inactivated by serum following multiple administrations.

 In some embodiments, a measure of immunogenicity for retroviral vector is serum inactivation. In an embodiment, repeated injections of a retroviral vector can lead to the
30 development of anti-retroviral vector antibodies, e.g., antibodies that recognize retroviral vectors.

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NOM DU FICHIER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

WHAT IS CLAIMED IS:

1. A fusosome comprising:
 - a) a lipid bilayer comprising a fusogen; and
 - b) a nucleic acid that comprises:
 - (i) a payload gene encoding an exogenous agent; and
 - (ii) a positive target cell-specific regulatory element operatively linked to the payload gene, wherein the positive target cell-specific regulatory element increases expression of the payload gene in a target cell relative to an otherwise similar fusosome lacking the positive target cell-specific regulatory element, wherein the target cell is a CNS cell.

2. The fusosome of claim 1, wherein the nucleic acid further comprises a non-target cell-specific regulatory element (NTCSRE), operatively linked to the payload gene, wherein the NTCSRE decreases expression of the payload gene in a non-target cell relative to an otherwise similar fusosome lacking the NTCSRE, wherein the target cell is a first type of CNS cell and the non-target cell is a second, different type of CNS cell or a non-CNS cell optionally wherein:
 - the target cell is a neuron and the non-target cell is a glial cell, optionally wherein the glial cell is an oligodendrocyte, an astrocyte, or a microglia cell, or
 - the target cell is a glial cell, optionally wherein the glial cell is an oligodendrocyte, an astrocyte, or a microglia cell, and the non-target cell is a neuron.

3. A fusosome comprising:
 - a) a lipid bilayer comprising a fusogen; and
 - b) a nucleic acid that comprises:
 - (i) a payload gene encoding an exogenous agent; and
 - (ii) a promoter operatively linked to the payload gene, wherein the promoter is chosen from a SYN, NSE, CaMKII, α Tubulin, PDGF, fSST, fNPY, GAD67, DLX5/6, VGLUT1, Dock10, ChAT, VACHT, Drd1a, TPH-2, GFAP, EAAT1, GS, CX3CR1, TMEM119, MBP, CNP, or CRFR2 β promoter.

4. A fusosome comprising:
- a) a lipid bilayer comprising a fusogen; and
 - b) a nucleic acid that comprises:
 - (i) a payload gene encoding an exogenous agent; and
 - (ii) a non-target cell-specific regulatory element (NTCSRE) operatively linked to the payload gene, wherein:

the NTCSRE decreases expression of the payload gene in a non-target cell or tissue relative to an otherwise similar fusosome lacking the NTCSRE, wherein the target cell is a first type of CNS cell and the non-target cell is a second, different type of CNS cell or a non-CNS cell; and

the NTCSRE comprises a non-target cell-specific miRNA recognition sequence, non-target cell-specific protease recognition site, non-target cell-specific ubiquitin ligase site, non-target cell-specific transcriptional repression site, or non-target cell-specific epigenetic repression site.

5. The fusosome of claim 4, wherein the nucleic acid further comprises a positive target cell-specific regulatory element operatively linked to the payload gene, wherein the positive target cell-specific regulatory element increases expression of the payload gene in a target cell relative to an otherwise similar fusosome lacking the positive target cell-specific regulatory element, wherein the target cell is a CNS cell.

6. The fusosome of any of claims 1-5, wherein the fusosome further comprises one or both of:

(i) a first exogenous or overexpressed immunosuppressive protein on the lipid bilayer; or

(ii) a first immunostimulatory protein that is absent or present at reduced levels, optionally wherein the reduced level is reduced by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% compared to a fusosome generated from an otherwise similar, unmodified source cell.

7. The fusosome of any of claims 1-6, wherein the payload gene is a gene that treats a lysosomal storage disease or disorder or a CNS disease or disorder, optionally wherein the disease or disorder is a genetic deficiency.

8. A fusosome comprising:

- a) a lipid bilayer comprising a fusogen;
- b) a nucleic acid that comprises a payload gene encoding an exogenous agent for treating a lysosomal storage disease or disorder or a CNS disease or disorder; and
- c) one or both of:
 - (i) a first exogenous or overexpressed immunosuppressive protein on the lipid bilayer; or
 - (ii) a first immunostimulatory protein that is absent or present at reduced levels (e.g., reduced by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%) compared to a fusosome generated from an otherwise similar, unmodified source cell.

9. The fusosome of any of claims 6-8, which comprises (i) and (ii).

10. The fusosome of any of claims 6-8, which comprises (i) and further comprises a second exogenous or overexpressed immunosuppressive protein on the lipid bilayer.

11. The fusosome of any of claims 6-10, which comprises (ii) and further comprises a second immunostimulatory protein that is absent or present at reduced levels, optionally wherein the reduced level is reduced by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% compared to a fusosome generated from an otherwise similar, unmodified source cell.

12. The fusosome of any of claims 8-11, wherein the nucleic acid further comprises a positive target cell-specific regulatory element operatively linked to the payload gene, wherein the positive target cell-specific regulatory element increases expression of the payload gene in a target cell relative to an otherwise similar fusosome lacking the positive target cell-specific regulatory element, wherein the target cell is a CNS cell.

13. The fusosome of any of claims 8-12, wherein the nucleic acid further comprises a non-target cell-specific regulatory element (NTCSRE) operatively linked to the payload gene, wherein the NTCSRE decreases expression of the payload gene in a non-target cell or tissue relative to an otherwise similar fusosome lacking the NTCSRE, wherein the target cell is a first type of CNS cell and the non-target cell is a second, different type of CNS cell or a non-CNS cell, optionally wherein:

the target cell is a neuron and the non-target cell is a glial cell, optionally wherein the glial cell is an oligodendrocyte, an astrocyte, or a microglia cell, or

the target cell is a glial cell, optionally wherein the glial cell is an oligodendrocyte, an astrocyte, or a microglia cell, and the non-target cell is a neuron.

14. The fusosome of any of claims 6-13, wherein, when administered to a subject, one or more of:

i) the fusosome does not produce a detectable antibody response or antibodies against the fusosome are present at a level of less than 10%, 5%, 4%, 3%, 2%, or 1% above a background level;

ii) the fusosome does not produce a detectable cellular immune response, or a cellular immune response against the fusosome is present at a level of less than 10%, 5%, 4%, 3%, 2%, or 1% above a background level;

iii) the fusosome does not produce a detectable innate immune response), or the innate immune response against the fusosome is present at a level of less than 10%, 5%, 4%, 3%, 2%, or 1% above a background level,;

iv) less than 10%, 5%, 4%, 3%, 2%, or 1% of fusosomes are inactivated by serum;

v) a target cell that has received the exogenous agent from the fusosome does not produce a detectable antibody response, or antibodies against the target cell are present at a level of less than 10%, 5%, 4%, 3%, 2%, or 1% above a background level; or

vi) a target cell that has received the exogenous agent from the fusosome does not produce a detectable cellular immune response, or a cellular response against the target cell is present at a level of less than 10%, 5%, 4%, 3%, 2%, or 1% above a background level.

15. The fusosome of claim 14, wherein the background level is the corresponding level in the same subject prior to administration of the fusosome.

16. The fusosome of any of claims 6-15, wherein the immunosuppressive protein is a complement regulatory protein or CD47.

17. The fusosome of any of claims 6-16, wherein the immunostimulatory protein is an MHC I or MHC II protein.

18. The fusosome of any of claims 1-17, wherein one or more of:

i) the fusosome fuses at a higher rate with the CNS target cell than with a non-target cell, optionally wherein the higher rate is 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;

ii) the fusosome fuses at a higher rate with the CNS target cell than with another fusosome, optionally wherein the higher rate is 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;

iii) the fusosome fuses with CNS target cells at a rate such that the exogenous agent in the fusosome is delivered to at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, of CNS target cells after 24, 48, or 72 hours;

iv) the fusosome delivers the nucleic acid to the CNS target cell at a higher rate than to a non-target cell, optionally wherein the higher rate is 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;

v) the fusosome delivers the nucleic acid to the CNS target cell at a higher rate than to another fusosome, optionally wherein the higher rate is 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; or

vi) the fusosome delivers the nucleic acid to the CNS target cell at a rate such that the exogenous 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.

19. The fusosome of any of claims 1-18, wherein the exogenous agent is chosen from: SYNE1, SETX, FMR1, SLC6A8, UBE3A, SOD1, TDP43, C9orf72, FXN, MECP2, ASPA, or ALDH7A1; or the exogenous agent is chosen from: TPP1, FUCA1, GALC, HEXA, HEXB, MANBA, ARSA, GNPTAB, or MCOLN1.

20. The fusosome of any of claims 1-19, wherein the payload gene is selected from among SYNE1, SETX, FMR1, SLC6A8, UBE3A, SOD1, TDP43, C9orf72, FXN, MECP2, ASPA and ALDH7A1.

21. The fusosome of any of claims 1-20, wherein the payload gene encodes an exogenous agent comprising the sequence set forth in any one of SEQ ID NOS: 134-145, a functional fragment thereof, or a functional variant thereof comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%, identity to an amino acid sequence set forth in any one of SEQ ID NOS: 134-145.

22. The fusosome of any of claims 1-19, wherein the payload gene is selected from TPP1, FUCA1, GALC, HEXA, HEXB, MANBA, ARSA, GNPTAB and MCOLN1.

23. The fusosome of any of claims 1-19 and 22, wherein the payload gene encodes an exogenous agent comprising the sequence set forth in any one of SEQ ID NOS: 146-154, a functional fragment thereof, or a functional variant thereof comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%, identity to an amino acid sequence set forth in any one of SEQ ID NOS: 146-154.

24. The fusosome of any of claims 1-23, wherein the fusogen targets a CNS cell, optionally wherein the CNS cell is a neuron or a glial cell, optionally wherein the CNS cell is a pan-neuronal cell, a GABAergic neuron, a Glutamatergic neuron, a Cholinergic neuron, a Dopaminergic neuron, a Serotonergic neuron, a glial cell, an astrocyte, a microglial cell, an oligodendrocyte, or a choroid plexus cell.

25. The fusosome of any of claims 1-24, wherein the fusogen is a viral envelope protein.

26. The fusosome of any of claims 1-25, wherein the fusogen comprises VSV-G.

27. The fusosome of any of claims 1-26, wherein the fusogen comprises a sequence chosen from Nipah virus F and G proteins, measles virus F and H proteins, tupaia paramyxovirus F and H proteins, paramyxovirus F and G proteins or F and H proteins or F and HN proteins, Hendra virus F and G proteins, Henipavirus F and G proteins, Morbilivirus F and H proteins, respirovirus F and HN protein, a Sendai virus F and HN protein, rubulavirus F and HN proteins, or avulavirus F and HN proteins, or a derivative thereof, or any combination thereof.

28. The fusosome of any of claims 1-24 and 27, wherein the fusogen comprises a domain of at least 100 amino acids in length having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to a wild-type paramyxovirus fusogen, optionally wherein the wild-type paramyxovirus fusogen is set forth in any one of SEQ ID NOS: 1-133.

29. The fusosome of claim 27, wherein the wild-type paramyxovirus is a Nipah virus, optionally wherein the Nipah virus is a henipavirus.

30. The fusosome of any of claims 1-29, wherein the fusogen is re-targeted for delivery to a CNS cell, optionally wherein the CNS cell is a neuron or a glial cell, optionally wherein the CNS cell is a pan-neuronal cell, a GABAergic neuron, a Glutamatergic neuron, a Cholinergic neuron, a Dopaminergic neuron, a Serotonergic neuron, a glial cell, an astrocyte, a microglial cell, an oligodendrocyte, or a choroid plexus cell.

31. The fusosome of any claims 1, 2, 5, 6, 7, and 12-30, wherein the positive target cell-specific regulatory element comprises a CNS cell-specific promoter, a CNS cell-specific enhancer, a CNS cell-specific splice site, a CNS cell-specific site extending half-life of an RNA

or protein, a CNS cell-specific mRNA nuclear export promoting site, a CNS cell-specific translational enhancing site, or a CNS cell-specific post-translational modification site.

32. The fusosome of any claims 1, 2, 5, 6, 7 and 12-31 wherein the positive target cell-specific regulatory element comprises a CNS cell-specific promoter.

33. The fusosome of claim 32, wherein the positive CNS cell-specific regulatory element comprises a promoter chosen from a SYN, NSE, CaMKII, α Tubulin, PDGF, fSST, fNPY, GAD67, DLX5/6, VGLUT1, Dock10, ChAT, VACHT, Drd1a, TPH-2, GFAP, EAAT1, GS, CX3CR1, TMEM119, MBP, CNP, or CRFR2 β promoter.

34. The fusosome of any of claims 2, 4-7, and 13-33, wherein the NTCSRE comprises a non-target cell-specific miRNA recognition sequence, non-target cell-specific protease recognition site, non-target cell-specific ubiquitin ligase site, non-target cell-specific transcriptional repression site, or non-target cell-specific epigenetic repression site.

35. The fusosome of any of claims 2, 4-7, and 13-34, wherein the NTCSRE comprises a tissue-specific miRNA recognition sequence, tissue-specific protease recognition site, tissue-specific ubiquitin ligase site, tissue-specific transcriptional repression site, or tissue-specific epigenetic repression site.

36. The fusosome of any of claims 2, 4-7, and 13-35, wherein the NTCSRE comprises a non-target cell-specific miRNA recognition sequence, non-target cell-specific protease recognition site, non-target cell-specific ubiquitin ligase site, non-target cell-specific transcriptional repression site, or non-target cell-specific epigenetic repression site.

37. The fusosome of any of claims 2, 4-7, and 13-35, wherein the NTCSRE comprises a non-target cell-specific miRNA recognition sequence and the miRNA recognition sequence is able to be bound by one or more of miR-338-3p, miR-9, miR-125b-5p, miR-342-3p, or miR-124; optionally wherein the miRNA is or comprises the sequence set forth in any one of SEQ ID NOS: 156-162.

38. The fusosome of any of claims 34-37, wherein the NTCSRE is situated or encoded within a transcribed region encoding the exogenous agent, optionally wherein an RNA produced by the transcribed region comprises the miRNA recognition sequence within a UTR or coding region.

39. The fusosome of any of claims 1-38, wherein the nucleic acid comprises one or more insulator elements.

40. The fusosome of claim 39, wherein the nucleic acid comprises two insulator elements, optionally wherein the two insulator elements comprise a first insulator element upstream of the payload gene and a second insulator element downstream of the payload gene, optionally wherein the first insulator element and second insulator element comprise the same or different sequences.

41. The fusosome of any of claims 1-40, wherein the fusosome is a retroviral vector particle.

42. The fusosome of any of claims 1-41, wherein the nucleic acid is capable of integrating into the genome of a CNS cell.

43. The fusosome of any of claims 1-42, wherein the target cell is chosen from a CNS cell, optionally wherein the CNS cell is a neuron or a glial cell, optionally wherein the CNS cell is a pan-neuronal cell, a GABAergic neuron, a Glutamatergic neuron, a Cholinergic neuron, a Dopaminergic neuron, a Serotonergic neuron, a glial cell, an astrocyte, a microglial cell, an oligodendrocyte, or a choroid plexus cell.

44. A pharmaceutical composition comprising the fusosome of any of any of claims 1-43, and a pharmaceutically acceptable carrier, diluent, or excipient.

45. A method of delivering an exogenous agent to a subject comprising administering to the subject the fusosome of any of claims 1-43 or the pharmaceutical composition of claim 44, thereby delivering the exogenous agent to the subject.

46. A method of modulating a function, in a subject , CNS tissue , or a CNS cell, comprising contacting the CNS tissue or the CNS cell of the subject with the fusosome of any of claims 1-43 or the pharmaceutical composition of claim 45.

47. The method of claim 46, wherein the CNS cell is neuron or a glial cell, optionally wherein the CNS cell is a pan-neuronal cell, a GABAergic neuron, a Glutamatergic neuron, a Cholinergic neuron, a Dopaminergic neuron, a Serotonergic neuron, a glial cell, an astrocyte, a microglial cell, an oligodendrocyte, or a choroid plexus cell.

48. The method of claim 46 or claim 47, wherein the CNS tissue or the CNS cell is present in a subject.

49. A method of treating a CNS disease or disorder or a lysosomal disease or disorder, comprising administering to the subject the fusosome of any of claims 1-43 or the pharmaceutical composition of claim 44.

50. The method of claim 49, wherein the CNS disease or disorder or the lysosomal disease or disorder is caused by a genetic deficiency.

51. A method of treating a genetic deficiency in a subject comprising administering to the subject the fusosome of any of claims 1-43 or the pharmaceutical composition of claim 44.

52. The method of claim 50 or claim 51, wherein the genetic deficiency is a genetic deficiency able to be treated by the payload gene encoding the exogenous agent.

53. The method of claim 49, claim 50 or claim 52, wherein the disease or disorder is selected from Spinocerebellar Ataxia; Autosomal Recessive, Type 1; Ataxia with Oculomotor Apraxia, Type 2; Fragile X Syndrome; Cerebral Creatine Deficiency Syndrome 1; Angelman Syndrome; Amyotrophic Lateral Sclerosis; Friedreich's Ataxia; Rett Syndrome; Canavan Disease; Pyridoxine-Dependent Epilepsy; Batten Disease, Fucosidosis; Krabbe Disease; Tay

Sachs Disease; Sandhoff Disease; Beta-mannosidosis; Metachromatic Leukodystrophy; Mucopolidosis Type IIIa; Mucopolidosis Type IIIb; or Mucopolidosis Type IV.

54. The method of any of claims 49-53, wherein the subject is a human subject.

55. A fusosome of any of claims 1-43 or the pharmaceutical composition of claim 44 for use in treating a subject with a CNS disease or disorder or a lysosomal disease or disorder.

56. Use of a fusosome of any of claims 1-43 or the pharmaceutical composition of claim 44 for manufacture of a medicament for use in treating a subject with a CNS disease or disorder or a lysosomal disease or disorder.

57. The fusosome or pharmaceutical composition for use of claim 55 or the use of claim 56, wherein the CNS disease or disorder or a lysosomal disease or disorder is caused by a genetic deficiency.

58. The fusosome or pharmaceutical composition for use of claim 55 or claim 57 or the use of claim 56 or claim 57, wherein the disease or disorder is selected from Spinocerebellar Ataxia; Autosomal Recessive, Type 1; Ataxia with Oculomotor Apraxia, Type 2; Fragile X Syndrome; Cerebral Creatine Deficiency Syndrome 1; Angelman Syndrome; Amyotrophic Lateral Sclerosis; Friedreich's Ataxia; Rett Syndrome; Canavan Disease; Pyridoxine-Dependent Epilepsy; Batten Disease, Fucosidosis; Krabbe Disease; Tay Sachs Disease; Sandhoff Disease; Beta-mannosidosis; Metachromatic Leukodystrophy; Mucopolidosis Type IIIa; Mucopolidosis Type IIIb; or Mucopolidosis Type IV.

59. A fusosome of any of claims 1-43 or the pharmaceutical composition of claim 44 for use in treating a genetic deficiency.

60. Use of a fusosome of any of claims 1-43 or the pharmaceutical composition of claim 44 for manufacture of a medicament for use in treating a genetic deficiency.

61. The fusosome or pharmaceutical composition for use of any of claims 57-59, or the use of claim 57, 58 and 60, wherein the genetic deficiency is a genetic deficiency able to be treated by the payload gene encoding the exogenous agent.

62. A method of making the fusosome of any of claims 1-43, comprising:

- a) providing a cell that comprises the nucleic acid and the fusogen;
- b) culturing the cell under conditions that allow for production of the fusosome,

and

- c) separating, enriching, or purifying the fusosome from the cell, thereby making the fusosome.

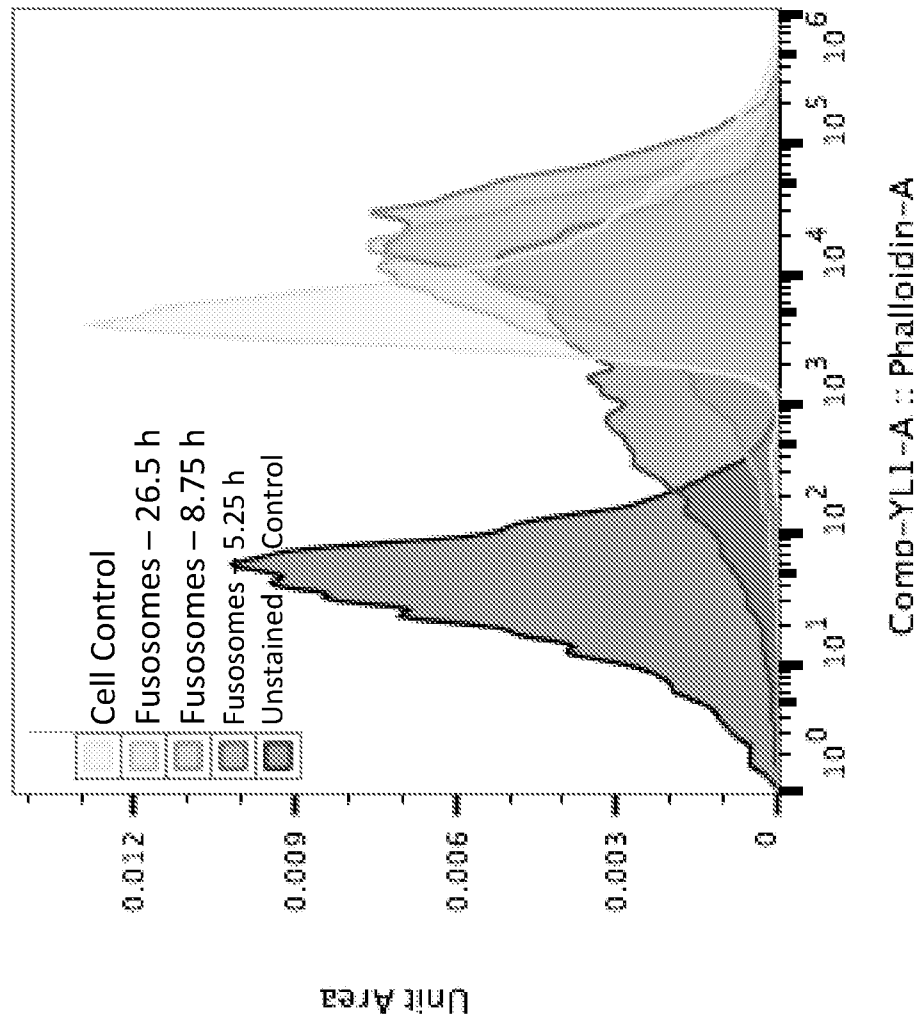


Fig. 1

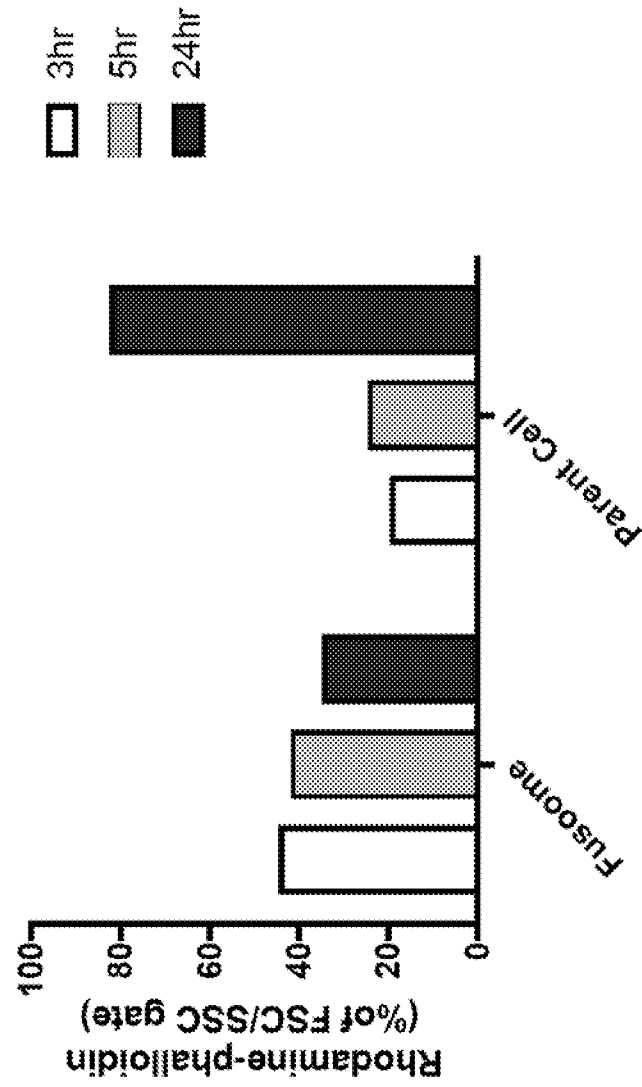


Fig. 2

	Fusosomes	Parental Cells
Average diameter (nm)	128.7	175.4
Min Diameter (nm)	14	29
Max Diameter (nm)	18720	19802
Median Diameter (nm)	134	99
10% Quantile (nm)	53	52
25% Quantile (nm)	88	66
75% Quantile (nm)	226	241
90% Quantile (nm)	4450	10649
Average volume (μm^3)	0.067	7.421

Fig. 3

	Fusosomes	Parental Cells
Average diameter (nm)	128.7	175.4
Average volume (μm^3)	0.067	7.421

Fig. 4

	[Protein] (mg/mL)		Ratio
	Insoluble	Soluble	
Cells	2.12	0.06	0.028
Fusosomes	1.28	0.72	0.563

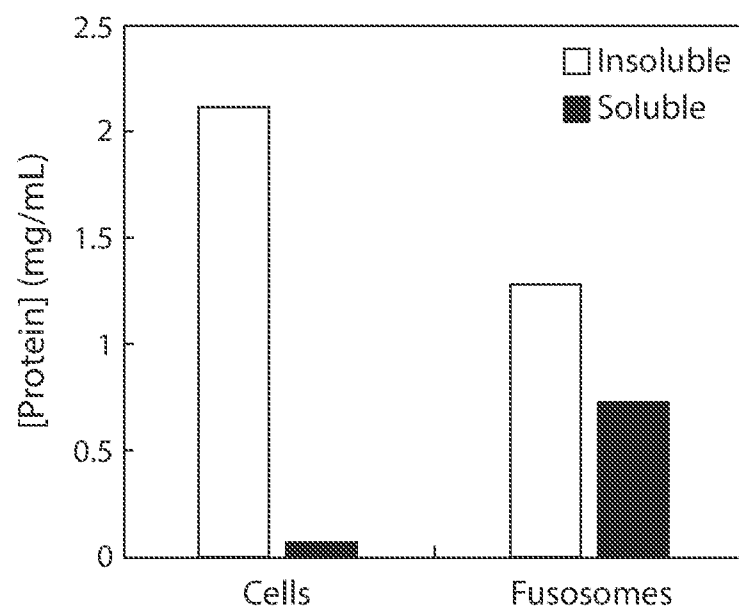
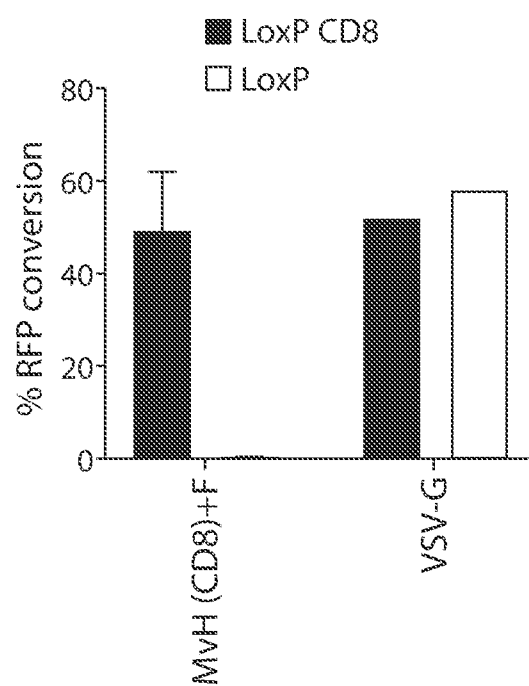


Fig. 5



Fusosome	Absolute amount of targeted fusion
MvH(CD8)+F	48.8%
VSV-G	0%

Fig. 6

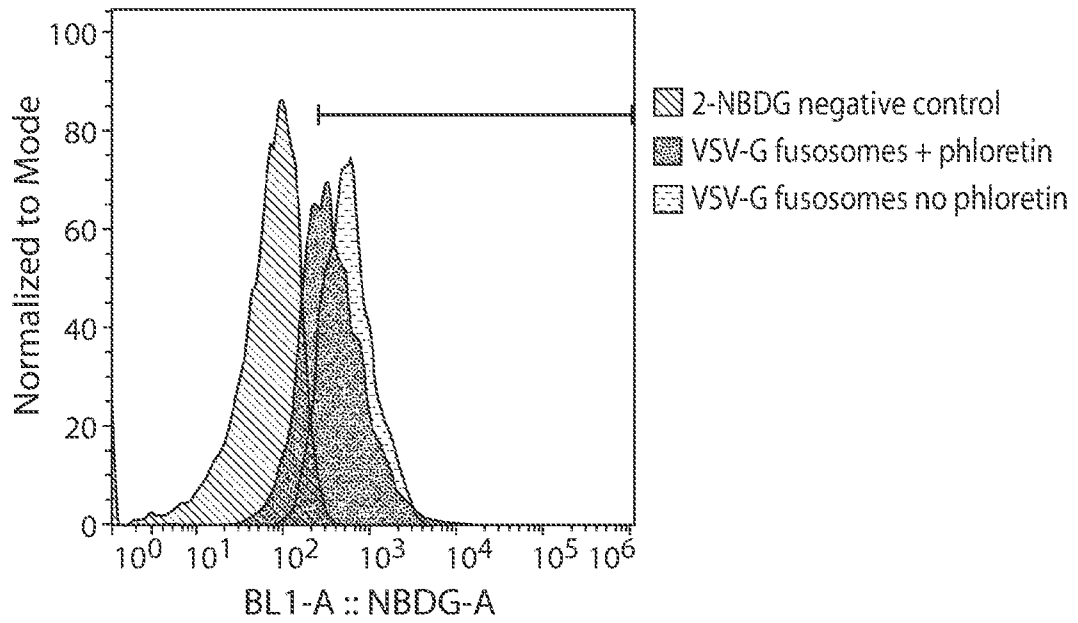


Fig. 7

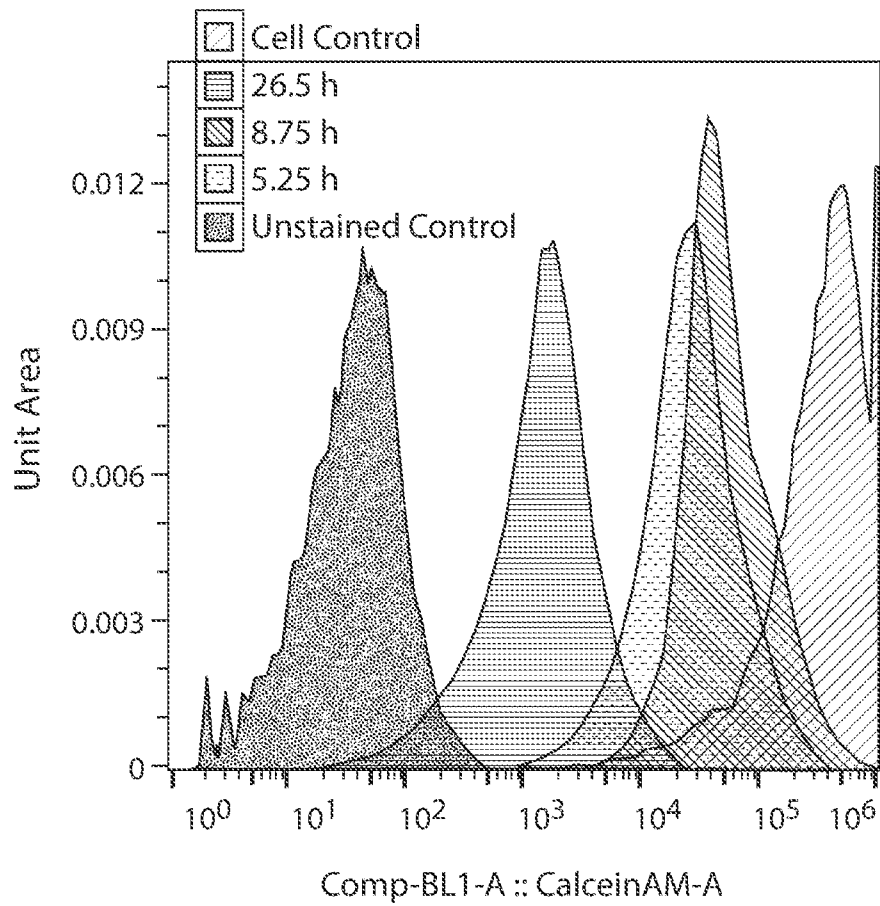


Fig. 8

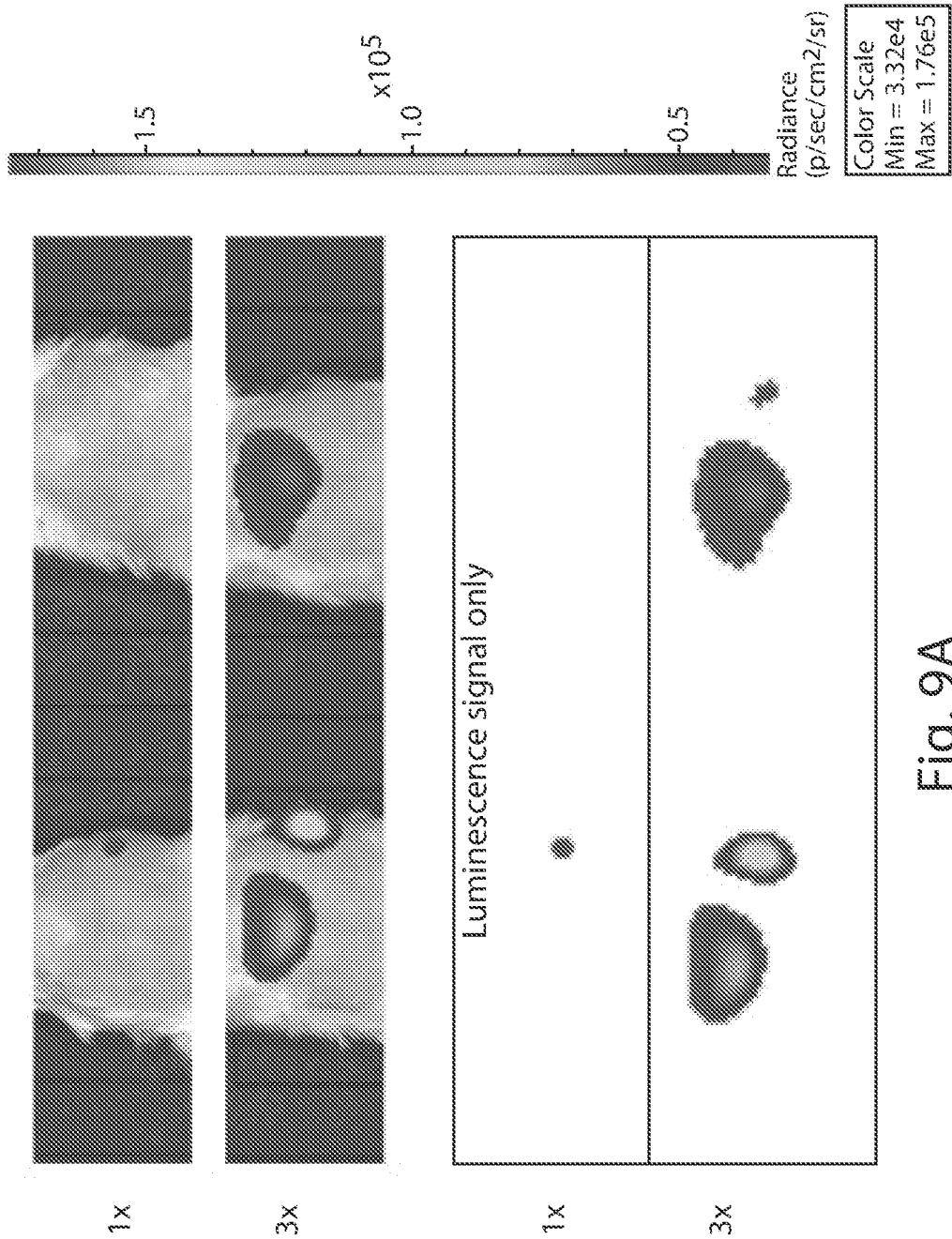


Fig. 9A

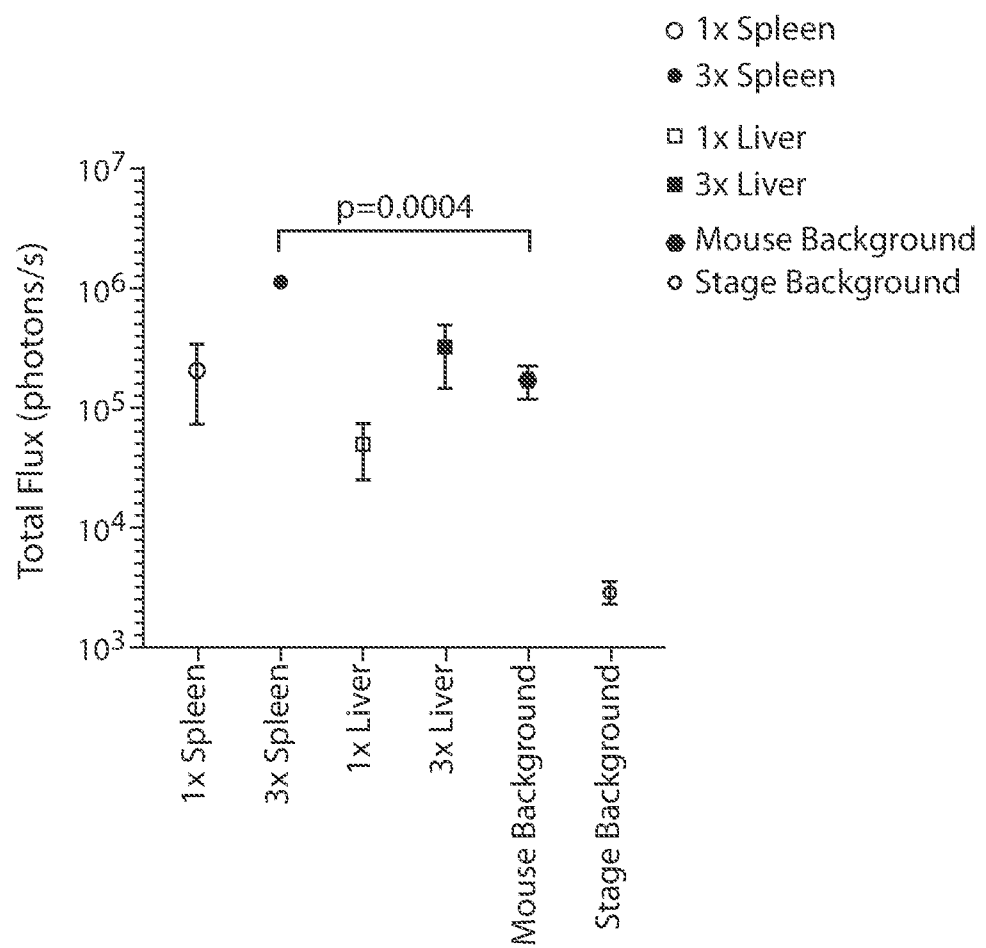


Fig. 9B

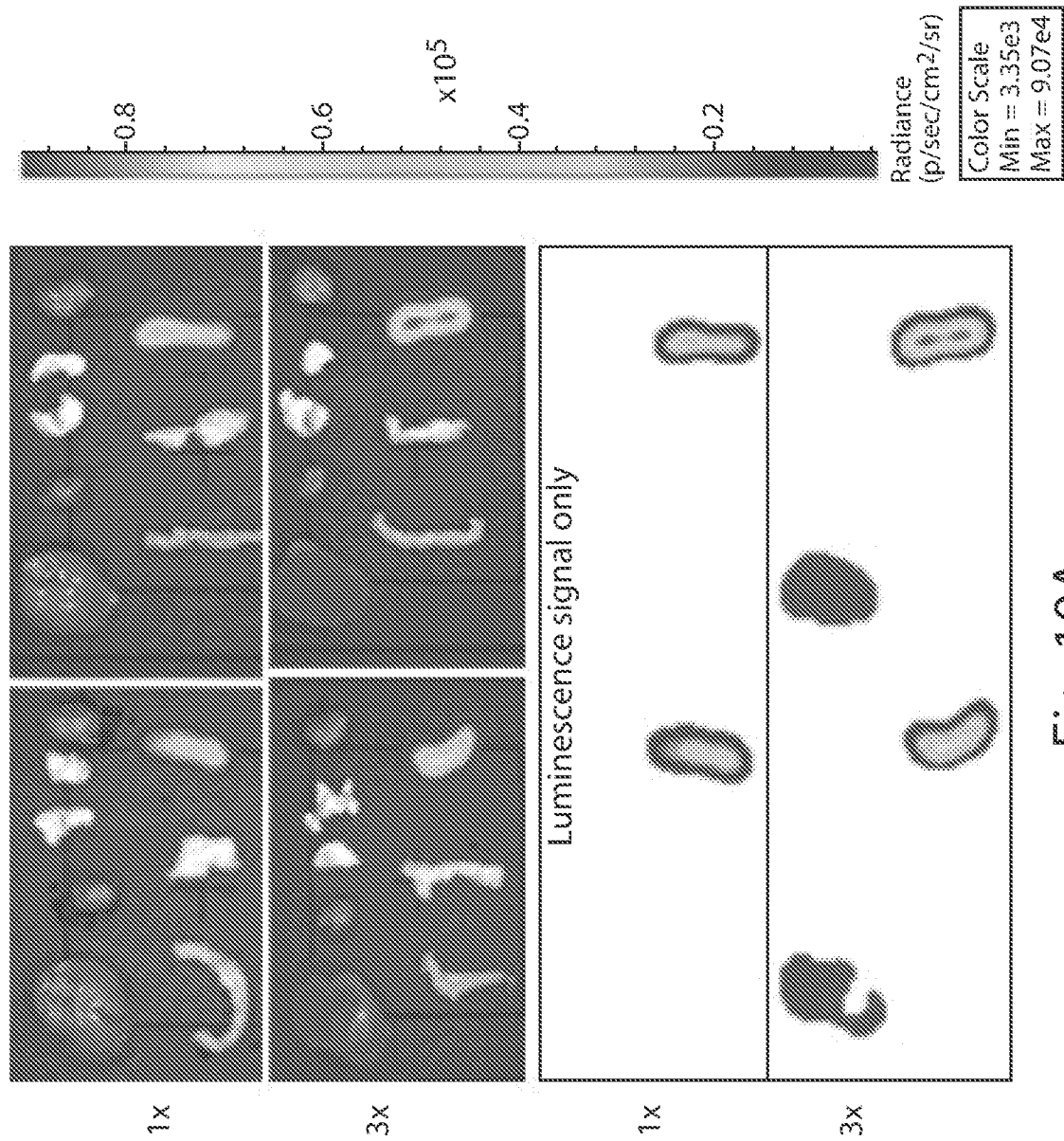


Fig. 10A

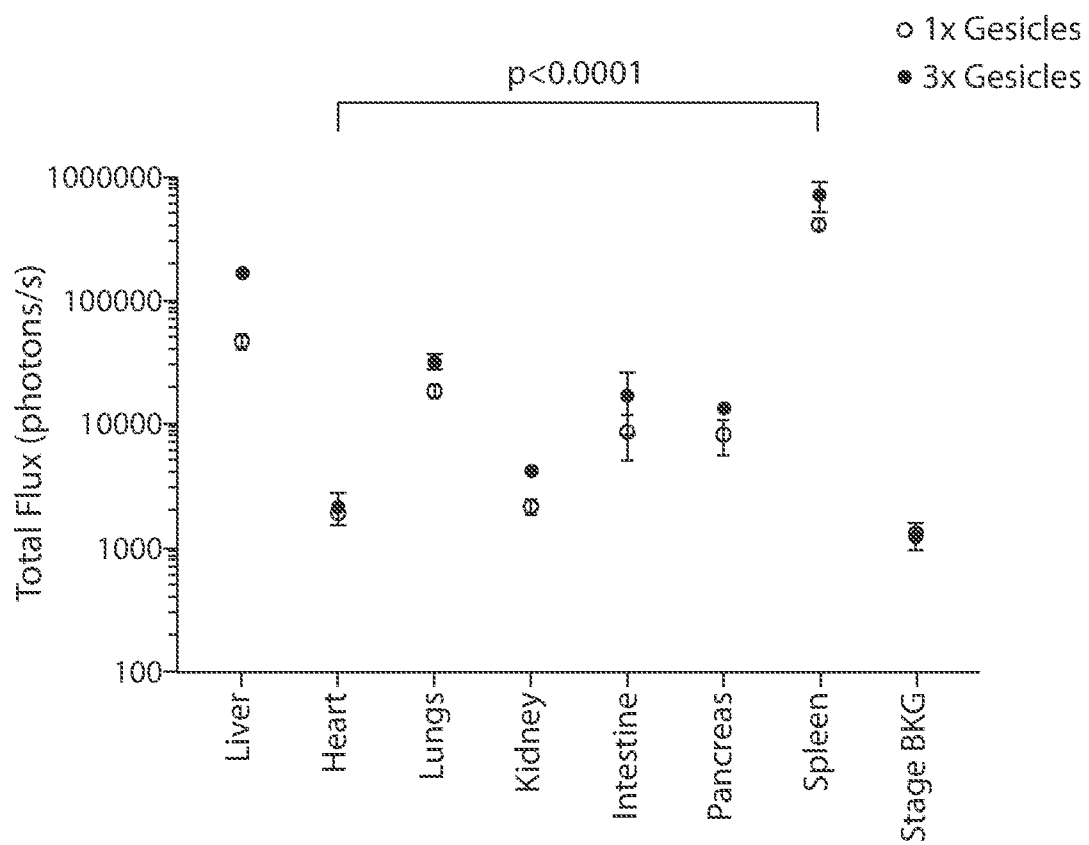


Fig. 10B

Group	% RFP conversion ($\pm$ SD)
Recipient + no fusosome	$0.4 \pm 0.2\%$
Recipient + NivG+F fusosome	$88.9 \pm 3.4\%$
Recipient + NivG+F fusosome + Baf	$68.1 \pm 2.7\%$

Fig. 11

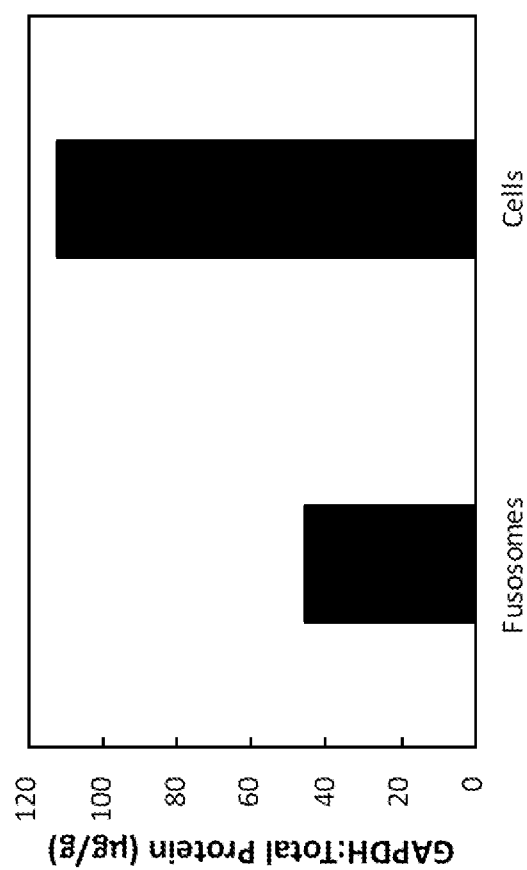


Fig. 12

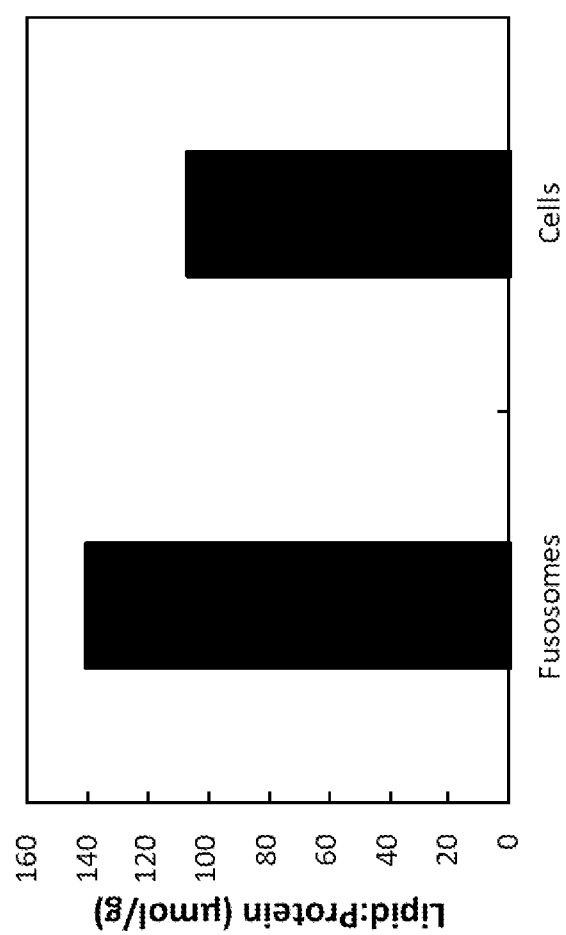


Fig. 13

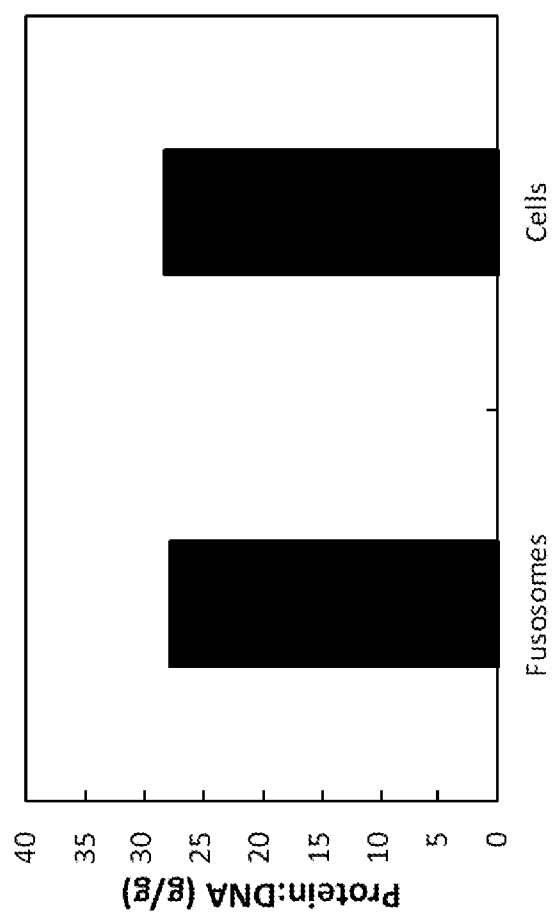


Fig. 14

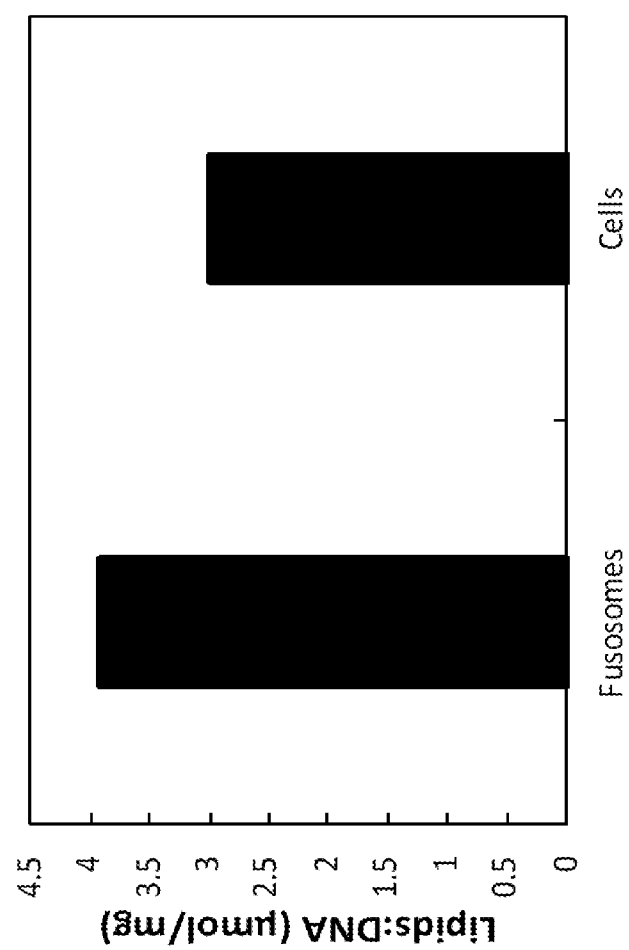


Fig. 15

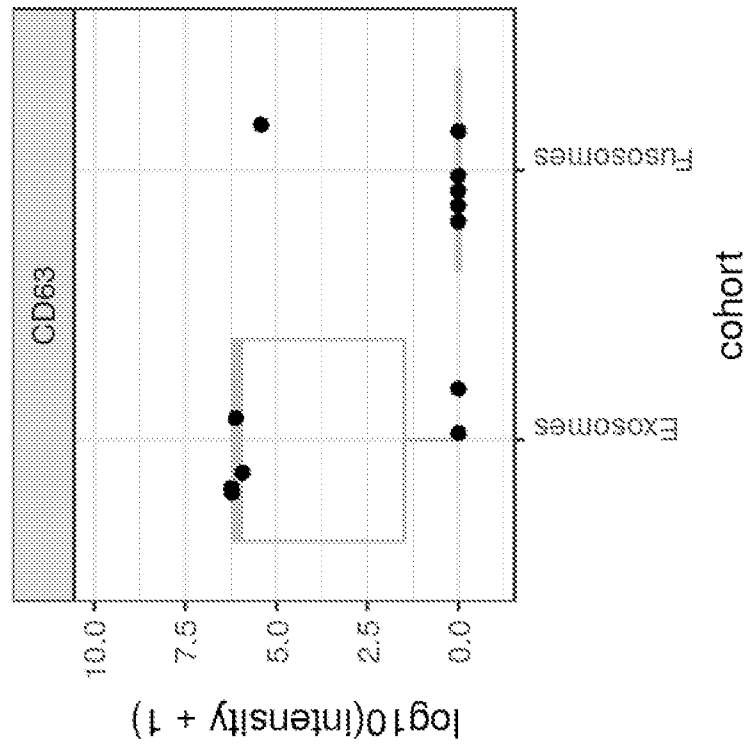


Fig. 16

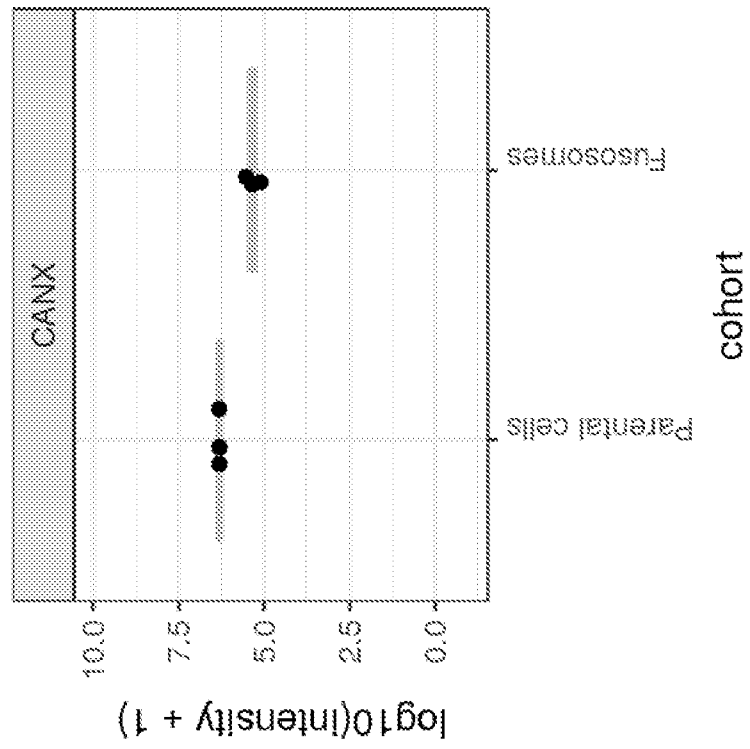


Fig. 17

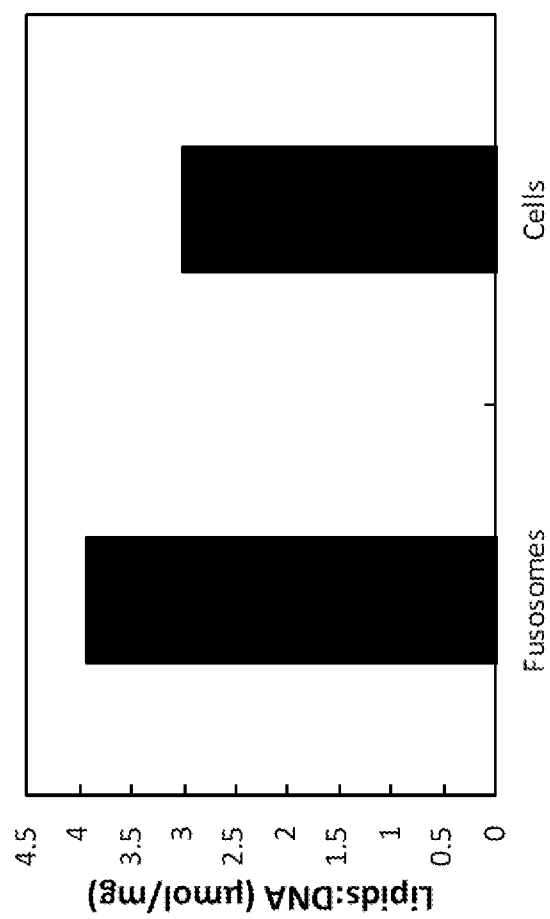


Fig. 18

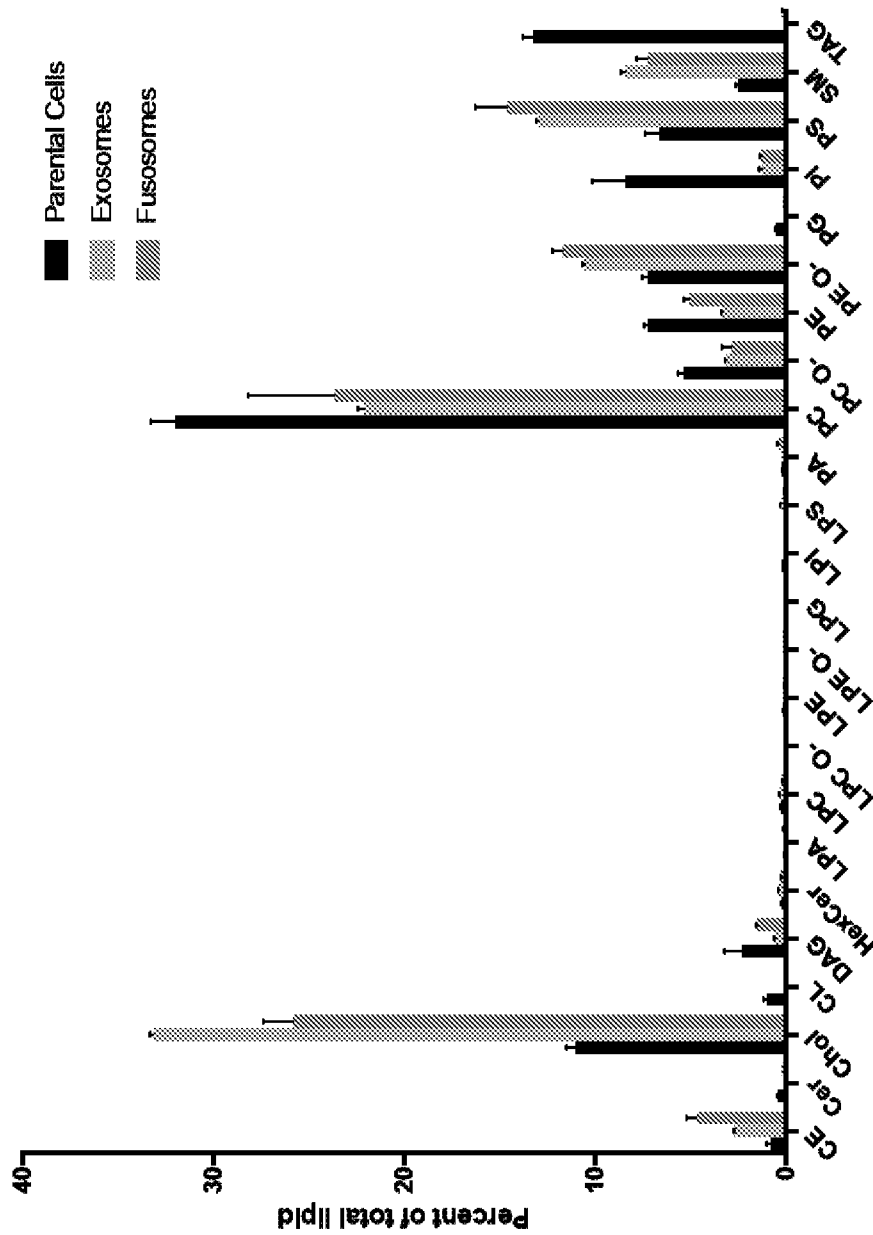


Fig. 19A

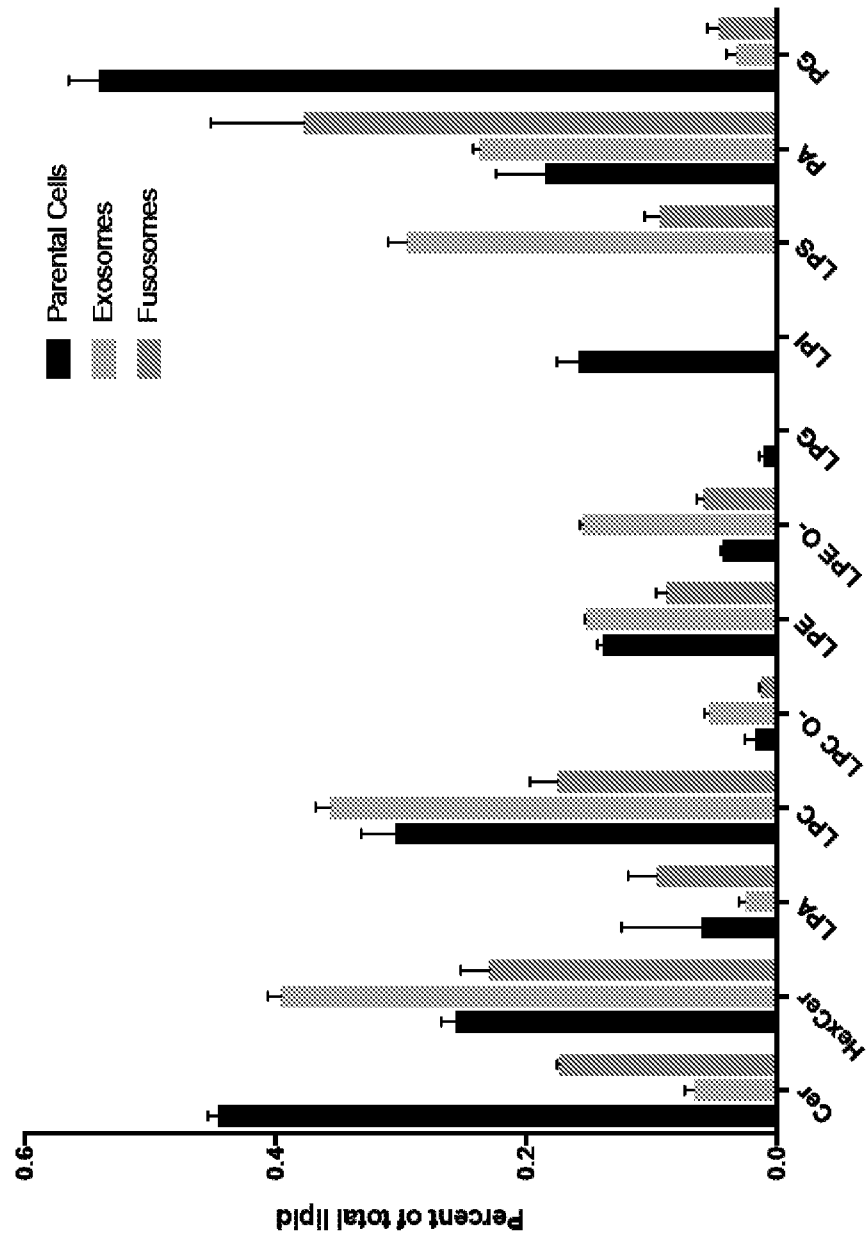


Fig. 19B

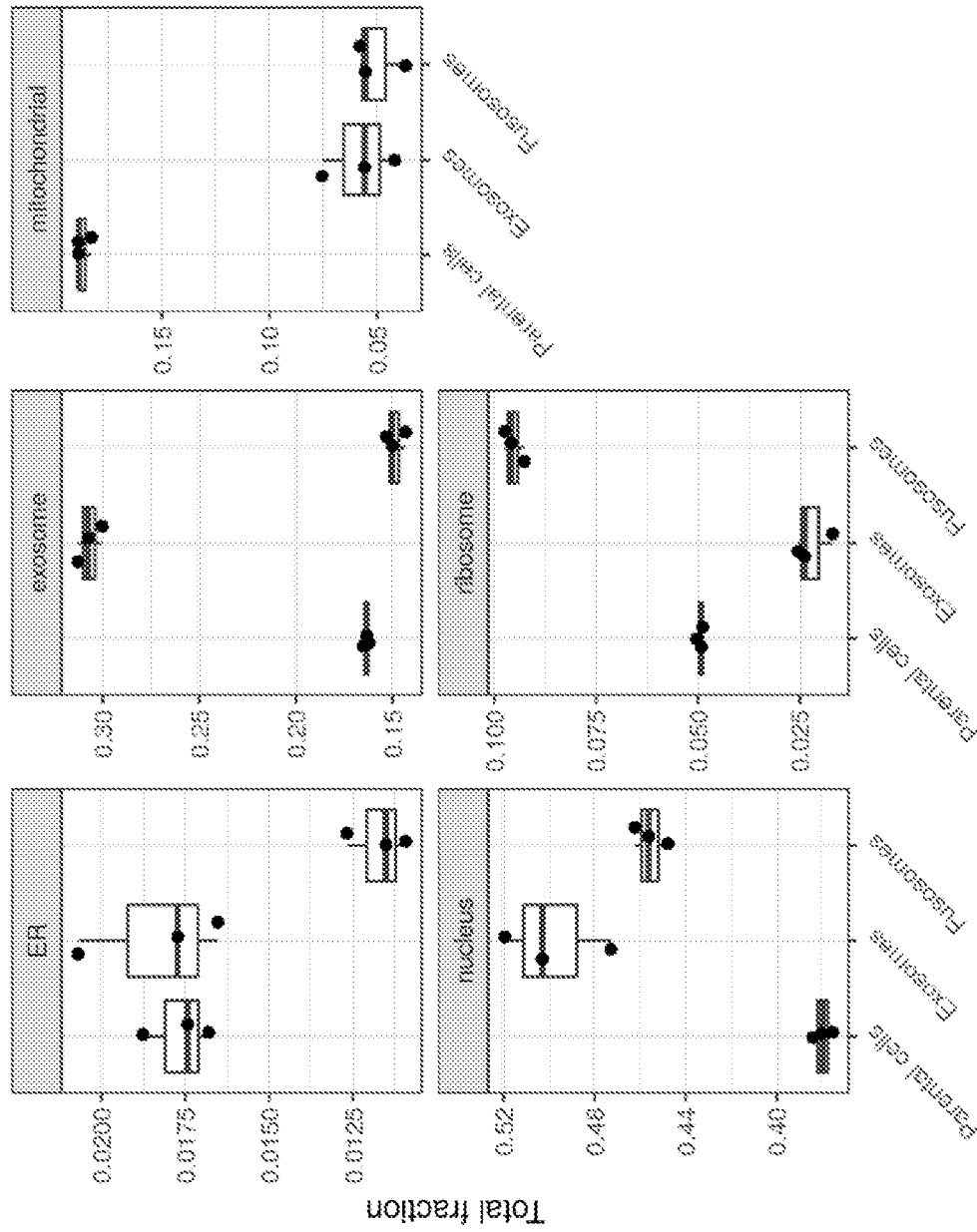


Fig. 20

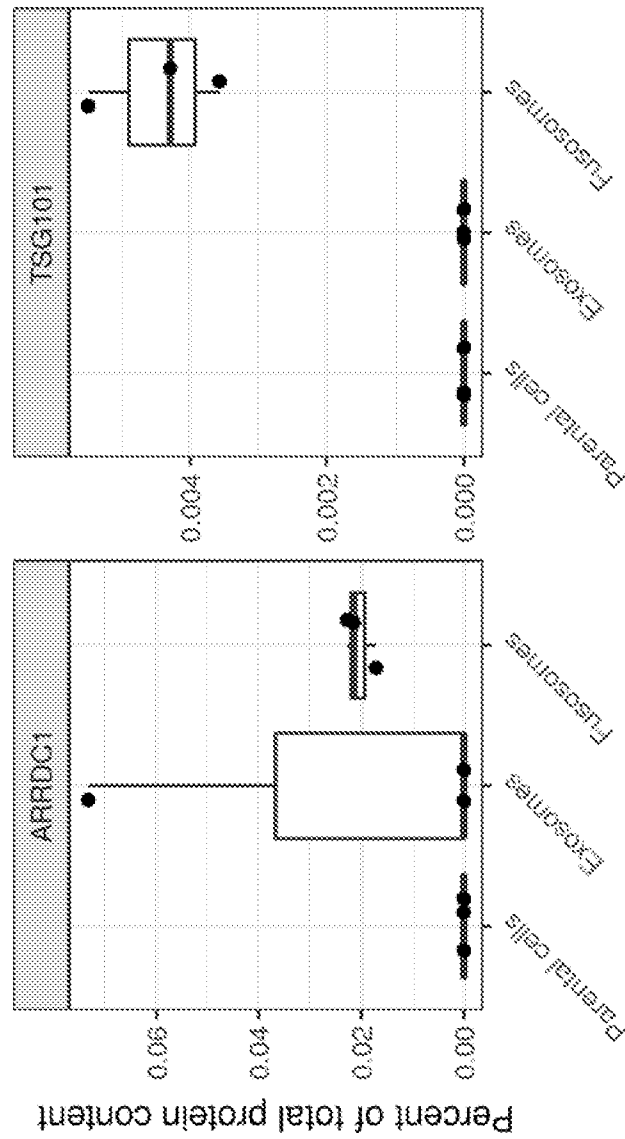


Fig. 21