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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
16 January 2020 (16.01.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/014209 A1

(51) International Patent Classification:
A61K 39/12 (2006.01) *C07K 14/005* (2006.01)
C12N 7/00 (2006.01) *C12N 15/88* (2006.01)

(21) International Application Number:
PCT/US2019/040978

(22) International Filing Date:
09 July 2019 (09.07.2019)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

62/695,537	09 July 2018 (09.07.2018)	US
62/695,650	09 July 2018 (09.07.2018)	US
62/767,261	14 November 2018 (14.11.2018)	US
62/767,241	14 November 2018 (14.11.2018)	US
62/848,305	15 May 2019 (15.05.2019)	US
62/848,284	15 May 2019 (15.05.2019)	US

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

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: FUSOSOME COMPOSITIONS AND USES THEREOF

(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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FUSOSOME COMPOSITIONS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority from U.S. provisional applications No. 62/695,537, filed July 9, 2018, entitled "FUSOSOME COMPOSITIONS AND USES THEREOF," No. 62/767,241, filed November 14, 2018, entitled "FUSOSOME COMPOSITIONS AND USES THEREOF", No. 62/848,284, filed May 15, 2019, entitled "FUSOSOME COMPOSITIONS AND USES THEREOF", No. 62/695,650, filed July 9, 2018, entitled "FUSOSOME COMPOSITIONS AND USES THEREOF," No. 62/767,261, filed November 14, 2018, entitled "FUSOSOME COMPOSITIONS AND USES THEREOF", and No. 62/848,305, filed May 15, 2019, entitled "FUSOSOME COMPOSITIONS AND USES THEREOF", the contents of which are incorporated by reference in their entireties.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled V2050-7024WO_SeqList.TXT, created on July 9, 2019, which is 2,549,164 bytes in size. The information in the electronic format of the Sequence Listing is incorporated by reference in its entirety.

BACKGROUND

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

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 fusosome.

Enumerated embodiments

Provided herein are fusosomes, including retroviral vectors or particles, such as lentiviral vectors or particles, that result in increased expression of a desired exogenous agent (e.g. therapeutic transgene) in liver target cells compared to non-target cells following introduction to cells in a subject. For example, in some cases the increase in expression is following in vivo adminstration of a provided fusosome (e.g. retroviral vectors or particle) to a subject, e.g. human subject. In particular, one of the major challenges for successful gene therapy is the ability to maintain stable, long-term expression of a therapeutic transgene (e.g. exogenous agent) from genetically modified cells *in vivo*. Transgene expression in non-target cells such as the antigen-presenting cells (APCs) can, in some aspects, result in activation of the adaptive immune response leading to generation of neutralizing antibodies against the transgene product by B-cells and/or elimination of transgene producing cells by T-cells. Thus, limiting transgene expression to target cells may substantially impact the durability of transgene expression by avoiding immune clearance. Furthermore, cell-type specific transgene expression may be very relevant to disease biology such as limiting expression of pro-apoptotic genes to target liver cells.

In particular, provided herein are fusosomes (e.g. retroviral vector or particles) that include expression of nucleic acid sequences under the control of or that are regulated by a a positive liver cell-specific regulatory element (e.g.liver-cell promoter) and/or a non-liver cell-specific regulatory element. In some embodiments, the non-liver cell-specific regulatory element is by miRNA-mediated gene silencing, such as by nucleic acid sequences complementatry to miRNA sequences in a non-liver cell. In some embodiments, the provided fusosomes (e.g. retroviral vectors or particles) can specifically drive transgene (exogenous agent) expression in a liver cell while restricting or limiting expression in non-target (non-liver) cells.

Among the provided embodiments are:

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, e.g. a payload gene encoding an exogneous agent of Table 5, optionally wherein the exogenous agent is set forth in any of SEQ ID NOS: 161-518 or is a functional fragment or functional variant thereof comprising at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOS: 161-518; and

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

2. The fusosome of embodiment 1, wherein the nucleic acid further comprises a non-liver cell-specific regulatory element (e.g., a non-liver cell-specific miRNA recognition sequence), operatively linked to the payload gene, wherein the non-liver cell-specific regulatory element decreases expression of the payload gene in a non-liver cell relative to an otherwise similar fusosome lacking the non-liver cell-specific regulatory element.

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, e.g., an exogenous agent of Table 5, optionally wherein the exogenous agent is set forth in any of SEQ ID NOS: 161-518 or is a functional fragment or functional variant thereof comprising at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOS: 161-518; and

(ii) a promoter operatively linked to the payload gene, wherein the promoter is chosen from an Apoa2, Cyp3a4, LP1B, MIR122, hemopexin, SERPINA1, or HLP promoter, e.g., according to a sequence of Table 3, or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto, optionally wherein the promoter comprises the sequence set forth in any of SEQ ID NOS: 133-136, or 519-525 or a sequence

having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to any one of SEQ ID NOS: 133-136, or 519-525.

4. A fusosome comprising:

a) a lipid bilayer comprising a fusogen; and

b) a nucleic acid that comprises:

(i) a payload gene encoding an exogneous agent, e.g. a payload gene encoding an exogenous agent of Table 5, optionally wherein the exogenous agent is set forth in any of SEQ ID NOS: 161-518 or is a functional fragment or functional variant thereof comprising at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOS: 161-518; 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 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, optionally wherein the exogenous agent is set forth in any of SEQ ID NOS: 161-518 or is a functional fragment or functional variant thereof comprising at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOS: 161-518; and

(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.

6. The fusosome of either embodiment 4 or 5, wherein the nucleic acid further comprises a positive liver cell-specific regulatory element (e.g., a liver-cell specific promoter) operatively linked to the payload gene, wherein the positive liver cell-specific regulatory element increases expression of the payload gene in a liver cell relative to an otherwise similar fusosome lacking the positive liver cell-specific regulatory element.

7. A fusosome comprising:

a) a lipid bilayer comprising a fusogen;

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, optionally wherein the exogenous agent is set forth in any of SEQ ID NOS: 161-518 or is a functional fragment or functional variant thereof comprising at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOS: 161-518; 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.

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

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.

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: OTC, CPS1, NAGS, BCKDHA, BCKDHB, DBT, DLD, MUT, MMAA, MMAB, MMACHC, MMADHC, MCEE, PCCA, PCCB, UGT1A1, ASS1, PAH, ATP8B1, ABCB11, ABCB4, TJP2, IVD, GCDH, ETFA, ETFB, ETFDH, ASL, D2HGDH, HMGCL, MCCC1, MCCC2, ABCD4, HCFC1, LMBRD1, ARG1, SLC25A15, SLC25A13, ALAD, CPOX, HMBS, PPOX, BTD, HLCS, PC, SLC7A7, CPT2, ACADM, ACADS, ACADVL, AGL, G6PC, GBE1, PHKA1, PHKA2, PHKB, PHKG2, SLC37A4, PMM2, CBS, FAH, TAT, GALT, GALK1, GALE, G6PD, SLC3A1, SLC7A9, MTHFR, MTR, MTRR, ATP7B, HPRT1, HJV, HAMP, JAG1, TTR, AGXT, LIPA, SERPING1, HSD17B4, UROD, HFE, LPL, GRHPR, HOGA1, or LDLR.

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

24. The fusosome of any embodiments 1, 2, 6, 15, 22, or 23, wherein the positive liver-specific regulatory element comprises a liver-specific promoter, a liver-specific enhancer, a liver-specific splice site, a liver-specific site extending half-life of an RNA or protein, a liver-

specific mRNA nuclear export promoting site, a liver-specific translational enhancing site, or a liver-specific post-translational modification site.

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

26. The fusosome of embodiment 25, wherein the hepatocyte-specific promoter comprises a motif of Table 3, optionally wherein the promoter is set forth in any of SEQ ID NOS: 133-136, or 519-525 or a sequence that has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOS: 133-136, or 519-525

27. The fusosome of embodiment 25 or 26, wherein the positive liver-specific regulatory element comprises a promoter selected from an enhanced transthyretin (ET), hAAT, Alb, Apoa2, Cyp3a4, LP1B, MIR122, hemopexin, SERPINA1, or HLP promoter.

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.

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

31. The fusosome of any of embodiments 4-6, 16-21, or 28-30, wherein the negative TCSRE or NTCSRE comprises a non-liver 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-142, mir-181a-2, mir-181b-1, mir-181c, mir-181a-1, mir-181b-2, mir-181d, miR-223, or miR-126.

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.

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

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.

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 hepatocyte, liver sinusoidal endothelial cell, cholangiocyte, stellate cell, liver-

resident antigen-presenting cell (e.g., Kupffer Cell), liver-resident immune lymphocyte (e.g., T cell, B cell, or NK cell), or portal fibroblast.

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, e.g., T cells);

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 liver-specific promoter that is at least 10%, 25%, 50%, 75%, 100%, 150%, 200%, 250%, 300%, 400%, 500%, 750%, 1000% or more active in a liver cell (e.g., hepatocyte) than a non-liver 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 hematopoietic cells compared to hepatocytes.

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., 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, a 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 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, a 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., wherien 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., 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, a 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 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, a 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 T cell, a CD3+ T cell, a CD4+ T cell, a CD8+ T cell, a hepatocyte, a haematepoietic stem cell, a CD34+ haematepoietic stem cell, a CD105+ haematepoietic stem cell, a CD117+ haematepoietic stem cell, a CD105+ endothelial cell, a B cell, a CD20+ B cell, a CD19+ B cell, a cancer cell, a CD133+ cancer cell, an EpCAM+ cancer cell, a CD19+ cancel cell, a Her2/Neu+ cancer cell, a GluA2+ neuron, a GluA4+ neuron, a NKG2D+ natural killer cell, a SLC1A3+ astrocyte, a SLC7A10+ adipocyte, or a CD30+ lung epithelial 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 T cell, a CD3+ T cell, a CD4+ T cell, a CD8+ T cell, a hepatocyte, a haematepoietic stem cell, a CD34+ haematepoietic stem cell, a CD105+ haematepoietic stem cell, a CD117+ haematepoietic stem cell, a CD105+ endothelial cell, a B cell, a CD20+ B cell, a CD19+ B cell, a cancer cell, a CD133+ cancer cell, an EpCAM+ cancer cell, a CD19+ cancel cell, a Her2/Neu+ cancer cell, a GluA2+ neuron, a GluA4+ neuron, a NKG2D+ natural killer cell, a SLC1A3+ astrocyte, a SLC7A10+ adipocyte, or a CD30+ lung epithelial 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 T cell, a CD3+ T cell, a CD4+ T cell, a CD8+ T cell, a hepatocyte, a haematepoietic stem cell, a CD34+ haematepoietic stem cell, a CD105+ haematepoietic stem cell, a CD117+ haematepoietic stem cell, a CD105+ endothelial cell, a B cell, a CD20+ B cell, a CD19+ B cell, a cancer cell, a CD133+ cancer cell, an EpCAM+ cancer cell, a CD19+ cancel cell, a Her2/Neu+ cancer cell, a GluA2+ neuron, a GluA4+ neuron, a NKG2D+ natural killer cell, a SLC1A3+ astrocyte, a SLC7A10+ adipocyte, or a CD30+ lung epithelial cell) comprise the exogenous agent.

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., 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 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 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 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 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.

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.

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.

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.

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.

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.

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 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%, 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 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 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 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.

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.

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.

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.

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.

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.

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.

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 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 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.,

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 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 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 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., 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) 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., 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.

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 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., serum from a mouse depleted of IgM and IgG, e.g., indicating that in 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, 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 (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 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.

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.

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.

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.

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.

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.

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 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.

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 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 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.

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.

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 a pharmaceutical composition of claim 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 (e.g., liver) or target cell (e.g., liver cell, e.g., hepatocyte), comprising contacting, e.g., 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.

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 embodiment 128.

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

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.

135. 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.

136. 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.

137. The fusosome or pharmaceutical composition for use of embodiment 135 or the use of embodiments 136, wherein the fusosome comprises a payload gene encoding an exogenous agent for treating the genetic deficiency.

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

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.

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 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 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 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 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 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 as detected by biolumniscent 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 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 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-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.

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.

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.

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.

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.

FIGS. 22A-22C show results for cell lines, including target human hepatoma cell lines (HepG2) and non-target (non-hepatic) cell lines, transduced with lentivirus (LV) encoding nucleic acid constructs containing positive TCSREs or NTCSREs. FIG. 22A shows GFP expression in human hepatoma cell line (HepG2), human embryonic kidney cell line (293LX), human T-cell line of hematopoietic origin (Molt4.8) and endothelial cell line derived from mouse brain (bEND.3) transduced with LV generated with miRT sequences (hPGK-eGFP+miRT) or without miRT sequences (hPGK-eGFP), under the control of the PGK promoter. FIG. 22B shows GFP expression in HepG2 and 293LX cells transduced with LV generated under the control of the PGK promoter (hPGK-eGFP) or LVs containing mirT sequences and GFP under the control of the hepatocyte specific promoter ApoE (hApoE-eGFP+miRT). FIG. 22C shows quantification of Phenylalanine (Phe) in supernatant of HepG2 and 293LX cells transduced with LVs containing the transgene phenylalanine ammonia lyase (PAL) under the control of the SFFV promoter (SFFV-PAL), or LVs containing mirT sequences and under the control of the hApoE promoter (hApoE-PAL+miRT).

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

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.

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 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 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 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 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 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 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 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 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, 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 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

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 modification of the protein. In some embodiments, the positive TCSRE decreases the level or activity of a downregulator or inhibitor of the exogenous agent. In some embodiments, the target cell is a liver cell and the positive target-cell-specific regulatory element is a positive liver cell-specific regulatory element.

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. In some embodiment, the non-target cell is a non-liver cell.

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. In some embodiments, the non-target cell is

a non-liver cell and the non-target cell-specific regulatory element is a non-liver cell-specific regulatory element. The terms "negative TCSRE" and "NTCSRE" are used interchangeably herein.

As used herein, a "non-liver cell specific regulatory element" refers to a non-target cell-specific regulatory element (NTCSRE), wherein the target cell is a liver cell. Thus, a non-liver cell specific regulatory element refers to a nucleic acid sequence that decreases the level of an exogenous agent in a non-liver cell (e.g., in an immune cell) or tissue compared to in a liver cell, wherein the nucleic acid encoding the exogenous agent is operably linked to the non-liver 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., an immune effector cell, e.g., a T 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.

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., neutrophiles 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. In accordance with one embodiment of the invention, a fusosome comprising a lipid bilayer comprises a retroviral vector comprising an envelope. 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, such as a desired transgene or encoding an exogenous agent, 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. 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.

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 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 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 Gammretrovirus. 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 protease4 (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 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. 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 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.

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.

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 increases expression of the exogenous agent. Transcription 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 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, ATTAAA, AGTAAA, a bovine growth hormone polyA sequence (BGHpA), a rabbit β-globin polyA sequence (rβgpA), or another suitable heterologous or endogenous polyA sequence.

In some embodiments, a retroviral or lentiviral vector further comprises one or more 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 of more accessory elements to 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 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 sequence (e.g., for polyadenylation and termination), and a U5 signal (e.g., for integration).

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.

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 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 are described in WO2012156839A2, all of which is included by reference.

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 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

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 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.

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 accessory genes selected from S2, rev and tat are disabled or absent from the retroviral nucleic acid.

Strategies for packaging a retroviral nucleic acid

Typically, modern retroviral vector systems consist of viral genomes bearing cis-acting 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 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. 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 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 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.

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.

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 phosphribosyltransferase (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 1 1:3374-3378, 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.

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: $DX_{39-58}DX_{35}E$ (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 DNAse1 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 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 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.

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.

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). 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 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 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 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 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 (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 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 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.

Fusogens and pseudotyping

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

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.

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 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, 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, Poxyiridae, and Iridoviridae) may be utilized. Representative examples include, FeLV, VEE, HFVW, WDSV, SFV, Rabies, ALV,

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, 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, 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), Bunyaviridiae such as Crimean-Congo 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 encephaliltis 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.

Exemplary Fusogens

In some embodiments, the fusosome includes one or more fusogens, e.g., to facilitate the fusion of the fusosome to a membrane, e.g., a cell membrane.

In some embodiments, the fusosome comprises one or more fusogens on its envelope to target a specific cell or tissue type. Fusogens include without limitation protein based, lipid based, and chemical based fusogens. In some embodiments, the fusosome includes a first fusogen which is a protein fusogen and a second fusogen which is a lipid fusogen or chemical fusogen. The fusogen may bind a fusogen binding partner on a target cell's surface. 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.

Protein Fusogens

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, the fusogen results in mixing between lipids in the fusosome and lipids in the target cell. In some embodiments, the fusogen results in formation of one or more pores between the interior of the fusosome and the cytosol of the target cell.

Mammalian Proteins

In some embodiments, the fusogen may include a mammalian protein, see Table 1A. Examples of mammalian fusogens may include, but are not limited to, a SNARE family protein such as vSNAREs and tSNAREs, a syncytin protein such as Syncytin-1 (DOI: 10.1128/JVI.76.13.6442–6452.2002), and Syncytin-2, myomaker (biorxiv.org/content/early/2017/04/02/123158, doi.org/10.1101/123158, doi: 10.1096/fj.201600945R, doi:10.1038/nature12343), myomixer (www.nature.com/nature/journal/v499/n7458/full/nature12343.html, doi:10.1038/nature12343), myomerger (science.sciencemag.org/content/early/2017/04/05/science.aam9361, DOI: 10.1126/science.aam9361), FGFRL1 (fibroblast growth factor receptor-like 1), Minion (doi.org/10.1101/122697), an isoform of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (e.g., as disclosed in US 6,099,857A), a gap junction protein such as connexin 43, connexin 40, connexin 45, connexin 32 or connexin 37 (e.g., as disclosed in US 2007/0224176, Hap2, any

protein capable of inducing syncytium formation between heterologous cells (see Table 2), any protein with fusogen properties (see Table 3), a homologue thereof, a fragment thereof, a variant thereof, and a protein fusion comprising one or more proteins or fragments thereof. In some embodiments, the fusogen is encoded by a human endogenous retroviral element (hERV) found in the human genome. Additional exemplary fusogens are disclosed in US 6,099,857A and US 2007/0224176, the entire contents of which are hereby incorporated by reference.

Table 1A: Non-limiting examples of human and non-human fusogens.

Human and Non-Human Fusogen Classes		
Fusogen Class	**Uniprot Protein Family ID**	**# of sequences**
EFF-AFF	PF14884	191
SNARE	PF05739	5977
DC-STAMP	PF07782	633
ENV	PF00429	312

Table 1B: Genes that encode proteins with fusogen properties.

Human genes with the gene ontology annotation of:	
Syncytium formation by plasma membrane fusion proteins	
ID	**Symbol**
A0A024R0I0	DYRK1B
A0A024R1N1	MYH9
A0A024R2D8	CAV3
A0A096LNV2	FER1L5
A0A096LPA8	FER1L5
A0A096LPB1	FER1L5
A0AVI2	FER1L5
A6NI61	TMEM8C (myomaker)
B3KSL7	-

B7ZLI3	FER1L5
H0YD14	MYOF
O43184	ADAM12
O60242	ADGRB3
O60500	NPHS1
O95180	CACNA1H
O95259	KCNH1
P04628	WNT1
P15172	MYOD1
P17655	CAPN2
P29475	NOS1
P35579	MYH9
P56539	CAV3
Q2NNQ7	FER1L5
Q4KMG0	CDON
Q53GL0	PLEKHO1
Q5TCZ1	SH3PXD2A
Q6YHK3	CD109
Q86V25	VASH2
Q99697	PITX2
Q9C0D5	TANC1
Q9H295	DCSTAMP
Q9NZM1	MYOF
Q9Y463	DYRK1B

Table 1C: Human Fusogen Candidates

Fusogen Class	Gene ID
SNARE	O15400
	Q16623

	K7EQB1
	Q86Y82
	E9PN33
	Q96NA8
	H3BT82
	Q9UNK0
	P32856
	Q13190
	O14662
	P61266
	O43752
	O60499
	Q13277
	B7ZBM8
	A0AVG3
	Q12846
DC-STAMP	Q9H295
	Q5T1A1
	Q5T197
	E9PJX3
	Q9BR26
ENV	Q9UQF0
	Q9N2K0
	P60507
	P60608
	B6SEH9
	P60508
	B6SEH8
	P61550

	P60509
	Q9N2J8
Muscle Fusion (Myomaker)	H0Y5B2
	H7C1S0
	Q9HCN3
	A6NDV4
	K4DI83
Muscle Fusion (Myomixer)	NP_001302423.1
	ACT64390.1
	XP_018884517.1
	XP_017826615.1
	XP_020012665.1
	XP_017402927.1
	XP_019498363.1
	ELW65617.1
	ERE90100.1
	XP_017813001.1
	XP_017733785.1
	XP_017531750.1
	XP_020142594.1
	XP_019649987.1
	XP_019805280.1
	NP_001170939.1
	NP_001170941.1
	XP_019590171.1
	XP_019062106.1
	EPQ04443.1

	EPY76709.1
	XP_017652630.1
	XP_017459263.1
	OBS58441.1
	XP_017459262.1
	XP_017894180.1
	XP_020746447.1
	ELK00259.1
	XP_019312826.1
	XP_017200354.1
	BAH40091.1
HA	P03452
	Q9Q0U6
	P03460
GAP JUNCTION	P36382
	P17302
	P36383
	P08034
	P35212
Other	FGFRL1
	GAPDH

In some embodiments, the fusosome comprises a curvature-generating protein, e.g., Epsin1, dynamin, or a protein comprising a BAR domain. See, e.g., Kozlovet al, CurrOp StrucBio 2015, Zimmerberget al. Nat Rev 2006, Richard et al, Biochem J 2011.

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Non-mammalian Proteins

Viral Proteins

In some embodiments, the fusogen may include a non-mammalian protein, e.g., a viral protein. In some embodiments, a viral fusogen is a Class I viral membrane fusion protein, a

Class 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 some embodiments, Class I viral membrane fusion proteins include, but are not limited to, Baculovirus F protein, e.g., F proteins of the nucleopolyhedrovirus (NPV) genera, e.g., Spodoptera exigua MNPV (SeMNPV) F protein and Lymantria dispar MNPV (LdMNPV), and paramyxovirus F proteins.

In some embodiments, Class II viral membrane proteins include, but are not limited to, tick bone encephalitis E (TBEV E), Semliki Forest Virus E1/E2.

In some embodiments, Class III viral membrane fusion proteins include, but are not limited to, rhabdovirus G (e.g., fusogenic protein G of the Vesicular Stomatatis Virus (VSV-G)), herpesvirus glycoprotein B (e.g., Herpes Simplex virus 1 (HSV-1) gB)), Epstein Barr Virus glycoprotein B (EBV gB), thogotovirus G, baculovirus gp64 (e.g., Autographa California multiple NPV (AcMNPV) gp64), and Borna disease virus (BDV) glycoprotein (BDV G).

Examples of other viral fusogens, e.g., membrane glycoproteins and viral fusion proteins, include, but are not limited to: viral syncytia proteins such as influenza hemagglutinin (HA) or mutants, or fusion proteins thereof; human immunodeficiency virus type 1 envelope protein (HIV-1 ENV), gp120 from HIV binding LFA-1 to form lymphocyte syncytium, HIV gp41, HIV gp160, or HIV Trans-Activator of Transcription (TAT); viral glycoprotein VSV-G, viral glycoprotein from vesicular stomatitis virus of the Rhabdoviridae family; glycoproteins gB and gH-gL of the varicella-zoster virus (VZV); murine leukaemia virus (MLV)-10A1; Gibbon Ape Leukemia Virus glycoprotein (GaLV); type G glycoproteins in Rabies, Mokola, vesicular stomatitis virus and Togaviruses; murine hepatitis virus JHM surface projection protein; porcine respiratory coronavirus spike- and membrane glycoproteins; avian infectious bronchitis spike glycoprotein and its precursor; bovine enteric coronavirus spike protein; the F and H, HN or G genes of Measles virus; canine distemper virus, Newcastle disease virus, human parainfluenza virus 3, simian virus 41, Sendai virus and human respiratory syncytial virus; gH of human herpesvirus 1 and simian varicella virus, with the chaperone protein gL; human, bovine and cercopithicine herpesvirus gB; envelope glycoproteins of Friend murine leukaemia virus and Mason Pfizer monkey virus; mumps virus hemagglutinin neuraminidase, and glyoproteins F1

and F2; membrane glycoproteins from Venezuelan equine encephalomyelitis; paramyxovirus F protein; SIV gp160 protein; Ebola virus G protein; or Sendai virus fusion protein, or a homologue thereof, a fragment thereof, a variant thereof, and a protein fusion comprising one or more proteins or fragments thereof.

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).

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 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, 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 resides in the natural fusogen protein sequence or viral envelope protein 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 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 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 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 1D, 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 1D. In some embodiments, a nucleic acid sequence described herein encodes an amino acid sequence of Table 1D, 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: 1-56, 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: 1-56. In some embodiments, a nucleic acid sequence described herein encodes an amino acid sequence set forth in any one of SEQ ID NOS: 1-56, 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 1D. Paramyxovirus F sequence clusters. Column 1, Genbank ID includes the Genbank ID of the whole genome sequence of the virus that is the centroid sequence of the cluster. Column 2, Nucleotides of CDS provides the nucleotides corresponding to the CDS of the gene in the whole genome. Column 3, Full Gene Name, provides the full name of the gene including Genbank ID, virus species, strain, and protein name. Column 4, Sequence, provides the amino acid sequence of the gene. Column 5, #Sequences/Cluster, provides the number of sequences that cluster with this centroid sequence.

Genbank ID	Nucleotides of CDS	Full Gene Name	Sequence	#Sequences/Cluster	SEQ ID NO
KP317927	5630-7399	gb:KP317927:5630-7399|Organism:Human respiratory syncytial virus|Strain Name:Kilifi_9465_7_RSVB_2011|Protein Name:fusion glycoprotein|Gene Symbol:F	MIPQARTELNLGQITMELLIHRSSAIFLTLAINALYLTSSQNITEEFYQSTCSAVSRGYLSALRTGWYTSVITIELSNIKETKCNGTDTKVKLIKQELDKYKNAVTELQLLMQNTPAANNRARREAPQYMNYTINTTGSLNVSISKKRKRRFLGFLLGVGSAIASGIAVSKVLHLEGEVNKIKNALLSTNKAVVSLSNGVSVLTSKVLDLKNYINNQLLPIVNQQSCRISNIETVIEFQQKNSRLLEITREFSVNAGVTTPLSTYMLTNSELLSLINDMPITNDQKKLMSSNVQIVRQQSYSIMSIIKEEVLAYVVQLPIYGVIDTPCWKLHTSPLCTTNIKEGSNICLTRTDRGWYCDNAGSVSFFPQADTCKVQSNRVFCDTMNSLTLPSEVSLCNTDIFNSKYDCKIMTSKTDISSSVITSLGAIVSCYGKTKCTASNKNRGIIKTFSNGCDYVSNKGVDTVSVGNTLYYVNKLEGKNLYVKGEPIINYYDPLVFPSDEFDASISQVNEKINQSLAFIRRSDELLHNVNTGKSTTNIMITAIIIVIVVLLSLIAIGLLLYCKAKNTPVTLSKDQLSGINNIAFSK	993	1
AB524405	4556-6217	gb:AB524405:4556-6217|Organism:Newcastle disease virus|Strain Name:Goose/Alaska/415/91|Protein Name:fusion protein|Gene Symbol:F	MDPKPSTSYLHAFPLIFVAISLVFMAGRASALDGRPLAAAGIVVTGDKAVNIYTSSQTGTIIIKLLPNMPKDKEQCAKSPLDAYNRTLTTLLAPLGDSIRRIQESVTTSGGERQERLVGAIIGGVALGVATAAQITAASALIQANQNAANILKLKESIAATNEAVHEVTSGLSQLAVAVGKMQQFVNDQFNKTAQEIDCIKITQQVGVELNLYLTELTTVFGPQITSPALTQLTIQALYNLAGGNMDYMLTKLGVGNNQLSSLISSGLISGNPILYDSQTQLLGIQVTLPSVGNLNNMRATYLETLSVSTNKGFASALVPKVVTQVGSVIEELDTSYCIETDLDLYCTRIVTFPMSPGIFSCLGGNTSACMYSKTEGALTTPYMTLKGSVIANCKMTTCRCADPPGIISQNYGEAVSLIDKKVCNILTLDGITLRLSGEFDATYQKNISIQDSQVVITGNLDISTELGNVNNSISNALDKLEESNSKLDKVNVRLTSTSALITYIVLTTIALICGIVSLVLACYIMYKQKAQQKTLLWLGNNTLDQMRATTKM	418	2
AF266286	4875-7247	gb:AF266286:4875-7247|Organism:Measles virus strain AIK-C|Strain Name:Measles virus strain Edmonston (AIK-C vaccine)|Protein Name:fusion protein|Gene Symbol:F	MSIMGLKVNVSAIFMAVLLTLQTPTGQIHWGNLSKIGVVGIGSASYKVMTRSSHQSLVIKLMPNITLLNNCTRVEIAEYRRLLRTVLEPIRDALNAMTQNIRPVQSVASSRRHKRFAGVVLAGAALGVATAAQITAGIALHQSMLNSQAIDNLRASLETTNQAIEAIRQAGQEMILAVQGVQDYINNELIPSMNQLSCDLIGQKLGLKLLRYYTEILSLFGPSLRDPISAEISIQALSYALGGDINKVLEKLGYSGGDLLGILESRGIKARITHVDTESYFIVLSIAYPTLSEIKGVIVHRLEGVSYNIGSQEWYTTVPKYVATQGYLISNFDESSCTFMPEGTVCSQNALYPMSPLLQECLRGYTKSCARTLVSGSFGNRFILSQGNLIANCASILCKCYTTGTIINQDPDKILTYIAADNCPVVEVNGVTIQVGSRRYPDAVYLHRIDLGPPILLERLDVGTNLGNAIA	128	3

Genbank ID	Nucleotides of CDS	Full Gene Name	Sequence	#Sequences/Cluster	SEQ ID NO
			KLEDAKELLESSDQILRSMKGLSSTCIVYILIAVCLGGLIG IPALICCCRGRCNKKGEQVGMSRPGLKPDLTGTSKSYVR SL		
AB503857	3068-4687	gb:AB503857:3068-4687|Organism:Human metapneumovirus|Strain Name:Jpn03-1|Protein Name:fusion glycoprotein precursor|Gene Symbol:F	MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRT GWYTNVFTLEVGDVENLTCSDGPSLIKTELDLTKSALRE LKTVSADQLAREEQIEKPRQSRFVLGAIALGVATAAAVT AGVAIAKTIRLESEVTAIKNALKTTNEAVSTLGNGVRVL ATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQF NRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNM PTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQL PIFGVIDTPCWIVKAAPSCSEKKGNYACLLREDQGWYC QNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKE CNINISTTNYPCKVSTGRHPISMVALSPLGALVACYKGV SCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLS KVEGEQHVIKGRPVSSSFDPIKFPEDQFNVALDQVFENIE NSQALVDQSNRILSSAEKGNTGFIIVIILIAVLGSSMILVSI FIIIKKTKKPTGAPPELSGVTNNGFIPHS	125	4
EU277658	5078-6700	gb:EU277658:5078-6700|Organism:Bovine parainfluenza virus 3|Strain Name:Q5592|Protein Name:fusion protein|Gene Symbol:F	MIIIVITMILSLTPSSLCQIDITKLQSVGVLVNSPKGIKISQ NFETRYLILSLIPKIEDSHSCGNQQIDQYKKLLDRLIIPLY DGLKLQKDVIVVNHESHNNTNLRTKRFFGEIIGTIAIGIA TSAQITAAVALVEAKQARSDIDKLKEAIKDTNKAVQSIQ SSVGNLIVAVKSVQDYVNNEIVPSITRLGCEAAGLQLGI ALTQHYSELTNIFGDNIGTLGEKGVKLQGIASLYRTNITE VFTTSTVDQYDIYDLLFTESIKMRVIDVDLSDYSITLQVR LPLLTKVSNTQIYKVDSISYNIQGKEWYIPLPHHIMTKGA FLGGADIKECIESFSNYICPSDPGFILNHEMENCLSGNITQ CPKTIVTSDIVPRYAFVDGGVIANCIPTTCTCNGIDNRIN QSPDQGIKIITYKECQIVGINGMLFKTNQEGTLAKYTFDN IKLNNSVALNPIDISLELNKAKSDLEESKRWIEKSNQKLD SIGSWHQSSVTIIIIIVMIVVLLIINAIIIMIMIRYLRDRNRH LNNKDSEPYVLTNRQ	93	5
AB040874	4546-6162	gb:AB040874:4546-6162|Organism:Mumps virus|Strain Name:Miyahara|Protein Name:fusion protein|Gene Symbol:F	MKVFLVTCLGFAVFSSSVCVNINILQQIGYIKQQVRQLS YYSQSSSSYIVVKLLPNIQPTDNSCEFKSVTQYNKTLSNL LLPIAENINNIASPSSGSRRHKRFAGIAIGIAALGVATAAQ VTAAVSLVQAQTNARAIAAMKNSIQATNRAVFEVKEGT QRLAIAVQAIQDHINTIMNTQLNNMSCQILDNQLATSLG LYLTELTTVFQPQLINPALSPISIQALRSLLGSMTPAVVQ ATLSTSISAAEILSAGLMEGQIVSVLLDEMQMIVKINIPTI VTQSNALVIDFYSISSFINNQESIIQLPDRILEIGNEQWSYP AKNCKLTRHHIFCQYNEAERLSLESKLCLAGNISACVFS PIAGSYMRRFVALDGTIVANCRSLTCLCKSPSYPIYQPDH HAVTTIDLTACQTLSLDGLDFSIVSLSNITYAENLTISLSQ TINTQPIDISTELSKVNASLQNAVKYIKESNHQLQSVNVN SKIGAIIVAALVLSILSIIISLLFCCWAYVATKEIRRINFKT NHINTISSSVDDLIRY	89	6
AB475097	4908-6923	gb:AB475097:4908-6923|Organism:Canine distemper virus|Strain Name:M25CR|Protein Name:fusion protein|Gene Symbol:F	MNPHEQTIPMHEKIPKRSKTQTHTQQDLPQQHSTKSAES KTSRARHSITSAQRSTHYDPRTADWPDYYIMKRTRSCK QASYRSDNIPAHGDHDGIIHHTPESVSQGAKSRLKMGQS NAVKSGSQCTWLVLWCIGVASLFLCSKAQIHWNNLSTI GIIGTDSVHYKIMTRPSHQYLVIKLMPNVSLIDNCTKAEL DEYEKLLSSILEPINQALTLMTKNVKPLQSVGSGRRQRR FAGVVLAGAALGVATAAQITAGIALHQSNLNAQAIQSL RTSLEQSNKAIEEIREATQETVIAVQGVQDYVNNELVPA MQHMSCELVGQRLGLKLLRYYTELLSIFGPSLRDPISAEI SIQALSYALGGEIHKILEKLGYSGNDMIAILESRGIKTKIT HVDLPGKFIILSVSYPTLSEVKGVIVHRLEAVSYNIGSQE	46	7

Genbank ID	Nucleotides of CDS	Full Gene Name	Sequence	#Sequences/Cluster	SEQ ID NO
			WYTTVPRYVATNGYLISNFDESSCVFVSESAICSQNSLY PMSPLLQQCIRGDTSSCARTLVSGTMGNKFILSKGNIVA NCASILCKCYSTSTIINQSPDKLLTFIASDTCPLVEIDGVTI QVGSRQYPDMVYESKVALGPAISLERLDVGTNLGNALK KLDDAKVLIDSSNQILETVRRSSFNFGSLLSVPILSCTALA LLLLICCCKRRYQQTHKQNTKVDPTFKPDLTGTSRSYVR SL		
AJ849636	5526-7166	gb:AJ849636:5526-7166|Organism:Peste-des-petits-ruminants virus|Strain Name:Turkey 2000|Protein Name:fusion protein|Gene Symbol:F	MTRVAILTFLFLFPNAVACQIHWGNLSKIGIVGTGSASY KVMTRPSHQTLVIKLMPNITAIDNCTKSEIAEYKRLLITV LKPVEDALSVITKNVRPIQTLTPGRRTRRFAGAVLAGVA LGVATAAQITAGVALHQSLMNSQAIESLKTSLEKSNQAI EEIRLANKETILAVQGVQDYINNELVPSVHRMSCELVGH KLGLKLLRYYTEILSIFGPSLRDPIAAEISIQALSYALGGDI NRILDKLGYSGGDFLAILESKGIKARVTYVDTRDYFIILSI AYPTLSEIKGVIVHKIEAITYNIGAQEWYTTIPKYVATQG YLISNFDETSCVFTPDGTVCSQNALYPMSPLLQECFQGS TKSCARTLVSGTISNRFILSKGNLIANCASVLCKCYTTET VISQDPDKLLTVVASDKCPVVEVDGVTIQVGSREYPDSV YLHKIDLGPAISLEKLDVGTNLGNAVTRLENAKELLDAS DQILKTVKGVPFGGNMYIALAACIGVSLGLVTLICCCKG RCKNKEVPISKINPGLKPDLTGTSKSYVRSL	34	8
AF017149	6618-8258	gb:AF017149|Organism:Hendra virus|Strain Name:UNKNOWN-AF017149|Protein Name:fusion|Gene Symbol:F	MATQEVRLKCLLCGIIVLVLSLEGLGILHYEKLSKIGLVK GITRKYKIKSNPLTKDIVIKMIPNVSNVSKCTGTVMENY KSRLTGILSPIKGAIELYNNNTHDLVGDVKLAGVVMAGI AIGIATAAQITAGVALYEAMKNADNINKLKSSIESTNEA VVKLQETAEKTVYVLTALQDYINTNLVPTIDQISCKQTE LALDLALSKYLSDLLFVFGPNLQDPVSNSMTIQAISQAF GGNYETLLRTLGYATEDFDDLLESDSIAGQIVYVDLSSY YIIVRVYFPILTEIQQAYVQELLPVSFNNDNSEWISIVPNF VLIRNTLISNIEVKYCLITKKSVICNQDYATPMTASVREC LTGSTDKCPRELVVSSHVPRFALSGGVLFANCISVTCQC QTTGRAISQSGEQTLLMIDNTTCTTVVLGNIIISLGKYLG SINYNSESIAVGPPVYTDKVDISSQISSMNQSLQQSKDYI KEAQKILDTVNPSLISMLSMIILYVLSIAALCIGLITFISFVI VEKKRGNYSRLDDRQVRPVSNGDLYYIGT	29	9
AB005795	4866-6563	gb:AB005795:4866-6563|Organism:Sendai virus|Strain Name:Ohita|Protein Name:fusion protein|Gene Symbol:F	MATYIQRVQCISALLSVVLTTLVSCQIPRDRLSNIGVIVD EGKSLKIAGSHESRYIVLSLVPGIDLENGCGTAQVIQYKS LLNRLLIPLRDALDLQEALITVTNDTMTGADVPQSRFFG AVIGTIALGVATSAQITAGIALAEAREAKRDIALIKESMT KTHKSIELLQNAVGEQILALKTLQDFVNDEIKPAISELGC ETAALRLGIKLTQHYSELLTAFGSNFGTIGEKSLTLQALS SLYSANITEIMTTIRTGQSNIYDVIYTEQIKGTVIDVDLER YMVTLSVKIPILSEVPGVLIHKASSISYNIDGEEWYVTVP SHILSRASFLGGANIADCVESRLTYICPRDPAQLIPDSQQ KCILGDTTRCPVTKVVDNIIPKFAFVNGGVVANCIASTC TCGTGRRPISQDRSKGVVFLTHDNCGLIGVNGIELYANR KGHDATWGVQNLTVGPAIAIRPVDISLNLAAATDFLQD SRAELEKARKILSEVGRWYNSGATLITIIVVMIVVLVVII VIVIVLYRLRRSMLMSNPAGRISRDTYTLEPKIRHMYTN GGFDAMTEKR	23	10
AF457102	5088-6755	gb:AF457102|Organism:Human parainfluenza virus 1 strain Washington/1964|Strain	MQKSEILFLVYSSLLLSSSLCQIPVEKLSNVGVIINEGKLL KIAGSYESRYIVLSLVPSIDLQDGCGTTQIIQYKNLLNRL LIPLKDALDLQESLITITNDTTVTNDNPQTRFFGAVIGTIA LGVATAAQITAGIALAEAREARKDIALIKDSIVKTHNSVE LIQRGIGEQIIALKTLQDFVNDEIRPAIGELRCETTALKLG IKLTQHYSELATAFSSNLGTIGEKSLTLQALSSLYSANITE	21	11

Genbank ID	Nucleotides of CDS	Full Gene Name	Sequence	#Sequences/Cluster	SEQ ID NO
		Name:Washington 1964|Protein Name:F glycoprotein|Gene Symbol:F	ILSTTKKDKSDIYDIIYTEQVKGTVIDVDLEKYMVTLLV KIPILSEIPGVLIYRASSISYNIEGEEWHVAIPNYIINKASSL GGADVTNCIESKLAYICPRDPTQLIPDNQQKCILGDVSK CPVTKVINNLVPKFAFINGGVVANCIASTCTCGTNRIPVN QDRSRGVTFLTYTNCGLIGINGIELYANKRGRDTTWGN QIIKVGPAVSIRPVDISLNLASATNFLEESKTELMKARAII SAVGGWHNTESTQIIMIIIVCILIIIICGILYYLYRVRRLLV MINSTHNSPVNAYTLESRMRNPYMGNNSN		
AB91030 9	4951-6582	gb:AB910309:4951-6582|Organism:Feline morbillivirus|Strain Name:SS1|Protein Name:fusion protein|Gene Symbol:F	MGKIRVIIISSLLLSNITTAQVGWDNLTSIGVISTKQYDYK ITTLNTNQLMVIKMVPNISSIINCTKPELMKYRELVLGVI RPINESLELMNSYINMRAGSERFIGAVIAGVALGVATAA QITSGIALHNSIMNKRQIQELRKALSTTNKAIDEIRIAGER TLIAVQGVQDYINNIIIPMQDKLQCDILSSQLAIALLRYY TNILTVFGPSIRDPVTSIISIQALSQAFNGNLQALLDGLGY TGRDLRDLLESRSITGQIIHADMTDLFLVLRINYPSITEM QGVTIYELNSITYHIGPEEWYTIMPNFIAVQGFLTSNFDE RKCSITKSSILCQQNSIYPMSTEMQRCIKGEIRFCPRSKAV GTLVNRFILTKGNLMANCLGVICRCYSSGQIITQDPSKLI TIISQEECKEVGVDGIRIMVGPRKLPDVIFNARLEVGVPIS LSKLDVGTDLAIASAKLNNSKALLEQSDKILDSMSKLDS INSRITGLILAIMAIFIITVTIIWIIYKRCRNKDNKFSTSIEPL YIPPSYNSPHSVVKSI	12	12
KT07175 5	4310-6070	gb:KT071755:4310-6070|Organism:Avian paramyxovirus 2|Strain Name:APMV-2/Procarduelis nipalensis/China/Suiling/53/2013|Protein Name:fusion protein|Gene Symbol:F	MIAALFISLFATCGALDNSVLAPVGIASAQEWQLAAYTN TLSGTIAVRFVPVLPGNLSTCAQATLAEYNKTVTNILGP LKENLETLLSEPTKTAARFVGAIIGTVALGVATSAQITAA VALNQAQENARNIWRLKESIRKTNEAVLELKDGLASTAI ALDKVQKFINEDIIPQIKEIDCQVVANKLGVYLSLYLTEL TTIFGAQITNPALTPLSYQALYNLCGGDMGKLTELIGVK AKDINSLYEANLITGQVIGYDSESQIILIQVSYPSVSEVTG VRATELVTVSVTTPKGEGRAIAPKYVAQSRVVTEELDTS TCRFSKTTLYCRSIITRPLPPLIANCLNGLYQDCQYTTEIG ALSSRFITVNGGIIANCRATICKCVNPPKIIVQSDASSLTVI DSAICKDVVLDNVQLRLEGKLSAQYFTNITIDLSQITTSG SLDISSEIGSINNTVNKVEELIAESNAWLQAVNPHLVNNT SIIVLCVLAAIFVVVWLVALTGCLAYYIKKSSATRMVGIG SSPAGNPYVAQSATKM	12	13
AY029299	4598-6265	gb:AY029299|Organism:Avian paramyxovirus 6|Strain Name:APMV-6/duck/Taiwan/Y1/98|Protein Name:fusion protein|Gene Symbol:F	MGARLGPLAMAPGRYVIIFNLILLHRVVSLDNSRLLQQG IMSATEREIKVYTNSITGSIAVRLIPNLPQEVLKCSAGQIK SYNDTLNRIFTPIKANLERLLATPSMLEDNQNPAPEPRLI GAIIGTAALGLATAAQVTAALALNQAQDNAKAILNLKE SITKTNEAVLELKDATGQIAIALDKTQRFINDNILPAINNL TCEVAGAKVGVELSLYLTELSTVFGSQITNPALSTLSIQA LMSLCGNDFNYLLNLMGAKHSDLGALYEANLINGRIIQ YDQASQIMVIQVSVPSISSISGLRLTELFTLSIETPVGEGK AVVPQFVVESGQLLEEIDTQACTLTDTTAYCTIVRTKPL PELVAQCLRGDESRCQYTTGIGMLESRFGVFDGLVIANC KATICRCLAPEMIITQNKGLPLTVISQETCKRILIDGVTLQ IEAQVSGSYSRNITVGNSQIAPSGPLDISSELGKVNQSLSN VEDLIDQSNQLLNRVNPNIVNNTAIIVTIVLLVLLVLWCL ALTISILYVSKHAVRMIKTVPNPYVMQAKSPGSATQF	11	14
AY141760	5028-6665	gb:AY141760|Organism:Fer-de-Lance paramyxovirus|Strain Name:ATCC VR-895|Protein	MTRITILQIILTLTLPVMCQVSFDNLEQVGVMFDKPKFLK ITGPASTATMIIKLIPTLGTMESCGTSAVNEYKKTLDTIL VPLRDTINKLSTDITVVEGTSNISNKREKRFVGIAIAVGA VALATSAQITAGIALSNTIKNAEAIESIKSSIQASNQAIQK VIDAQGRTVTVINGIQDHINSVINPALNQLGCDVAKNTL AISLTQYFSKLSLLFGPNLRNPVEQPLSVQAIAGLMDGDI	8	15

Genbank ID	Nucleotides of CDS	Full Gene Name	Sequence	#Sequences/Cluster	SEQ ID NO
		Name:fusion protein F|Gene Symbol:F	NAVVSQLGYTQSDLLDLLSTESIVGTVTAIDMVNYMIQI EMSFPQYITIPDTKVLEGHKITFNDKGSEWQTQVPSTIAV RDILIAGVDPDGCSITSTSYICKNDPTYAMSEVLTNCFRG NTQECPRARITSTFATRFAIARSTVIANCVAAVCLCGDPG IPVVQKAEVTLTAMTLDQCSLITVDGLQIKPSKSIANVTA NFGNITLGPVVSVGDLDLSAELTKVQSDLKEAQDKLDE SNAILQGINNKILTAPTSIALIVVSVVVILLIIGMISWLVW LTKAVRRSNTRSERVTPSAYNNLGFIK		
EU877976	4330-6410	gb:EU877976:4330-6410|Organism:Avian paramyxovirus 4|Strain Name:APMV-4/KR/YJ/06|Protein Name:fusion protein|Gene Symbol:F	MRLSRTILTLILGTLTGYLMGAHSTNVNEGPKSEGIRGD LIPGAGIFVTQVRQLQIYQQSGYHDLVIRLLPLLPAELND CQREVVTEYNNTVSQLLQPIKTNLDTLLADGGTRDADI QPRFIGAIIATGALAVATVAEVTAAQALSQSKTNAQNIL KLRDSIQATNQAVFEISQGLEATATVLSKLQTELNENIIP SLNNLSCAAMGNRLGVSLSLYLTLMTTLFGDQITNPVLT PISYSTLSAMAGGHIGPVMSKILAGSVTSQLGAEQLIASG LIQSQVVGYDSQYQLLVIRVNLVRIQEVQNTRVVSLRTL AVNRDGGLYRAQVPPEVVERSGIAERFYADDCVLTTTD YICSSIRSSRLNPELVKCLSGALDSCTFERESALLSTPFFV YNKAVVANCKAATCRCNKPPSIIAQYSASALVTITTDTC ADLEIEGYRFNIQTESNSWVAPNFTVSTSQIVSVDPIDISS DIAKINSSIEAAREQLELSNQILSRINPRIVNDESLIAIIVTI VVLSLLVIGLIVVLGVMYKNLKKVQRAQAAMMMQQM SSSQPVTTKLGTPF	8	16
AB176531	4793-6448	gb:AB176531:4793-6448|Organism:Human parainfluenza virus 2|Strain Name:Nishio|Protein Name:fusion protein|Gene Symbol:F	MHHLHPMIVCIFVMYTGIVGSDAIAGDQLLNIGVIQSKIR SLMYYTDGGASFIVVKLLPNLPPSNGTCNITSLDAYNVT LFKLLTPLIENLSKISTVTDTKTRQKRFAGVVVGLAALG VATAAQITAAVAIVKANANAAAINNLASSIQSTNKAVSD VIDASRTIATAVQAIQDRINGAIVNGITSASCRAHDALIG SILNLYLTELTTIFHNQITNPALTPLSIQALRILLGSTLPIVI ESKLNTNFNTAELLSSGLLTGQIISISPMYMQMLIQINVPT FIMQPGAKVIDLIAISANHKLQEVVVQVPNRILEYANEL QNYPANDCVVTPNSVFCRYNEGSPIPESQYQCLRGNLNS CTFTPIIGNFLKRFAFANGVLYANCKSLLCRCADPPHVV SQDDTQGISIIDIKRCSEMMLDTFSFRITSTFNATYVTDFS MINANIVHLSPLDLSNQINSINKSLKSAEDWIADSNFFAN QARTAKTLYSLSAIALILSVITLVVVGLLIAYIIKLVSQIH QFRSLAATTMFHRENPAFFSKNNHGNIYGIS	7	17
BK005918	4677-6302	gb:BK005918|Organism:Porcine rubulavirus|Strain Name:UNKNOWN-BK005918|Protein Name:fusion protein|Gene Symbol:F	MPQQQVAHTCVMLWGIISTVSGINTEALSQYGVVVTNV RQLTYYTQAGSTYLAVRLLPSLASPDQSCALHSIINYNA TLQAILSPIAENLNLISTALREQHRKKRFAGVAIGLTALG VATAAQATAAVALVRANKNAEKVEQLSQALGETNAAI SDLIDATKNLGFAVQAIQNQINTAILPQIHNLSCQVIDAQ LGNILSLYLTELTTVFQPQLTNPALSPLTIQALRAVLGTT LPALLSEKLKSNIPLGDLMSSGLLKGQLVGLNLQNMLMI IELYIPTLSTHSTAKVLDLVTISSHVNGREVEIQVPNRVLE LGSEVLGYGGSECALTMSHILCPFNDARVLSTDMKYCL QGNITHCIFSPVVGSFLRRFALVNGVVIANCADMSCVCF DPQEIIYQNFQEPTTVIDIKKCGKVQLDTLTFTISTFANRT YGPPAYVPPDNIIQSEPLDISGNLIAVNNSLSSALNHLATS EILRNEQIWTSSLGISTIVALVIIGILIICLVVTWAALWALL KEVRGLNSAVNSQLSSYVMGDKFIRY	7	18
KC237063	4530-6185	gb:KC237063:4530-6185|Organism:Parainfluenza virus 5|Strain Name:08-	MGTRIQFLMVSCLLAGTGSLDPAALMQIGVIPTNVRQL MYYTEASSAFIVVKLMPTIDSPISGCNITSISSYNATMTK LLQPIGENLETIRYQLIPTRRRRRFVGVVIGLAALGVATA AQVTAAVALVKANKNAAAILNLKNAIQKTNAAVADVV QATQSLGTAVQAVQDHINSVVSPAITAANCKAQDAIIGS	7	19

Genbank ID	Nucleotides of CDS	Full Gene Name	Sequence	#Sequences/Cluster	SEQ ID NO
		1990|Protein Name:fusion protein|Gene Symbol:F|Segment: 4	ILNLYLTELTTIFHNQITNPALSPITIQALRILLGSTLPTVV RKSFNTQISAAELLSSGLLTGQIVGLDLTYMQMVIKIELP TLTVQPATQIIDLVTISAFINNREVMAQLPTRIIVTGSLIQ AYPASQCTITPNTVYCRYNDAQVLSDDTMACLQGNLTR CTFSPVVGSFLTRFVLFDGIVYANCRSMLCKCMQPAAVI LQPSSSPVTVIDMHKCVSLQLDNLRFTITQLANITYNSTI KLETSQILPIDPLDISQNLAAVNKSLSDALQHLAQSDTYL SAITSATTTSVLSIIAICLGSLGLILIILISVVVWKLLTIVAA NRNRMENFVYHNSAFHHSRSDLSEKNQPATLGTR		
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	MIPGRIFLVLLVIFNTKPIHPNTLTEKFYESTCSVETAGYK SALRTGWHMTVMSIKLSQINIESCKSSNSLLAHELAIYSS AVDELRTLSSNALKSKRKKRFLGLILGLGAAVTAGVAL AKTVQLESEIALIRDAVRNTNEAVVSLTNGMSVLAKVV DDLKNFISKELLPKINRVSCDVHDITAVIRFQQLNKRLLE VSREFSSNAGLTHTVSSFMLTDRELTSIVGGMAVSAGQK EIMLSSKAIMRRNGLAILSSVNADTLVYVIQLPLFGVMD TDCWVIRSSIDCHNIADKYACLARADNGWYCHNAGSLS YFPSPTDCEIHNGYAFCDTLKSLTVPVTSRECNSNMYTT NYDCKISTSKTYVSTAVLTTMGCLVSCYGHNSCTVINN DKGIIRTLPDGCHYISNKGVDRVQVGNTVYYLSKEVGK SIVVRGEPLVLKYDPLSFPDDKFDVAIRDVEHSINQTRTF LKASDQLLDLSENRENKNLNKSYILTTLLFVVMLIIIMAV IGFILYKVLKMIRDNKLKSKSTPGLTVLS	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	MGVKGLSLIMIGLLISPITNLDITHLMNLGTVPTAIRSLV YYTYTKPSYLTVDLIPNLKNLDQNCNYSSLNYYNKTAL SLIQPIADNINRLTKPITSSEIQSRFFGAVIGTIALGVATAA QVTAAIGLAKAQENAKLILTLKKAATETNEAVRDLANS NKIVVKMISAIQNQINTIIQPAIDQINCQIKDLQVANILNL YLTEITTVFHNQLTNPALESISIQALKSLLGPTLPEVLSKL DLNNISAASVMASGLIKGQIIAVDIPTMTLVLMVQIPSISP LRQAKIIDLTSITIHTNSQEVQAVVPARFLEIGSEILGFDG SVCQITKDTIFCPYNDAYELPIQQKRCLQGQTRDCVFTP VAGTFPRRFLTTYGTIVANCRDLVCSCLRPPQIIYQPDEN PVTIIDKDLCTTLTLDSITIEIQKSINSTFRREVVLESTQVR SLTPLDLSTDLNQYNQLLKSAEDHIQRSTDYLNSINPSIV NNNAIIILIILCILLILTVTICIIWLKYLTKEVKNVARNQRL NRDADLFYKIPSQIPVPR	5	21
AF298895	4834-6450	gb:AF298895|Organism:Tioman virus|Strain Name:UNKNOWN-AF298895|Protein Name:fusion protein|Gene Symbol:F	MRIALTAVIVSIHFDLAFPMNKNSLLSVGLVHKSVKNLY FYSQGSPSYIVVKLVPTLGNVPGNCTLNSLVRYKSTVSS LLSPLAENLEYLQKTLTVSRGGRRRRFAGVAIGLAALGV AAAAQATAAVALVEARQNAAQIQSLSEAIQNTNLAVNE LKTAIGASATAIQAIQTQINEVINPAINRLSCEILDAQLAS MLNLYLIHLTTVFQNQLTNPALTPLSIQSLQSLLQGTSSV LTNITSSSKLALNDALVTGLITGQVVGLNMTSLQIVIAAY VPSVAKLSNAVVHNFIRITTSVNGTEVIIQSPTIIMEQNEV MYDLKTGHCTESDLNIYCPYVDAQLLSPGMTNCINGRL NDCTFSKVVGSFPTRFAAVEGAILANCKYLQCNCLTPPY IITPLNGEMISMIDLSKCQRLDLGTIVFDINNPVNVTFNG NYRADVGQMIVTNPLDISAELNQINTSLSNAQGFLSKSD AWLHVSQWVTNSGTIFIILIIGLIVGIVYMIINTYVVVQIIK EINRMRTSDRAHLLKGSISSIST	5	22
FJ215863	4499-6130	gb:FJ215863:4499-6130|Organism:Avian paramyxovirus 8|Strain	MGQISVYLINSVLLLLVYPVNSIDNTLIAPIGVASANEWQ LAAYTTSLSGTIAVRFLPVLPDNMTTCLRETITTYNNTV NNILGPLKSNLDALLSSETYPQTRLIGAVIGSIALGVATS AQITAAVALKQAQDNARNILALKEALSKTNEAVKELSS GLQQTAIALGKIQSFVNEEILPSINQLSCEVTANKLGVYL	5	23

Genbank ID	Nucleotides of CDS	Full Gene Name	Sequence	#Sequences/Cluster	SEQ ID NO
		Name:goose/Delaware/1053/76|Protein Name:fusion protein|Gene Symbol:F	SLYLTELTTIFGAQLTNPALTSLSYQALYNLCGGNMAM LTQKIGIKQQDVNSLYEAGLITGQVIGYDSQYQLLVIQV NYPSISEVTGVRATELVTVSVTTDKGEGKAIVPQFVAES RVTIEELDVASCKFSSTTLYCRQVNTRALPPLVASCLRG NYDDCQYTTEIGALSSRYITLDGGVLVNCKSIVCRCLNP SKIISQNTNAAVTYVDATICKTIQLDDIQLQLEGSLSSVY ARNISIEISQVTTSGSLDISSEIGNINNTVNRVEDLIHQSEE WLAKVNPHIVNNTTLIVLCVLSALAVIWLAVLTAIIIYLR TKLKTISALAVTNTIQSNPYVNQTKRESKF		
JN689227	4689-6521	gb:JN689227:4689-6521|Organism:Tailam virus|Strain Name:TL8K|Protein Name:fusion protein|Gene Symbol:F	MKLSVVYTTLLVSTFYSDLARSQLALSELTKIGVIPGRSY DLKISTQASYQYMVVKLIPNLTGLNNCTNGTIEAYKKM LNRLLSPIDAALRKMKDAVNDKPPESVGNVKFWGAVIG GVALGVATSAQITAGVALHNSIQNANAILALKDSIRQSN KAIQELQTAMSTTVVVLNALQDQINNQLVPAINSLGCQ VVANTLGLKLNQYFSEISLVFGPNLRDPTSETLSIQALSR AFNGDFDSMLSKLKYDDSDFLDLLESDSIRGRIIDVSLSD YLITIQIEYPALLSIKDAVIQTFNLISYNTRGTEWISIFPKQ LLVRGTYISNIDISQCVIAATSIICKSDTSTPISSATWSCAT GNITNCARTRVVNAHVPRFALYGGVVFANCAPVVCKC QDPLYSINQEPKVTNVMVDVDACKEMYLDGLYITLGKT QISRAMYAEDVSLGGPISVDPIDLGNEINSINSAINRSEEH LNHANELLDKVNPRIVNVKTFGVMIGLLVLVVLWCVIT LVWLICLTKQLARTAYAGSMGSRASTVNSLSGFVG	5	24
JX857409	4831-6615	gb:JX857409:4831-6615|Organism:Porcine parainfluenza virus 1|Strain Name:S206N|Protein Name:fusion protein|Gene Symbol:F	MQVTTLRPAIILSIALLVTGQVPRDKLANLGIIIKDSKAL KIAGSYENRYIVLSLVPTIDNVNGCGSIQIAKYKEMLERL LIPIKDALDLQESLIVIDNETVNNNYSPQYRFVGAIIGTIA LGVATAAQVTAGVALMEAREAKRDISMLKEAIEKTQNS IEKLQNSAGEQILALKMLQDYVNGEIKPAIEELGCETAA LKLGIALTQHYTELTNAFGSNLGSIGEKSLTLQALSSLYK TNITNILTATNLGKTDIYDIIYAEQVKGRVIDVDLKRYM VTISVKIPILSEIPGVLIYEVSSISYNIDGAEWYAAVPDHIL SKSAYIGGADISDCIESRLTYICPQDPAQIIADNQQQCFFG HLDKCPITKVIDNLVPKFAFINGGVVANCIASTCTCGEER IQVSQDRNKGVTFLTHNNCGLIGINGIEFHANKKGSDAT WNVSPIGVGPAVSLRPVDISLQIVAATNFLNSSRKDLMK AKEILNQVGNLKDLTTITIINIVIIIILLICVIGLGILYHQLRS ALGMRDKMSVLNNSSYSLEPRTAQVQVIKPTSFMG	5	25
AY640317	2932-4571	gb:AY640317:2932-4571|Organism:Avian metapneumovirus|Strain Name:LAHA|Protein Name:F|Gene Symbol:F	MDVRICLLLFLISNPSSCIQETYNEESCSTVTRGYKSVLR TGWYTNVFNLEIGNVENITCNDGPSLIDTELVLTKNALR ELKTVSADQVAKESRLSSPRRRRFVLGAIALGVATAAA VTAGVALAKTIRLEGEVKAIKNALRNTNEAVSTLGNGV RVLATAVNDLKEFISKKLTPAINQNKCNIADIKMAISFGQ NNRRFLNVVRQFSDSAGITSAVSLDLMTDDELVRAINR MPTSSGQISLMLNNRAMVRRKGFGILIGVYDGTVVYMV QLPIFGVIETPCWRVVAAPLCRKRRGNYACILREDQGW YCTNAGSTAYYPNKDDCEVRDDYVFCDTAAGINVALE VDQCNYNISTSKYPCKVSTGRHPVSMVALTPLGGLVSC YESVSCSIGSNKVGIIKQLGKGCTHIPNNEADTITIDNTV YQLSKVVGEQRTIKGAPVVNNFNPILFPVDQFNVALDQ VFESIDRSQDLIDKSNDLLGADAKSKAGIAIAIVVLVILGI FFLLAVIYYCSRVRKTKPKHDYPATTGHSSMAYVS	4	26
KU646513	4641-6498	gb:KU646513:4641-6498|Organism:Avian paramyxovirus 13	MARFSWEIFRLSTILLIAQTCQGSIDGRLTLAAGIVPVGD RPISIYTSSQTGIIVVKLIPNLPDNKKDCAKQSLQSYNETL SRILTPLATAMSAIRGNSTTQVRENRLVGAIIGSVALGVA TAAQITAATALIQANQNAANIARLANSIAKTNEAVTDLT EGLGTLAIGVGKLQDYVNEQFNNTAVAIDCLTLESRLGI	4	27

Genbank ID	Nucleotides of CDS	Full Gene Name	Sequence	#Sequences/Cluster	SEQ ID NO
		goose/Kazakhstan/5751/2013|Strain Name:APMV-13/white fronted goose/Northern Kazakhstan/5751/2013|Protein Name:fusion protein|Gene Symbol:F	QLSLYLTELMGVFGNQLTSPALTPITIQALYNLAGGNLN ALLSRLGASETQLGSLINSGLIKGMPIMYDDANKLLAVQ VELPSIGKLNGARSTLLETLAVDTTRGPSSPIIPSAVIEIGG AMEELDLSPCITTDLDMFCTKIISYPLSQSTLSCLNGNLS DCVFSRSEGVLSTPYMTIKGKIVANCKQVICRCMDPPQI LSQNYGEALLLIDENTCRSLELSGVILKLAGTYESEYTRN LTVDPSQVIITGPLDISAELSKVNQSIDSAKENIAESNKFL SQVNVKLLSSSAMITYIVATVVCLIIAITGCVIGIYTLTKL KSQQKTLLWLGNNAEMHGSRSKTSF		
AF326114	4818-6482	gb:AF326114|Organism:Menangle virus|Strain Name:UNKNOWN-AF326114|Protein Name:fusion protein|Gene Symbol:F	MMPRVLGMIVLYLTHSQILCINRNTLYQIGLIHRSVKKV NFYSQGSPSYIVVKLVPTLAAIPPNCSIKSLQRYKETVTS LVQPISDNLGYLQDKLVTGQSRRRRRFAGVAIGLAALG VAAAAQATAAVALVETRENAGKIQALSESIQNTNQAVH SLKTALGFSATAIQAIQNQVNEVINPAINKLSCEVLDSQL ASMLNLYLIHLTTVFQTQLTNPALTPLSIQALTSVLQGTS GVLMNSTNSTLTQPIDLLATGLITGQIISVNMTSLQLIIAT FMPSIAELPNAVLHSFFRITTSVNLTEVMIQSPEFIMEQN GVFYDFNTAHCQLGDNNVYCPYIDAARLSSMMTNCING NLGECVFSRVIGSFPSRFVSLNGAILANCKFMRCNCLSPE KIITPLDGEMISLIDLRVCQKLTLGTITFEISQPVNVSFQG GFVANAGQIIVTNPFDISAELGQINNSLNDAQGFLDQSN NWLKVSGWINNSGSLFIAGIVVIGLIVLCIVIIIYINVQIIRE VNRLRSFIYRDYVLDHDKAPYSPESSSPHRKSLKTVS	3	28
GU206351	5441-7468	gb:GU206351:5441-7468|Organism:Avian paramyxovirus 5|Strain Name:budgerigar/Kunitachi/74|Protein Name:fusion protein|Gene Symbol:F	MLQLPLTILLSILSAHQSLCLDNSKLIHAGIMSTTEREVN VYAQSITGSIVVRLIPNIPSNHKSCATSQIKLYNDTLTRLL TPIKANLEGLISAVSQDQSQNSGKRKKRFVGAVIGAAAL GLATAAQVTATVALNQAQENARNILRLKNSIQKTNEAV MELKDAVGQTAVAIDKTQAFINNQILPAISNLSCEVLGN KIGVQLSLYLTELTTVFGNQLTNPALTTLSLQALYNLCG DDFNYLINLLNAKNRNLASLYEANLIQGRITQYDSMNQ LLIIQVQIPSISTVSGMRVTELFTLSVDTPIGEGKALVPKY VLSSGRIMEEVDLSSCAITSTSVFCSSIISRPLPLETINCLN GNVTQCQFTANTGTLESRYAVIGGLVIANCKAIVCRCLN PPGVIAQNLGLPITIISSNTCQRINLEQITLSLGNSILSTYSA NLSQVEMNLAPSNPLDISVELNRVNTSLSKVESLIKESNS ILDSVNPQILNVKTVIILAVIIGLIVVWCFILTCLIVRGFML LVKQQKFKGLSVQNNPYVSNNSH	3	29
JQ001776	6129-8166	gb:JQ001776:6129-8166|Organism:Cedar virus|Strain Name:CG1a|Protein Name:fusion glycoprotein|Gene Symbol:F	MSNKRTTVLIIISYTLFYLNNAAIVGFDFDKLNKIGVVQG RVLNYKIKGDPMTKDLVLKFIPNIVNITECVREPLSRYNE TVRRLLLPIHNMLGLYLNNTNAKMTGLMIAGVIMGGIAI GIATAAQITAGFALYEAKKNTENIQKLTDSIMKTQDSID KLTDSVGTSILILNKLQTYINNQLVPNLELLSCRQNKIEF DLMLTKYLVDLMTVIGPNINNPVNKDMTIQSLSLLFDG NYDIMMSELGYTPQDFLDLIESKSITGQIIYVDMENLYV VIRTYLPTLIEVPDAQIYEFNKITMSSNGGEYLSTIPNFILI RGNYMSNIDVATCYMTKASVICNQDYSLPMSQNLRSCY QGETEYCPVEAVIASHSPRFALTNGVIFANCINTICRCQD NGKTITQNINQFVSMIDNSTCNDVMVDKFTIKVGKYMG RKDINNINIQIGPQIIIDKVDLSNEINKMNQSLKDSIFYLRE AKRILDSVNISLISPSVQLFLIIISVLSFIILLIIIVYLYCKSK HSYKYNKFIDDPDYYNDYKRERINGKASKSNNIYYVGD	3	30
LC168749	4869-7235	gb:LC168749:4869-7235|Organism:Rinderpest	MGILFAALLAMTNPHLATGQIHWGNLSKIGVVGTGSAS YKVMTQSSHQSLVIKLMPNVTAIDNCTKTEIMEYKRLL GTVLKPIREALNAITKNIKPIQSSTTSRRHKRFAGVVLAG AALGVATAAQITAGIALHQSMMNSQAIESLKASLETTN	2	31

Genbank ID	Nucleotides of CDS	Full Gene Name	Sequence	#Sequences/Cluster	SEQ ID NO
		morbillivirus|Strain Name:Lv|Protein Name:F protein|Gene Symbol:F	QAIEEIRQAGQEMVLAVQGVQDYINNELVPAMGQLSCE IVGQKLGLKLLRYYTEILSLFGPSLRDPVSAELSIQALSY ALGGDINKILEKLGYSGSDLLAILESKGIKAKITYVDIESY FIVLSIAYPSLSEIKGVIVHRLESVSYNIGSQEWYTTVPRY VATQGYLISNFDDTPCAFTPEGTICSQNALYPMSPLLQEC FRGSTRSCARTLVSGSIGNRFILSKGNLIANCASILCKCYT TGSIISQDPDKILTYIAADQCPVVEVGGVTIQVGSREYSD AVYLHEIDLGPPISLEKLDVGTNLWNAVTKLEKAKDLL DSSDLILENIKGVSVTNTGYILVGVGLIAVVGILIITCCCK KRRSDNKVSTMVLNPGLRPDLTGTSKSYVRSL		
LC187310	6250-7860	gb:LC187310:6250-7860|Organism:Avian paramyxovirus 10|Strain Name:rAPMV-10-FI324/YmHA|Protein Name:fusion protein|Gene Symbol:F	MTRTRLLFLLTCYIPGAVSLDNSILAPAGIISASERQIAIY TQTLQGTIALRFIPVLPQNLSSCAKDTLESYNSTVSNLLL PIAENLNALLKDADKPSQRIIGAIIGSVALGVATTAQVTA ALAMTQAQQNARNIWKLKESIKNTNQAVLELKDGLQQ SAIALDKVQSFINSEILPQINQLGCEVAANKLGIFLSLYLT EITTVFKNQITNPALSTLSYQALYNLCGGNMAALTKQIG IKDTEINSLYEAELITGQVIGYDSADQILLIQVSYPSVSRV QGVRAVELLTVSVATPKGEGKAIAPSFIAQSNIIAEELDT QPCKFSKTTLYCRQVNTRTLPVRVANCLKGKYNDCQYT TEIGALASRYVTITNGVVANCRSIICRCLDPEGIVAQNSD AAITVIDRSTCKLIQLGDITLRLEGKLSSSYSKNITIDISQV TTSGSLDISSELGSINNTITKVEDLISKSNDWLSKVNPTLI SNDTIIALCVIAGIVVIWLVIITILSYYILIKLKNVALLSTM PKKDLNPYVNNTKF	2	32
NC_005283	5277-6935	gb:NC_005283:5277-6935|Organism:Dolphin morbillivirus|Strain Name:UNKNOWN-NC_005283|Protein Name:fusion protein|Gene Symbol:F	MAASNGGVMYQSFLTIIILVIMTEGQIHWGNLSKIGIVGT GSASYKVMTRPNHQYLVIKLMPNVTMIDNCTRTEVTEY RKLLKTVLEPVKNALTVITKNIKPIQSLTTSRRSKRFAGV VLAGVALGVATAAQITAGVALHQSIMNSQSIDNLRTSLE KSNQAIEEIRQASQETVLAVQGVQDFINNELIPSMHQLSC EMLGQKLGLKLLRYYTEILSIFGPSLRDPVSAEISIQALSY ALGGDINKILEKLGYSGADLLAILESRGIKAKVTHVDLE GYFIVLSIAYPTLSEVKGVIVHKLEAVSYNLGSQEWYTT LPKYVATNGYLISNFDESSCAFMSEVTICSQNALYPMSP LLQQCLRGSTASCARSLVSGTIGNRFILSKGNLIANCASV LCKCYSTGTIISQDPDKLLTFVAADKCPLVEVDGITIQVG SREYPDSVYVSRIDLGPAISLEKLDVGTNLGSALTKLDN AKDLLDSSNQILENVRRSSFGGAMYIGILVCAGALVILC VLVYCCRRHCRKRVQTPPKATPGLKPDLTGTTKSYVRS L	2	33
NC_005339	5374-7602	gb:NC_005339:5374-7602|Organism:Mossman virus|Strain Name:UNKNOWN-NC_005339|Protein Name:fusion protein|Gene Symbol:F	MSNYFPARVIIIVSLITAVSCQISFQNLSTIGVFKFKEYDY RVSGDYNEQFLAIKMVPNVTGVENCTASLIDEYRHVIY NLLQPINTTLTASTSNVDPYAGNKKFFGAVIAGVALGVA TAAQVTAGVALYEARQNAAAIAEIKESLHYTHKAIESLQ ISQKQTVVAIQGIQDQINTNIIPQINALTCEIANQRLRLML LQYYTEMLSSFGPIIQDPLSGHITVQALSQAAGGNITGLM RELGYSSKDLRYILSVNGISANIIDADPEIGSIILRIRYPSMI KIPDVAVMELSYLAYHAAGGDWLTVGPRFILKRGYSLS NLDITSCTIGEDFLLCSKDVSSPMSLATQSCLRGDTQMC SRTAVQDREAPRFLLLQGNLIVNCMSVNCKCEDPEETIT QDPAYPLMVLGSDTCKIHYIDGIRIKLGKVQLPPITVLNT LSLGPIVVLNPIDVSNQLSLVETTVKESEDHLKNAIGALR SQSRVGGVGIVAIVGLIIATVSLVVLVISGCCLVKYFSRT ATLESSLTTIEHGPTLAPKSGPIIPTYINPVYRHD	2	34
NC_007454	4635-6384	gb:NC_007454:4635-6384|Organism:J-	MKPVALIYLTILAFTVKVRSQLALSDLTKIGIIPAKSYEL KISTQAAQQLMVIKLIPNVNGLTNCTIPVMDSYKKMLD RILKPIDDALNHVKNAIQDKQGDGVPGVRFWGAIIGGV	2	35

Genbank ID	Nucleotides of CDS	Full Gene Name	Sequence	#Sequences/Cluster	SEQ ID NO
		virus|Strain Name:UNKNOWN-NC_007454|Protein Name:fusion protein|Gene Symbol:F	ALGVATSAQITAGVALHNSIQNANAILQLKESIRNSNKAI EELQAGLQSTVLVINALQDQINSQLVPAINTLGCSVIANT LGLRLNQYFSEISLVFGPNLRDPTSQTLSIQAIAKAFNGD FDSMMKKMHYTDSDFLDLLESDSIRGRIISVSLEDYLIIIQ IDYPGLTTIPNSVVQTFNLITYNYKGTEWESIFPRELLIRG SYISNIDISQCVGTSKSMICKSDTSTTISPATWACATGNLT SCARTRVVNSHSTRFALSGGVLFANCAPIACRCQDPQYS INQEPKTTNVMVTSEDCKELYIDGFYLTLGKKMLDRAM YAEDVALGGSVSVDPIDIGNELNSINESINKSHEYLDKA NELLEQVNPNIVNVSSFSFILVISILLIIWFIVTLVWLIYLT KHMNFIVGKVAMGSRSSTVNSLSGFVG		
NC_009489	4620-6500	gb:NC_009489:4620-6500|Organism:Mapuera virus|Strain Name:BeAnn 370284|Protein Name:fusion protein|Gene Symbol:F	MRSSLFLVLTLLVPFAHSIDSITLEQYGTVITSVRSLAYFL ETNPTYISVRLMPAIQTDSSHCSYHSIENYNLTLTKLLLP LQENLHQITDSLSSRRRKKRFAGVAVGLAALGVATAAQ VTAAIAVVKAKENSAKIAQLTSAISETNRAVQDLIEGSK QLAVAVQAIQDQINNVIQPQLTNLSCQVADAQVGTILN MYLTELTTVFHPQITNSALTPITIQALRSLLGSTLPQVVTS TIKTDVPLQDLLTSGLLKGQIVYLDLQSMIMVVSVSVPTI ALHSMAKVYTLKAISAHVNNAEVQMQVPSRVMELGSEI MGYDIDQCEETSRYLFCPYNGGSILSATMKMCLNGNISQ CVFTPIYGSFLQRFVLVDGVIVANCRDMTCACKSPSKIIT QPDSLPVTIIDSTSCSNLVLDTLELPIISINNATYRPVQYV GPNQIIFSQPLDLLSQLGKINSSLSDAIEHLAKSDEILEQIQ WDSPQGYTLIALTSVLAFVVVAIVGLLISTRYLIFEIRRIN TTLTQQLSSYVLSNKIIQY	2	36
NC_017937	4534-6330	gb:NC_017937:4534-6330|Organism:Nariva virus|Strain Name:UNKNOWN-NC_017937|Protein Name:fusion protein|Gene Symbol:F	MAEQEKTPLRYKILLIIIVINHYNITNVFGQIHLANLSSIG VFVTKTLDYRTTSDPTEQLLVINMLPNISNIQDCAQGVV NEYKHLISSLLTPINDTLDLITSNINPYSGRNKLFGEIIAG AALTVATSAQITAGVALYEARQNAKDIAAIKESLGYAY KAIDKLTTATREITVVINELQDQINNRLIPRINDLACEVW ATRLQAMLLQYYAEIFSVIGPNLQDPLSGKISIQALARAA GGNIKLMVDELNYSGQDLSRLVKVGAIKGQIIDADPSLG VVIIKMRYPNIIKIPNVAISELSYVSYSSDGQDWITTGPNY IVTRGYSIANIQTSSCSVGDDFVLCDRDMTYPMSQVTQD CLRGNIALCSRMVVRDREAPRYLILQGNMVANCMSITC RCEEPESEIYQSPDQPLTLLTRDTCDTHVVDGIRIRLGVR KLPTISVINNITLGPIITTDPIDVSNQLNAVVSTIDQSAELL HQAQRVLSERARGARDHILATAAIVICVVLAVLILVLLIG LVYLYRTQNEILVKTTMLEQVPTFAPKSFPMESQIYSGK TNKGYDPAE	2	37
NC_025256	6865-8853	gb:NC_025256:6865-8853|Organism:Bat Paramyxovirus Eid_hel/GH-M74a/GHA/2009|Strain Name:BatPV/Eid_hel/GH-M74a/GHA/2009|Protein Name:fusion protein|Gene Symbol:F	MKKKTDNPTISKRGHNHSRGIKSRALLRETDNYSNGLIV ENLVRNCHHPSKNNLNYTKTQKRDSTIPYRVEERKGHY PKIKHLIDKSYKHIKRGKRRNGHNGNIITIILLLILILKTQ MSEGAIHYETLSKIGLIKGITREYKVKGTPSSKDIVIKLIP NVTGLNKCTNISMENYKEQLDKILIPINNIIELYANSTKS APGNARFAGVIIAGVALGVAAAAQITAGIALHEARQNA ERINLLKDSISATNNAVAELQEATGGIVNVITGMQDYIN TNLVPQIDKLQCSQIKTALDISLSQYYSEILTVFGPNLQN PVTTSMSIQAISQSFGGNIDLLLNLLGYTANDLLDLLESK SITGQITYINLEHYFMVIRVYYPIMTTISNAYVQELIKISF NVDGSEWVSLVPSYILIRNSYLSNIDISECLITKNSVICRH DFAMPMSYTLKECLTGDTEKCPREAVVTSYVPRFAISG GVIYANCLSTTCQCYQTGKVIAQDGSQTLMMIDNQTCSI VRIEEILISTGKYLGSQEYNTMHVSVGNPVFTDKLDITSQ ISNINQSIEQSKFYLDKSKAILDKINLNLIGSVPISILFIIAIL	2	38

Genbank ID	Nucleotides of CDS	Full Gene Name	Sequence	#Sequences/Cluster	SEQ ID NO
			SLILSIITFVIVMIIVRRYNKYTPLINSDPSSRRSTIQDVYIIP NPGEHSIRSAARSIDRDRD		
NC_025347	4471-6386	gb:NC_025347:4471-6386|Organism:Avian paramyxovirus 7|Strain Name:APMV-7/dove/Tennessee/4/75|Protein Name:fusion protein|Gene Symbol:F	MRVRPLIIILVLLVLLWLNILPVIGLDNSKIAQAGIISAQE YAVNVYSQSNEAYIALRTVPYIPPHNLSCFQDLINTYNT TIQNIFSPIQDQITSITSASTLPSSRFAGLVVGAIALGVATS AQITAAVALTKAQQNAQEIIRLRDSIQNTINAVNDITVGL SSIGVALSKVQNYLNDVINPALQNLSCQVSALNLGIQLN LYLTEITTIFGPQITNPSLTPLSIQALYTLAGDNLMQFLTR YGYGETSVSSILESGLISAQIVSFDKQTGIAILYVTLPSIAT LSGSRVTKLMSVSVQTGVGEGSAIVPSYVIQQGTVIEEFI PDSCIFTRSDVYCTQLYSKLLPDSILQCLQGSMADCQFT RSLGSFANRFMTVAGGVIANCQTVLCRCYNPVMIIPQN NGIAVTLIDGSLCKELELEGIRLTMADPVFASYSRDLIIN GNQFAPSDALDISSELGQLNNSISSATDNLQKAQESLNK SIIPAATSSWLIILLFVLVSISLVIGCISIYFIYKHSTTNRSR NLSSDIISNPYIQKAN	2	39
NC_025348	4790-6570	gb:NC_025348:4790-6570|Organism:Tuhoko virus 2|Strain Name:UNKNOWN-NC_025348|Protein Name:fusion protein|Gene Symbol:F	MAPCVLFLSSLLLISTISPSHGINQPALRRIGAIVSSVKQL KFYSKTKPNYIIVKLLPTINLSKSNCNLTSINRYKESVIEII KPLADNIDNLNQKLLPKNRRKRMAGVAIGLAALGVAA AAQATAAVALVEARKNTQMIQSLADSIQDTNAAVQAV NIGLQNSAVAIQAIQNQINNVINPALDRLNCEVLDAQIAS ILNLYLIKSVTIFQNQLTNPALQQLSIQMLSIVMQDTAKI LGNFTIGDKFDQHDLLGSGLITGQVVGVNLTNLQLIIAA FIPSIAPLPQAYIIDLISITISVNDTEAVIQIPERIMEHGSSIY QFGGKQCVYGQFSAYCPFSDAVLMTQDLQLCMKGNIE HCIFSSVLGSFPNRFASVDGVFYANCKYMSCACSDPLQV IHQDDSVNLMVIDSSVCRSLTLGHVTFPIIAFSNVSYQMK TNISIEQMIVTSPLDLSTELKQINNSVNIANTFLDSSNRAL KTSIFGTSSQIILIVLLIFTCLLILYVIFLTYIIKILIKEVKRLR DGNSRTGSKLSFINPDV	2	40
NC_025350	4663-6428	gb:NC_025350:4663-6428|Organism:Tuhoko virus 3|Strain Name:UNKNOWN-NC_025350|Protein Name:fusion protein|Gene Symbol:F	MLWLTILIALVGNHESTCMNINFLQSLGQINSQKRFLNF YTQQPPSYMVIRLVPTLQLSANNCTLGSIVRYRNAIKELI QPMDENLRWLSSNLIPQRRGKRFAGVAVGLAALGVAV AAQATAAVALVEARANAEKIASMSQSIQETNKAVTSLS QAVSASGIAIQAIQNEINNVIHPILNQVQCDVLDARVGNI LNLYLIKVTTIFQNQLTNPALQRLSTQALSMLMQSTSSY LRNLSSSESAINADLSMTNLIEAQIVGINMTNLQLVLAVF IPSIARLNGALLYDFISITISSNQTEVMLQIPHRVLEIGNSL YTFEGTQCEMTKLNAYCLYSDAIPVTESLRDCMNGLFS QCGFVRIIGSFANRFASVNGVIYANCKHLTCSCLQPDEII TQDTNVPLTIIDTKRCTKISLGHLTFTIREYANVTYSLRTE IANSQITVVSPLDLSSQLTTINNSLADATNHIMNSDRILD RLNSGLYSKWVIIFLICASIVSLIGLVFLGFLIRGLILELRS KHRSNLNKASTYSIDSSIGLT	2	41
NC_025352	5950-8712	gb:NC_025352:5950-8712|Organism:Mojiang virus|Strain Name:Tongguan1|Protein Name:fusion protein|Gene Symbol:F	MALNKNMFSSLFLGYLLVYATTVQSSIHYDSLSKVGVIK GLTYNYKIKGSPSTKLMVVKLIPNIDSVKNCTQKQYDEY KNLVRKALEPVKMAIDTMLNNVKSGNNKYRFAGAIMA GVALGVATAATVTAGIALHRSNENAQAIANMKSAIQNT NEAVKQLQLANKQTLAVIDTIRGEINNNIIPVINQLSCDTI GLSVGIRLTQYYSEIITAFGPALQNPVNTRITIQAISSVFN GNFDELLKIMGYTSGDLYEILHSELIRGNIIDVDVDAGYI ALEIEFPNLTLVPNAVVQELMPISYNIDGDEWVTLVPRF VLTRTTLLSNIDTSRCTITDSSVICDNDYALPMSHELIGCL QGDTSKCAREKVVSSYVPKFALSDGLVYANCLNTICRC MDTDTPISQSLGATVSLLDNKRCSVYQVGDVLISVGSYL GDGEYNADNVELGPPIVIDKIDIGNQLAGINQTLQEAED	2	42

Genbank ID	Nucleotides of CDS	Full Gene Name	Sequence	#Sequences/Cluster	SEQ ID NO
			YIEKSEEFLKGVNPSIITLGSMVVLYIFMILIAIVSVIALVL SIKLTVKGNVVRQQFTYTQHVPSMENINYVSH		
NC_025363	4622-6262	gb:NC_025363:4622-6262|Organism:Avian paramyxovirus 12|Strain Name:Wigeon/Italy/3920_1/2005|Protein Name:fusion protein|Gene Symbol:F	MAIPVPSSTALMIFNILVSLAPASALDGRLLLGAGIVPTG DRQVNVYTSSQTGIIALKLLPNLPKDKENCAEVSIRSYN ETLTRILTPLAQSMAAIRGNSTVSTRGREPRLVGAIIGGV ALGVATAAQITAATALIQANQNAENIARLAKGLAATNE AVTDLTKGVGSLAIGVGKLQDYVNEQFNRTGEAIECLTI ESRVGVQLSLYLTEVIGVFGDQITSPALSDISIQALYNLA GGNLNVLLQKMGIEGTQLGSLINSGLIKGRPIMYDDGNK ILGIQVTLPSVGRINGARATLLEAIAVATPKGNASPLIPRA VISVGSLVEELDMTPCVLTPTDIFCTRILSYPLSDSLTTCL KGNLSSCVFSRTEGALSTPYVSVHGKIVANCKSVVCRC VEPQQIISQNYGEALSLIDESLCRILELNGVILKMDGQFTS EYTKNITIDPVQVIISGPIDISSELSQVNQSLDSALENIKES NSYLSKVNVKLISSSAMITYIVITVICLILTFVALVLGIYS YTKIRSQQKTLIWMGNNIARSKEGNRF	2	43
NC_025373	4617-6582	gb:NC_025373:4617-6582|Organism:Avian paramyxovirus 3|Strain Name:turkey/Wisconsin/68|Protein Name:fusion protein|Gene Symbol:F	MASPMVPLLIITVVPALISSQSANIDKLIQAGIIMGSGKEL HIYQESGSLDLYLRLLPVIPSNLSHCQSEVITQYNSTVTR LLSPIAKNLNHLLQPRPSGRLFGAVIGSIALGVATSAQIS AAIALVRAQQNANDILALKAAIQSSNEAIKQLTYGQEKQ LLAISKIQKAVNEQVIPALTALDCAVLGNKLAAQLNLYL IEMTTIFGDQINNPVLTPIPLSYLLRLTGSELNDVLLQQTR SSLSLIHLVSKGLLSGQIIGYDPSVQGIIIRIGLIRTQRIDRS LVFXPYVLPITISSNIATPIIPDCVVKKGVIIEGMLKSNCIE LERDIICKTINTYQITKETRACLQGNITMCKYQQSRTQLS TPFITYNGVVIANCDLVSCRCIRPPMIITQVKGYPLTIINR NLCTELSVDNLILNIETNHNFSLNPTIIDSQSRLIATSPLEI DALIQDAQHHAAAALLKVEESNAHLLRVTGLGSSSWHII LILTLLVCTIAWLIGLSIYVCRIKNDDSTDKEPTTQSSNRG IGVGSIQYMT	2	44
NC_025386	5548-7206	gb:NC_025386:5548-7206|Organism:Salem virus|Strain Name:UNKNOWN-NC_025386|Protein Name:fusion protein|Gene Symbol:F	MNPLNQTLIAKVLGFLLLSSSFTVGQIGFENLTRIGVHQV KQYGYKLAHYNSHQLLLIRMIPTVNGTHNCTHQVITRY REMVREIITPIKGALDIMKKAVSPDLVGARIFGAIVAGAA LGIATSAQITAGVALHRTKLNGQEISKLKEAVSLTNEAV EQLQYSQGKSILAIQGIQDFINFNVVPLLEEHTCGIAKLH LEMALMEYFQKLILVFGPNLRDPIGSTIGIQALATLFQNN MFEVSLRLGYAGDDLEDVLQSNSIRANIIEAEPDSGFIVL AIRYPTLTLVEDQVITELAHITFNDGPQEWVATIPQFVTY RGLVLANIDVSTCTFTERNVICARDQTYPMIIDLQLCMR GNIAKCGRTRVTGSTASRFLLKDGNMYANCIATMCRC MSSSSIINQEPSHLTTLIVKETCSEVMIDTIRITLGERKHPP IDYQTTITLGQPIALAPLDVGTELANAVSYLNKSKVLLE HSNEVLSSVSTAHTSLTATIVLGIVVGGLAILIVVMFLFL EAQVIKVQRAMMLCPITNHGYLPNEDLLTRGHSIPTIG	2	45
NC_025390	4805-6460	gb:NC_025390:4805-6460|Organism:Avian paramyxovirus 9|Strain Name:duck/New York/22/1978|Protein Name:fusion protein|Gene Symbol:F	MGYFHLLLILTAIAISAHLCYTTTLDGRKLLGAGIVITEE KQVRVYTAAQSGTIVLRSFRVVSLDRYSCMESTIESYNK TVYNILAPLGDAIRRIQASGVSVERIREGRIFGAILGGVA LGVATAAQITAAIALIQANENAKNILRIKDSITKTNEAVR DVTNGVSQLTIAVGKLQDFVNKEFNKTTEAINCVQAAQ QLGVELSLYLTEITTVFGPQITSPALSKLTIQALYNLAGV SLDVLLGRLGADNSQLSSLVSSGLITGQPILYDSESQILA LQVSLPSISDLRGVRATYLDTLAVNTAAGLASAMIPKVV IQSNNIVEELDTTACIAAEADLYCTRITTFPIASAVSACIL GDVSQCLYSKTNGVLTTPYVAVKGKIVANCKHVTCRC VDPTSIISQNYGEAATLIDDQLCKVINLDGVSIQLSGTFES TYVRNVSISANKVIVSSSIDISNELENVNSSLSSALEKLDE	2	46

Genbank ID	Nucleotides of CDS	Full Gene Name	Sequence	#Sequences/Cluster	SEQ ID NO
			SDAALSKVNVHLTSTSAMATYIVLTVIALILGFVGLGLG CFAMIKVKSQAKTLLWLGAHADRSYILQSKPAQSST		
NC_025403	4826-6649	gb:NC_025403:4826-6649|Organism:Achimota virus 1|Strain Name:UNKNOWN-NC_025403|Protein Name:fusion protein|Gene Symbol:F	MWIMIILSLFQIIPGVTPINSKVLTQLGVITKHTRQLKFYS HSTPSYLVVKLVPTINTESTVCNFTSLSRYKDSVRELITP LAKNIDNLNSILTIPKRRKRMAGVVIGLAALGVAAAAQ ATAAVALIEAKKNTEQIQALSESIQNTNKAVSSIEKGLSS AAIAVQAIQNQINNVINPALTALDCGVTDAQLGNILNLY LIKTLTVFQKQITNPALQPLSIQALNIIMQETSSVLRNFTK TDEIEHTDLLTSGLITGQVVGVNLTNLQLIIAAFIPSIAPL NQAYILDFIRITVNINNSESMIQIPERIMEHGISLYQFGGD QCTFSDWSAYCPYSDATLMAPGLQNCFRGQAADCVFST VMGSFPNRFVSVQGVFYVNCKFIRCACTQPQRLITQDDS LSLTQIDAKTCRMLTLGFVQFSINEYANVTYSFKNNVTA GQLIMTNPIDLSTEIKQMNDSVDEAARYIEKSNAALNKL MYGGRSDIVTTVLLVGFILLVVYVIFVTYILKILMKEVA RLRNSNHPDLIKPYNYPM	2	47
NC_025404	4772-6647	gb:NC_025404:4772-6647|Organism:Achimota virus 2|Strain Name:UNKNOWN-NC_025404|Protein Name:fusion protein|Gene Symbol:F	MLNSFYQIICLAVCLTTYTVISIDQHNLLKAGVIVKSIKG LNFYSRGQANYIIVKLIPNVNVTDTDCDIGSIKRYNETVY SLIKPLADNIDYLRTQFAPTKRKKRFAGVAIGLTALGVA TAAQVTAAVALVKAQENARKLDALADSIQATNEAVQD LSTGLQAGAIAIQAIQSEINHVINPALERLSCEIIDTRVASI LNLYLIRLTTVFHRQLVNPALTPLSIQALNHLLQGETEGL VKNESKMTDSKIDLLMSGLITGQVVGVNIKHMQLMIAV FVPTTAQLPNAYVINLLTITANINNSEVLVQLPNQILERS GIIYQFRGKDCVSSPNHMYCPYSDASILSPELQLCLQGRL EMCLFTQVVGSFPTRFASDKGIVYANCRHLQCACSEPEG IIYQDDTSAITQIDASKCSTLKLDMLTFKLSTYANKTFDA SFSVGKDQMLVTNLLDLSAELKTMNASVAHANKLIDKS NLLIQSNALIGHSNTIFIVVIVILAVMVLYLIIVTYIIKVIM VEVSRLKRMNIYSIDK	2	48
NC_025410	4958-6751	gb:NC_025410:4958-6751|Organism:Tuhoko virus 1|Strain Name:UNKNOWN-NC_025410|Protein Name:fusion protein|Gene Symbol:F	MVTIIKPLILLVTVILQISGHIDTTALTSIGAVIASSKEIMY YAQSTPNYIVIKLIPNLPNIPSQCNFSSIAYYNKTLLDLFT PISDNINMLHQRLSNTGRNRRFAGVAIGLAALGVATAA QVTAAFALVEAKSNTAKIAQIGQAIQNTNAAINSLNAGI GGAVTAIQAIQTQINGIITDQINAATCTALDAQIGTLLNM YLLQLTTTFQPQIQNPALQPLSIQALHRIMQGTSIVLSNL TDSSKYGLNDALSAGLITGQIVSVDLRLMQITIAANVPTL SRLENAIAHDIMRITTNVNNTEVIVQLPETIMEHAGRLY QFNKDHCLSSTQRFFCPYSDAKLLTSKISSCLSGIRGDCIF SPVVGNFATRFISVKGVIIANCKFIRCTCLQPEGIISQLDD HTLTVIDLKLCNKLDLGLIQFDLQVLSNISYEMTLNTSQ NQLILTDPLDLSSELQTMNQSINNAANFIEKSNSLLNSST YEFNRSVALLVALILLSLTILYVIVLTCVVKLLVHEVSKN RRHIQDLESHHK	2	49
NC_028249	4850-7055	gb:NC_028249:4850-7055|Organism:Phocine distemper virus|Strain Name:PDV/Wadden_Sea.NLD/1988|Protein Name:fusion protein|Gene Symbol:F	MTRVKKLPVPTNPPMHHSLDSPFLNPEHATGKISITDDT SSQLTNFLYHKYHKTTINHLSRTISGTDPPSAKLNKFGSP ILSTYQIRSALWWIAMVILVHCVMGQIHWTNLSTIGIIGT DSSHYKIMTRSSHQYLVLKLMPNVSIIDNCTKAELDEYE KLLNSVLEPINQALTLMTKNVKSLQSLGSGRRQRRFAG VVIAGAALGVATAAQITAGVALYQSNLNAQAIQSLRAS LEQSNKAIDEVRQASQNIIIAVQGVQDYVNNEIVPALQH MSCELIGQRLGLKLLRYYTELLSVFGPSLRDPVSAEISIQ ALSYALGGEIHKILEKLGYSGNDMVAILETKGIRAKITH VDLSGKFIVLSISYPTLSEVKGVVVHRLEAVSYNIGSQE WYTTVPRYVATNGYLISNFDESSCVFVSESAICSQNSLY PMSPILQQCLRGETASCARTLVSGTLGNKFILSKGNIIAN	2	50

Genbank ID	Nucleotides of CDS	Full Gene Name	Sequence	#Sequences/Cluster	SEQ ID NO
			CASILCKCHSTSKIINQSPDKLLTFIASDTCSLVEIDGVTIQ VGSRQYPDVVYASKVILGPAISLERLDVGTNLGSALKKL NDAKVLIESSDQILDTVKNSYLSLGTLIALPVSIGLGLILL LLICCCKKRYQHLFSQSTKVAPVFKPDLTGTSKSYVRSL		
NC_028362	5217-6842	gb:NC_028362:5217-6842|Organism:Caprine parainfluenza virus 3|Strain Name:JS2013|Protein Name:fusion protein|Gene Symbol:F	MIKKIICIFSMPILLSFCQVDIIKLQRVGILVSKPKSIKISQN FETRYLVLNLIPNIENAQSCGDQQIKQYKKLLDRLIIPLY DGLRLQQDIIVVDNNLKNNTNHRAKRFFGEIIGTIALGV ATSAQITAAVALVEAKQARSDIERVKNAVRDTNKAVQS IQGSVGNLIVAVKSVQDYVNNEIVPSIKRLGCEAAGLQL GIALTQHYSELTNIFGDNIGTLKEKGIKLQGIASLYHTNIT EIFTTSTVDQYDIYDLLFTESIKMRVIDVDLNDYSITLQV RLPLLTKISDAQIYNVDSVSYNIGGTEWYIPLPRNIMTKG AFLGGANLQDCIESFSDYICPSDPGFILNRDIENCLSGNIT QCPKTLVISDIVPRYAFVDGGVIANCLSTTCTCNGIDNRI NQAPDQGIKIITYKDCQTIGINGMLFKTNQEGTLAAYTP VDITLNNSVNLDPIDLSIELNRARSDLAESKEWIKRSEAK LDSVGSWYQSSTTEIIQIVMIIVLFIINIIVLIVLIKYSRSQN QSMNNHMNEPYILTNKVQ	2	51
AF079780	5919-7580	gb:AF079780|Organism:Tupaia paramyxovirus|Strain Name:UNKNOWN-AF079780|Protein Name:fusion protein|Gene Symbol:F	MASLLKTICYIYLITYAKLEPTPKSQLDLDSLASIGVVDA GKYNYKLMTTGSEKLMVIKLVPNITYATNCNLTAHTAY TKMIERLLTPINQSLYEMRSVITERDGGTIFWGAIIAGAA LGVATAAAITAGVALHRAEQNARNIAALKDALRNSNEA IQHLKDAQGHTVLAIQGLQEQINNNIIPKLKESHCLGVN NQLGLLLNQYYSEILTVFGPNLQNPVSASLTIQAIAKAFN GDFNSLMTNLNYDPTDLLDILESNSINGRIIDVNLNEKYI ALSIEIPNFITLTDAKIQTFNRITYGYGSNEWLTLIPDNILE YGNLISNVDLTSCVKTKSSYICNQDTSYPISSELTRCLRG DTSSCPRTPVVNSRAPTFALSGGHIYANCAKAACRCEKP PMAIVQPATSTLTFLTEKECQEVVIDQINIQLAPNRLNKT IITDGIDLGPEVIINPIDVSAELGNIELEMDKTQKALDRSN KILDSMITEVTPDKLLIAMIVVFGILLLWLFGVSYYAFKI WSKLHFLDSYVYSLRNPSHHRSNGHQNHSFSTDISG	1	52
EU403085	4664-6585	gb:EU403085:4664-6585|Organism:Avian paramyxovirus 3|Strain Name:APMV3/PKT/Netherland/449/75|Protein Name:fusion protein|Gene Symbol:F	MQPGSALHLPHLYIIIALVSDGTLGQTAKIDRLIQAGIVL GSGKELHISQDSGTLDLFVRLLPVLPSNLSHCQLEAITQY NKTVTRLLAPIGKNLEQVLQARPRGRLFGPIIGSIALGVA TSAQITAAIALVRAQQNANDILALKNALQSSNEAIRQLT YGQDKQLLAISKIQKAVNEQILPALDQLDCAVLGTKLA VQLNLYLIEMTTIFGEQINNPVLATIPLSYILRLTGAELNN VLMKQARSSLSLVQLVSKGLLSGQVIGYDPSVQGLIIRV NLMRTQKIDRALVYQPYVLPITLNSNIVTPIAPECVIQKG TIIEGMSRKDCTELEQDIICRTVTTYTLARDTRLCLQGNI SSCRYQQSGTQLHTPFITYNGAVIANCDLVSCRCLRPPMI ITQVKGYPLTIITRSVCQELSVDNLVLNIETHHNFSLNPTI IDPLTRVIATTPLEIDSLIQEAQDHANAALAKVEESDKYL RAVTGGNYSNWYIVLVIVLLFGNLGWSLLLTVLLCRSR KQQRRYQQDDSVGSERGVGVGTIQYMS	1	53
KX258200	4443-6068	gb:KX258200:4443-6068|Organism:Avian paramyxovirus 14|Strain Name:APMV14/duck/Japan/11OG0352/2011|Protein Name:fusion	MEKGTVLFLAALTLYNVKALDNTKLLGAGIASGKEHEL KIYQSSVNGYIAVKLIPFLPSTKRECYNEQLKNYNATINR LMGPINDNIKLVLSGVKTRTREGKLIGAIIGTAALGLATA AQVTAAIALEQAQDNARAILTLKESIRNTNNAVSELKTG LSEVSIALSKTQDYINTQIMPALSNLSCEIVGLKIGIQLSQ YLTEVTAVFGNQITNPALQPLSMQALYQLCGGDFSLLL DKIGADRNELESLYEANLVTGRIVQYDTADQLVIIQVSIP SVSTLSGYRVTELQSISVDMDHGEGKAVIPRYIVTSGRVI EEMDISPCVLTATAVYCNRLLTTSLPESVLKCLDGDHSS CTYTSNSGVLETRYIAFDGMLIANCRSIVCKCLDPPYIIP	1	54

Genbank ID	Nucleotides of CDS	Full Gene Name	Sequence	#Sequences/Cluster	SEQ ID NO
		protein|Gene Symbol:F	QNKGKPLTIISKEVCKKVTLDGITLLIDAEFTGEYGLNITI GPDQFAPSGALDISTELGKLNNSINKAEDYIDKSNELLNR VNVDIVNDTAVIVLCVMSALVVVWCIGLTVGLIYVSKN TLRAVAIKGTSIENPYVSSGKHAKNSS		
KY51104 4	4592-6247	gb:KY511044:4592-6247|Organism:Avian paramyxovirus UPO216|Strain Name:APMV-15/WB/Kr/UPO216/2014|Protein Name:fusion protein|Gene Symbol:F	MIFTMYHVTVLLLLSLLTLPLGIQLARASIDGRQLAAAGI VVTGEKAINLYTSSQTGTIVVKLLPNVPQGREACMRDPL TSYNKTLTSLLSPLGEAIRRIHESTTETAGLVQARLVGAII GSVALGVATSAQITAAAALIQANKNAENILKLKQSIAAT NEAVHEVTDGLSQLAVAVGKMQDFINTQFNNTAQEIDC IRISQQLGVELNLYLTELTTVFGPQITSPALSPLSIQALYN LAGGNLDVLLSKIGVGNNQLSALISSGLISGSPILYDSQT QLLGIQVTLPSVSSLNNMRAIFLETLSVSTDKGFAAALIP KVVTTVGTVTEELDTSYCIETDIDLFCTRIVTFPMSPGIY ACLNGNTSECMYSKTQGALTTPYMSVKGSIVANCKMT TCRCADPASIISQNYGEAVSLIDSSVCRVITLDGVTLRLS GSFDSTYQKNITIRDSQVIITGSLDISTELGNVNNSINNAL DKIEESNQILESVNVSLTSTNALIVYIICTALALICGITGLI LSCYIMYKMRSQQKTLMWLGNNTLDQMRAQTKM	1	55
NC_025360	6104-8123	gb:NC_025360:6104-8123|Organism:Atlantic salmon paramyxovirus|Strain Name:ASPV/Yrkje371/95|Protein Name:fusion protein|Gene Symbol:F	MDGPKFRFVLLILLTAPARGQVDYDKLLKVGIFEKGTA NLKISVSSQQRYMVIKMMPNLGPMNQCGIKEVNLYKES ILRLITPISTTLNYIKSEIQVEREVALQPNGTIVRFFGLIVA AGALTLATSAQITAGIALHNSLENAKAIKGLTDAIKESNL AIQKIQDATAGTVIALNALQDQVNTNIIPAINTLGCTAAG NTLGIALTRYYSELIMIFGPSLGNPVEAPLTIQALAGAFN GDLHGMIREYGYTPSDIEDILRTNSVTGRVIDVDLVGMN IVLEINLPTLYTLRDTKIVNLGKITYNVDGSEWQTLVPE WLAIRNTLMGGVDLSRCVVSSRDLICKQDPVFSLDTSIIS CLNGNTESCPRNRVVNSVAPRYAVIRGNILANCISTTCL CGDPGVPIIQKGDNTLTAMSINDCKLVGVDGYVFRPGP KAVNVTFNLPHLNLGPEVNVNPVDISGALGKVEQDLAS SRDHLAKSEKILSGINPNIINTEMVLVAVILSLVCAMVVI GIVCWLSILTKWVRSCRADCRRPNKGPDLGPIMSSQDNL SF	1	56

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: 57-132, 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: 57-132. In some embodiments, a nucleic acid sequence described herein encodes an amino acid sequence set forth in any one of SEQ ID NOS: 57-132, 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 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/TH_10506/2014|Protein Name:attachment glycoprotein|Gene Symbol:G	MSKTKDQRTAKTLERTWDTLNHLLFISSCLYKLNLKSIAQITLSILAMIISTSLIIAAIIFIASANHKVTLTTAIIQDATNQIKNTTPTYLTQNPQLGISFSNLSGTTLQSTTILASTTPSAESTPQSTTVKIINTTTTQILPSKPTTKQRQNKPQNKPNNDFHFEVFNFVPCSICSNNPTCWAICKRIPNKKPGKKTTTKPTKKPTLKTTKKDPKPQTTKPKEALTTKPTGKPTINTTKTNIRTTLLTSNTKGNPEHTSQEETLHSTTSEGYLSPSQVYTTSGQEETLHSTTSEGYLSPSQVYTTSEYLSQSLSSSNTTK	706	57

Genbank ID	Nucleotides of CDS	Full sequence ID	Sequence	#Sequences/Cluster	SEQ ID NO
AB524405	6424-8274	gb:AB524405:6424-8274|Organism:Newcastle disease virus|Strain Name:Goose/Alaska/415/91|Protein Name:hemagglutinin-neuraminidase protein|Gene Symbol:HN	MERGVSQVALENDEREAKNTWRLVFRVTVLFLTIVTLA ISAAALAFSMNASTPQDLEGIPVAISKVEDKITSALGASQ DVMDRIYKQVALESPLALLNTESTIMNALTSLSYQINGA ANASGCGAPVPDPDYIGGIGKELIVDDTSDVTSFYPSAF QEHLNFIPAPTTGSGCTRIPSFDMSATHYCYTHNVILSGC RDHSHSHQYLALGVLRTSATGRVFFSTLRSINLDDTQNR KSCSVSATPLGCDMLCSKVTETEEEDYQSTDPTLMVHG RLGFDGQYHERDLDVHTLFGDWVANYPGVGGGSFINN RVWFPVYGGLKPGSPTDKRQEGQYAIYKRYNDTCPDD QEYQVRMAKSAYKPNRFGGKRVQQAILSIGVSTTLADD PVLTVTSNTITLMGAEGRVMTVGTSHYLYQRGSSYYSP AILYPLTIANKTATLQDPYKFNAFTRPGSVPCQASARCP NSCVTGVYTDPYPIVFHKNHTLRGVFGTMLDDEQARLN PVSAVFDSIARSRVTRVSSSSTKAAYTTSTCFKVVKTGK VYCLSIAEISNTLFGEFRIVPLLVEILRDEGRSEARSALTT QGHPGWNDEVVDPIFCAVTNQTDHRQKLEEYAQSWP	418	58
JQ582844	4686-5636	gb:JQ582844:4686-5636|Organism:Human respiratory syncytial virus|Strain Name:NH1067|Protein Name:receptor-binding glycoprotein|Gene Symbol:G	MSKNKNQRTARTLEKTWDTLNHLIVISSCLYKLNLKSIA QIALSVLAMIISTSLIIAAIIFIISANHKVTLTTVTVQTIKNH TEKNITTYLTQVSPERVSPSKQPTTTPPIHTNSATISPNTK SETHHTTAQTKGRTTTPTQNNKPSTKPRPKNPPKKPKDD YHFEVFNFVPCSICGNNQLCKSICKTIPNNKPKKKPTTKP TNKPPTKTTNKRDPKTPAKTLKKETTINPTTKKPTPKTTE RDTSTPQSTVLDTTTSKHTERDTSTPQSTVLDTTTSKHTI QQQSLHSITPENTPNSTQTPTASEPSTSNSTQKL	278	59
AB254456	7271-9136	gb:AB254456:7271-9136|Organism:Measles virus|Strain Name:SSPE-Kobe-1|Protein Name:Hemagglutinin|Gene Symbol:H	MSPHRDRINAFYRDNPHPKGSRIVINREHLMIDRPYVLL AVLFVMFLSLIGLLAIAGIRLHRAAIYTAEIHKSLSTNLD VTNSIEHQVKDVLTPLFKIIGDEVGLRTPQRFTDLVKFIS DKIKFLNPDREYDFRDLTWCINPPERIKLDYDQYCADVA AEELMNALVNSTLLEARATNQFLAVSKGNCSGPTTIRG QFSNMSLSLLDLYLSRGYNVSSIVTMTSQGMYGGTYLV GKPNLSSKGSELSQLSMHRVFEVGVIRNPGLGAPVFHM TNYFEQPVSNDFSNCMVALGELRFAALCHREDSVTVPY QGSGKGVSFQLVKLGVWKSPTDMQSWVPLSTDDPVID RLYLSSHRGVIADNQAKWAVPTTRTDDKLRMETCFQQ ACKGKNQALCENPEWAPLKDNRIPSYGVLSVNLSLTVE LKIKIASGFGPLITHGSGMDLYKTNHDNVYWLTIPPMKN LALGVINTLEWIPRFKVSPNLFTVPIKEAGEDCHAPTYLP AEVDGDVKLSSNLVILPGQDLQYVLATYDTSRVEHAVV YYVYSPSRSFSYFYPFRLPIKGVPIELQVECFTWDQKLW CRHFCVLADSESGGHITHSGMVGMGVSCTVTREDGTNR RQGCQ	128	60
AB040874	6614-8362	gb:AB040874:6614-8362|Organism:Mumps virus|Strain Name:Miyahara|Protein Name:hemagglutinin-neuraminidase|Gene Symbol:HN	MEPSKLFTMSDNATFAPGPVINAADKKTFRTCFRILVLS VQAVTLILVIVTLGELVRMINDQGLSNQLSSIADKIRESA TMIASAVGVMNQVIHGVTVSLPLQIEGNQNQLLSTLATI CTGKKQVSNCSTNIPLVNDLRFINGINKFIIEDYATHDFSI GHPLNMPSFIPTATSPNGCTRIPSFSLGKTHWCYTHNVIN ANCKDHTSSNQYISMGILVQTASGYPMFKTLKIQYLSDG LNRKSCSIATVPDGCAMYCYVSTQLETDDYAGSSPPTQ KLTLLFYNDTVTERTISPTGLEGNWATLVPGVGSGIYFE NKLIFPAYGGVLPNSSLGVKSAREFFRPVNPYNPCSGPQ QDLDQRALRSYFPSYFSNRRVQSAFLVCAWNQILVTNC ELVVPSNNQTLMGAEGRVLLINNRLLYYQRSTSWWPYE LLYEISFTFTNSGQSSVNMSWIPIYSFTRPGSGNCSGENV CPTACVSGVYLDPWPLTPYSHQSGINRNFYFTGALLNSS	87	61

Genbank ID	Nucleotides of CDS	Full sequence ID	Sequence	#Sequences/Cluster	SEQ ID NO
			TTRVNPTLYVSALNNLKVLAPYGNQGLFASYTTTTCFQ DTGDASVYCVYIMELASNIVGEFQILPVLTRLTIT		
AB736166	6709-8427	gb:AB736166:6709-8427|Organism:Human respirovirus 3|Strain Name:ZMLS/2011|Protein Name:hemagglutinin-neuraminidase|Gene Symbol:HN	MEYWKHTNHGKDAGNELETATATHGNRLTNKITYILW TITLVLLSIVFIIVLINSIKSEKAHESLLQDINNEFMEVTEK IQVASDNTNDLIQSGVNTRLLTIQSHVQNYIPISLTQQISD LRKFISEITIRNDNQEVPPQRITHDVGIKPLNPDDFWRCTS GLPSLMRTPKIRLMPGPGLLAMPTTVDGCVRTPSLVIND LIYAYTSNLITRGCQDIGKSYQVLQIGIITVNSDLVPDLNP RISHTFNINDNRKSCSLALLNTDVYQLCSTPKVDERSDY ASSGIEDIVLDIVNYDGSISTTRFKNNNISFDQPYAALYPS VGPGIYYKGKIIFLGYGGLEHPINENAICNTTGCPGKTQR DCNQASHSPWFSDRRMVNSIIVVDKGLNSVPKLKVWTI SMRQNYWGSEGRLLLLGNKIYIYTRSTSWHSKLQLGIID ITDYSDIRIKWTWHNVLSRPGNNECPWGHSCPDGCITGV YTDAYPLNPTGSIVSSVILDSQKSRVNPVITYSTATERVN ELAIRNKTLSAGYTTTSCITHYNKGYCFHIVEINHKSLNT FQPMLFKTEIPKSCS	78	62
KJ627396	6166-6885	gb:KJ627396:6166-6885|Organism:Human metapneumovirus|Strain Name:HMPV/Homo sapiens/PER/FLI1305/2010/A|Protein Name:attachment glycoprotein G|Gene Symbol:G	MEVKVENIRAIDMLKARVKNRVARSKCFKNASLILIGIT TLSIALNIYLIINYTIQKTTSESEHHTSSPPTESNKETSTIPI DNPDITPNSQHPTQQSTESLTLYPASSMSPSETEPASTPGI TNRLSLADRSTTQPSESRTKTNSTVHKKNKKNISSTISRT QSPPRTTAKAVSRTTALRMSSTGERPTTTSVQSDSSTTA QNHEETGPANPQASVSTM	71	63
AB475097	7079-8902	gb:AB475097:7079-8902|Organism:Canine distemper virus|Strain Name:M25CR|Protein Name:hemagglutinin|Gene Symbol:H	MLSYQDKVGAFYKDNARANSSKLSLVTEEQGGRRPPYL LFVLLILLVGILALLAIAGVRFRQVSTSNVEFGRLLKDDL EKSEAVHHQVMDVLTPLFKIIGDEIGLRLPQKLNEIKQFI LQKTNFFNPNREFDFRDLHWCINPPSKIKVNFTNYCDAI GVRKSIASAANPILLSALSGGRGDIFPPYRCSGATTSVGR VFPLSVSLSMSLISKTSEIISMLTAISDGVYGKTYLLVPDY IEREFDTQKIRVFEIGFIKRWLNDMPLLQTTNYMVLPENS KAKVCTIAVGELTLASLCVDESTVLLYHDSNGSQDSILV VTLGIFGATPMNQVEEVIPVAHPSVERIHITNHRGFIKDS VATWMVPALVSEQQEGQKNCLESACQRKSYPMCNQTS WEPFGGVQLPSYGRLTLPLDASIDLQLNISFTYGPVILNG DGMDYYENPLLDSGWLTIPPKNGTILGLINKASRGDQFT VTPHVLTFAPRESSGNCYLPIQTSQIMDKDVLTESNLVV LPTQNFRYVVATYDISRENHAIVYYVYDPIRTISYTYPFR LTTKGRPDFLRIECFVWDDDLWCHQFYRFESDITNSTTS VEDLVRIRFSCNRSKP	45	64
AJ849636	7326-9155	gb:AJ849636:7326-9155|Organism:Peste-des-petits-ruminants virus|Strain Name:Turkey 2000|Protein Name:haemagglutinin|Gene Symbol:H	MSAQRERINAFYKDNPHNKNHRVILDRERLVIERPYILL GVLLVMFLSLIGLLAIAGIRLHRATVGTSEIQSRLNTNIEL TESIDHQTKDVLTPLFKIIGDEVGIRIPQKFSDLVKFISDKI KFLNPDREYDFRDLRWCMNPPERVKINFDQFCEYKAAV KSIEHIFESPLNKSKKLQSLTLGPGTGCLGRTVTRAHFSE LTLTLMDLDLEMKHNVSSVFTVVEEGLFGRTYTVWRSD ARDPSTDLGIGHFLRVFEIGLVRDLGLGPPVFHMTNYLT VNMSDDYRRCLLAVGELKLTALCSSSETVTLGERGVPK REPLVVVILNLAGPTLGGELYSVLPTSDLMVEKLYLSSH RGIIKDDEANWVVPSTDVRDLQNKGECLVEACKTRPPS FCNGTGSGPWSEGRIPAYGVIRVSLDLASDPGVVITSVF	34	65

Genbank ID	Nucleotides of CDS	Full sequence ID	Sequence	#Sequences/Cluster	SEQ ID NO
			GPLIPHLSGMDLYNNPFSRAVWLAVPPYEQSFLGMINTI GFPNRAEVMPHILTTEIRGPRGRCHVPIELSRRVDDDIKI GSNMVILPTIDLRYITATYDVSRSEHAIVYYIYDTGRSSS YFYPVRLNFKGNPLSLRIECFPWRHKVWCYHDCLIYNTI TDEEVHTRGLTGIEVTCNPV		
AB005795	6693-8420	gb:AB005795:6693-8420|Organism:Sendai virus|Strain Name:Ohita|Protein Name:hemagglutinin-neuraminidase protein|Gene Symbol:HN	MDGDRSKRDSYWSTSPGGSTTKLVSDSERSGKVDTWLL ILAFTQWALSIATVIICIVIAARQGYSMERYSMTVEALNT SNKEVKESLTSLIRQEVITRAANIQSSVQTGIPVLLNKNS RDVIRLIEKSCNRQELTQLCDSTIAVHHAEGIAPLEPHSF WRCPAGEPYLSSDPEVSLLPGPSLLSGSTTISGCVRLPSLS IGEAIYAYSSNLITQGCADIGKSYQVLQLGYISLNSDMFP DLNPVVSHTYDINDNRKSCSVVATGTRGYQLCSMPIVD ERTDYSSDGIEDLVLDILDLKGRTKSHRYSNSEIDLDHPF SALYPSVGSGIATEGSLIFLGYGGLTTPLQGDTKCRIQGC QQVSQDTCNEALKITWLGGKQVVSVLIQVNDYLSERPRI RVTTIPITQNYLGAEGRLLKLGDQVYIYTRSSGWHSQLQ IGVLDVSHPLTISWTPHEALSRPGNEDCNWYNTCPKECI SGVYTDAYPLSPDAANVATVTLYANTSRVNPTIMYSNT TNIINMLRIKDVQLEAAYTTTSCITHFGKGYCFHIIEINQK SLNTLQPMLFKTSIPKLCKAES	23	66
AF457102	6903-8630	gb:AF457102|Organism:Human parainfluenza virus 1 strain Washington/1964|Strain Name:Washington 1964|Protein Name:HN glycoprotein|Gene Symbol:HN	MAEKGKTNSSYWSTTRNDNSTVNTHINTPAGRTHIWLL IATTMHTVLSFIIMILCIDLIIKQDTCMKTNIMTVSSMNES AKIIKETITELIRQEVISRTINIQSSVQSGIPILLNKQSRDLT QLIEKSCNRQELAQICENTIAIHHADGISPLDPHDFWRCP VGEPLLSNNPNISLLPGPSLLSGSTTISGCVRLPSLSIGDAI YAYSSNLITQGCADIGKSYQVLQLGYISLNSDMYPDLNP VISHTYDINDNRKSCSVIAAGTRGYQLCSLPTVNETTDY SSEGIEDLVFDILDLKGKTKSHRYKNEDITFDHPFSAMYP SVGSGIKIENTLIFLGYGGLTTPLQGDTKCVINRCTNVNQ SVCNDALKITWLKKRQVVNVLIRINNYLSDRPKIVVETIP ITQNYLGAEGRLLKLGKKIYIYTRSSGWHSNLQIGSLDIN NPMTIKWAPHEVLSRPGNQDCNWYNRCPRECISGVYTD AYPLSPDAVNVATTTLYANTSRVNPTIMYSNTSEIINML RLKNVQLEAAYTTTSCITHFGKGYCFHIVEINQASLNTL QPMLFKTSIPKICKITS	21	67
KJ627397	6146-6888	gb:KJ627397:6146-6888|Organism:Human metapneumovirus|Strain Name:HMPV/Homo sapiens/PER/FPP00098/2010/B|Protein Name:attachment glycoprotein G|Gene Symbol:G	MEVRVENIRAIDMFKAKMKNRIRSSKCYRNATLILIGLT ALSMALNIFLIIDYATLKNMTKVEHCVNMPPVEPSKKSP MTSAADLNTKLNPQQATQLTTEDSTSLAATSENHLHTE TTPTSDATISQQATDEHTTLLRPINRQTTQTTTEKKPTGA TTKKDKEKETTTRTTSTAATQTLNTTNQTSNGREATTTS ARSRNGATTQNSDQTIQAADPSSKPYHTQTNTTTAHNT DTSSLSS	21	68
AF017149	8913-10727	gb:AF017149|Organism:Hendra virus|Strain Name:UNKNOWN-AF017149|Protein Name:glycoprotein|Gene Symbol:G	MMADSKLVSLNNNLSGKIKDQGKVIKNYYGTMDIKKIN DGLLDSKILGAFNTVIALLGSIIIIVMNIMIIQNYTRTTDN QALIKESLQSVQQQIKALTDKIGTEIGPKVSLIDTSSTITIP ANIGLLGSKISQSTSSINENVNDKCKFTLPPLKIHECNISC PNPLPFREYRPISQGVSDLVGLPNQICLQKTTSTILKPRLI SYTLPINTREGVCITDPLLAVDNGFFAYSHLEKIGSCTRG IAKQRIIGVGEVLDRGDKVPSMFMTNVWTPPNPSTIHHC SSTYHEDFYYTLCAVSHVGDPILNSTSWTESLSLIRLAVR PKSDSGDYNQKYIAITKVERGKYDKVMPYGPSGIKQGD	14	69

Genbank ID	Nucleotides of CDS	Full sequence ID	Sequence	#Sequences/Cluster	SEQ ID NO
			TLYFPAVGFLPRTEFQYNDSNCPIIHCKYSKAENCRLSM GVNSKSHYILRSGLLKYNLSLGGDIILQFIEIADNRLTIGS PSKIYNSLGQPVFYQASYSWDTMIKLGDVDTVDPLRVQ WRNNSVISRPGQSQCPRFNVCPEVCWEGTYNDAFLIDR LNWVSAGVYLNSNQTAENPVFAVFKDNEILYQVPLAED DTNAQKTITDCFLLENVIWCISLVEIYDTGDSVIRPKLFA VKIPAQCSES		
AF212302	8943-10751	gb:AF212302|Organism:Nipah virus|Strain Name:UNKNOWN-AF212302|Protein Name:attachment glycoprotein|Gene Symbol:G	MPAENKKVRFENTTSDKGKIPSKVIKSYYGTMDIKKINE GLLDSKILSAFNTVIALLGSIVIIVMNIMIIQNYTRSTDNQ AVIKDALQGIQQQIKGLADKIGTEIGPKVSLIDTSSTITIPA NIGLLGSKISQSTASINENVNEKCKFTLPPLKIHECNISCP NPLPFREYRPQTEGVSNLVGLPNNICLQKTSNQILKPKLI SYTLPVVGQSGTCITDPLLAMDEGYFAYSHLERIGSCSR GVSKQRIIGVGEVLDRGDEVPSLFMTNVWTPPNPNTVY HCSAVYNNEFYYVLCAVSTVGDPILNSTYWSGSLMMTR LAVKPKSNGGGYNQHQLALRSIEKGRYDKVMPYGPSGI KQGDTLYFPAVGFLVRTEFKYNDSNCPITKCQYSKPENC RLSMGIRPNSHYILRSGLLKYNLSDGENPKVVFIEISDQR LSIGSPSKIYDSLGQPVFYQASFSWDTMIKFGDVLTVNPL VVNWRNNTVISRPGQSQCPRFNTCPEICWEGVYNDAFLI DRINWISAGVFLDSNQTAENPVFTVFKDNEILYRAQLAS EDTNAQKTITNCFLLKNKIWCISLVEIYDTGDNVIRPKLF AVKIPEQCT	14	70
EU439428	6751-8638	gb:EU439428:6751-8638|Organism:Swine parainfluenza virus 3|Strain Name:92-7783_ISU-92|Protein Name:hemagglutinin-neuraminidase HN|Gene Symbol:HN	MEYWKHTNSTKDTNNELGTTRDRHSSKATNIIMYIFWT TTSTILSVIFIMILINLIQENNHNKLMLQEIKKEFAVIDTKI QKTSDDISTSIQSGINTRLLTIQSHVQNYIPLSLTQQMSDL RKFINDLTTKREHQEVPIQRMTHDSGIEPLNPDKFWRCT SGNPSLTSSPKIRLIPGPGLLATSTTVNGCIRIPSLAINNLI YAYTSNLITQGCQDIGKSYQVLQIGIITINSDLVPDLNPR VTHTFNIDDNRKSCSLALLNTDVYQLCSTPKVDERSDY ASTGIEDIVLDIVTSNGLIITTRFTNNNITFDKPYAALYPS VGPGIYYKDKVIFLGYGGLEHEENGDVICNTTGCPGKTQ RDCNQASYSPWFSNRRMVNSIIVVDKSIDTTFSLRVWTI PMRQNYWGSEGRLLLLGDRIYIYTRSTSWHSKLQLGVI DISDYNNIRINWTWHNVLSRPGNDECPWGHSCPDGCIT GVYTDAYPLNPSGSVVSSVILDSQKSRENPIITYSTATNR VNELAIYNRTLPAAYTTTNCITHYDKGYCFHIVEINHRSL NTFQPMLFKTEVPKNCS	14	71
KF530164	6157-6906	gb:KF530164:6157-6906|Organism:Human metapneumovirus|Strain Name:HMPV/AUS/172832788/2004/B|Protein Name:attachment glycoprotein G|Gene Symbol:G	MEVRVENIRAIDMFKAKIKNRIRSSRCYRNATLILIGLTA LSMALNIFLIIDHATLRNMIKTENCANMPSAEPSKKTPM TSTAGPSTKPNPQQATQWTTENSTSPAATLEGHPYTGTT QTPDTTAPQQTTDKHTALPKSTNEQITQTTTEKKTTRAT TQKREKRKENTNQTTSTAATQTTNTTNQTRNASETITTS DGPRIDTTTQSSEQTARATEPGSSPYHARRGAGPR	14	72
AB910309	6960-8747	gb:AB910309:6960-8747|Organism:Feline morbillivirus|Strain Name:SS1|Protein	MKNINIKYYKDSNRYLGKILDEHKIVNSQLYSLSIKVITII AIIVSLIATIMTIINATSGRTTLNSNTDILLNQRDEIHSIHE MIFDRVYPLITAMSTELGLHIPTLLDELTKAIDQKIKIMN PPVDTVTSDLSWCIKPPNGIIIDPKGYCESMELSKTYKLL LDQLDVSRKKSLTINRKNINQCQLVDDSEIIFATVNIQST PRFLNFGHTVSNQRITFGQGTYSSTYILTIQEDGITDVQY	12	73

Genbank ID	Nucleotides of CDS	Full sequence ID	Sequence	#Sequences/Cluster	SEQ ID NO
		Name:hemagglutinin protein|Gene Symbol:H	RVFEIGYISDQFGVFPSLIVSRVLPIRMVLGMESCTLTSD RQGGYFLCMNTLTRSIYDYVNIRDLKSLYITLPHYGKVN YTYFNFGKIRSPHEIDKLWLTSDRGQIISGYFAAFVTITIR NYNNYPYKCLNNPCFDNSENYCRGWYKNITGTDDVPIL AYLLVEMYDEEGPLITLVAIPPYNYTAPSHNSLYYDDKI NKLIMTTSHIGYIQINEVHEVIVGDNLKAILLNRLSDEHP NLTACRLNQGIKEQYKSDGMIISNSALIDIQERMYITVKA IPPVGNYNFTVELHSRSNTSYILLPKQFNAKYDKLHLECF NWDKSWWCALIPQFSLSWNESLSVDTAIFNLINCK		
AB759118	7116-8957	gb:AB759118:7116-8957|Organism:Avian paramyxovirus 6|Strain Name:red-necked stint/Japan/8KS0813/2008|Protein Name:hemagglutinin-neuraminidase|Gene Symbol:HN	MASPSELNRSQATLYEGDPNSKRTWRTVYRASTLILDL AILCVSIVAIVRMSTLTPSDVTDSISSSITSLSDTYQSVWS DTHQKVNSIFKEVGISIPVTLDKMQVEMGTAVNIITDAV RQLQGVNGSAGFSITNSPEYSGGIDALIYPQKSLNGKSLA ISDLLEHPSFIPAPTTSHGCTRIPTFHLGYRHWCYSHNTIE SGCHDAGESIMYLSMGAVGVGHQGKPVFTTSAAVILDD GKNRKSCSVVANPNGCDVLCSLVKQTEDQDYADPTPTP MIHGRLHFNGTYTESMLDQSLFTGHWVAQYPAVGSGS VSHGRLFFPLYGGISKSSSLFPKLRAHAYFTHNEELECKN LTSKQREDLFNAYMPGKIAGSLWAQGIVICNLTTLADC KIAVANTSTMMMAAEGRLQLVQDKVVLYQRSSSWWP VLIYYDILVSELVNARHLDIVNWVPYPQSKFPRPTWTKG LCEKPSICPAVCVTGVYQDVWVVSVGDFSNETVVIGGY LEAASERKDPWIAAANQYNWLTRRQLFTAQTEAAYSST TCFRNTHQDKVFCLTIMEVTDNLLGDWRIAPLLYEVTV VDRQQSSRKAVAMSEAHRTRFKYYSPENKFTPQH	11	74
AY141760	6791-8485	gb:AY141760|Organism:Fer-de-Lance paramyxovirus|Strain Name:ATCC VR-895|Protein Name:hemagglutinin-neuraminidase protein HN|Gene Symbol:HN	MDPKSYYCNEDLRSDGGEKSPGGDLYKGIILVSTVISLII AIISLAFIIDNKINIQSLDPLRGLEDSYLVPIKDKSESISQDI QEGIFPRLNLITAATTTTIPRSIAIQTKDLSDLIMNRCYPS VVNNDTSCDVLAGAIHSNLFSQLDPSTYWTCSSGTPTM NQTVKLLPDNSQIPGSTYSTGCVRIPTFSLGSMIYSYSHN VIYEGCNDHSKSSQYWQLGYISTSKTGEPLQQVSRTLTL NNGLNRKSCSTVAQGRGAYLLCTNVVEDERTDYSTEGI QDLTLDYIDIFGAERSYRYTNNEVDLDRPYAALYPSVGS GTVYNDRILFLGYGGLMTPYGDQAMCQAPECTSATQE GCNSNQLIGYFSGRQIVNCIIEIITVGTEKPIIRVRTIPNSQ VWLGAEGRIQTLGGVLYLYIRSSGWHALAQTGIILTLDP IRISWIVNTGYSRPGNGPCSASSRCPAQCITGVYTDIFPLS QNYGYLATVTLLSGVDRVNPVISYGTSTGRVADSQLTSS SQVAAYTTTTCFTFNQKGYCYHIIELSPATLGIFQPVLVV TEIPKICS	8	75
EU877976	6248-8161	gb:EU877976:6248-8161|Organism:Avian paramyxovirus 4|Strain Name:APMV-4/KR/YJ/06|Protein Name:hemagglutinin-neuraminidase|Gene Symbol:HN	MQGNMEGSRDNLTVDDELKTTWRLAYRVVSLLLMVS ALIISIVILTRDNSQSIITAINQSSDADSKWQTGIEGKITSI MTDTLDTRNAALLHIPLQLNTLEANLLSALGGNTGIGPG DLEHCRYPVHDTAYLHGVNRLLINQTADYTAEGPLDHV NFIPAPVTTTGCTRIPSFSVSSSIWCYTHNVIETGCNDHSG SNQYISMGVIKRAGNGLPYFSTVVSKYLTDGLNRKSCSV AAGSGHCYLLCSLVSEPEPDDYVSPDPTPMRLGVLTWD GSYTEQAVPERIFKNIWSANYPGVGSGAIVGNKVLFPFY GGVRNGSTPEVMNRGRYYYIQDPNDYCPDPLQDQILRA EQSYYPTRFGRRMVMQGVLACPVSNNSTIASQCQSYYF NNSLGFIGAESRIYYLNGNIYLYQRSSSWWPHPQIYLLDS RIASPGTQNIDSGVNLKMLNVTVITRPSSGFCNSQSRCPN DCLFGVYSDIWPLSLTSDSIFAFTMYLQGKTTRIDPAWA LFSNHAIGHEARLFNKEVSAAYSTTTCFSDTIQNQVYCL SILEVRSELLGAFKIVPFLYRVL	8	76

Genbank ID	Nucleotides of CDS	Full sequence ID	Sequence	#Sequences/Cluster	SEQ ID NO
AB176531	6821-8536	gb:AB176531:6821-8536|Organism:Human parainfluenza virus 2|Strain Name:Nishio|Protein Name:hemagglutinin-neuraminidase protein|Gene Symbol:HN	MEDYSNLSLKSIPKRTCRIIFRTATILGICTLIVLCSSILHEI IHLDVSSGLMDSDDSQQGIIQPIIESLKSLIALANQILYNV AIIIPLKIDSIETVIFSALKDMHTGSMSNTNCTPGNLLLHD AAYINGINKFLVLKSYNGTPKYGPLLNIPSFIPSATSPNGC TRIPSFSLIKTHWCYTHNVMLGDCLDFTTSNQYLAMGII QQSAAAFPIFRTMKTIYLSDGINRKSCSVTAIPGGCVLYC YVATRSEKEDYATTDLAELRLAFYYYNDTFIERVISLPN TTGQWATINPAVGSGIYHLGFILFPVYGGLISGTPSYNKQ SSRYFIPKHPNITCAGNSSEQAAAARSSYVIRYHSNRLIQ SAVLICPLSDMHTARCNLVMFNNSQVMMGAEGRLYVI DNNLYYYQRSSSWWSASLFYRINTDFSKGIPPIIEAQWV PSYQVPRPGVMPCNATSFCPANCITGVYADVWPLNDPE PTSQNALNPNYRFAGAFLRNESNRTNPTFYTASASALLN TTGFNNTNHKAAYTSSTCFKNTGTQKIYCLIIIEMGSSLL GEFQIIPFLRELIP	7	77
AF052755	6584-8281	gb:AF052755|Organism:Parainfluenza virus 5|Strain Name:W3A|Protein Name:hemagglutinin-neuraminidase protein|Gene Symbol:HN	MVAEDAPVRATCRVLFRTTTLIFLCTLLALSISILYESLIT QKQIMSQAGSTGSNSGLGSITDLLNNILSVANQIIYNSAV ALPLQLDTLESTLLTAIKSLQTSDKLEQNCSWSAALIND NRYINGINQFYFSIAEGRNLTLGPLLNMPSFIPTATTPEGC TRIPSFSLTKTHWCYTHNVILNGCQDHVSSNQFVSMGIIE PTSAGFPFFRTLKTLYLSDGVNRKSCSISTVPGGCMMYC FVSTQPERDDYFSAAPPEQRIIIMYYNDTIVERIINPPGVL DVWATLNPGTGSGVYYLGWVLFPIYGGVIKGTSLWNN QANKYFIPQMVAALCSQNQATQVQNAKSSYYSSWFGN RMIQSGILACPLRQDLTNECLVLPFSNDQVLMGAEGRLY MYGDSVYYYQRSNSWWPMTMLYKVTITFTNGQPSAIS AQNVPTQQVPRPGTGDCSATNRCPGFCLTGVYADAWL LTNPSSTSTFGSEATFTGSYLNTATQRINPTMYIANNTQII SSQQFGSSGQEAAYGHTTCFRDTGSVMVYCIYIIELSSSL LGQFQIVPFIRQVTLS	7	78
BK005918	6560-8290	gb:BK005918|Organism:Porcine rubulavirus|Strain Name:UNKNOWN-BK005918|Protein Name:attachment protein|Gene Symbol:HN	MSQLGTDQIMHLAQPAIARRTWRLCFRIFALFILIAIVITQ IFMLTFDHTLLTTTQFLTSIGNLQSTITSWTPDVQAMLSIS NQLIYTTSITLPLKISTTEMSILTAIRDHCHCPDCSSACPT RQMLLNDPRYMSGVNQFIGAPTESINITFGPLFGIPSFIPT STTTQGCTRIPSFALGPSHWCYTHNFITAGCADGGHSNQ YLAMGTIQSASDGSPLLITARSYYLSDGVNRKSCSIAVV PGGCAMYCYVATRSETDYYAGNSPPQQLLTLVFSNDTII ERTIHPTGLANGWVMLVPGVGSGTLYNEYLLFPAYGG MQQILANQSGEINQFFTPYNATVRCAMAQPQFSQRAAA SYYPRYFSNRWIRSAIVACPYRAIYQTQCTLIPLPNRMV MMGSEGRIFTLGDRLFYYQRSSSWWPYPLLYQVGLNFL TTPPSVSSMTQVPLEHLARPGKGGCPGNSHCPATCVTG VYADVWPLTDPRSGVGGTSLVAAGGLDSTSERMAPVN YLAIGESLLSKTYLLSKTQPAAYTTTTCFRDTDTGKIYCI TIAELGKVLLGEFQIVPFLREIKIQSRY	7	79
EU338414	6015-7913	gb:EU338414:6015-7913|Organism:Avian paramyxovirus 2|Strain Name:APMV-2/Chicken/California/Yucaipa/56|Protein Name:hemagglutinin-	MDFPSRENLAAGDISGRKTWRLLFRILTLSIGVVCLAINI ATIAKLDHLDNMASNTWTTTEADRVISSITTPLKVPVNQ INDMFRIVALDLPLQMTSLQKEITSQVGFLAESINNVLSK NGSAGLVLVNDPEYAGGIAVSLYQGDASAGLNFQPISLI EHPSFVPGPTTAKGCIRIPTFHMGPSHWCYSHNIIASGCQ DASHSSMYISLGVLKASQTGSPIFLTTASHLVDDNINRKS CSIVASKYGCDILCSIVIETENEDYRSDPATSMIIGRLFFN GSYTESKINTGSIFSLFSANYPAVGSGIVVGDEAAFPIYG GVKQNTWLFNQLKDFGYFTHNDVYKCNRTDIQQTILDA YRPPKISGRLWVQGILLCPVSLRPDPGCRLKVFNTSNVM MGAEARLIQVGSTVYLYQRSSSWWVVGLTYKLDVSEIT	7	80

Genbank ID	Nucleotides of CDS	Full sequence ID	Sequence	#Sequences/Cluster	SEQ ID NO
		neuraminidase|Gene Symbol:HN	SQTGNTLNHVDPIAHTKFPRPSFRRDACARPNICPAVCV SGVYQDIWPISTATNNSNIVWVGQYLEAFYSRKDPRIGI ATQYEWKVTNQLFNSNTEGGYSTTTCFRNTKRDKAYC VVISEYADGVFGSYRIVPQLIEIRTTTGKSE		
KC403973	6234-6964	gb:KC403973:6234-6964|Organism:Human metapneumovirus|Strain Name:HMPV/USA/TN-82-518/1982/A|Protein Name:attachment glycoprotein G|Gene Symbol:G|Segment:8	MEVKVENIRTIDMLKARVKNRVARSKCFKNASLILIGIT TLSIALNIYLIINYTMQENTSESEHHTSSSPMESSRETPTV PIDNSDTNPSSQYPTQQSTEGSTLYFAASASSPETEPTSTP DTTSRPPFVDTHTTPPSASRTKTSPAVHTKNNPRISSRTH SPPWAMTRTVRRTTTLRTSSIRKRSSTASVQPDSSATTH KHEEASPVSPQTSASTTRPQRKSMEASTSTTYNQTS	6	81
KF015281	4511-5844	gb:KF015281:4511-5844|Organism:Canine pneumovirus|Strain Name:dog/Bari/100-12/ITA/2012|Protein Name:attachment protein|Gene Symbol:G	MRPAEQLIQENYKLTSLSMGRNFEVSGSTTNLNFERTQY PDTFRAVVKVNQMCKLIAGVLTSAAVAVCVGVIMYSV FTSNHKANSMQNATIRNSTSAPPQPTAGPPTTEQGTTPK FTKPPTKTTTHHEITEPAKMVTPSEDPYQCSSNGYLDRP DLPEDFKLVLDVICKPPGPEHHSTNCYEKREINLGSVCP DLVTMKANMGLNNGGGEEAAPYIEVITLSTYSNKRAM CVHNGCDQGFCFFLSGLSTDQKRAVLELGGQQAIMELH YDSYWKHYWSNSNCVVPRTNCNLTDQTVILFPSFNNKN QSQCTTCADSAGLDNKFYLTCDGLSRNLPLVGLPSLSPQ AHKAALKQSTGTTTAPTPETRNPTPAPRRSKPLSRKKRA LCGVDSSREPKPTMPYWCPMLQLFPRRSNS	6	82
KF973339	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	MSKTKDQRAAKTLEKTWDTLNHLLFISSCLYKSNLKSIA QITLSILAMTIPTSLIIVATTFIASANNKVTPTTAIIQDATS QIKNTTPTHLTQNPQPGISFFNLSGTISQTTAILAPTTPSVE PILQSTTVKTKNTTTTQIQPSKLTTKQRQNKPPNKPNDDF HFEVFNFVPCSICSNNPTCWAICKRIPSKKPGKKTTTKPT KKQTIKTTKKDLKPQTTKPKEAPTT	6	83
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	MSNIASSLENIVEQDSRKTTWRAIFRWSVLLITTGCLALS IVSIVQIGNLKIPSVGDLADEVVTPLKTTLSDTLRNPINQI NDIFRIVALDIPLQVTSIQKDLASQFSMLIDSLNAIKLGNG TNLIIPTSDKEYAGGIGNPVFTVDAGGSIGFKQFSLIEHPS FIAGPTTTRGCTRIPTFHMSESHWCYSHNIIAAGCQDASA SSMYISMGVLHVSSSGTPIFLTTASELIDDGVNRKSCSIV ATQFGCDILCSIVIEKEGDDYWSDTPTPMRHGRFSFNGS FVETELPVSSMFSSFSANYPAVGSGEIVKDRILFPIYGGIK QTSPEFTELVKYGLFVSTPTTVCQSSWTYDQVKAAYRP DYISGRFWAQVILSCALDAVDLSSCIVKIMNSSTVMMA AEGRIIKIGIDYFYYQRSSSWWPLAFVTKLDPQELADTN SIWLTNSIPIPQSKFPRPSYSENYCTKPAVCPATCVTGVY SDIWPLTSSSSLPSIIWIGQYLDAPVGRTYPRFGIANQSH WYLQEDILPTSTASAYSTTTCFKNTARNRVFCVTIAEFA DGLFGEYRITPQLYELVRNN	5	84

Genbank ID	Nucleotides of CDS	Full sequence ID	Sequence	#Sequences/Cluster	SEQ ID NO
JX857409	6619-8542	gb:JX857409:6619-8542|Organism:Porcine parainfluenza virus 1|Strain Name:S206N|Protein Name:haemagglutinin protein|Gene Symbol:H	MEETKVKTSEYWARSPQIHATNHPNVQNREKIKEILTILI SFISSLSLVLVIAVLIMQSLHNGTILRCKDVGLESINKSTY SISNAILDVIKQELITRIINTQSSVQVALPILINKKIQDLSLII EKSSKVHQNSPTCSGVAALTHVEGIKPLDPDDYWRCPS GEPYLEDELTLSLIPGPSMLAGTSTIDGCVRLPSLAIGKSL YAYSSNLITKGCQDIGKSYQVLQLGIITLNSDLHPDLNPII SHTYDINDNRKSCSVAVSETKGYQLCSMPRVNEKTDYT SDGIEDIVFDVLDLKGSSRSFKFSNNDINFDHPFSALYPS VGSGIIWKNELYFLGYGALTTALQGNTKCNLMGCPGAT QDNCNKFISSSWLYSKQMVNVLIQVKGYLSSKPSIIVRTI PITENYVGAEGKLVGTRERIYIYTRSTGWHTNLQIGVLNI NHPITITWTDHRVLSRPGRSPCAWNNKCPRNCTTGVYT DAYPISPDANYVATVTLLSNSTRNNPTIMYSSSDRVYNM LRLRNTELEAAYTTTSCIVHFDRGYCFHIIEINQKELNTL QPMLFKTAIPKACRISNL	5	85
KF908238	7510-9249	gb:KF908238:7510-9249|Organism:Human parainfluenza virus 4b|Strain Name:QLD-01|Protein Name:hemagglutinin-neuraminidase protein|Gene Symbol:HN	MQDSRGNTQIFSQANSMVKRTWRLLFRIVTLILLISIFVL SLIIVLQSTPGNLQSDVDIIRKELDELMENFETTSKSLLSV ANQITYDVSVLTPIRQEATETNIIAKIKDHCKDRVVKGES TCTLGHKPLHDVSFLNGFNKFYFTYRDNVQIRLNPLLDY PNFIPTATTPHGCIRIPSFSLSQTHWCYTHNTILRGCEDTA SSKQYVSLGTLQTLENGDPYFKVEYSHYLNDRKNRKSC SVVAVLDGCLLYCVIMTKNETENFKDPQLATQLLTYISY NGTIKERIINPPGSSRDWVHISPGVGSGILYSNYIIFPLYG GLMENSMIYNNQSGKYFFPNSTKLPCSNKTSEKITGAKD SYTITYFSKRLIQSAFLICDLRQFLSEDCEILIPSNDHMLV GAEGRLYNIENNIFYYQRGSSWWPYPSLYRIKLNSNKK YPRIIEIKFTKIEIAPRPGNKDCPGNKACPKECITGVYQDI WPLSYPNTAFPHKKRAYYTGFYLNNSLARRNPTFYTAD NLDYHQQERLGKFNLTAGYSTTTCFKQTTTARLYCLYIL EVGDSVIGDFQIFPFLRSIDQAIT	5	86
KT071757	6066-7962	gb:KT071757:6066-7962|Organism:Avian paramyxovirus 2|Strain Name:APMV-2/Emberiza spodocephala/China/Daxing'anling/974/2013|Protein Name:hemagglutinin-neuraminidase protein|Gene Symbol:HN	MDALSRENLTEISQGGRRTWRMLFRILTLVLTLVCLAINI ATIAKLDSIDTSKVQTWTTTESDRVIGSLTDTLKIPINQV NDMFRIVALDLPLQMTTLQKEIASQVGFLAESINNFLSK NGSAGSVLVNDPEYAGGIGTSLFHGDSASGLDFEAPSLI EHPSFIPGPTTAKGCIRIPTFHMSASHWCYSHNIIASGCQ DAGHSSMYISMGVLKATQAGSPSFLTTASQLVDDKLNR KSCSIISTTYGCDILCSLVVENEDADYRSDPPTDMILGRL FFNGTYSESKLNTSAIFQLFSANYPAVGSGIVLGDEIAFP VYGGVKQNTWLFNQLKDYGYFAHNNVYKCNNSNIHQ TVLNAYRPPKISGRLWSQVVLICPMRLFINTDCRIKVFNT STVMMGAEARLIQVGSDIYLYQRSSSWWVVGLTYKLD FQELSSKTGNILNNVSPIAHAKFPRPSYSRDACARPNICP AVCVSGVYQDIWPISTAHNLSQVVWVGQYLEAFYARK DPWIGIATQYDWKKNVRLFNANTEGGYSTTTCFRNTKR DKAFCVIISEYADGVFGSYRIVPQLIEIRTTSKKGLPS	5	87
LC041132	6605-8437	gb:LC041132:6605-8437|Organism:Avian paramyxovirus goose/Shimane/67/2000|Strain Name:goose/Shimane/67/2000|Protein Name:hemagglutinin-	MQPGISEVSFVNDERSERGTWRLLFRILTIVLCLTSIGIGI PALIYSKEAATSGDIDKSLEAVKTGMSTLSSKIDESINTE QKIYRQVILEAPVSQLNMESNILSAITSLSYQIDGTSNSSG CGSPMHDQDFVGGINKEIWTTDNVNLGEITLTPFLEHLN FIPAPTTGNGCTRIPSFDLGLTHWCYTHNVILSGCQDYSS SFQYIALGVLKISATGHVFLSTMRSINLDDERNRKSCSIS ATSIGCDIICSLVTEREVDDYNSPAATPMIHGRLDFSGKY NEVDLNVGQLFGDWSANYPGVGGGSFLNGRVWFPIYG GVKEGTPTFKENDGRYAIYTRYNDTCPDSESEQVSRAKS SYRPSYFGGKLVQQAVLSIKIDDTLGLDPVLTISNNSITL MGAESRVLQIEEKLYFYQRGTSWFPSLIMYPLTVDDKM	4	88

Genbank ID	Nucleotides of CDS	Full sequence ID	Sequence	#Sequences/Cluster	SEQ ID NO
		neuraminidase|Gene Symbol:HN	VRFEPPTIFDQFTRPGNHPCSADSRCPNACVTGVYTDGY PIVFHNNHSIAAVYGMQLNDVTNRLNPRSAVWYGVSM SNVIRVSSSTTKAAYTTSTCFKVKKTQRVYCLSIGEIGNT LFGEFRIVPLLLEVYSEKGKSLKSSFDGWEDISINNPLRPL DNHRVDPILISNYTSSWP		
AF092942	4705-5478	gb:AF092942|Organism:Bovine respiratory syncytial virus|Strain Name:ATue51908|Protein Name:attachment glycoprotein|Gene Symbol:G	MSNHTHHLKFKTLKRAWKASKYFIVGLSCLYKFNLKSL VQTALTTLAMITLTSLVITAIIYISVGNAKAKPTSKPTIQQ TQQPQNHTSPFFTEHNYKSTHTSIQSTTLSQLPNTDTTRE TTYSHSINETQNRKIKSQSTLPATRKPPINPSGSNPPENHQ DHNNSQTLPYVPCSTCEGNLACLSLCQIGPERAPSRAPTI TLKKTPKPKTTKKPTKTTIHHRTSPEAKLQPKNNTAAPQ QGILSSPEHHTNQSTTQI	3	89
AF326114	6691-847	gb:AF326114|Organism:Menangle virus|Strain Name:UNKNOWN-AF326114|Protein Name:attachment protein|Gene Symbol:HN	MWNSIPQLVSDHEEAKGKFTDIPLQDDTDSQHPSGSKST CRTLFRTVSIILSLVILVLGVTSTMFSAKYSGGCATNSQL LGVSNLINQIQKSIDSLISEVNQVSITTAVTLPIKIMDFGK SVTDQVTQMIRQCNTVCKGPGQKPGSQNVRIMPSNNLS TFQNINMSARGIAYQDVPLTFVRPIKNPQSCSRFPSYSVS FGVHCFANAVTDQTCELNQNTFYRVVLSVSKGNISDPSS LETKAETRTPKGTPVRTCSIISSVYGCYLLCSKATVPESE EMKTIGFSQMFILYLSMDSKRIIYDNIVSSTSAIWSGLYP GEGAGIWHMGQLFFPLWGGIPFLTPLGQKILNSTLDIPEV GSKCKSDLTSNPAKTKDMLFSPYYGENVMVFGFLTCYL LSNVPTNCHADYLNSTVLGFGSKAQFYDYRGIVYMYIQ SAGWYPFTQIFRITLQLKQNRLQAKSIKRIEVTSTTRPGN RECSVLRNCPYICATGLFQVPWIVNSDAITSKEVDNMVF VQAWAADFTEFRKGILSLCSQVSCPINDLLSKDNSYMR DTTTYCFPQTVPNILSCTSFVEWGGDSGNPINILEIHYEVI FVAS	3	90
GU206351	7500-9714	gb:GU206351:7500-9714|Organism:Avian paramyxovirus 5|Strain Name:budgerigar/Kunitachi/74|Protein Name:hemagglutinin neuraminidase protein|Gene Symbol:HN	MDKSYYTEPEDQRGNSRTWRLLFRLIVLTLLCLIACTSV SQLFYPWLPQVLSTLISLNSSIITSSNGLKKEILNQNIKED LIYREVAINIPLTLDRVTVEVGTAVNQITDALRQLQSVN GSAAFALSNSPDYSGGIEHLVFQRNTLINRSVSVSDLIEH PSFIPTPTTQHGCTRIPTFHLGTRHWCYSHNIIGQGCADS GASMMYISMGALGVSSLGTPTFTTSATSILSDSLNRKSCS IVATTEGCDVLCSIVTQTEDQDYADHTPTPMIHGRLWFN GTYTERSLSQSLFLGTWAAQYPAVGSGIMTPGRVIFPFY GGVIPNSPLFLDLERFALFTHNGDLECRNLTQYQKEAIY SAYKPPKIRGSLWAQGFIVCSVGDMGNCSLKVINTSTV MMGAEGRLQLVGDSVMYYQRSSSWWPVGILYRLSLVD IIARDIQVVINSEPLPLSKFPRPTWTPGVCQKPNVCPAVC VTGVYQDLWAISAGETLSEMTFFGGYLEASTQRKDPWI GVANQYSWFMRRRLFKTSTEAAYSSSTCFRNTRLDRNF CLLIFELTDNLLGDWRIVPLLFELTIV	3	91
JQ001776	8170-10275	gb:JQ001776:8170-10275|Organism:Cedar virus|Strain Name:CG1a|Protein Name:attachment glycoprotein|Gene Symbol:G	MLSQLQKNYLDNSNQQGDKMNNPDKKLSVNFNPLELD KGQKDLNKSYYVKNKNYNVSNLLNESLHDIKFCIYCIFS LLIIITIINIITISIVITRLKVHEENNGMESPNLQSIQDSLSSL TNMINTEITPRIGILVTATSVTLSSSINYVGTKTNQLVNEL KDYITKSCGFKVPELKLHECNISCADPKISKSAMYSTNA YAELAGPPKIFCKSVSKDPDFRLKQIDYVIPVQQDRSICM NNPLLDISDGFFTYIHYEGINSCKKSDSFKVLLSHGEIVD RGDYRPSLYLLSSHYHPYSMQVINCVPVTCNQSSFVFCH ISNNTKTLDNSDYSSDEYYITYFNGIDRPKTKKIPINNMT ADNRYIHFTFSGGGGVCLGEEFIIPVTTVINTDVFTHDYC	3	92

Genbank ID	Nucleotides of CDS	Full sequence ID	Sequence	#Sequences/Cluster	SEQ ID NO
			ESFNCSVQTGKSLKEICSESLRSPTNSSRYNLNGIMIISQN NMTDFKIQLNGITYNKLSFGSPGRLSKTLGQVLYYQSSM SWDTYLKAGFVEKWKPFTPNWMNNTVISRPNQGNCPR YHKCPEICYGGTYNDIAPLDLGKDMYVSVILDSDQLAE NPEITVFNSTTILYKERVSKDELNTRSTTTSCFLFLDEPW CISVLETNRFNGKSIRPEIYSYKIPKYC		
KP271123	6644-8431	gb:KP271123:6644-8431|Organism:Teviot virus|Strain Name:Geelong|Protein Name:attachment protein|Gene Symbol:HN	MWSTQASKHPAMVNSATNLVDIPLDHPSSAQFPINRKR TGRLIYRLFSILCNLILISILISLVVIWSRSSRDCAKSDGLS SVDNQLSSLSRSINSLITEVNQISVTTAINLPIKLSEFGKSV VDQVTQMIRQCNAACKGPGEKPGIQNVRINIPNNFSTYS ELNRTANSLNFQSRTALFARPNPYPKTCSRFPSYSVYFGI HCFSHAVTDSSCELSDSTYYRLVIGVADKNLSDPADVK YIGETTTPVRVQTRGCSVVSSIYGCYLLCSKSNQDYQDD FREQGFHQMFILFLSRELKTTFFDDMVSSTTVTWNGLYP GEGSGIWHMGHLVFPLWGGIRFGTHASEGILNSTLELPP VGPSCKRSLADNGLINKDVLFSPYFGDSVMVFAYLSCY MLSNVPTHCQVETMNSSVLGFGSRAQFYDLKGIVYLYI QSAGWFSYTQLFRLSLQSKGYKLSVKQIKRIPISSTSRPG TEPCDIIHNCPYTCATGLFQAPWIVNGDSIRDRDVRNMA FVQAWSGAINTFQRPFMSICSQYSCPLSELLDSESSIMRS TTTYCFPSLTESILQCVSFIEWGGPVGNPISINEVYSSISFR PD	3	93
AY286409	7644-9542	gb:AY286409|Organism:Mossman virus|Strain Name:UNKNOWN-AY286409|Protein Name:attachment glycoprotein|Gene Symbol:G	MVDPPAVSYYTGTGRNDRVKVVTTQSTNPYWAHNPNQ GLRRLIDMVVNVIMVTGVIFALINIILGIVIISQSAGSRQD TSKSLDIIQHVDSSVAITKQIVMENLEPKIRSILDSVSFQIP KLLSSLLGPGKTDPPIALPTKASTPVIPTEYPSLNTTTCLR IEESVTQNAAALFNISFDLKTVMYELVTRTGGCVTLPSY SELYTRVRTFSTAIRNPKTCQRAGQETDLNLIPAFIGTDT GILINSCVRQPVIATGDGIYALTYLTMRGTCQDHRHAVR HFEIGLVRRDAWWDPVLTPIHHFTEPGTPVFDGCSLTVQ NQTALALCTLTTDGPETDIHNGASLGLALVHFNIRGEFS KHKVDPRNIDTQNQGLHLVTTAGKSAVKKGILYSFGYM VTRSPEPGDSKCVTEECNQNNQEKCNAYSKTTLDPDKP RSMIIFQIDVGAEYFTVDKVVVVPRTQYYQLTSGDLFYT GEENDLLYQLHNKGWYNKPIRGRVTFDGQVTLHEHSRT YDSLSNQRACNPRLGCPSTCELTSMASYFPLDKDFKAA VGVIALRNGMTPIITYSTDDWRNHWKYIKNADLEFSESS LSCYSPNPPLDDYVLCTAVITAKVMSNTNPQLLATSWY QYDKCHT	2	94
AY900001	7809-9938	gb:AY900001|Organism:J-virus|Strain Name:UNKNOWN-AY900001|Protein Name:attachment glycoprotein|Gene Symbol:G	MNPVAMSNFYGINQADHLREKGDQPEKGPSVLTYVSLI TGLLSLFTIIALNVTNIIYLTGSGGTMATIKDNQQSMSGS MRDISGMLVEDLKPKTDLINSMVSYTIPSQISAMSAMIK NEVLRQCTPSFMFNNTICPIAEHPVHTSYFEEVGIEAISM CTGTNRKLVVNQGINFVEYPSFIPGSTKPGGCVRLPSFSL GLEVFAYAHAITQDDCTSSSTPDYYFSVGRIADHGTDVP VFETLAEWFLDDKMNRRSCSVTAAGKGGWLGCSILVG SFTDELTSPEVNRISLSYMDTFGKKKDWLYTGSEVRAD QSWSALFFSVGSGVVIGDTVYFLVWGGLNHPINVDAMC RAPGCQSPTQSLCNYAIKPQEWGGNQIVNGILHFKHDTN EKPTLHVRTLSPDNNWMGAEGRLFHFHNSGKTFIYTRSS TWHTLPQVGILTLGWPLSVQWVDITSISRPGQSPCEYDN RCPHQCVTGVYTDLFPLGVSYEYSVTAYLDQVQSRMNP KIALVGAQEKIYEKTITTNTQHADYTTTSCFAYKLRVWC VSIVEMSPGVITTRQPVPFLYHLNLGCQDTSTGSLTPLDA HGGTYLNTDPVGNKVDCYFVLHEGQIYFGMSVGPINYT YSIVGRSREIGANMNVSLNQLCHSVYTEFLKEKEHPGTR	2	95

Genbank ID	Nucleotides of CDS	Full sequence ID	Sequence	#Sequences/Cluster	SEQ ID NO
			NNIDVEGWLLKRIETLNGTKIFGLDDLEGSGPGHQSGPE DPSIAPIGHN		
EF199772	6150-6944	gb:EF199772:6150-6944|Organism:Avian metapneumovirus|Strain Name:PL-2|Protein Name:attachment glycoprotein|Gene Symbol:G	MEVKVENVGKSQELKVKVKNFIKRSDCKKKLFALILGL VSFELTMNIMLSVMYVESNEALSLCRIQGTPAPRDNKTN TENATKETTLHTTTTTRDPEVRETKTTKPQANEGATNPS RNLTTKGDKHQTTRATTEAELEKQSKQTTEPGTSTQKH TPARPSSKSPTTTQATAQPTTPTAPKASTAPKNRQATTK KTETDTTTASRARNTNNPTETATTTPKATTETGKGKEGP TQHTTKEQPETTARETTTPQPRRTAGASPRAS	2	96
JF424833	5981-7156	gb:JF424833:5981-7156|Organism:Avian metapneumovirus|Strain Name:IT/Ty/A/259-01/03|Protein Name:attachment protein|Gene Symbol:G	MGSKLYMVQGTSAYQTAVGFWLDIGRRYILAIVLSAFG LTCTVTIALTVSVIVEQSVLEECRNYNGGDRDWWSTTQ EQPTTAPSATPAGNYGGLQTARTRKSESCLHVQISYGD MYSRSDTVLGGFDCMGLLVLCKSGPICQRDNQVDPTAL CHCRVDLSSVDCCKVNKISTNSSTTSEPQKTNPAWPSQD NTDSDPNPQGITTSTATLLSTSLGLMLTSKTGTHKSGPPQ ALPGSNTNGKTTTDRELGSTNQPNSTTNGQHNKHTQRM TLPPSYDNTRTILQHTTPWEKTFSTYKPTHSPTNESDQSL PTTQNSINCEHFDPQGKEKICYRVGSYNSNITKQCRIDVP LCSTYNTVCMKTYYTEPFNCWRRIWRCLCDDGVGLVE WCCTS	2	97
JN689227	7918-12444	gb:JN689227:7918-12444|Organism:Tailam virus|Strain Name:TL8K|Protein Name:attachment glycoprotein|Gene Symbol:G	MSQLAAHNLAMSNFYGIHQGGQSTSQKEEEQPVQGVIR YASMIVGLLSLFTIIALNVTNIIYMTESGGTMQSIKNAQG SIDGSMKDLSGTIMEDIKPKTDLINSMVSYNIPAQLSMIH QIIKNDVLKQCTPSFMFNNTICPLAENPTHSRYFEEVNLD SISECSGNEMSLELGTEPEFIEYPSFAPGSTKPGSCVRLPS FSLSSTVFAYTHTIMGHGCSELDVGDHYLAIGRIADAGH EIPQFETISSWFINDKINRRSCTVAAGVMETWMGCVIMT ETFYDDLDSLDTGKITISYLDVFGRKKEWIYTRSEILYDY TYTSVYFSIGSGVVVGDTVYFLLWGSLSSPIEETAYCYA PGCSNYNQRMCNEAQRPAKFGHRQMANAILRFKTNSM GKPSISVRTLSPTVIPFGTEGRLIYSDFTKIIYLYLRSTSWY VLPLTGLLILGPPVSISWVTQEAVSRPGEYPCGASNRCPK DCITGVYTDLFPLGARYEYAVTVYLNAETYRVNPTLALI DRSKIIARKKITTESQKAGYTTTTCFVFKLRIWCMSVVEL APATMTAFEPVPFLYQLDLTCKRNNGTTAMQFSGQDG MYKSGRYKSPRNECFFEKVSNKYYFVVSTPEGIQPYEVR DLTPERVSHVIMYISDVCAPALSAFKKLIPAMRPITTLTIG NWQFRPVDISGGLRVNIYRNLTRYGDLSMSAPEDPGTD TFPGTHAPSKGHEEVGHYTLPNEKLSEVTTAAVKTKESL NLIPDTKDTRGEEENGSGLNEIITGHTTPGHIKTHPAETK VTKHTVIIPQIEEDGSGATTSTELQDETGYHTEDYNTTNT NGSLTAPNERNNYTSGDHTVSGEDITHTITVSDRTKTTQ TLPTDNTFNQTPTKIQEGSPKSESTPKDYTAIESEDSHFT DPTLIRSTPEGTIVQVIGDQFHSAVTQLGESNAIGNSEPID QGNNLIPTTDRGTMDNTSSQSHSSTTSTQGSHSAGHGSQ SNMNLTALADTDSVTDQSTSTQEIDHEHENVSSILNPLS RHTRVMRDTVQEALTGAWGFIRGMIP	2	98
KC562242	6178-6926	gb:KC562242:6178-6926|Organism:Human metapneumovirus|Strain Name:HMPV/USA/	MEVRVENIRAIDMFKAKIKNRIRNSRCYRNATLILIGLTA LSMALNIFLIIDHATLRNMIKTENCANMPSAEPSKKTPM TSIAGPSTKPNPQQATQWTTENSTSPAATLEGHPYTGTT QTPDTTAPQQTTDKHTALPKSTNEQITQTTTEKKTTRAT TQKRKKEKKTQTKPQVQLQPKQPTPPTKSEMQVRQSQH PTDPELTPLPKAVNRQPGQQNQAPHHIMHGEVQDPGER NTQVSHPSS	2	99

Genbank ID	Nucleotides of CDS	Full sequence ID	Sequence	#Sequences/Cluster	SEQ ID NO
		C1-334/2004/B|Protein Name:attachment glycoprotein G|Gene Symbol:G			
KC915036	6154-7911	gb:KC915036:6154-7911|Organism:Avian metapneumovirus type C|Strain Name:GDY|Protein Name:attachment glycoprotein|Gene Symbol:G	MEVKIENVGKSQELRVKVKNFIKRSDCKKKLFALILGLI SFDITMNIMLSVMYVESNEALSSCRVQGTPAPRDNRTNT ENTAKETTLHTMTTTRNTEAGGTKTTKPQADERATSPS KNPTIGADKHKTTRATTEAEQEKQSKQTTEPGTSTPKHI PARPSSKSPATTKTTTQPTTPTVAKGGTAPKNRQTTTKK TEADTPTTSRAKQTNKPTGTETTPPRATTETDKDKEGPT QHTTKEQPETTAGGTTTPQPRRTTSRPAPTTNTKEGAET TGTRTTKSTQTSASPPRPTRSTPSKTATGTNKRATTTKGP NTASTDRRQQTRTTPKQDQQTQTKAKTTTNKAHAKAA TTPEHNTDTTDSMKENSKEDKTTRDPSSKATTKQENTS KGTTATNLGNNTEAGARTPPTTTPTRHTTEPATSTAGGH TKARTTRWKSTAARQPTRNNTTADTKTAQSKQTTPAQL GNNTTPENTTPPDNKSNSQTNVAPTEEIEIGSSLWRRRY VYGPCRENALEHPMNPCLKDNTTWIYLDNGRNLPAGY YDSKTDKIICYGIYRGNSYCYGRIECTCKNGTGLLSYCC NSYNWS	2	100
LC168749	7239-9196	gb:LC168749:7239-9196|Organism:Rinderpest morbillivirus|Strain Name:Lv|Protein Name:H protein|Gene Symbol:H	MSSPRDRVNAFYKDNLQFKNTRVVLNKEQLLIERPYML LAVLFVMFLSLVGLLAIAGIRLHRAAVNTAEINSGLTTSI DITKSIEYQVKDVLTPLFKIIGDEVGLRTPQRFTDLTKFIS DKIKFLNPDKEYDFRDINWCISPPERIKINYDQYCAHTAA EELITMLVNSSLAGTAVLRTSLVNLGRSCTGSTTTKGQF SNMSLALSGIYSGRGYNISSMITITEKGMYGSTYLVGKH NQGARRPSTAWQRDYRVFEVGIIRELGVGTPVFHMTNY LELPRQPELEICMLALGEFKLAALCLADNSVALHYGGLR DDHKIRFVKLGVWPSPADSDTLATLSAVDPTLDGLYITT HRGIIAAGKAVWAVPVTRTDDQRKMGQCRREACREKP PPFCNSTDWEPLEAGRIPAYGILTIRLGLADKPEIDIISEFG PLITHDSGMDLYTPLDGNEYWLTIPPLQNSALGTVNTLV LEPSLKISPNILTLPIRSGGGDCYTPTYLSDLADDDVKLSS NLVILPSRNLQYVSATYDTSRVEHAIVYYIYSTGRLSSYY YPVKLPIKGDPVSLQIGCFPWGLKLWCHHFCSVIDSGTG KQVTHTGAVGIEITCNSR	2	101
LC187310	8144-9871	gb:LC187310:8144-9871|Organism:Avian paramyxovirus 10|Strain Name:rAPMV-10-FI324/YmHA|Protein Name:hemagglutinin-neuraminidase|Gene Symbol:HN	MDSSQMNILDAMDRESSKRTWRGVFRVTTIIMVVTCVV LSAITLSKVAHPQGFDTNELGNGIVDRVSDKITEALTVP NNQIGEIFKIVALDLHVLVSSSQQAIAGQIGMLAESINSIL SQNGSASTILSSSPEYAGGIGVPLFSNKLTNGTVIKPITLIE HPSFIPGPTTIGGCTRIPTFHMASSHWCYSHNIIEKGCKDS GISSMYISLGVLQVLKKGTPVFLVTASAVLSDDRNRKSC SIISSRFGCEILCSLVTEAESDDYKSDTPTGMVHGRLYFN GTYREGLVDTETIFRDFSANYPGVGSGEIVEGHIHFPIYG GVKQNTGLYNSLTPYWLDAKNKYDYCKLPYTNQTIQN SYKPPFIHGRFWAQGILSCELDLFNLGNCNLKIIRSDKVM MGAESRLMLVGSKLLMYQRASSWWPLGITQEIDIAELH SSNTTILREVKPILSSKFPRPSYQPNYCTKPSVCPAVCVT GVYTDMWPISITGNISDYAWISHYLDAPTSRQQPRIGIAN QYFWIHQTTIFPTNTQSSYSTTTCFRNQVRSRMFCLSIAE FADGVFGEFRIVPLLYELRV	2	102
NC_004074	6590-8563	gb:NC_004074:6590-8563|Organism:Tioman virus|Strain	MWATSESKAPIPANSTLNLVDVPLDEPQTITKHRKQKRT GRLVFRLLSLVLSLMTVILVLVILASWSQKINACATKEG FNSLDLQISGLVKSINSLITEVNQISITTAINLPIKLSDFGK SIVDQVTQMIRQCNAVCKGPGEKPGIQNIRINIPNNFSTY	2	103

Genbank ID	Nucleotides of CDS	Full sequence ID	Sequence	#Sequences/Cluster	SEQ ID NO
		Name:UNKNOWN-NC_004074|Protein Name:attachment protein|Gene Symbol:HN	LELNNTVKSIELQRRPALLARPNPIPKSCSRFPSYSVNFGI HCFAHAITDQSCELSDKTYYRLAIGISDKNLSDPSDVKYI GEAFTPMGLQARGCSVISSIYGCYLLCSKSNQGYEADFQ TQGFHQMYILFLSRDLKTTLFNDMISSTTVVWNGLYPGE GAGIWHMGYLIFPLWGGIKIGTPASTSILNSTLDLPLVGP SCKSTLEENNLINKDVLFSPYFGESVMVFGFLSCYMLSN VPTHCQVEVLNSSVLGFGSRSQLMDLKGIVYLYIQSAG WYSYTQLFRLSLQSRGYKLTVKQIRRIPISSTTRPGTAPC DVVHNCPYTCATGLFQAPWIVNGDSILDRDVRNLVFVQ AWSGNFNTFQKGLISICNQYTCPLTTLLDNDNSIMRSTT TYCYPSLSEYNLQCQSFIEWGGPVGNPIGILEVHYIIKFK	2	104
NC_005283	7091-8905	gb:NC_005283:7091-8905|Organism:Dolphin morbillivirus|Strain Name:UNKNOWN-NC_005283|Protein Name:haemagglutinin protein|Gene Symbol:H	MSSPRDKVDAFYKDIPRPRNNRVLLDNERVIIERPLILVG VLAVMFLSLVGLLAIAGVRLQKATTNSIEVNRKLSTNLE TTVSIEHHVKDVLTPLFKIIGDEVGLRMPQKLTEIMQFIS NKIKFLNPDREYDFNDLHWCVNPPDQVKIDYAQYCNHI AAEELIVTKFKELMNHSLDMSKGRIFPPKNCSGSVITRG QTIKPGLTLVNIYTTRNFEVSFMVTVISGGMYGKTYFLK PPEPDDPFEFQAFRIFEVGLVRDVGSREPVLQMTNFMVI DEDEGLNFCLLSVGELRLAAVCVRGRPVVTKDIGGYKD EPFKVVTLGIIGGGLSNQKTEIYPTIDSSIEKLYITSHRGII RNSKARWSVPAIRSDDKDKMEKCTQALCKSRPPPSCNS SDWEPLTSNRIPAYAYIALEIKEDSGLELDITSNYGPLIIH GAGMDIYEGPSSNQDWLAIPPLSQSVLGVINKVDFTAGF DIKPHTLTTAVDYESGKCYVPVELSGAKDQDLKLESNL VVLPTKDFGYVTATYDTSRSEHAIVYYVYDTARSSSYFF PFRIKARGEPIYLRIECFPWSRQLWCHHYCMINSTVSNEI VVVDNLVSINMSCSR	2	105
NC_007803	7978-12504	gb:NC_007803:7978-12504|Organism:Beilong virus|Strain Name:Li|Protein Name:attachment glycoprotein|Gene Symbol:G	MSQLAAHNLAMSNFYGTHQGDLSGSQKGEEQQVQGVI RYVSMIVSLLSLFTIIALNVTNIIYMTESGGTMQSIKTAQ GSIDGSMREISGVIMEDVKPKTDLINSMVSYNIPAQLSMI HQIIKNDVPKQCTPSFMFNNTICPLAENPTHSRYFEEVNL DSISECSGPDMHLGLGVNPEFIEFPSFAPGSTKPGSCVRL PSFSLSTTVFAYTHTIMGHGCSELDVGDHYFSVGRIADA GHEIPQFETISSWFINDKINRRSCTVAAGAMEAWMGCVI MTETFYDDRNSLDTGKLTISYLDVFGRKKEWIYTRSEIL YDYTYTSVYFSVGSGVVVGDTVYFLIWGSLSSPIEETAY CFAPDCSNYNQRMCNEAQRPSKFGHRQMVNGILKFKTT STGKPLLSVGTLSPSVVPFGSEGRLMYSEITKIIYLYLRST SWHALPLTGLFVLGPPTSISWIVQRAVSRPGEFPCGASN RCPKDCVTGVYTDLFPLGSRYEYAATVYLNSETYRVNP TLALINQTNIIASKKVTTESQRAGYTTTTCFVFKLRVWCI SVVELAPSTMTAYEPIPFLYQLDLTCKGKNGSLAMRFA GKEGTYKSGRYKSPRNECFFEKVSNKYYFIVSTPEGIQP YEIRDLTPDRMPHIIMYISDVCAPALSAFKKLLPAMRPIT TLTIGNWQFRPVEVSGGLRVNIGRNLTKEGDLTMSAPE DPGSNTFPGNHIPGNGILDAGYYTVEYPKE	2	106
NC_009489	6559-8512	gb:NC_009489:6559-8512|Organism:Mapuera virus|Strain Name:BeAnn 370284|Protein Name:attachment protein|Gene Symbol:HN	MASLQSEPGSQKPHYQSDDQLVKRTWRSFFRFSVLVVTI TSLALSIITLIGVNRISTAKQISNAFAAIQANILSSIPDIRPIN SLLNQLVYTSSVTLPLRISSLESNVLAAIQEACTYRDSQS SCSATMSVMNDQRYIEGIQVYSGSFLDLQKHTLSPPIAFP SFIPTSTTTVGCTRIPSFSLTKTHWCYTHNYIKTGCRDAT QSNQYIALGTIYTDPDGTPGFSTSRSQYLNDGVNRKSCSI SAVPMGCALYCFISVKEEVDYYKGTVPPAQTLILFFFNG TVHEHRIVPSSMNSEWVMLSPGVGSGVFYNNYIIFPLYG GMTKDKAEKRGELTRFFTPKNSRSLCKMNDSVFSNAAQ SAYYPPYFSSRWIRSGLLACNWNQIITTNCEILTFSNQVM	2	106

Genbank ID	Nucleotides of CDS	Full sequence ID	Sequence	#Sequences/Cluster	SEQ ID NO
			MMGAEGRLILINDDLFYYQRSTSWWPRPLVYKLDIELN YPDSHIQRVDQVEVTFPTRPGWGGCVGNNFCPMICVSG VYQDVWPVTNPVNTTDSRTLWVGGTLLSNTTRENPAS VVTSGGSISQTVSWFNQTVPGAYSTTTCFNDQVQGRIFC LIIFEVGGGLLGEYQIVPFLKELKYQGAVHA		
NC_017937	6334-8544	gb:NC_017937:6334-8544|Organism:Nariva virus|Strain Name:UNKNOWN-NC_017937|Protein Name:attachment protein|Gene Symbol:H	MAPINYPASYYTNNAERPVVITTKSTESKGQRPLPLGNA RFWEYFGHVCGTLTFCMSLIGIIVGIIALANYSSDKDWK GRIGGDIQVTRMATEKTVKLILEDTTPKLRNILDSVLFQL PKMLASIASKINTQTPPPPTTSGHSTALATQCSSNCENRP EIGYDYLRQVEQSLQRITNISIQLLEASEIHSMAGAYPNA LYKIRTQDSWSVTAKECPLQAFQPNLNLIPAMIGTATGA LIRNCVRQPVIVVDDGVYMLTYLAMRGSCQDHQKSVR HFEMGVITSDPFGDPVPTPLRHWTKRALPAYDGCALAV KGHAGFALCTETSVGPLRDRTAKRKPNIVLFKASLVGEL SERVIPPQSWLSGFSFFSVYTVAGKGYAYHSKFHAFGNV VRVGQSEYQAKCRGTGCPTANQDDCNTAQRVSQEDNT YLHQAILSVDIDSVIDPEDVVYVIERDQYYQASAGDLYR VPETGEILYNLHNGGWSNEVQVGRIQPSDRFYMREIQLT STRVPAPNGCNRVKGCPGGCVAVISPAFTPMHPEFNVG VGIFPMNQPHNPSIMHVQQQTELFWKPIVGGNITLHESSI ACYSTVPPNPSYDLCIGVMTLLLHQGQLPQFQALSWYQ PTMCNGNAPQNRRALIPVIVEDSKAMSVSSDAPRTP	2	107
NC_025256	9117-11015	gb:NC_025256:9117-11015|Organism:Bat Paramyxovirus Eid_hel/GH-M74a/GHA/2009|Strain Name:BatPV/Eid_hel/GH-M74a/GHA/2009|Protein Name:glycoprotein|Gene Symbol:G	MPQKTVEFINMNSPLERGVSTLSDKKTLNQSKITKQGYF GLGSHSERNWKKQKNQNDHYMTVSTMILEILVVLGIMF NLIVLTMVYYQNDNINQRMAELTSNITVLNLNLNQLTN KIQREIIPRITLIDTATTITIPSAITYILATLTTRISELLPSINQ KCEFKTPTLVLNDCRINCTPPLNPSDGVKMSSLATNLVA HGPSPCRNFSSVPTIYYYRIPGLYNRTALDERCILNPRLTI SSTKFAYVHSEYDKNCTRGFKYYELMTFGEILEGPEKEP RMFSRSFYSPTNAVNYHSCTPIVTVNEGYFLCLECTSSDP LYKANLSNSTFHLVILRHNKDEKIVSMPSFNLSTDQEYV QIIPAEGGGTAESGNLYFPCIGRLLHKRVTHPLCKKSNCS RTDDESCLKSYYNQGSPQHQVVNCLIRIRNAQRDNPTW DVITVDLTNTYPGSRSRIFGSFSKPMLYQSSVSWHTLLQ VAEITDLDKYQLDWLDTPYISRPGGSECPFGNYCPTVC WEGTYNDVYSLTPNNDLFVTVYLKSEQVAENPYFAIFS RDQILKEFPLDAWISSARTTTISCFMFNNEIWCIAALEITR LNDDIIRPIYYSFWLPTDCRTPYPHTGKMTRVPLRSTYN Y	2	108
NC_025347	6398-8418	gb:NC_025347:6398-8418|Organism:Avian paramyxovirus 7|Strain Name:APMV-7/dove/Tennessee/4/75|Protein Name:hemagglutinin-neuraminidase|Gene Symbol:HN	MESIGKGTWRTVYRVLTILLDVVIIILSVIALISLGLKPGE RIINEVNGSIHNQLVPLSGITSDIQAKVSSIYRSNLLSIPLQ LDQINQAISSSARQIADTINSFLALNGSGTFIYTNSPEFAN GFNRAMFPTLNQSLNMLTPGNLIEFTNFIPTPTTKSGCIRI PSFSMSSSHWCYTHNIIASGCQDHSTSSEYISMGVVEVT DQAYPNFRTTLSITLADNLNRKSCSIAATGFGCDILCSVV TETENDDYQSPEPTQMIYGRLFFNGTYSEMSLNVNQMF ADWVANYPAVGSGVELADFVIFPLYGGVKITSTLGASLS QYYYIPKVPTVNCSETDAQQIEKAKASYSPPKVAPNIWA QAVVRCNKSVNLANSCEILTFNTSTMMMGAEGRLLMIG KNVYFYQRSSSYWPVGIIYKLDLQELTTFSSNQLLSTIPIP FEKFPRPASTAGVCSKPNVCPAVCQTGVYQDLWVLYDL GKLENTTAVGLYLNSAVGRMNPFIGIANTLSWYNTTRL FAQGTPASYSTTTCFKNTKIDTAYCLSILELSDSLLGSWR ITPLLYNITLSIMS	2	109

Genbank ID	Nucleotides of CDS	Full sequence ID	Sequence	#Sequences/Cluster	SEQ ID NO
NC_025348	6590-8548	gb:NC_025348:6590-8548|Organism:Tuhoko virus 2|Strain Name:UNKNOWN-NC_025348|Protein Name:hemagglutinin-neuraminidase|Gene Symbol:HN	MPPVPTVSQSIDEGSFTDIPLSPDDIKHPLSKKTCRKLFRI VTLIGVGLISILTIISLAQQTGILRKVDSSDFQSYVQESFK QVLNLMKQFSSNLNSLIEITSVTLPFRIDQFGTDIKTQVA QLVRQCNAVCRGPIKGPTTQNIVYPALYETSLNKTLETK NVRIQEVRQEVDPVPGPGLSNGCTRNPSFSVYHGVWCY THATSIGNCNGSLGTSQLFRIGNVLEGDGGAPYHKSLAT HLLTTRNVSRQCSATASYYGCYFICSEPVLTERDDYETP GIEPITIFRLDPDGNWVVFPNINRFTEYSLKALYPGIGSGV LFQGKLIFPMYGGIDKERLSALGLGNIGLIERRMADTCN HTEKELGRSFPGAFSSPYYHDAVMLNFLLICEMIENLPG DCDLQILNPTNMSMGSESQLSVLDNELFLYQRSASWWP YTLIYRLNMRYTGKYLKPKSIIPMVIKSNTRPGYEGCNH ERVCPKVCVTGVFQAPWILSIGRDHKERVSNVTYMVA WSMDKSDRTYPAVSVCGSDTCKLTVPLGDSKVHSAYS VTRCYLSRDHMSAYCLVIFELDARPWAEMRIQSFLYKLI LT	2	110
NC_025350	6451-8341	gb:NC_025350:6451-8341|Organism:Tuhoko virus 3|Strain Name:UNKNOWN-NC_025350|Protein Name:hemagglutinin-neuraminidase|Gene Symbol:HN	MHNRTQSVSSIDTSSDVYLPRRKKAVTKFTFKKIFRVLIL TLLLSIIIIIAVIFPKIDHIRETCDNSQILETITNQNSEIKNLI NSAITNLNVLLTSTTVDLPIKLNNFGKSIVDQVTMMVRQ CNAVCRGPGDRPTQNIELFKGLYHTSPPSNTSTKLSMITE ASNPDDIVPRPGKLLGCTRFPSFSVHYGLWCYGHMAST GNCSGSSPSVQIIRIGSIGTNKDGTPKYVIIASASLPETTRL YHCSVTMTSIGCYILCTTPSVSETDDYSTMGIEKMSISFL SLDGYLTQLGQPTGLDNQNLYALYPGPGSGVIFRDFLIF PMMGGIRLMDAQKMLNRNITYRGFPPSETCTESELKLK QEVANMLTSPYYGEVLVLNFLYVCSLLDNIPGDCSVQLI PPDNMTLGAESRLYVLNGSLIMYKRGSSWWPYTELYQI NYRVNNRAFRVRESVRINTTSTSRPGVQGCNLEKVCPK VCVSGIYQSPGIISAPVNPTRQEEGLLYFLVWTSSMSSRT GPLSSLCDHSTCRITYPIGDDTIFIGYTDSSCFMSSIKEGIY CIAFLELDNQPYSMMAIRSLSYIIN	2	111
NC_025352	8716-11257	gb:NC_025352:8716-11257|Organism:Mojiang virus|Strain Name:Tongguan1|Protein Name:attachment glycoprotein|Gene Symbol:G	MATNRDNTITSAEVSQEDKVKKYYGVETAEKVADSISG NKVFILMNTLLILTGAIITITLNITNLTAAKSQQNMLKIIQ DDVNAKLEMFVNLDQLVKGEIKPKVSLINTAVSVSIPGQ ISNLQTKFLQKYVYLEESITKQCTCNPLSGIFPTSGPTYPP TDKPDDDTTDDDKVDTTIKPIEYPKPDGCNRTGDHFTM EPGANFYTVPNLGPASSNSDECYTNPSFSIGSSIYMFSQEI RKTDCTAGEILSIQIVLGRIVDKGQQGPQASPLLVWAVP NPKIINSCAVAAGDEMGWVLCSVTLTAASGEPIPHMFD GFWLYKLEPDTEVVSYRITGYAYLLDKQYDSVFIGKGG GIQKGNDLYFQMYGLSRNRQSFKALCEHGSCLGTGGGG YQVLCDRAVMSFGSEESLITNAYLKVNDLASGKPVIIGQ TFPPSDSYKGSNGRMYTIGDKYGLYLAPSSWNRYLRFGI TPDISVRSTTWLKSQDPIMKILSTCTNTDRDMCPEICNTR GYQDIFPLSEDSEYYTYIGITPNNGGTKNFVAVRDSDGHI ASIDILQNYYSITSATISCFMYKDEIWCIAITEGKKQKDNP QRIYAHSYKIRQMCYNMKSATVTVGNAKNITIRRY	2	112
NC_025363	6503-8347	gb:NC_025363:6503-8347|Organism:Avian paramyxovirus 12|Strain Name:Wigeon/Italy/3920_1/2005|Protein Name:hemagglutini	MESATSQVSFENDKTSDRRTWRAVFRVLMIILALSSLCV TVAALIYSAKAAIPGNIDASEQRILSSVEAVQVPVSRLED TSQKIYRQVILEAPVTQLNMETNILNAITSLSYQIDASAN SSGCGAPVHDSDFTGGVGRELLQEAEVNLTIIRPSKFLEH LNFIPAPTTGNGCTRIPSFDLGQTHWCYTHNVVLNGCRD RGHSFQYVALGILRTSATGSVFLSTLRSVNLDDDRNRKS CSVSATPIGCEMLCSLVTETEEGDYDSIDPTPMVHGRLG FDGKYREVDLSEKEIFADWRANYPAVGGGAFFGNRVW FPVYGGLKEGTQSERDAEKGYAIYKRFNNTCPDDNTTQI	2	113

Genbank ID	Nucleotides of CDS	Full sequence ID	Sequence	#Sequences/Cluster	SEQ ID NO
		n-neuraminidase|Gene Symbol:HN	ANAKASYRPSRFGGRFIQQGILSFKVEGNLGSDPILSLTD NSITLMGAEARVMNIENKLYLYQRGTSWFPSALVYPLD VANTAVKVRAPYIFDKFTRPGGHPCSASSRCPNVCVTG VYTDAYPLVFSRSHDIVAVYGMQLAAGTARLDPQAAI WYGNEMSTPTKVSSSTTKAAYTTSTCFKVTKTKRIYCISI AEIGNTLFGEFRIVPLLIEVQKTPLTRRSELRQQMPQPPID LVIDNPFCAPSGNLSRKNAIDEYANSWP		
NC_025373	6619-8605	gb:NC_025373:6619-8605|Organism:Avian paramyxovirus 3|Strain Name:turkey/Wisconsin/68|Protein Name:hemagglutinin|Gene Symbol:HN	MEPTGSKVDIVPSQGTKRTCRTFYRLLILILNLIIIILTIISIY VSISTDQHKLCNNEADSLLHSIVEPITVPLGTDSDVEDEL REIRRDTGINIPIQIDNTENIILTTLASINSNIARLHNATDES PTCLSPVNDPRFIAGINKITKGSMIYRNFSNLIEHVNFIPSP TTLSGCTRIPSFSLSKTHWCYSHNVISTGCQDHAASSQYI SIGIVDTGLNNEPYLRTMSSRLLNDGLNRKSCSVTAGAG VCWLLCSVVTESESADYRSRAPTAMILGRFNFYGDYTE SPVPASLFSGRFTANYPGVGSGTQLNGTLYFPIYGGVVN DSDIELSNRGKSFRPRNPTNPCPDPEVTQSQRAQASYYP TRFGRLLIQQAILACRISDTTCTDYYLLYFDNNQVMMG AEARIYYLNNQMYLYQRSSSWWPHPLFYRFSLPHCEPM SVCMITDTHLILTYATSRPGTSICTGASRCPNNCVDGVY TDVWPLTEGTTQDPDSYYTVFLNSPNRRISPTISIYSYNQ KISSRLAVGSEIGAAYTTSTCFSRTDTGALYCITIIEAVNT IFGQYRIVPILVQLISD	2	114
NC_025386	7541-9403	gb:NC_025386:7541-9403|Organism:Salem virus|Strain Name:UNKNOWN-NC_025386|Protein Name:attachment glycoprotein|Gene Symbol:G	MKAMHYYKNDFADPGTNDNSSDLTTNPFISNQIKSNLSP PVLAEGHLSPSPIPKFRKILLTISFVSTIVVLTVILLVLTIRI LTIIEASAGDEKDIHTILSSLLNTFMNEYIPVFKNLVSIISL QIPQMLIDLKTSSTQMMQSLKTFPRDLETLSTVTQSVAV LLEKAKSTIPDINKFYKNVGKVTFNDPNIKVLTLEVPAW LPIVRQCLKQDFRQVISNSTGFALIGALPSQLFNEFEGYP SLAIVSEVYAITYLKGVMFENQENFLYQYFEIGTISPDGY NKPYFLRHTSVMLSTFKLSGKCTAAVDYRGGIFLCTPSP KIPKILQNPPDLPTLTVVSIPFDGRYTIRNISLMLTDEADII YDLDTLQGRGVLQAMRFYALVRVISSSSPRHFPFCKNS WCPTADDKICDQSRRLGADGNYPVMYGLISIPAHSSYQ GNVSLKLIDPKYYAYTRDASLFYNSMTDTYHYSFGTRG WVSRPIIGELLLGDDIVLTRYTVRSVSRATAGDCTTVSM CPQACSGGMNSIFYPLNFDKPQVTGVAIRQYERQQEGII VVTMNDHYYYSVPIIKNGTLLISSVTDCFWLMGDLWCM SLMEKNNLPLGVRSLAHLTWNIHWSCS	2	115
NC_025390	6647-8386	gb:NC_025390:6647-8386|Organism:Avian paramyxovirus 9|Strain Name:duck/New York/22/1978|Protein Name:hemagglutinin-neuraminidase|Gene Symbol:HN	MESGISQASLVNDNIELRNTWRTAFRVVSLLLGFTSLVL TACALHFALNAATPADLSSIPVAVDQSHHEILQTLSLMS DIGNKIYKQVALDSPVALLNTESTLMSAITSLSYQINNAA NNSGCGAPVHDKDFINGVAKELFVGSQYNASNYRPSRF LEHLNFIPAPTTGKGCTRIPSFDLAATHWCYTHNVILNG CNDHAQSYQYISLGILKVSATGNVFLSTLRSINLDDDEN RKSCSISATPLGCDLLCAKVTEREEADYNSDAATRLVHG RLGFDGVYHEQALPVESLFSDWVANYPSVGGGSYFDNR VWFGVYGGIRPGSQTDLLQSEKYAIYRRYNNTCPDNNP TQIERAKSSYRPQRFGQRLVQQAILSIRVEPSLGNDPKLS VLDNTVVLMGAEARIMTFGHVALMYQRGSSYFPSALL YPLSLTNGSAAASKPFIFEQYTRPGSPPCQATARCPNSCV TGVYTDAYPLFWSEDHKVNGVYGMMLDDITSRLNPVA AIFDRYGRSRVTRVSSSSTKAAYTTNTCFKVVKTKRVY CLSIAEIENTLFGEFRITPLLSEIIFDPNLEPSDTSRN	2	116
NC_025403	6692-8645	gb:NC_025403:6692-8645|Organism:Ach	MATNLSTITNGKFSQNSDEGSLTELPFFEHNRKVATTKR TCRFVFRSVITLCNLTILIVTVVVLFQQAGFIKRTESNQV CETLQNDMHGVVTMSKGVITTLNNLIEITSVNLPFQMK	2	117

Genbank ID	Nucleotides of CDS	Full sequence ID	Sequence	#Sequences/Cluster	SEQ ID NO
		imota virus 1|Strain Name:UNKNOWN-NC_025403|Protein Name:attachment protein|Gene Symbol:HN	QFGQGIVTQVTQMVRQCNAVCKGPTIGPDIQNIVYPASY ESMIKHPVNNSNILLSEIRQPLNFVPNTGKLNGCTRTPSF SVYNGFWCYTHAESDWNCNGSSPYMQVFRVGVVTSDY DYNVIHKTLHTKTSRLANVTYQCSTISTGYECYFLCSTP NVDEITDYKTPGIESLQIYKIDNRGTFAKFPITDQLNKEL LTALYPGPGNGVLYQGRLLFPMHGGMQSSELNKVNLN NTVLSQFNDNKGCNATEIKLESEFPGTFTSPYYSNQVML NYILICEMIENLPGNCDLQIVAPKNMSMGSESQLYSINN KLYLYQRSSSRWPYPLIYEVGTRLTNRQFRLRAINRFLIK STTRPGSEGCNIYRVCPKVCVTGVYQAPWILHVSKAGS QSIAKVLYAVAWSKDHMSRKGPLFSICDNDTCFLTKSL ASEHVHSGYSITRCYLENSERHIICVVIMELDASPWAEM RIQSVIYNITLPS		
NC_025404	6655-8586	gb:NC_025404:6655-8586|Organism:Achimota virus 2|Strain Name:UNKNOWN-NC_025404|Protein Name:attachment protein|Gene Symbol:HN	MDNSMSISTISLDAQPRIWSRHESRRTWRNIFRITSLVLL GVTVIICIWLCCEVARESELELLASPLGALIMAINTIKSSV VKMTTELNQVTFTTSIILPNKVDQFGQNVVSQVAQLVK QCNAVCRGHQDTPELEQFINQKNPTWILQPNYTTKLTN LHEIDSIIPLVDYPGFSKSCTRFPSFSEGSKFWCFTYAVVK EPCSDISSSIQVVKYGAIKANHSDGNPYLVLGTKVLDDG KFRRGCSITSSLYGCYLLCSTANVSEVNDYAHTPAYPLT LELISKDGITTDLSPTYTVQLDKWSALYPGIGSGVIFKGY LMFPVYGGLPFKSPLISASWVGPGNKWPVDFSCSEDQY STFNFSNPYSALYSPHFSNNIVVSALFVCPLNENLPYSCE VQVLPQGNLTIGAEGRLYVIDQDLYYYQRSTSWWPYLQ LYKLNIRITNRVFRVRSLSLLPIKSTTRPGYGNCTYFKLC PHICVTGVYQSPWLISIRDKRPHEEKNILYFIGWSPDEQIR QNPLVSLCHETACFINRSLATNKTHAGYSESHCVQSFER NKLTCTVFYELTAKPWAEMRVQSLLFQVDFL	2	118
NC_025410	6799-8869	gb:NC_025410:6799-8869|Organism:Tuhoko virus 1|Strain Name:UNKNOWN-NC_025410|Protein Name:hemagglutinin-neuraminidase|Gene Symbol:HN	MDSRSDSFTDIPLDNRIERTVTSKKTWRSIFRVTAIILLIIC VVVSSISLNQHNDAPLNGAGNQATSGFMDAIKSLEKLM SQTINELNQVVMTTSVQLPNRITKFGQDILDQVTQMVR QCNAVCRGPGVGPSIQNYVIQGHAPTVSFDPISAEYQKF VFGITEKTLITAYHNPWECLRFPSQHLFDTTWCVSYQILT QNCSDHGPRITVIQLGEIMIANNLSTVFRDPVIKYIRHHI WLRSCSVVAYYSQCTIFCTSTNKSEPSDYADTGYEQLFL ATLQSDGTFTEHSMHGVNIVHQWNAIYGGVGNGVIIGR NMLIPLYGGINYYDHNTTIVQTVDLRPYPIPDSCSQTDN YQTNYLPSMFTNSYYGTNLVVSGYLSCRLMAGTPTSCSI RVIPIENMTMGSEGQFYLINNQLYYYKRSSNWIRDTQVY LLSYSDKGNIIEITSAERYIFKSVTSPDEGDCVTNHGCPSN CIGGLFQAPWILNDFKLCGSNITCPKIVTVWADQPDKRS NPMLSIAETDKLLLHKSYINYHTAVGYSTVLCFDSPKLN LKTCVVLQELMSDDKLLIRISYSIVSIMVE	2	119
NC_028249	7059-9010	gb:NC_028249:7059-9010|Organism:Phocine distemper virus|Strain Name:PDV/Wadden_Sea.NLD/1988|Protein Name:hemagglutinin protein|Gene Symbol:H	MFSHQDKVGAFYKNNARANSSKLSLVTDEVEERRSPWF LSILLILLVGILILLAITGIRFHQVVKSNLEFNKLLIEDMEK TKAVHHQVKDVLTPLFKIIGDEVGLRLPQKLNEIKQFIV QKTNFFNPNREFDFRELHWCINPPSKVKVNFTQYCEITE FKEATRSVANSILLLTLYRGRDDIFPPYKCRGATTSMGN VFPLAVSLSMSLISKPSEVINMLTAISEGIYGKTYLLVTD DTEENFETPEIRVFEIGFINRWLGDMPLFQTTNYRIISNNS NTKICTIAVGELALASLCTKESTILLNLGDEESQNSVLVV ILGLFGATHMDQLEEVIPVAHPSIEKIHITNHRGFIKDSVA TWMVPALALSEQGEQINCLRSACKRRTYPMCNQTSWEP FGDKRLPSYGRLTLSLDVSTDLSINVSVAQGPIIFNGDGM DYYEGTLLNSGWLTIPPKNGTILGLINQASKGDQFIVTPH ILTFAPRESSTDCHLPIQTYQIQDDDVLLESNLVVLPTQSF	2	120

Genbank ID	Nucleotides of CDS	Full sequence ID	Sequence	#Sequences/Cluster	SEQ ID NO
			EYVVATYDVSRSDHAIVYYVYDPARTVSYTYPFRLRTK GRPDILRIECFVWDGHLWCHQFYRFQLDATNSTSVVEN LIRIRFSCDRLDP		
NC_028362	6951-8675	gb:NC_028362:6951-8675|Organism:Caprine parainfluenza virus 3|Strain Name:JS2013|Protein Name:hemagglutinin-neuraminidase|Gene Symbol:HN	MEYWGHTNNPDKINRKVGVDQVRDRSKTLKIITFIISM MTSIMSTVALILILIMFIQNNNNNRIILQELRDETDAIEARI QKASNDIGVSIQSGINTRLLTIQNHVQNYIPLALTQQVSS LRESINDVITKREETQSKMPIQRMTHDDGIEPLIPDNFWK CPSGIPTISASPKIRLIPGPGLLATSTTINGCIRLPSLVINNLI YAYTSNLITQGCQDIGKSYQVLQIGIITINSDLVPDLNPRI THTFDIDDNRKSCSLALRNADVYQLCSTPKVDERSDYSS IGIEDIVLDIVTSEGTVSTTRFTNNNITFDKPYAALYPSVG PGIYYDNKIIFLGYGGLEHEENGDVICNITGCPGKTQHDC NQASYSPWFSNRRMVNAIILVNKGLNKVPSLQVWTIPM RQNYWGSEGRLLLLGNKIYIYTRSTSWHSKLQLGTLDIS NYNDIRIRWTHHDVLSRPGSEECPWGNTCPRGCITGVY NDAYPLNPSGSVVSSVILDSRTSRENPIITYSTDTSRVNEL AIRNNTLSAAYTTTNCVTHYGKGYCFHIIEINHKSLNTL QPMLFKTEIPKSCN	2	121
AB548428	5999-7261	gb:AB548428:5999-7261|Organism:Avian metapneumovirus|Strain Name:VCO3/60616|Protein Name:attachment glycoprotein|Gene Symbol:G	MGSELYIIEGVSSSEIVLKQVLRRSKKILLGLVLSALGLT LTSTIVISICISVEQVKLRQCVDTYWAENGSLHPGQSTEN TSTRGKTTTKDPRRLQATGAGKFESCGYVQVVDGDMH DRSYAVLGGVDCLGLLALCESGPICQGDTWSEDGNFCR CTFSSHGVSCCKKPKSKATTAQRNSKPANSKSTPPVHSD RASKEHNPSQGEQPRRGPTSSKTTIASTPSTEDTAKPTIS KPKLTIRPSQRGPSGSTKAASSTPSHKTNTRGTSKTTDQR PRTGPTPERPRQTHSTATPPPTTPIHKGRAPTPKPTTDLK VNPREGSTSPTAIQKNPTTQSNLVDCTLSDPDEPQRICYQ VGTYNPSQSGTCNIEVPKCSTYGHACMATLYDTPFNCW RRTRRCICDSGGELIEWCCTSQ	1	122
AF079780	8118-10115	gb:AF079780|Organism:Tupaia paramyxovirus|Strain Name:UNKNOWN-AF079780|Protein Name:hemagglutinin|Gene Symbol:H	MDYHSHTTQTGSNETLYQDPLQSQSGSRDTLDGPPSTL QHYSNPPPYSEEDQGIDGPQRSQPLSTPHQYDRYYGVNI QHTRVYNHLGTIYKGLKLAFQILGWVSVIITMIITVTTLK KMSDGNSQDSAMLKSLDENFDAIQEVANLLDNEVRPKL GVTMTQTTFQLPKELSEIKRYLLRLERNCPVCGTEATPQ GSKGNASGDTAFCPPCLTRQCSEDSTHDQGPGVEGTSR NHKGKINFPHILQSDDCGRSDNLIVYSINLVPGLSFIQLPS GTKHCIIDVSYTFSDTLAGYLIVGGVDGCQLHNKAIIYLS LGYYKTKMIYPPDYIAIATYTYDLVPNLRDCSIAVNQTS LAAICTSKKTKENQDFSTSGVHPFYIFTLNTDGIFTVTVIE QSQLKLDYQYAALYPATGPGIFIGDHLVFLMWGGLMTK AEGDAYCQASGCNDAHRTSCNIAQMPSAYGHRQLVNG LLMLPIKELGSHLIQPSLETISPKINWAGGHGRLYYNWEI NTTYIYIEGKTWRSRPNLGIISWSKPLSIRWIDHSVARRP GARPCDSANDCPEDCLVGGYYDMFPMSSDYKTAITIIPT HHQWPSSPALKLFNTNREVRVVMILRPPNNVKKTTISCI RIMQTNWCLGFIIFKEGNNAWGQIYSYIYQVESTCPNTK	1	123
AY590688	6138-7935	gb:AY590688:6138-7935|Organism:Avian metapneumovirus|Strain Name:Colorado|Protein Name:attachment	MEVKVENVGKSQELKVKVKNFIKRSDCKKKLFALILGL VSFELTMNIMLSVMYVESNEALSLCRIQGTPAPRDNKTN TENATKETTLHTTTTTRDPEVRETKTTKPQANEGATNPS RNLTTKGDKHQTTRATTEAELEKQSKQTTEPGTSTQKH TPTRPSSKSPTTTQAIAQLTTPTTPKASTAPKNRQATTKK TETDTTTASRARNTNNPTETATTTPKATTETGKSKEGPT QHTTKEQPETTAGETTTPQPRRTASRPAPTTKIEEEAETT KTRTTKSTQTSTGPPRPTGGAPSGAATEGSGRAAAAGGP SAASAGGRRRTEAAAERDRRTRAGAGPTAGGARARTA AASERGADTAGSAGGGPGGDGATGGLSGGAPAEREDA	1	124

Genbank ID	Nucleotides of CDS	Full sequence ID	Sequence	#Sequences/Cluster	SEQ ID NO
		glycoprotein|Gene Symbol:G	SGGTAAAGPGDGTEADGRAPPAAALAGRTTESAAGAA GDSGRAGTAGWGSAADGRSTGGNAAAEAGAAQSGRA APRQPSGGTAPESTAPPNSGGSGRADAAPTEEVGVGSGL WRGRYVCGPCGESVPEHPMNPCFGDGTAWICSDDGGS LPAGCYDGGTDGVVCCGVCGGNSCCCGRVECTCGGGA GLLSCCCGSYSWS		
EU403085	6620-8593	gb:EU403085:6620-8593|Organism:Avian paramyxovirus 3|Strain Name:APMV3/PKT/Netherland/449/75|Protein Name:hemagglutinin-neuraminidase protein|Gene Symbol:HN	MESPPSGKDAPAFREPKRTCRLCYRATTLSLNLTIVVLSI ISIYVSTQTGANNSCVNPTIVTPDYLTGSTTGSVEDLADL ESQLREIRRDTGINLPVQIDNTENLILTTLASINSNLRFLQ NATTESQTCLSPVNDPRFVAGINRIPAGSMAYNDFSNLIE HVNFIPSPTTLSGCTRIPSFSLSKTHWCYTHNVISNGCLD HAASSQYISIGIVDTGLNNEPYFRTMSSKSLNDGLNRKS CSVTAAANACWLLCSVVTEYEAADYRSRTPTAMVLGR FDFNGEYTEIAVPSSLFDGRFASNYPGVGSGTQVNGTLY FPLYGGVLNGSDIETANKGKSFRPQNPKNRCPDSEAIQS FRAQDSYYPTRFGKVLIQQAIIACRISNKSCTDFYLLYFD NNRVMMGAEARLYYLNNQLYLYQRSSSWWPHPLFYSI SLPSCQALAVCQITEAHLTLTYATSRPGMSICTGASRCPN NCVDGVYTDVWPLTKNDAQDPNLFYTVYLNNSTRRISP TISLYTYDRRIKSKLAVGSDIGAAYTTSTCFGRSDTGAV YCLTIMETVNTIFGQYRIVPILLRVTSR	1	125
FJ977568	6139-7936	gb:FJ977568:6139-7936|Organism:Avian metapneumovirus|Strain Name:aMPV/MN/turkey/2a/97|Protein Name:attachment glycoprotein|Gene Symbol:G	MEVKVENVGKSQELKVKVKNFIKRSDCKKKLFALILGL VSFELTMNIMLSVMYVESNEALSLCRIQGTPAPRDNKTN TENATKETTLHTTTTTRDPEVRETKTTKPQANEGATNPS RNLTTKGDKHQTTRATTEAELEKQSKQTTEPGTSTQKH TPARPSSKSPTTTQATAQPTTPTAPKASTAPKNRQATTK KTETDTTTASRARNTNNPTETATTTPKATTETGKGKEGP TQHTTKEQPETTARETTTPQPRRTASRPAPTTKIEEEAET TKTRTTKNTQTSTGPPRPTRSTPSKTATENNKRTTTTKRP NTASTDSRQQTRTTAEQDQQTQTRAKPTTNGAHPQTTT TPEHNTDTTNSTKGSPKEDKTTRDPSSKTPTEQEDASKG TAAANPGGSAEADRRAPPATTPTGRTTESAAGTTGDDS GAETTRRRSAADRRPTGGSTAAEAGTAQSGRATPKQPS GGTAAGNTAPPNNESSGRADAAPAEEAGVGPSIRRGRH ACGPRRESAPEHPTNPCPGDGTAWTRSDGGGNLPAGRH DSGADGAARRGARGGNPRRRGRAERTRGGGAGPPSCR CGSHNRS	1	126
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	MGAKLYAISGASDAQLMKKTCAKLLEKVVPIIILAVLGI TGTTTIALSISISIERAVLSDCTTQLRNGTTSGSLSNPTRST TSTAVTTRDIRGLQTTRTRELKSCSNVQIAYGYLHDSSN PVLDSIGCLGLLALCESGPFCQRNYNPRDRPKCRCTLRG KDISCCKEPPTAVTTSKTTPWGTEVHPTYPTQVTPQSQP ATMAHQTATANQRSSTTEPVGSQGNTTSSNPEQQTEPPP SPQHPPTTTSQDQSTETADGQEHTPTRKTPTATSNRRSPT PKRQETGRATPRNTATTQSGSSPPHSSPPGVDANMEGQC KELQAPKPNSVCKGLDIYREALPRGCDKVLPLCKTSTIM CVDAYYSKPPICFGYNQRCFCMETFGPIEFCCKS	1	127
JN032116	4659-5252	gb:JN032116:4659-5252|Organism:Respiratory syncytial virus|Strain Name:B/WI/629-12/06-07|Protein Name:attachment	MSKNKNQRTARTLEKTWDTLNHLIVISSCLYKLNLKSIA QIALSVLAMIISTSLIIAAIIFIISANHKVTLTTVTVQTIKNH TEKNITTYLTQVSPERVSPSKQPTTTPPIHTNSATISPNTK SEIHHTTAQTKGRTSTPTQNNKPNTKPRPKNPPKKDDYH FEVFNFVPCSICGNNQLCKSICKTIPSNKPRKNQP	1	128

Genbank ID	Nucleotides of CDS	Full sequence ID	Sequence	#Sequences/Cluster	SEQ ID NO
		glycoprotein|Gene Symbol:G			
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	MEGSRTVIYQGDPNEKNTWRLVFRTLTLILNLAILSVTIA SIIITSKITLSEVTTLKTEGVEEVITPLMATLSDSVQQEKM IYKEVAISIPLVLDKIQTDVGTSVAQITDALRQIQGVNGT QAFALSNAPEYSGGIEVPLFQIDSFVNKSMSISGLLEHAS FIPSPTTLHGCTRIPSFHLGPRHWCYTHNIIGSRCRDEGFS SMYISIGAITVNRDGNPLFITTASTILADDNNRKSCSIIASS YGCDLLCSIVTESENDDYANPNPTKMVHGRFLYNGSYV EQALPNSLFQDKWVAQYPGVGSGITTHGKVLFPIYGGIK KNTQLFYELSKYGFFAHNKELECKNMTEEQIRDIKAAY LPSKTSGNLFAQGIIYCNISKLGDCNVAVLNTSTTMMGA EGRLQMMGEYVYYYQRSSSWWPVGIVYKKSLAELMN GINMEVLSFEPIPLSKFPRPTWTAGLCQKPSICPDVCVTG VYTDLFSVTIGSTTDKDTYFGVYLDSATERKDPWVAAA DQYEWRNRVRLFESTTEAAYTTSTCFKNTVNNRVFCVS IVELRENLLGDWKIVPLLFQIGVSQGPPPK	1	129
KX940961	7978-12504	gb:KX940961:7978-12504|Organism:Beilong virus|Strain Name:ERN081008_1S|Protein Name:attachment glycoprotein|Gene Symbol:G	MSQLAAHNLAMSNFYGTHQGDLSGSQKGEEQQVQGVI RYVSMIVGLLSLFTIIALNVTNIIYMTESGGTMQSIKTAQ GSIDGSMREISGVIMEDVKPKTDLINSMVSYNIPAQLSMI HQIIKNDVLKQCTPSFMFNNTICPLAENPTHSRYFEEVNL DSISECSGPDMHLGLGVNPEFIEFPSFAPGSTKPGSCVRL PSFSLSTTVFAYTHTIMGHGCSELDVGDHYFSVGRIADA GHEIPQFETISSWFINDKINRRSCTVAAGAMEAWMGCVI MTETFYDDLNSLDTGKLTISYLDVFGRKKEWIYTRSEIL YDYTYTSVYFSVGSGVVVGDTVYFLIWGSLSSPIEETAY CFAPDCSNYNQRMCNEAQRPSKFGHRQMVNGILKFKTT STGKPLLSVGTLSPSVVPFGSEGRLMYSEITKIIYLYLRST SWHALPLTGLFVLGPPTSISWIVQRAVSRPGEFPCGASN RCPKDCVTGVYTDLFPLGSRYEYAATVYLNSETYRVNP TLALINQTNIIASKKVTTESQRAGYTTTTCFVFKLRVWCI SVVELAPSTMTAYEPIPFLYQLDLTCKGKNGSLAMRFTG KEGTYKSGRYKSPRNECFFEKVSNKYYFIVSTPEGIQPYE IRDLTPDRMPHIIMYISDVCAPALSAFKKLLPAMRPITTL TIGNWQFRPVEVSGGLRVSIGRNLTKEGDLTMSAPEDPG SNTFPGGHIPGNGLFDAGYYTVEYPKEWKQTTPKPSEG GNIIDKNKTPVIPSRDNPTSDSSIPHRESIEPVRPTREVLKS SDYVTIVSTDSGSGSGDFATGVPWTGVSPKAPQNGINLP GTELPHPTVLDRINTPAPSDPKVSADSDHTRDTIDPTALS KPLNHDTTGDTDTRINTGTATYGFTPGREATSSGKLAND LTNSTSVPSEAHPSASTSEASKPEKNTDNRVTQDPTSGT AERPTTNAPVDGKHSTQLTDARPNTADPERTSQHSSSTT RDEVKPSLPSTTEASTHQRTEAATPPELVNNTLNPPSTQ VRSVRSLMQDAIAQAWNFVRGVTP	1	130
KY511044	6454-8310	gb:KY511044:6454-8310|Organism:Avian paramyxovirus UPO216|Strain Name:APMV-15/WB/Kr/UPO216/2014|Protein Name:hemagglutinin-neuraminidase protein|Gene Symbol:HN	MERGISEVALANDRTEEKNTWRLIFRITVLVVSVITLGLT AASLVYSMNAAQPADFDGIIPAVQQVGTSLTNSIGGMQ DVLDRTYKQVALESPLTLLNMESTIMNAITSLSYKINNG GNSSGCGAPIHDPEYIGGIGKELLIDDNVDVTSFYPSAFK EHLNFIPAPTTGAGCTRIPSFDLSATHYCYTHNVILSGCQ DHSHSHQYIALGVLKLSDTGNVFFSTLRSINLDDTANRK SCSISATPLGCDILCSKVTETELEDYKSEEPTPMVHGRLS FDGTYSEKDLDVNNLFSDWTANYPSVGGGSYIGNRVW YAVYGGLKPGSNTDQSQRDKYVIYKRYNNTCPDPEDY QINKAKSSYTPSYFGSKRVQQAILSIAVSPTLGSDPVLTP LSNDVVLMGAEGRVMHIGGYTYLYQRGTSYYSPALLY PLNIQDKSATASSPYKFDAFTRPGSVPCQADARCPQSCV	1	131

Genbank ID	Nucleotides of CDS	Full sequence ID	Sequence	#Sequences/Cluster	SEQ ID NO
			TGVYTDPYPLIFAKDHSIRGVYGMMLNDVTARLNPIAA VFSNISRSQITRVSSSSTKAAYTTSTCFKVIKTNRIYCMSI AEISNTLFGEFRIVPLLVEILSNGGNTARSAGGTPVKESP KGWSDAIAEPLFCTPTNVTRYNADIRRYAYSWP		
NC_025360	8127-10158	gb:NC_025360:8127-10158|Organism:Atlantic salmon paramyxovirus|Strain Name:ASPV/Yrkje371/95|Protein Name:hemagglutinin-neuraminidase protein|Gene Symbol:HN	MPPAPSPVHDPSSFYGSSLFNEDTASRKGTSEEIHLLGIR WNTVLIVLGLILAIIGIGIGASSFSASGITGNTTKEIRLIVE EMSYGLVRISDSVRQEISPKVTLLQNAVLSSIPALVTTET NTIINAVKNHCNSPPTPPPPTEAPLKKHETGMAPLDPTTY WTCTSGTPRFYSSPNATFIPGPSPLPHTATPGGCVRIPSM HIGSEIYAYTSNLIASGCQDIGKSYQNVQIGVLDRTPEGN PEMSPMLSHTFPINDNRKSCSIVTLKRAAYIYCSQPKVTE FVDYQTPGIEPMSLDHINANGTTKTWIYSPTEVVTDVPY ASMYPSVGSGVVIDGKLVFLVYGGLLNGIQVPAMCLSP ECPGIDQAACNASQYNQYLSGRQVVNGIATVDLMNGQ KPHISVETISPSKNWFGAEGRLVYMGGRLYIYIRSTGWH SPIQIGVIYTMNPLAITWVTNTVLSRPGSAGCDWNNRCP KACLSGVYTDAYPISPDYNHLATMILHSTSTRSNPVMVY SSPTNMVNYAQLTTTAQIAGYTTTSCFTDNEVGYCATA LELTPGTLSSVQPILVMTKIPKECV	1	132

Other Proteins

In some embodiments, the fusogen may include a pH dependent protein, a homologue thereof, a fragment thereof, and a protein fusion comprising one or more proteins or fragments thereof. Fusogens may mediate membrane fusion at the cell surface or in an endosome or in another cell-membrane bound space.

In some embodiments, the fusogen includes a EFF-1, AFF-1, gap junction protein, e.g., a connexin (such as Cn43, GAP43, CX43) (DOI: 10.1021/jacs.6b05191), other tumor connection proteins, a homologue thereof, a fragment thereof, a variant thereof, and a protein fusion comprising one or more proteins or fragments thereof.

Lipid Fusogens

In some embodiments, the fusosome can comprise one or more fusogenic lipids, such as saturated fatty acids. In some embodiments, the saturated fatty acids have between 10-14 carbons. In some embodiments, the saturated fatty acids have longer-chain carboxylic acids. In some embodiments, the saturated fatty acids are mono-esters.

In some embodiments, the fusosome can comprise one or more unsaturated fatty acids. In some embodiments, the unsaturated fatty acids have between C16 and C18 unsaturated fatty

acids. In some embodiments, the unsaturated fatty acids include oleic acid, glycerol mono-oleate, glycerides, diacylglycerol, modified unsaturated fatty acids, and any combination thereof.

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., exogenous negative curvature lipids, in the membrane. In embodiments, the negative curvature lipid or a precursor thereof is added to media comprising source cells or fusosomes. In embodiments, the source cell is engineered to express or overexpress one or more lipid synthesis genes. The negative curvature lipid can be, e.g., diacylglycerol (DAG), cholesterol, phosphatidic acid (PA), phosphatidylethanolamine (PE), or fatty acid (FA).

Without wishing to be bound by theory, in some embodiments positive curvature lipids inhibit membrane fusion. In some embodiments, the fusosome comprises reduced levels of one or more positive curvature lipids, e.g., exogenous positive curvature lipids, in the membrane. In embodiments, the levels are reduced by inhibiting synthesis of the lipid, e.g., by knockout or knockdown of a lipid synthesis gene, in the source cell. The positive curvature lipid can be, e.g., lysophosphatidylcholine (LPC), phosphatidylinositol (PtdIns), lysophosphatidic acid (LPA), lysophosphatidylethanolamine (LPE), or monoacylglycerol (MAG).

Chemical Fusogens

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

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 two membranes 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 Ca2+, Mg2+, Mn2+, Zn2+, La3+, Sr3+, and H+.

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

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.

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.

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 exogenous actin, e.g., wild-type actin or actin comprising a mutation that promotes polymerization. In embodiments, the fusosome comprises ATP or phosphocreatine, e.g., exogenous ATP or phosphocreatine.

Small Molecule Fusogens

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

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

Modifications to Protein Fusogens

Protein fusogens or viral envelope proteins may be re-targeted by mutating amino acid residues in a fusion protein or a targeting protein (e.g. the hemagglutinin protein). In some 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, 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).

Protein fusogens may be re-targeted by covalently conjugating a targeting-moiety to the fusion protein or targeting protein (e.g. the hemagglutinin protein). In some embodiments, the fusogen and targeting moiety are covalently conjugated by expression of a chimeric protein comprising the fusogen linked to the targeting moiety. A target includes any peptide (e.g. a receptor) that is displayed on a target cell. In some examples the target is expressed at higher levels on a target cell than non-target cells. For example, single-chain variable fragment (scFv) can be conjugated to fusogens to redirect fusion activity towards cells that display the scFv binding target (doi:10.1038/nbt1060, DOI 10.1182/blood-2012-11-468579, doi:10.1038/nmeth.1514, doi:10.1006/mthe.2002.0550, HUMAN GENE THERAPY 11:817– 826, doi:10.1038/nbt942, doi:10.1371/journal.pone.0026381, DOI 10.1186/s12896-015-0142-z). For example, designed ankyrin repeat proteins (DARPin) can be conjugated to fusogens to redirect fusion activity towards cells that display the DARPin binding target (doi:10.1038/mt.2013.16, doi:10.1038/mt.2010.298, doi: 10.4049/jimmunol.1500956), as well as combinations of different DARPins (doi:10.1038/mto.2016.3). For example, receptor ligands and antigens can be conjugated to fusogens to redirect fusion activity towards cells that display the target receptor (DOI: 10.1089/hgtb.2012.054, DOI: 10.1128/JVI.76.7.3558–3563.2002). A targeting protein can also include, e.g., an antibody or an antigen-binding fragment thereof (e.g., Fab, Fab', F(ab')2, Fv fragments, scFv antibody fragments, disulfide-linked Fvs (sdFv), a Fd

fragment consisting of the VH and CH1 domains, linear antibodies, single domain antibodies such as sdAb (either VL or VH), nanobodies, or camelid VHH domains), an antigen-binding fibronectin type III (Fn3) scaffold such as a fibronectin polypeptide minibody, a ligand, a cytokine, a chemokine, or a T cell receptor (TCRs). Protein fusogens may be re-targeted by non-covalently conjugating a targeting moiety to the fusion protein or targeting protein (e.g. the hemagglutinin protein). For example, the fusion protein can be engineered to bind the Fc region of an antibody that targets an antigen on a target cell, redirecting the fusion activity towards cells that display the antibody's target (DOI: 10.1128/JVI.75.17.8016–8020.2001, doi:10.1038/nm1192). Altered and non-altered fusogens may be displayed on the same retroviral vector or VLP (doi: 10.1016/j.biomaterials.2014.01.051).

A targeting moiety may comprise, e.g., a humanized antibody molecule, intact IgA, IgG, IgE or IgM antibody; bi- or multi- specific antibody (e.g., Zybodies®, etc); antibody fragments such as Fab fragments, Fab' fragments, F(ab')2 fragments, Fd' fragments, Fd fragments, and isolated CDRs or sets thereof; single chain Fvs; polypeptide-Fc fusions; single domain antibodies (e.g., shark single domain antibodies such as IgNAR or fragments thereof); cameloid antibodies; masked antibodies (e.g., Probodies®); Small Modular ImmunoPharmaceuticals ("SMIPsTM"); single chain or Tandem diabodies (TandAb®); VHHs; Anticalins®; Nanobodies®; minibodies; BiTE®s; ankyrin repeat proteins or DARPINs®; Avimers®; DARTs; TCR-like antibodies;, Adnectins®; Affilins®; Trans-bodies®; Affibodies®; TrimerX®; MicroProteins; Fynomers®, Centyrins®; and KALBITOR®s.

In embodiments, the re-targeted fusogen binds a cell surface marker on the target cell, e.g., a protein, glycoprotein, receptor, cell surface ligand, agonist, lipid, sugar, class I transmembrane protein, class II transmembrane protein, or class III transmembrane protein.

Fusosomes may display targeting moieties that are not conjugated to protein fusogens in order to redirect the fusion activity towards a cell that is bound by the targeting moiety, or to affect homing.

The targeting moiety added to the fusosome may be modulated to have different binding strengths. For example, scFvs and antibodies with various binding strengths may be used to alter the fusion activity of the fusosome towards cells that display high or low amounts of the target antigen (doi:10.1128/JVI.01415-07, doi:10.1038/cgt.2014.25, DOI: 10.1002/jgm.1151). For

example DARPins with different affinities may be used to alter the fusion activity of the retroviral vector or VLP towards cells that display high or low amounts of the target antigen (doi:10.1038/mt.2010.298). Targeting moieties may also be modulated to target different regions on the target ligand, which will affect the fusion rate with cells displaying the target (doi: 10.1093/protein/gzv005).

In some embodiments protein fusogens can be altered to reduce immunoreactivity, e.g., as described herein. For instance, protein fusogens may be decorated with molecules that reduce immune interactions, such as PEG (DOI: 10.1128/JVI.78.2.912–921.2004). Thus, in some embodiments, the fusogen comprises PEG, e.g., is a PEGylated polypeptide. Amino acid residues in the fusogen that are targeted by the immune system may be altered to be unrecognized by the immune system (doi: 10.1016/j.virol.2014.01.027, doi:10.1371/journal.pone.0046667). In some embodiments the protein sequence of the fusogen is altered to resemble amino acid sequences found in humans (humanized). In some embodiments the protein sequence of the fusogen is changed to a protein sequence that binds MHC complexes less strongly. In some embodiments, the protein fusogens are derived from viruses or organisms that do not infect humans (and which humans have not been vaccinated against), increasing the likelihood that a patient's immune system is naïve to the protein fusogens (e.g., there is a negligible humoral or cell-mediated adaptive immune response towards the fusogen) (doi:10.1006/mthe.2002.0550, doi:10.1371/journal.ppat.1005641, doi:10.1038/gt.2011.209, DOI 10.1182/blood-2014-02-558163). In some embodiments, glycosylation of the fusogen may be changed to alter immune interactions or reduce immunoreactivity. Without wishing to be bound by theory, in some embodiments, a protein fusogen derived from a virus or organism that do not infect humans does not have a natural fusion targets in patients, and thus has high specificity.

Positive target cell-specific regulatory element

In some embodiments, a retroviral nucleic acid described herein 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.

A retroviral nucleic acid described herein 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 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.

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, 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 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 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 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.

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.

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 (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) 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-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 liver cells, e.g., hepatocytes, liver sinusoidal endothelial cells, cholangiocytes, stellate cells, liver-resident antigen-presenting cells (e.g., Kupffer Cells), liver-resident immune lymphocytes (e.g., T cell, B cell, or NK cell), or portal fibroblasts. Various suitable liver-specific promoters (e.g., hepatocyte-specific promoters and liver sinusoidal endothelial cell promoters) are described in Table 3 below. Table 3 also lists several ubiquitous promoters which are not specific to liver cells. In some embodiments, a fusosome (e.g., viral vector) described herein comprises, in its nucleic acid, a promoter having a sequence of 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 (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.

In some embodiments, a fusosome (e.g., viral vector) described herein comprises, in its nucleic acid, a promoter having a sequence set forth in any one of SEQ ID NOS: 133-142 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.

In some embodiments, the promoter is a promoter that drives expression in liver cells, e.g., hepatocytes, liver sinusoidal endothelial cells, cholangiocytes, stellate cells, liver-resident antigen-presenting cells (e.g., Kupffer Cells), liver-resident immune lymphocytes (e.g., T cell, B cell, or NK cell), or portal fibroblasts. In some embodiments, a fusosome (e.g., viral vector) described herein comprises, in its nucleic acid, a promoter having a sequence set forth in any one of SEQ ID NOS: 133-136 or 519-525 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, the promoter is a a hepatocyte-specific human (ApoE.HCR-hAAT (hApoE) promoter. In some embodiments, the promoter has a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO:133. In some embodiments, the promoter has the sequence set forth in SEQ ID NO:133.

Table 3. Exemplary promoters, e.g., hepatocyte-specific promoters

Specificity	Promoter Name	Source of cis-regulatory elements	Exemplary sequence	SEQ ID NO
Hepatocytes	hAAT (serpin A1	α1 antitrypsin gene (Serpina 1 gene)	AGATCTTGCTACCAGTGGAACAGCCACTAAGG ATTCTGCAGTGAGAGCAGAGGGCCAGCTAAGT GGTACTCTCCCAGAGACTGTCTGACTCACGCCA CCCCCTCCACCTTGGACACAGGACGCTGTGGTT TCTGAGCCAGGTACAATGACTCCTTTCGGTAAG TGCAGTGGAAGCTGTACACTGCCCAGGCAAAG CGTCCGGGCAGCGTAGGCGGGCGACTCAGATC CCAGCCAGTGGACTTAGCCCCTGTTTGCTCCTC CGATAACTGGGGTGACCTTGGTTAATATTCACC AGCAGCCTCCCCCGTTGCCCCTCTGGATCCACT GCTTAAATACGGACGAGGACAGGGCCCTGTCT	519

Specificity	Promoter Name	Source of cis-regulatory elements	Exemplary sequence	SEQ ID NO
			CCTCAGCTTCAGGCACCACCACTGACCTGGGA CAGTGAATGTCCCCCTGATCTGCGGCCGTGACT CTCTTAAGGTAGCCTTGCAGAAGTTGGTCGTGA GGCACTGGGCAGGTAAGTATCAAGGTTACAAG ACAGGTTTAAGGAGACCAATAGAAACTGGGCT TGTCGAGACAGAGAAGACTCTTGCGTTTCTGAT AGGCACCTATTGGTCTTACTGACATCCACTTTG CCTTTCTCTCCACAGGTGTCCACTCCCAGTTCA ATTACAGCT	
Hepatocytes	ApoE. HCR-hAAT	Apolipoprotein E/C-I gene, α1 antitrypsin gene	gttaggctcagaggcacacaggagtttctgggctcaccctgccccttccaac ccctcagttcccatcctccagcagctgtttgtgtgctgcctctgaagtccacact gaacaaacttcagcctactcatgtccctaaaatgggcaaacattgcaagcagc aaacagcaaacacacagccctccctgcctgctgaccttggagctggggcag aggtcagagacctctctgggcccatgccacctccaacatccactcgacccctt ggaatttcggtggagaggagcagaggttgtcctggcgtggtttaggtagtgtg agaggggtacccggggatcttgctaccagtggaacagccactaaggattctg cagtgagagcagagggccagctaagtggtactctcccagagactgtctgact cacgccacccccctccaccttggacacaggacgctgtggtttctgagccaggt acaatgactcctttcggtaagtgcagtggaagctgtacactgcccaggcaaa gcgtccgggcagcgtaggcgggcgactcagatcccagccagtggacttag cccctgtttgctcctccgataactggggtgaccttggttaatattcaccagcagc ctcccccgttgcccctctggatccactgcttaaatacggacgaggacagggc cctgtctcctcagcttcaggcaccaccactgacctgggacagtgaatgatccc cctgatctgcggcctcgacggtatcgataagcttgatatcgaattctagtcgtc gaccactttcacaatctgctagcaacctgaggaggttatcgtacgaaattcgct gtctgcgagggccagctgttggggtgagtactccctctcaaaagcgggcatg acttctgcgctaagattgtcagtttccaaaaacgaggaggatttgatattcacct ggcccgcggtgatgcctttgagggtggccgcgtccatctggtcagaaaaga caatctttttgttgtcaagcttgaggtgtggcaggcttgagatcgatctgaccat acacttgagtgacaatgacatccactttgcctttctctccacaggtgtccactcc caggtccaac	133
Hepatocytes	Enhanced transthyretin	Transthyretin gene	CAAATGACTTAGTTTGGCTAAAATGTAGGCTTT TAAAAATGTGAGCACTGCCAAGGGTTTTTCCTT GTTGACCCATGGATCCATCAAGTGCAAACATTT TCTAATGCACTATATTTAAGCCTGTGCAGCTAG ATGTCATTCAACATGAAATACATTATTACAACT TGCATCTGTCTAAAATCTTGCATCTAAAATGAG AGACAAAAAATCTATAAAAATGGAAAACATGC ATAGAAATATGTGAGGGAGGAAAAAATTACCC CCAAGAATGTTAGTGCACGCAGTCACACAGGG AGAAGACTATTTTTGTTTTGTTTTGATTGTTTTG TTTTGTTTTGGTTGTTTTGTTTTGGTGACCTAAC TGGTCAAATGACCTATTAAGAATATTTCATAGA ACGAATGTTCCGATGCTCTAATCTCTCTAGACA AGGTTCATATTTGTATGGGTTACTTATTCTCTCT	520

Specificity	Promoter Name	Source of cis-regulatory elements	Exemplary sequence	SEQ ID NO
			TTGTTGACTAAGTCAATAATCAGAATCAGCAG GTTTGCAGTCAGATTGGCAGGGATAAGCAGCC TAGCTCAGG	
Hepatocytes	Alb	Albumin gene	ccaccgcggtggcggccgctctagcttccttagcatgacgttccacttttttcta aggtggagcttacttctttgatttgatcttttgtgaaacttttggaaattacccatct tcctaagcttctgcttctctcagttttctgcttgctcattccacttttccagctgacc ctgccccctaccaacattgctccacaagcacaaattcatccagagaaaataaa ttctaagttttatagttgtttggatcgcataggtagctaaagaggtggcaaccca cacatccttaggcatgagcttgatttttttgatttagaaccttcccctctctgttcc tagactacactacacattctgcaagcatagcacagagcaatgttctactttaatt actttcattttcttgtatcctcacagcctagaaaataacctgcgttacagcatcca ctcagtatcccttgagcatgaggtgacactacttaacatagggacgagatggt actttgtgtctcctgctctgtcagcagggcactgtacttgctgataccagggaat gtttgttcttaaataccatcattccggacgtgtttgccttggccagttttccatgta catgcagaaagaagtttggactgatcaatacagtcctctgcctttaaagcaata ggaaaaggccaacttgtctacgtttagtatgtggctgtagaaagggtatagata taaaaattaaaactaatgaaatggcagtcttacacattttggcagcttatttaaa gtcttggtgttaagtacgctggagctgtcacagctaccaatcaggcatgtctgg gaatgagtacacggggaccataagttactgacattcgtttcccattccatttgaa tacacacttttgtcatggtattgcttgctgaaattgttttgcaaaaaaaaccccttc aaattcatatatattatttttaataaatgaattttaatttatctcaatgttataaaaaagt caattttaataattaggtacttatatacccaataatatctaacaatcatttttaaaca tttgtttattgagcttattatggatgaatctatctctatatactctatatactctaaaa aagaagaaagaccatagacaatcatctatttgatatgtgtaaagtttacatgtga gtagacatcagatgctccatttctcactgtaataccatttatagttacttgcaaaa ctaactggaattctaggacttaaatattttaagttttagctgggtgactggttgga aaatttaggtaagtactgaaaccaagagattataaaacaataaattctaaagttt tagaagtgatcataatcaaatattaccctctaatgaaaatattccaaagttgagct acagaaatttcaacataagataatttagctgtaacaatgtaatttgttgtctatttt cttttgagatacagttttttctgtctagctttggctgtcctggaccttgctctgtaga ccaggttggtcttgaactcagagatctgcttgcctctgccttgcaagtgctagg attaaaagcatgtgccaccactgcctggctacaatctatgttttataagagattat aaagctctggctttgtgacattaatctttcagataataagtcttttggattgtgtctg gagaacatacagactgtgagcagatgttcagaggtatatttgcttaggggtga attcaatctgcagcaataattatgagcagaattactgacacttccatttatacatt ctacttgctgatctatgaaacatagataagcatgcaggcattcatcatagttttct ttatctggaaaaacattaaatatgaaagaagcactttattaatacagtttagatgt gttttgccatcttttaatttcttaagaaatactaagctgatgcagagtgaagagtg tgtgaaaagcagtggtgcagcttggcttgaactcgttctccagcttgggatcga cctgcaggcatgcttccatgccaaggcccacactgaaatgctcaaatgggag acaaagagattaagctcttatgtaaaatttgctgttttacataactttaatgaatgg acaaagtcttgtgcatgggggtgggggtggggttagaggggaacagctcca gatggcaaacatacgcaagggatttagtcaaacaacttttggcaaagatggt atgattttgtaatggggtaggaaccaatgaaatgcgaggtaagtatggttaatg atctacagttattggttaaagaagtatattagagcgagtctttctgcacacagat	134

Specificity	Promoter Name	Source of cis-regulatory elements	Exemplary sequence	SEQ ID NO
			cacctttcctatcaaccccgggatccccgggctgcaggaattcgatatcaag cttatcgataccgtcgacctcgaggggggggcccggtac	
Hepatocytes (e.g., hepatocytes from hepatocyte progenitors)	Apoa2	Apolipoprotein A-II gene	CCGGGCGTGGTGGCGCATGTCTGTAATCCCAG CTACTTGGGATGCTGAGGCAGGAGAATCCTTG AACCCGGGAGGTGGAGGTTGCAGTGAGCCGAG ATCATGCCATTACGCTCCAGCCTGAGCAACAA GAGCAAAACTCCGTCTCAGGAAAACAAACAAA AAAACCTGCACATATACTTCTGAATTTAAAACA AAAGTTAAAAAACAAAGATTTCTTGGTCTCTG GTCACTACCTCCCTCATCAGCTTTGCGCCTCCA CTGTCACCCTCAGGAATGTTCCACATACTCAGC GAGTATGCTTGGGGGGCAAAAGGGTGAAAGAT ACAAAAGCTTCTGATATCTATTTAACTGATTTC ACCCAAATGCTTTGAACCTGGGAATGTACCTCT CCCCCTCCCCCACCCCCAACAGGAGTGAGACA AGGGCCAGGGCTATTGCCCCTGCTGACTCAAT ATTGGCTAATCACTGCCTAGAACTGATAAGGT GATCAAATGACCAGGTGCCTTCAACCTTTACCC TGGTAGAAGCCTCTTATTCACCTCTTTTCCTGC CAGAGCCCTCCATTGGGAGGGGACGGGCGGAA GCTGTTTTCTGAATTTGTTTTACTGGGGGTAGG GTATGTTCAGTGATCAGCATCCAGGTCATTCTG GGCTCTCCTGTTTTCTCCCCGTCTCATTACACAT TAACTCAAAAACGGACAAGATCATTTACACTT GCCCTCTTACCCGACCCTCATTCCCCTAACCCC CATAGCCCTCAACCCTGTCCCTGATTTCAATTC CTTTCTCCTTTCTTCTGCTCCCCAATATCTCTCT GCCAAGTTGCAGTAAAGTGGGATAAGGTTGAG AGATGAGATCTACCCATAATGGAATAAAGACA CCATGAGCTTTCCATGGTATGATGGGTTGATGG TATTCCATGGGTTGATATGTCAGAGCTTTCCAG AGAAATAACTTGGAATCCTGCTTCCTGTTGCAC TCAAGTCCAAGGACCTCAGATCTCAAAAGAAT GAACCTCAAATATACCTGAAGTGTACCCCCTTA GCCTCCACTAAGAGCTGTACCCCCTGCCTCTCA CCCCATCACCATGAGTCTTCCATGTGCTTGTCC TCTCCTCCCCCATTTCTCCAACTTGTTTATCCTC ACATAATCCCTGCCCCACTGGGCCCATCCATAG TCCCTGTCACCTGACAGGGGGTGGGTAAACAG ACAGGTATATAGCCCCTTCCTCTCCAGCCAGGG CAGGCACAGACACCAAGGACAGAGACGCTGGC TAGGTAAGATAAGGAGGCAAGATGTGTGAGCA GCATCCAAAGAGGCCTGGGCTTCAGTTGTGGA GAGGGAGAGAGCCAGGTTGGAATGGGCAGCA GGTAGGGAGATCCCTGGGGAGGAGCTGAAGCC	521

Specificity	Promoter Name	Source of cis-regulatory elements	Exemplary sequence	SEQ ID NO
			CATTTGGCTTCAGTGTCCCCCAAACCCCCACCACCCT	
Hepatocytes (e.g., mature hepatocytes)	Cyp3a4	Cyp3a4 gene	AGCTCCTGGGGCCTGCCCTCCTCCCATTAGAAAATCCTCCACTTGTCAAAAAGGAAGCCATTTGCTTTGAACTCCAATTCCACCCCCAAGAGGCTGGGACCATCTTATTGGAGTCCTTGATGCTGTGTGACCTGCAGTGACCACTGCCCCATCATTGCTGGCTGAGGTGGTTGGGGTCCATCTGGCTATCTGGGCAGCTGTTCTCTTCTCTCCTTTCTCTCCTGTTTCCAGACATGCAGTATTTCCAGAGAGAAGGGGCCACTCTTTGGCAAAGAACCTGTCTAACTTGCTATCTATGGCAGGACCTTTGAAGGGTTCACAGGAAGCAGCACAAATTGATACTATTCCACCAAGCCATCAGCTCCATCTCATCCATGCCCTGTCTCTCCTTTAGGGGTCCCCTTGCCAACAGAATCACAGAGGACCAGCCTGAAAGTGCAGAGACAGCAGCTGAGGCACAGCCAAGAGCTCTGGCTGTATTAATGACCTAAGAAGTCACCAGAAAGTCAGAAGGGATGACATGCAGAGGCCCAGCAATCTCAGCTAAGTCAACTCCACCAGCCTTTCTAGTTGCCCACTGTGTGTACAGCACCCTGGTAGGGACCAGAGCCATGACAGGGAATAAGACTAGACTATGCCCTTGAGGAGCTCACCTCTGTTCAGGGAAACAGGCGTGGAAACACAATGGTGGTAAAGAGGAAAGAGGACAATAGGATTGCATGAAGGGGATGGAAAGTGCCCAGGGGAGGAAATGGTTACATCTGTGTGAGGAGTTTGGTGAGGAAAGACTCTAAGAGAAGGCTCTGTCTGTCTGGGTTTGGAAGGATGTGTAGGAGTCTTCTAGGGGGCACAGGCACACTCCAGGCATAGGTAAAGATCTGTAGGTGTGGCTTGTTGGGATGAATTTCAAGTATTTTGGAATGAGGACAGCCATAGAGACAAGGGCAGGAGAGAGGCGATTTAATAGATTTTATGCCAATGGCTCCACTTGAGTTTCTGATAAGAACCCAGAACCCTTGGACTCCCCAGTAACATTGATTGAGTTGTTTATGATACCTCATAGAATATGAACTCAAAGGAGGTCAGTGAGTGGTGTGTGTGTGATTCTTTGCCAACTTCCAAGGTGGAGAAGCCTCTTCCAACTGCAGGCAGAGCACAGGTGGCCCTGCTACTGGCTGCAGCTCCAGCCCTGCCTCCTTCTCTAGCATATAAACAATCCAACAGCCTCACTGAATCACTGCTGTGCAGGGCAGGAAAGCTCCATGCA	522
Hepatocytes	LP1B	Apolipoprotein E/C-I gene, α1	cggcctctagactcgagccctaaaatgggcaaacattgcaagcagcaaacagcaaacacacagccctccctgcctgctgaccttggagctggggcagaggtcagagacctctctgggcccatgccacctccaacatccactcgacccttggaat	135

Specificity	Promoter Name	Source of cis-regulatory elements	Exemplary sequence	SEQ ID NO
		antitrypsin gene	ttcggtggagaggagcagaggttgtcctggcgtggtttaggtagtgtgagag ggtggacacaggacgctgtggtttctgagccaggggggcgactcagatccca gccagtggacttagcccctgtttgctcctccgataactggggtgaccttggtta atattcaccagcagcctcccccgttgcccctctggatccactgcttaaatacgg acgaggacagggccctgtctcctcagcttcaggcaccaccactgacctggg acagtgaatccggactctaaggtaaatataaaatttttaagtgtataatgtgttaa actactgattctaattgtttctctcttttagattccaacctttggaactgaaccggt	
Hepatocytes (e.g., hepatocytes from early stage embryonic liver cells and endoderm)	MIR122	microRNA-122	GAATGCATGGTTAACTACGTCAGAAATGACCA GTTCAAGAGGAGAATGAGATTGGCTTCCAAAT GTTGGTCAAGAGCTCTACGTAGCATGAGCCAA GGATCTATTGAACTTAGTAGGCTCCTGTGACCG GTGACTCTTCTGTCTCTAGAAATCTGGGGAGGT GACCAGGTCATACATGGCAGTCTTCCCGTGAG GAACGTTAAACTGGTTGGAAGTTGGGGTTCTG AGGGGAAGATGTATTCACTAGGTGACCTGTCTT CTCTGCCTCGGTGGCCTCCATGGCTGCCTGCTG GCCGCACACCCCCACTCAGCAGAGGAATGGAC TTTCCAATCTTGCTGAGTGTGTTTGACCAAAGG TGGTGCTGACTTAGTGGCCTAAGGTCGTGCCCT CCCTCCCCCACTGAATCGATAAATAATGCGACT TATCAGAAAGAGAAAGAATTGTTTACTTTTAA ACCCTGGATCCCATAAAGGGAGAGGGGAGAGG CCTAAAGCCACAGAAGCTGTGGAAGGCGCCAT CCTGCCTGCCACAGGAAGGGCCTTGGACTGAG AGGACCGGAGCTGACTGGGGGTAAGTGCGGCT CTCCCCCGGCGCCTGCCGACCCCCCTGAGTGAT CAGGCCGTTCTTTGGGGTGGCCGCTGACCGAG AAATGACGGGAGG See Li et al., 2011, J. Hepatol., 55:602-611	523
Hepatocytes	hemopexin	Hemopexin gene	GCAGCTTTGGGAGTGGGCCCAGGAAGTACTGA GGATAGCAGGTGAGATCCCAGGAAGAGATGGA TGTGGGGCCGAGACACTGGAGAGAGAAACAG GACTGTCAGATAAAGGGCGTCTGTGACTCCTA GATCTCATTATGCCTACTACCATAACCTACCCC CAATTCCTAATATTCTCCTACCCTAGAGGGGGG GAAATTGTCAGAAATTTGGCTGCAACACTAGC AACACTACTCAGTACTTGAAATGCATTTTTGCA TTTTTTTCATTCAACAAATATTTCTGGAACAAC TCTTATATGCCAGGCACTATTTTAGGAGTCAGG GATATATAATGGTAAACAAGACAGGCAAAACA AAGCAAAGCAACAACAACCATCACCAGATAAG TAGACAGATGAAAGAATTTCAAGTTTTAGTAA GTAAAATAAAACAAGCAAGGGTCTGAAATGGC TAGATAAGGTGGTCAAGAAAGGCTTCATTGAG AAGGTAGCATTTAAGCAGGAGTCAGCTAGAAA	524

Specificity	Promoter Name	Source of cis-regulatory elements	Exemplary sequence	SEQ ID NO
			TATTGTGAAATTCCAGTTACAGTTCTATTTGTT CTGGGTTGGTTAAATAAAGCTTTTTCCCCCAAG GTGGAAACTACCAAGAAAGACTAATTACTAGT AGTGGTGGTGCTCTCTGGAAGAGAGACACCTC CTGTTTCTGCCTCATTACTGTCAACCCTTCACTT CCAGGCACTTTTTGCAAAGCCCTTTGCCAGTCA GGGAAGGCGAGAGGCTGGGCATGGGGCTTGGA CATTTGACAACAGTGAGACATTATTGTCCCCAG ACTCACTAGCCCAAGGGTAAAGCTGAAGAGGC TTGGGCATGCCCCAGAAAGGCCCCTGATGAAG CTTGGAAAAAGCTGTTCTCTGAGTATTTCTAAG TAAGTTTATCTGTGTGTGTGGTTACTAAAAGTA GTAAGTATTGCTGTCTCTAGCTGCCTTAGAGCA GGGCTTGACACAGTACACAGCAATATTAGTTC CCTCCTTTTCTCACCTCCCCCATTGTGGAGATA AACTCAATCACAAAAGGTGATCCTCAGTCTACT CACTTCCCTGACTTATGGATGCCTGGACCCATT GCCAGTGTGAGAGTCACAGCTGGACGTCAGCA GTGTAGCCCAGTTACTGCTTGAAAATTGCTGAA GGGGGTTGGGGGGCAGCTGCCGGGAAAAAGG AGTCTTGGATTCAGATTTCTGTCCAGACCCTGA CCTTATTTGCAGTGATGTAATCAGCCAATATTG GCTTAGTCCTGGGAGACAGCACATTCCCAGTA GAGTTGGAGGTGGGGGTGGTGCTGCTGCCAAC T	
Hepatocytes	HLP	Apolipoprotein, SERINA1	tgtttgctgcttgcaatgtttgcccattttagggtggacacaggacgctgtggttt ctgagccaggggcgactcagatcccagccagtggacttagcccctgtttgc tcctccgataactggggtgaccttggttaatattcaccagcagcctcccccgtt gcccctctggatccactgcttaaatacggacgaggacagggccctgtctcctc agcttcaggcaccaccactgacctgggacagtgaatc	136
liver sinusoidal endothelial cells	VEC	Vascular endothelial cadherin gene	CCCCTGCCCTCCTCCTCTGCCCTCTCCTGGCATT CCTCCTTCATCATGGGACCCTCTTCTAATGGAT CCCCAAATGTCAGAGGGTCCAAGTCCTCCCTCC CTCCAAGCTCATCCATGCCCATGGCCTCAGATG CCAGCCATAAGCTGTTGGGTTCCAAACCTCGAC TCCAGGCTGGACTCACCCCTGTCTCCCCCACCA GCCTGACACCTCCACCTGGGTATCTAACGAGC ATCTCAAACTCAACCTGCCTGAGACAGAGGAA TCACTATCCCCTCCTCCTCCAAAAATATCCTTC CATCACACTCCCCATCTTGTGCTCTGATTTACT AAACGGCCCTGGGCCCTCTCTTTCTCAGGGTCT CTGCTTGCCCAGCTATATAATAAAACAAGTTTG GGACTTCCCAACCATTCACCCATGGAAAAACA GAAGCAACTCTTCAAAGGACAGATTCCCAGGA TCTGCCCTGGGAGATTCCAAATCAGTTGATCTG	525

Specificity	Promoter Name	Source of cis-regulatory elements	Exemplary sequence	SEQ ID NO
			GGGTGAGCCCAGTCCTCTGTAGTTTTTAGAAGC TCCTCCTATGTCTCTCCTGGTCAGCAGAATCTT GGCCCCTCCCTTCCCCCCAGCCTCTTGGTTCTTC TGGGCTCTGATCCAGCCTCAGCGTCACTGTCTT CCACGCCCCTCTTTGATTCTCGTTTATGTCAAA AGCCTTGTGAGGATGAGGCTGTGATTATCCCCA TTTTACAGATGAGGAAACTGTGGCTCCAGGAT GACACAACTGGCCAGAGGTCACATCAGAAGCA GAGCTGGGTCACTTGACTCCACCCAATATCCCT AAATGCAAACATCCCCTACAGACCGAGGCTGG CACCTTAGAGCTGGAGTCCATGCCCGCTCTGAC CAGGAGAAGCCAACCTGGTCCTCCAGAGCCAA GAGCTTCTGTCCCTTTCCCATCTCCTGAAGCCT CCCTGTCACCTTTAAAGTCCATTCCCACAAAGA CATCATGGGATCACCACAGAAAATCAAGCTCT GGGGCTAGGCTGACCCCAGCTAGATTTTTGGCT CTTTTATACCCCAGCTGGGTGGACAAGCACCTT AAACCCGCTGAGCCTCAGCTTCCCGGGCTATA AAATGGGGGTGATGACACCTGCCTGTAGCATT CCAAGGAGGGTTAAATGTGATGCTGCAGCCAA GGGTCCCCACAGCCAGGCTCTTTGCAGGTGCTG GGTTCAGAGTCCCAGAGCTGAGGCCGGGAGTA GGGGTTCAAGTGGGGTGCCCCAGGCAGGGTCC AGTGCCAGCCCTCTGTGGAGACAGCCATCCGG GGCCGAGGCAGCCGCCCACCGCAGGGCCTGCC TATCTGCAGCCAGCCCAGCCCTCACAAAGGAA CAATAACAGGAAACCATCCCAGGGGGAAGTGG GCCAGGGCCAGCTGGAAAACCTGAAGGGGAG GCAGCCAGGCCTCCCTCGCCAGCGGGGTGTGG CTCCCCTCCAAAGACGGTCGGCTGACAGGCTC CACAGAGCTCCACTCACGCTCAGCCCTGGACG GACAGGCAGTCCAACGGAACAGAAACATCCCT CAGCCCACAGGCACGGTGAGTGGGGGCTCCCA CACTCCCCTCCACCCCAAACCCGCCACCCTGCG	
ubiquitous	EF1a core promoter	EF1α gene	gggcagagcgcacatcgcccacagtccccgagaagttgggggggaggggt cggcaattgaacgggtgcctagagaaggtggcgcggggtaaactgggaaa gtgatgtcgtgtactggctccgccttttcccgagggtgggggagaaccgtat ataagtgcagtagtcgccgtgaacgttcttttcgcaacgggtttgccgccaga acacag	137
ubiquitous	EF1a	EF1α gene	ggctccggtgcccgtcagtgggcagagcgcacatcgcccacagtccccga gaagttgggggggaggggtcggcaattgaaccggtgcctagagaaggtggc gcggggtaaactgggaaagtgatgtcgtgtactggctccgccttttcccgag ggtgggggagaaccgtatataagtgcagtagtcgccgtgaacgttcttttcg caacgggtttgccgccagaacacaggtaagtgccgtgtgtggttcccgcgg gcctggcctctttacgggttatggcccttgcgtgccttgaattacttccacctgg	138

Specificity	Promoter Name	Source of cis-regulatory elements	Exemplary sequence	SEQ ID NO
			ctccagtacgtgattcttgatcccgagctggagccaggggcgggccttgcgc tttaggagccccttcgcctcgtgcttgagttgaggcctggcctgggcgctggg gccgccgcgtgcgaatctggtggcaccttcgcgcctgtctcgctgctttcgat aagtctctagccatttaaaatttttgatgacctgctgcgacgctttttttctggcaa gatagtcttgtaaatgcgggccaggatctgcacactggtatttcggttttgggc ccgcggccggcgacggggcccgtgcgtcccagcgcacatgttcggcgag gcggggcctgcgagcgcggccaccgagaatcggacgggggtagtctcaa gctggccggcctgctctggtgcctggcctcgcgccgccgtgtatcgccccgc cctgggcggcaaggctggcccggtcggcaccagttgcgtgagcggaaaga tggccgcttcccggccctgctccagggggctcaaaatggaggacgcggcg ctcgggagagcgggcgggtgagtcacccacacaaaggaaaagggcctttc cgtcctcagccgtcgcttcatgtgactccacggagtaccgggcgccgtccag gcacctcgattagttctggagcttttggagtacgtcgtctttaggttgggggga ggggttttatgcgatggagtttccccacactgagtgggtggagactgaagtta ggccagcttggcacttgatgtaattctccttggaatttggccttttgagtttggat cttggttcattctcaagcctcagacagtggttcaaagttttttttcttccatttcaggt gtcgtga	
ubiquitous	hPGK	PGK gene	ggggttggggttgcgccttttccaaggcagccctgggtttgcgcagggacgc ggctgctctgggcgtggttccgggaaacgcagcggcgccgaccctgggtct cgcacattcttcacgtccgttcgcagcgtcacccggatcttcgccgctaccctt gtgggcccccccggcgacgcttcctgctccgcccctaagtcgggaaggttcct tgcggttcgcggcgtgccggacgtgacaaacggaagccgcacgtctcacta gtaccctcgcagacggacagcgccagggagcaatggcagcgcgccgacc gcgatgggctgtggccaatagcggctgctcagcggggcgcgccgagagca gcggccgggaaggggcggtgcgggaggcggggtgtggggcggtagtgt gggccctgttcctgcccgcgcggtgttccgcattctgcaagcctccggagcg cacgtcggcagtcggctccctcgttgaccgaatcaccgacctctctcccca	139
ubiquitous	mCMV	Cytomegalovirus	ggtaggcgtgtacggtgggaggcctatataagcagagct	140
ubiquitous	Ubc	Ubiquitin C gene	gtctaacaaaaaagccaaaaacggccagaatttagcggacaatttactagtct aacactgaaaattacatattgacccaaatgattacatttcaaaaggtgcctaaaa aacttcacaaaacacactcgccaaccccgagcgcatagttcaaaaccggag cttcagctacttaagaagataggtacataaaaccgaccaaagaaactgacgc ctcacttatccctcccctcaccagaggtccggcgcctgtcgattcaggagagc ctaccctaggcccgaaccctgcgtcctgcgacggagaaaagcctaccgcac acctaccggcaggtggccccaccctgcattataagccaacagaacgggtga cgtcacgacacgacgagggcgcgcgctcccaaaggtacgggtgcactgcc caacggcaccgccataactgccgcccccgcaacagacgacaaaccgagtt ctccagtcagtgacaaacttcacgtcagggtccccagatggtgccccagccc atctcacccgaataagagctttcccgcattagcgaaggcctcaagaccttggg ttcttgccgcccaccatgccccccaccttgtttcaacgacctcacagcccgcct cacaagcgtcttccattcaagactcgggaacagccgccattttgctgcgctcc ccccaaccccccagttcagggcaaccttgctcgcggacccagactacagccc ttggcggtctctccacacgcttccgtcccaccgagcggcccggcggccacg aaagccccggccagcccagcagcccgctactcaccaagtgacgatcacag	141

Specificity	Promoter Name	Source of cis-regulatory elements	Exemplary sequence	SEQ ID NO
			cgatccacaaacaagaaccgcgacccaaatcccggctgcgacggaactag ctgtgccacacccggcgcgtccttatataatcatcggcgttcaccgccccacg gagatccctccgcagaatcgccgagaagggactacttttcctcgcctgttccg ctctctggaaagaaaaccagtgccctagagtcacccaagtcccgtcctaaaat gtccttctgctgatactggggttctaaggccgagtcttatgagcagcgggccg ctgtcctgagcgtccgggcggaaggatcaggacgctcgctgcgcccttcgtc tgacgtggcagcgctcgccgtgaggagggggcgcccgcgggaggcgc caaaacccggcgcggaggcc	
ubiquitous	SFFV	Spleen focus-forming virus	gtaacgccattttgcaaggcatggaaaaataccaaaccaagaatagagaagt tcagatcaagggcgggtacatgaaaatagctaacgttgggccaaacaggata tctgcggtgagcagtttcggccccggcccggggccaagaacagatggtcac cgcagtttcggccccggcccgaggccaagaacagatggtccccagatatgg cccaaccctcagcagtttcttaagacccatcagatgtttccaggctcccccaag gacctgaaatgaccctgcgccttatttgaattaaccaatcagcctgcttctcgct tctgttcgcgcgcttctgcttcccgagctctataaaagagctcacaacccctca ctcggcgcgccagtcctccgacagactgagtcgcccggg	142

Various consensus sequences within liver-specific cis-regulatory modules (e.g., promoters) have been described. In some embodiments, a liver-specific cis-regulatory module comprises a binding site for one or more of HNF1α, C/EBP, LEF1, FOX, IRF, LEF1/TCF, Tal1β/E47, and MyoD. In some embodiments, a liver-specific cis-regulatory module comprises a sequence set out in Figure 1 or Table 1 of Chuah et al, "Liver-Specific Transcriptional Modules Identified by Genome-Wide In Silico Analysis Enable Efficient Gene Therapy in Mice and Non-Human Primates" Mol Ther. 2014 Sep; 22(9): 1605–1613, which is herein incorporated by reference in its entirety, including the sequences of Figure 1 and Table 1 therein. In some embodiment, a liver-specific cis-regulatory module comprises a human sequence of HS-CRM1, HS-CRM2, HS-CRM3, HS-CRM4, HS-CRM5, HS-CRM6, HS-CRM7, HS-CRM8, HS-CRM9, HS-CRM10, HS-CRM11, HS-CRM12, HS-CRM13, or HS-CRM14 as described in Chuah et al supra. Additional cell specific promoters and cis-regulatory elements, for example liver specific promoters or cis-regulatory elements, may be identified using methods described in Chuah et al., supra.

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 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 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 (Do), referred to as Tet-OFF system, modification of four amino acids within the transactivator protein resulted in a reverse tTA (rtTA), which only 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 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: TII) variant displays a high dynamic range and low background activity.

Conditional expression can also be achieved by using a site specific DNA recombinase. According to certain embodiments, the 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 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, ΦC31, 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 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 (PI, P2, P3) with intervening loop/junction elements (Jl-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 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).

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. The retroviral nucleic acid (e.g., the gene encoding the exogenous agent) may comprise a recognition sequence for that miRNA. Thus, if the 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. 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, 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 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 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.

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 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.)

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 Med. 12(5):585-91., and WO2007/000668, each of which is herein incorporated by reference in its entirety. In some embodiments, the retroviral nucleic acid comprises one or more of (e.g., a plurality of) tissue-specific miRNA recognition sequences. In some 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 exogenous agent in a target cell comprising contacting a plurality of 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.

For example, the 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 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 maintaining expression and therapeutic efficacy in the target cells.

In some embodiments, the negative TSCRE or NTSCRE comprises an miRNA recognition site, e.g., a miRNA recognition site that is bound by an miRNA endogenous to hematopoietic cells. 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%, 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 embodiments, the seed sequence is at least 6, 7, 8, 9, or 10 nucleotides in length.

In some embodiments, the nucleic acid (e.g., fusosome nucleic acid or retroviral nucleic acid) comprises a sequence that is complementary to a miRNA set forth in any one of SEQ ID NOS:143-160, or a sequence that has at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 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 set forth in any

one of SEQ ID NOS: 143-160. In embodiments, the seed sequence is at least 6, 7, 8, 9, or 10 nucleotides in length.

Table 4. Exemplary miRNA sequences.

Silenced cell type	miRNA name	Mature miRNA	miRNA sequence	SEQ ID NO
hematopoietic cells	miR-142	hsa-miR-142-3p	uguaguguuuccuacuuuaugga	143
hematopoietic cells	miR-142	hsa-miR-142-5p	cauaaaguagaaagcacuacu	144
hematopoietic cells	mir-181a-2	hsa-miR-181a-5p	aacauucaacgcugucggugagu	145
hematopoietic cells	mir-181a-2	hsa-miR-181a-2-3p	accacugaccguugacuguacc	146
hematopoietic cells	mir-181b-1	hsa-miR-181b-5p	aacauucauugcugucgguggguu	147
hematopoietic cells	mir-181b-1	hsa-miR-181b-3p	cucacugaacaaugaaugcaa	148
hematopoietic cells	mir-181c	hsa-miR-181c-5p	aacauucaaccugucggugagu	149
hematopoietic cells	mir-181c	hsa-miR-181c-3p	aaccaucgaccguugaguggac	150
hematopoietic cells	mir-181a-1	hsa-miR-181a	aacauucaacgcugucggugagu	151
hematopoietic cells	mir-181a-1	hsa-miR-181a-3p	accaucgaccguugauuguacc	152
hematopoietic cells	mir-181b-2	hsa-miR-181b-5p	aacauucauugcugucgguggguu	153
hematopoietic cells	mir-181b-2	hsa-miR-181b-2-3p	cucacugaucaaugaaugca	154
hematopoietic cells	mir-181d	hsa-miR-181d-5p	aacauucauuguugucgguggguu	155
hematopoietic cells	mir-181d	hsa-miR-181d-3p	ccaccgggggaugaaugucac	156
hematopoietic cells	miR-223	hsa-miR-223-5p	cguguauuugacaagcugaguu	157
hematopoietic cells	miR-223	hsa-miR-223-3p	ugucaguuugucaaauacccca	158
pDCs	miR-126	hsa-miR-126-5p	cauuauuacuuuugguacgcg	159
pDCs	miR-126	hsa-miR-126-3p	ucguaccgugaguaauaaugcg	160

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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-142-s, 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-142-a, miR-223, miR-142, miR-124a, miR-190, miR-149, miR-193, miR-181, 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., fusosome nucleic acid or retroviral nucleic acid) comprises two or more miRNA recognition sites. In some embodiments, each of the two or more miRNA recognition sites are recognized by an miRNA of any as described herein, such as any set forth in Table 4. In some embodiments, each of the two or more miRNA recognition sites are recognized by an miRNA set forth in any one of SEQ ID NOS: 143-160. In some embodiments, the two or more miRNA recognition sites can include 2, 3, 4, 5, 6, 7, 8, 9, 10 or more miRNA recognition sites. The two or more miRNA recognition sites can be positioned in tandem in the nucleic acid to provide multiple, tandem-binding sites for a miRNA.

In some embodiments, the two or more miRNA recognition sites can include at least one first miRNA recognition site, such as 1, 2, 3, 4, 5, 6 or more first miRNA recognition sites, and at least one second miRNA recognition site, such as 1, 2, 3, 4, 5, 6 or more second miRNA recognition sites. In some embodiments, the nucleic acid contains two or more first miRNA recognition site and each of the first miRNA recognition sites are present in tandem in the nucleic acid to provide multiple, tandem-binding sites for a first miRNA and/or the nucleic acid contains two more second miRNA recognition site and each of the second miRNA recognition sites are present in tandem in the nucleic acid to provide multiple, tandem-binding sites for a second miRNA. 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 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 miRNAs of any as described, such as any set forth in Table 4. In some embodiments, one or both of the first miRNA recognition site and second miRNA recognition site are recognized by an miRNAs set forth in any one of SEQ ID NOS: 143-160.

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 miRNA recognition sites on the fusosome nucleic acid (e.g., retroviral nucleic acid) are situated downstream of the WPRE.

Immune modulation

In some embodiments, a retroviral vector or VLP described herein comprises elevated CD47. See, e.g., US Pat. 9,050,269, which is herein incorporated by reference in its entirety. In

some embodiments, 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 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., US20170165348, which is herein incorporated by reference in its entirety.

Sometimes 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, 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 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, 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 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 SIRPα (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-SIRPα

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 retroviral vector or VLP (e.g., a viral particle having a radius of less than about 1 μm, less than about 400 nm, or less than about 150 nm), comprises at least a biologically active portion of CD47, e.g., on an exposed surface of the retroviral vector or VLP. In some embodiments, the retroviral vector (e.g., lentivirus) or VLP includes a lipid coat. In embodiments, the amount of the biologically active CD47 in the 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 retroviral vector or VLP so that, when the retroviral vector or VLP comprising the CD47 is exposed to a phagocytic cell, the viral particle evades phacocytosis by the phagocytic cell, or shows decreased phagocytosis compared to an otherwise similar unmodified retroviral vector or VLP. In some embodiments, the half-life of the retroviral vector or VLP in a subject is extended compared to an otherwise similar unmodified 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 β2-microglobulin (β2M). 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 β2-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 β2-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 i*n 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 an anti-CD3 antibody, e.g. OKT3, or IL7.

In some embodiments, 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.

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

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 CD8+ T-cells and then assessed for lysis of the cells by the CD8+ T-cells.

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%, 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 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 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;

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, or a HEK cell, or a reference cell described herein;

c. expression of surface proteins which suppress macrophage engulfment e.g., CD47, e.g., detectable expression by a method described herein, e.g., more than 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, or more expression

of the surface protein which suppresses macrophage engulfment, e.g., CD47, compared to a reference 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;

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;

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 TCRα/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 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.

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,

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 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 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 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-a;
d. endogenous immune-stimulatory antigen, e.g., Zg16 or Hormad1;
e. T-cell receptors (TCR);
f. The genes encoding ABO blood groups, e.g., *ABO* gene;
g. transcription factors which drive immune activation, e.g., NFkB;
h. transcription factors that control MHC expression e.g., class II trans-activator (CIITA), regulatory factor of the Xbox 5 (RFX5), RFX-associated protein (RFXAP), or RFX ankyrin repeats (RFXANK; also known as RFXB); or
i. TAP proteins, e.g., TAP2, TAP1, or TAPBP, which reduce MHC class I expression.

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;
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;
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

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 compelement pathway CD/C5 convertase enzymes, e.g. proteins that regulate MAC assembly; e.g. 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 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:

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 i*n 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., Ciclosporin 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.

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.

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

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.

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.

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 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 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 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 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 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 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 i*n 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%, 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%, 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 i*n 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%, 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%, 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 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 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 i*n 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 i*n 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:

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;

c. wherein the source cell is obtained is from an allogeneic cell source is which is HLA matched with a recipient's HLA, e.g., at one or more alleles;

d. wherein the source cell is obtained is from an allogeneic cell source which is an HLA homozygote;

e. wherein the source cell is obtained is from an allogeneic cell source which lacks (or has reduced levels compared to a reference cell) MHC class I and II; or

f. wherein the source cell is obtained is from a cell source which is known to be substantially non-immunogenic including but not limited to a stem cell, a mesenchymal stem cell, an induced pluripotent stem cell, an embryonic stem cell, a sertoli cell, or a retinal pigment epithelial cell.

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.

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 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.

Exogenous agents

In some embodiments, a retroviral vector, VLP, or pharmaceutical composition described herein encodes an exogenous agent.

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 OTC, CPS1, NAGS, BCKDHA, BCKDHB, DBT, DLD, MUT, MMAA, MMAB, MMACHC, MMADHC, MCEE, PCCA, PCCB, UGT1A1, ASS1, PAH, PAL, ATP8B1, ABCB11, ABCB4, TJP2, IVD, GCDH, ETFA, ETFB, ETFDH, ASL, D2HGDH, HMGCL, MCCC1, MCCC2, ABCD4, HCFC1, LNBRD1, ARG1, SLC25A15, SLC25A13, ALAD, CPOX, HMBS, PPOX, BTD, HLCS, PC, SLC7A7, CPT2, ACADM, ACADS, ACADVL, AGL, G6PC, GBE1, PHKA1, PHKA2, PHKB, PHKG2, SLC37A4, PMM2, CBS, FAH, TAT, GALT, GALK1, GALE, G6PD, SLC3A1, SLC7A9, MTHFR, MTR, MTRR, ATP7B, HPRT1, HJV, HAMP, JAG1, TTR, AGXT, LIPA, SERPING1, HSD17B4, UROD, HFE, LPL,GRHPR, HOGA1, LDLR, ACAD8, ACADSB, ACAT1, ACSF3, ASPA, AUH, DNAJC19, ETHE1, FBP1, FTCD, GSS, HIBCH, IDH2, L2HGDH, MLYCD, OPA3, OPLAH, OXCT1, POLG, PPM1K, SERAC1, SLC25A1, SUCLA2, SUCLG1, TAZ, AGK, CLPB, TMEM70, ALDH18A1, OAT, CA5A, GLUD1, GLUL, UMPS, SLC22A5, CPT1A, HADHA, HADH, SLC52A1, SLC52A2, SLC52A3, HADHB, GYS2, PYGL, SLC2A2, ALG1, ALG2, ALG3, ALG6, ALG8, ALG9, ALG11, ALG12, ALG13, ATP6V0A2, B3GLCT, CHST14, COG1, COG2, COG4, COG5, COG6, COG7, COG8, DOLK, DHDDS, DPAGT1, DPM1, DPM2, DPM3, G6PC3, GFPT1, GMPPA,

GMPPB, MAGT1, MAN1B1, MGAT2, MOGS, MPDU1, MPI, NGLY1, PGM1, PGM3, RFT1, SEC23B, SLC35A1, SLC35A2, SLC35C1, SSR4, SRD5A3, TMEM165, TRIP11, TUSC3, ALG14, B4GALT1, DDOST, NUS1, RPN2, SEC23A, SLC35A3, ST3GAL3, STT3A, STT3B, AGA, ARSA, ARSB, ASAH1, ATP13A2, CLN3, CLN5, CLN6, CLN8, CTNS, CTSA, CTSD, CTSF, CTSK, DNAJC5, FUCA1, GAA, GALC, GALNS, GLA, GLB1, GM2A, GNPTAB, GNPTG, GNS, GRN, GUSB, HEXA, HEXB, HGSNAT, HYAL1, IDS, IDUA, KCTD7, LAMP2, MAN2B1, MANBA, MCOLN1, MFSD8, NAGA, NAGLU, NEU1 NPC1, NPC2, SGSH, PPT1, PSAP, SLC17A5, SMPD1, SUMF1, TPP1, AHCY, GNMT, MAT1A, GCH1, PCBD1, PTS, QDPR, SPR, DNAJC12, ALDH4A1, PRODH, HPD, GBA, HGD, AMN, CD320, CUBN, GIF, TCN1, TCN2, PREPL, PHGDH, PSAT1, PSPH, AMT,GCSH, GLDC, LIAS, NFU1, SLC6A9, SLC2A1, ATP7A, AP1S1, CP, SLC33A1, PEX7 PHYH, AGPS, GNPAT, ABCD1, ACOX1, PEX1, PEX2, PEX3, PEX5, PEX6, PEX10, PEX12, PEX13, PEX14, PEX16, PEX19, PEX26, AMACR, ADA, ADSL, AMPD1, GPHN, MOCOS, MOCS1, PNP, XDH, SUOX, OGDH, SLC25A19, DHTKD1, SLC13A5, FH, DLAT, MPC1, PDHA1, PDHB, PDHX, PDP1, ABCC2, SLCO1B1, SLCO1B3, HFE2, ADAMTS13, PYGM , COL1A2, TNFRSF11B, TSC1, TSC2, DHCR7, PGK1, VLDLR, KYNU, F5, C3, COL4A1, CFH, SLC12A2, GK, SFTPC, CRTAP, P3H1, COL7A1, PKLR, TALDO1, TF, EPCAM, VHL, GC, SERPINA1, ABCC6, F8, F9, ApoB, PCSK9, LDLRAP1,ABCG5, ABCG8, LCAT, SPINK5, or GNE.

In some embodiments, the exogenous agent is encoded by a gene from among OTC, CPS1, NAGS, BCKDHA, BCKDHB, DBT, DLD, MUT, MMAA, MMAB, MMACHC, MMADHC, MCEE, PCCA, PCCB, UGT1A1, ASS1, PAL, PAH, ATP8B1, ABCB11, ABCB4, TJP2, IVD, GCDH, ETFA, ETFB, ETFDH, ASL, D2HGDH, HMGCL, MCCC1, MCCC2, ABCD4, HCFC1, LMBRD1, ARG1, SLC25A15, SLC25A13, ALAD, CPOX, HMBS, PPOX, BTD, HLCS, PC, SLC7A7, CPT2, ACADM, ACADS, ACADVL, AGL, G6PC, GBE1, PHKA1, PHKA2, PHKB, PHKG2, SLC37A4, PMM2, CBS, FAH, TAT, GALT, GALK1, GALE, G6PD, SLC3A1, SLC7A9, MTHFR, MTR, MTRR, ATP7B, HPRT1, HJV, HAMP, JAG1, TTR, AGXT, LIPA, SERPING1, HSD17B4, UROD, HFE, LPL, GRHPR, HOGA1, or LDLR. In some embodiments, the exogenous agent is the enzyme phenylalanine ammonia lyase (PAL).

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 4 of Table 5 or an amino acid sequence of column 5 of Table 5. In some embodiments, the payload gene encoding an exogenous agent 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 5. In some embodiments, the payload gene encoding an exogenous agent has a nucleic acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%, identity to a nucleic acid sequence of Table 5, e.g., an Ensemble Gene Accession Number of column 3 of Table 5.

In some embodiments, the exogenous agent comprises an amino acid sequence set forth in any one of SEQ ID NOS: 161-518. In some embodiments, the exogenous agent comprises the wild-type human sequence set forth in any one of SEQ ID NOS: 161-518, 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 set forth in any one of SEQ ID NOS: 161-518. In some embodiments, the payload gene encoding an exogenous agent encodes an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%, identity to any one of SEQ ID NOS: 161-518. In some embodiments, the payload gene encoding an exogenous agent encodes the amino acid sequence set forth in any one of SEQ ID NOS: 161-518.

Table 5. The first column lists exogenous agents that can be delivered to treat the indications in the sixth column, according to the methods and uses herein. Each Uniprot accession number of Table 5 is herein incorporated by reference in its entirety.

Gene	Entrez Accession Number	Ensembl Gene(s) Accession Number (ENSG0000+ number shown)	Uniprot Protein(s) Accession Number	Amino Acid Sequence (first Uniprot Accession Number) [SEQ ID NO]	Disease / Disorder	Category
OTC	5009	0036473	P00480	MLFNLRILLNNAAFRNGHNFMVRNFRCGQPL QNKVQLKGRDLLTLKNFTGEEIKYMLWLSA DLKFRIKQKGEYLPLLQGKSLGMIFEKRSTRT RLSTETGFALLGGHPCFLTTQDIHLGVNESLT DTARVLSSMADAVLARVYKQSDLDTLAKEA SIPIINGLSDLYHPIQILADYLTLQEHYSSLKGL TLSWIGDGNNILHSIMMSAAKFGMHLQAATP KGYEPDASVTKLAEQYAKENGTKLLLTNDPL EAAHGGNVLITDTWISMGQEEEKKKRLQAFQ GYQVTMKTAKVAASDWTFLHCLPRKPEEVD DEVFYSPRSLVFPEAENRKWTIMAVMVSLLT DYSPQLQKPKF [SEQ ID NO:161]	ornithine transcarbamylase (OTC) deficiency	Urea cycle disorder
CPS 1	1373	0021826	P31327, Q6PEK7, B7ZAW0, A0A024R454	MTRILTAFKVVRTLKTGFGFTNVTAHQKWKF SRPGIRLLSVKAQTAHIVLEDGTKMKGYSFG HPSSVAGEVVFNTGLGGYPEAITDPAYKGQIL TMANPIIGNGGAPDTTALDELGLSKYLESNGI KVSGLLVLDYSKDYNHWLATKSLGQWLQEE KVPAIYGVDTRMLTKIIRDKGTMLGKIEFEGQ PVDFVDPNKQNLIAEVSTKDVKVYGKGNPTK VVAVDCGIKNNVIRLLVKRGAEVHLVPWNH DFTKMEYDGILIAGGPGNPALAEPLIQNVRKI LESDRKEPLFGISTGNLITGLAAGAKTYKMSM ANRGQNQPVLNITNKQAFITAQNHGYALDNT LPAGWKPLFVNVNDQTNEGIMHESKPFFAVQ FHPEVTPGPIDTEYLFDSFFSLIKKGKATTITSV LPKPALVASRVEVSKVLILGSGGLSIGQAGEF DYSGSQAVKAMKEENVKTVLMNPNIASVQT NEVGLKQADTVYFLPITPQFVTEVIKAEQPDG LILGMGGQTALNCGVELFKRGVLKEYGVKV LGTSVESIMATEDRQLFSDKLNEINEKIAPSFA VESIEDALKAADTIGYPVMIRSAYALGGLGSG ICPNRETLMDLSTKAFAMTNQILVEKSVTGW KEIEYEVVRDADDNCVTVCNMENVDAMGV HTGDSVVVAPAQTLSNAEFQMLRRTSINVVR HLGIVGECNIQFALHPTSMEYCIIEVNARLSRS SALASKATGYPLAFIAAKIALGIPLPEIKNVVS GKTSACFEPSLDYMVTKIPRWDLDRFHGTSS RIGSSMKSVGEVMAIGRTFEESFQKALRMCH PSIEGFTPRLPMNKEWPSNLDLRKELSEPSSTR IYAIAKAIDDNMSLDEIEKLTYIDKWFLYKMR DILNMEKTLKGLNSESMTEETLKRAKEIGFSD KQISKCLGLTEAQTRELRLKKNIHPWVKQIDT LAAEYPSVTNYLYVTYNGQEHDVNFDDHGM MVLGCGPYHIGSSVEFDWCAVSSIRTLRQLG KKTVVVNCNPETVSTDFDECDKLYFEELSLE RILDIYHQEACGGCIISVGGQIPNNLAVPLYKN GVKIMGTSPLQIDRAEDRSIFSAVLDELKVAQ APWKAVNTLNEALEFAKSVDYPCLLRPSYVL SGSAMNVVFSEDEMKKFLEEATRVSQEHPVV LTKFVEGAREVEMDAVGKDGRVISHAISEHV EDAGVHSGDATLMLPTQTISQGAIEKVKDAT RKIAKAFAISGPFNVQFLVKGNDVLVIECNLR ASRSFPFVSKTLGVDFIDVATKVMIGENVDEK	carbamoyl phosphate synthetase I (CPSI) deficiency	Urea cycle disorder

				HLPTLDHPIIPADYVAIKAPMFSWPRLRDADPI LRCEMASTGEVACFGEGIHTAFLKAMLSTGF KIPQKGILIGIQQSFRPRFLGVAEQLHNEGFKL FATEATSDWLNANNVPATPVAWPSQEGQNP SLSSIRKLIRDGSIDLVINLPNNNTKFVHDNYV IRRTAVDSGIPLLTNFQVTKLFAEAVQKSRKV DSKSLFHYRQYSAGKAA [SEQ ID NO:162]		
NAGS	162417	0161653	Q8N159, Q2NKP2	MATALMAVVLRAAAVAPRLRGRGGTGGAR RLSCGARRRAARGTSPGRRLSTAWSQPQPPP EEYAGADDVSQSPVAEEPSWVPSPRPPVPHES PEPPSGRSLVQRDIQAFLNQCGASPGEARHW LTQFQTCHHSADKPFAVIEVDEEVLKCQQGV SSLAFALAFLQRMDMKPLVVLGLPAPTAPSG CLSFWEAKAQLAKSCKVLVDALRHNAAAAV PFFGGGSVLRAAEPAPHASYGGIVSVETDLLQ WCLESGSIPILCPIGETAARRSVLLDSLEVTAS LAKALRPTKIIFLNNTGGLRDSSHKVLSNVNL PADLDLVCNAEWVSTKERQQMRLIVDVLSRL PHHSSAVITAASTLLTELFSNKGSGTLFKNAE RMLRVRSLDKLDQGRLVDLVNASFGKKLRD DYLASLRPRLHSIYVSEGYNAAAILTMEPVLG GTPYLDKFVVSSSRQGQGSGQMLWECLRRD LQTLFWRSRVTNPINPWYFKHSDGSFSNKQW IFFWFGLADIRDSYELVNHAKGLPDSFHKPAS DPGS [SEQ ID NO:163]	N-acetylglutamate synthase (NAGS) deficiency	Urea cycle disorder
BCKDHA	593	0248098	A0A024R0K3, P12694, Q59EI3	MAVAIAAARVWRLNRGLSQAALLLLRQPGA RGLARSHPPRQQQQFSSLDDKPQFPGASAEFI DKLEFIQPNVISGIPIYRVMDRQGQIINPSEDPH LPKEKVLKLYKSMTLLNTMDRILYESQRQGR ISFYMTNYGEEGTHVGSAAALDNTDLVFGQY REAGVLMYRDYPLELFMAQCYGNISDLGKG RQMPVHYGCKERHFVTISSPLATQIPQAVGA AYAAKRANANRVVICYFGEGAASEGDAHAG FNFAATLECPIIFFCRNNGYAISTPTSEQYRGD GIAARGPGYGIMSIRVDGNDVFAVYNATKEA RRRAVAENQPFLIEAMTYRIGHHSTSDDSSAY RSVDEVNYWDKQDHPISRLRHYLLSQGWWD EEQEKAWRKQSRRKVMEAFEQAERKPKPNP NLLFSDVYQEMPAQLRKQQESLARHLQTYGE HYPLDHFDK [SEQ ID NO:164]	maple syrup urine disease (MSUD); Classic Maple Syrup Urine Disease (CMSUD)	Organic acidemia
BCKDHB	594	0083123	A0A140VKB3, P21953, B4E2N3, B7ZB80	MAVVAAAAGWLLRLRAAGAEGHWRRLPGA GLARGFLHPAATVEDAAQRRQVAHFTFQPDP EPREYGQTQKMNLFQSVTSALDNSLAKDPTA VIFGEDVAFGGVFRCTVGLRDKYGKDRVFNT PLCEQGIVGFGIGIAVTGATAIAEIQFADYIFPA FDQIVNEAAKYRYRSGDLFNCGSLTIRSPWG CVGHGALYHSQSPEAFFAHCPGIKVVIPRSPF QAKGLLLSCIEDKNPCIFFEPKILYRAAAEEVP IEPYNIPLSQAEVIQEGSDVTLVAWGTQVHVI REVASMAKEKLGVSCEVIDLRTIIPWDVDTIC KSVIKTGRLLISHEAPLTGGFASEISSTVQEEC FLNLEAPISRVCGYDTPFPHIFEPFYIPDKWKC YDALRKMINY [SEQ ID NO:165]	maple syrup urine disease (MSUD); Classic Maple Syrup Urine Disease (CMSUD)	Organic acidemia
DBT	1629	0137992	P11182	MAAVRMLRTWSRNAGKLICVRYFQTCGNVH VLKPNYVCFFGYPSFKYSHPHHFLKTTAALR GQVVQFKLSDIGEGIREVTVKEWYVKEGDTV SQFDSICEVQSDKASVTITSRYDGVIKKLYYN LDDIAYVGKPLVDIETEALKDSEEDVVETPAV SHDEHTHQEIKGRKTLATPAVRRLAMENNIK LSEVVGSGKDGRILKEDILNYLEKQTGAILPPS	maple syrup urine disease (MSUD); Classic Maple Syrup	Organic acidemia

				PKVEIMPPPPKPKDMTVPILVSKPPVFTGKDK TEPIKGFQKAMVKTMSAALKIPHFGYCDEIDL TELVKLREELKPIAFARGIKLSFMPFFLKAASL GLLQFPILNASVDENCQNITYKASHNIGIAMD TEQGLIVPNVKNVQICSIFDIATELNRLQKLGS VGQLSTTDLTGGTFTLSNIGSIGGTFAKPVIMP PEVAIGALGSIKAIPRFNQKGEVYKAQIMNVS WSADHRVIDGATMSRFSNLWKSYLENPAFM LLDLK [SEQ ID NO:166]	Urine Disease (CMSUD)	
DLD	1738	0091140	A0A024R 713, P09622, E9PEX6	MQSWSRVYCSLAKRGHFNRISHGLQGLSAVP LRTYADQPIDADVTVIGSGPGGYVAAIKAAQ LGFKTVCIEKNETLGGTCLNVGCIPSKALLNN SHYYHMAHGKDFASRGIEMSEVRLNLDKMM EQKSTAVKALTGGIAHLFKQNKVVHVNGYG KITGKNQVTATKADGGTQVIDTKNILIATGSE VTPFPGITIDEDTIVSSTGALSLKKVPEKMVVI GAGVIGVELGSVWQRLGADVTAVEFLGHVG GVGIDMEISKNFQRILQKQGFKFKLNTKVTG ATKKSDGKIDVSIEAASGGKAEVITCDVLLVC IGRRPFTKNLGLEELGIELDPRGRIPVNTRFQT KIPNIYAIGDVVAGPMLAHKAEDEGIICVEGM AGGAVHIDYNCVPSVIYTHPEVAWVGKSEEQ LKEEGIEYKVGKFPFAANSRAKTNADTDGMV KILGQKSTDRVLGAHILGPGAGEMVNEAALA LEYGASCEDIARVCHAHPTLSEAFREANLAAS FGKSINF [SEQ ID NO:167]	maple syrup urine disease (MSUD) Dihydrolip oamide dehydrogen ase deficiency	Urea cycle disorder
MUT	4594	0146085	A0A024R D82, B2R6K1, P22033	MLRAKNQLFLLSPHYLRQVKESSGSRLIQQRL LHQQQPLHPEWAALAKKQLKGKNPEDLIWH TPEGISIKPLYSKRDTMDLPEELPGVKPFTRGP YPTMYTFRPWTIRQYAGFSTVEESNKFYKDNI KAGQQGLSVAFDLATHRGYDSDNPRVRGDV GMAGVAIDTVEDTKILFDGIPLEKMSVSMTM NGAVIPVLANFIVTGEEQGVPKEKLTGTIQND ILKEFMVRNTYIFPPEPSMKIIADIFEYTAKHM PKFNSISISGYHMQEAGADAILELAYTLADGL EYSRTGLQAGLTIDEFAPRLSFFWGIGMNFY MEIAKMRAGRRLWAHLIEKMFQPKNSKSLLL RAHCQTSGWSLTEQDPYNNIVRTAIEAMAAV FGGTQSLHTNSFDEALGLPTVKSARIARNTQII IQEESGIPKVADPWGGSYMMECLTNDVYDA ALKLINEIEEMGGMAKAVAEGIPKLRIEECAA RRQARIDSGSEVIVGVNKYQLEKEDAVEVLAI DNTSVRNRQIEKLKKIKSSRDQALAERCLAAL TECAASGDGNILALAVDASRARCTVGEITDA LKKVFGEHKANDRMVSGAYRQEFGESKEITS AIKRVHKFMEREGRRPRLLVAKMGQDGHDR GAKVIATGFADLGFDVDIGPLFQTPREVAQQ AVDADVHAVGISTLAAGHKTLVPELIKELNS LGRPDILVMCGGVIPPQDYEFLFEVGVSNVFG PGTRIPKAAVQVLDDIEKCLEKKQQSV [SEQ ID NO:168]	methylmalo nic acidemia due to methylmalo nyl-CoA mutase deficiency	Organic acidemia
MM AA	166785	0151611	Q8IVH4	MPMLLPHPHQHFLKGLLRAPFRCYHFIFHSST HLGSGIPCAQPFNSLGLHCTKWMLLSDGLKR KLCVQTTLKDHTEGLSDKEQRFVDKLYTGLI QGQRACLAEAITLVESTHSRKKELAQVLLQK VLLYHREQEQSNKGKPLAFRVGLSGPPGAGK STFIEYFGKMLTERGHKLSVLAVDPSSCTSGG SLLGDKTRMTELSRDMNAYIRPSPTRGTLGG VTRTTNEAILLCEGAGYDIILIETVGVGQSEFA VADMVDMFVLLLPPAGGDELQGIKRGIIEMA	cobalamin A deficiency (methylmal onic acidemia)	Organic acidemia

				DLVAVTKSDGDLIVPARRIQAEYVSALKLLR KRSQVWKPKVIRISARSGEGISEMWDKMKDF QDLMLASGELTAKRRKQQKVWMWNLIQES VLEHFRTHPTVREQIPLLEQKVLIGALSPGLA ADFLLKAFKSRD [SEQ ID NO:169]		
MM AB	326625	0139428	Q96EY8	MAVCGLGSRLGLGSRLGLRGCFGAARLLYPR FQSRGPQGVEDGDRPQPSSKTPRIPKIYTKTG DKGFSSTFTGERRPKDDQVFEAVGTTDELSSA IGFALELVTEKGHTFAEELQKIQCTLQDVGSA LATPCSSAREAHLKYTTFKAGPILELEQWIDK YTSQLPPLTAFILPSGGKISSALHFCRAVCRRA ERRVVPLVQMGETDANVAKFLNRLSDYLFTL ARYAAMKEGNQEKIYMKNDPSAESEGL [SEQ ID NO:170]	cobalamin B deficiency (methylmal onic acidemia)	Organic acidemia
MM ACH C	25974	0132763	A0A0C4 DGU2, Q9Y4U1	MFDRALKPFLQSCHLRMLTDPVDQCVAYHL GRVRESLPELQIEIIADYEVHPNRRPKILAQTA AHVAGAAYYYQRQDVEADPWGNQRISGVCI HPRFGGWFAIRGVVLLPGIEVPDLPPRKPHDC VPTRADRIALLEGFNFHWRDWTYRDAVTPQE RYSEEQKAYFSTPPAQRLALLGLAQPSEKPSS PSPDLPFTTPAPKKPGNPSRARSWLSPRVSPPA SPGP [SEQ ID NO:171]	cobalamin C deficiency (methylmal onic acidemia); Methylmal onic Acidemia with Homocysti nuria	Organic acidemia
MM ADH C	27249	0168288	Q9H3L0	MANVLCNRARLVSYLPGFCSLVKRVVNPKA FSTAGSSGSDESHVAAAPPDICSRTVWPDETM GPFGPQDQRFQLPGNIGFDCHLNGTASQKKS LVHKTLPDVLAEPLSSERHEFVMAQYVNEFQ GNDAPVEQEINSAETYFESARVECAIQTCPEL LRKDFESLFPEVANGKLMILTVTQKTKNDMT VWSEEVEIEREVLLEKFINGAKEICYALRAEG YWADFIDPSSGLAFFGPYTNNTLFETDERYRH LGFSVDDLGCCKVIRHSLWGTHVVVGSIFTN ATPDSHIMKKLSGN [SEQ ID NO:172]	cobalamin D deficiency (methylmal onic acidemia); Methylmal onic Acidemia with Homocysti nuria; Homocysti nuria; Cobalamin C Deficiency	Organic acidemia
MCE E	84693	0124370	Q96PE7	MARVLKAAAANAVGLFSRLQAPIPTVRASST SQPLDQVTGSVWNLGRLNHVAIAVPDLEKA AAFYKNILGAQVSEAVPLPEHGVSVVFVNLG NTKMELLHPLGRDSPIAGFLQKNKAGGMHHI CIEVDNINAAVMDLKKKKIRSLSEEVKIGAHG KPVIFLHPKDCGGVLVELEQA [SEQ ID NO:173]	methylmalo nic acidemia; Cobalamin D Deficiency	Organic acidemia
PCC A	5095	0175198	P05165	MAGFWVGTAPLVAAGRRGRWPPQQLMLSA ALRTLKHVLYYSRQCLMVSRNLGSVGYDPN EKTFDKILVANRGEIACRVIRTCKKMGIKTVA IHSDVDASSVHVKMADEAVCVGPAPTSKSYL NMDAIMEAIKKTRAQAVHPGYGFLSENKEFA RCLAAEDVVFIGPDTHAIQAMGDKIESKLLA KKAEVNTIPGFDGVVKDAEEAVRIAREIGYPV MIKASAGGGGKGMRIAWDDEETRDGFRLSS QEAASSFGDDRLLIEKFIDNPRHIEIQVLGDKH GNALWLNERECSIQRRNQKVVEEAPSIFLDAE	propionic acidemia	Organic acidemia

				TRRAMGEQAVALARAVKYSSAGTVEFLVDS KKNFYFLEMNTRLQVEHPVTECITGLDLVQE MIRVAKGYPLRHKQADIRINGWAVECRVYA EDPYKSFGLPSIGRLSQYQEPLHLPGVRVDSGI QPGSDISIYYDPMISKLITYGSDRTEALKRMA DALDNYVIRGVTHNIALLREVIINSRFVKGDIS TKFLSDVYPDGFKGHMLTKSEKNQLLAIASS LFVAFQLRAQHFQENSRMPVIKPDIANWELS VKLHDKVHTVVASNNGSVFSVEVDGSKLNV TSTWNLASPLLSVSVDGTQRTVQCLSREAGG NMSIQFLGTVYKVNILTRLAAELNKFMLEKV TEDTSSVLRSPMPGVVVAVSVKPGDAVAEGQ EICVIEAMKMQNSMTAGKTGTVKSVHCQAG DTVGEGDLLVELE [SEQ ID NO:174]		
PCC B	5096	0114054	P05166	MAAALRVAAVGARLSVLASGLRAAVRSLCS QATSVNERIENKRRTALLGGGQRRIDAQHKR GKLTARERISLLLDPGSFVESDMFVEHRCADF GMAADKNKFPGDSVVTGRGRINGRLVYVFS QDFTVFGGSLSGAHAQKICKIMDQAITVGAP VIGLNDSGGARIQEGVESLAGYADIFLRNVTA SGVIPQISLIMGPCAGGAVYSPALTDFTFMVK DTSYLFITGPDVVKSVTNEDVTQEELGGAKT HTTMSGVAHRAFENDVDALCNLRDFFNYLPL SSQDPAPVRECHDPSDRLVPELDTIVPLESTK AYNMVDIIHSVVDEREFFEIMPNYAKNIIVGF ARMNGRTVGIVGNQPKVASGCLDINSSVKGA RFVRFCDAFNIPLITFVDVPGFLPGTAQEYGGI IRHGAKLLYAFAEATVPKVTVITRKAYGGAY DVMSSKHLCGDTNYAWPTAEIAVMGAKGA VEIIFKGHENVEAAQAEYIEKFANPFPAAVRG FVDDIIQPSSTRARICCDLDVLASKKVQRPWR KHANIPL [SEQ ID NO:175]	propionic acidemia	Organic acidemia
UGT 1A1	54658	0241635	P22309, Q5DT03	MAVESQGGRPLVLGLLLCVLGPVVSHAGKIL LIPVDGSHWLSMLGAIQQLQQRGHEIVVLAP DASLYIRDGAFYTLKTYPVPFQREDVKESFVS LGHNVFENDSFLQRVIKTYKKIKKDSAMLLS GCSHLLHNKELMASLAESSFDVMLTDPFLPC SPIVAQYLSLPTVFFLHALPCSLEFEATQCPNP FSYVPRPLSSHSDHMTFLQRVKNMLIAFSQNF LCDVVYSPYATLASEFLQREVTVQDLLSSAS VWLFRSDFVKDYPRPIMPNMVFVGGINCLHQ NPLSQEFEAYINASGEHGIVVFSLGSMVSEIPE KKAMAIADALGKIPQTVLWRYTGTRPSNLAN NTILVKWLPQNDLLGHPMTRAFITHAGSHGV YESICNGVPMVMMPLFGDQMDNAKRMETK GAGVTLNVLEMTSEDLENALKAVINDKSYKE NIMRLSSLHKDRPVEPLDLAVFWVEFVMRHK GAPHLRPAAHDLTWYQYHSLDVIGFLLAVVL TVAFITFKCCAYGYRKCLGKKGRVKKAHKS KTH [SEQ ID NO:176]	Crigler-Najjar syndrome type 1 Crigler-Najjar syndrome type 2, Gilbert syndrome	
ASS 1	445	0130707	P00966, Q5T6L4	MSSKGSVVLAYSGGLDTSCILVWLKEQGYD VIAYLANIGQKEDFEEARKKALKLGAKKVFIE DVSREFVEEFIWPAIQSSALYEDRYLLGTSLA RPCIARKQVEIAQREGAKYVSHGATGKGNDQ VRFELSCYSLAPQIKVIAPWRMPEFYNRFKGR NDLMEYAKQHGIPIPVTPKNPWSMDENLMHI SYEAGILENPKNQAPPGLYTKTQDPAKAPNTP DILEIEFKKGVPVKVTNVKDGTTHQTSLELFM YLNEVAGKHGVGRIDIVENRFIGMKSRGIYET PAGTILYHAHLDIEAFTMDREVRKIKQGLGLK	citrullinemia type I	Urea cycle disorder

				FAELVYTGFWHSPECEFVRHCIAKSQERVEG KVQVSVLKGQVYILGRESPLSLYNEELVSMN VQGDYEPTDATGFININSLRLKEYHRLQSKVT AK [SEQ ID NO:177]		
PAH	5053	0171759	A0A024R BG4, P00439	MSTAVLENPGLGRKLSDFGQETSYIEDNCNQ NGAISLIFSLKEEVGALAKVLRLFEENDVNLT HIESRPSRLKKDEYEFFTHLDKRSLPALTNIIKI LRHDIGATVHELSRDKKKDTVPWFPRTIQEL DRFANQILSYGAELDADHPGFKDPVYRARRK QFADIAYNYRHGQPIPRVEYMEEEKKTWGTV FKTLKSLYKTHACYEYNHIFPLLEKYCGFHED NIPQLEDVSQFLQTCTGFRLRPVAGLLSSRDF LGGLAFRVFHCTQYIRHGSKPMYTPEPDICHE LLGHVPLFSDRSFAQFSQEIGLASLGAPDEYIE KLATIYWFTVEFGLCKQGDSIKAYGAGLLSSF GELQYCLSEKPKLLPLELEKTAIQNYTVTEFQ PLYYVAESFNDAKEKVRNFAATIPRPFSVRYD PYTQRIEVLDNTQQLKILADSINSEIGILCSAL QKIK [SEQ ID NO:178]	Phenylalanine hydroxylase deficiency	Aminoacidopathy
PAL				MAKTLSQAQSKTSSQQFSFTGNSSANVIIGNQ KLTINDVARVARNGTLVSLTNNTDILQGIQAS CDYINNAVESGEPIYGVTSGFGGMANVAISRE QASELQTNLVWFLKTGAGNKLPLADVRAAM LLRANSHMRGASGIRLELIKRMEIFLNAGVTP YVYEFGSIGASGDLVPLSYITGSLIGLDPSFKV DFNGKEMDAPTALRQLNLSPLTLLPKEGLAM MNGTSVMTGIAANCVYDTQILTAIAMGVHA LDIQALNGTNQSFHPFIHNSKPHPGQLWAAD QMISLLANS QLVRDELDGKHDYRDHELIQDRYSLRCLPQY LGPIVDGISQIAKQIEIEINSVTDNPLIDVDNQA SYHGGNFLGQYVGMGMDHLRYYIGLLAKHL DVQIALLASPEFSNGLPPSLLGNRERKVNMGL KGLQICGNSIMPLLTFYGNSIADRFPTHAEQF NQNINSQGYTSATLARRSVDIFQNYVAIALMF GVQAVDLRTYKKTGHYDARASLSPATERLYS AVRHVVGQKPTSDRPYIWNDNEQGLDEHIAR ISADIAAGGVIVQAVQDILPSLH [SEQ ID NO:179]	Phenylalanine hydroxylase deficiency	Aminoacidopathy
ATP 8B1	5205	0081923	O43520	MSTERDSETTFDEDSQPNDEVVPYSDDETED ELDDQGSAVEPEQNRVNREAEENREPFRKEC TWQVKANDRKYHEQPHFMNTKFLCIKESKY ANNAIKTYKYNAFTFIPMNLFEQFKRAANLY FLALLILQAVPQISTLAWYTTLVPLLVVLGVT AIKDLVDDVARHKMDKEINNRTCEVIKDGRF KVAKWKEIQVGDVIRLKKNDFVPADILLLSSS EPNSLCYVETAELDGETNLKFKMSLEITDQYL QREDTLATFDGFIECEEPNNRLDKFTGTLFWR NTSFPLDADKILLRGCVIRNTDFCHGLVIFAG ADTKIMKNSGKTRFKRTKIDYLMNYMVYTIF VVLILLSAGLAIGHAYWEAQVGNSSWYLYD GEDDTPSYRGFLIFWGYIIVLNTMVPISLYVSV EVIRLGQSHFINWDLQMYYAEKDTPAKARTT TLNEQLGQIHYIFSDKTGTLTQNIMTFKKCCI NGQIYGDHRDASQHNHNKIEQVDFSWNTYA DGKLAFYDHYLIEQIQSGKEPEVRQFFFLLAV CHTVMVDRTDGQLNYQAASPDEGALVNAAR NFGFAFLARTQNTITISELGTERTYNVLAILDF NSDRKRMSIIVRTPEGNIKLYCKGADTVIYER LHRMNPTKQETQDALDIFANETLRTLCLCYK	Progressive familial intrahepatic cholestasis Type 1	

				EIEEKEFTEWNKKFMAASVASTNRDEALDKV YEEIEKDLILLGATAIEDKLQDGVPETISKLAK ADIKIWVLTGDKKETAENIGFACELLTEDTTI CYGEDINSLLHARMENQRNRGGVYAKFAPP VQESFFPPGGNRALIITGSWLNEILLEKKTKRN KILKLKFPRTEEERRMRTQSKRRLEAKKEQR QKNFVDLACECSAVICCRVTPKQKAMVVDL VKRYKKAITLAIGDGANDVNMIKTAHIGVGIS GQEGMQAVMSSDYSFAQFRYLQRLLLVHGR WSYIRMCKFLRYFFYKNFAFTLVHFWYSFFN GYSAQTAYEDWFITLYNVLYTSLPVLLMGLL DQDVSDKLSLRFPGLYIVGQRDLLFNYKRFF VSLLHGVLTSMILFFIPLGAYLQTVGQDGEAP SDYQSFAVTIASALVITVNFQIGLDTSYWTFV NAFSIFGSIALYFGIMFDFHSAGIHVLFPSAFQF TGTASNALRQPYIWLTIILAVAVCLLPVVAIRF LSMTIWPSESDKIQKHRKRLKAEEQWQRRQQ VFRRGVSTRRSAYAFSHQRGYADLISSGRSIR KKRSPLDAIVADGTAEYRRTGDS [SEQ ID NO:180]		
ABC B11	8647	0073734,02 76582	O95342	MSDSVILRSIKKFGEENDGFESDKSYNNDKKS RLQDEKKGDGVRVGFFQLFRFSSSTDIWLMF VGSLCAFLHGIAQPGVLLIFGTMTDVFIDYDV ELQELQIPGKACVNNTIVWTNSSLNQNMTNG TRCGLLNIESEMIKFASYYAGIAVAVLITGYIQ ICFWVIAAARQIQKMRKFYFRRIMRMEIGWF DCNSVGELNTRFSDDINKINDAIADQMALFIQ RMTSTICGFLLGFFRGWKLTLVIISVSPLIGIGA ATIGLSVSKFTDYELKAYAKAGVVADEVISS MRTVAAFGGEKREVERYEKNLVFAQRWGIR KGIVMGFFTGFVWCLIFLCYALAFWYGSTLV LDEGEYTPGTLVQIFLSVIVGALNLGNASPCL EAFATGRAAATSIFETIDRKPIIDCMSEDGYKL DRIKGEIEFHNVTFHYPSRPEVKILNDLNMVI KPGEMTALVGPSGAGKSTALQLIQRFYDPCE GMVTVDGHDIRSLNIQWLRDQIGIVEQEPVLF STTIAENIRYGREDATMEDIVQAAKEANAYN FIMDLPQQFDTLVGEGGGQMSGGQKQRVAIA RALIRNPKILLLDMATSALDNESEAMVQEVLS KIQHGHTIISVAHRLSTVRAADTIIGFEHGTAV ERGTHEELLERKGVYFTLVTLQSQGNQALNE EDIKDATEDDMLARTFSRGSYQDSLRASIRQR SKSQLSYLVHEPPLAVVDHKSTYEEDRKDKD IPVQEEVEPAPVRRILKFSAPEWPYMLVGSVG AAVNGTVTPLYAFLFSQILGTFSIPDKEEQRSQ INGVCLLFVAMGCVSLFTQFLQGYAFAKSGE LLTKRLRKFGFRAMLGQDIAWFDDLRNSPGA LTTRLATDASQVQGAAGSQIGMIVNSFTNVT VAMIIAFSFSWKLSLVILCFFPFLALSGATQTR MLTGFASRDKQALEMVGQITNEALSNIRTVA GIGKERRFIEALETELEKPFKTAIQKANIYGFC FAFAQCIMFIANSASYRYGGYLISNEGLHFSY VFRVISAVVLSATALGRAFSYTPSYAKAKISA ARFFQLLDRQPPISVYNTAGEKWDNFQGKID FVDCKFTYPSRPDSQVLNGLSVSISPGQTLAF VGSSGCGKSTSIQLLERFYDPDQGKVMIDGH DSKKVNVQFLRSNIGIVSQEPVLFACSIMDNI KYGDNTKEIPMERVIAAAKQAQLHDFVMSLP EKYETNVGSQGSQLSRGEKQRIAIARAIVRDP KILLLDEATSALDTESEKTVQVALDKAREGR	Progressive familial intrahepatic cholestasis Type 2; Progressive Familial Intrahepatic Cholestasis Type 3	

				TCIVIAHRLSTIQNADIIAVMAQGVVIEKGTHE ELMAQKGAYYKLVTTGSPIS [SEQ ID NO:181]		
ABC B4	5244	0005471	P21439	MDLEAAKNGTAWRPTSAEGDFELGISSKQKR KKTKTVKMIGVLTLFRYSDWQDKLFMSLGTI MAIAHGSGLPLMMIVFGEMTDKFVDTAGNFS FPVNFSLSLLNPGKILEEEMTRYAYYYSGLGA GVLVAAYIQVSFWTLAAGRQIRKIRQKFFHAI LRQEIGWFDINDTTELNTRLTDDISKISEGIGD KVGMFFQAVATFFAGFIVGFIRGWKLTLVIM AISPILGLSAAVWAKILSAFSDKELAAYAKAG AVAEEALGAIRTVIAFGGQNKELERYQKHLE NAKEIGIKKAISANISMGIAFLLIYASYALAFW YGSTLVISKEYTIGNAMTVFFSILIGAFSVGQA APCIDAFANARGAAYVIFDIIDNNPKIDSFSER GHKPDSIKGNLEFNDVHFSYPSRANVKILKGL NLKVQSGQTVALVGSSGCGKSTTVQLIQRLY DPDEGTINIDGQDIRNFNVNYLREIIGVVSQEP VLFSTTIAENICYGRGNVTMDEIKKAVKEAN AYEFIMKLPQKFDTLVGERGAQLSGGQKQRI AIARALVRNPKILLLDEATSALDTESEAEVQA ALDKAREGRTTIVIAHRLSTVRNADVIAGFED GVIVEQGSHSELMKKEGVYFKLVNMQTSGS QIQSEEFELNDEKAATRMAPNGWKSRLFRHS TQKNLKNSQMCQKSLDVETDGLEANVPPVSF LKVLKLNKTEWPYFVVGTVCAIANGGLQPAF SVIFSEIIAIFGPGDDAVKQQKCNIFSLIFLFLGI ISFFTFFLQGFTFGKAGEILTRRLRSMAFKAM LRQDMSWFDDHKNSTGALSTRLATDAAQVQ GATGTRLALIAQNIANLGTGIIISFIYGWQLTL LLLAVVPIIAVSGIVEMKLLAGNAKRDKKELE AAGKIATEAIENIRTVVSLTQERKFESMYVEK LYGPYRNSVQKAHIYGITFSISQAFMYFSYAG CFRFGAYLIVNGHMRFRDVILVFSAIVFGAVA LGHASSFAPDYAKAKLSAAHLFMLFERQPLI DSYSEEGLKPDKFEGNITFNEVVFNYPTRANV PVLQGLSLEVKKGQTLALVGSSGCGKSTVVQ LLERFYDPLAGTVFVDFGFQLLDGQEAKKLN VQWLRAQLGIVSQEPILFDCSIAENIAYGDNS RVVSQDEIVSAAKAANIHPFIETLPHKYETRV GDKGTQLSGGQKQRIAIARALIRQPQILLLDE ATSALDTESEKVVQEALDKAREGRTCIVIAHR LSTIQNADLIVVFQNGRVKEHGTHQQLLAQK GIYFSMVSVQAGTQNL [SEQ ID NO:184]		
TJP2	9414	0119139	B7Z2R3, Q9UDY2, B7Z954	MPVRGDRGFPPRRELSGWLRAPGMEELIWEQ YTVTLQKDSKRGFGIAVSGGRDNPHFENGET SIVISDVLPGGPADGLLQENDRVVMVNGTPM EDVLHSFAVQQLRKSGKVAAIVVKRPRKVQ VAALQASPPLDQDDRAFEVMDEFDGRSFRSG YSERSRLNSHGGRSRSWEDSPERGRPHERAR SRERDLSRDRSRGRSLERGLDQDHARTRDRS RGRSLERGLDHDFGPSRDRDRDRSRGRSIDQ DYERAYHRAYDPDYERAYSPEYRRGARHDA RSRGPRSRSREHPHSRSPSPEPRGRPGPIGVLL MKSRANEEYGLRLGSQIFVKEMTRTGLATKD GNLHEGDIILKINGTVTENMSLTDARKLIEKS RGKLQLVVLRDSQQTLINIPSLNDSDSEIEDIS EIESNRSFSPEERRHQYSDYDYHSSSEKLKERP SSREDTPSRLSRMGATPTPFKSTGDIAGTVVP ETNKEPRYQEDPPAPQPKAAPRTFLRPSPEDE AIYGPNTKMVRFKKGDSVGLRLAGGNDVGIF		

				VAGIQEGTSAEQEGLQEGDQILKVNTQDFRG LVREDAVLYLLEIPKGEMVTILAQSRADVYR DILACGRGDSFFIRSHFECEKETPQSLAFTRGE VFRVVDTLYDGKLGNWLAVRIGNELEKGLIP NKSRAEQMASVQNAQRDNAGDRADFWRMR GQRSGVKKNLRKSREDLTAVVSVSTKFPAYE RVLLREAGFKRPVVLFGPIADIAMEKLANELP DWFQTAKTEPKDAGSEKSTGVVRLNTVRQII EQDKHALLDVTPKAVDLLNYTQWFPIVIFFNP DSRQGVKTMRQRLNPTSNKSSRKLFDQANKL KKTCAHLFTATINLNSANDSWFGSLKDTIQH QQGEAVWVSEGKMEGMDDDPEDRMSYLTA MGADYLSCDSRLISDFEDTDGEGGAYTDNEL DEPAEEPLVSSITRSSEPVQHEESIRKPSPEPRA QMRRAASSDQLRDNSPPPAFKPEPPKAKTQN KEESYDFSKSYEYKSNPSAVAGNETPGASTK GYPPPVAAKPTFGRSILKPSTPIPPQEGEEVGE SSEEQDNAPKSVLGKVKIFEKMDHKARLQR MQELQEAQNARIEIAQKHPDIYAVPIKTHKPD PGTPQHTSSRPPEPQKAPSRPYQDTRGSYGSD AEEEEYRQQLSEHSKRGYYGQSARYRDTEL [SEQ ID NO:183]		
IVD	3712	0128928	P26440, A0A0A0 MT83	MATATRLLGWRVASWRLRPPLAGFVSQRAH SLLPVDDAINGLSEEQRQLRQTMAKFLQEHL APKAQEIDRSNEFKNLREFWKQLGNLGVLGI TAPVQYGGSGLGYLEHVLVMEEISRASGAVG LSYGAHSNLCINQLVRNGNEAQKEKYLPKLIS GEYIGALAMSEPNAGSDVVSMKLKAEKKGN HYILNGNKFWITNGPDADVLIVYAKTDLAAV PASRGITAFIVEKGMPGFSTSKKLDKLGMRGS NTCELIFEDCKIPAANILGHENKGVYVLMSGL DLERLVLAGGPLGLMQAVLDHTIPYLHVREA FGQKIGHFQLMQGKMADMYTRLMACRQYV YNVAKACDEGHCTAKDCAGVILYSAECATQ VALDGIQCFGGNGYINDFPMGRFLRDAKLYE IGAGTSEVRRLVIGRAFNADFH [SEQ ID NO:184]	isovaleric acidemia (IVD)	Organic acidemia
GCD H	2639	0105607	A0A024R 7F9, Q92947	MALRGVSVRLLSRGPGLHVLRTWVSSAAQT EKGGRTQSQLAKSSRPEFDWQDPLVLEEQLT TDEILIRDTFRTYCQERLMPRILLANRNEVFH REIISEMGELGVLGPTIKGYGCAGVSSVAYGL LARELERVDSGYRSAMSVQSSLVMHPIYAYG SEEQRQKYLPQLAKGELLGCFGLTEPNSGSDP SSMETRAHYNSSNKSYTLNGTKTWITNSPMA DLFVVWARCEDGCIRGFLLEKGMRGLSAPRI QGKFSLRASATGMIIMDGVEVPEENVLPGASS LGGPFGCLNNARYGIAWGVLGASEFCLHTAR QYALDRMQFGVPLARNQLIQKKLADMLTEIT LGLHACLQLGRLKDQDKAAPEMVSLLKRNN CGKALDIARQARDMLGGNGISDEYHVIRHA MNLEAVNTYEGTHDIHALILGRAITGIQAFTA SK [SEQ ID NO:185]	glutaric acidemia type I	Organic acidemia
ETF A	2108	0140374	A0A0S2Z 3L0, P13804	MFRAAAPGQLRRAASLLRFQSTLVIAEHAND SLAPITLNTITAATRLGGEVSCLVAGTKCDKV AQDLCKVAGIAKVLVAQHDVYKGLLPEELTP LILATQKQFNYTHICAGASAFGKNLLPRVAA KLEVAPISDIIAIKSPDTFVRTIYAGNALCTVK CDEKVKVFSVRGTSFDAAATSGGSASSEKAS STSPVEISEWLDQKLTKSDRPELTGAKVVVSG GRGLKSGENFKLLYDLADQLHAAVGASRAA	multiple acyl-CoA dehydrogen ase deficiency (a.k.a. glutaric	Organic acidemia

				VDAGFVPNDMQVGQTGKIVAPELYIAVGISG AIQHLAGMKDSKTIVAINKDPEAPIFQVADYG IVADLFKVVPEMTEILKKK [SEQ ID NO:186]	aciduria type II)	
ETF B	2109	0105379	P38117	MAELRVLVAVKRVIDYAVKIRVKPDRTGVV TDGVKHSMNPFCEIAVEEAVRLKEKKLVKEV IAVSCGPAQCQETIRTALAMGADRGIHVEVPP AEAERLGPLQVARVLAKLAEKEKVDLVLLG KQAIDDDCNQTGQMTAGFLDWPQGTFASQV TLEGDKLKVEREIDGGLETLRLKLPAVVTAD LRLNEPRYATLPNIMKAKKKKIEVIKPGDLGV DLTSKLSVISVEDPPQRTAGVKVETTEDLVAK LKEIGRI [SEQID NO:187]	multiple acyl-CoA dehydrogen ase deficiency (a.k.a. glutaric aciduria type II)	Organic acidemia
ETF DH	2110	0171503	B4DEQ0, Q16134	MLVPLAKLSCLAYQCFHALKIKKNYLPLCAT RWSSTSTVPRITTHYTIYPRDKDKRWEGVNM ERFAEEADVVIVGAGPAGLSAAVRLKQLAVA HEKDIRVCLVEKAAQIGAHTLSGACLDPGAF KELFPDWKEKGAPLNTPVTEDRFGILTEKYRI PVPILPGLPMNNHGNYIVRLGHLVSWMGEQA EALGVEVYPGYAAAEVLFHDDGSVKGIATN DVGIQKDGAPKATFERGLELHAKVTIFAEGC HGHLAKQLYKKFDLRANCEPQTYGIGLKEL WVIDEKNWKPGRVDHTVGWPLDRHTYGGSF LYHLNEGEPLVALGLVVGLDYQNPYLSPFRE FQRWKHHPSIRPTLEGGKRIAYGARALNEGG FQSIPKLTFPGGLLIGCSPGFMNVPKIKGTHTA MKSGILAAESIFNQLTSENLQSKTIGLHVTEYE DNLKNSWVWKELYSVRNIRPSCHGVLGVYG GMIYTGIFYWILRGMEPWTLKHKGSDFERLK PAKDCTPIEYPKPDGQISFDLLSSVALSGTNHE HDQPAHLTLRDDSIPVNRNLSIYDGPEQRFCP AGVYEFVPVEQGDGFRLQINAQNCVHCKTC DIKDPSQNINWVVPEGGGGPAYNGM [SEQ ID NO:188]	multiple acyl-CoA dehydrogen ase deficiency (a.k.a. glutaric aciduria type II)	Organic acidemia
ASL	435	0126522	A0A024R DL8, P04424, A0A0S2Z 316	MASESGKLWGGRFVGAVDPIMEKFNASIAYD RHLWEVDVQGSKAYSRGLEKAGLLTKAEMD QILHGLDKVAEEWAQGTFKLNSNDEDIHTAN ERRLKELIGATAGKLHTGRSRNDQVVTDLRL WMRQTCSTLSGLLWELIRTMVDRAEAERDV LFPGYTHLQRAQPIRWSHWILSHAVALTRDS ERLLEVRKRINVLPLGSGAIAGNPLGVDRELL RAELNFGAITLNSMDATSERDFVAEFLFWAS LCMTHLSRMAEDLILYCTKEFSFVQLSDAYST GSSLMPQKKNPDSLELIRSKAGRVFGRCAGL LMTLKGLPSTYNKDLQEDKEAVFEVSDTMSA VLQVATGVISTLQIHQENMGQALSPDMLATD LAYYLVRKGMPFRQAHEASGKAVFMAETKG VALNQLSLQELQTISPLFSGDVICVWDYGHSV EQYGALGGTARSSVDWQIRQVRALLQAQQA [SEQ ID NO:189]	argininosuc cinate lyase (ASL) deficiency	Urea cycle disorder
D2H GDH	728294	0180902	B3KSR6, B4E3K7, B5MCV2, Q8N465	MVGGSVPVFDEIILSTARMNRVLSFHSVSGIL VCQAGCVLEELSRYVEERDFIMPLDLGAKGS CHIGGNVATNAGGLRFLRYGSLHGTVLGLEV VLADGTVLDCLTSLRKDNTGYDLKQLFIGSE GTLGIITTVSILCPPKPRAVNVAFLGCPGFAEV LQTFSTCKGMLGEILSAFEFMDAVCMQLVGR HLHLASPVQESPFYVLIETSGSNAGHDAEKLG HFLEHALGSGLVTDGTMATDQRKVKMLWA LRERITEALSRDGYVYKYDLSLPVERLYDIVT DLRARLGPHAKHVVGYGHLGDGNLHLNVTA EAFSPSLLAALEPHVYEWTAGQQGSVSAEHG	D-2-hydroxyglu taric aciduria type I	Organic acidemia

				VGFRKRDVLGYSKPPGALQLMQQLKALLDP KGILNPYKTLPSQA [SEQ ID NO:190]		
HM GCL	3155	0117305	P35914	MAAMRKALPRRLVGLASLRAVSTSSMGTLP KRVKIVEVGPRDGLQNEKNIVSTPVKIKLIDM LSEAGLSVIETTSFVSPKWVPQMGDHTEVLK GIQKFPGINYPVLTPNLKGFEAAVAAGAKEV VIFGAASELFTKKNINCSIEESFQRFDAILKAA QSANISVRGYVSCALGCPYEGKISPAKVAEVT KKFYSMGCYEISLGDTIGVGTPGIMKDMLSA VMQEVPLAALAVHCHDTYGQALANTLMAL QMGVSVVDSSVAGLGGCPYAQGASGNLATE DLVYMLEGLGIHTGVNLQKLLEAGNFICQAL NRKTSSKVAQATCKL [SEQ ID NO:191]	3-hydroxy-3-methylglutaryl-CoA lyase (3HMG) deficiency	Organic academia Urea cycle disorder
MCC C1	56922	0078070	Q68D27, Q96RQ3, A0A0S2Z693, E9PHF7	MAAASAVSVLLVAAERNRWHRLPSLLLPPRT WVWRQRTMKYTTATGRNITKVLIANRGEIAC RVMRTAKKLGVQTVAVYSEADRNSMHVDM ADEAYSIGPAPSQQSYLSMEKIIQVAKTSAAQ AIHPGCGFLSENMEFAELCKQEGIIFIGPPPSAI RDMGIKSTSKSIMAAAGVPVVEGYHGEDQSD QCLKEHARRIGYPVMIKAVRGGGGKGMRIV RSEQEFQEQLESARREAKKSFNDDAMLIEKF VDTPRHVEVQVFGDHHGNAVYLFERDCSVQ RRHQKIIEEAPAPGIKSEVRKKLGEAAVRAAK AVNYVGAGTVEFIMDSKHNFCFMEMNTRLQ VEHPVTEMITGTDLVEWQLRIAAGEKIPLSQE EITLQGHAFEARIYAEDPSNNFMPVAGPLVHL STPRADPSTRIETGVRQGDEVSVHYDPMIAKL VVWAADRQAALTKLRYSLRQYNIVGLHTNI DFLLNLSGHPEFEAGNVHTDFIPQHHKQLLLS RKAAAKESLCQAALGLILKEKAMTDTFTLQA HDQFSPFSSSSGRRLNISYTRNMTLKDGKNNV AIAVTYNHDGSYSMQIEDKTFQVLGNLYSEG DCTYLKCSVNGVASKAKLIILENTIYLFSKEG SIEIDIPVPKYLSSVSSQETQGGPLAPMTGTIEK VFVKAGDKVKAGDSLMVMIAMKMEHTIKSP KDGTVKKVFYREGAQANRHTPLVEFEEEESD KRESE [SEQID NO:192]	3-methylcrotonyl-CoA carboxylase (3MCC) deficiency	Organic acidemia
MCC C2	64087	0131844,0281742,0275300	A0A140VK29, Q9HCC0	MWAVLRLALRPCARASPAGPRAYHGDSVAS LGTQPDLGSALYQENYKQMKALVNQLHERV EHIKLGGGEKARALHISRGKLLPRERIDNLIDP GSPFLELSQFAGYQLYDNEEVPGGGIITGIGR VSGVECMIIANDATVKGGAYYPVTVKKQLR AQEIAMQNRLPCIYLVDSGGAYLPRQADVFP DRDHFGRTFYNQAIMSSKNIAQIAVVMGSCT AGGAYVPAMADENIIVRKQGTIFLAGPPLVK AATGEEVSAEDLGGADLHCRKSGVSDHWAL DDHHALHLTRKVVRNLNYQKKLDVTIEPSEE PLFPADELYGIVGANLKRSFDVREVIARIVDG SRFTEFKAFYGDTLVTGFARIFGYPVGIVGNN GVLFSESAKKGTHFVQLCCQRNIPLLFLQNIT GFMVGREYEAEGIAKDGAKMVAAVACAQV PKITLIIGGSYGAGNYGMCGRAYSPRFLYIWP NARISVMGGEQAANVLATITKDQRAREGKQF SSADEAALKEPIIKKFEEEGNPYYSSARVWDD GIIDPADTRLVLGLSFSAALNAPIEKTDFGIFR M [SEQ ID NO:193]	3-methylcrotonyl-CoA carboxylase (3MCC) deficiency	Organic acidemia
ABC D4	5826	0119688	A0A024R6B9, O14678,	MAVAGPAPGAGARPRLDLQFLQRFLQILKVL FPSWSSQNALMFLTLLCLTLLEQFVIYQVGLI PSQYYGVLGNKDLEGFKTLTFLAVMLIVLNS TLKSFDQFTCNLLYVSWRKDLTEHLHRLYFR	methylmalonic acidemia with	Organic acidemia

			A0A024R6C8	GRAYYTLNVLRDDIDNPDQRISQDVERFCRQLSSMASKLIISPFTLVYYTYQCFQSTGWLGPVSIFGYFILGTVVNKTLMGPIVMKLVHQEKLEGDFRFKHMQIRVNAEPAAFYRAGHVEHMRTDRRLQRLLQTQRELMSKELWLYIGINTFDYLGSILSYVVIAIPIFSGVYGDLSPAELSTLVSKNAFVCIYLISCFTQLIDLSTTLSDVAGYTHRIGQLRETLLDMSLKSQDCEILGESEWGLDTPPGWPAAEPADTAFLLERVSISAPSSDKPLIKDLSLKISEGQSLLITGNTGTGKTSLLRVLGGLWTSTRGSVQMLTDFGPHGVLFLPQKPFFTDGTLREQVIYPLKEVYPDSGSADDERILRFLELAGLSNLVARTEGLDQQVDWNWYDVLSPGEMQRLSFARLFYLQPKYAVLDEATSALTEEVESELYRIGQQLGMTFISVGHRQSLEKFHSLVLKLCGGGRWELMRIKVE [SEQ ID NO:194]	homocystinuria	
HCFC1	3054	0172534	P51610, A6NEM2	MASAVSPANLPAVLLQPRWKRVVGWSGPVPRPRHGHRAVAIKELIVVFGGGNEGIVDELHVYNTATNQWFIPAVRGDIPPGCAAYGFVCDGTRLLVFGGMVEYGKYSNDLYELQASRWEWKRLKAKTPKNGPPPCPRLGHSFSLVGNKCYLFGGLANDSEDPKNNIPRYLNDLYILELRPGSGVVAWDIPITYGVLPPPRESHTAVVYTEKDNKKSKLVIYGGMSGCRLGDLWTLDIDTLTWNKPSLSGVAPLPRSLHSATTIGNKMYVFGGWVPLVMDDVKVATHEKEWKCTNTLACLNLDTMAWETILMDTLEDNIPRARAGHCAVAINTRLYIWSGRDGYRKAWNNQVCCKDLWYLETEKPPPPARVQLVRANTNSLEVSWGAVATADSYLLQLQKYDIPATAATATSPTPNPVPSVPANPPKSPAPAAAAPAVQPLTQVGITLLPQAAPAPPTTTTIQVLPTVPGSSISVPTAARTQGVPAVLKVTGPQATTGTPLVTMRPASQAGKAPVTVTSLPAGVRMVVPTQSAQGTVIGSSPQMSGMAALAAAAAAATQKIPPSSAPTVLSVPAGTTIVKTMAVTPGTTTLPATVKVASSPVMVSNPATRMLKTAAAQVGTSVSSATNTSTRPIITVHKSGTVTVAQQAQVVTTVVGGVTKTITLVKSPISVPGGSALISNLGKVMSVVQTKPVQTSAVTGQASTGPVTQIIQTKGPLPAGTILKLVTSADGKPTTIITTTQASGAGTKPTILGISSVSPSTTKPGTTTIIKTIPMSAIITQAGATGVTSSPGIKSPITIITTKVMTSGTGAPAKIITAVPKIATGHGQQGVTQVVLKGAPGQPGTILRTVPMGGVRLVTPVTVSAVKPAVTTLVVKGTTGVTTLGTVTGTVSTSLAGAGGHSTSASLATPITTLGTIATLSSQVINPTAITVSAAQTTLTAAGGLTTPTITMQPVSQPTQVTLITAPSGVEAQPVHDLPVSILASPTTEQPTATVTIADSGQGDVQPGTVTLVCSNPPCETHETGTTNTATTTVVANLGGHPQPTQVQFVCDRQEAAASLVTSTVGQQNGSVVRVCSNPPCETHETGTTNTATTATSNMAGQHGCSNPPCETHETGTTNTATTAMSSVGANHQRDARRACAAGTPAVIRISVATGALEAAQGSKSQCQTRQTSATSTTMTVMATGAPCSAGPLLGPSMAREPGGRSPAFVQLAPLSSKVRLSSPSIKDLPAGRHSHAVSTAAMTRSSVGAGEPRMAPVCESLQGGSPSTTVTVTALEALLCPSATVTQVCSNPPCETHETGTTNTATTSNAGSAQRVCSNPPCETHETGTTHTATTATSNGGTGQPEGGQQPPAGRPCETHQTTSTGTTMSVSVGALLPD	homocystinuria	Organic acidemia

				ATSSHRTVESGLEVAAAPSVTPQAGTALLAPF PTQRVCSNPPCETHETGTTHTATTVTSNMSSN QDPPPAASDQGEVESTQGDSVNITSSSAITTTV SSTLTRAVTTVTQSTPVPGPSVPPPEELQVSPG PRQQLPPRQLLQSASTALMGESAEVLSASQTP ELPAAVDLSSTGEPSSGQESAGSAVVATVVV QPPPPTQSEVDQLSLPQELMAEAQAGTTTLM VTGLTPEELAVTAAAEAAAQAAATEEAQAL AIQAVLQAAQQAVMGTGEPMDTSEAAATVT QAELGHLSAEGQEGQATTIPIVLTQQELAALV QQQQLQEAQAQQQHHHLPTEALAPADSLND PAIESNCLNELAGTVPSTVALLPSTATESLAPS NTFVAPQPVVVASPAKLQAAATLTEVANGIE SLGVKPDLPPPPSKAPMKKENQWFDVGVIKG TNVMVTHYFLPPDDAVPSDDDLGTVPDYNQ LKKQELQPGTAYKFRVAGINACGRGPFSEISA FKTCLPGFPGAPCAIKISKSPDGAHLTWEPPSV TSGKIIEYSVYLAIQSSQAGGELKSSTPAQLAF MRVYCGPSPSCLVQSSSLSNAHIDYTTKPAIIF RIAARNEKGYGPATQVRWLQETSKDSSGTKP ANKRPMSSPEMKSAPKKSKADGQ [SEQ ID NO:195]		
LMB RD1	55788	0168216	Q9NUN5	MATSGAASAELVIGWCIFGLLLLAILAFCWIY VRKYQSRRESEVVSTITAIFSLAIALITSALLPV DIFLVSYMKNQNGTFKDWANANVSRQIEDT VLYGYYTLYSVILFCVFFWIPFVYFYYEEKDD DDTSKCTQIKTALKYTLGFVVICALLLLVGAF VPLNVPNNKNSTEWEKVKSLFEELGSSHGLA ALSFSISSLTLIGMLAAITYTAYGMSALPLNLI KGTRSAAYERLENTEDIEEVEQHIQTIKSKSK DGRPLPARDKRALKQFEERLRTLKKRERHLE FIENSWWTKFCGALRPLKIVWGIFFILVALLF VISLFLSNLDKALHSAGIDSGFIIFGANLSNPL NMLLPLLQTVFPLDYILITIIIMYFIFTSMAGIR NIGIWFFWIRLYKIRRGRTRPQALLFLCMILLL IVLHTSYMIYSLAPQYVMYGSQNYLIETNITS DNHKGNSTLSVPKRCDADAPEDQCTVTRTYL FLHKFWFFSAAYYFGNWAFLGVFLIGLIVSCC KGKKSVIEGVDEDSDISDDEPSVYSA [SEQID NO:196]	methylmalonic acidemia with homocystinuria	Organic acidemia
ARG 1	383	0118520	P05089	MSAKSRTIGIIGAPFSKGQPRGGVEEGPTVLR KAGLLEKLKEQECDVKDYGDLPFADIPNDSP FQIVKNPRSVGKASEQLAGKVAEVKKNGRIS LVLGGDHSLAIGSISGHARVHPDLGVIWVDA HTDINTPLTTTSGNLHGQPVSFLLKELKGKIP DVPGFSWVTPCISAKDIVYIGLRDVDPGEHYI LKTLGIKYFSMTEVDRLGIGKVMEETLSYLLG RKKRPIHLSFDVDGLDPSFTPATGTPVVGGLT YREGLYITEEIYKTGLLSGLDIMEVNPSLGKTP EEVTRTVNTAVAITLACFGLAREGNHKPIDYL NPPK [SEQ ID NO:197]	arginase (ARG1) deficiency	Urea cycle disorder
SLC 25A1 5	10166	0102743	Q9Y619	MKSNPAIQAAIDLTAGAAGGTACVLTGQPFD TMKVKMQTFPDLYRGLTDCCLKTYSQVGFR GFYKGTSPALIANIAENSVLFMCYGFCQQVV RKVAGLDKQAKLSDLQNAAAGSFASAFAAL VLCPTELVKCRLQTMYEMETSGKIAKSQNTV WSVIKSILRKDGPLGFYHGLSSTLLREVPGYF FFFGGYELSRSFFASGRSKDELGPVPLMLSGG VGGICLWLAVYPVDCIKSRIQVLSMSGKQAG FIRTFINVVKNEGITALYSGLKPTMIRAFPANG	hyperammonemia-hyperornithinemia-homocitrullinuria (HHH) syndrome	Urea cycle disorder

				ALFLAYEYSRKLMMNQLEAY [SEQ ID NO:198]		
SLC25A13	10165	0004864	Q9UJS0	MAAAKVALTKRADPAELRTIFLKYASIEKNG EFFMSPNDFVTRYLNIFGESQPNPKTVELLSG VVDQTKDGLISFQEFVAFESVLCAPDALFMV AFQLFDKAGKGEVTFEDVKQVFGQTTIHQHI PFNWDSEFVQLHFGKERKRHLTYAEFTQFLL EIQLEHAKQAFVQRDNARTGRVTAIDFRDIM VTIRPHVLTPFVEECLVAAAGGTTSHQVSFSY FNGFNSLLNNMELIRKIYSTLAGTRKDVEVTK EEFVLAAQKFGQVTPMEVDILFQLADLYEPR GRMTLADIERIAPLEEGTLPFNLAEAQRQKAS GDSARPVLLQVAESAYRFGLGSVAGAVGAT AVYPIDLVKTRMQNQRSTGSFVGELMYKNSF DCFKKVLRYEGFFGLYRGLLPQLLGVAPEKA IKLTVNDFVRDKFMHKDGSVPLAAEILAGGC AGGSQVIFTNPLEIVKIRLQVAGEITTGPRVSA LSVVRDLGFFGIYKGAKACFLRDIPFSAIYFPC YAHVKASFANEDGQVSPGSLLLAGAIAGMPA ASLVTPADVIKTRLQVAARAGQTTYSGVIDC FRKILREEGPKALWKGAGARVFRSSPQFGVT LLTYELLQRWFYIDFGGVKPMGSEPVPKSRIN LPAPNPDHVGGYKLAVATFAGIENKFGLYLP LFKPSVSTSKAIGGGP [SEQ ID NO:199]	Citrin deficiency Citrullinemia type II	Urea cycle disorder
ALAD	210	0148218	P13716	MQPQSVLHSGYFHPLLRAWQTATTTLNASNL IYPIFVTDVPDDIQPITSLPGVARYGVKRLEEM LRPLVEEGLRCVLIFGVPSRVPKDERGSAADS EESPAIEAIHLLRKTFPNLLVACDVCLCPYTSH GHCGLLSENGAFRAEESRQRLAEVALAYAKA GCQVVAPSDMMDGRVEAIKEALMAHGLGN RVSVMSYSAKFASCFYGPFRDAAKSSPAFGD RRCYQLPPGARGLALRAVDRDVREGADMLM VKPGMPYLDIVREVKDKHPDLPLAVYHVSGE FAMLWHGAQAGAFDLKAAVLEAMTAFRRA GADIIITYYTPQLLQWLKEE [SEQ ID NO:200]	Acute Hepatic porphyria	Porphyria
CPOX	1371	0080819	P36551	MALQLGRLSSGPCWLVARGGCGGPRAWSQC GGGGLRAWSQRSAAGRVCRPPGPAGTEQSR GLGHGSTSRGGPWVGTGLAAALAGLVGLAT AAFGHVQRAEMLPKTSGTRATSLGRPEEEED ELAHRCSSFMAPPVTDLGELRRRPGDMKTK MELLILETQAQVCQALAQVDGGANFSVDRW ERKEGGGGISCVLQDGCVFEKAGVSISVVHG NLSEEAAKQMRSRGKVLKTKDGKLPFCAMG VSSVIHPKNPHAPTIHFNYRYFEVEEADGNKQ WWFGGGCDLTPTYLNQEDAVHFHRTLKEAC DQHGPDLYPKFKKWCDDYFFIAHRGERRGIG GIFFDDLDSPSKEEVFRFVQSCARAVVPSYIPL VKKHCDDSFTPQEKLWQQLRRGRYVEFNLL YDRGTKFGLFTPGSRIESILMSLPLTARWEYM HSPSENSKEAEILEVLRHPRDWVR [SEQ ID NO:201]	Acute Hepatic porphyria	Porphyria
HMBS	3145	0256269,0281702	P08397	MSGNGNAAATAEENSPKMRVIRVGTRKSQL ARIQTDSVVATLKASYPGLQFEIIAMSTTGDKI LDTALSKIGEKSLFTKELEHALEKNEVDLVVH SLKDLPTVLPPGFTIGAICKRENPHDAVVFHP KFVGKTLETLPEKSVVGTSSLRRAAQLQRKFP HLEFRSIRGNLNTRLRKLDEQQEFSAIILATAG LQRMGWHNRVGQILHPEECMYAVGQGALG VEVRAKDQDILDLVGVLHDPETLLRCIAERAF LRHLEGGCSVPVAVHTAMKDGQLYLTGGV	Acute Hepatic porphyria; Acute Intermittent Porphyria	Porphyria

				WSLDGSDSIQETMQATIHVPAQHEDGPEDDP QLVGITARNIPRGPQLAAQNLGISLANLLLSK GAKNILDVARQLNDAH [SEQ ID NO:202]		
PPOX	5498	0143224	P50336, B4DY76	MGRTVVVLGGGISGLAASYHLSRAPCPPKVV LVESSERLGGWIRSVRGPNGAIFELGPRGIRPA GALGARTLLLVSELGLDSEVLPVRGDHPAAQ NRFLYVGGALHALPTGLRGLLRPSPPFSKPLF WAGLRELTKPRGKEPDETVHSFAQRRLGPEV ASLAMDSLCRGVFAGNSRELSIRSCFPSLFQA EQTHRSILLGLLLGAGRTPQPDSALIRQALAE RWSQWSLRGGLEMLPQALETHLTSRGVSVL RGQPVCGLSLQAEGRWKVSLRDSSLEADHVI SAIPASVLSELLPAEAAPLARALSAITAVSVAV VNLQYQGAHLPVQGFGHLVPSSEDPGVLGIV YDSVAFPEQDGSPPGLRVTVMLGGSWLQTLE ASGCVLSQELFQQRAQEAAATQLGLKEMPSH CLVHLHKNCIPQYTLGHWQKLESARQFLTAH RLPLTLAGASYEGVAVNDCIESGRQAAVSVL GTEPNS [SEQ ID NO:203]	Acute Hepatic porphyria	Porphyria
BTD	686	0169814	P43251	MAHAHIQGGRRAKSRFVVCIMSGARSKLALF LCGCYVVALGAHTGEESVADHHEAEYYVAA VYEHPSILSLNPLALISRQEALELMNQNLDIYE QQVMTAAQKDVQIIVFPEDGIHGFNFTRTSIY PFLDFMPSPQVVRWNPCLEPHRFNDTEVLQR LSCMAIRGDMFLVANLGTKEPCHSSDPRCPK DGRYQFNTNVVFSNNGTLVDRYRKHNLYFE AAFDVPLKVDLITFDTPFAGRFGIFTCFDILFF DPAIRVLRDYKVKHVVYPTAWMNQLPLLAA IEIQKAFAVAFGINVLAANVHHPVLGMTGSGI HTPLESFWYHDMENPKSHLIIAQVAKNPVGLI GAENATGETDPSHSKFLKILSGDPYCEKDAQE VHCDEATKWNVNAPPTFHSEMMYDNFTLVP VWGKEGYLHVCSNGLCCYLLYERPTLSKELY ALGVFDGLHTVHGTYYIQVCALVRCGGLGF DTCGQEITEATGIFEFHLWGNFSTSYIFPLFLT SGMTLEVPDQLGWENDHYFLRKSRLSSGLVT AALYGRLYERD [SEQ ID NO:204]	Biotinidase Deficiency	Organic acidemia
HLCS	3141	0159267	P50747	MEDRLHMDNGLVPQKIVSVHLQDSTLKEVK DQVSNKQAQILEPKPEPSLEIKPEQDGMEHVG RDDPKALGEEPKQRRGSASGSEPAGDSDRGG GPVEHYHLHLSSCHECLELENSTIESVKFASA ENIPDLPYDYSSSLESVADETSPEREGRRVNL TGKAPNILLYVGSDSQEALGRFHEVRSVLAD CVDIDSYILYHLLEDSALRDPWTDNCLLLVIA TRESIPEDLYQKFMAYLSQGGKVLGLSSSFTF GGFQVTSKGALHKTVQNLVFSKADQSEVKLS VLSSGCRYQEGPVRLSPGRLQGHLENEDKDR MIVHVPFGTRGGEAVLCQVHLELPPSSNIVQT PEDFNLLKSSNFRRYEVLREILTTLGLSCDMK QVPALTPLYLLSAAEEIRDPLMQWLGKHVDS EGEIKSGQLSLRFVSSYVSEVEITPSCIPVVTN MEAFSSEHFNLEIYRQNLQTKQLGKVILFAEV TPTTMRLLDGLMFQTPQEMGLIVIAARQTEG KGRGGNVWLSPVGCALSTLLISIPLRSQLGQR IPFVQHLMSVAVVEAVRSIPEYQDINLRVKWP NDIYYSDLMKIGGVLVNSTLMGETFYILIGCG FNVTNSNPTICINDLITEYNKQHKAELKPLRA DYLIARVVTVLEKLIKEFQDKGPNSVLPLYYR YWVHSGQQVHLGSAEGPKVSIVGLDDSGFLQ	Holocarboxylase Synthetase Deficiency	Organic acidemia

				VHQEGGEVVTVHPDGNSFDMLRNLILPKRR [SEQ ID NO:205]		
PC	5091	0173599	P11498 A0A024R5C5	MLKFRTVHGGLRLLGIRRTSTAPAASPNVRR LEYKPIKKVMVANRGEIAIRVFRACTELGIRT VAIYSEQDTGQMHRQKADEAYLIGRGLAPVQ AYLHIPDIIKVAKENNVDAVHPGYGFLSERAD FAQACQDAGVRFIGPSPEVVRKMGDKVEAR AIAIAAGVPVVPGTDAPITSLHEAHEFSNTYG FPIIFKAAYGGGGRGMRVVHSYEELEENYTR AYSEALAAFGNGALFVEKFIEKPRHIEVQILG DQYGNILHLYERDCSIQRRHQKVVEIAPAAH LDPQLRTRLTSDSVKLAKQVGYENAGTVEFL VDRHGKHYFIEVNSRLQVEHTVTEEITDVDL VHAQIHVAEGRSLPDLGLRQENIRINGCAIQC RVTTEDPARSFQPDTGRIEVFRSGEGMGIRLD NASAFQGAVISPHYDSLLVKVIAHGKDHPTA ATKMSRALAEFRVRGVKTNIAFLQNVLNNQ QFLAGTVDTQFIDENPELFQLRPAQNRAQKLL HYLGHVMVNGPTTPIPVKASPSPTDPVVPAVP IGPPPAGFRDILLREGPEGFARAVRNHPGLLL MDTTFRDAHQSLLATRVRTHDLKKIAPYVAH NFSKLFSMENWGGATFDVAMRFLYECPWRR LQELRELIPNIPFQMLLRGANAVGYTNYPDN VVFKFCEVAKENGMDVFRVFDSLNYLPNML LGMEAAGSAGGVVEAAISYTGDVADPSRTK YSLQYYMGLAEELVRAGTHILCIKDMAGLLK PTACTMLVSSLRDRFPDLPLHIHTHDTSGAGV AAMLACAQAGADVVDVAADSMSGMTSQPS MGALVACTRGTPLDTEVPMERVFDYSEYWE GARGLYAAFDCTATMKSGNSDVYENEIPGG QYTNLHFQAHSMGLGSKFKEVKKAYVEANQ MLGDLIKVTPSSKIVGDLAQFMVQNGLSRAE AEAQAEELSFPRSVVEFLQGYIGVPHGGFPEP FRSKVLKDLPRVEGRPGASLPPLDLQALEKEL VDRHGEEVTPEDVLSAAMYPDVFAHFKDFT ATFGPLDSLNTRLFLQGPKIAEEFEVELERGK TLHIKALAVSDLNRAGQRQVFFELNGQLRSIL VKDTQAMKEMHFHPKALKDVKGQIGAPMPG KVIDIKVVAGAKVAKGQPLCVLSAMKMETV VTSPMEGTVRKVHVTKDMTLEGDDLILEIE [SEQ ID NO:206]	Pyruvate Carboxylase Deficiency	Urea cycle disorder
SLC7A7	9056	0155465	Q9UM01 A0A0S2Z502	MVDSTEYEVASQPEVETSPLGDGASPGPEQV KLKKEISLLNGVCLIVGNMIGSGIFVSPKGVLI YSASFGLSLVIWAVGGLFSVFGALCYAELGT TIKKSGASYAYILEAFGGFLAFIRLWTSLLIIEP TSQAIIAITFANYMVQPLFPSCFAPYAASRLLA AACICLLTFINCAYVKWGTLVQDIFTYAKVL ALIAVIVAGIVRLGQGASTHFENSFEGSSFAV GDIALALYSALFSYSGWDTLNYVTEEIKNPER NLPLSIGISMPIVTIIYILTNVAYYTVLDMRDIL ASDAVAVTFADQIFGIFNWIIPLSVALSCFGGL NASIVAASRLFFVGSREGHLPDAICMIHVERF TPVPSLLFNGIMALIYLCVEDIFQLINYYSFSY WFFVGLSIVGQLYLRWKEPDRPRPLKLSVFFP IVFCLCTIFLVAVPLYSDTINSLIGIAIALSGLPF YFLIIRVPEHKRPLYLRRIVGSATRYLQVLCM SVAAEMDLEDGGEMPKQRDPKSN [SEQ ID NO:207]	Lysinuric Protein Intolerance	Urea cycle disorder
CPT2	1376	0157184	P23786	MVPRLLLRAWPRGPAVGPGAPSRPLSAGSGP GQYLQRSIVPTMHYQDSLPRLPIPKLEDTIRR	Carnitine Palmitoyltr	Fatty Acid Oxidation

			A0A140VK13 A0A1B0GTB8	YLSAQKPLLNDGQFRKTEQFCKSFENGIGKELHEQLVALDKQNKHTSYISGPWFDMYLSARDSVVLNFNPFMAFNPDPKSEYNDQLTRATNMTVSAIRFLKTLRAGLLEPEVFHLNPAKSDTITFKRLIRFVPSSLSWYGAYLVNAYPLDMSQYFRLFNSTRLPKPSRDELFTDDKARHLLVLRKGNFYIFDVLDQDGNIVSPSEIQAHLKYILSDSSPAPEFPLAYLTSENRDIWAELRQKLMSSGNEESLRKVDSAVFCLCLDDFPIKDLVHLSHNMLHGDGTNRWFDKSFNLIIAKDGSTAVHFEHSWGDGVAVLRFFNEVFKDSTQTPAVTPQSQPATTDSTVTVQKLNFELTDALKTGITAAKEKFDATMKTLTIDCVQFQRGGKEFLKKQKLSPDAVAQLAFQMAFLRQYGQTVATYESCSTAAFKHGRTETIRPASVYTKRCSEAFVREPSRHSAGELQQMMVECSKYHGQLTKEAAMGQGFDRHLFALRHLAAAKGIILPELYLDPAYGQINHNVLSTSTLSSPAVNLGGFAPVVSDGFGVGYAVHDNWIGCNVSSYPGRNAREFLQCVEKALEDMFDALEGKSIKS [SEQ ID NO:208]	ansferase Type II (CPT II) Deficiency	
ACADM	34	0117054	P11310 A0A0S2Z366, B7Z911, Q5HYG7, Q5T4U5, B4DJE7	MAAGFGRCCRVLRSISRFHWRSQHTKANRQREPGLGFSFEFTEQQKEFQATARKFAREEIIPVAAEYDKTGEYPVPLIRRAWELGLMNTHIPENCGGLGLGTFDACLISEELAYGCTGVQTAIEGNSLGQMPIIIAGNDQQKKKYLGRMTEEPLMCAYCVTEPGAGSDVAGIKTKAEKKGDEYIINGQKMWITNGGKANWYFLLARSDPDPKAPANKAFTGFIVEADTPGIQIGRKELNMGQRCSDTRGIVFEDVKVPKENVLIGDGAGFKVAMGAFDKTRPVVAAGAVGLAQRALDEATKYALERKTFGKLLVEHQAISFMLAEMAMKVELARMSYQRAAWEVDSGRRNTYYASIAKAFAGDIANQLATDAVQILGGNGFNTEYPVEKLMRDAKIYQIYEGTSQIQRLIVAREHIDKYKN [SEQID NO:209]	Medium Chain Acyl-CoA Dehydrogenase (MCAD) Deficiency	Fatty Acid Oxidation
ACADS	35	0122971	P16219 E5KSD5, B4DUH1, E9PE82	MAAALLARASGPARRALCPRAWRQLHTIYQSVELPETHQMLLQTCRDFAEKELFPIAAQVDKEHLFPAAQVKKMGGLGLLAMDVPEELGGAGLDYLAYAIAMEEISRGCASTGVIMSVNNSLYLGPILKFGSKEQKQAWVTPFTSGDKIGCFALSEPGNGSDAGAASTTARAEGDSWVLNGTKAWITNAWEASAAVVFASTDRALQNKGISAFLVPMPTPGLTLGKKEDKLGIRGSSTANLIFEDCRIPKDSILGEPGMGFKIAMQTLDMGRIGIASQALGIAQTALDCAVNYAENRMAFGAPLTKLQVIQFKLADMALALESARLLTWRAAMLKDNKKPFIKEAAMAKLAASEAATAISHQAIQILGGMGYVTEMPAERHYRDARITEIYEGTSEIQRLVIAGHLLRSYRS [SEQ ID NO:210]	Short Chain Acyl-CoA (SCAD) Dehydrogenase Deficiency	Fatty acid oxidation
ACADVL	37	0072778	P49748 B3KPA6	MQAARMAASLGRQLLRLGGGSSRLTALLGQPRPGPARRPYAGGAAQLALDKSDSHPSDALTRKKPAKAESKSFAVGMFKGQLTTDQVFPYPSVLNEEQTQFLKELVEPVSRFFEEVNDPAKNDALEMVEETTWQGLKELGAFGLQVPSELGGVGLCNTQYARLVEIVGMHDLGVGITLGAHQSIGFKGILLFGTKAQKEKYLPKLASGETVAAFCLTEPSSGSDAASIRTSAVPSPCGKYYTLNGSKLWISNGGLADIFTVFAKTPVTDPATGAVKEKITAFVVERGFGGITHGPPEKKMGIKASNTAEVFFDGVRVPSENVLGEVGSGFKVAMHILNNGRFGMAAALAGTMRGIIAKAVDHATNRTQFGEKI		

				HNFGLIQEKLARMVMLQYVTESMAYMVSAN MDQGATDFQIEAAISKIFGSEAAWKVTDECIQ IMGGMGFMKEPGVERVLRDLRIFRIFEGTNDI LRLFVALQGCMDKGKELSGLGSALKNPFGN AGLLLGEAGKQLRRRAGLGSGLSLSGLVHPE LSRSGELAVRALEQFATVVEAKLIKHKKGIV NEQFLLQRLADGAIDLYAMVVVLSRASRSLS EGHPTAQHEKMLCDTWCIEAAARIREGMAA LQSDPWQQELYRNFKSISKALVERGGVVTSN PLGF[SEQID NO:211]		
AGL	178	0162688	P35573 A0A0S2A 4E4	MGHSKQIRILLLNEMEKLEKTLFRLEQGYELQ FRLGPTLQGKAVTVYTNYPFPGETFNREKFRS LDWENPTEREDDSDKYCKLNLQQSGSFQYYF LQGNEKSGGGYIVVDPILRVGADNHVLPLDC VTLQTFLAKCLGPFDEWESRLRVAKESGYNM IHFTPLQTLGLSRSCYSLANQLELNPDFSRPNR KYTWNDVGQLVEKLKKEWNVICITDVVYNH TAANSKWIQEHPECAYNLVNSPHLKPAWVL DRALWRFSCDVAEGKYKEKGIPALIENDHHM NSIRKIIWEDIFPKLKLWEFFQVDVNKAVEQF RRLLTQENRRVTKSDPNQHLTIIQDPEYRRFG CTVDMNIALTTFIPHDKGPAAIEECCNWFHKR MEELNSEKHRLINYHQEQAVNCLLGNVFYER LAGHGPKLGPVTRKHPLVTRYFTFPFEEIDFS MEESMIHLPNKACFLMAHNGWVMGDDPLR NFAEPGSEVYLRRELICWGDSVKLRYGNKPE DCPYLWAHMKKYTEITATYFQGVRLDNCHS TPLHVAEYMLDAARNLQPNLYVVAELFTGSE DLDNVFVTRLGISSLIREAMSAYNSHEEGRLV YRYGGEPVGSFVQPCLRPLMPAIAHALFMDIT HDNECPIVHRSAYDALPSTTIVSMACCASGST RGYDELVPHQISVVSEERFYTKWNPEALPSNT GEVNFQSGIIAARCAISKLHQELGAKGFIQVY VDQVDEDIVAVTRHSPSIHQSVVAVSRTAFR NPKTSFYSKEVPQMCIPGKIEEVVLEARTIERN TKPYRKDENSINGTPDITVEIREHIQLNESKIV KQAGVATKGPNEYIQEIEFENLSPGSVIIFRVS LDPHAQVAVGILRNHLTQFSPHFKSGSLAVD NADPILKIPFASLASRLTLAELNQILYRCESEE KEDGGGCYDIPNWSALKYAGLQGLMSVLAEI RPKNDLGHPFCNNLRSGDWMIDYVSNRLISR SGTIAEVGKWLQAMFFYLKQIPRYLIPCYFDA ILIGAYTTLLDTAWKQMSSFVQNGSTFVKHL SLGSVQLCGVGKFPSLPILSPALMDVPYRLNE ITKEKEQCCVSLAAGLPHFSSGIFRCWGRDTFI ALRGILLITGRYVEARNIILAFAGTLRHGLIPN LLGEGIYARYNCRDAVWWWLQCIQDYCKM VPNGLDILKCPVSRMYPTDDSAPLPAGTLDQP LFEVIQEAMQKHMQGIQFRERNAGPQIDRNM KDEGFNITAGVDEETGFVYGGNRFNCGTWM DKMGESDRARNRGIPATPRDGSAVEIVGLSK SAVRWLLELSKKNIFPYHEVTVKRHGKAIKV SYDEWNRKIQDNFEKLFHVSEDPSDLNEKHP NLVHKRGIYKDSYGASSPWCDYQLRPNFTIA MVVAPELFTTEKAWKALEIAEKKLLGPLGM KTLDPDDMVYCGIYDNALDNDNYNLAKGFN YHQGPEWLWPIGYFLRAKLYFSRLMGPETTA KTIVLVKNVLSRHYVHLERSPWKGLPELTNE NAQYCPFSCETQAWSIATILETLYDL [SEQ ID NO:212]	GSD III (Cori / Forbe Disease or Debrancher)	Liver glycogen storage disorder

G6PC	2538	0131482	P35575	MEEGMNVLHDFGIQSTHYLQVNYQDSQDWF ILVSVIADLRNAFYVLFPIWFHLQEAVGIKLL WVAVIGDWLNLVFKWILFGQRPYWWVLDT DYYSNTSVPLIKQFPVTCETGPGSPSGHAMGT AGVYYVMVTSTLSIFQGKIKPTYRFRCLNVIL WLGFWAVQLNVCLSRIYLAAHFPHQVVAGV LSGIAVAETFSHIHSIYNASLKKYFLITFFLFSF AIGFYLLLKGLGVDLLWTLEKAQRWCEQPE WVHIDTTPFASLLKNLGTLFGLGLALNSSMY RESCKGKLSKWLPFRLSSIVASLVLLHVFDSL KPPSQVELVFYVLSFCKSAVVPLASVSVIPYC LAQVLGQPHKKSL[SEQID NO:213]	GSDIa (Von Gierke Disease)	Liver glycogen storage disorder
GBE1	2632	0114480	Q04446 Q59ET0	MAAPMTPAARPEDYEAALNAALADVPELAR LLEIDPYLKPYAVDFQRRYKQFSQILKNIGEN EGGIDKFSRGYESFGVHRCADGGLYCKEWAP GAEGVFLTGDFNGWNPFSYPYKKLDYGKWE LYIPPKQNKSVLVPHGSKLKVVITSKSGEILYR ISPWAKYVVREGDNVNYDWIHWDPEHSYEF KHSRPKKPRSLRIYESHVGISSHEGKVASYKH FTCNVLPRIKGLGYNCIQLMAIMEHAYYASF GYQITSFFAASSRYGTPEELQELVDTAHSMGII VLLDVVHSHASKNSADGLNMFDGTDSCYFH SGPRGTHDLWDSRLFAYSSWEILRFLLSNIRW WLEEYRFDGFRFDGVTSMLYHHHGVGQGFS GDYSEYFGLQVDEDALTYLMLANHLVHTLC PDSITIAEDVSGMPALCSPISQGGGGFDYRLA MAIPDKWIQLLKEFKDEDWNMGDIVYTLTN RRYLEKCIAYAESHDQALVGDKSLAFWLMD AEMYTNMSVLTPFTPVIDRGIQLHKMIRLITH GLGGEGYLNFMGNEFGHPEWLDFPRKGNNE SYHYARRQFHLTDDDLLRYKFLNNFDRDMN RLEERYGWLAAPQAYVSEKHEGNKIIAFERA GLLFIFNFHPSKSYTDYRVGTALPGKFKIVLD SDAAEYGGHQRLDHSTDFFSEAFEHNGRPYS LLVYIPSRVALILQNVDLPN [SEQ ID NO:214]	GSD IV (Andersen Disease, Brancher Enzyme)	Liver glycogen storage disorder
PHKA1	5255	0067177	P46020	MRSRSNSGVRLDGYARLVQQTILCHQNPVTG LLPASYDQKDAWVRDNVYSILAVWGLGLAY RKNADRDEDKAKAYELEQSVVKLMRGLLHC MIRQVDKVESFKYSQSTKDSLHAKYNTKTCA TVVGDDQWGHLQLDATSVYLLFLAQMTASG LHIIIHSLDEVNFIQNLVFYIEAAYKTADFGIWE RGDKTNQGISELNASSVGMAKAALEALDELD LFGVKGGPQSVIHVLADEVQHCQSILNSLLPR ASTSKEVDASLLSVVSFPAFAVEDSQLVELTK QEIITKLQGRYGCCRFLRDGYKTPKEDPNRLY YEPAELKLFENIECEWPLFWTYFILDGVFSGN AEQVQEYKEALEAVLIKGKNGVPLLPELYSV PPDRVDEEYQNPHTVDRVPMGKLPHMWGQS LYILGSLMAEGFLAPGEIDPLNRRFSTVPKPD VVVQVSILAETEEIKTILKDKGIYVETIAEVYPI RVQPARILSHIYSSLGCNNRMKLSGRPYRHM GVLGTSKLYDIRKTIFTFTPQFIDQQQFYLALD NKMIVEMLRTDLSYLCSRWRMTGQPTITFPIS HSMLDEDGTSLNSSILAALRKMQDGYFGGAR VQTGKLSEFLTTSCCTHLSFMDPGPEGKLYSE DYDDNYDYLESGNWMNDYDSTSHARCGDE VARYLDHLLAHTAPHPKLAPTSQKGGLDRFQ AAVQTTCDLMSLVTKAKELHVQNVHMYLPT KLFQASRPSFNLLDSPHPRQENQVPSVRVEIH LPRDQSGEVDFKALVLQLKETSSLQEQADILY	GSD IXa	

				MLYTMKGPDWNTELYNERSATVRELLTELY GKVGEIRHWGLIRYISGILRKKVEALDEACTD LLSHQKHLTVGLPPEPREKTISAPLPYEALTQL IDEASEGDMSISILTQEIMVYLAMYMRTQPGL FAEMFRLRIGLIIQVMATELAHSLRCSAEEAT EGLMNLSPSAMKNLLHHILSGKEFGVERSVR PTDSNVSPAISIHEIGAVGATKTERTGIMQLKS EIKQVEFRRLSISAESQSPGTSMTPSSGSFPSA YDQQSSKDSRQGQWQRRRRLDGALNRVPVG FYQKVWKVLQKCHGLSVEGFVLPSSTTREMT PGEIKFSVHVESVLNRVPQPEYRQLLVEAILV LTMLADIEIHSIGSIIAVEKIVHIANDLFLQEQK TLGADDTMLAKDPASGICTLLYDSAPSGRFG TMTYLSKAAATYVQEFLPHSICAMQ [SEQ ID NO:215]		
PHK A2	0044446 5256	5256 0044446	P46019	MRSRSNSGVRLDGYARLVQQTILCYQNPVTG LLSASHEQKDAWVRDNIYSILAVWGLGMAY RKNADRDEDKAKAYELEQNVVKLMRGLLQ CMMRQVAKVEKFKHTQSTKDSLHAKYNTAT CGTVVGDDQWGHLQVDATSLFLLFLAQMTA SGLRIIFTLDEVAFIQNLVFYIEAAYKVADYG MWERGDKTNQGIPELNASSVGMAKAALEAI DELDLFGAHGGRKSVIHVLPDEVEHCQSILFS MLPRASTSKEIDAGLLSIISFPAFAVEDVNLVN VTKNEIISKLQGRYGCCRFLRDGYKTPREDPN RLHYDPAELKLFENIECEWPVFWTYFIIDGVF SGDAVQVQEYREALEGILIRGKNGIRLVPELY AVPPNKVDEEYKNPHTVDRVPMGKVPHLWG QSLYILSSLLAEGFLAAGEIDPLNRRFSTSVKP DVVVQVTVLAENNHIKDLLRKHGVNVQSIA DIHPIQVQPGRILSHIYAKLGRNKNMNLSGRP YRHIGVLGTSKLYVIRNQIFTFTPQFTDQHHF YLALDNEMIVEMLRIELAYLCTCWRMTGRPT LTFPISRTMLTNDGSDIHSAVLSTIRKLEDGYF GGARVKLGNLSEFLTTSFYTYLTFLDPDCDEK LFDNASEGTFSPDSDSDLVGYLEDTCNQESQ DELDHYINHLLQSTSLRSYLPPLCKNTEDRHV FSAIHSTRDILSVMAKAKGLEVPFVPMTLPTK VLSAHRKSLNLVDSPQPLLEKVPESDFQWPR DDHGDVDCEKLVEQLKDCSNLQDQADILYIL YVIKGPSWDTNLSGQHGVTVQNLLGELYGK AGLNQEWGLIRYISGLLRKKVEVLAEACTDL LSHQKQLTVGLPPEPREKIISAPLPPEELTKLIY EASGQDISIAVLTQEIVVYLAMYVRAQPSLFV EMLRLRIGLIIQVMATELARSLNCSGEEASESL MNLSPFDMKNLLHHILSGKEFGVERSVRPIHS STSSPTISIHEVGHTGVTKTERSGINRLRSEMK QMTRRFSADEQFFSVGQAASSSAHSSKSARSS TPSSPTGTSSSDSGGHHIGWGERQGQWLRRR RLDGAINRVPVGFYQRVWKILQKCHGLSIDG YVLPSSTTREMTPHEIKFAVHVESVLNRVPQP EYRQLLVEAIMVLTLLSDTEMTSIGGIIHVDQI VQMASQLFLQDQVSIGAMDTLEKDQATGICH FFYDSAPSGAYGTMTYLTRAVASYLQELLPN SGCQMQ [SEQ ID NO:216]	GSD IXa	Liver glycogen storage disorder
PHK B	5257	0102893	Q93100	MAGAAGLTAEVSWKVLERRARTKRSGSVYE PLKSINLPRPDNETLWDKLDHYYRIVKSTLLL YQSPTTGLFPTKTCGGDQKAKIQDSLYCAAG AWALALAYRRIDDDKGRTHELEHSAIKCMR GILYCYMRQADKVQQFKQDPRPTTCLHSVFN	GSD IXb	Liver glycogen storage disorder

				VHTGDELLSYEEYGHLQINAVSLYLLYLVEM ISSGLQIIYNTDEVSFIQNLVFCVERVYRVPDF GVWERGSKYNNGSTELHSSSVGLAKAALEAI NGFNLFGNQGCSWSVIFVDLDAHNRNRQTLC SLLPRESRSHNTDAALLPCISYPAFALDDEVLF SQTLDKVVRKLKGKYGFKRFLRDGYRTSLED PNRCYYKPAEIKLFDGIECEFPIFFLYMMIDGV FRGNPKQVQEYQDLLTPVLHHTTEGYPVVPK YYYVPADFVEYEKNNPGSQKRFPSNCGRDG KLFLWGQALYIIAKLLADELISPKDIDPVQRY VPLKDQRNVSMRFSNQGPLENDLVVHVALIA ESQRLQVFLNTYGIQTQTPQQVEPIQIWPQQE LVKAYLQLGINEKLGLSGRPDRPIGCLGTSKI YRILGKTVVCYPIIFDLSDFYMSQDVFLLIDDI KNALQFIKQYWKMHGRPLFLVLIREDNIRGS RFNPILDMLAALKKGIIGGVKVHVDRLQTLIS GAVVEQLDFLRISDTEELPEFKSFEELEPPKHS KVKRQSSTPSAPELGQQPDVNISEWKDKPTH EILQKLNDCSCLASQAILLGILLKREGPNFITK EGTVSDHIERVYRRAGSQKLWLAVRYGAAF TQKFSSSIAPHITTFLVHGKQVTLGAFGHEEE VISNPLSPRVIQNIIYYKCNTHDEREAVIQQEL VIHIGWIISNNPELFSGMLKIRIGWIIHAMEYE LQIRGGDKPALDLYQLSPSEVKQLLLDILQPQ QNGRCWLNRRQIDGSLNRTPTGFYDRVWQIL ERTPNGIIVAGKHLPQQPTLSDMTMYEMNFS LLVEDTLGNIDQPQYRQIVVELLMVVSIVLER NPELEFQDKVDLDRLVKEAFNEFQKDQSRLK EIEKQDDMTSFYNTPPLGKRGTCSYLTKAVM NLLLEGEVKPNNDDPCLIS [SEQ ID NO:217]		
PHKG2	5261	0156873	P15735	MTLDVGPEDELPDWAAAKEFYQKYDPKDVI GRGVSSVVRRCVHRATGHEFAVKIMEVTAER LSPEQLEEVREATRRETHILRQVAGHPHIITLI DSYESSSFMFLVFDLMRKGELFDYLTEKVAL SEKETRSIMRSLLEAVSFLHANNIVHRDLKPE NILLDDNMQIRLSDFGFSCHLEPGEKLRELCG TPGYLAPEILKCSMDETHPGYGKEVDLWACG VILFTLLAGSPPFWHRRQILMLRMIMEGQYQF SSPEWDDRSSTVKDLISRLLQVDPEARLTAEQ ALQHPFFERCEGSQPWNLTPRQRFRVAVWTV LAAGRVALSTHRVRPLTKNALLRDPYALRSV RHLIDNCAFRLYGHWVKKGEQQNRAALFQH RPPGPFPIMGPEEEGDSAAITEDEAVLVLG [SEQ ID NO:218]	GSD IXc	Liver glycogen storage disorder
SLC37A4	2542	0281500 0137700	O43826 A0A024R3H9, A8K0S7, A0A024R3L1, B4DUH2	MAAQGYGYYRTVIFSAMFGGYSLYYFNRKT FSFVMPSLVEEIPLDKDDLGFITSSQSAAYAIS KFVSGVLSDQMSARWLFSSGLLLVGLVNIFF AWSSTVPVFAALWFLNGLAQGLGWPPCGKV LRKWFEPSQFGTWWAILSTSMNLAGGLGPIL ATILAQSYSWRSTLALSGALCVVVSFLCLLLI HNEPADVGLRNLDPMPSEGKKGSLKEESTLQ ELLLSPYLWVLSTGYLVVFGVKTCCTDWGQF FLIQEKGQSALVGSSYMSALEVGGLVGSIAA GYLSDRAMAKAGLSNYGNPRHGLLLFMMA GMTVSMYLFRVTVTSDSPKLWILVLGAVFGF SSYGPIALFGVIANESAPPNLCGTSHAIVGLM ANVGGFLAGLPFSTIAKHYSWSTAFWVAEVI CAASTAAFFLLRNIRTKMGRVSKKAE [SEQ ID NO:219]	GSDIb. c, d	Liver glycogen storage disorder

PMM2	5373	0140650	O15305, A0A0S2Z4J6, Q59F02	MAAPGPALCLFDVDGTLTAPRQKITKEMDDFLQKLRQKIKIGVVGGSDFEKVQEQLGNDVVEKYDYVFPENGLVAYKDGKLLCRQNIQSHLGEALIQDLINYCLSYIAKIKLPKKRGTFIEFRNGMLNVSPIGRSCSQEERIEFYELDKKENIRQKFVADLRKEFAGKGLTFSIGGQISFDVFPDGWDKRYCLRHVENDGYKTIYFFGDKTMPGGNDHEIFTDPRTMGYSVTAPEDTRRICELLFS [SEQ ID NO:220]	PMM2-CDG	Glycosylation disorder
CBS	102724560, 875	0160200	P35520, P0DN79, Q9NTF0, B7Z2D6	MPSETPQAEVGPTGCPHRSGPHSAKGSLEKGSPEDKEAKEPLWIRPDAPSRCTWQLGRPASESPHHHTAPAKSPKILPDILKKIGDTPMVRINKIGKKFGLKCELLAKCEFFNAGGSVKDRISLRMIEDAERDGTLKPGDTIIEPTSGNTGIGLALAAAVRGYRCIIVMPEKMSSEKVDVLRALGAEIVRTPTNARFDSPESHVGVAWRLKNEIPNSHILDQYRNASNPLAHYDTTADEILQQCDGKLDMLVASVGTGGTITGIARKLKEKCPGCRIIGVDPEGSILAEPEELNQTEQTTYEVEGIGYDFIPTVLDRTVVDKWFKSNDEEAFTFARMLIAQEGLLCGGSAGSTVAVAVKAAQELQEGQRCVVILPDSVRNYMTKFLSDRWMLQKGFLKEEDLTEKKPWWWHLRVQELGLSAPLTVLPTITCGHTIEILREKGFDQAPVVDEAGVILGMVTLGNMLSSLLAGKVQPSDQVGKVIYKQFKQIRLTDTLGRLSHILEMDHFALVVHEQIQYHSTGKSSQRQMVFGVVTAIDLLNFVAAQERDQK [SEQ ID NO:221]	Cystathionine Beta-Synthase Deficiency (Classic Homocystinuria); Homocystinuria	Aminoacidopathy
FAH	2184	0103876	P16930	MSFIPVAEDSDFPIHNLPYGVFSTRGDPRPRIGVAIGDQILDLSIIKHLFTGPVLSKHQDVFNQPTLNSFMGLGQAAWKEARVFLQNLLSVSQARLRDDTELRKCAFISQASATMHLPATIGDYTDFYSSRQHATNVGIMFRDKENALMPNWLHLPVGYHGRASSVVVSGTPIRRPMGQMKPDDSKPPVYGACKLLDMELEMAFFVGPGNRLGEPIPISKAHEHIFGMVLMNDWSARDIQKWEYVPLGPFLGKSFGTTVSPWVVPMDALMPFAVPNPKQDPRPLPYLCHDEPYTFDINLSVNLKGEGMSQAATICKSNFKYMYWTMLQQLTHHSVNGCNLRPGDLLASGTISGPEPENFGSMLELSWKGTKPIDLGNGQTRKFLLDGDEVIITGYCQGDGYRIGFGQCAGKVLPALLPS [SEQ ID NO:222]	Tyrosinemia Type I	Aminoacidopathy
TAT	6898	0198650	P17735, A0A140VKB7	MDPYMIQMSSKGNLPSILDVHVNVGGRSSVPGKMKGRKARWSVRPSDMAKKTFNPIRAIVDNMKVKPNPNKTMISLSIGDPTVFGNLPTDPEVTQAMKDALDSGKYNGYAPSIGFLSSREEIASYYHCPEAPLEAKDVILTSGCSQAIDLCLAVLANPGQNILVPRPGFSLYKTLAESMGIEVKLYNLLPEKSWEIDLKQLEYLIDEKTACLIVNNPSNPCGSVFSKRHLQKILAVAARQCVPILADEIYGDMVFSDCKYEPLATLSTDVPILSCGGLAKRWLVPGWRLGWILIHDRRDIFGNEIRDGLVKLSQRILGPCTIVQGALKSILCRTPGEFYHNTLSFLKSNADLCYGALAAIPGLRPVRPSGAMYLMVGIEMEHFPEFENDVEFTERLVAEQSVHCLPATCFEYPNFIRVVITVPEVMMLEACSRIQEFCEQHYHCAEGSQEECDK [SEQ ID NO:223]	Tyrosinemia Type II Tyrosinemia Type III	Aminoacidopathy
GALT	2592	0213930	P07902, A0A0S2Z3Y7, B2RAT6	MSRSGTDPQQRQQASEADAAAATFRANDHQHIRYNPLQDEWVLVSAHRMKRPWQGQVEPQLLKTVPRHDPLNPLCPGAIRANGEVNPQYDSTFLFDNDFPALQPDAPSPGPSDHPLFQAKSARG	Galactosemia due to galactose-	Carbohydrate disorder

				VCKVMCFHPWSDVTLPLMSVPEIRAVVDAW ASVTEELGAQYPWVQIFENKGAMMGCSNPH PHCQVWASSFLPDIAQREERSQQAYKSQHGE PLLMEYSRQELLRKERLVLTSEHWLVLVPFW ATWPYQTLLLPRRHVRRLPELTPAERDDLASI MKKLLTKYDNLFETSFPYSMGWHGAPTGSE AGANWNHWQLHAHYYPPLLRSATVRKFMV GYEMLAQAQRDLTPEQAAERLRALPEVHYH LGQKDRETATIA [SEQ ID NO:224]	1-phosphate uridylyltransferase (GALT) deficiency	
GALK1	2584	0108479	P51570	MAALRQPQVAELLAEARRAFREEFGAEPELA VSAPGRVNLIGEHTDYNQGLVLPMALELMTV LVGSPRKDGLVSLLTTSEGADEPQRLQFPLPT AQRSLEPGTPRWANYVKGVIQYYPAAPLPGF SAVVVSSVPLGGGLSSSASLEVATYTFLQQLC PDSGTIAARAQVCQQAEHSFAGMPCGIMDQF ISLMGQKGHALLIDCRSLETSLVPLSDPKLAV LITNSNVRHSLASSEYPVRRRQCEEVARALGK ESLREVQLEELEAARDLVSKEGFRRARHVVG EIRRTAQAAAALRRGDYRAFGRLMVESHRSL RDDYEVSCPELDQLVEAALAVPGVYGSRMT GGGFGGCTVTLLEASAAPHAMRHIQEHYGGT ATFYLSQAADGAKVLCL [SEQ ID NO:225]	Galactosemia	Carbohydrate disorder
GALE	2582	0117308	Q14376	MAEKVLVTGGAGYIGSHTVLELLEAGYLPVV IDNFHNAFRGGGSLPESLRRVQELTGRSVEFE EMDILDQGALQRLFKKYSFMAVIHFAGLKAV GESVQKPLDYYRVNLTGTIQLLEIMKAHGVK NLVFSSSATVYGNPQYLPLDEAHPTGGCTNP YGKSKFFIEEMIRDLCQADKTWNAVLLRYFN PTGAHASGCIGEDPQGIPNNLMPYVSQVAIGR REALNVFGNDYDTEDGTGVRDYIHVVDLAK GHIAALRKLKEQCGCRIYNLGTGTGYSVLQM VQAMEKASGKKIPYKVVARREGDVAACYAN PSLAQEELGWTAALGLDRMCEDLWRWQKQ NPSGFGTQA [SEQ ID NO:226]	Galactosemia	Carbohydrate disorder
G6PD	2539	0160211	P11413	MAEQVALSRTQVCGILREELFQGDAFHQSDT HIFIIMGASGDLAKKKIYPTIWWLFRDGLLPE NTFIVGYARSRLTVADIRKQSEPFFKATPEEK LKLEDFFARNSYVAGQYDDAASYQRLNSHM NALHLGSQANRLFYLALPPTVYEAVTKNIHE SCMSQIGWNRIIVEKPFGRDLQSSDRLSNHISS LFREDQIYRIDHYLGKEMVQNLMVLRFANRI FGPIWNRDNIACVILTFKEPFGTEGRGGYFDE FGIIRDVMQNHLLQMLCLVAMEKPASTNSDD VRDEKVKVLKCISEVQANNVVLGQYVGNPD GEGEATKGYLDDPTVPRGSTTATFAAVVLYV ENERWDGVPFILRCGKALNERKAEVRLQFHD VAGDIFHQQCKRNELVIRVQPNEAVYTKMM TKKPGMFFNPEESELDLTYGNRYKNVKLPDA YERLILDVFCGSQMHFVRSDELREAWRIFTPL LHQIELEKPKPIPYIYGSRGPTEADELMKRVG FQYEGTYKWVNPHKL [SEQ ID NO:227]	Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency	Carbohydrate disorder
SLC3A1	6519	0138079	Q07837, A0A0S2Z4E1, B8ZZK1	MAEDKSKRDSIEMSMKGCQTNNGFVHNEDIL EQTPDPGSSTDNLKHSTRGILGSQEPDFKGVQ PYAGMPKEVLFQFSGQARYRIPREILFWLTVA SVLVLIAATIAIIALSPKCLDWWQEGPMYQIY PRSFKDSNKDGNGDLKGIQDKLDYITALNIKT VWITSFYKSSLKDFRYGVEDFREVDPIFGTME DFENLVAAIHDKGLKLIIDFIPNHTSDKHIWFQ LSRTRTGKYTDYYIWHDCTHENGKTIPPNNW LSVYGNSSWHFDEVRNQCYFHQFMKEQPDL	Cystinuria	Aminoacidopathy

				NFRNPDVQEEIKEILRFWLTKGVDGFSLDAV KFLLEAKHLRDEIQVNKTQIPDTVTQYSELYH DFTTTQVGMHDIVRSFRQTMDQYSTEPGRYR FMGTEAYAESIDRTVMYYGLPFIQEADFPFNN YLSMLDTVSGNSVYEVITSWMENMPEGKWP NWMIGGPDSSRLTSRLGNQYVNVMNMLLFT LPGTPITYYGEEIGMGNIVAANLNESYDINTL RSKSPMQWDNSSNAGFSEASNTWLPTNSDY HTVNVDVQKTQPRSALKLYQDLSLLHANELL LNRGWFCHLRNDSHYVVYTRELDGIDRIFIV VLNFGESTLLNLHNMISGLPAKMRIRLSTNSA DKGSKVDTSGIFLDKGEGLIFEHNTKNLLHRQ TAFRDRCFVSNRACYSSVLNILYTSC [SEQ ID NO:228]		
SLC 7A9	11136	0021488	P82251	MGDTGLRKRREDEKSIQSQEPKTTSLQKELG LISGISIIVGTIIGSGIFVSPKSVLSNTEAVGPCLI IWAACGVLATLGALCFAELGTMITKSGGEYP YLMEAYGPIPAYLFSWASLIVIKPTSFAIICLSF SEYVCAPFYVGCKPPQIVVKCLAAAAILFIST VNSLSVRLGSYVQNIFTAAKLVIVAIIISGLVL LAQGNTKNFDNSFEGAQLSVGAISLAFYNGL WAYDGWNQLNYITEELRNPYRNLPLAIIIGIP LVTACYILMNVSYFTVMTATELLQSQAVAVT FGDRVLYPASWIVPLFVAFSTIGAANGTCFTA GRLIYVAGREGHMLKVLSYISVRRLTPAPAIIF YGIIATIYIIPGDINSLVNYFSFAAWLFYGLTIL GLIVMRFTRKELERPIKVPVVIPVLMTLISVFL VLAPIISKPTWEYLYCVLFILSGLLFYFLFVHY KFGWAQKISKPITMHLQMLMEVVPPEEDPE [SEQ ID NO:229]	Cystinuria	Aminoaci dopathy
MTH FR	4524	0177000	P42898, Q59GJ6, Q81U67	MVNEARGNSSLNPCLEGSASSGSESSKDSSRC STPGLDPERHERLREKMRRRLESGDKWFSLE FFPPRTAEGAVNLISRFDRMAAGGPLYIDVT WHPAGDPGSDKETSSMMIASTAVNYCGLETI LHMTCCRQRLEEITGHLHKAKQLGLKNIMAL RGDPIGDQWEEEEGGFNYAVDLVKHIRSEFG DYFDICVAGYPKGHPEAGSFEADLKHLKEKV SAGADFIITQLFFEADTFFRFVKACTDMGITCP IVPGIFPIQGYHSLRQLVKLSKLEVPQEIKDVIE PIKDNDAAIRNYGIELAVSLCQELLASGLVPG LHFYTLNREMATTEVLKRLGMWTEDPRRPLP WALSAHPKRREEDVRPIFWASRPKSYIYRTQE WDEFPNGRWGNSSSPAFGELKDYYLFYLKSK SPKEELLKMWGEELTSEESVFEVFVLYLSGEP NRNGHKVTCLPWNDEPLAAETSLLKEELLRV NRQGILTINSQPNINGKPSSDPIVGWGPSGGY VFQKAYLEFFTSRETAEALLQVLKKYELRVN YHLVNVKGENITNAPELQPNAVTWGIFPGREI IQPTVVDPVSFMFWKDEAFALWIERWGKLYE EESPSRTIIQYIHDNYFLVNLVDNDFPLDNCL WQVVEDTLELLNRPTQNARETEAP [SEQ ID NO:230]	Homocysti nuria	Aminoaci dopathy
MTR	4548	0116984	Q99707	MSPALQDLSQPEGLKKTLRDEINAILQKRIMV LDGGMGTMIQREKLNEEHFRGQEFKDHARPL KGNNDILSITQPDVIYQIHKEYLLAGADIIETN TFSSTSIAQADYGLEHLAYRMNMCSAGVARK AAEEVTLQTGIKRFVAGALGPTNKTLSVSPSV ERPDYRNITFDELVEAYQEQAKGLLDGGVDI LLIETIFDTANAKAALFALQNLFEEKYAPRPIF ISGTIVDKSGRTLSGQTGEGFVISVSHGEPLCI	Homocysti nuria	Aminoaci dopathy

				GLNCALGAAEMRPFIEIIGKCTTAYVLCYPNA GLPNTFGDYDETPSMMAKHLKDFAMDGLVN IVGGCCGSTPDHIREIAEAVKNCKPRVPPATA FEGHMLLSGLEPFRIGPYTNFVNIGERCNVAG SRKFAKLIMAGNYEEALCVAKVQVEMGAQV LDVNMDDGMLDGPSAMTRFCNLIASEPDIAK VPLCIDSSNFAVIEAGLKCCQGKCIVNSISLKE GEDDFLEKARKIKKYGAAMVVMAFDEEGQA TETDTKIRVCTRAYHLLVKKLGFNPNDIIFDP NILTIGTGMEEHNLYAINFIHATKVIKETLPGA RISGGLSNLSFSFRGMEAIREAMHGVFLYHAI KSGMDMGIVNAGNLPVYDDIHKELLQLCEDL IWNKDPEATEKLLRYAQTQGTGGKKVIQTDE WRNGPVEERLEYALVKGIEKHIIEDTEEARLN QKKYPRPLNIIEGPLMNGMKIVGDLFGAGKM FLPQVIKSARVMKKAVGHLIPFMEKEREETR VLNGTVEEEDPYQGTIVLATVKGDVHDIGKN IVGVVLGCNNFRVIDLGVMTPCDKILKAALD HKADIIGLSGLITPSLDEMIFVAKEMERLAIRIP LLIGGATTSKTHTAVKIAPRYSAPVIHVLDAS KSVVVCSQLLDENLKDEYFEEIMEEYEDIRQD HYESLKERRYLPLSQARKSGFQMDWLSEPHP VKPTFIGTQVFEDYDLQKLVDYIDWKPFFDV WQLRGKYPNRGFPKIFNDKTVGGEARKVYD DAHNMLNTLISQKKLRARGVVGFWPAQSIQD DIHLYAEAAVPQAAEPIATFYGLRQQAEKDS ASTEPYYCLSDFIAPLHSGIRDYLGLFAVACF GVEELSKAYEDDGDDYSSIMVKALGDRLAE AFAEELHERVRRELWAYCGSEQLDVADLRR LRYKGIRPAPGYPSQPDHTEKLTMWRLADIE QSTGIRLTESLAMAPASAVSGLYFSNLKSKYF AVGKISKDQVEDYALRKNISVAEVEKWLGPI LGYDTD [SEQ ID NO:231]		
MTRR	4552	0124275	Q9UBK8	MGAASVRAGARLVEVALCSFTVTCLEVMRR FLLLYATQQGQAKAIAEEICEQAVVHGFSAD LHCISESDKYDLKTETAPLVVVVSTTGTGDPP DTARKFVKEIQNQTLPVDFFAHLRYGLLGLG DSEYTYFCNGGKIIDKRLQELGARHFYDTGH ADDCVGLELVVEPWIAGLWPALRKHFRSSRG QEEISGALPVASPASSRTDLVKSELLHIESQVE LLRFDDSGRKDSEVLKQNAVNSNQSNVVIED FESSLTRSVPPLSQASLNIPGLPPEYLQVHLQE SLGQEESQVSVTSADPVFQVPISKAVQLTTND AIKTTLLVELDISNTDFSYQPGDAFSVICPNSD SEVQSLLQRLQLEDKREHCVLLKIKADTKKK GATLPQHIPAGCSLQFIFTWCLEIRAIPKKAFL RALVDYTSDSAEKRRLQELCSKQGAADYSRF VRDACACLLDLLLAFPSCQPPLSLLLEHLPKL QPRPYSCASSSLFHPGKLHFVFNIVEFLSTATT EVLRKGVCTGWLALLVASVLQPNIHASHEDS GKALAPKISISPRTTNSFHLPDDPSIPIIMVGPG TGIAPFIGFLQHREKLQEQHPDGNFGAMWLF FGCRHKDRDYLFRKELRHFLKHGILTHLKVS FSRDAPVGEEEAPAKYVQDNIQLHGQQVARI LLQENGHIYVCGDAKNMAKDVHDALVQIISK EVGVEKLEAMKTLATLKEEKRYLQDIWS [SEQ ID NO:232]	Homocystinuria	Aminoacidopathy
ATP7B	540	0123191	P35670, A0A024RDX3,	MPEQERQITAREGASRKILSKLSLPTRAWEPA MKKSFAFDNVGYEGGLDGLGPSSQVATSTVR ILGMTCQSCVKSIEDRISNLKGIISMKVSLEQG	Wilson Disease	Metal transport disorder

			B7ZLR4, B7ZLR3, E7ET55	SATVKYVPSVVCLQQVCHQIGDMGFEASIAE GKAASWPSRSLPAQEAVVKLRVEGMTCQSC VSSIEGKVRKLQGVVRVKVSLSNQEAVITYQ PYLIQPEDLRDHVNDMGFEAAIKSKVAPLSLG PIDIERLQSTNPKRPLSSANQNFNNSETLGHQ GSHVVTLQLRIDGMHCKSCVLNIEENIGQLLG VQSIQVSLENKTAQVKYDPSCTSPVALQRAIE ALPPGNFKVSLPDGAEGSGTDHRSSSSHSPGS PPRNQVQGTCSTTLIAIAGMTCASCVHSIEGM ISQLEGVQQISVSLAEGTATVLYNPSVISPEEL RAAIEDMGFEASVVSESCSTNPLGNHSAGNS MVQTTDGTPTSVQEVAPHTGRLPANHAPDIL AKSPQSTRAVAPQKCFLQIKGMTCASCVSNIE RNLQKEAGVLSVLVALMAGKAEIKYDPEVIQ PLEIAQFIQDLGFEAAVMEDYAGSDGNIELTIT GMTCASCVHNIESKLTRTNGITYASVALATSK ALVKFDPEIIGPRDIIKIIEEIGFHASLAQRNPN AHHLDHKMEIKQWKKSFLCSLVFGIPVMAL MIYMLIPSNEPHQSMVLDHNIIPGLSILNLIFFI LCTFVQLLGGWYFYVQAYKSLRHRSANMDV LIVLATSIAYVYSLVILVVAVAEKAERSPVTFF DTPPMLFVFIALGRWLEHLAKSKTSEALAKL MSLQATEATVVTLGEDNLIIREEQVPMELVQ RGDIVKVVPGGKFPVDGKVLEGNTMADESLI TGEAMPVTKKPGSTVIAGSINAHGSVLIKATH VGNDTTLAQIVKLVEEAQMSKAPIQQLADRF SGYFVPFIIIMSTLTLVVWIVIGFIDFGVVQRYF PNPNKHISQTEVIIRFAFQTSITVLCIACPCSLG LATPTAVMVGTGVAAQNGILIKGGKPLEMA HKIKTVMFDKTGTITHGVPRVMRVLLLGDVA TLPLRKVLAVVGTAEASSEHPLGVAVTKYCK EELGTETLGYCTDFQAVPGCGIGCKVSNVEGI LAHSERPLSAPASHLNEAGSLPAEKDAVPQTF SVLIGNREWLRRNGLTISSDVSDAMTDHEMK GQTAILVAIDGVLCGMIAIADAVKQEAALAV HTLQSMGVDVVLITGDNRKTARAIATQVGIN KVFAEVLPSHKVAKVQELQNKGKKVAMVG DGVNDSPALAQADMGVAIGTGTDVAIEAAD VVLIRNDLLDVVASIHLSKRTVRRIRINLVLAL IYNLVGIPIAAGVFMPIGIVLQPWMGSAAMA ASSVSVVLSSLQLKCYKKPDLERYEAQAHGH MKPLTASQVSVHIGMDDRWRDSPRATPWDQ VSYVSQVSLSSLTSDKPSRHSAAADDDGDKW SLLLNGRDEEQYI [SEQ ID NO:233]	Copper Metabolism Disorder	
HPRT1	3251	0165704	P00492, A0A140VJL3	MATRSPGVVISDDEPGYDLDLFCIPNHYAEDL ERVFIPHGLIMDRTERLARDVMKEMGGHHIV ALCVLKGGYKFFADLLDYIKALNRNSDRSIP MTVDFIRLKSYCNDQSTGDIKVIGGDDLSTLT GKNVLIVEDIIDTGKTMQTLLSLVRQYNPKM VKVASLLVKRTPRSVGYKPDFVGFEIPDKFV VGYALDYNEYFRDLNHVCVISETGKAKYKA [SEQ ID NO:234]	Lesch-Nyhan Syndrome Purine Metabolism Disorder	Purine Metabolism Disorder
HJV	148738	0168509	Q6ZVN8	MGEPGQSPSPRSSHGSPPTLSTLTLLLLLCGH AHSQCKILRCNAEYVSSTLSLRGGGSSGALRG GGGGGRGGGVGSGGLCRALRSYALCTRRTA RTCRGDLAFHSAVHGIEDLMIQHNCSRQGPT APPPPRGPALPGAGSGLPAPDPCDYEGRFSRL HGRPPGFLHCASFGDPHVRSFHHHFHTCRVQ GAWPLLDNDFLFVQATSSPMALGANATATR KLTIIFKNMQECIDQKVYQAEVDNLPVAFED	Hemochromatosis, Type 2A	

				GSINGGDRPGGSSLSIQTANPGNHVEIQAAYI GTTIIIRQTAGQLSFSIKVAEDVAMAFSAEQDL QLCVGGCPPSQRLSRSERNRRGAITIDTARRL CKEGLPVEDAYFHSCVFDVLISGDPNFTVAA QAALEDARAFLPDLEKLHLFPSDAGVPLSSAT LLAPLLSGLFVLWLCIQ [SEQ ID NO:235]		
HAMP	57817	0105697	P81172	MALSSQIWAACLLLLLLLASLTSGSVFPQQTG QLAELQPQDRAGARASWMPMFQRRRRRDTH FPICIFCCGCCHRSKCGMCCKT [SEQ ID NO:236]	Hemochromatosis Type 2B: Primary Hemochromatosis	
JAG1	182	0101384	P78504, Q99740	MRSPRTRGRSGRPLSLLLALLCALRAKVCGA SGQFELEILSMQNVNGELQNGNCCGGARNPG DRKCTRDECDTYFKVCLKEYQSRVTAGGPCS FGSGSTPVIGGNTFNLKASRGNDRNRIVLPFSF AWPRSYTLLVEAWDSSNDTVQPDSIIEKASHS GMINPSRQWQTLKQNTGVAHFEYQIRVTCDD YYYGFGCNKFCRPRDDFFGHYACDQNGNKT CMEGWMGPECNRAICRQGCSPKHGSCKLPG DCRCQYGWQGLYCDKCIPHPGCVHGICNEP WQCLCETNWGGQLCDKDLNYCGTHQPCLN GGTCSNTGPDKYQCSCPEGYSGPNCEIAEHA CLSDPCHNRGSCKETSLGFECECSPGWTGPTC STNIDDCSPNNCSHGGTCQDLVNGFKCVCPP QWTGKTCQLDANECEAKPCVNAKSCKNLIA SYYCDCLPGWMGQNCDININDCLGQCQNDA SCRDLVNGYRCICPPGYAGDHCERDIDECAS NPCLNGGHCQNEINRFQCLCPTGFSGNLCQL DIDYCEPNPCQNGAQCYNRASDYFCKCPEDY EGKNCSHLKDHCRTTPCEVIDSCTVAMASND TPEGVRYISSNVCGPHGKCKSQSGGKFTCDC NKGFTGTYCHENINDCESNPCRNGGTCIDGV NSYKCICSDGWEGAYCETNINDCSQNPCHNG GTCRDLVNDFYCDCKNGWKGKTCHSRDSQC DEATCNNGGTCYDEGDAFKCMCPGGWEGTT CNIARNSSCLPNPCHNGGTCVVNGESFTCVC KEGWEGPICAQNTNDCSPHPCYNSGTCVDGD NWYRCECAPGFAGPDCRININECQSSPCAFGA TCVDEINGYRCVCPPGHSGAKCQEVSGRPCIT MGSVIPDGAKWDDDCNTCQCLNGRIACSKV WCGPRPCLLHKGHSECPSGQSCIPILDDQCFV HPCTGVGECRSSSLQPVKTKCTSDSYYQDNC ANITFTFNKEMMSPGLTTEHICSELRNLNILK NVSAEYSIYIACEPSPSANNEIHVAISAEDIRD DGNPIKEITDKIIDLVSKRDGNSSLIAAVAEVR VQRRPLKNRTDFLVPLLSSVLTVAWICCLVT AFYWCLRKRRKPGSHTHSASEDNTTNNVRE QLNQIKNPIEKHGANTVPIKDYENKNSKMSKI RTHNSEVEEDDMDKHQQKARFAKQPAYTLV DREEKPPNGTPTKHPNWTNKQDNRDLESAQS LNRMEYIV [SEQ ID NO:237]		
TTR	7276	0118271	P02766, E9KL36	MASHRLLLLCLAGLVFVSEAGPTGTGESKCP LMVKVLDAVRGSPAINVAVHVFRKAADDTW EPFASGKTSESGELHGLTTEEEFVEGIYKVEID TKSYWKALGISPFHEHAEVVFTANDSGPRRY TIAALLSPYSYSTTAVVTNPKE [SEQ ID NO:238]	Familial TTR Amyloidosis; Familial amyloid polyneuropathy	

AGXT	189	0172482	P21549	MASHKLLVTPPKALLKPLSIPNQLLLGPGPSNLPPRIMAAGGLQMIGSMSKDMYQIMDEIKEGIQYVFQTRNPLTLVISGSGHCALEAALVNVLEPGDSFLVGANGIWGQRAVDIGERIGARVHPMTKDPGGHYTLQEVEEGLAQHKPVLLFLTHGESSTGVLQPLDGFGELCHRYKCLLLVDSVASLGGTPLYMDRQGIDILYSGSQKALNAPPGTSLISFSDKAKKKMYSRKTKPFSFYLDIKWLANFWGCDDQPRMYHHTIPVISLYSLRESLALIAEQGLENSWRQHREAAAYLHGRLQALGLQLFVKDPALRLPTVTTVAVPAGYDWRDIVSYVIDHFDIEIMGGLGPSTGKVLRIGLLGCNATRENVDRVTEALRAALQHCPKKKL [SEQ ID NO:239]	Primary Hyperoxaluria Type I	
LIPA	3988	0107798	P38571 A0A0A0MT32	MKMRFLGLVVCLVLWTLHSEGSGGKLTAVDPETNMNVSEIISYWGFPSEEYLVETEDGYILCLNRIPHGRKNHSDKGPKPVVFLQHGLLADSSNWVTNLANSSLGFILADAGFDVWMGNSRGNTWSRKHKTLSVSQDEFWAFSYDEMAKYDLPASINFILNKTGQEQVYYVGHSQGTTIGFIAFSQIPELAKRIKMFFALGPVASVAFCTSPMAKLGRLPDHLIKDLFGDKEFLPQSAFLKWLGTHVCTHVILKELCGNLCFLLCGFNERNLNMSRVDVYTTHSPAGTSVQNMLHWSQAVKFQKFQAFDWGSSAKNYFHYNQSYPPTYNVKDMLVPTAVWSGGHDWLADVYDVNILLTQITNLVFHESIPEWEHLDFIWGLDAPWRLYNKIINLMRKYQ [SEQ ID NO:240]	Lysosomal Acid Lipase Deficiency	Lyososomal storage disorder
SERPING1	710	0149131	P05155, A0A0S2Z4J1, B2R659, E7EWE5, B3KSP2, G5E9S2	MASRLTLLTLLLLLLAGDRASSNPNATSSSSQDPESLQDRGEGKVATTVISKMLFVEPILEVSSLPTTNSTTNSATKITANTTDEPTTQPTTEPTTQPTIQPTQPTTQLPTDSPTQPTTGSFCPGPVTLCSDLESHSTEAVLGDALVDFSLKLYHAFSAMKKVETNMAFSPFSIASLLTQVLLGAGENTKTNLESILSYPKDFTCVHQALKGFTTKGVTSVSQIFHSPDLAIRDTFVNASRTLYSSSPRVLSNNSDANLELINTWVAKNTNNKISRLLDSLPSDTRLVLLNAIYLSAKWKTTFDPKKTRMEPFHFKNSVIKVPMMNSKKYPVAHFIDQTLKAKVGQLQLSHNLSLVILVPQNLKHRLEDMEQALSPSVFKAIMEKLEMSKFQPTLLTLPRIKVTTSQDMLSIMEKLEFFDFSYDLNLCGLTEDPDLQVSAMQHQTVLELTETGVEAAAASAISVARTLLVFEVQQPFLFVLWDQQHKFPVFMGRVYDPRA [SEQ ID NO:241]	Hereditary Angioedma	
HSD17B4	3295	0133835	P51659	MGSPLRFDGRVVLVTGAGAGLGRAYALAFAERGALVVVNDLGGDFKGVGKGSLAADKVVEEIRRRGGKAVANYDSVEEGEKVVKTALDAFGRIDVVVNNAGILRDRSFARISDEDWDIIHRVHLRGSFQVTRAAWEHMKKQKYGRIIMTSSASGIYGNFGQANYSAAKLGLLGLANSLAIEGRKSNIHCNTIAPNAGSRMTQTVMPEDLVEALKPEYVAPLVLWLCHESCEENGGLFEVGAGWIGKLRWERTLGAIVRQKNHPMTPEAVKANWKKICDFENASKPQSIQESTGSIIEVLSKIDSEGGVSANHTSRATSTATSGFAGAIGQKLPPFSYAYTELEAIMYALGVGASIKDPKDLKFIYEGSSDFSCLPTFGVIIGQKSMMGGGLAEIPGLSINFAKVLHGEQYLELYKPLPRAGKLKCEAVVADVLDKGSGVVIIMDVYSYSEKELICHNQFSLFLVGSGGFGGKRTSDKVKVAVAIPNRPPDAVLTDTTS	D-Bifunctional Protein Deficiency X-linked Adrenoleukodystrophy	Peroxisomal disorders

				LNQAALYRLSGDWNPLHIDPNFASLAGFDKPI LHGLCTFGFSARRVLQQFADNDVSRFKAIKA RFAKPVYPGQTLQTEMWKEGNRIHFQTKVQ ETGDIVISNAYVDLAPTSGTSAKTPSEGGKLQ STFVFEEIGRRLKDIGPEVVKKVNAVFEWHIT KGGNIGAKWTIDLKSGSGKVYQGPAKGAAD TTIILSDEDFMEVVLGKLDPQKAFFSGRLKAR GNIMLSQKLQMILKDYAKL [SEQ ID NO:242]		
URO D	7389	0126088	P06132	MEANGLGPQGFPELKNDTFLRAAWGEETDY TPVWCMRQAGRYLPEFRETRAAQDFFSTCRS PEACCELTLQPLRRFPLDAAIIFSDILVVPQAL GMEVTMVPGKGPSFPEPLREEQDLERLRDPE VVASELGYVFQAITLTRQRLAGRVPLIGFAGA PWTLMTYMVEGGGSSTMAQAKRWLYQRPQ ASHQLLRILTDALVPYLVGQVVAGAQALQLF ESHAGHLGPQLFNKFALPYIRDVAKQVKARL REAGLAPVPMIIFAKDGHFALEELAQAGYEV VGLDWTVAPKKARECVGKTVTLQGNLDPCA LYASEEEIGQLVKQMLDDFGPHRYIANLGHG LYPDMDPEHVGAFVDAVHKHSRLLRQN [SEQ ID NO:243]	Porphyria Cutanea Tarda	
HFE	3077	0010704	Q30201	MGPRARPALLLLMLLQTAVLQGRLLRSHSLH YLFMGASEQDLGLSLFEALGYVDDQLFVFYD HESRRVEPRTPWVSSRISSQMWLQLSQSLKG WDHMFTVDFWTIMENHNHSKESHTLQVILG CEMQEDNSTEGYWKYGYDGQDHLEFCPDTL DWRAAEPRAWPTKLEWERHKIRARQNRAYL ERDCPAQLQQLLELGRGVLDQQVPPLVKVTH HVTSSVTTLRCRALNYYPQNITMKWLKDKQP MDAKEFEPKDVLPNGDGTYQGWITLAVPPGE EQRYTCQVEHPGLDQPLIVIWEPSPSGTLVIG VISGIAVFVVILFIGILFIILRKRQGSRGAMGHY VLAERE [SEQ ID NO:244]	Porphyria Cutanea Tarda	
LPL	4023	0175445	P06858, A0A1B1RVA9	MESKALLVLTLAVWLQSLTASRGGVAAADQ RRDFIDIESKFALRTPEDTAEDTCHLIPGVAES VATCHFNHSSKTFMVIHGWTVTGMYESWVP KLVAALYKREPDSNVIVVDWLSRAQEHYPVS AGYTKLVGQDVARFINWMEEEFNYPLDNVH LLGYSLGAHAAGIAGSLTNKKVNRITGLDPA GPNFEYAEAPSRLSPDDADFVDVLHTFTRGSP GRSIGIQKPVGHVDIYPNGGTFQPGCNIGEAIR VIAERGLGDVDQLVKCSHERSIHLFIDSLLNE ENPSKAYRCSSKEAFEKGLCLSCRKNRCNNL GYEINKVRAKRSSKMYLKTRSQMPYKVFHY QVKIHFSGTESETHTNQAFEISLYGTVAESENI PFTLPEVSTNKTYSFLIYTEVDIGELLMLKLK WKSDSYFSWSDWWSSPGFAIQKIRVKAGETQ KKVIFCSREKVSHLQKGKAPAVFVKCHDKSL NKKSG [SEQ ID NO:245]	Lipoprotein Lipase Deficiency (“hyperlipoproteinemia type Ia; Buerger-Gruetz syndrome, or Familial hyperchylomicronemia)	
GRHPR	9380	0137106	Q9UBQ7	MRPVRLMKVFVTRRIPAEGRVALARAADCE VEQWDSDEPIPAKELERGVAGAHGLLCLLSD HVDKRILDAAGANLKVISTMSVGIDHLALDEI KKRGIRVGYTPDVLTDTTAELAVSLLLTTCRR LPEAIEEVKNGGWTSWKPLWLCGYGLTQST VGIIGLGRIGQAIARRLKPFGVQRFLYTGRQP RPEEAAEFQAEFVSTPELAAQSDFIVVACSLT PATEGLCNKDFFQKMKETAVFINISRGDVVN QDDLYQALASGKIAAAGLDVTSPEPLPTNHP LLTLKNCVILPHIGSATHRTRNTMSLLAANNL LAGLRGEPMPSELKL [SEQ ID NO:246]	Primary Hyperoxaluria Type II	

HOGA1	112817	0241935	Q86XE5	MLGPQVWSSVRQGLSRSLSRNVGVWASGEGKKVDIAGIYPPVTTPFTATAEVDYGKLEENLHKLGTFPFRGFVVQGSNGEFPFLTSSERLEVVSRVRQAMPKNRLLLAGSGCESTQATVEMTVSMAQVGADAAMVVTPCYYRGRMSSAALIHHYTKVADLSPIPVVLYSVPANTGLDLPVDAVVTLSQHPNIVGMKDSGGDVTRIGLIVHKTRKQDFQVLAGSAGFLMASYALGAVGGVCALANVLGAQVCQLERLCCTGQWEDAQKLQHRLIEPNAAVTRRFGIPGLKKIMDWFGYYGGPCRAPLQELSPAEEEALRMDFTSNGWL [SEQ ID NO:247]	Primary Hyperoxaluria Type III	
LDLR	3949	0130164	P01130, A0A024R7D5	MGPWGWKLRWTVALLLAAAGTAVGDRCERNEFQCQDGKCISYKWVCDGSAECQDGSDESQETCLSVTCKSGDFSCGGRVNRCIPQFWRCDGQVDCDNGSDEQGCPPKTCSQDEFRCHDGKCISRQFVCDSDRDCLDGSDEASCPVLTCGPASFQCNSSTCIPQLWACDNDPDCEDGSDEWPQRCRGLYVFQGDSSPCSAFEFHCLSGECIHSSWRCDGGPDCKDKSDEENCAVATCRPDEFQCSDGNCIHGSRQCDREYDCKDMSDEVGCVNVTLCEGPNKFKCHSGECITLDKVCNMARDCRDWSDEPIKECGTNECLDNNGGCSHVCNDLKIGYECLCPDGFQLVAQRRCEDIDECQDPDTCSQLCVNLEGGYKCQCEEGFQLDPHTKACKAVGSIAYLFFTNRHEVRKMTLDRSEYTSLIPNLRNVVALDTEVASNRIYWSDLSQRMICSTQLDRAHGVSSYDTVISRDIQAPDGLAVDWIHSNIYWTDSVLGTVSVADTKGVKRKTLFRENGSKPRAIVVDPVHGFMYWTDWGTPAKIKKGGLNGVDIYSLVTENIQWPNGITLDLLSGRLYWVDSKLHSISSIDVNGGNRKTILEDEKRLAHPFSLAVFEDKVFWTDIINEAIFSANRLTGSDVNLLAENLLSPEDMVLFHNLTQPRGVNWCERTTLSNGGCQYLCLPAPQINPHSPKFTCACPDGMLLARDMRSCLTEAEAAVATQETSTVRLKVSSTAVRTQHTTTRPVPDTSRLPGATPGLTTVEIVTMSHQALGDVAGRGNEKKPSSVRALSIVLPIVLLVFLCLGVFLLWKNWRLKNINSINFDNPVYQKTTEDEVHICHNQDGYSYPSRQMVSLEDDVA [SEQ ID NO:248]		
ACAD8	27034	0151498	Q9UKU7	MLWSGCRRFGARLGCLPGGLRVLVQTGHRSLTSCIDPSMGLNEEQKEFQKVAFDFAAREMAPNMAEWDQKELFPVDVMRKAAQLGFGGVY IQTDVGGSGLSRLDTSVIFEALATGCTSTTAYISIHNMCAWMIDSFGNEEQRHKFCPPLCTMEKFASYCLTEPGSGSDAASLLTSAKKQGDHYILNGSKAFISGAGESDIYVVMCRTGGPGPKGISCIVVEKGTPGLSFGKKEKKVGWNSQPTRAVIFEDCAVPVANRIGSEGQGFLIAVRGLNGGRINIASCSLGAAHASVILTRDHLNVRKQFGEPLASNQYLQFTLADMATRLVAARLMVRNAAVALQEERKDAVALCSMAKLFATDECFAICNQALQMHGGYGYLKDYAVQQYVRDSRVHQILEGSNEVMRILISRSLLQE [SEQ ID NO:249]	isobutyryl-CoA dehydrogenase (IBD) deficiency	Organic acidemia
ACADSB	36	0196177	P45954, A0A0S2Z3P9	MEGLAVRLLRGSRLLRRNFLTCLSSWKIPPHVSKSSQSEALLNITNNGIHFAPLQTFTDEEMMIKSSVKKFAQEQIAPLVSTMDENSKMEKSVIQGLFQQGLMGIEVDPEYGGTGASFLSTVLVIEE	short-branched chain acyl-CoA	Organic acidemia

				LAKVDASVAVFCEIQNTLINTLIRKHGTEEQK ATYLPQLTTEKVGSFCLSEAGAGSDSFALKTR ADKEGDYYVLNGSKMWISSAEHAGLFLVMA NVDPTIGYKGITSFLVDRDTPGLHIGKPENKL GLRASSTCPLTFENVKVPEANILGQIGHGYKY AIGSLNEGRIGIAAQMLGLAQGCFDYTIPYIKE RIQFGKRLFDFQGLQHQVAHVATQLEAARLL TYNAARLLEAGKPFIKEASMAKYYASEIAGQ TTSKCIEWMGGVGYTKDYPVEKYFRDAKIGT IYEGASNIQLNTIAKHIDAEY [SEQ ID NO:250]	dehydrogenase (SBCAD) deficiency	
ACAT1	38	0075239	A0A140VJX1, P24752	MAVLAALLRSGARSRSPLLRRLVQEIRYVERS YVSKPTLKEVVIVSATRTPIGSFLGSLSLLPAT KLGSIAIQGAIEKAGIPKEEVKEAYMGNVLQG GEGQAPTRQAVLGAGLPISTPCTTINKVCASG MKAIMMASQSLMCGHQDVMVAGGMESMSN VPYVMNRGSTPYGGVKLEDLIVKDGLTDVY NKIHMGSCAENTAKKLNIARNEQDAYAINSY TRSKAAWEAGKFGNEVIPVTVTVKGQPDVV VKEDEEYKRVDFSKVPKLKTVFQKENGTVTA ANASTLNDGAAALVLMTADAAKRLNVTPLA RIVAFADAAVEPIDFPIAPVYAASMVLKDVGL KKEDIAMWEVNEAFSLVVLANIKMLEIDPQK VNINGGAVSLGHPIGMSGARIVGHLTHALKQ GEYGLASICNGGGGASAMLIQKL [SEQ ID NO:251]	beta-ketothiolase deficiency	Organic acidemia
ACSF3	197322	0176715	Q4G176, F5H5A1	MLPHVVLTFRRLGCALASCRLAPARHRGSGL LHTAPVARSDRSAPVFTRALAFGDRIALDQH GRHTYRELYSRSLRLSQEICRLCGCVGGDLRE ERVSFLCANDASYVVAQWASWMSGGVAVP LYRKHPAAQLEYVICDSQSSVVLASQEYLELL SPVVRKLGVPLLPLTPAIYTGAVEEPAEVPVP EQGWRNKGAMIIYTSGTTGRPKGVLSTHQNI RAVVTGLVHKWAWTKDDVILHVLPLHHVH GVVNALLCPLWVGATCVMMPEFSPQQVWE KFLSSETPRINVFMAVPTIYTKLMEYYDRHFT QPHAQDFLRAVCEEKIRLMVSGSAALPLPVL EKWKNITGHTLLERYGMTEIGMALSGPLTTA VRLPGSVGTPLPGVQVRIVSENPQREACSYTI HAEGDERGTKVTPGFEEKEGELLVRGPSVFR EYWNKPEETKSAFTLDGWFKTGDTVVFKDG QYWIRGRTSVDIIKTGGYKVSALEVEWHLLA HPSITDVAVIGVPDMTWGQRVTAVVTLREGH SLSHRELKEWARNVLAPYAVPSELVLVEEIPR NQMGKIDKKALIRHFHPS [SEQ ID NO:252]	combined malonic and methylmalonic aciduria	Organic acidemia
ASPA	443	0108381	P45381, Q6FH48	MTSCHIAEEHIQKVAIFGGTHGNELTGVFLVK HWLENGAEIQRTGLEVKPFITNPRAVKKCTR YIDCDLNRIFDLENLGKKMSEDLPYEVRRAQ EINHLFGPKDSEDSYDIIFDLHNTTSNMGCTLI LEDSRNNFLIQMFHYIKTSLAPLPCYVYLIEHP SLKYATTRSIAKYPVGIEVGPQPQGVLRADIL DQMRKMIKHALDFIHHFNEGKEFPPCAIEVY KIIEKVDYPRDENGEIA AIIHPNLQDQDWKPLHPGDPMFLTLDGKTIPL GGDCTVYPVFVNEAAYYEKKEAFAKTTKLT LNAKSIRCCLH [SEQ ID NO:253]	Canavan disease	Organic acidemia

AUH	549	0148090	Q13825, B4DYI6	MAAAVAAAPGALGSLHAGGARLVAACSAW LCPGLRLPGSLAGRRAGPAIWAQGWVPAAG GPAPKRGYSSEMKTEDELRVRHLEEENRGIV VLGINRAYGKNSLSKNLIKMLSKAVDALKSD KKVRTIIIRSEVPGIFCAGADLKERAKMSSSEV GPFVSKIRAVINDIANLPVPTIAAIDGLALGGG LELALACDIRVAASSAKMGLVETKLAIIPGGG GTQRLPRAIGMSLAKELIFSARVLDGKEAKA VGLISHVLEQNQEGDAAYRKALDLAREFLPQ GPVAMRVAKLAINQGMEVDLVTGLAIEEAC YAQTIPTKDRLEGLLAFKEKRPPRYKGE [SEQ ID NO:254]	3-methylglutaconic acidemia type I	Organic acidemia
DNAJC19	131118	0205981	Q96DA6, A0A0S2Z5X1	MASTVVAVGLTIAAAGFAGRYVLQAMKHM EPQVKQVFQSLPKSAFSGGYYRGGFEPKMTK REAALILGVSPTANKGKIRDAHRRIMLLNHPD KGGSPYIAAKINEAKDLLEGQAKK [SEQ ID NO:255]	dilated cardiomyopathy with ataxia syndrome (causes 3-methylglutaconic aciduria)	Organic acidemia
ETHE1	23474	0105755	A0A0S2Z580, O95571, A0A0S2Z5N8, A0A0S2Z5B3, B2RCZ7	MAEAVLRVARRQLSQRGGSGAPILLRQMFEP VSCTFTYLLGDRESREAVLIDPVLETAPRDAQ LIKELGLRLLYAVNTHCHADHITGSGLLRSLL PGCQSVISRLSGAQADLHIEDGDSIRFGRFALE TRASPGHTPGCVTFVLNDHSMAFTGDALLIR GCGRTDFQQGCAKTLYHSVHEKIFTLPGDCLI YPAHDYHGFTVSTVEEERTLNPRLTLSCEEFV KIMGNLNLPKPQQIDFAVPANMRCGVQTPTA [SEQ ID NO:256]	ethylmalonic encephalopathy	Organic acidemia
FBP1	2203	0165140	P09467, Q2TU34	MADQAPFDTDVNTLTRFVMEEGRKARGTGE LTQLLNSLCTAVKAISSAVRKAGIAHLYGIAG STNVTGDQVKKLDVLSNDLVMNMLKSSFAT CVLVSEEDKHAIIVEPEKRGKYVVCFDPLDGS SNIDCLVSVGTIFGIYRKKSTDEPSEKDALQPG RNLVAAGYALYGSATMLVLAMDCGVNCFM LDPAIGEFILVDKDVKIKKKGKIYSLNEGYAR DFDPAVTEYIQRKKFPPDNSAPYGARYVGSM VADVHRTLVYGGIFLYPANKKSPNGKLRLLY ECNPMAYVMEKAGGMATTGKEAVLDVIPTD IHQRAPVILGSPDDVLEFLKVYEKHSAQ[SEQ ID NO:257]	fructose 1,6-Bisphosphatase deficiency	Organic acidemia
FTCD	10841	0160282,0281775	O95954	MSQLVECVPNFSEGKNQEVIDAISGAITQTPG CVLLDVDAGPSTNRTVYTFVGPPECVVEGAL NAARVASRLIDMSRHQGEHPRMGALDVCPFI PVRGVSVDECVLCAQAFGQRLAEELDVPVYL YGEAARMDSRRTLPAIRAGEYEALPKKLQQA DWAPDFGPSSFVPSWGATATGARKFLIAFNIN LLGTKEQAHRIALNLREQGRGKDQPGRLKKV QGIGWYLDEKNLAQVSTNLLDFEVTALHTVY EETCREAQELSLPVVGSQLVGLVPLKALLDA AAFYCEKENLFILEEEQRI RLVVSRLGLDSLCPFSPKERIIEYLVPERGPER GLGSKSLRAFVGEVGARSAAPGGGSVAAAA AAMGAALGSMVGLMTYGRRQFQSLDTTMR RLIPPFREASAKLTTLVDADAEAFTAYLEAMR LPKNTPEEKDRRTAALQEGLRRAVSVPLTLA ETVASLWPALQELARCGNLACRSDLQVAAK	glutamate formiminotransferase deficiency (FIGLU	Organic acidemia

				ALEMGVFGAYFNVLINLRDITDEAFKDQIHH RVSSLLQEAKTQAALVLDCLETRQE [SEQ ID NO:258]		
GSS	2937	0100983	P48637, V9HWJ1	MATNWGSLLQDKQQLEELARQAVDRALAEG VLLRTSQEPTSSEVVSYAPFTLFPSLVPSALLE QAYAVQMDFNLLVDAVSQNAAFLEQTLSSTI KQDDFTARLFDIHKQVLKEGIAQTVFLGLNRS DYMFQRSADGSPALKQIEINTISASFGGLASR TPAVHRHVLSVLSKTKEAGKILSNNPSKGLAL GIAKAWELYGSPNALVLLIAQEKERNIFDQRA IENELLARNIHVIRRTFEDISEKGSLDQDRRLF VDGQEIAVVYFRDGYMPRQYSLQNWEARLL LERSHAAKCPDIATQLAGTKKVQQELSRPGM LEMLLPGQPEAVARLRATFAGLYSLDVGEEG DQAIAEALAAPSRFVLKPQREGGGNNLYGEE MVQALKQLKDSEERASYILMEKIEPEPFENCL LRPGSPARVVQCISELGIFGVYVRQEKTLVMN KHVGHLLRTKAIEHADGGVAAGVAVLDNPY PV [SEQ ID NO:259]	glutathione synthetase deficiency	Organic acidemia
HIB CH	26275	0198130	A0A140V JL0, Q6NVY1	MGQREMWRLMSRFNAFKRTNTILHHLRMSK HTDAAEEVLLEKKGCTGVITLNRPKFLNALT LNMIRQIYPQLKKWEQDPETFLIIIKGAGGKA FCAGGDIRVISEAEKAKQKIAPVFFREEYMLN NAVGSCQKPYVALIHGITMGGGVGLSVHGQF RVATEKCLFAMPETAIGLFPDVGGGYFLPRL QGKLGYFLALTGFRLKGRDVYRAGIATHFVD SEKLAMLEEDLLALKSPSKENIASVLENYHTE SKIDRDKSFILEEHMDKINSCFSANTVEEIIENL QQDGSSFALEQLKVINKMSPTSLKITLRQLME GSSKTLQEVLTMEYRLSQACMRGHDFHEGV RAVLIDKDQSPKWKPADLKEVTEEDLNNHFK SLGSSDLKF [SEQ ID NO:260]	3-hyroxyisobutyryl-CoA hydrolase deficiency	Organic acidemia
IDH 2	3418	0182054	P48735, B4DSZ6	MAGYLRVVRSLCRASGSRPAWAPAALTAPTS QEQPRRHYADKRIKVAKPVVEMDGDEMTRII WQFIKEKLILPHVDIQLKYFDLGLPNRDQTDD QVTIDSALATQKYSVAVKCATITPDEARVEEF KLKKMWKSPNGTIRNILGGTVFREPIICKNIPR LVPGWTKPITIGRHAHGDQYKATDFVADRAG TFKMVFTPKDGSGVKEWEVYNFPAGGVGMG MYNTDESISGFAHSCFQYAIQKKWPLYMSTK NTILKAYDGRFKDIFQEIFDKHYKTDFDKNKI WYEHRLIDDMVAQVLKSSGGFVWACKNYD GDVQSDILAQGFGSLGLMTSVLVCPDGKTIE AEAAHGTVTRHYREHQKGRPTSTNPIASIFA WTRGLEHRGKLDGNQDLIRFAQMLEKVCVE TVESGAMTKDLAGCIHGLSNVKLNEHFLNTT DFLDTIKSNLDRALGRQ [SEQ ID NO:261]	D-2-hydroxyglutaric aciduria type II	Organic acidemia
L2H GDH	79944	0087299	Q9H9P8	MVPALRYLVGACGRARGLFAGGSPGACGFA SGRPRPLCGGSRSASTSSFDIVIVGGGIVGLAS ARALILRHPSLSIGVLEKEKDLAVHQTGHNSG VIHSGIYYKPESLKAKLCVQGAALLYEYCQQ KGISYKQCGKLIVAVEQEEIPRLQALYEKGLQ NGVPGLRLIQQEDIKKKEPYCRGLMAIDCPHT GIVDYRQVALSFAQDFQEAGGSVLTNFEVKG IEMAKESPSRSIDGMQYPIVIKNTKGEEIRCQY VVTCAGLYSDRISELSGCTPDPRIVPFRGDYL	L-2-hydroxyglutaric aciduria	Organic acidemia

				LLKPEKCYLVKGNIYPVPDSRFPFLGVHFTPR MDGSIWLGPNAVLAFKREGYRPFDFSATDV MDIIINSGLIKLASQNFSYGVTEMYKACFLGA TVKYLQKFIPEITISDILRGPAGVRAQALDRDG NLVEDFVFDAGVGDIGNRILHVRNAPSPAATS SIAISGMIADEVQQRFEL [SEQ ID NO:262]		
MLY CD	23417	0103150	O95822	MRGFGPGLTARRLLPLRLPPRPPGPRLASGQA AGALERAMDELLRRAVPPTPAYELREKTPAP AEGQCADFVSFYGGLAETAQRAELLGRLARG FGVDHGQVAEQSAGVLHLRQQQREAAVLLQ AEDRLRYALVPRYRGLFHHISKLDGGVRFLV QLRADLLEAQALKLVEGPDVREMNGVLKGM LSEWFSSGFLNLERVTWHSPCEVLQKISEAEA VHPVKNWMDMKRRVGPYRRCYFFSHCSTPG EPLVVLHVALTGDISSNIQAIVKEHPPSETEEK NKITAAIFYSISLTQQGLQG VELGTFLIKRVVKELQREFPHLGVFSSLSPIPG FTKWLLGLLNSQTKEHGRNELFTDSECKEISE ITGGPINETLKLLLSSSEWVQSEKLVRALQTPL MRLCAWYLYGEKHRGYALNPVANFHLQNG AVLWRINWMADVSLRGITGSCGLMANYRYF LEETGPNSTSYLGSKIIKASEQVLSLVAQFQK NSKL [SEQ ID NO:263]	malonic acidemia	Organic acidemia
OPA 3	80207	0125741	Q9H6K4, B4DK77	MVVGAFPMAKLLYLGIRQVSKPLANRIKEAA RRSEFFKTYICLPPAQLYHWVEMRTKMRIMG FRGTVIKPLNEEAAAELGAELLGEATIFIVGG GCLVLEYWRHQAQQRHKEEEQRAAWNALR DEVGHLALALEALQAQVQAAPPQGALEELRT ELQEVRAQLCNPGRSASHAVPASKK [SEQ ID NO:264]	Costeff syndrome / 3-methylglutaconic aciduria type III	Organic acidemia
OPL AH	26873	0178814	O14841	MGSPEGRFHFAIDRGGTFTDVFAQCPGGHVR VLKLLSEDPANYADAPTEGIRRILEQEAGMLL PRDQPLDSSHIASIRMGTTVATNALLERKGER VALLVTRGFRDLLHIGTQARGDLFDLAVPMP EVLYEEVLEVDERVVLHRGEAGTGTPVKGRT GDLLEVQQPVDLGALRGKLEGLLSRGIRSLA VVLMHSYTWAQHEQQVGVLARELGFTHVSL SSEAMPMVRIVPRGHTACADAYLTPAIQRYV QGFCRGFQGQLKDVQVLFMRSDGGLAPMDT FSGSSAVLSGPAGGVVGYSATTYQQEGGQPV IGFDMGGTSTDVSRYAGEFEHVFEASTAGVT LQAPQLDINTVAAGGGSRLFFRSGLFVVGPES AGAHPGPACYRKGGPVTVTDANLVLGRLLP ASFPCIFGPGENQPLSPEASRKALEAVATEVN SFLTNGPCPASPLSLEEVAMGFVRVANEAMC RPIRALTQARGHDPSAHVLACFGGAGGQHAC AIARALGMDTVHIHRHSGLLSALGLALADVV HEAQEPCSLLYAPETFVQLDQRLSRLEEQCV DALQAQGFPRSQISTESFLHLRYQGTDCALM VSAHQHPATA RSPRAGDFGAAFVERYMREFGFVIPERPVVV DDVRVRGTGRSGLRLEDAPKAQTGPPRVDK MTQCYFEGGYQETPVYLLAELGYGHKLHGP CLIIDSNSTILVEPGCQAEVTKTGDICISVGAE VPGTVGPQLDPIQLSIFSHRFMSIAEQMGRILQ RTAISTNIKERLDFSCALFGPDGGLVSNAPHIP VHLGAMQETVQFQIQHLGADLHPGDVLLSN HPSAGGSHLPDLTVITPVFWPGQTRPVFYVAS	5-oxoprolinase deficiency	Organic acidemia

				RGHHADIGGITPGSMPPHSTMLQQEGAVFLSF KLVQGGVFQEEAVTEALRAPGKVPNCSGTRN LHDNLSDLRAQVAANQKGIQLVGELIGQYGL DVVQAYMGHIQANAELAVRDMLRAFGTSRQ ARGLPLEVSSEDHMDDGSPIRLRVQISLSQGS AVFDFSGTGPEVFGNLNAPRAVTLSALIYCLR CLVGRDIPLNQGCLAPVRVVIPRGSILDPSPEA AVVGGNVLTSQRVVDVILGAFGACAASQGC MNNVTLGNAHMGYYETVAGGAGAGPSWHG RSGVHSHMTNTRITDPEILESRYPVILRRFELR RGSGGRGRFRGGDGVTRELLFREEALLSVLT ERRAFRPYGLHGGEPGARGLNLLIRKNGRTV NLGGKTSVTVYPGDVFCLHTPGGGGYGDPE DPAPPPGSPPQALAFPEHGSVYEYRRAQEAV [SEQ ID NO:265]		
OXC T1	5019	0083720	A0A024R 040, P55809	MAALKLLSSGLRLCASARGSGATWYKGCVC SFSTSAHRHTKFYTDPVEAVKDIPDGATVLV GGFGLCGIPENLIDALLKTGVKGLTAVSNNA GVDNFGLGLLLRSKQIKRMVSSYVGENAEFE RQYLSGELEVELTPQGTLAERIRAGGAGVPAF YTPTGYGTLVQEGGSPIKYNKDGSVAIASKPR EVREFNGQHFILEEAITGDFALVKAWKADRA GNVIFRKSARNFNLPMCKAAETTVVEVEEIV DIGAFAPEDIHIPQIYVHRLIKGEKYEKRIERLS IRKEGDGEAKSAKPGDDVRERIIKRAALEFED GMYANLGIGIPLLASNFISPNITVHLQSENGVL GLGPYPRQHEADADLINAGKETVTILPGASFF SSDESFAMIRGGHVDLTMLGAMQVSKYGDL ANWMIPGKMVKGMGGAMDLVSSAKTKVVV TMEHSAKGNAHKIMEKCTLPLTGKQCVNRII TEKAVFDVDKKKGLTLIELWEGLTVDDVQKS TGCDFAVSPKLMPMQQIAN [SEQ ID NO:266]	SCOT defi ciency	Organic acidemia
POL G	5428	0140521	E5KNU5, P54098	MSRLLWRKVAGATVGPGPVPAPGRWVSSSV PASDPSDGQRRRQQQQQQQQQQQQPQQPQ VLSSEGGQLRHNPLDIQMLSRGLHEQIFGQGG EMPGEAAVRRSVEHLQKHGLWGQPAVPLPD VELRLPPLYGDNLDQHFRLLAQKQSLPYLEA ANLLLQAQLPPKPPAWAWAEGWTRYGPEGE AVPVAIPEERALVFDVEVCLAEGTCPTLAVAI SPSAWYSWCSQRLVEERYSWTSQLSPADLIPL EVPTGASSPTQRDWQEQLVVGHNVSFDRAHI REQYLIQGSRMRFLDTMSMHMAISGLSSFQR SLWIAAKQGKHKVQPPTKQGQKSQRKARRG PAISSWDWLDISSVNSLAEVHRLYVGGPPLEK EPRELFVKGTMKDIRENFQDLMQYCAQDVW ATHEVFQQQLPLFLERCPHPVTLAGMLEMGV SYLPVNQNWERYLAEAQGTYEELQREMKKS LMDLANDACQLLSGERYKEDPWLWDLEWD LQEFKQKKAKKVKKEPATASKLPIEGAGAPG DPMDQEDLGPCSEEEEFQQDVMARACLQKL KGTTELLPKRPQHLPGHPGWYRKLCPRLDDP AWTPGPSLLSLQMRVTPKLMALTWDGFPLH YSERHGWGYLVPGRRDNLAKLPTGTTLESAG VVCPYRAIESLYRKHCLEQGKQQLMPQEAGL AEEFLLTDNSAIWQTVEELDYLEVEAEAKME NLRAAVPGQPLALTARGGPKDTQPSYHHGN GPYNDVDIPGCWFFKLPHKDGNSCNVGSPFA KDFLPKMEDGTLQAGPGGASGPRALEINKMI SFWRNAHKRISSQMVVWLPRSALPRAVIRHP	3-methylgluta conic aciduria	Organic acidemia

				DYDEEGLYGAILPQVVTAGTITRRAVEPTWL TASNARPDRVGSELKAMVQAPPGYTLVGAD VDSQELWIAAVLGDAHFAGMHGCTAFGWM TLQGRKSRGTDLHSKTATTVGISREHAKIFNY GRIYGAGQPFAERLLMQFNHRLTQQEAAEKA QQMYAATKGLRWYRLSDEGEWLVRELNLPV DRTEGGWISLQDLRKVQRETARKSQWKKWE VVAERAWKGGTESEMFNKLESIATSDIPRTPV LGCCISRALEPSAVQEEFMTSRVNWVVQSSA VDYLHLMLVAMKWLFEEFAIDGRFCISIHDE VRYLVREEDRYRAALALQITNLLTRCMFAYK LGLNDLPQSVAFFSAVDIDRCLRKEVTMDCK TPSNPTGMERRYGIPQGEALDIYQIIELTKGSL EKRSQPGP [SEQ ID NO:267]		
PPM 1K	152926	0163644	Q8N3J5	MSTAALITLVRSGGNQVRRRVLLSSRLLQDD RRVTPTCHSSTSEPRCSRFDPDGSGSPATWDN FGIWDNRIDEPILLPPSIKYGKPIPKISLENVGC ASQIGKRKENEDRFDFAQLTDEVLYFAVYDG HGGPAAADFCHTHMEKCIMDLLPKEKNLETL LTLAFLEIDKAFSSHARLSADATLLTSGTTAT VALLRDGIELVVASVGDSRAILCRKGKPMKL TIDHTPERKDEKERIKKCGGFVAWNSLGQPH VNGRLAMTRSIGDLDLKTSGVIAEPETKRIKL HHADDSFLVLTTDGINFMVNSQEICDFVNQC HDPNEAAHAVTEQAIQYGTEDNSTAVVVPFG AWGKYKNSEINFSFSRSFASSGRWA [SEQ ID NO:268]	maple syrup urine disease (MSUD), variant type	Organic acidemia
SER AC1	84947	0122335	Q96JX3	MSLAAYCVICCRRIGTSTSPPKSGTHWRDIRN IIKFTGSLILGGSLFLTYEVLALKKAVTLDTQV VEREKMKSYIYVHTVSLDKGENHGIAWQAR KELHKAVRKVLATSAKILRNPFADPFSTVDIE DHECAVWLLLRKSKSDDKTTRLEAVREMSE THHWHDYQYRIIAQACDPKTLIGLARSEESDL RFFLLPPPLPSLKEDSSTEEELRQLLASLPQTE LDECIQYFTSLALSESSQ SLAAQKGGLWCFGGNGLPYAESFGEVPSATV EMFCLEAIVKHSEISTHCDKIEANGGLQLLQR LYRLHKDCPKVQRNIMRVIGNMALNEHLHSS IVRSGWVSIMAEAMKSPHIMESSHAARILANL DRETVQEKYQDGVYVLHPQYRTSQPIKADVL FIHGLMGAAFKTWRQQDSEQAVIEKPMEDED RYTTCWPKTWLAKDCPALRIISVEYDTSLSD WRARCPMERKSIAFRSNELLRKLRAAGVGDR PVVWISHSMGGLLVKKMLLEASTKPEMSTVI NNTRGIIFYSVPHHGSRLAEYSVNIRYLLFPSL EVKELSKDSPALKTLQDDFLEFAKDKNFQVL NFVETLPTYIGSMIKLHVVPVESADLGIGDLIP VDVNHLNICKPKKKDAFLYQRTLQFIREALA KDLEN [SEQ ID NO:269]	Megdel Syndrome	Organic acidemia
SLC 25A1	6576	0100075	D9HTE9, B4DP62, P53007	MPAPRAPRALAAAAPASGKAKLTHPGKAILA GGLAGGIEICITFPTEYVKTQLQLDERSHPPRY RGIGDCVRQTVRSHGVLGLYRGLSSLLYGSIP KAAVRFGMFEFLSNHMRDAQGRLDSTRGLL CGLGAGVAEAVVVVCPMETIKVKFIHDQTSP NPKYRGFFHGVREIVREQGLKGTYQGLTATV LKQGSNQAIRFFVMTSLRNWYRGDNPNKPM NPLITGVFGAIAGAASVFGNTPLDVIKTRMQG LEAHKYRNTWDCGLQILKKEGLKAFYKGTV PRLGRVCLDVAIVFVIYDEV VKLLNKVWKTD [SEQ ID NO:270]	D,L-2-hydroxyglutaric aciduria	Organic acidemia

SUCLA2	8803	0136143	E5KS60, Q9P2R7, Q9Y4T0	MAASMFYGRLVAVATLRNHRPRTAQRAAAQVLGSSGLFNNHGLQVQQQQQRNLSLHEYMSMELLQEAGVSVPKGYVAKSPDEAYAIAKKLGSKDVVIKAQVLAGGRGKGTFESGLKGGVKIVFSPEEAKAVSSQMIGKKLFTKQTGEKGRICNQVLVCERKYPRREYYFAITMERSFQGPVLIGSSHGGVNIEDVAAESPEAIIKEPIDIEEGIKKEQALQLAQKMGFPPNIVESAAENMVKLYSLFLKYDATMIEINPMVEDSDGAVLCMDAKINFDSNSAYRQKKIFDLQDWTQEDERDKDAAKANLNYIGLDGNIGCLVNGAGLAMATMDIIKLHGGTPANFLDVGGGATVHQVTEAFKLITSDKKVLAILVNIFGGIMRCDVIAQGIVMAVKDLEIKIPVVVRLQGTRVDDAKALIADSGLKILACDDLDEAARMVVKLSEIVTLAKQAHVDVKFQLPI [SEQ ID NO:271]	succinate-CoA ligase deficiency, methylmalonic aciduria	Organic acidemia
SUCLG1	8802	0163541	P53597	MTATLAAAADIATMVSGSSGLAAARLLSRSFLLPQNGIRHCSYTASRQHLYVDKNTKIICQGFTGKQGTFHSQQALEYGTKLVGGTTPGKGGQTHLGLPVFNTVKEAKEQTGATASVIYVPPPFAAAAINEAIEAEIPLVVCITEGIPQQDMVRVKHKLLRQEKTRLIGPNCPGVINPGECKIGIMPGHIHKKGRIGIVSRSGTLTYEAVHQTTQVGLGQSLCVGIGGDPFNGTDFIDCLEIFLNDSATEGIILIGEIGGNAEENAAEFLKQHNSGPNSKPVVSFIAGLTAPPGRRMGHAGAIIAGGKGGAKEKISALQSAGVVVSMSPAQLGTTIYKEFEKRKML [SEQ ID NO:272]	succinate-CoA ligase deficiency, methylmalonic aciduria	Organic acidemia
TAZ	6901	0102125	A0A0S2Z4K0, Q16635, A6XNE1, A0A0S2Z4E6, A0A0S2Z4K9, A0A0S2Z4F4	MPLHVKWPFPAVPPLTWTLASSVVMGLVGTYSCFWTKYMNHLTVHNREVLYELIEKRGPATPLITVSNHQSCMDDPHLWGILKLRHIWNLKLMRWTPAAADICFTKELHSHFFSLGKCVPVCRGAEFFQAENEGKGVLDTGRHMPGAGKRREKGDGVYQKGMDFILEKLNHGDWVHIFPEGKVNMSSEFLRFKWGIGRLIAECHLNPIILPLWHVGMNDVLPNSPPYFPRFGQKITVLIGKPFSALPVLERLRAENKSAVEMRKALTDFIQEEFQHLKTQAEQLHNHLQPGR [SEQ ID NO:273]	Barth syndrome	Organic acidemia
AGK	55750	0006530,0262327	A4D1U5, Q53H12	MTVFFKTLRNHWKKTTAGLCLLTWGGHWLYGKHCDNLLRRAACQEAQVFGNQLIPPNAQVKKATVFLNPAACKGKARTLFEKNAAPILHLSGMDVTIVKTDYEGQAKKLLELMENTDVIIVAGGDGTLQEVVTGVLRRTDEATFSKIPIGFIPLGETSSLSHTLFAESGNKVQHITDATLAIVKGETVPLDVLQIKGEKEQPVFAMTGLRWGSFRDAGVKVSKYWYLGPLKIKAAHFFSTLKEWPQTHQASISYTGPTERPPNEPEETPVQRPSLYRRILRRLASYWAQPQDALSQEVSPEVWKDVQLSTIELSITTRNNQLDPTSKEDFLNICIEPDTISKGDFITIGSRKVRNPKLHVEGTECLQASQCTLLIPEGAGGSFSIDSEEYEAMPVEVKLLPRKLQFFCDPRKREQMLTSPTQ [SEQ ID NO:274]	3-methylglutaconic aciduria	Organic acidemia
CLPB	81570	0162129	Q9H078, A0A140VK11	MLGSLVLRRKALAPRLLLRLLRSPTLRGHGGASGRNVTTGSLGEPQWLRVATGGRPGTSPALFSGRGAATGGRQGGRFDTKCLAAATWGRLPGPEETLPGQDSWNGVPSRAGLGMCALAAALVVHCYSKSPSNKDAALLEAARANNMQEVSRLLSEGADVNAKHRLGWTALMVAAINRNNSVVQVLLAAGADPNLGDDFSSVYKTAKEQGIHSLEDGGQDGASRHITNQWTSALEFRRWLGLPA	3-methylglutaconic aciduria	Organic acidemia

				GVLITREDDFNNRLNNRASFKGCTALHYAVL ADDYRTVKELLDGGANPLQRNEMGHTPLDY AREGEVMKLLRTSEAKYQEKQRKREAEERR RFPLEQRLKEHIIGQESAIATVGAA IRRKENGWYDEEHPLVFLFLGSSGIGKTELAK QTAKYMHKDAKKGFIRLDMSEFQERHEVAK FIGSPPGYVGHEEGGQLTKKLKQCPNAVVLF DEVDKAHPDVLTIMLQLFDEGRLTDGKGKTI DCKDAIFIMTSNVASDEIAQHALQLRQEALE MSRNRIAENLGDVQISDKITISKNFKENVIRPI LKAHFRRDEFLGRINEIVYFLPFCHSELIQLVN KELNFWAKRAKQRHNITLLWDREVADVLVD GYNVHYGARSIKHEVERRVVNQLAAAYEQD LLPGGCTLRITVEDSDKQLLKSPELPSPQAEK RLPKLRLEIIDKDSKTRRLDIRAPLHPEKVCNT I [SEQ ID NO:275]		
TME M70	54968	0175606	Q9BUB7	MLFLALGSPWAVELPLCGRRTALCAAAALR GPRASVSRASSSSGPSGPVAGWSTGPSGAARL LRRPGRAQIPVYWEGYVRFLNTPSDKSEDGR LIYTGNMARAVFGVKCFSYSTSLIGLTFLPYIF TQNNAISESVPLPIQIIFYGIMGSFTVITPVLLH FITKGYVIRLYHEATTDTYKAITYNAMLAETS TVFHQNDVKIPDAKHVFTTFYAKTKSLLVNP VLFPNREDYIHLMGYDKEEFILYMEETSEEKR HKDDK [SEQ ID NO:276]	3-methylglutaconic aciduria	Organic acidemia
ALDH18A1	5832	0059573	P54886	MLSQVYRCGFQPFNQHLLPWVKCTTVFRSHC IQPSVIRHVRSWSNIPFITVPLSRTHGKSFAHR SELKHAKRIVVKLGSAVVTRGDECGLALGRL ASIVEQVSVLQNQGREMMLVTSGAVAFGKQ RLRHEILLSQSVRQALHSGQNQLKEMAIPVLE ARACAAAGQSGLMALYEAMFTQYSICAAQIL VTNLDFHDEQKRRNLNGTLHELLRMNIVPIV NTNDAVVPPAEPNSDLQGVNVISVKDNDSLA ARLAVEMKTDLLIVLSDVEGLFDSPPGSDDA KLIDIFYPGDQQSVTFGTKSRVGMGGMEAKV KAALWALQGGTSVVIANGTHPKVSGHVITDI VEGKKVGTFFSEVKPAGPTVEQQGEMARSG GRMLATLEPEQRAEIIHHLADLLTDQRDEILL ANKKDLEEAEGRLAAPLLKRLSLSTSKLNSL AIGLRQIAASSQDSVGRVLRRTRIAKNLELEQ VTVPIGVLLVIFESRPDCLPQVAALAIASGNGL LLKGGKEAAHSNRILHLLTQEALSIHGVKEA VQLVNTREEVEDLCRLDKMIDLIIPRGSSQLV RDIQKAAKGIPVMGHSEGICHMYVDSEASVD KVTRLVRDSKCEYPAACNALETLLIHRDLLR TPLFDQIIDMLRVEQVKIHAGPKFASYLTFSPS EVKSLRTEYGDLELCIEVVDNVQDAIDHIHKY GSSHTDVIVTEDENTAEFFLQHVDSACVFWN ASTRFSDGYRFGLGAEVGISTSRIHARGPVGL EGLLTTKWLLRGKDHVVSDFSEHGSLKYLHE NLPIPQRNTN [SEQ ID NO:277]	ALDH18A1-related cutis laxa	Urea cycle disorder
OAT	4942	0065154	A0A140VJQ4, P04181	MFSKLAHLQRFAVLSRGVHSSVASATSVATK KTVQGPPTSDDIFEREYKYGAHNYHPLPVAL ERGKGIYLWDVEGRKYFDFLSSYSAVNQGHC HPKIVNALKSQVDKLTLTSRAFYNNVLGEYE EYITKLFNYHKVLPMNTGVEAGETACKLARK WGYTVKGIQKYKAKIVFAAGNFWGRTLSAIS SSTDPTSYDGFGPFMPGFDIIPYNDLPALERAL QDPNVAAFMVEPIQGEAGVVVPDPGYLMGV	gyrate atrophy (OAT)	Urea cycle disorder

				RELCTRHQVLFIADEIQTGLARTGRWLAVDY ENVRPDIVLLGKALSGGLYPVSAVLCDDDIM LTIKPGEHGSTYGGNPLGCRVAIAALEVLEEE NLAENADKLGIILRNELMKLPSDVVTAVRGK GLLNAIVIKETKDWDAWKVCLRLRDNGLLA KPTHGDIIRFAPPLVIKEDELRESIEIINKTILSF [SEQ ID NO:278]		
CA5A	763	0174990	P35218	MLGRNTWKTSAFSFLVEQMWAPLWSRSMRP GRWCSQRSCAWQTSNNTLHPLWTVPVSVPG GTRQSPINIQWRDSVYDPQLKPLRVSYEAASC LYIWNTGYLFQVEFDDATEASGISGGPLENH YRLKQFHFHWGAVNEGGSEHTVDGHAYPAE LHLVHWNSVKYQNYKEAVVGENGLAVIGVF LKLGAHHQTLQRLVDILPEIKHKDARAAMRP FDPSTLLPTCWDYWTYAGSLTTPPLTESVTWI IQKEPVEVAPSQLSAFRTLLFSALGEEEKMMV NNYRPLQPLMNRKVWASFQATNEGTRS [SEQ ID NO:279]	carbonic anhydrase deficiency	Urea cycle disorder
GLUD1	2746	0148672	P00367, E9KL48	MYRYLGEALLLSRAGPAALGSASADSAALLG WARGQPAAAPQPGLALAARRHYSEAVADRE DDPNFFKMVEGFFDRGASIVEDKLVEDLRTR ESEEQKRNRVRGILRIIKPCNHVLSLSFPIRRD DGSWEVIEGYRAQHSQHRTPCKGGIRYSTDV SVDEVKALASLMTYKCAVVDVPFGGAKAGV KINPKNYTDNELEKITRRFTMELAKKGFIGPGI DVPAPDMSTGEREMSWIADTYASTIGHYDIN AHACVTGKPISQGGIHGRISATGRGVFHGIEN FINEASYMSILGMTPGFG DKTFVVQGFGNVGLHSMRYLHRFGAKCIAV GESDGSIWNPDGIDPKELEDFKLQHGSILGFP KAKPYEGSILEADCDILIPAASEKQLTKSNAPR VKAKIIAEGANGPTTPEADKIFLERNIMVIPDL YLNAGGVTVSYFEWLKNLNHVSYGRLTFKY ERDSNYHLLMSVQESLERKFGKHGGTIPIVPT AEFQDRISGASEKDIVHSGLAYTMERSARQIM RTAMKYNLGLDLRTAAYVNAIEKVFKVYNE AGVTFT[SEQ ID NO:280]	glutamate dehydrogenase deficiency	Urea cycle disorder
GLUL	2752	0135821	A8YXX4, P15104	MTTSASSHLNKGIKQVYMSLPQGEKVQAMYI WIDGTGEGLRCKTRTLDSEPKCVEELPEWNF DGSSTLQSEGSNSDMYLVPAAMFRDPFRKDP NKLVLCEVFKYNRRPAETNLRHTCKRIMDM VSNQHPWFGMEQEYTLMGTDGHPFGWPSNG FPGPQGPYYCGVGADRAYGRDIVEAHYRAC LYAGVKIAGTNAEVMPAQWEFQIGPCEGISM GDHLWVARFILHRVCEDFGVIATFDPKPIPGN WNGAGCHTNFSTKAMREENGLKYIEEAIEKL SKRHQYHIRAYDPKGGLDNARRLTGFHETSN INDFSAGVANRSASIRIPRTVGQEKKGYFEDR RPSANCDPFSVTEALIRTCLLNETGDEPFQYK N [SEQ ID NO:281]	glutamine synthetase deficienc	Urea cycle disorder
UMPS	7372	0114491	A8K5J1, P11172	MAVARAALGPLVTGLYDVQAFKFGDFVLKS GLSSPIYIDLRGIVSRPRLLSQVADILFQTAQN AGISFDTVCGVPYTALPLATVICSTNQIPMLIR RKETKDYGTKRLVEGTINPGETCLIIEDVVTS GSSVLETVEVLQKEGLKVTDAIVLLDREQGG KDKLQAHGIRLHSVCTLSKMLEILEQQKKVD AETVGRVKRFIQENVFVAANHNGSPLSIKEAP KELSFGARAELPRIHPVA SKLLRLMQKKETNLCLSADVSLARELLQLAD ALGPSICMLKTHVDILNDFTLDVMKELITLAK	Orotic Aciduria	Urea cycle disorder

				CHEFLIFEDRKFADIGNTVKKQYEGGIFKIAS WADLVNAHVVPGSGVVKGLQEVGLPLHRGC LLIAEMSSTGSLATGDYTRAAVRMAEEHSEF VVGFISGSRVSMKPEFLHLTPGVQLEAGGDN LGQQYNSPQEVIGKRGSDIIIVGRGIISAADRL EAAEMYRKAAWEAYLSRLGV [SEQ ID NO:282]		
SLC 22A5	6584	0197375	O76082	MRDYDEVTAFLGEWGPFQRLIFFLLSASIIPN GFTGLSSVFLIATPEHRCRVPDAANLSSAWRN HTVPLRLRDGREVPHSCRRYRLATIANFSALG LEPGRDVDLGQLEQESCLDGWEFSQDVYLST IVTEWNLVCEDDWKAPLTISLFFVGVLLGSFI SGQLSDRFGRKNVLFVTMGMQTGFSFLQIFS KNFEMFVVLFVLVGMGQISNYVAAFVLGTEI LGKSVRIIFSTLGVCIFYAFGYMVLPLFAYFIR DWRMLLVALTMPGVLCVALWWFIPESPRWL ISQGRFEEAEVIIRKAAKANGIVVPSTIFDPSEL QDLSSKKQQSHNILDLLRTWNIRMVTIMSIML WMTISVGYFGLSLDTPNLHGDIFVNCFLSAM VEVPAYVLAWLLLQYLPRRYSMATALFLGG SVLLFMQLVPPDLYYLATVLVMVGKFGVTA AFSMVYVYTAELYPTVVRNMGVGVSSTASR LGSILSPYFVYLGAYDRFLPYILMGSLTILTAI LTLFLPESFGTPLPDTIDQMLRVKGMKHRKTP SHTR MLKDGQERPTILKSTAF [SEQ ID NO:283]	carnitine-acylcarnitine translocase (CACT) deficiency	Fatty acid oxidation
CPT 1A	1374	0110090	P50416, A0A024R5F4, B2RAQ8, Q8WZ48	MAEAHQAVAFQFTVTPDGIDLRLSHEALRQI YLSGLHSWKKKFIRFKNGIITGVYPASPSSWLI VVVGVMTTMYAKIDPSLGIIAKINRTLETANC MSSQTKNVVSGVLFGTGLWVALIVTMRYSL KVLLSYHGWMFTEHGKMSRATKIWMGMVK IFSGRKPMLYSFQTSLPRLPVPAVKDTVNRYL QSVRPLMKEEDFKRMTALAQDFAVGLGPRL QWYLKLKSWWATNYVSDWWEEYIYLRGRG PLMVNSNYYAMDLLYILPTHIQAARAGNAIH AILLYRRKLDREEIKPIRLLGSTIPLCSAQWER MFNTSRIPGEETDTIQHMRDSKHIVVYHRGR YFKVWLYHDGRLLKPREMEQQMQRILDNTS EPQPGEARLAALTAGDRVPWARCRQAYFGR GKNKQSLDAVEKAAFFVTLDETEEGYRSEDP DTSMDSYAKSLLHGRCYDRWFDKSFTFVVF KNGKMGLNAEHSWADAPIVAHLWEYVMSID SLQLGYAEDGHCKGDINPNIPYPTRLQWDIPG ECQEVIETSLNTANLLANDVDFHSFPFVAFGK GIIKKCRTSPDAFVQLALQLAHYKDMGKFCL TYEASMTRLFREGRTETVRSCTTESCDFVRA MVDPAQTVEQRLKLFKLASEKHQHMYRLAM TGSGIDRHLFCLYVVSKYLAVESPFLKEVLSE PWRLSTSQTPQQQVELFDLENNPEYVSSGGG FGPVADDGYGVSYILVGENLINFHISSKFSCPE TDSHRFGRHLKEAMTDIITLFGLSSNSKK [SEQ ID NO:284]	carnitine palmitoyltransferase type I (CPT I) deficiency	Fatty acid oxidation
HADHA	3030	0084754	E9KL44, P40939	MVACRAIGILSRFSAFRILRSRGYICRNFTGSS ALLTRTHINYGVKGDVAVVRINSPNSKVNTL SKELHSEFSEVMNEIWASDQIRSAVLISSKPGC FIAGADINMLAACKTLQEVTQLSQEAQRIVEK LEKSTKPIVAAINGSCLGGGLEVAISCQYRIAT KDRKTVLGTPEVLLGALPGAGGTQRLPKMV GVPAALDMMLTGRSIRADRAKKMGLVDQLV EPLGPGLKPPEERTIEYLEEVAITFAKGLADK	long chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency	Fatty acid oxidation

				KISPKRDKGLVEKLTAYAMTIPFVRQQVYKK VEEKVRKQTKGLYPAPLKIIDVVKTGIEQGSD AGYLCESQKFGELVMTKESKALMGLYHGQV LCKKNKFGAPQKDVKHLAILGAGLMGAGIA QVSVDKGLKTILKDATLTALDRGQQQVFKGL NDKVKKKALTSFERDSIFSNLTGQLDYQGFE KADMVIEAVFEDLSLKHRVLKEVEAVIPDHCI FASNTSALPISEIAAVSKRPEKVIGMHYFSPVD KMQLLEIITTEKTSKDTSASAVAVGLKQGKVI IVVK DGPGFYTTRCLAPMMSEVIRILQEGVDPKKL DSLTTSFGFPVGAATLVDEVGVDVAKHVAED LGKVFGERFGGGNPELLTQMVSKGFLGRKSG KGFYIYQEGVKRKDLNSDMDSILASLKLPPKS EVSSDEDIQFRLVTRFVNEAVMCLQEGILATP AEGDIGAVFGLGFPPCLGGPFRFVDLYGAQKI VDRLKKYEAAYGKQFTPCQLLADHANSPNK KFYQ [SEQ ID NO:285]		
HADH	3033	0138796	Q16836, B3KTT6	MAFVTRQFMRSVSSSSTASASAKKIIVKHVTV IGGGLMGAGIAQVAAATGHTVVLVDQTEDIL AKSKKGIEESLRKVAKKKFAENLKAGDEFVE KTLSTIATSTDAASVVHSTDLVVEAIVENLKV KNELFKRLDKFAAEHTIFASNTSSLQITSIANA TTRQDRFAGLHFFNPVPVMKLVEVIKTPMTS QKTFESLVDFSKALGKHPVSCKDTPGFIVNRL LVPYLMEAIRLYERGDASKEDIDTAMKLGAG YPMGPFELLDYVGLDTTKFIVDGWHEMDAE NPLHQPSPSLNKLVAENKFGKKTGEGFYKYK [SEQ ID NO:286]	medium/short chain acyl-CoA dehydrogenase (M/SCHAD) deficiency	Fatty acid oxidation
SLC52A1	55065	0132517	Q9NWF4	MAAPTLGRLVLTHLLVALFGMGSWAAVNGI WVELPVVVKDLPEGWSLPSYLSVVVALGNL GLLVVTLWRQLAPGKGEQVPIQVVQVLSVV GTALLAPLWHHVAPVAGQLHSVAFLTLALV LAMACCTSNVTFLPFLSHLPPPFLRSFFLGQG LSALLPCVLALVQGVGRLECPPAPTNGTSGPP LDFPERFPASTFFWALTALLVTSAAAFRGLLL LLPSLPSVTTGGSGPELQLGSPGAEEEEKEEEE ALPLQEPPSQAAGTIPGPDPEAHQLFSAHGAF LLGLMAFTSAVTNGVLPSVQSFSCLPYGRLA YHLAVVLGSAANPLACFLAMGVLCRSLAGL VGLSLLGMLFGAYLMALAILSPCPPLVGTTA GVVLVVLSWVLCLCVFSYVKVAASSLLHGG GRPALLAAGVAIQVGSLLGAGAMFPPTSIYH VFQSRKDCVDPCGP [SEQ ID NO:287]	Riboflavin transporter deficiency	Fatty acid oxidation
SLC52A2	79581	0185803	Q9HAB3	MAAPTPARPVLTHLLVALFGMGSWAAVNGI WVELPVVVKELPEGWSLPSYVSVLVALGNL GLLVVTLWRRLAPGKDEQVPIRVVQVLGMV GTALLASLWHHVAPVAGQLHSVAFLALAFV LALACCASNVTFLPFLSHLPPRFLRSFFLGQGL SALLPCVLALVQGVGRLECPPAPINGTPGPPL DFLERFPASTFFWALTALLVASAAAFQGLLLL LPPPPSVPTGELGSGLQVGAPGAEEEVEESSPL QEPPSQAAGTTPGPDPKAYQLLSARSACLLGL LAATNALTNGVLPAVQSFSCLPYGRLAYHLA VVLGSAANPLACFLAMGVLCRSLAGLGGLSL LGVFCGGYLMALAVLSPCPPLVGTSAGVVLV VLSWVLCLGVFSYVKVAASSLLHGGGRPALL AAGVAIQVGSLLGAVAMFPPTSIYHVFHSRK DCADPCDS [SEQ ID NO:288]	Riboflavin transporter deficiency	Fatty acid oxidation

SLC 52A3	113278	0101276	K0A6P4, Q9NQ40	MAFLMHLLVCVFGMGSWVTINGLWVELPLL VMELPEGWYLPSYLTVVIQLANIGPLLVTLLH HFRPSCLSEVPIIFTLLGVGTVTCIIFAFLWNM TSWVLDGHHSIAFLVLTFFLALVDCTSSVTFL PFMSRLPTYYLTTFFVGEGLSGLLPALVALAQ GSGLTTCVNVTEISDSVPSPVPTRETDIAQGVP RALVSALPGMEAPLSHLESRYLPAHFSPLVFF LLLSIMMACCLVAFFV LQRQPRCWEASVEDLLNDQVTLHSIRPREEN DLGPAGTVDSSQGQGYLEEKAAPCCPAHLAF IYTLVAFVNALTNGMLPSVQTYSCLSYGPVA YHLAATLSIVANPLASLVSMFLPNRSLLFLGV LSVLGTCFGGYNMAMAVMSPCPLLQGHWG GEVLIVASWVLFSGCLSYVKVMLGVVLRDLS RSALLWCGAAVQLGSLLGALLMFPLVNVLR LFSSADFCNLHCPA [SEQ ID NO:289]	Riboflavin transporter deficiency	Fatty acid oxidation
HAD HB	3032	0138029	P55084, F5GZQ3	MTILTYPFKNLPTASKWALRFSIRPLSCSSQLR AAPAVQTKTKKTLAKPNIRNVVVVDGVRTPF LLSGTSYKDLMPHDLARAALTGLLHRTSVPK EVVDYIIFGTVIQEVKTSNVAREAALGAGFSD KTPAHTVTMACISANQAMTTGVGLIASGQCD VIVAGGVELMSDVPIRHSRKMRKLMLDLNK AKSMGQRLSLISKFRFNFLAPELPAVSEFSTSE TMGHSADRLAAAFAVSRLEQDEYALRSHSLA KKAQDEGLLSDVVPFKVPGKDTVTKDNGIRP SSLEQMAKLKPAFIKPY GTVTAANSSFLTDGASAMLIMAEEKALAMG YKPKAYLRDFMYVSQDPKDQLLLGPTYATP KVLEKAGLTMNDIDAFEFHEAFSGQILANFK AMDSDWFAENYMGRKTKVGLPPLEKFNNW GGSLSLGHPFGATGCRLVMAAANRLRKEGG QYGLVAACAAGGQGHAMIVEAYPK [SEQ ID NO:290]	Trifunctional protein deficiency	Fatty acid oxidation
GYS 2	2998	0111713	P54840	MLRGRSLSVTSLGGLPQWEVEELPVEELLLFE VAWEVTNKVGGIYTVIQTKAKTTADEWGEN YFLIGPYFEHNMKTQVEQCEPVNDAVRRAVD AMNKHGCQVHFGRWLIEGSPYVVLFDIGYSA WNLDRWKGDLWEACSVGIPYHDREANDMLI FGSLTAWFLKEVTDHADGKYVVAQFHEWQA GIGLILSRARKLPIATIFTTHATLLGRYLCAANI DFYNHLDKFNIDKEAGERQIYHRYCMERASV HCAHVFTTVSEITAIEAEHMLKRKPDVVTPN GLNVKKFSAVHEFQNLHAMYKARIQDFVRG HFYGHLDFDLEKTLFLFIAGRYEFSNKGADIF LESLSRLNFLLRMHKSDITVMVFFIMPAKTNN FNVETLKGQAVRKQLWDVAHSVKEKFGKKL YDALLRGEIPDLNDILDRDDLTIMKRAIFSTQ RQSLPPVTTHNMIDDSTDPILSTIRRIGLFNNR TDRVKVILHPEFLSSTSPLLPMDYEEFVRGCH LGVFPSYYEPWGYTPAECTVMGIPSVTTNLS GFGCFMQEHVADPTAYGIYIVDRRFRSPDDS CNQLTKFLYGFCKQSRRQRIIQRNRTERLSDL LDWRYLGRYYQHARHLTLSRAFPDKFHVEL TSPPTTEGFKYPRPSSVPPSPSGSQASSPQSSD VEDEVEDERYDEEEEAERDRLNIKSPFSLSHV PHGKKKLHGEYKN [SEQ ID NO:291]	GSD 0 (Glycogen synthase, liver isoform)	Liver glycogen storage disorder
PYG L	5836	0100504	P06737	MAKPLTDQEKRRQISIRGIVGVENVAELKKSF NRHLHFTLVKDRNVATTRDYYFALAHTVRD HLVGRWIRTQQHYYDKCPKRVYYLSLEFYM GRTLQNTMINLGLQNACDEAIYQLGLDIEELE	GSD VI (Hers disease)	Liver glycogen storage disorder

				EIEEDAGLGNGGLGRLAACFLDSMATLGLAA YGYGIRYEYGIFNQKIRDGWQVEEADDWLR YGNPWEKSRPEFMLPVHFYGKVEHTNTGTK WIDTQVVLALPYDTPVPGYMNNTVNTMRLW SARAPNDFNLRDFNVGDYIQAVLDRNLAENI SRVLYPNDNFFEGKELRLKQEYFVVAATLQD IIRRFKASKFGSTRGAGTVFDAFPDQVAIQLN DTHPALAIPELMRIFVDIEKL PWSKAWELTQKTFAYTNHTVLPEALERWPV DLVEKLLPRHLEIIYEINQKHLDRIVALFPKDV DRLRRMSLIEEEGSKRINMAHLCIVGSHAVN GVAKIHSDIVKTKVFKDFSELEPDKFQNKTNG ITPRRWLLLCNPGLAELIAEKIGEDYVKDLSQ LTKLHSFLGDDVFLRELAKVKQENKLKFSQF LETEYKVKINPSSMFDVQVKRIHEYKRQLLN CLHVITMYNRIKKDPKKLFVPRTVIIGGKAAP GYHMAKMIIKLITSVADVVNNDPMVGSKLK VIFLENYRVSLAEKVIPATDLSEQISTAGTEAS GTGNMKFMLNGALTIGTMDGANVEMAEEA GEENLFIFGMRIDDVAALDKKGYEAKEYYEA LPELKLVIDQIDNGFFSPKQPDLFKDIINMLFY HDRFKVFADYEAYVKCQDKVSQLYMNPKA WNTMVLKNIAASGKFSSDRTIKEYAQNIWNV EPSDLKISLSNESNKVNGN [SEQ ID NO:292]		
SLC 2A2	6514	0163581	P11168, Q6PAU8	MTEDKVTGTLVFTVITAVLGSFQFGYDIGVIN APQQVIISHYRHVLGVPLDDRKAINNYVINST DELPTISYSMNPKPTPWAEEETVAAAQLITML WSLSVSSFAVGGMTASFFGGWLGDTLGRIKA MLVANILSLVGALLMGFSKLGPSHILIIAGRSI SGLYCGLISGLVPMYIGEIAPTALRGALGTFH QLAIVTGILISQIIGLEFILGNYDLWHILLGLSG VRAILQSLLLFFCPESPRYLYIKLDEEVKAKQS LKRLRGYDDVTKDINEMRKEREEASSEQKVS IIQLFTNSSYRQPILVALMLHVAQQFSGINGIF YYSTSIFQTAGISKPVYATIGVGAVNMVFTAV SVFLVEKAGRRSLFLIGMSGMFVCAIFMSVGL VLLNKFSWMSYVSMIAIFLFVSFFEIGPGPIPW FMVAEFFSQGPRPAALAIAAFSNWTCNFIVAL CFQYIADFCGPYVFFLFAGVLLAFTLFTFFKV PETKGKSFEEIAAEFQKKSGSAHRPKAAVEM KFLGATETV [SEQ ID NO:293]	Fanconi-Bickel syndrome	Liver glycogen storage disorder
ALG 1	56052	0033011	Q9BT22	MAASCLVLLALCLLLPLLLLGGWKRWRRGR AARHVVAVVLGDVGRSPRMQYHALSLAMH GFSVTLLGFCNSKPHDELLQNNRIQIVGLTEL QSLAVGPRVFQYGVKVVLQAMYLLWKLMW REPGAYIFLQNPPGLPSIAVCWFVGCLCGSKL VIDWHNYGYSIMGLVHGPNHPLVLLAKWYE KFFGRLSHLNLCVTNAMREDLADNWHIRAV TVYDKPASFFKETPLDLQHRLFMKLGSMHSP FRARSEPEDPVTERSAFTERDAGSGLVTRLRE RPALLVSSTSWTEDEDFSILLAALEKFEQLTL DGHNLPSLVCVITGKGPLREYYSRLIHQKHFQ HIQVCTPWLEAEDYPLLLGSADLGVCLHTSSS GLDLPMKVVDMFGCCLPVCAVNFKCLHELV KHEENGLVFEDSEELAAQLQMLFSNFPDPAG KLNQFRKNLRESQQLRWDESWVQTVLPLVM DT [SEQ ID NO:294]	ALG1-CDG	Glycosylation disorder
ALG 2	85365	0119523	A0A024R184, Q9H553	MAEEQGRERDSVPKPSVLFLHPDLGVGGAER LVLDAALALQARGCSVKIWTAHYDPGHCFA ESRELPVRCAGDWLPRGLGWGGRGAAVCAY	ALG2-associated	Glycosylation disorder

				VRMVFLALYVLFLADEEFDVVVCDQVSACIP VFRLARRRKKILFYCHFPDLLLTKRDSFLKRL YRAPIDWIEEYTTGMADCILVNSQFTAAVFKE TFKSLSHIDPDVLYPSLNVTSFDSVVPEKLDD LVPKGKKFLLLSINRYERKKNLTLALEALVQL RGRLTSQDWERVHLIVAGGYDERVLENVEH YQELKKMVQQSDLGQYVTFLRSFSDKQKISL LHSCTCVLYTPSNEHFGIVPLEAMYMQCPVIA VNSGGPLESIDHSVTGFLCEPDPVHFSEAIEKF IREPSLKATMGLAGRARVKEKFSPEAFTEQLY RYVTKLLV [SEQ ID NO:295]	myasthenic syndrome	
ALG 3	10195	0214160	Q92685, C9J7S5	MAAGLRKRGRSGSAAQAEGLCKQWLQRAW QERRLLLREPRYTLLVAACLCLAEVGITFWVI HRVAYTEIDWKAYMAEVEGVINGTYDYTQL QGDTGPLVYPAGFVYIFMGLYYATSRGTDIR MAQNIFAVLYLATLLLVFLIYHQTCKVPPFVF FFMCCASYRVHSIFVLRLFNDPVAMVLLFLSI NLLLAQRWGWGCCFFSLAVSVKMNVLLFAP GLLFLLLTQFGFRGALPKLGICAGLQVVLGLP FLLENPSGYLSRSFDLGRQFLFHWTVNWRFL PEALFLHRAFHLALLTAHLTL LLLFALCRWHRTGESILSLLRDPSKRKVPPQP LTPNQIVSTLFTSNFIGICFSRSLHYQFYVWYF HTLPYLLWAMPARWLTHLLRLLVLGLIELSW NTYPSTSCSSAALHICHAVILLQLWLGPQPFP KSTQHSKKAH [SEQ ID NO:296]	ALG3-CDG	Glycosylation disorder
ALG 6	29929	0088035	Q9Y672	MEKWYLMTVVVLIGLTVRWTVSLNSYSGAG KPPMFGDYEAQRHWQEITFNLPVKQWYFNSS DNNLQYWGLDYPPLTAYHSLLCAYVAKFINP DWIALHTSRGYESQAHKLFMRTTVLIADLLIY IPAVVLYCCCLKEISTKKKIANALCILLYPGLI LIDYGHFQYNSVSLGFALWGVLGISCDCDLL GSLAFCLAINYKQMELYHALPFFCFLLGKCFK KGLKGKGFVLLVKLACIVVASFVLCWLPFFT EREQTLQVLRRLFPVDRGLFEDKVANIWCSF NVFLKIKDILPRHIQLIMSFCSTFLSLLPACIKLI LQPSSKGFKFTLVSCALSFFLFSFQVHEKSILL VSLPVCLVLSEIPFMSTWFLLVSTFSMLPLLLK DELLMPSVVTTMAFFIACVTSFSIFEKTSEEEL QLKSFSISVRKYLPCFTFLSRIIQYLFLISVITM VLLTLMTVTLDPPQKLPDLFSVLVCFVSCLNF LFFLVYFNIIIMWDSKSGRNQKKIS [SEQ ID NO:297]	ALG6-CDG	Glycosylation disorder
ALG 8	79053	0159063	Q9BVK2, A0A024R5K5	MAALTIATGTGNWFSALALGVTLLKCLLIPT YHSTDFEVHRNWLAITHSLPISQWYYEATSE WTLDYPPFFAWFEYILSHVAKYFDQEMLNVH NLNYSSSRTLLFQRFSVIFMDVLFVYAVRECC KCIDGKKVGKELTEKPKFILSVLLLWNFGLLI VDHIHFQYNGFLFGLMLLSIARLFQKRHMEG AFLFAVLLHFKHIYLYVAPAYGVYLLRSYCF TANKPDGSIRWKSFSFVRVISLGLVVFLVSAL SLGPFLALNQLPQVFSRLFPFKRGLCHAYWA PNFWALYNALDKVLSVIGLKLKFLDPNNIPK ASMTSGLVQQFQHTVLPSVTPLATLICTLIAIL PSIFCLWFKPQGPRGFLRCLTLCALSSFMFGW HVHEKAILLAILPMSLLSVGKAGDASIFLILTT TGHYSLFPLLFTAPELPIKILLMLLFTIYSISSLK TLFRKEKPLFNWMETFYLLGLGPLEVCCEFV FPFTSWKVKYPFIPLLLTSVYCAVGITYAWFK LYVSVLIDSAIGKTKKQ [SEQ ID NO:298]	ALG8-CDG	Glycosylation disorder

ALG9	79796	0086848	Q9H6U8	MASRGARQRLKGSGASSGDTAPAADKLREL LGSREAGGAEHRTELSGNKAGQVWAPEGST AFKCLLSARLCAALLSNISDCDETFNYWEPTH YLIYGEGFQTWEYSPAYAIRSYAYLLLHAWP AAFHARILQTNKILVFYFLRCLLAFVSCICELY FYKAVCKKFGLHVSRMMLAFLVLSTGMFCS SSAFLPSSFCMYTTLIAMTGWYMDKTSIAVL GVAAGAILGWPFSAALGLPIAFDLLVMKHRW KSFFHWSLMALILFLVPVVVIDSYYYGKLVIA PLNIVLYNVFTPHGPDLYGT EPWYFYLINGFLNFNVAFALALLVLPLTSLME YLLQRFHVQNLGHPYWLTLAPMYIWFIIFFIQ PHKEERFLFPVYPLICLCGAVALSALQKCYHF VFQRYRLEHYTVTSNWLALGTVFLFGLLSFS RSVALFRGYHGPLDLYPEFYRIATDPTIHTVP EGRPVNVCVGKEWYRFPSSFLLPDNWQLQFI PSEFRGQLPKPFAEGPLATRIVPTDMNDQNLE EPSRYIDISKCHYLVDLDTMRETPREPKYSSN KEEWISLAYRPFLDASRSSKLLRAFYVPFLSD QYTVYVNYTILKPRKAKQIRKKSGG [SEQ ID NO:299]	ALG9-CDG	Glycosylation disorder
ALG11	440138	0253710	Q2TAA5	MAAGERSWCLCKLLRFFYSLFFPGLIVCGTLC VCLVIVLWGIRLLLQRKKKLVSTSKNGKNQM VIAFFHPYCNAGGGGERVLWCALRALQKKY PEAVYVVYTGDVNVNGQQILEGAFRRFNIRLI HPVQFVFLRKRYLVEDSLYPHFTLLGQSLGSI FLGWEALMQCVPDVYIDSMGYAFTLPLFKYI GGCQVGSYVHYPTISTDMLSVVKNQNIGFNN AAFITRNPFLSKVKLIYYYLFAFIYGLVGSCSD VVMVNSSWTLNHILSLWKVGNCTNIVYPPCD VQTFLDIPLHEKKMTPGHLLVSVGQFRPEKN HPLQIRAFAKLLNKKMVESPPSLKLVLIGGCR NKDDELRVNQLRRLSEDLGVQEYVEFKINIPF DELKNYLSEATIGLHTMWNEHFGIGVVECMA AGTIILAHNSGGPKLDIVVPHEGDITGFLAESE EDYAETIAHILSMSAEKRLQIRKSARASVSRFS DQEFEVTFLSSVEKLFK [SEQ ID NO:300]	ALG11-CDG	Glycosylation disorder
ALG12	79087	0182858	A0A024R4V6, Q9BV10	MAGKGSSGRRPLLLGLLVAVATVHLVICPYT KVEESFNLQATHDLLYHWQDLEQYDHLEFP GVVPRTFLGPVVIAVFSSPAVYVLSLLEMSKF YSQLIVRGVLGLGVIFGLWTLQKEVRRHFGA MVATMFCWVTAMQFHLMFYCTRTLPNVLA LPVVLLALAAWLRHEWARFIWLSAFAIIVFR VELCLFLGLLLLLALGNRKVSVVRALRHAVP AGILCLGLTVAVDSYFWRQLTWPEGKVLWY NTVLNKSSNWGTSPLLWYFYSALPRGLGCSL LFIPLGLVDRRTHAPTVLALGFMALYSLLPHK ELRFIIYAFPMLNITAARGCSYLLNNYKKSWL YKAGSLLVIGHLVVNAAYSATALYVSHFNYP GGVAMQRLHQLVPPQTDVLLHIDVAAAQTG VSRFLQVNSAWRYDKREDVQPGTGMLAYTH ILMEAAPGLLALYRDTHRVLASVVGTTGVSL NLTQLPPFNVHLQTKLVLLERLPRPS [SEQ ID NO:301]	ALG12-CDG	Glycosylation disorder
ALG13	79868	0101901	Q9NP73, A0A087WX43, A0A087WT15	MKCVFVTVGTTSFDDLIACVSAPDSLQKIESL GYNRLILQIGRGTVVPEPFSTESFTLDVYRYK DSLKEDIQKADLVISHAGAGSCLETLEKGKPL VVVINEKLMNNHQLELAKQLHKEGHLFYCT CRVLTCPGQAKSIASAPGKCQDSAALTSTAFS GLDFGLLSGYLHKQALVTATHPTCTLLFPSCH	ALG13-CDG	Glycosylation disorder

				AFFPLPLTPTLYKMHKGWKNYCSQKSLNEAS MDEYLGSLGLFRKLTAKDASCLFRAISEQLFC SQVHHLEIRKACVSYMRENQQTFESYVEGSF EKYLERLGDPKESAGQLEIRALSLIYNRDFILY RFPGKPPTYVTDNGYEDKILLCYSSSGHYDSV YSKQFQSSAAVCQAVLYEILYKDVFVVDEEE LKTAIKLFRSGSKKNRNNAVTGSEDAHTDYK SSNQNRMEEWGACYNAENIPEGYNKGTEET KSPENPSKMPFPYKVLKALDPEIYRNVEFDV WLDSRKELQKSDYMEYAGRQYYLGDKCQV CLESEGRYYNAHIQEVGNENNSVTVFIEELAE KHVVPLANLKPVTQVMSVPAWNAMPSRKGR GYQKMPGGYVPEIVISEMDIKQQKKMFKKIR GKEVYM TMAYGKGDPLLPPRLQHSMHYGHDPPMHYS QTAGNVMSNEHFHPQHPSPRQGRGYGMPRN SSRFINRHNMPGPKVDFYPGPGKRCCQSYDN FSYRSRSFRRSHRQMSCVNKESQYGFTPGNG QMPRGLEETITFYEVEEGDETAYPTLPNHGGP STMVPATSGYCVGRRGHSSGKQTLNLEEGNG QSENGRYHEEYLYRAEPDYETSGVYSTTAST ANLSLQDRKSCSMSPQDTVTSYNYPQKMMG NIAAVAASCANNVPAPVLSNGAAANQAISTT SVSSQNAIQPLFVSPPTHGRPVIASPSYPCHSAI PHAGASLPPPPPPPPPPPPPPPPPPPPPPPPA LDVGETSNLQPPPPLPPPPYSCDPSGSDLPQDT KVLQYYFNLGLQCYYHSYWHSMVYVPQMQ QQLHVENYPVYTEPPLVDQTVPQCYSEVRRE DGIQAEASANDTFPNADSSSVPHGAVYYPVM SDPYGQPPLPGFDSCLPVVPDYSCVPPWHPV GTAYGGSSQIHGAINPGPIGCIAPSPPASHYVP QGM [SEQ ID NO:302]		
ATP 6V0 A2	23545	0185344	Q9Y487	MGSLFRSETMCLAQLFLQSGTAYECLSALGE KGLVQFRDLNQNVSSFQRKFVGEVKRCEELE RILVYLVQEINRADIPLPEGEASPPAPPLKQVL EMQEQLQKLEVELREVTKNKEKLRKNLLELI EYTHMLRVTKTFVKRNVEFEPTYEEFPSLESD SLLDYSCMQRLGAKLGFVSGLINQGKVEAFE KMLWRVCKGYTIVSYAELDESLEDPETGEVI KWYVFLISFWGEQIGHKVKKICDCYHCHVYP YPNTAEERREIQEGLNTRIQDLYTVLHKTEDY LRQVLCKAAESVYSRVIQVKKMKAIYHMLN MCSFDVTNKCLIAEVWCPEADLQDLRRALEE GSRESGATIPSFMNIIPTKETPPTRIRTNKFTEG FQNIVDAYGVGSYREVNPALFTIITFPFLFAV MFGDFGHGFVMFLFALLLVLNENHPRLNQSQ EIMRMFFNGRYILLLMGLFSVYTGLIYNDCFS KSVNLFGSGWNVSAMYSSSHPPAEHKKMVL WNDSVVRHNSILQLDPSIPGVFRGPYPLGIDPI WNLATNRLTFLNSFKMKMSVILGIIHMTFGVI LGIFNHLHFRKKFNIYLVSIPELLFMLCIFGYLI FMIFYKWLVFSAETSRVAPSILIEFINMFLFPA SKTSGLYTGQEYVQRVLLVVTALSVPVLFLG KPLFLLWLHNGRSCFGVNRSGYTLIRKDSEEE VSLLGSQDIEEGNHQVEDGCREMACEEFNFG EILMTQVIHSIEYCLGCISNTASYLRLWALSLA HAQLSDVLWAMLMRVGLRVDTTYGVLLLLP VIALFAVLTIFILLIMEGLSAFLHAIRLHWVEF QNKFYVGAGTKFVPF	ATP6V0A 2- associated cutis laxa	Glycosyla tion disorder

				SFSLLSSKFNNDDSVA [SEQ ID NO:303]		
B3GLCT	145173	0187676	Q6Y288	MRPPACWWLLAPPALLALLTCSLAFGLASED TKKEVKQSQDLEKSGISRKNDIDLKGIVFVIQ SQSNSFHAKRAEQLKKSILKQAADLTQELPSV LLLHQLAKQEGAWTILPLLPHFSVTYSRNSS WIFFCEEETRIQIPKLLETLRRYDPSKEWFLGK ALHDEEATIIHHYAFSENPTVFKYPDFAAGW ALSIPLVNKLTKRLKSESLKSDFTIDLKHEIAL YIWDKGGGPPLTPVPEF CTNDVDFYCATTFHSFLPLCRKPVKKKDIFVA VKTCKKFHGDRIPIVKQTWESQASLIEYYSDY TENSIPTVDLGIPNTDRGHCGKTFAILERFLNR SQDKTAWLVIVDDDTLISISRLQHLLSCYDSG EPVFLGERYGYGLGTGGYSYITGGGGMVFSR EAVRRLLASKCRCYSNDAPDDMVLGMCFSG LGIPVTHSPLFHQARPVDYPKDYLSHQVPISF HKHWNIDPVKVYFTWLAPSDEDKARQETQK GFREEL [SEQ ID NO:304]	B3GLCT-CDG	Glycosylation disorder
CHST14	113189	0169105	Q8NCH0	MFPRPLTPLAAPNGAEPLGRALRRAPLGRAR AGLGGPPLLLPSMLMFAVIVASSGLLLMIERG ILAEMKPLPLHPPGREGTAWRGKAPKPGGLS LRAGDADLQVRQDVRNRTLRAVCGQPGMPR DPWDLPVGQRRTLLRHILVSDRYRFLYCYVP KVACSNWKRVMKVLAGVLDSVDVRLKMDH RSDLVFLADLRPEEIRYRLQHYFKFLFVREPL ERLLSAYRNKFGEIREYQQRYGAEIVRRYRA GAGPSPAGDDVTFPEFLRYLVDEDPERMNEH WMPVYHLCQPCAVHYDFVGSYERLEADANQ VLEWVRAPPHVRFPARQAWYRPASPESLHY HLCSAPRALLQDVLPKYILDFSLFAYPLPNVT KEACQQ [SEQ ID NO:305]	CHST14-CDG	Glycosylation disorder
COG1	9382	0166685	Q8WTW3	MATAATSPALKRLDLRDPAALFETHGAEEIR GLERQVRAEIEHKKEELRQMVGERYRDLIEA ADTIGQMRRCAVGLVDAVKATDQYCARLRQ AGSAAPRPPRAQQPQQPSQEKFYSMAAQIKL LLEIPEKIWSSMEASQCLHATQLYLLCCHLHS LLQLDSSSSRYSPVLSRFPILIRQVAAASHFRS TILHESKMLLKCQGVSDQAVAEALCSIMLLE ESSPRQALTDFLLARKATIQKLLNQPHHGAGI KAQICSLVELLATTLKQAHALFYTLPEGLLPD PALPCGLLFSTLETITGQHPAGKGTGVLQEEM KLCSWFKHLPASIVEFQPTLRTLAHPISQEYL KDTLQKWIHMCNEDIKNGITNLLMYVKSMK GLAGIRDAMWELLTNESTNHSWDVLCRRLL EKPLLFWEDMMQQLFLDRLQTLTKEGFDSIS SSSKELLVSALQELESSTSNSPSNKHIIHFEYNM SLFLWSESPNDLPSDAAWVSVANRGQFASSG LSMKAQAISPCVQNFCSALDSKLKVKLDDLL AYLPSDD SSLPKDVSPTQAKSSAFDRYADAGTVQEMLR TQSVACIKHIVDCIRAELQSIEEGVQGQQDAL NSAKLHSVLFMARLCQSLGELCPHLKQCILG KSESSEKPAREFRALRKQGKVKTQEIIPTQAK WQEVKEVLLQQSVMGYQVWSSAVVKVLIH GFTQSLLLDDAGSVLATATSWDELEIQEEAES GSSVTSKIRLPAQPSWYVQSFLFSLCQEINRV GGHALPKVTLQEMLKSCMVQVVAAYEKLSE	COG1-CDG	Glycosylation disorder

				EKQIKKEGAFPVTQNRALQLLYDLRYLNIVLT AKGDEVKSGRSKPDSRIEK VTDHLEALIDPFDLDVFTPHLNSNLHRLVQRT SVLFGLVTGTENQLAPRSSTFNSQEPHNILPLA SSQIRFGLLPLSMTSTRKAKSTRNIETKAQVV PPARSTAGDPTVPGSLFRQLVSEEDNTSAPSL FKLGWLSSMTK [SEQ ID NO:306]		
COG 2	22796	0135775	Q14746, B1ALW7	MEKSRMNLPKGPDTLCFDKDEFMKEDFDVD HFVSDCRKRVQLEELRDDLELYYKLLKTAM VELINKDYADFVNLSTNLVGMDKALNQLSVP LGQLREEVLSLRSSVSEGIRAVDERMSKQEDI RKKKMCVLRLIQVIRSVEKIEKILNSQSSKETS ALEASSPLLTGQILERIATEFNQLQFHAVQSK GMPLLDKVRPRIAGITAMLQQSLEGLLLEGL QTSDVDIIRHCLRTYATIDKTRDAEALVGQVL VKPYIDEVIIEQFVESHPNGLQVMYNKLLEFV PHHCRLLREVTGGAISSEKGNTVPGYDFLVNS VWPQIVQGLEEKLPSLFNPGNPDAFHEKYTIS MDFVRRLERQCGSQASVKRLRAHPAYHSFN KKWNLPVYFQIRFREIAGSLEAALTDVLEDAP AESPYCLLASHRTWSSLRRCWSDEMFLPLLV HRLWRLTLQILARYSVFVNELSLRPISNESPKE IKKPLVTGSKEPSITQGNTEDQGSGPSETKPV VSISRTQLVYVVADLDKLQEQLPELLEIIKPKL EMIGFKNFSSISAALEDSQSSFSACVPSLSSKII QDLSDSCFGFLKSALEVPRLYRRTNKEVPTTA SSYVDSALKPLFQLQSGHKDKLKQAIIQQWL EGTLSESTHKYYETVSDVLNSVKKMEESLKR LKQARKTTPANPVGPSGGMSDDDKIRLQLAL DVEYLGEQIQKLGLQASDIKSFSALAELVAAA KDQATAEQP [SEQ ID NO:307]	COG2-CDG	Glycosylation disorder
COG 4	25839	0103051	A0A0A0MS45, Q8N8L9, Q9H9E3, J3KNI1	MADLDSPPKLSGVQQPSEGVGGGRCSEISAEL IRSLTELQELEAVYERLCGEEKVVERELDALL EQQNTIESKMVTLHRMGPNLQLIEGDAKQLA GMITFTCNLAENVSSKVRQLDLAKNRLYQAI QRADDILDLKFCMDGVQTALRSEDYEQAAA HTHRYLCLDKSVIELSRQGKEGSMIDANLKL LQEAEQRLKAIVAEKFAIATKEGDLPQVERFF KIFPLLGLHEEGLRKFSEYLCKQVASKAEENL LMVLGTDMSDRRAAVIFADTLTLLFEGIARIV ETHQPIVETYYGPGRLYTLIKYLQVECDRQVE KVVDKFIKQRDYHQQFRHVQNNLMRNSTTE KIEPRELDPILTEVTLMNARSELYLRFLKKRIS SDFEVGDSMASEEVKQEHQKCLDKLLNNCLL SCTMQELIGLYVTMEEYFMRETVNKAVALD TYEKGQLTSSMVDDVFYIVKKCIGRALSSSSI DCLCAMINLATTELESDFRDVLCNKLRMGFP ATTFQDIQRGVTSAVNIMHSSLQQGKFDTKGI ESTDEAKMSFLVTLNNVEVCSENISTLKKTLE SDCTKLFSQGIGGEQAQAKFDSCLSDLAAVS NKFRDLLQEGLTELNSTAIKPQVQPWINSFFS VSHNIEEEEFNDYEANDPWVQQFILNLEQQM AEFKASLSPVIYDSLTGLMTSLVAVELEKVVL KSTFNRLGGLQFDKELRSLIAYLTTVTTWTIR DKFARLSQMATILNLERVTEILDYWGPNSGPL TWRLTPAEVRQVLALRIDFRSEDIKRLRL [SEQ ID NO:308]	COG4-CDG	Glycosylation disorder
COG 5	10466	0164597,0284369	Q9UP83	MGWVGGRRRDSASPPGRSRSAADDINPAPAN MEGGGGSVAVAGLGARGSGAAAATVRELLQ DGCYSDFLNEDFDVKTYTSQSIHQAVIAEQLA	COG5-CDG	Glycosylation disorder

				KLAQGISQLDRELHLQVVARHEDLLAQATGI ESLEGVLQMMQTRIGALQGAVDRIKAKIVEP YNKIVARTAQLARLQVACDLLRRIIRILNLSK RLQGQLQGGSREITKAAQSLNELDYLSQGIDL SGIEVIENDLLFIARARLEVENQAKRLLEQGL ETQNPTQVGTALQVFYNLGTLKDTITSVVDG YCATLEENINSALDIKVLTQPSQSAVRGGPGR STMPTPGNTAALRASFWTNMEKLMDHIYAV CGQVQHLQKVLAKKRDPVSHICFIEEIVKDG QPEIFYTFWNSVTQALSSQFHMATNSSMFLK QAFEGEYPKLLRLYNDLWKRLQQYSQHIQG NFNASGTTDLYVDLQHMEDDAQDIFIPKKPD YDPEKALKDSLQPYEAAYLSKSLSRLFDPINL VFPPGGRNPPSSDELDGIIKTIASELNVAAVDT NLTLAVSKNVAKTIQLYSVKSEQLLSTQGDA SQVIGPLTEGQRRNVAVVNSLYKLHQSVTKA IHALMENAVQPLLTSVGDAIEAIIITMHQEDFS GSLSSSGKPDVPCSLYMKELQGFIARVMSDY FKHFECLDFVFDNTEAIAQRAVELFIRHASLIR PLGEGGKMRLAADFAQMELAVGPFCRRVSD LGKSYRMLRSFRPLLFQASEHVASSPALGDVI PFSIIIQFLFTRAPAELKSPFQRAEWSHTRFSQ WLDDHPSEKDRLLLIRGALEAYVQSVRSREG KEFAPVYPIMVQLLQKAMSALQ [SEQ ID NO:309]		
COG 6	57511	0133103	A0A140V JG7, Q9Y2V7, A0A024R DW5	MAEGSGEVVAVSATGAANGLNNGAGGTSAT TCNPLSRKLHKILETRLDNDKEMLEALKALST FFVENSLRTRRNLRGDIERKSLAINEEFVSIFK EVKEELESISEDVQAMSNCCQDMTSRLQAAK EQTQDLIVKTTKLQSESQKLEIRAQVADAFLS KFQLTSDEMSLLRGTREGPITEDFFKALGRVK QIHNDVKVLLRTNQQTAGLEIMEQMALLQET AYERLYRWAQSECRTLTQESCDVSPVLTQA MEALQDRPVLYKYTLDEFGTARRSTVVRGFI DALTRGGPGGTPRPIEMHSHDPLRYVGDMLA WLHQATASEKEHLEALLKHVTTQGVEENIQE VVGHITEGVCRPLKVRIEQVIVAEPGAVLLYK ISNLLKFYHHTISGIVGNSATALLTTIEEMHLL SKKIFFNSLSLHASKLMDKVELPPPDLGPSSA LNQTLMLLREVLASHDSSVVPLDARQADFVQ VLSCVLDPLLQMCTVSASNLGTADMATFMV NSLYMMKTTLALFEFTDRRLEMLQFQIEAHL DTLINEQASYVLTRVGLSYIYNTVQQHKPEQ GSLANMPNLDSVTLKAAMVQFDRYLSAPDN LLIPQLNFLLSATVKEQIVKQSTELVCRAYGE VYAAVMNPINEYKDPENILHRSPQQVQTLLS [SEQ ID NO:310]	COG6-CDG	Glycosylation disorder
COG 7	91949	0168434	A0A0S2Z 652, P83436	MDFSKFLADDFDVKEWINAAFRAGSKEAAS GKADGHAATLVMKLQLFIQEVNHAVEETSH QALQNMPKVLRDVEALKQEASFLKEQMILV KEDIKKFEQDTSQSMQVLVEIDQVKSRMQLA AESLQEADKWSTLSADIEETFKTQDIAVISAK LTGMQNSLMMLVDTPDYSEKCVHLEALKNR LEALASPQIVAAFTSQAVDQSKVFVKVFTEID RMPQLLAYYYKCHKVQLLAAWQELCQSDLS LDRQLTGLYDALLGAWHTQIQWATQVFQKP HEVVMVLLIQTLGALMPSLPSCLSNGVERAG PEQELTRLLEFYDATAHFAKGLEMALLPHLH EHNLVKVTELVDAVYDPYKPYQLKYGDMEE SNLLIQMSAVPLEHGEVIDCVQELSHSVNKLF	COG7-CDG	Glycosylation disorder

				GLASAAVDRCVRFTNGLGTCGLLSALKSLFA KYVSDFTSTLQSIRKKCKLDHIPPNSLFQEDW TAFQNSIRIIATCGELLRHCGDFEQQLANRILS TAGKYLSDSCSPRSLAGFQESILTDKKNSAKN PWQEYNYLQKDNPAEYASLMEILYTLKEKGS SNHNLLAAPRAALTRLNQQAHQLAFDSVFLR IKQQLLLISKMDSWNTAGIGETLTDELPAFSL TPLEYISNIGQYIMSLPLNLEPFVTQEDSALEL ALHAGKLPFPPEQGDELPELDNMADNWLGSI ARATMQTYCDAILQIPELSPHSAKQLATDIDY LINVMDALGLQPSRTLQHIVTLLKTRPEDYRQ VSKGLPRRLATTVATMRSVNY [SEQ ID NO:311]		
COG 8	84342	0272617	A0A024R 6Z6, Q96MW5	MATAATIPSVATATAAALGEVEDEGLLASLF RDRFPEAQWRERPDVGRYLRELSGSGLERLR REPERLAEERAQLLQQTRDLAFANYKTFIRG AECTERIHRLFGDVEASLGRLLDRLPSFQQSC RNFVKEAEEISSNRRMNSLTLNRHTEILEILEIP QLMDTCVRNSYYEEALELAAYVRRLERKYSS IPVIQGIVNEVRQSMQLMLSQLIQQLRTNIQLP ACLRVIGYLRRMDVFTEAELRVKFLQARDA WLRSILTAIPNDDPYFHITKTIEASRVHLFDIIT QYRAIFSDEDPLLPPAMGEHTVNESAIFHGW VLQKVSQFLQVLETDLYRGIGGHLDSLLGQC MYFGLSFSRVGADFRGQLAPVFQRVAISTFQ KAIQETVEKFQEEMNSYMLISAPAILGTSNMP AAVPATQPGTLQPPMVLLDFPPLACFLNNILV AFNDLRLCCPVALAQDVTGALEDALAKVTKI ILAFHRAEEAAFSSGEQELFVQFCTVFLEDLV PYLNRCLQVLFPPAQIAQTLGIPPTQLSKYGN LGHVNIGAIQEPLAFILPKRETLFTLDDQALGP ELTAPAPEPPAEEPRLEPAGPACPEGGRAETQ AEPPSVGP [SEQ ID NO:312]	COG8-CDG	Glycosylation disorder
DOLK	22845	0175283	A0A0S2Z 597, Q9UPQ8	DRLLQQGSAVFQFRMSANSGLLPASMVMPLL GLVMKERCQTAGNPFFERFGIVVAATGMAV ALFSSVLALGITRPVPTNTCVILGLAGGVIIYI MKHSLSVGEVIEVLEVLLIFVYLNMILLYLLP RCFTPGEALLVLGGISFVLNQLIKRSLTLVESQ GDPVDFFLLVVVVGMVLMGIFFSTLFVFMDS GTWASSIFFHLMTCVLSLGVVLPWLHRLIRR NPLLWLLQFLFQTDTRIYLLAYWSLLATLAC LVVLYQNAKRSSSESKKHQAPTIARKYFHLIV VATYIPGIIFDRPLLYVAATVCLAVFIFLEYVR YFRIKPLGHTLRSFLSLFLDERDSGPLILTHIYL LLGMSLPIWLIPRPCTQKGSLGGARALVPYAG VLAVGVGDTVASIFGSTMGEIRWPGTKKTFE GTMTSIFAQIISVALILIFDSGVDLNYSYAWIL GSISTVSLLEAYTTQIDNLLLPLYLLILLMA [SEQ ID NO:313]	DOLK-CDG	Glycosylation disorder
DHDDS	79947	0117682	Q86SQ9	MSWIKEGELSLWERFCANIIKAGPMPKHIAFI MDGNRRYAKKCQVERQEGHSQGFNKLAETL RWCLNLGILEVTVYAFSIENFKRSKSEVDGL MDLARQKFSRLMEEKEKLQKHGVCIRVLGD LHLLPLDLQELIAQAVQATKNYNKCFLNVCF AYTSRHEISNAVREMAWGVEQGLLDPSDISE SLLDKCLYTNRSPHPDILIRTSGEVRLSDFLLW QTSHSCLVFQPVLWPEYTFWNLFEAILQFQM NHSVLQKARDMYAEERKRQQLERDQATVTE QLLREGLQASGDAQLRRTRLHKLSARREERV	DHDDS-CDG	Glycosylation disorder

				QGFLQALELKRADWLARLGTASA [SEQ ID NO:314]		
DPAGT1	1798	0172269	A0A024R3H8, Q9H3H5	MWAFSELPMPLLINLIVSLLGFVATVTLIPAFR GHFIAARLCGQDLNKTSRQQIPESQGVISGAV FLIILFCFIPFPFLNCFVKEQCKAFPHHEFVALI GALLAICCMIFLGFADDVLNLRWRHKLLLPT AASLPLLMVYFTNFGNTTIVVPKPFRPILGLH LDLGILYYVYMGLLAVFCTNAINILAGINGLE AGQSLVISASIIVFNLVELEGDCRDDHVFSLYF MIPFFFTTLGLLYHNWYPSRVFVGDTFCYFA GMTFAVVGILGHFSKTMLLFFMPQVFNFLYS LPQLLHIIPCPRHRIPRLNIKTGKLEMSYSKFK TKSLSFLGTFILKVAESLQLVTVHQSETEDGE FTECNNMTLINLLLKVLGPIHERNLTLLLLLL QILGSAITFSIRYQLVRLFYDV [SEQ ID NO:315]	DPAGT1-CDG	Glycosylation disorder
DPM1	8813	0000419	O60762, Q5QPK2, A0A0S2Z4Y5	MASLEVSRSPRRSRRELEVRSPRQNKYSVLLP TYNERENLPLIVWLLVKSFSESGINYEIIIIDDG SPDGTRDVAEQLEKIYGSDRILLRPREKKLGL GTAYIHGMKHATGNYIIIMDADLSHHPKFIPE FIRKQKEGNFDIVSGTRYKGNGGVYGWDLK RKIISRGANFLTQILLRPGASDLTGSFRLYRKE VLEKLIEKCVSKGYVFQMEMIVRARQLNYTI GEVPISFVDRVYGESK LGGNEIVSFLKGLLTLFATT [SEQ ID NO:316]	DPM1-CDG	Glycosylation disorder
DPM2	8818	0136908	O94777	MATGTDQVVGLGLVAVSLIIFTYYTAWVILL PFIDSQHVIHKYFLPRAYAVAIPLAAGLLLLLF VGLFISYVMLKTKRVTKKAQ [SEQ ID NO:317]	DPM2-CDG	Glycosylation disorder
DPM3	54344	0179085	A0A140VJI4, Q9P2X0, Q86TM7	MTKLAQWLWGLAILGSTWVALTTGALGLEL PLSCQEVLWPLPAYLLVSAGCYALGTVGYRV ATFHDCEDAARELQSQIQEARADLARRGLRF [SEQ ID NO:318]	DPM3-CDG	Glycosylation disorder
G6PC3	92579	0141349	Q9BUM1	MESTLGAGIVIAEALQNQLAWLENVWLWITF LGDPKILFLFYFPAAYYASRRVGIAVLWISLIT EWLNLIFKWFLFGDRPFWWVHESGYYSQAP AQVHQFPSSCETGPGSPSGHCMITGAALWPI MTALSSQVATRARSRWVRVMPSLAYCTFLL AVGLSRIFILAHFPHQVLAGLITGAVLGWLMT PRVPMERELSFYGLTALALMLGTSLIYWTLFT LGLDLSWSISLAFKWCERPEWIHVDSRPFASL SRDSGAALGLGIALHSPCYAQVRRAQLGNGQ KIACLVLAMGLLGPLDWLGHPPQISLFYIFNF LKYTLWPCLVLALVPWAVHMFSAQEAPPIHSS [SEQ ID NO:319]	Congenital neutropenia	Glycosylation disorder
GFPT1	2673	0198380	Q06210	MCGIFAYLNYHVPRTRREILETLIKGLQRLEY RGYDSAGVGFDGGNDKDWEANACKIQLIKK KGKVKALDEEVHKQQDMDLDIEFDVHLGIA HTRWATHGEPSPVNSHPQRSDKNNEFIVIHN GIITNYKDLKKFLESKGYDFESETDTETIAKLV KYMYDNRESQDTSFTTLVERVIQQLEGAFAL VFKSVHFPGQAVGTRRGSPLLIGVRSEHKLST DHIPILYRTARTQIGSKFTRWGSQGERGKDKK GSCNLSRVDSTTCLFPVEEKAVEYYFASDAS AVIEHTNRVIFLEDDDVAAVVDGRLSIHRIKR TAGDHPGRAVQTLQMELQQIMKGNFSSFMQ KEIFEQPESVVNTMRGRVNFDDYTVNLGGLK DHIKEIQRCRRLILIACGTSYHAGVATRQVLE ELTELPVMVELASDFLDRNTPVFRDDVCFFLS QSGETADTLMGLRYCKERGALTVGITNTVGS SISRETDCGVHINAGPEIGVASTKAYTSQFVSL	Congenital myasthenic syndrome	Glycosylation disorder

				VMFALMMCDDRISMQERRKEIMLGLKRLPD LIKEVLSMDDEIQKLATELYHQKSVLIMGRG YHYATCLEGALKIKEITYMHSEGILAGELKHG PLALVDKLMPVIMIIMRDHTYAKCQNALQQV VARQGRPVVICDKEDTETIKNTKRTIKVPHSV DCLQGILSVIPLQLLAFHLAVLRGYDVDFPRN LAKSVTVE [SEQ ID NO:320]		
GMPPA	29926	0144591	A0A024R482, Q96IJ6	MLKAVILIGGPQKGTRFRPLSFEVPKPLFPVA GVPMIQHHIEACAQVPGMQEILLIGFYQPDEP LTQFLEAAQQEFNLPVRYLQEFAPLGTGGGL YHFRDQILAGSPEAFFVLNADVCSDFPLSAML EAHRRQRHPFLLLGTTANRTQSLNYGCIVENP QTHEVLHYVEKPSTFISDIINCGIYLFSPEALKP LRDVFQRNQQDGQLEDSPGLWPGAGTIRLEQ DVFSALAGQGQIYVHL TDGIWSQIKSAGSALYASRLYLSRYQDTHPER LAKHTPGGPWIRGNVYIHPTAKVAPSAVLGP NVSIGKGVTVGEGVRLRESIVLHGATLQEHT CVLHSIVGWGSTVGRWARVEGTPSDPNPNDP RARMDSESLFKDGKLLPAITILGCRVRIPAEV LILNSIVLPHKELSRSFTNQIIL [SEQ ID NO:321]	GMPPA-CDG	Glycosylation disorder
GMPPB	29925	0173540	Q9Y5P6	MKALILVGGYGTRLRPLTLSTPKPLVDFCNKP ILLHQVEALAAAGVDHVILAVSYMSQVLEKE MKAQEQRLGIRISMSHEEEPLGTAGPLALAR DLLSETADPFFVLNSDVICDFPFQAMVQFHRH HGQEGSILVTKVEEPSKYGVVVCEADTGRIH RFVEKPQVFVSNKINAGMYILSPAVLQRIQLQ PTSIEKEVFPIMAKEGQLYAMELQGFWMDIG QPKDFLTGMCLFLQSLRQKQPERLCSGPGIVG NVLVDPSARIGQNCSIGPNVSLGPGVVVEDG VCIRRCTVLRDARIRSHSWLESCIVGWRCRV GQWVRMENVTVLGEDVIVNDELYLNGASVL PHKSIGESVPEPRIIM [SEQ ID NO:322]	Congenital muscular dystrophy, congenital myasthenic syndrome, and dystroglycanopathy	Glycosylation disorder
MAGT1	84061	0102158	A0A087WU53, Q9H0U3	MAARWRFWCVSVTMVVALLIVCDVPSASAQ RKKEMVLSEKVSQLMEWTNKRPVIRMNGDK FRRLVKAPPRNYSVIVMFTALQLHRQCVVCK QADEEFQILANSWRYSSAFTNRIFFAMVDFDE GSDVFQMLNMNSAPTFINFPAKGKPKRGDTY ELQVRGFSAEQIARWIADRTDVNIRVIRPPNY AGPLMLGLLLAVIGGLVYLRRSNMEFLFNKT GWAFAALCFVLAMTSGQMWNHIRGPPYAHK NPHTGHVNYIIHGSSQAQFVAETHIVLLFNGG VTLGMVLLCEAATSDMDIGKRKIMCVAGIGL VVLFFSWMLSIFRSKYHGYPYSFLMS [SEQ ID NO:323]	MAGT1-CDG; X-linked immunodeficiency with magnesium defect, Epstein-Barr virus infection and neoplasia (XMEN) syndrome	Glycosylation disorder
MAN1B1	11253	0177239	Q9UKM7	MAACEGRRSGALGSSQSDFLTPPVGGAPWA VATTVVMYPPPPPPPHRDFISVTLSFGENYDN SKSWRRRSCWRKWKQLSRLQRNMILFLLAFL LFCGLLFYINLADHWKALAFRLEEEQKMRPEI AGLKPANPPVLPAPQKADTDPENLPEISSQKT QRHIQRGPPHLQIRPPSQDLKDGTQEEATKRQ EAPVDPRPEGDPQRTVISWRGAVIEPEQGTEL PSRRAEVPTKPPLPPARTQGTPVHLNYRQKG VIDVFLHAWKGYRKFAWGHDELKPVSRSFSE WFGLGLTLIDALDTMWILGLRKEFEEARKWV SKKLHFEKDVDVNLFESTIRILGGLLSAYHLS	MAN1B1-CDG	Glycosylation disorder

				GDSLFLRKAEDFGNRLMPAFRTPSKIPYSDVN IGTGVAHPPRWTSDSTVAEVTSIQLEFRELSR LTGDKKFQEAVEKVTQHIHGLSGKKDGLVP MFINTHSGLFTHLGVFTLGARADSYYEYLLK QWIQGGKQETQLLEDYVEAIEGVRTHLLRHS EPSKLTFVGELAHGRFSAKMDHLVCFLPGTL ALGVYHGLPASHMELAQELMETCYQMNRQ METGLSPEIVHFNLYPQPGRRDVEVKPADRH NLLRPETVESLFYLYRVTGDRKYQDWGWEIL QSFSRFTRVPSGGYSSINNVQDPQKPEPRDKM ESFFLGETLKYLFLLFSDDPNLLSLDAYVFNT EAHPLPIWTPA [SEQ ID NO:324]		
MG AT2	4247	0168282	Q10469	MRFRIYKRKVLILTLVVAACGFVLWSSNGRQ RKNEALAPPLLDAEPARGAGGRGGDHPSVA VGIRRVSNVSAASLVPAVPQPEADNLTLRYRS LVYQLNFDQTLRNVDKAGTWAPRELVLVVQ VHNRPEYLRLLLDSLRKAQGIDNVLVIFSHDF WSTEINQLIAGVNFCPVLQVFFPFSIQLYPNEF PGSDPRDCPRDLPKNAALKLGCINAEYPDSFG HYREAKFSQTKHHWWWKLHFVWERVKILR DYAGLILFLEEDHYLAPDFYHVFKKMWKLK QQECPECDVLSLGTYSASRSF YGMADKVDVKTWKSTEHNMGLALTRNAYQ KLIECTDTFCTYDDYNWDWTLQYLTVSCLPK FWKVLVPQIPRIFHAGDCGMHHKKTCRPSTQ SAQIESLLNNNKQYMFPETLTISEKFTVVAISP PRKNGGWGDIRDHELCKSYRRLQ [SEQ ID NO:325]	MGAT2-CDG	Glycosylation disorder
MO GS	7841	0115275	Q13724, Q58F09	MARGERRRRAVPAEGVRTAERAARGGPGRR DGRGGGPRSTAGGVALAVVVLSLALGMSGR WVLAWYRARRAVTLHSAPPVLPADSSSPAV APDLFWGTYRPHVYFGMKTRSPKPLLTGLM WAQQGTTPGTPKLRHTCEQGDGVGPYGWEF HDGLSFGRQHIQDGALRLTTEFVKRPGGQHG GDWSWRVTVEPQDSGTSALPLVSLFFYVVTD GKEVLLPEVGAKGQLKFISGHTSELGDFRFTL LPPTSPGDTAPKYGSYNVFWTSNPGLPLLTE MVKSRLNSWFQHRPPGAPPERYLGLPGSLKW EDRGPSGQGQGQFLIQQVTLKIPISIEFVFESGS AQAGGNQALPRLAGSLLTQALESHAEGFRER FEKTFQLKEKGLSSGEQVLGQAALSGLLGGIG YFYGQGLVLPDIGVEGSEQKVDPALFPPVPLF TAVPSRSFFPRGFLWDEGFHQLVVQRWDPSL TREALGHWLGLLNADGWIGREQILGDEARA RVPPEFLVQRAVHANPPTLLLPVAHMLEVGD PDDLAFLRKALPRLHAWFSWLHQSQAGPLPL SYRWRGRDPALPTLLNPKTLPSGLDDYPRAS HPSVTERHLDLRCWVALGARVLTRLAEHLGE AEVAAELGPLAASLEAAESLDELHWAPELGV FADFGNHTKAVQLKPRPPQGLVRVVGRPQPQ LQYVDALGYVSLFPLLLRLLDPTSSRLGPLLD ILADSRHLWSPFGLRSLAASSSFYGQRNSEHD PPYWRGAVWLNVNYLALGALHHYGHLEGP HQARAAKLHGELRANVVGNVWRQYQATGF LWEQYSDRDGRGMGCRPFHGWTSLVLLAM AEDY [SEQ ID NO:326]	MOGS-CDG	Glycosylation disorder
MPD U1	9526	0129255	J3QW43, O75352, A0A0S2Z	MAAEADGPLKRLLVPILLPEKCYDQLFVQWD LLHVPCLKILLSKGLGLGIVAGSLLVKLPQVF KILGAKSAEGLSLQSVMLELVALTGTMVYSIT NNFPFSSWGEALFLMLQTITICFLVMHYRGQT	MPDU1-CDG	Glycosylation disorder

			4W8, B4DLH7	VKGVAFLACYGLVLLVLLSPLTPLTVVTLLQ ASNVPAVVVGRLLQAATNYHNGHTGQLSAIT VFLLFGGSLARIFTSIQETGDPLMAGTFVVSSL CNGLIAAQLLFYWNAKPPHKQKKAQ [SEQ ID NO:327]		
MPI	4351	0178802	H3BPP3, Q8NHZ6, B4DW50, F5GX71, P34949, H3BPB8	MAAPRVFPLSCAVQQYAWGKMGSNSEVARL LASSDPLAQIAEDKPYAELWMGTHPRGDAKI LDNRISQKTLSQWIAENQDSLGSKVKDTFNG NLPFLFKVLSVETPLSIQAHPNKELAEKLHLQ APQHYPDANHKPEMAIALTPFQGLCGFRPVE EIVTFLKKVPEFQFLIGDEAATHLKQTMSHDS QAVASSLQSCFSHLMKSEKKVVVEQLNLLVK RISQQAAAGNNMEDIFGELLLQLHQQYPGDI GCFAIYFLNLLTLKPGEAMFLEANVPHAYLK GDCVECMACSDNTVRAGLTP KFIDVPTLCEMLSYTPSSSKDRLFLPTRSQEDP YLSIYDPPVPDFTIMKTEVPGSVTEYKVLALD SASILLMVQGTVIASTPTTQTPIPLQRGGVLFI GANESVSLKLTEPKDLLIFRACCLL [SEQ ID NO:328]	MPI-CDG	Glycosylation disorder
NGLY1	55768	0151092	Q96IV0	MAAAALGSSSGSASPAVAELCQNTPETFLEA SKLLLTYADNILRNPNDEKYRSIRIGNTAFSTR LLPVRGAVECLFEMGFEEGETHLIFPKKASVE QLQKIRDLIAIERSSRLDGSNKSHKVKSSQQP AASTQLPTTPSSNPSGLNQHTRNRQGQSSDPP SASTVAADSAILEVLQSNIQHVLVYENPALQE KALACIPVQELKRKSQEKLSRARKLDKGINIS DEDFLLLELLHWFKEE FFHWVNNVLCSKCGGQTRSRDRSLLPSDDEL KWGAKEVEDHYCDACQFSNRFPRYNNPEKL LETRCGRCGEWANCFTLCCRAVGFEARYVW DYTDHVWTEVYSPSQQRWLHCDACEDVCD KPLLYEIGWGKKLSYVIAFSKDEVVDVTWRY SCKHEEVIARRTKVKEALLRDTINGLNKQRQ LFLSENRRKELLQRIIVELVEFISPKTPKPGELG GRISGSVAWRVARGEMGLQRKETLFIPCENE KISKQLHLCYNIVKDRYVRVSNNNQTISGWE NGVWKMESIFRKVETDWHMVYLARKEGSSF AYISWKFECGSVGLKVDSISIRTSSQTFQTGTV EWKLRSDTAQVELTGDNSLHSYADFSGATEV ILEAELSRGDGDVAWQHTQLFRQSLNDHEEN CLEIIIKFSDL [SEQ ID NO:329]	NGLY1-CDG	Glycosylation disorder
PGM1	5236	0079739	B7Z6C2, P36871, B4DDQ8	MVKIVTVKTQAYQDQKPGTSGLRKRVKVFQ SSANYAENFIQSIISTVEPAQRQEATLVVGGD GRFYMKEAIQLIARIAAANGIGRLVIGQNGILS TPAVSCIIRKIKAIGGIILTASHNPGGPNGDFGI KFNISNGGPAPEAITDKIFQISKTIEEYAVCPDL KVDLGVLGKQQFDLENKFKPFTVEIVDSVEA YATMLRSIFDFSALKELLSGPNRLKIRIDAMH GVVGPYVKKILCEELGAPANSAVNCVPLEDF GGHHPDPNLTYAADLVETMKSGEHDFGAAF DGDGDRNMILGKHGFFVNPSDSVAVIAANIFS IPYFQQTGVRGFARSMPTSGALDRVASATKIA LYETPTGWKFFGNLMDASKLSLCGEESFGTG SDHIREKDGLWAVLAWLSILATRKQSVEDIL KDHWQKYGRNFFTRYDYEEVEAEGANKMM KDLEALMFDRSFVGKQFSANDKVYTVEKAD NFEYSDPVDGSISRNQGLRLIFTDGSRIVFRLS GTGSAGATIRLYIDSYEKDVAKINQDPQVML	PGM1-CDG	Glycosylation disorder

				APLISIALKVSQLQERTGRTAPTVIT [SEQ ID NO:330]		
PGM3	5238	0013375	O95394, A0A087WT27	MDLGAITKYSALHAKPNGLILQYGTAGFRTKAEHLDHVMFRMGLLAVLRSKQTKSTIGVMVTASHNPEEDNGVKLVDPLGEMLAPSWEEHATCLANAEEQDMQRVLIDISEKEAVNLQQDAFVVIGRDTRPSSEKLSQSVIDGVTVLGGQFHDYGLLTTPQLHYMVYCRNTGGRYGKATIEGYYQKLSKAFVELTKQASCSGDEYRSLKVDCANGIGALKLREMEHYFSQGLSVQLFNDGSKGKLNHLCGADFVKSHQKPPQGMEIKSNERCCSFDGDADRIVYYYHDADGHFHLIDGDKIATLISSFLKELLVEIGESLNIGVVQTAYANGSSTRYLEEVMKVPVYCTKTGVKHLHHKAQEFDIGVYFEANGHGTALFSTAVEMKIKQSAEQLEDKKRKAAKMLENIIDLFNQAAGDAISDMLVIEAILALKGLTVQQWDALYTDLPNRQLKVQVADRRVISTTDAERQAVTPPGLQEAINDLVKKYKLSRAFVRPSGTEDVVRVYAEADSQESADHLAHEVSLAVFQLAGGIGERPQPGF [SEQ ID NO:331]	PGM3-CDG	Glycosylation disorder
RFT1	91869	0163933	Q96AA3	MGSQEVLGHAARLASSGLLLQVLFRLITFVLNAFILRFLSKEIVGVVNVRLTLLYSTTLFLAREAFRRACLSGGTQRDWSQTLNLLWLTVPLGVFWSLFLGWIWLQLLEVPDPNVVPHYATGVVLFGLSAVVELLGEPFWVLAQAHMFVKLKVIAESLSVILKSVLTAFLVLWLPHWGLYIFSLAQLFYTTVLVLCYVIYFTKLLGSPESTKLQTLPVSRITDLLPNITRNGAFINWKEAKLTWSFFKQSFLKQILTEGERYVMTFLNVLNFGDQGVYDIVNNLGSLVARLIFQPIEESFYIFFAKVLERGKDATLQKQEDVAVAAAVLESLLKLALLAGLTITVFGFAYSQLALDIYGGTMLSSGSGPVLLRSYCLYVLLLAINGVTECFTFAAMSKEEVDRYNFVMLALSSSFLVLSYLLTRWCGSVGFILANCFNMGIRITQSLCFIHRYYRRSPHRPLAGLHLSPVLLGTFALSGGVTAVSEVFLCCEQGWPARLAHIAVGAFCLGATLGTAFLTETKLIHFLRTQLGVPRRTDKMT [SEQ ID NO:332]	RFT1-CDG	Glycosylation disorder
SEC23B	10483	0101310	Q15437, B4DJW8	MATYLEFIQQNEERDGVRFSWNVWPSSRLEATRMVVPLACLLTPLKERPDLPPVQYEPVLCSRPTCKAVLNPLCQVDYRAKLWACNFCFQRNQFPPAYGGISEVNQPAELMPQFSTIEYVIQRGAQSPLIFLYVVDTCLEEDDLQALKESLQMSLSLLPPDALVGLITFGRMVQVHELSCEGISKSYVFRGTKDLTAKQIQDMLGLTKPAMPMQQARPAQPQEHPFASSRFLQPVHKIDMNLTDLLGELQRDPWPVTQGKRPLRSTGVALSIAVGLLEGTFPNTGARIMLFTGGPPTQGPGMVVGDELKIPIRSWHDIEKDNARFMKKATKHYEMLANRTAANGHCIDIYACALDQTGLLEMKCCANLTGGYMVMGDSFNTSLFKQTFQRIFTKDFNGDFRMAFGATLDVKTSRELKIAGAIGPCVSLNVKGPCVSENELGVGGTSQWKICGLDPTSTLGIYFEVVNQHNTPIPQGGRGAIQFVTHYQHSSTQRRIRVTTIARNWADVQSQLRHIEAAFDQEAAAVLMARLGVFRAESEEGPDVLRWLDRQLIRLCQKFGQYNKEDPTSFRLSDSFSLYPQFMFHLRRSPFLQVFNNSPDESSYYRHHFARQDLTQSLIMIQPILYSYSFHGPPEPVLLDSSSILADRILLMDTFFQIVIYLGETIAQWRKAGYQDMPEYENFKHLLQAPLDD	SEC23B-CDG	Glycosylation disorder

				AQEILQARFPMPRYINTEHGGSQARFLLSKVN PSQTHNNLYAWGQETGAPILTDDVSLQVFMD HLKKLAVSSAC [SEQ ID NO:333]		
SLC 35A1	10559	0164414	P78382	MAAPRDNVTLLFKLYCLAVMTLMAAVYTIA LRYTRTSDKELYFSTTAVCITEVIKLLLSVGIL AKETGSLGRFKASLRENVLGSPKELLKLSVPS LVYAVQNNMAFLALSNLDAAVYQVTYQLKI PCTALCTVLMLNRTLSKLQWVSVFMLCAGV TLVQWKPAQATKVVVEQNPLLGFGAIAIAVL CSGFAGVYFEKVLKSSDTSLWVRNIQMYLSG IIVTLAGVYLSDGAEIKEKGFFYGYTYYVWF VIFLASVGGLYTSVVVKYTDNIMKGFSAAAA IVLSTIASVMLFGLQITLTFALGTLLVCVSIYL YGLPRQDTTSIQQGETASKERVIGV [SEQ ID NO:334]	SLC35A1-CDG	Glycosylation disorder
SLC 35A2	7355	0102100	P78381, A6NFI1, A6NKM8, B4DE15	MAAVGAGGSTAAPGPGAVSAGALEPGTASA AHRRLKYISLAVLVVQNASLILSIRYARTLPG DRFFATTAVVMAEVLKGLTCLLLLFAQKRGN VKHLVLFLHEAVLVQYVDTLKLAVPSLIYTL QNNLQYVAISNLPAATFQVTYQLKILTTALFS VLMLNRSLSRLQWASLLLLFTGVAIVQAQQA GGGGPRPLDQNPGAGLAAVVASCLSSGFAGV YFEKILKGSSGSVWLRNLQLGLFGTALGLVG LWWAEGTAVATRGFFFGYTPAVWGVVLNQ AFGGLLVAVVVKYADNILKGFATSLSIVLSTV ASIRLFGFHVDPLFALGAGLVIGAVYLYSLPR GAAKAIASASASASGPCVHQQPPGQPPPPQLS SHRGDLITEPFLPKLLTKVKGS [SEQ ID NO:335]	SLC35A2-CDG	Glycosylation disorder
SLC 35C1	55343	0181830	Q96A29, B3KQH0	MNRAPLKRSRILHMALTGASDPSAEAEANGE KPFLLRALQIALVVSLYWVTSISMVFLNKYLL DSPSLRLDTPIFVTFYQCLVTTLLCKGLSALA ACCPGAVDFPSLRLDLRVARSVLPLSVVFIGM ITFNNLCLKYVGVAFYNVGRSLTTVFNVLLS YLLLKQTTSFYALLTCGIIIGGFWLGVDQEGA EGTLSWLGTVFGVLASLCVSLNAIYTTKVLP AVDGSIWRLTFYNNVNACILFLPLLLLLGELQ ALRDFAQLGSAHFWGMMTLGGLFGFAIGYV TGLQIKFTSPLTHNVSG TAKACAQTVLAVLYYEETKSFLWWTSNMM VLGGSSAYTWVRGWEMKKTPEEPSPKDSEK SAMGV [SEQ ID NO:336]	SLC35C1-CDG	Glycosylation disorder
SSR 4	6748	0180879	P51571	MAAMASLGALALLLLSSLSRCSAEACLEPQIT PSYYTTSDAVISTETVFIVEISLTCKNRVQNM ALYADVGGKQFPVTRGQDVGRYQVSWSLDH KSAHAGTYEVRFFDEESYSLLRKAQRNNEDIS IIPPLFTVSVDHRGTWNGPWVSTEVLAAAIGL VIYYLAFSAKSHIQA [SEQ ID NO:337]	SSR4-CDG	Glycosylation disorder
SRD 5A3	79644	0128039	Q9H8P0	MAPWAEAEHSALNPLRAVWLTLTAAFLLTL LLQLLPPGLLPGCAIFQDLIRYGKTKCGEPSRP AACRAFDVPKRYFSHFYIISVLWNGFLLWCL TQSLFLGAPFPSWLHGLLRILGAAQFQGGELA LSAFLVLVFLWLHSLRRLFECLYVSVFSNVMI HVVQYCFGLVYYVLVGLTVLSQVPMDGRNA YITGKNLLMQARWFHILGMMMFIWSSAHQY KCHVILGNLRKNKAGVVIHCNHRIPFGDWFE YVSSPNYLAELMIYVSMAVTFGFHNLTWWL VVTNVFFNQALSAFLSHQFYKSKFVSYPKHR KAFLPFLF [SEQ ID NO:338]	SRD5A3-CDG	Glycosylation disorder

TMEM165	55858	0134851	Q9HC07	MAAAAPGNGRASAPRLLLLFLVPLLWAPAA VRAGPDEDLSHRNKEPPAPAQQLQPQPVAVQ GPEPARVEKIFTPAAPVHTNKEDPATQTNLGF IHAFVAAISVIIVSELGDKTFFIAAIMAMRYNR LTVLAGAMLALGLMTCLSVLFGYATTVIPRV YTYYVSTVLFAIFGIRMLREGLKMSPDEGQEE LEEVQAELKKKDEEFQRTKLLNGPGDVETGT SITVPQKKWLHFISPIFVQALTLTFLAEWGDR SQLTTIVLAAREDPYGVAVGGTVGHCLCTGL AVIGGRMIAQKISVRTVTIIGGIVFLAFAFSAL FISPDSGF [SEQ ID NO:339]	TMEM165-CDG	Glycosylation disorder
TRIP11	9321	0100815	Q15643	MSSWLGGLGSGLGQSLGQVGGSLASLTGQIS NFTKDMLMEGTEEVEAELPDSRTKEIEAIHAI LRSENERLKKLCTDLEEKHEASEIQIKQQSTS YRNQLQQKEVEISHLKARQIALQDQLLKLQS AAQSVPSGAGVPATTASSSFAYGISHHPSAFH DDDMDFGDIISSQQEINRLSNEVSRLESEVGH WRHIAQTSKAQGTDNSDQSEICKLQNIIKELK QNRSQEIDDHQHEMSVLQNAHQQKLTEISRR HREELSDYEERIEELENLLQQGGSGVIETDLS KIYEMQKTIQVLQIEKVESTKKMEQLEDKIKD INKKLSSAENDRDILRREQEQLNVEKRQIMEE CENLKLECSKLQPSAVKQSDTMTEKERILAQS ASVEEVFRLQQALSDAENEIMRLSSLNQDNSL AEDNLKLKMRIEVLEKEKSLLSQEKEELQMS LLKLNNEYEVIKSTATRDISLDSELHDLRLNL EAKEQELNQSISEKETLIAEIEELDRQNQEATK HMILIKDQLSKQQNEGDSIISKLKQDLNDEKK RVHQLEDDKMDITKELDVQKEKLIQSEVALN DLHLTKQKLEDKVENLVDQLNKSQESNVSIQ KENLELKEHIRQNEEELSRIRNELMQSLNQDS NSNFKDTLLKEREAEVRNLKQNLSELEQLNE NLKKVAFDVKMENEKLVLACEDVRHQLEEC LAGNNQLSLEKNTIVETLKMEKGEIEAELCW AKKRLLEEANKYEKTIEELSNARNLNTSALQ LEHEHLIKLNQKKDMEIAELKKNIEQMDTDH KETKDVLSSSLEEQKQLTQLINKKEIFIEKLKE RSSKLQEELDKYSQALRKNEILRQTIEEKDRS LGSMKEENNHLQEELERLREEQSRTAPVADP KTLDSVTELASEVSQLNTIKEHLEEEIKHHQKI IEDQNQSKMQLLQSLQEQKKEMDEFRYQHE QMNATHTQLFLEKDEEIKSLQKTIEQIKTQLH EERQDIQTDNSDIFQETKVQSLNIENGSEKHD LSKAETERLVKGIKERELEIKLLNEKNISLTKQ IDQLSKDEVGKLTQIIQQKDLEIQALHARISST SHTQDVVYLQQQLQAYAMEREKVFAVLNEK TRENSHLKTEYHKMMDIVAAKEAALIKLQDE NKKLSTRFESSGQDMFRETIQNLSRIIREKDIEI DALSQKCQTLLAVLQTSSTGNEAGGVNSNQF EELLQERDKLKQQVKKMEEWKQQVMTTVQ NMQHESAQLQEELHQLQAQVLVDSDNNSKL QVDYTGLIQSYEQNETKLKNFGQELAQVQHS IGQLCNTKDLLLGKLDIISPQLSSASLLTPQSA ECLRASKSEVLSESSELLQQELEELRKSLQEK DATIRTLQENNHRLSDSIAATSELERKEHEQT DSEIKQLKEKQDVLQKLLKEKDLLIKAKSDQ LLSSNENFTNKVNENELLRQAVTNLKERILIL EMDIGKLKGENEKIVETYRGKETEYQALQET NMKFSMMLREKEFECHSMKEKALAFEQLLK EKEQGKTGELNQLLNAVKSMQEKTVVFQQE	TRIP11-CDG	Glycosylation disorder

				RDQVMLALKQKQMENTALQNEVQRLRDKEF RSNQELERLRNHLLESEDSYTREALAAEDRE AKLRKKVTVLEEKLVSSSNAMENASHQASV QVESLQEQLNVVSKQRDETALQLSVSQEQVK QYALSLANLQMVLEHFQQEEKAMYSAELEK QKQLIAEWKKNAENLEGKVISLQECLDEANA ALDSASRLTEQLDVKEEQIEELKRQNELRQE MLDDVQKKLMSLANSSEGKVDKVLMRNLFI GHFHTPKNQRHEVLRLMGSILGVRREEMEQL FHDDQGGVTRWMTGWLGGGSKSVPNTPLRP NQQSVVNSSFSELFVKFLETESHPSIPPPKLSV HDMKPLDSPGRRKRDTNAPESFKDTAESRSG RRTDVNPFLAPRSAAVPLINPAGLGPGGPGHL LLKPISDVLPTFTPLPALPDNSAGVVLKDLLK Q [SEQ ID NO:340]		
TUSC3	7991	0104723	Q13454	MGARGAPSRRRQAGRRLRYLPTGSFPFLLLL LLLCIQLGGGQKKKENLLAEKVEQLMEWSSR RSIFRMNGDKFRKFIKAPPRNYSMIVMFTALQ PQRQCSVCRQANEEYQILANSWRYSSAFCNK LFFSMVDYDEGTDVFQQLNMNSAPTFMHFPP KGRPKRADTFDLQRIGFAAEQLAKWIADRTD VHIRVFRPPNYSGTIALALLVSLVGGLLYLRR NNLEFIYNKTGWAMVSLCIVFAMTSGQMWN HIRGPPYAHKNPHNGQVSYIHGSSQAQFVAE SHIILVLNAAITMGMVLLNE AATSKGDVGKRRIICLVGLGLVVFFFSFLLSIF RSKYHGYPYSDLDFE [SEQ ID NO:341]	TUSC3-CDG	Glycosylation disorder
ALG14	199857	0172339	Q96F25	MVCVLVLAAAAGAVAVFLILRIWVVLRSMD VTPRESLSILVVAGSGGHTTEILRLLGSLSNAY SPRHYVIADTDEMSANKINSFELDRADRDPSN MYTKYYIHRIPRSREVQQSWPSTVFTTLHSM WLSFPLIHRVKPDLVLCNGPGTCVPICVSALL LGILGIKKVIIVYVESICRVETLSMSGKILFHLS DYFIVQWPALKEKYPKSVYLGRIV [SEQ ID NO:342]	ALG14-CDG	Glycosylation disorder
B4GALT1	2683	0086062	P15291, W6MEN3	MRLREPLLSGSAAMPGASLQRACRLLVAVCA LHLGVTLVYYLAGRDLSRLPQLVGVSTPLQG GSNSAAAIGQSSGELRTGGARPPPPLGASSQP RPGGDSSPVVDSGPGPASNLTSVPVPHTTALS LPACPEESPLLVGPMLIEFNMPVDLELVAKQN PNVKMGGRYAPRDCVSPHKVAIIIPFRNRQEH LKYWLYYLHPVLQRQQLDYGIYVINQAGDTI FNRAKLLNVGFQEALKDYDYTCFVFSDVDLI PMNDHNAYRCFSQPRHISVAMDKFGFSLPYV QYFGGVSALSKQQFLTINGFPNNYWGWGGE DDDIFNRLVFRGMSISRPNAVVGRCRMIRHSR DKKNEPNPQRFDRIAHTKETMLSDGLNSLTY QVLDVQRYPLYTQITVDIGTPS [SEQ ID NO:343]	B4GALT1-CDG	Glycosylation disorder
DDOST	1650	0244038	A0A024RAD5, P39656	MGYFRCARAGSFGRRRKMEPSTAARAWALF WLLLPLLGAVCASGPRTLVLLDNLNVRETHS LFFRSLKDRGFELTFKTADDPSLSLIKYGEFLY DNLIIFSPSVEDFGGNINVETISAFIDGGGSVLV AASSDIGDPLRELGSECGIEFDEEKTAVIDHH NYDISDLGQHTLIVADTENLLKAPTIVGKSSL NPILFRGVGMVADPDNPLVLDILTGSSTSYSF FPDKPITQYPHAVGKNTLLIAGLQARNNARVI FSGSLDFFSDSFFNSAVQKAAPGSQRYSQTGN YELAVALSRWVFKEEGVLRVGPVSHHRVGE TAPPNAYTVTDLVEYSIVIQQLSNGKWVPFD	DDOST-CDG	Glycosylation disorder

				GDDIQLEFVRIDPFVRTFLKKKGGKYSVQFKL PDVYGVFQFKVDYNRLGYTHLYSSTQVSVRP LQHTQYERFIPSAYPYYASAFSMMLGLFIFSIV FLHMKEKEKSD [SEQ ID NO:344]		
NUS 1	116150	0153989	Q96E22	MTGLYELVWRVLHALLCLHRTLTSWLRVRF GTWNWIWRRCCRAASAAVLAPLGFTLRKPP AVGRNRRHHRHPRGGSCLAAAHHRMRWRA DGRSLEKLPVHMGLVITEVEQEPSFSDIASLV VWCMAVGISYISVYDHQGIFKRNNSRLMDEI LKQQQELLGLDCSKYSPEFANSNDKDDQVLN CHLAVKVLSPEDGKADIVRAAQDFCQLVAQ KQKRPTDLDVDTLASLLSSNGCPDPDLVLKF GPVDSTLGFLPWHIRLTEIVSLPSHLNISYEDF FSALRQYAACEQRLGK [SEQ ID NO:345]	NUS1-CDG	Glycosylation disorder
RPN 2	6185	0118705	P04844	MAPPGSSTVFLLALTIIASTWALTPTHYLTKH DVERLKASLDRPFTNLESAFYSIVGLSSLGAQ VPDAKKACTYIRSNLDPSNVDSLFYAAQASQ ALSGCEISISNETKDLLLAAVSEDSSVTQIYHA VAALSGFGLPLASQEALSALTARLSKEETVLA TVQALQTASHLSQQADLRSIVEEIEDLVARLD ELGGVYLQFEEGLETTALFVAATYKLMDHV GTEPSIKEDQVIQLMNAIFSKKNFESLSEAFSV ASAAAVLSHNRYHVPVVVVPEGSASDTHEQ AILRLQVTNVLSQPLTQATVKLEHAKSVASR ATVLQKTSFTPVGDVFELNFMNVKFSSGYYD FLVEVEGDNRYIANTVELRVKISTEVGITNVD LSTVDKDQSIAPKTTRVTYPAKAKGTFIADSH QNFALFFQLVDVNTGAELTPHQTFVRLHNQK TGQEVVFVAEPDNKNVYKFELDTSERKIEFDS ASGTYTLYLIIGDATLKNPILWNVADVVIKFP EEEAPSTVLSQNLFTPKQEIQHLFREPEKRPPT V VSNTFTALILSPLLLLFALWIRIGANVSNFTFA PSTIIFHLGHAAMLGLMYVYWTQLNMFQTLK YLAILGSVTFLAGNRMLAQQAVKRTAH [SEQ ID NO:346]	RPN2-CDG	Glycosylation disorder
SEC 23A	10484	0100934	Q15436	MTTYLEFIQQNEERDGVRFSWNVWPSSRLEA TRMVVPVAALFTPLKERPDLPPIQYEPVLCSR TTCRAVLNPLCQVDYRAKLWACNFCYQRNQ FPPSYAGISELNQPAELLPQFSSIEYVVLRGPQ MPLIFLYVVDTCMEDEDLQALKESMQMSLSL LPPTALVGLITFGRMVQVHELGCEGISKSYVF RGTKDLSAKQLQEMLGLSKVPLTQATRGPQV QQPPPSNRFLQPVQKIDMNLTDLLGELQRDP WPVPQGKRPLRSSGVALSIAVGLLECTFPNTG ARIMMFIGGPATQGPGM VVGDELKTPIRSWHDIDKDNAKYVKKGTKH FEALANRAATTGHVIDIYACALDQTGLLEMK CCPNLTGGYMVMGDSFNTSLFKQTFQRVFTK DMHGQFKMGFGGTLEIKTSREIKISGAIGPCV SLNSKGPCVSENEIGTGGTCQWKICGLSPTTT LAIYFEVVNQHNAPIPQGGRGAIQFVTQYQHS SGQRRIRVTTIARNWADAQTQIQNIAASFDQE AAAILMARLAIYRAETEEGPDVLRWLDRQLI RLCQKFGEYHKDDPSSFRFSETFSLYPQFMFH LRRSSFLQVFNNSPDESSYYRHHFMRQDLTQ SLIMIQPILYAYSFSGPPEPVLLDSSSILADRILL MDTFFQILIYHGETIAQWRKSGYQDMPEYEN FRHLLQAPVDDAQEILHSRFPMPRYIDTEHGG	SEC23A-CDG	Glycosylation disorder

				SQARFLLSKVNPSQTHNNMYAWGQESGAPIL TDDVSLQVFMDHLKKLAVSSAA [SEQ ID NO:347]		
SLC 35A3	23443	0117620	Q9Y2D2, A0A1W2 PRT7, A0A1W2 PSD1, A0A1W2 PQL8	MFANLKYVSLGILVFQTTSLVLTMRYSRTLK EEGPRYLSSTAVVVAELLKIMACILLVYKDSK CSLRALNRVLHDEILNKPMETLKLAIPSGIYTL QNNLLYVALSNLDAATYQVTYQLKILTTALF SVSMLSKKLGVYQWLSLVILMTGVAFVQWP SDSQLDSKELSAGSQFVGLMAVLTACFSSGF AGVYFEKILKETKQSVWIRNIQLGFFGSIFGL MGVYIYDGELVSKNGFFQGYNRLTWIVVVL QALGGLVIAAVIKYADNILKGFATSLSIILSTLI SYFWLQDFVPTSVFFLGAILVITATFLYGYDP KPAGNPTKA [SEQ ID NO:348]	SLC35A3-CDG	Glycosylation disorder
ST3 GAL 3	6487	0126091	Q11203	MGLLVFVRNLLLALCLFLVLGFLYYSAWKLH LLQWEEDSNSVVLSFDSAGQTLGSEYDRLGF LLNLDSKLPAELATKYANFSEGACKPGYASA LMTAIFPRFSKPAPMFLDDSFRKWARIREFVP PFGIKGQDNLIKAILSVTKEYRLTPALDSLRCR RCIIVGNGGVLANKSLGSRIDDYDIVVRLNSA PVKGFEKDVGSKTTLRITYPEGAMQRPEQYE RDSLFVLAGFKWQDFKWLKYIVYKERVSAS DGFWKSVATRVPKEPPEIRILNPYFIQEAAFTL IGLPFNNGLMGRGNIPTLGSVAVTMALHGCD EVAVAGFGYDMSTPNAPLHYYETVRMAAIK ESWTHNIQREKEFLRKLVKARVITDLSSGI [SEQ ID NO:349]	ST3GAL3-CDG	Glycosylation disorder
STT 3A	3703	0134910	P46977	MTKFGFLRLSYEKQDTLLKLLILSMAAVLSFS TRLFAVLRFESVIHEFDPYFNYRTTRFLAEEGF YKFHNWFDDRAWYPLGRIIGGTIYPGLMITSA AIYHVLHFFHITIDIRNVCVFLAPLFSSFTTIVT YHLTKELKDAGAGLLAAAMIAVVPGYISRSV AGSYDNEGIAIFCMLLTYYMWIKAVKTGSIC WAAKCALAYFYMVSSWGGYVFLINLIPLHVL VLMLTGRFSHRIYVAYCTVYCLGTILSMQISF VGFQPVLSSEHMAAFGVFGLCQIHAFVDYLR SKLNPQQFEVLFRSVISLVGFVLLTVGALLML TGKISPWTGRFYSLLDPSYAKNNIPIIASVSEH QPTTWSSYYFDLQLLVFMFPVGLYYCFSNLS DARIFIIMYGVTSMYFSAVMVRLMLVLAPVM CILSGIGVSQVLSTYMKNLDISRPDKKSKKQQ DSTYPIKNEVASGMILVMAFFLITYTFHSTWV TSEAYSSPSIVLSARGGDGSRIIFDDFREAYYW LRHNTPEDAKVMSWWDYGYQITAMANRTIL VDNNTWNNTHISRVGQAMASTEEKAYEIMR ELDVSYVLVIFGGLTGYSSDDINKFLWMVRIG GSTDTGKHIKENDYYTPTGEFRVDREGSPVLL NCLMYKMCYYRFGQVYTEAKRPPGFDRVRN AEIGNKDFELDVLEEAYTTEHWLVRIYKVKD LDNRGLSRT [SEQ ID NO:350]	STT3A-CDG	Glycosylation disorder
STT 3B	201595	0163527	Q8TCJ2	MAEPSAPESKHKSSLNSSPWSGLMALGNSRH GHHGPGAQCAHKAAGGAAPPKPAPAGLSGG LSQPAGWQSLLSFTILFLAWLAGFSSRLFAVI RFESIIHEFDPWFNYRSTHHLASHGFYEFLNW FDERAWYPLGRIVGGTVYPGLMITAGLIHWIL NTLNITVHIRDVCVFLAPTFSGLTSISTFLLTRE LWNQGAGLLAACFIAIVPGYISRSVAGSFDNE GIAIFALQFTYYLWVKSVKTGSVFWTMCCCL SYFYMVSAWGGYVFIINLIPLHVFVLLLMQR YSKRVYIAYSTFYIVGLILSMQIPFVGFQPIRTS	STT3B-CDG	Glycosylation disorder

				EHMAAAGVFALLQAYAFLQYLRDRLTKQEF QTLFFLGVSLAAGAVFLSVIYLTYTGYIAPWS GRFYSLWDTGYAKIHIPIIASVSEHQPTTWVSF FFDLHILVCTFPAGLWFCIKNINDERVFVALY AISAVYFAGVMVRLMLTLTPVVCMLSAIAFS NVFEHYLGDDMKRENPPVEDSSDEDDKRNQ GNLYDKAGKVRKHATEQEKTEEGLGPNIKSI VTMLMLMLLMMFAVHCTWVTSNAYSSPSV VLASYNHDGTRNILDDFREAYFWLRQNTDEH ARVMSWWDYGYQIAGMANRTTLVDNNTW NNSHIALVGKAMSSNETAAYKIMRTLDVDY VLVIFGGVIGYSGDDINKFLWMVRIAEGEHPK DIRESDYFTPQGEFRVDKAGSPTLLNCLMYK MSYYRFGEMQLDFRTPPGFDRTRNAEIGNKD IKFKHLEEAFTSEHWLVRIYKVKAPDNRETLD HKPRVTNIFPKQKYLSKKTTKRKRGYIKNKL VFKKGKKISKKTV [SEQ ID NO:351]		
AGA	175	0038002	P20933	MARKSNLPVLLVPFLLCQALVRCSSPLPLVV NTWPFKNATEAAWRALASGGSALDAVESGC AMCEREQCDGSVGFGGSPDELGETTLDAMIM DGTTMDVGAVGDLRRIKNAIGVARKVLEHT THTLLVGESATTFAQSMGFINEDLSTTASQAL HSDWLARNCQPNYWRNVIPDPSKYCGPYKPP GILKQDIPIHKETEDDRGHDTIGMVVIHKTGHI AAGTSTNGIKFKIHGRVGDSPIPGAGAYADDT AGAAAATGNGDILMRFLPSYQAVEYMRRGE DPTIACQKVISRIQKHFPEF FGAVICANVTGSYGAACNKLSTFTQFSFMVY NSEKNQPTEEKVDCI [SEQ ID NO:352]	Aspartylglucosaminuria	Lysosomal storage disorder
ARSA	410	0100299	A0A0C4DFZ2, B4DVI5, P15289	MGAPRSLLLALAAGLAVARPPNIVLIFADDLG YGDLGCYGHPSSTTPNLDQLAAGGLRFTDFY VPVSLCTPSRAALLTGRLPVRMGMYPGVLVP SSRGGLPLEEVTVAEVLAARGYLTGMAGKW HLGVGPEGAFLPPHQGFHRFLGIPYSHDQGPC QNLTCFPPATPCDGGCDQGLVPIPLLANLSVE AQPPWLPGLEARYMAFAHDLMADAQRQDRP FFLYYASHHTHYPQFSGQSFAERSGRGPFGDS LMELDAAVGTLMTAIGDLGLLEETLVIFTAD NGPETMRMSRGGCSGLLRC GKGTTYEGGVREPALAFWPGHIAPGVTHELA SSLDLLPTLAALAGAPLPNVTLDGFDLSPLLL GTGKSPRQSLFFYPSYPDEVRGVFAVRTGKY KAHFFTQGSAHSDTTADPACHASSSLTAHEPP LLYDLSKDPGENYNLLGGVAGATPEVLQALK QLQLLKAQLDAAVTFGPSQVARGEDPALQIC CHPGCTPRPACCHCPDPHA [SEQ ID NO:353]	Metachromatic leukodystrophy	Lysosomal storage disorder
ARSB	411	0113273	A0A024RAJ9, P15848, A8K4A0	MGPRGAASLPRGPGPRRLLLPVVLPLLLLLLL APPGSGAGASRPPHLVFLLADDLGWNDVGFH GSRIRTPHLDALAAGGVLLDNYYTQPLCTPSR SQLLTGRYQIRTGLQHQIIWPCQPSCVPLDEK LLPQLLKEAGYTTHMVGKWHLGMYRKECLP TRRGFDTYFGYLLGSEDYYSHERCTLIDALN VTRCALDFRDGEEVATGYKNMYSTNIFTKRA IALITNHPPEKPLFLYLALQSVHEPLQVPEEYL KPYDFIQDKNRHHYAGMVSLMDEAVGNVTA ALKSSGLWNNTVFIFSTDNGGQTLAGGNNWP LRGRKWSLWEGGVRGVGFVASPLLKQKGVK NRELIHISDWLPTLVKLARGHTNGTKPLDGFD VWKTISEGSPSPRIELLHNIDPNFVDSSPCPRN SMAPAKDDSSLPEYSAFNTSVHAAIRHGNWK	Mucopolysaccharidosis type VI	Lysosomal storage disorder

				LLTGYPGCGYWFPPPSQYNVSEIPSSDPPTKT LWLFDIDRDPEERHDLSREYPHIVTKLLSRLQ FYHKHSVPVYFPAQDPRCDPKATGVWGPWM [SEQ ID NO:354]		
ASAH1	427	0104763	A8K0B6, Q13510, Q53H01	MPGRSCVALVLLAAAVSCAVAQHAPPWTED CRKSTYPPSGPTYRGAVPWYTINLDLPPYKR WHELMLDKAPVLKVIVNSLKNMINTFVPSGK IMQVVDEKLPGLLGNFPGPFEEEMKGIAAVT DIPLGEIISFNIFYELFTICTSIVAEDKKGHLIHG RNMDFGVFLGWNINNDTWVITEQLKPLTVNL DFQRNNKTVFKASSFAGYVGMLTGFKPGLFS LTLNERFSINGGYLGILEWILGKKDVMWIGFL TRTVLENSTSYEEAKNLLTKTKILAPAYFILG GNQSGEGCVITRDRKESLDVYELDAKQGRW YVVQTNYDRWKHPFFLDDRRTPAKMCLNRT SQENISFETMYDVLSTKPVLNKLTVYTTLIDV TKGQFETYLRDCPDPCIGW [SEQ ID NO:355]	Farber disease	Lysosomal storage disorder
ATP13A2	23400	0159363	Q8N4D4, Q9NQ11, Q8NBS1	MSADSSPLVGSTPTGYGTLTIGTSIDPLSSSVS SVRLSGYCGSPWRVIGYHVVVWMMAGIPLL LFRWKPLWGVRLRLRPCNLAHAETLVIEIRD KEDSSWQLFTVQVQTEAIGEGSLEPSPQSQAE DGRSQAAVGAVPEGAWKDTAQLHKSEEAVS VGQKRVLRYYLFQGQRYIWIETQQAFYQVSL LDHGRSCDDVHRSRHGLSLQDQMVRKAIYG PNVISIPVKSYPQLLVDEALNPYYGFQAFSIAL WLADHYYWYALCIFLISSISICLSLYKTRKQS QTLRDMVKLSMRVCVCRPGGEEEWVDSSEL VPGDCLVLPQEGGLMPCDAALVAGECMVNE SSLTGESIPVLKTALPEGLGPYCAETHRRHTLF CGTLILQARAYVGPHVLAVVTRTGFCTAKGG LVSSILHPRPINFKFYKHSMKFVAALSVLALL GTIYSIFILYRNRVPLNEIVIRALDLVTVVVPP ALPAAMTVCTLYAQSRLRRQGIFCIHPLRINL GGKLQLVCFDKTGTLTEDGLDVMGVVPLKG QAFLPLV PEPRRLPVGPLLRALATCHALSRLQDTPVGDP MDLKMVESTGWVLEEEPAADSAFGTQVLAV MRPPLWEPQLQAMEEPPVPVSVLHRFPFSSAL QRMSVVVAWPGATQPEAYVKGSPELVAGLC NPETVPTDFAQMLQSYTAAGYRVVALASKPL PTVPSLEAAQQLTRDTVEGDLSLLGLLVMRN LLKPQTTPVIQALRRTRIRAVMVTGDNLQTA VTVARGCGMVAPQEHLIIVHATHPERGQPAS LEFLPMESPTAVNGVKDPDQAASYTVEPDPR SRHLALSGPTFGIIVKHFPKL LPKVLVQGTVFARMAPEQKTELVCELQKLQ YCVGMCGDGANDCGALKAADVGISLSQAEA SVVSPFTSSMASIECVPMVIREGRCSLDTSFSV FKYMALYSLTQFISVLILYTINTNLGDLQFLAI DLVITTTVAVLMSRTGPALVLGRVRPPGALLS VPVLSSLLLQMVLVTGVQLGGYFLTLAQPWF VPLNRTVAAPDNLPNYENTVVFSLSSFQYLIL AAAVSKGAPFRRPLYTNVPFLVALALLSSVL VGLVLVPGLLQGPLALRNITDTGFKLLLLGLV TLNFVGAFMLESVLDQCLPACLRRLRPKRAS KKRFKQLERELAEQPWPPLPAGPLR [SEQ ID NO:356]	Neuronal ceroid lipofuscinosis 12 (CLN12), Kufor-Rakeb syndrome (KRS)	Lysosomal storage disorder
CLN3	1201	0188603,0261832	A0A024QZB8, Q13286,	MGGCAGSRRRFSDSEGEETVPEPRLPLLDHQ GAHWKNAVGFWLLGLCNNFSYVVMLSAAH DILSHKRTSGNQSHVDPGPTPIPHNSSSRFDCN	Neuronal ceroid lipofuscino	Lysosomal storage disorder

			B4DMY6, Q2TA70, B4DFF3	SVSTAAVLLADILPTLVIKLLAPLGLHLLPYSP RVLVSGICAAGSFVLVAFSHSVGTSLCGVVFA SISSGLGEVTFLSLTAFYPRAVISWWSSGTGG AGLLGALSYLGLTQAGLSPQQTLLSMLGIPAL LLASYFLLLTSPEAQDPGGEEEAESAARQPLI RTEAPESKPGSSSSLSLRERWTVFKGLLWYIV PLVVVYFAEYFINQGLFELLFFWNTSLSHAQQ YRWYQMLYQAGVFASRSSLRCCRIRFTWAL ALLQCLNLVFLLADVWFGFLPSIYLVFLIILYE GLLGGAAYVNTFHNIALETSDEHREFAMAAT CISDTLGISLSGLLALPLHDFLCQLS [SEQ ID NO:357]	sis 3 (CLN3)	
CLN 5	1203	0102805	A0A024R 644, O75503	MAQEVDTAQGAEMRRGAGAARGRASWCW ALALLWLAVVPGWSRVSGIPSRRHWPVPYK RFDFRPKPDPYCQAKYTFCPTGSPIPVMEGDD DIEVFRLQAPVWEFKYGDLLGHLKIMHDAIG FRSTLTGKNYTMEWYELFQLGNCTFPHLRPE MDAPFWCNQGAACFFEGIDDVHWKENGTLV QVATISGNMFNQMAKWVKQDNETGIYYETW NVKASPEKGAETWFDSYDCSKFVLRTFNKLA EFGAEFKNIETNYTRIFLYSGEPTYLGNETSVF GPTGNKTLGLAIKRFYYPFKPHLPTKEFLLSL LQIFDAVIVHKQFYLFYNFEYWFLPMKFPFIKI TYEEIPLPIRNKTLSGL [SEQ ID NO:358]	Neuronal ceroid lipofuscino sis 5 (CLN5)	Lyososom al storage disorder
CLN 6	54982	0128973	A0A024R 601, Q9NWW 5	MEATRRRQHLGATGGPGAQLGASFLQARHG SVSADEAARTAPFHLDLWFYFTLQNWVLDF GRPIAMLVFPLEWFPLNKPSVGDYFHMAYNV ITPFLLLKLIERSPRTLPRSITYVSIIIFIMGASIH LVGDSVNHRLLFSGYQHHLSVRENPIIKNLKP ETLIDSFELLYYYDEYLGHCMWYIPFFLILFM YFSGCFTASKAESLIPGPALLLVAPSGLYYWY LVTEGQIFILFIFTFFAMLALVLHQKRKRLFLD SNGLFLFSSFALTLLLVALWVAWLWNDPVLR KKYPGVIYVPEPWAFYTLHVSSRH [SEQ ID NO:359]	Neuronal ceroid lipofuscino sis 6 (CLN6)	Lyososom al storage disorder
CLN 8	2055	0182372,02 78220	A0A024Q Z57, Q9UBY8	MNPASDGGTSESIFDLDYASWGIRSTLMVAG FVFYLGVFVVCHQLSSSLNATYRSLVAREKV FWDLAATRAVFGVQSTAAGLWALLGDPVLH ADKARGQQNWCWFHITTATGFFCFENVAVH LSNLIFRTFDLFLVIHHLFAFLGFLGCLVNLQA GHYLAMTTLLLEMSTPFTCVSWMLLKAGWS ESLFWKLNQWLMIHMFHCRMVLTYHMWW VCFWHWDGLVSSLYLPHLTLFLVGLALLTLII NPYWTHKKTQQLLNPVDWNFAQPEAKSRPE GNGQLLRKKRP [SEQ ID NO:360]	Neuronal ceroid lipofuscino sis 8 (CLN8)	Lyososom al storage disorder
CTN S	1497	0040531	A0A0S2Z 3I9, O60931, A0A0S2Z 3K3	MIRNWLTIFILFPLKLVEKCESSVSLTVPPVVK LENGSSTNVSLTLRPPLNATLVITFEITFRSKNI TILELPDEVVVPPGVTNSSFQVTSQNVGQLTV YLHGNHSNQTGPRIRFLVIRSSAISIINQVIGWI YFVAWSISFYPQVIMNWRRKSVIGLSFDFVAL NLTGFVAYSVFNIGLLWVPYIKEQFLLKYPNG VNPVNSNDVFFSLHAVVLTLIIIVQCCLYERG GQRVSWPAIGFLVLAWLFAFVTMIVAAVGVT TWLQFLFCFSYIKLAVTLVKYFPQAYMNFYY KSTEGWSIGNVLLDFTGGSFSLLQMFLQSYN NDQWTLIFGDPTKFGLGVFSIVFDVVFFIQHF CLYRKRPGYDQLN [SEQ ID NO:361]	cystinosis	Lyososom al storage disorder
CTS A	5476	0064601	P10619, X6R8A1,	MIRAAPPPLFLLLLLLLLLVSWASRGEAAPDQ DEIQRLPGLAKQPSFRQYSGYLKGSGSKHLH YWFVESQKDPENSPVVLWLNGGPGCSSLDGL	Galactosiali dosis	Lyososom al storage disorder

			B4E324, X6R5C5	LTEHGPFLVQPDGVTLEYNPYSWNLIANVLY LESPAGVGFSYSDDKFYATNDTEVAQSNFEA LQDFFRLFPEYKNNKLFLTGESYAGIYIPTLA VLVMQDPSMNLQGLAVGNGLSSYEQNDNSL VYFAYYHGLLGNRLWSSLQTHCCSQNKCNF YDNKDLECVTNLQEVARIVGNSGLNIYNLYA PCAGGVPSHFRYEKDTVVVQD LGNIFTRLPLKRMWHQALLRSGDKVRMDPPC TNTTAASTYLNNPYVRKALNIPEQLPQWDMC NFLVNLQYRRLYRSMNSQYLKLLSSQKYQIL LYNGDVDMACNFMGDEWFVDSLNQKMEVQ RRPWLVKYGDSGEQIAGFVKEFSHIAFLTIKG AGHMVPTDKPLAAFTMFSRFLNKQPY [SEQ ID NO:362]		
CTSD	1509	0117984	P07339, V9HWI3	MQPSSLLPLALCLLAAPASALVRIPLHKFTSIR RTMSEVGGSVEDLIAKGPVSKYSQAVPAVTE GPIPEVLKNYMDAQYYGEIGIGTPPQCFTVVF DTGSSNLWVPSIHCKLLDIACWIHHKYNSDK SSTYVKNGTSFDIHYGSGSLSGYLSQDTVSVP CQSASSASALGGVKVERQVFG EATKQPGITFIAAKFDGILGMAYPRISVNNVL PVFDNLMQQKLVDQNIFSFYLSRDPDAQPGG ELMLGGTDSKYYKGSLSYLNVTRKAYWQVH LDQVEVASGLTLCKEGCEAIVDTGTSLMVGP VDEVRELQKAIGAVPLIQGEYMIPCEKVSTLP AITLKLGGKGYKLSPEDYTLKVSQAGKTLCL SGFMGMDIPPPSGPLWILGDVFIGRYYTVFDR DNNRVGFAEAARL [SEQ ID NO:363]	Neuronal ceroid lipofuscinosis 10 (CLN10)	Lysosomal storage disorder
CTSF	8722	0174080	Q9UBX1	MAPWLQLLSLLGLLPGAVAAPAQPRAASFQA WGPPSPELLAPTRFALEMFNRGRAAGTRAVL GLVRGRVRRAGQGSLYSLEATLEEPPCNDPM VCRLPVSKKTLLCSFQVLDELGRHVLLRKDC GPVDTKVPGAGEPKSAFTQGSAMISSLSQNHP DNRNETFSSVISLLNEDPLSQDLPVKMASIFK NFVITYNRTYESKEEARWRLSVFVNNMVRA QKIQALDRGTAQYGVTKFSDLTEEEFRTIYLN TLLRKEPGNKMKQAKSVGDLAPPEWDWRSK GAVTKVKDQGMCGSCWAFSVTGNVEGQWF LNQGTLLSLSEQELLDCDKMDKACMGGLPS NAYSAIKNLGGLETEDDYSYQGHMQSCNFSA EKAKVYINDSVELSQNEQKLAAWLAKRGPIS VAINAFGMQFYRHGISRPLRPLCSPWLIDHAV LLVGYGNRSDVPFWAIKNSWGTDWGEKGYY YLHRGSGACGVNTMASSAVVD [SEQ ID NO:364]	Neuronal ceroid lipofuscinosis 13 (CLN13)	Lysosomal storage disorder
CTSK	1513	0143387	P43235	MWGLKVLLLPVVSFALYPEEILDTHWELWK KTHRKQYNNKVDEISRRLIWEKNLKYISIHNL EASLGVHTYELAMNHLGDMTSEEVVQKMTG LKVPLSHSRSNDTLYIPEWEGRAPDSVDYRK KGYVTPVKNQGQCGSCWAFSSVGALEGQLK KKTGKLLNLSPQNLVDCVSENDGCGGGYMT NAFQYVQKNRGIDSEDAYPYVGQEESCMYN PTGKAAKCRGYREIPEGNEKALKRAVARVGP VSVAIDASLTSFQFYSKGVYYDESCNSDNLN HAVLAVGYGIQKGNKHWIIKNSWGENWGNK GYILMARNKNNACGIANLASFPKM [SEQ ID NO:365]	Pycnodysostosis	Lysosomal storage disorder

DNAJC5	80331	0101152	Q6AHX3, Q9H3Z4	MADQRQRSLSTSGESLYHVLGLDKNATSDDIKKSYRKLALKYHPDKNPDNPEAADKFKEINNAHAILTDATKRNIYDKYGSLGLYVAEQFGEENVNTYFVLSSWWAKALFVFCGLLTCCYCCCCLCCCFNCCCGKCKPKAPEGEETEFYVSPEDLEAQLQSDEREATDTPIVIQPASATETTQLTADSHPSYHTDGFN [SEQ ID NO:366]	Neuronal ceroid lipofuscinosis 4 (CLN4)	Lysosomal storage disorder
FUCA1	2517	0179163	P04066, B5MDC5	MRAPGMRSRPAGPALLLLLLFLGAAESVRRAQPPRRYTPDWPSLDSRPLPAWFDEAKFGVFIHWGVFSVPAWGSEWFWWHWQGEGRPQYQRFMRDNYPPGFSYADFGPQFTARFFHPEEWADLFQAAGAKYVVLTTKHHEGFTNWPSPVSWNWNSKDVGPHRDLVGELGTALRKRNIRYGLYHSLLEWFHPLYLLDKKNGFKTQHFVSAKTMPELYDLVNSYKPDLIWSDGEWECPDTYWNSTNFLSWLYNDSPVKDEVVVNDRWGQNCSCHHGGYYNCEDKFKPQSLPDHKWEMCTSIDKFSWGYRRDMALSDVTEESEIISELVQTVSLGGNYLLNIGPTKDGLIVPIFQERLLAVGKWLSINGEAIYASKPWRVQWEKNTTSVWYTSKGSAVYAIFLHWPENGVLNLESPITTSTTKITMLGIQGDLKWSTDPDKGLFISLPQLPPSAVPAEFAWTIKLTGVK[SEQ ID NO:367]	Fucosidosis	Lysosomal storage disorder
GAA	2548	0171298	P10253	MGVRHPPCSHRLLAVCALVSLATAALLGHILLHDFLLVPRELSGSSPVLEETHPAHQQGASRPGPRDAQAHPGRPRAVPTQCDVPPNSRFDCAPDKAITQEQCEARGCCYIPAKQGLQGAQMGQPWCFFPPSYPSYKLENLSSSEMGYTATLTRTTPTFFPKDILTLRLDVMMETENRLHFTIKDPANRRYEVPLETPHVHSRAPSPLYSVEFSEEPFGVIVRRQLDGRVLLNTTVAPLFFADQFLQLSTSLPSQYITGLAEHLSPLMLSTSWTRITLWNRDLAPTPGANLYGSHPFYLALEDGGSAHGVFLLNSNAMDVVLQPSPALSWRSTGGILDVYIFLGPEPKSVVQQYLDVVGYPFMPPYWGLGFHLCRWGYSSTAITRQVVENMTRAHFPLDVQWNDLDYMDSRRDFTFNKDGFRDFPAMVQELHQGGRRYMMIVDPAISSSGPAGSYRPYDEGLRRGVFITNETGQPLIGKVWPGSTAFPDFTNPTALAWWEDMVAEFHDQVPFDGMWIDMNEPSNFIRGSEDGCPNNELENPPYVPGVVGGTLQAATICASSHQFLSTHYNLHNLYGLTEAIASHRALVKARGTRPFVISRSTFAGHGRYAGHWTGDVWSSWEQLASSVPEILQFNLLGVPLVGADVCGFLGNTSEELCVRWTQLGAFYPFMRNHNSLLSLPQEPYSFSEPAQQAMRKALTLRYALLPHLYTLFHQAHVAGETVARPLFLEFPKDSSTWTVDHQLLWGEALLITPVLQAGKAEVTGYFPLGTWYDLQTVPVEALGSLPPPPAAPREPAIHSEGQWVTLPAPLDTINVHLRAGYIIPLQGPGLTTTESRQQPMALAVALTKGGEARGELFWDDGESLEVLERGAYTQVIFLARNNTIVNELVRVTSEGAGLQLQKVTVLGVATAPQQVLSNGVPVSNFTYSPDTKVLDICVSLLMGEQFLVSWC [SEQ ID NO:368]	Pompe disease	Lysosomal storage disorder
GALC	2581	0054983	A0A0A0MQV0, P54803	MAEWLLSASWQRRAKAMTAAAGSAGRAAVPLLLCALLAPGGAYVLDDSDGLGREFDGIGAVSGGGATSRLLVNYPEPYRSQILDYLFKPNFGASLHILKVEIGGDGQTTDGTEPSHMHYALDENYFRGYEWWLMKEAKKRNPNITLIGLPWSFPGWLGKGFDWPYVNLQLTAYYVVTWIVGAK	Krabbe disease	Lysosomal storage disorder

				RYHDLDIDYIGIWNERSYNANYIKILRKMLNY QGLQRVKIIASDNLWESISASMLLDAELFKVV DVIGAHYPGTHSAKDAKLTGKKLWSSEDFST LNSDMGAGCWGRILNQNYINGYMTSTIAWN LVASYYEQLPYGRCGLMTAQEPWSGHYVVE SPVWVSAHTTQFTQPGWYYLKTVGHLEKGG SYVALTDGLGNLTIIIETMSHKHSKCIRPFLPY FNVSQQFATFVLKGSFSEIPELQVWYTKLGKT SERFLFKQLDSLWLLDSDGSFTLSLHEDELFT LTTLTTGRKGSYPLPPKSQPFPSTYKDDFNVD YPFFSEAPNFADQTGVFEYFTNIEDPGEHHFT LRQVLNQRPITWAADASNTISIIGDYNWTNLT IKCDVYIETPDTGGVFIAGRVNKGGILIRSARG IFFWIFANGSYRVTGDLAGWIIYALGRVEVTA KKWYTLTLTIKGHFTSGMLNDKSLWTDIPVN FPKNGWAAIGTHSFEFAQFDNFLVEATR [SEQ ID NO:369]		
GAL NS	2588	0141012	P34059, Q96I49, Q6YL38	MAAVVAATRWWQLLLVLSAAGMGASGAPQ PPNILLLLMDDMGWGDLGVYGEPSRETPNLD RMAAEGLLFPNFYSANPLCSPSRAALLTGRLP IRNGFYTTNAHARNAYTPQEIVGGIPDSEQLL PELLKKAGYVSKIVGKWHLGHRPQFHPLKHG FDEWFGSPNCHFGPYDNKARPNIPVYRDWE MVGRYYEEFPINLKTGEANLTQIYLQEALDFI KRQARHHPFFLYWAVDATHAPVYASKPFLG TSQRGRYGDAVREIDDSIGKILELLQDLHVAD NTFVFFTSDNGAALISAPEQGGSNGPFLCGKQ TTFEGGMREPALAWWPGHVTAGQVSHQLGS IMDLFTTSLALAGLTPPSDRAIDGLNLLPTLLQ GRLMDRPIFYYRGDTLMAATLGQHKAHFWT WTNSWENFRQGIDFCPGQNVSGVTTHNLED HTKLPLIFHLGRDPGERFPLSFASAEYQEALSR ITSVVQQHQEALVPAQPQLNVCNWAVMNW APPGCEKLGKCLTPPESIPKKCLWSH [SEQ ID NO:370]	Mucopolysaccharidosis type IVa	Lysosomal storage disorder
GLA	2717	0102393	P06280, Q53Y83	MQLRNPELHLGCALALRFLALVSWDIPGARA LDNGLARTPTMGWLHWERFMCNLDCQEEPD SCISEKLFMEMAELMVSEGWKDAGYEYLCID DCWMAPQRDSEGRLQADPQRFPHGIRQLAN YVHSKGLKLGIYADVGNKTCAGFPGSFGYYD IDAQTFADWGVDLLKFDGCYCDSLENLADG YKHMSLALNRTGRSIVYSCEWPLYMWPFQK PNYTEIRQYCNHWRNFADIDDSWKSIKSILD WTSFNQERIVDVAGPGGWNDPDMLVIGNFG LSWNQQVTQMALWAIMAAPLFMSNDLRHIS PQAKALLQDKDVIAINQDPLGKQGYQLRQGD NFEVWERPLSGLAWAVAMINRQEIG GPRSYTIAVASLGKGVACNPACFITQLLPVKR KLGFYEWTSRLRSHINPTGTVLLQLENTMQM SLKDLL [SEQ ID NO:371]	Fabry disease	Lysosomal storage disorder
GLB 1	2720	0170266	P16278, B7Z6Q5	MPGFLVRILPLLLVLLLLGPTRGLRNATQRMF EIDYSRDSFLKDGQPFRYISGSIHYSRVPRFYW KDRLLKMKMAGLNAIQTYVPWNFHEPWPGQ YQFSEDHDVEYFLRLAHELGLLVILRPGPYIC AEWEMGGLPAWLLEKESILLRSSDPDYLAAV DKWLGVLLPKMKPLLYQNGGPVITVQVENE YGSYFACDFDYLRFLQKRFRHHLGDDVVLFT TDGAHKTFLKCGALQGLYTTVDFGTGSNITD AFLSQRKCEPKGPLINSEFYTGWLDHWGQPH STIKTEAVASSLYDILARG	GM1 gangliosidosis, Mucopolysaccharidosis IVb	Lysosomal storage disorder

				ASVNLYMFIGGTNFAYWNGANSPYAAQPTS YDYDAPLSEAGDLTEKYFALRNIIQKFEKVPE GPIPPSTPKFAYGKVTLEKLKTVGAALDILCPS GPIKSLYPLTFIQVKQHYGFVLYRTTLPQDCS NPAPLSSPLNGVHDRAYVAVDGIPQGVLERN NVITLNITGKAGATLDLLVENMGRVNYGAYI NDFKGLVSNLTLSSNILTDWTIFPLDTEDAVR SHLGGWGHRDSGHHDEAWAHNSSNYTLPAF YMGNFSIPSGIPDLPQDTFIQFPGWTKGQVWI NGFNLGRYWPARGPQLTLFVPQHILMTSAPN TITVLELEWAPCSSDDPELCAVTFVDRPVIGSS VTYDHPSKPVEKRLMPPPPQKNKDSWLDHV [SEQ ID NO:372]		
GM2A	2760	0196743	P17900	MQSLMQAPLLIALGLLLAAPAQAHLKKPSQL SSFSWDNCDEGKDPAVIRSLTLEPDPIIVPGNV TLSVMGSTSVPLSSPLKVDLVLEKEVAGLWI KIPCTDYIGSCTFEHFCDVLDMLIPTGEPCPEP LRTYGLPCHCPFKEGTYSLPKSEFVVPDLELP SWLTTGNYRIESVLSSSGKRLGCIKIAASLKGI [SEQ ID NO:373]	GM2-gangliosidosis, AB variant	Lysosomal storage disorder
GNPTAB	79158	0111670	Q3T906	MLFKLLQRQTYTCLSHRYGLYVCFLGVVVTI VSAFQFGEVVLEWSRDQYHVLFDSYRDNIAG KSFQNRLCLPMPIDVVYTWVNGTDLELLKEL QQVREQMEEEQKAMREILGKNTTEPTKKSEK QLECLLTHCIKVPMLVLDPALPANITLKDLPS LYPSFHSASDIFNVAKPKNPSTNVSVVVFDST KDVEDAHSGLLKGNSRQTVWRGYLTTDKEV PGLVLMQDLAFLSGFPPTFKETNQLKTKLPEN LSSKVKLLQLYSEASVALLKLNNPKDFQELN KQTKKNMTIDGKELTISPA YLLWDLSAISQSKQDEDISASRFEDNEELRYS LRSIERHAPWVRNIFIVTNGQIPSWLNLDNPR VTIVTHQDVFRNLSHLPTFSSPAIESHIHRIEGL SQKFIYLNDDVMFGKDVWPDDFYSHSKGQK VYLTWPVPNCAEGCPGSWIKDGYCDKACNN SACDWDGGDCSGNSGGSRYIAGGGGTGSIGV GQPWQFGGGINSVSYCNQGCANSWLADKFC DQACNVLSCGFDAGDCGQDHFHELYKVILLP NQTHYIIPKGECLPYFSFAEVAKRGVEGAYSD NPIIRHASIANKWKTIHLIMHSGMNATTIHFNL TFQNTNDEEFKMQITVEVDTREGPKLNSTAQ KGYENLVSPITLLPEAEILFEDIPKEKRFPKFK RHDVNSTRRAQEEVKIPLVNISLLPKDAQLSL NTLDLQLEHGDITLKGYNLSKSALLRSFLMNS QHAKIKNQAIITDETNDSLVAPQEKQVHKSIL PNSLGVSERLQRLTFPAVSVKVNGHDQGQNP PLDLETTARFRVETHTQKTIGGNVTKEKPPSLI VPLESQMTKEKKITGKEKENSRMEENAENHI GVTEVLLGRKLQHYTDSYLGFLPWEKKKYF QDLLDEEESLKTQLAYFTDSKNTGRQLKDTF ADSLRYVNKILNSKFGFTSRKVPAHMPHMID RIVMQELQDMFPEEFDKTSFHKVRHSEDMQF AFSYFYYLMSAVQPLNISQVFDEVDTDQSGV LSDREIRTLATRIHELPLSLQDLTGLEHMLINC SKMLPADITQLNNIPPTQESYYDPNLPPVTKS LVTNCKPVTDKIHKAYKDKNKYRFEIMGEEE IAFKMIRTNVSHVVGQLDDIRKNPRKFVCLN DNIDHNHKDAQTVKAVLRDFYESMFPIPSQF ELPREYRNRFLHMHELQEWRAYRDKLKFWT	Mucolipidosis type II alpha/beta, Mucolipidosis III alpha/beta	Lysosomal storage disorder

				HCVLATLIMFTIFSFFAEQLIALKRKIFPRRRIH KEASPNRIRV[SEQ ID NO:374]		
GNPTG	84572	0090581	Q9UJJ9	MAAGLARLLLLLGLSAGGPAPAGAAKMKVV EEPNAFGVNNPFLPQASRLQAKRDPSPVSGPV HLFRLSGKCFSLVESTYKYEFCPFHNVTQHEQ TFRWNAYSGILGIWHEWEIANNTFTGMWMR DGDACRSRSRQSKVELACGKSNRLAHVSEPS TCVYALTFETPLVCHPHALLVYPTLPEALQRQ WDQVEQDLADELITPQGHEKLLRTLFEDAGY LKTPEENEPTQLEGGPDSLGFETLENCRKAHK ELSKEIKRLKGLLTQHGIPYTRPTETSNLEHLG HETPRAKSPEQLRGDPG LRGSL [SEQ ID NO:375]	Mucolipidosis III gamma	Lysosomal storage disorder
GNS	2799	0135677	A0A024RBC5, P15586, Q7Z3X3	MRLLPLAPGRLRRGSPRHLPSCSPALLLLVLG GCLGVFGVAAGTRRPNVVLLLTDDQDEVLG GMTPLKKTKALIGEMGMTFSSAYVPSALCCP SRASILTGKYPHNHHVVNNTLEGNCSSKSWQ KIQEPNTFPAILRSMCGYQTFFAGKYLNEYGA PDAGGLEHVPLGWSYWYALEKNSKYYNYTL SINGKARKHGENYSVDYLTDVLANVSLDFLD YKSNFEPFFMMIATPAPHSPWTAAPQYQKAF QNVFAPRNKNFNIHGTNKHWLIRQAKTPMTN SSIQFLDNAFRKRWQTLLSVD DLVEKLVKRLEFTGELNNTYIFYTSDNGYHT GQFSLPIDKRQLYEFDIKVPLLVRGPGIKPNQT SKMLVANIDLGPTILDIAGYDLNKTQMDGMS LLPILRGASNLTWRSDVLVEYQGEGRNVTDP TCPSLSPGVSQCFPDCVCEDAYNNTYACVRT MSALWNLQYCEFDDQEVFVEVYNLTADPDQ ITNIAKTIDPELLGKMNYRLMMLQSCSGPTCR TPGVFDPGYRFDPRLMFSNRGSVRTRRFSKH LL [SEQ ID NO:376]	Mucopolysaccharidosis type IIID	Lysosomal storage disorder
GRN	2896	0030582	P28799	MWTLVSWVALTAGLVAGTRCPDGQFCPVAC CLDPGGASYSCCRPLLDKWPTTLSRHLGGPC QVDAHCSAGHSCIFTVSGTSSCCPFPEAVACG DGHHCCPRGFHCSADGRSCFQRSGNNSVGAI QCPDSQFECPDFSTCCVMVDGSWGCCPMPQ ASCCEDRVHCCPHGAFCDLVHTRCITPTGTHP LAKKLPAQRTNRAVALSSSVMCPDARSRCPD GSTCCELPSGKYGCCPMPNATCCSDHLHCCP QDTVCDLIQSKCLSKENATTDLLTKLPAHTV GDVKCDMEVSCPDGYTCCRLQSGAWGCCPF TQAVCCEDHIHCCPAGFTCDTQKGTCEQGPH QVPWMEKAPAHLSLPDPQALKRDVPCDNVS SCPSSDTCCQLTSGEWGCCPIPEAVCCSDHQH CCPQGYTCVAEGQCQRGSEIVAGLEKMPARR ASLSHPRDIGCDQHTSCPVGQTCCPSLGGSW ACCQLPHAVCCEDRQHCCPAGYTCNVKARS CEKEVVSAQPATFLARSPHVGVKDVECGEGH FCHDNQTCCRDNRQGWACCPYRQGVCCAD RRHCCPAGFRCAARGTKCLRREAPRWDAPL RDPALRQLL [SEQ ID NO:377]	Neuronal ceroid lipofuscinosis 11 (CLN11), frontotemporal dementia	Lysosomal storage disorder
GUSB	2990	0169919	P08236	MARGSAVAWAALGPLLWGCALGLQGGMLY PQESPSRECKELDGLWSFRADFSDNRRRGFEE QWYRRPLWESGPTVDMPVPSSFNDISQDWRL RHFVGWVWYEREVILPERWTQDLRTRVVLRI GSAHSYAIVWVNGVDTLEHEGGYLPFEADIS NLVQVGPLPSRLRITIAINNTLTPTTLPPGTIQY LTDTSKYPKGYFVQNTYFDFFNYAGLQRSVL LYTTPTTYIDDITVTTSVEQDSGLVNYQISVK	Mucopolysaccharidosis type VII	Lysosomal storage disorder

				GSNLFKLEVRLLDAENKVVANGTGTQGQLK VPGVSLWWPYLMHERPAYL YSLEVQLTAQTSLGPVSDFYTLPVGIRTVAVT KSQFLINGKPFYFHGVNKHEDADIRGKGFDW PLLVKDFNLLRWLGANAFRTSHYPYAEEVM QMCDRYGIVVIDECPGVGLALPQFFNNVSLH HHMQVMEEVVRRDKNHPAVVMWSVANEPA SHLESAGYYLKMVIAHTKSLDPSRPVTFVSNS NYAADKGAPYVDVICLNSYYSWYHDYGHLE LIQLQLATQFENWYKKYQKPIIQSEYGAETIA GFHQDPPLMFTEEYQKSLLEQYHLGLDQKRR KYVVGELIWNFADFMTEQSPTRVLGNKKGIF TRQRQPKSAAFLLRERYWKIANETRYPHSVA KSQCLENSLFT [SEQ ID NO:378]		
HEX A	3073	0213614	A0A0S2Z 3W3, P06865, B4DVA7, H3BP20	MTSSRLWFSLLLAAAFAGRATALWPWPQNF QTSDQRYVLYPNNFQFQYDVSSAAQPGCSVL DEAFQRYRDLLFGSGSWPRPYLTGKRHTLEK NVLVVSVVTPGCNQLPTLESVENYTLTINDD QCLLLSETVWGALRGLETFSQLVWKSAEGTF FINKTEIEDFPRFPHRGLLLDTSRHYLPLSSILD TLDVMAYNKLNVFHWHLVDDPSFPYESFTFP ELMRKGSYNPVTHIYTAQDVKEVIEYARLRG IRVLAEFDTPGHTLSWGPGIPGLLTPCYSGSEP SGTFGPVNPSLNNTYEFMSTFFLEVSSVFPDF YLHLGGDEVDFTCWKSNPEIQDFMRKKGFGE DFKQLESFYIQTLLDIVSSYGKGYVVWQEVF DNKVKIQPDTIIQVWREDIPVNYMKELELVTK AGFRALLSAPWYLNRISYGPDWKDFYIVEPL AFEGTPEQKALVIGGEACMWGEYVDNTNLV PRLWPRAGAVAERLWSNKLTSDLTFAYERLS HFRCELLRRGVQAQPLNVGFCEQEFEQT [SEQ ID NO:379]	Tay-Sachs disease	Lyososomal storage disorder
HEX B	3074	0049860	A0A024R AJ6, P07686, Q5URX0	MELCGLGLPRPPMLLALLLATLLAAMLALLT QVALVVQVAEAARAPSVSAKPGPALWPLPLS VKMTPNLLHLAPENFYISHSPNSTAGPSCTLL EEAFRRYHGYIFGFYKWHHEPAEFQAKTQVQ QLLVSITLQSECDAFPNISSDESYTLLVKEPVA VLKANRVWGALRGLETFSQLVYQDSYGTFTI NESTIIDSPRFSHRGILIDTSRHYLPVKIILKTLD AMAFNKFNVLHWHIVDDQSFPYQSITFPELSN KGSYSLSHVYTPNDVRMVIEYARLRGIRVLPE FDTPGHTLSWGKGQKDLLTPCYSRQNKLDSF GPINPTLNTTYSFLTTFFKEISEVFPDQFIHLGG DEVEFKCWESNPKIQDFMRQKGFGTDFKKLE SFYIQKVLDIIATINKGSIVWQEVFDDKAKLA PGTIVEVWKDSAYPEELSRVTASGFPVILSAP WYLDLISYGQDWRKYYKVEPLDFGGTQKQK QLFIGGEACLWGEYVDATNLTPRLWPRASAV GERLWSSKDVRDMDDAYDRLTRHRCRMVE RG IAAQPLYAGYCNHENM [SEQ ID NO:380]	Sandhoff diseaase	Lyososomal storage disorder
HGS NAT	138050	0165102	Q68CP4, Q8IVU6	MTGARASAAEQRRAGRSGQARAAERAAGM SGAGRALAALLLAASVLSAALLAPGGSSGRD AQAAPPRDLDKKRHAELKMDQALLLIHNELL WTNLTVYWKSECCYHCLFQVLVNVPQSPKA GKPSAAAASVSTQHGSILQLNDTLEEKEVCRL EYRFGEFGNYSLLVKNIHNGVSEIACDLAVNE DPVDSNLPVSIAFLIGLAVIIVISFLRLLLSLDD FNNWISKAISSRETDRLINSELGSPSRTDPLDG DVQPATWRLSALPPRLRSVDTFRGIALILMVF	Mucopolysaccharidosis type IIIC	Lyososomal storage disorder

				VNYGGGKYWYFKHASWNGLTVADLVFPWF VFIMGSSIFLSMTSILQRGCSKFRLLGKIAWRS FLLICIGIIIVNPNYCLGPLSWDKVRIPGVLQRL GVTYFVVAVLELLFAKPVPEHCASERSCLSLR DITSSWPQWLLILVLEGLWLGLTFLLPVPGCP TGYLGPGGIGDFGKYPNCTGGAAGYIDRLLL GDDHLYQHPSSAVLYHTEVAYDPEGILGTINS IVMAFLGVQAGKILLYYKARTKDILIRFTAWC C ILGLISVALTKVSENEGFIPVNKNLWSLSYVTT LSSFAFFILLVLYPVVDVKGLWTGTPFFYPGM NSILVYVGHEVFENYFPFQWKLKDNQSHKEH LTQNIVATALWVLIAYILYRKKIFWKI [SEQ ID NO:381]		
HYAL1	3373	0114378	A0A024R2X3, Q12794, B3KUI5, A0A0S2Z3Q0	MAAHLLPICALFLTLLDMAQGFRGPLLPNRPF TTVWNANTQWCLERHGVDVDVSVFDVVAN PGQTFRGPDMTIFYSSQLGTYPYYTPTGEPVF GGLPQNASLIAHLARTFQDILAAIPAPDFSGLA VIDWEAWRPRWAFNWDTKDIYRQRSRALVQ AQHPDWPAPQVEAVAQDQFQGAARAWMAG TLQLGRALRPRGLWGFYGFPDCYNYDFLSPN YTGQCPSGIRAQNDQLGWLWGQSRALYPSIY MPAVLEGTGKSQMYVQHRVAEAFRVAVAA GDPNLPVLPYVQIFYDTTNHFLPLDELEHSLG ESAAQGAAGVVLWVSWENTRTKESCQAIKE YMDTTLGPFILNVTSGALLCSQ ALCSGHGRCVRRTSHPKALLLLNPASFSIQLT PGGGPLSLRGALSLEDQAQMAVEFKCRCYPG WQAPWCERKSMW [SEQ ID NO:382]	Mucopolysaccharidosis type IX	Lysosomal storage disorder
IDS	3423	0010404	P22304, B4DGD7	MPPPRTGRGLLWLGLVLSSVCVALGSETQAN STTDALNVLLIIVDDLRPSLGCYGDKLVRSPNI DQLASHSLLFQNAFAQQAVCAPSRVSFLTGR RPDTTRLYDFNSYWRVHAGNFSTIPQYFKEN GYVTMSVGKVFHPGISSNHTDDSPYSWSFPP YHPSSEKYENTKTCRGPDGELHANLLCPVDV LDVPEGTLPDKQSTEQAIQLLEKMKTSASPFF LAVGYHKPHIPFRYPKEFQKLYPLENITLAPD PEVPDGLPPVAYNPWMDIRQREDVQALNISV PYGPIPVDFQRKIRQSYFASVSYLDTQVGRLL SALDDLQLANSTIIAFTSDHGWALGEHGEWA KYSNFDVATHVPLIFYVPGRTASLPEAGEKLF PYLDPFDSASQLMEPGRQSMDLVELVSLFPTL AGLAGLQVPPRCPVPSFHVELCREGKNLLKH FRFRDLEEDPYLPGNPRELIAYSQYPRPSDIPQ WNSDKPSLKDIKIMGYSIRTIDYRYTVWVGF NPDEFLANFSDIHAGELYFVDSDPLQDHNMY NDSQGGDLFQLLMP [SEQ ID NO:383]	Mucopolysaccharidosis type II	Lysosomal storage disorder
IDUA	3425	0127415	P35475	MRPLRPRAALLALLASLLAAPPVAPAEAPHL VHVDAARALWPLRRFWRSTGFCPPLPHSQAD QYVLSWDQQLNLAYVGAVPHRGIKQVRTH WLLELVTTRGSTGRGLSYNFTHLDGYLDLLR ENQLLPGFELMGSASGHFTDFEDKQQVFEWK DLVSSLARRYIGRYGLAHVSKWNFETWNEPD HHDFDNVSMTMQGFLNYYDACSEGLRAASP ALRLGGPGDSFHTPPRSPLSWGLLRHCHDGT NFFTGEAGVRLDYISLHRKGARSSISILEQEKV VAQQIRQLFPKFADTPIYNDEADPLVGWSLPQ PWRADVTYAAMVVKVIAQHQNLLLANTTSA FPYALLSNDNAFLSYHPHPFAQRTLTARFQV NNTRPPHVQLLRKPVLTAMGLLALLDEEQL	Mucopolysaccharidosis type I	Lysosomal storage disorder

				WAEVSQAGTVLDSNHTVGVLASAHRPQGPA DAWRAAVLIYASDDTRAHPNRSVAVTLRLR GVPPGPGLVYVTRYLDNGLCSPDGEWRRLG RPVFPTAEQFRRMRAAEDPVAAAPRPLPAGG RLTLRPALRLPSLLLVHVCARPEKPPGQVTRL RALPLTQGQLVLVWSDEHVGSKCLWTYEIQF SQDGKAYTPVSRKPSTFNLFVFSPDTGAVSGS YRVRALDYWARPGPFSDPVPYLEVPVPRGPP SPGNP [SEQ ID NO:384]		
KCT D7	154881	0243335	Q96MP8, A0A024R DN7	MVVVTGREPDSRRQDGAMSSSDAEDDFLEP ATPTATQAGHALPLLPQEFPEVVPLNIGGAHF TTRLSTLRCYEDTMLAAMFSGRHYIPTDSEG RYFIDRDGTHFGDVLNFLRSGDLPPRERVRA VYKEAQYYAIGPLLEQLENMQPLKGEKVRQ AFLGLMPYYKDHLERIVEIARLRAVQRKARF AKLKVCVFKEEMPITPYECPLLNSLRFERSES DGQLFEHHCEVDVSFGPWEAVADVYDLLHC LVTDLSAQGLTVDHQCIGVCDKHLVNHYYC KRPIYEFKITWW [SEQ ID NO:385]	Neuronal ceroid lipofuscinosis 14 (CLN14)	Lysosomal storage disorder
LAM P2	3920	0005893	P13473	MVCFRLFPVPGSGLVLVCLVLGAVRSYALEL NLTDSENATCLYAKWQMNFTVRYETTNKTY KTVTISDHGTVTYNGSICGDDQNGPKIAVQFG PGFSWIANFTKAASTYSIDSVSFSYNTGDNTT FPDAEDKGILTVDELLAIRIPLNDLFRCNSLST LEKNDVVQHYWDVLVQAFVQNGTVSTNEFL CDKDKTSTVAPTIHTTVPSPTTTPTPKEKPEA GTYSVNNGNDTCLLATMGLQLNITQDKVAS VININPNTTHSTGSCRSHTALLRLNSSTIKYLD FVFAVKNENRFYLKEVNISMYLVNGSVFSIA NNNLSYWDAPLGSSYMCNKEQTVSVSGAFQI NTFDLRVQPFNVTQGKYSTAQDCSADDDNFL VPIAVGAALAGVLILVLLAYFIGLKHHHAGY EQF [SEQ ID NO:386]	Danon disease	Lysosomal storage disorder
MA N2B 1	4125	0104774	O00754, A8K6A7	MGAYARASGVCARGCLDSAGPWTMSRALRP PLPPLCFFLLLLAAAGARAGGYETCPTVQPN MLNVHLLPHTHDDVGWLKTVDQYFYGIKND IQHAGVQYILDSVISALLADPTRRFIYVEIAFFS RWWHQQTNATQEVVRDLVRQGRLEFANGG WVMNDEAATHYGAIVDQMTLGLRFLEDTFG NDGRPRVAWHIDPFGHSREQASLFAQMGFD GFFFGRLDYQDKWVRMQKLEMEQVWRAST SLKPPTADLFTGVLPNGYNPPRNLCWDVLCV DQPLVEDPRSPEYNAKELVDYFLNVATAQGR YYRTNHTVMTMGSDFQYENANMWFKNLDK LIRLVNAQQAKGSSVHVLYSTPACYLWELNK ANLTWSVKHDDFFPYADGPHQFWTGYFSSRP ALKRYERLSYNFLQVCNQLEALVGLAANVG PYGSGDSAPLNEAMAVLQHHDAVSGTSRQH VANDYARQLAAGWGPCEVLLSNALARLRGF KDHFTFCQQLNISICPLSQTAARFQVIVYNPLG RKVNWMVRLPVSEGVFVVKDPNGRTVPSDV VIFPSSDSQAHPPELLFSASLPALGFSTYSVAQ VPRWKPQARAPQPIPRRSWSPALTIENEHIRA TFDPDTGLLMEIMNMNQQLLLPVRQTFFWY NASIGDNESDQASGAYIFRPNQQKPLPVSRW AQIHLVKTPLVQEVHQNFSAWCSQVVRLYPG QRHLELEWSVGPIPVGDTWGKEVISRFDTPLE TKGRFYTDSNGREILERRRDYRPTWKLNQTE PVAGNYYPVNTRIYITDGNMQLTVLTDRSQG GSSLRDGSLELMVHRRLLKDDGRGVSEPLME	alpha-mannosidosis	Lysosomal storage disorder

				NGSGAWVRGRHLVLLDTAQAAAAGHRLLAE QEVLAPQVVLAPGGGAAYNLGAPPRTQFSGL RRDLPPSVHLLTLASWGPEMVLLRLEHQFAV GEDSGRNLSAPVTLNLRDLFSTFTITRLQETTL VANQLREAASRLKWTTNTGPTPHQTPYQLDP ANITLEPMEIRTFLASVQWKEVDG [SEQ ID NO:387]		
MA NBA	4126	0109323	O00462	MRLHLLLLLALCGAGTTAAELSYSLRGNWSI CNGNGSLELPGAVPGCVHSALFQQGLIQDSY YRFNDLNYRWVSLDNWTYSKEFKIPFEISKW QKVNLILEGVDTVSKILFNEVTIGETDNMFNR YSFDITNVVRDVNSIELRFQSAVLYAAQQSKA HTRYQVPPDCPPLVQKGECHVNFVRKEQCSF SWDWGPSFPTQGIWKDVRIEAYNICHLNYFT FSPIYDKSAQEWNLEIESTFDVVSSKPVGGQV IVAIPKLQTQQTYSIELQPGKRIVELFVNISKNI TVETWWPHGHGNQTGYNMTVLFELDGGLNI EKSAKVYFRTVELIEEPIKGSPGLSFYFKINGF PIFLKGSNWIPADSFQDRVTSELLRLLLQSVV DANMNTLRVWGGGIYEQDEFYELCDELGIM VWQDFMFACALYPTDQGFLDSVTAEVAYQI KRLKSHPSIIIWSGNNENEEALMMNWYHISFT DRPIYIKDYVTLYVKNIRELVLAGDKSRPFITS SPTNGAETVAEAWVSQNPNSNYFGDVHFYD YISDC WNWKVFPKARFASEYGYQSWPSFSTLEKVSS TEDWSFNSKFSLHRQHHEGGNKQMLYQAGL HFKLPQSTDPLRTFKDTIYLTQVMQAQCVKT ETEFYRRSRSEIVDQQGHTMGALYWQLNDIW QAPSWASLEYGGKWKMLHYFAQNFFAPLLP VGFENENTFYIYGVSDLHSDYSMTLSVRVHT WSSLEPVCSRVTERFVMKGGEAVCLYEEPVS ELLRRCGNCTRESCVVSFYLSADHELLSPTNY HFLSSPKEAVGLCKAQITAIISQQGDIFVFDLE TSAVAPFVWLDVGSIPGRFSDNGFLMTEKTR TILFYPWEPTSKNELEQSFHVTSLTDIY [SEQ ID NO:388]	Mucolipidosis type IV	Lysosomal storage disorder
MC OLN 1	57192	0090674	Q9GZU1	MTAPAGPRGSETERLLTPNPGYGTQAGPSPAP PTPPEEEDLRRRLKYFFMSPCDKFRAKGRKPC KLMLQVVKILVVTVQLILFGLSNQLAVTFREE NTIAFRHLFLLGYSDGADDTFAAYTREQLYQ AIFHAVDQYLALPDVSLGRYAYVRGGGDPW TNGSGLALCQRYYHRGHVDPANDTFDIDPM VVTDCIQVDPPERPPPPPSDDLTLLESSSSYKN LTLKFHKLVNVTIHFRLKTINLQSLINNEIPDC YTFSVLITFDNKAHSGRIPISLETQAHIQECKH PSVFQHGDNSFRLLFDVVVILTCSLSFLLCARS LLRGFLLQNEFVGFMWRQRGRVISLWERLEF VNGWYILLVTSDVLTISGTIMKIGIEAKNLAS YDVCSILLGTSTLLVWVGVIRYLTFFHNYNILI ATLRVALPSVMRFCCCVAVIYLGYCFCGWIV LGPYHVKFRSLSMVSECLFSLINGDDMFVTFA AMQAQQGRSSLVWLFSQLYLYSFISLFIYMV LSLFIALITGAYDTIKHPGGAGAEESELQAYIA QCQDSPTSGKFRRGSGSACSLLCCCGRDPSEE HSLLVN [SEQ ID NO:389]	Mucolipidosis type IV	Lysosomal storage disorder
MFS D8	256471	0164073	Q8NHS3	MAGLRNESEQEPLLGDTPGSREWDILETEEH YKSRWRSIRILYLTMFLSSVGFSVVMMSIWPY LQKIDPTADTSFLGWVIASYSLGQMVASPIFG LWSNYRPRKEPLIVSILISVAANCLYAYLHIPA	Neuronal ceroid lipofuscino	Lysosomal storage disorder

				SHNKYYMLVARGLLGIGAGNVAVVRSYTAG ATSLQERTSSMANISMCQALGFILGPVFQTCF TFLGEKGVTWDVIKLQINMYTTPVLLSAFLGI LNIILILAILREHRVDDS GRQCKSINFEEASTDEAQVPQGNIDQVAVVAI NVLFFVTLFIFALFETIITPLTMDMYAWTQEQ AVLYNGIILAALGVEAVVIFLGVKLLSKKIGE RAILLGGLIVVWVGFFILLPWGNQFPKIQWED LHNNSIPNTTFGEIIIGLWKSPMEDDNERPTGC SIEQAWCLYTPVIHLAQFLTSAVLIGLGYPVC NLMSYTLYSKILGPKPQGVYMGWLTASGSG ARILGPMFISQVYAHWGPRWAFSLVCGIIVLT ITLLGVVYKRLIALSVRYGRIQE[SEQ ID NO:390]	sis 7 (CLN7)	
NAG A	4668	0198951	A0A024R 1Q5, P17050	MLLKTVLLLGHVAQVLMLDNGLLQTPPMG WLAWERFRCNINCDEDPKNCISEQLFMEMAD RMAQDGWRDMGYTYLNIDDCWIGGRDASG RLMPDPKRFPHGIPFLADYVHSLGLKLGIYAD MGNFTCMGYPGTTLDKVVQDAQTFAEWKV DMLKLDGCFSTPEERAQGYPKMAAALNATG RPIAFSCSWPAYEGGLPPRVNYSLLADICNLW RNYDDIQDSWWSVLSILNWFVEHQDILQPVA GPGHWNDPDMLLIGNFGLSLEQSRAQMALW TVLAAPLLMSTDLRTISAQNMDILQNPLMIKI NQDPLGIQGRRIHKEKSLIEVYMRPLSNKASA LVFFSCRTDMPYRYHSSLGQLNFTGSVIYEAQ DVYSGDIISGLRDETNFTVIINPSGVVMWYLY PIKNLEMSQQ[SEQ ID NO:391]	Schindler disease	Lysosomal storage disorder
NAG LU	4669	0108784	A0A140V JE4, P54802	MEAVAVAAAVGVLLLAGAGGAAGDEAREA AAVRALVARLLGPGPAADFSVSVERALAAKP GLDTYSLGGGGAARVRVRGSTGVAAAAGLH RYLRDFCGCHVAWSGSQLRLPRPLPAVPGEL TEATPNRYRYYQNVCTQSYSFVWWDWARW EREIDWMALNGINLALAWSGQEAIWQRVYL ALGLTQAEINEFFTGPAFLAWGRMGNLHTW DGPLPPSWHIKQLYLQHRVLDQMRSFGMTPV LPAFAGHVPEAVTRVFPQVNVTKMGSWGHF NCSYSCSFLLAPEDPIFPIIGSLFLRELIKEFGTD HIYGADTFNEMQPPSSEPSYLAAATTAVYEA MTAVDTEAVWLLQGWLFQHQPQF WGPAQIRAVLGAVPRGRLLVLDLFAESQPVY TRTASFQGQPFIWCMLHNFGGNHGLFGALEA VNGGPEAARLFPNSTMVGTGMAPEGISQNEV VYSLMAELGWRKDPVPDLAAWVTSFAARRY GVSHPDAGAAWRLLLRSVYNCSGEACRGHN RSPLVRRPSLQMNTSIWYNRSDVFEAWRLLL TSAPSLATSPAFRYDLLDLTRQAVQELVSLYY EEARSAYLSKELASLLRAGGVLAYELLPALD EVLASDSRFLLGSWLEQARAAAVSEAEADFY EQNSRYQLTLWGPEGNILDYANKQLAGLVA NYYTPRWRLFLEALVDSVAQGIPFQQHQFDK NVFQLEQAFVLSKQRYPSQPRGDTVDLAKKI FLKYYPRWVAGSW [SEQ ID NO:392]	Mucopolysaccharidosis IIIB	Lysosomal storage disorder
NEU 1	4758	0204386,02 27315,0227 129,022395 7,0234846, 0184494,02 28691,0234 343	Q5JQI0, Q99519	MTGERPSTALPDRRWGPRILGFWGGCRVWV FAAIFLLLSLAASWSKAENDFGLVQPLVTME QLLWVSGRQIGSVDTFRIPLITATPRGTLLAFA EARKMSSSDEGAKFIALRRSMDQGSTWSPTA FIVNDGDVPDGLNLGAVVSDVETGVVFLFYS LCAHKAGCQVASTMLVWSKDDGVSWSTPR NLSLDIGTEVFAPGPGSGIQKQREPRKGRLIVC	Mucolipidosis type I, Sialidosis I	Lysosomal storage disorder

				GHGTLERDGVFCLLSDDHGASWRYGSGVSGI PYGQPKQENDFNPDECQPYELPDGSVVINAR NQNNYHCHCRIVLRSYDACDTLRPRDVTFDP ELVDPVVAAGAVVTSSGIVFFSNPAHPEFRVN LTLRWSFSNGTSWRKET VQLWPGPSGYSSLATLEGSMDGEEQAPQLYV LYEKGRNHYTESISVAKISVYGTL [SEQ ID NO:393]		
NPC 1	4864	0141458	O15118	MTARGLALGLLLLLLCPAQVFSQSCVWYGEC GIAYGDKRYNCEYSGPPKPLPKDGYDLVQEL CPGFFFGNVSLCCDVRQLQTLKDNLQLPLQF LSRCPSCFYNLLNLFCELTCSPRQSQFLNVTA TEDYVDPVTNQTKTNVKELQYYVGQSFANA MYNACRDVEAPSSNDKALGLLCGKDADACN ATNWIEYMFNKDNGQAPFTITPVFSDFPVHG MEPMNNATKGCDESVDEVTAPCSCQDCSIVC GPKPQPPPPPAPWTILGLDAMYVIMWITYMA FLLVFFGAFFAVWCYRKRYFVSEYTPIDSNIA FSVNASDKGEASCCDPVSAAFEGCLRRLFTR WGSFCVRNPGCVIFFSLVFITACSSGLVFVRV TTNPVDLWSAPSSQARLEKEYFDQHFGPFFRT EQLIIRAPLTDKHIYQPYPSGADVPFGPPLDIQI LHQVLDLQIAIENITASYDNETVTLQDICLAPL SPYNTNCTILSVLNYFQNSHSVLDHKKGDDFF VYADYHTHFLYCVRAPASLNDTSLLHDPCLG TFGGPVFPWLVLGGYDDQNYNNATALVITFP VNNYYNDTEKLQRAQAWEKEFINFVKNYKN PNLTISFTAERSIEDELNRESDSDVFTVVISYAI MFLYISLALGHMKSCRRLLVDSKVSLGIAGIL IVLSSVACSLGVFSYIGLPLTLIVIEVIPFLVLA VGVDNIFILVQAYQRDERLQGETLDQQLGRV LGEVAPSMFLSSFSETVAFFLGALSVMPAVHT FSLFAGLAVFIDFLLQITCFV SLLGLDIKRQEKNRLDIFCCVRGAEDGTSVQA SESCLFRFFKNSYSPLLLKDWMRPIVIAIFVGV LSFSIAVLNKVDIGLDQSLSMPDDSYMVDYF KSISQYLHAGPPVYFVLEEGHDYTSSKGQNM VCGGMGCNNDSLVQQIFNAAQLDNYTRIGFA PSSWIDDYFDWVKPQSSCCRVDNITDQFCNA SVVDPACVRCRPLTPEGKQRPQGGDFMRFLP MFLSDNPNPKCGKGGHAAYSSAVNILLGHGT RVGATYFMTYHTVLQTSADFIDALKKARLIA SNVTETMGINGSAYRVFPYSVFYVFYEQYLTI IDDTIFNLGVSLGAIFLVTMVLLGCELWSAVI MCATIAMVLVNMFGVMWLWGISLNAVSLV NLVMSCGISVEFCSHITRAFTVSMKGSRVERA EEALAHMGSSVFSGITLTKFGGIVVLAFAKSQ IFQIFYFRMYLAMVLLGATHGLIFLPVLLSYIG PSVNKAKSCATEERYKGTERERLLNF [SEQ ID NO:394]	Niemann-Pick type C	Lysosomal storage disorder
NPC 2	10577	0119655	A0A024R6C0, P61916, G3V3E8	MRFLAATFLLLALSTAAQAEPVQFKDCGSVD GVIKEVNVSPCPTQPCQLSKGQSYSVNVTFTS NIQSKSSKAVVHGILMGVPVPFPIPEPDGCKS GINCPIQKDKTYSYLNKLPVKSEYPSIKLVVE WQLQDDKNQSLFCWEIPVQIVSHL [SEQ ID NO:395]	Niemann-Pick type C	Lysosomal storage disorder
SGSH	6448	0181523	P51688	MSCPVPACCALLLVLGLCRARPRNALLLLAD DGGFESGAYNNSAIATPHLDALARRSLLFRN AFTSVSSCSPSRASLLTGLPQHQNGMYGLHQ DVHHFNSFDKVRSLPLLLSQAGVRTGIIGKKH	Mucopolysaccharidosis IIIA	Lysosomal storage disorder

				VGPETVYPFDFAYTEENGSVLQVGRNITRIKL LVRKFLQTQDDRPFFLYVAFHDPHRCGHSQP QYGTFCEKFGNGESGMGRIPDWTPQAYDPLD VLVPYFVPNTPAARADLAAQYTTVGRMDQG VGLVLQELRDAGVLNDTLVIFTSDNGIPFPSG RTNLYWPGTAEPLLVSSPE HPKRWGQVSEAYVSLLDLTPTILDWFSIPYPS YAIFGSKTIHLTGRSLLPALEAEPLWATVFGS QSHHEVTMSYPMRSVQHRHFRLVHNLNFKM PFPIDQDFYVSPTFQDLLNRTTAGQPTGWYK DLRHYYYRARWELYDRSRDPHETQNLATDP RFAQLLEMLRDQLAKWQWETHDPWVCAPD GVLEEKLSPQCQPLHNEL [SEQ ID NO:396]		
PPT1	5538	0131238	P50897	MASPGCLWLLAVALLPWTCASRALQHLDPP APLPLVIWHGMGDSCCNPLSMGAIKKMVEK KIPGIYVLSLEIGKTLMEDVENSFFLNVNSQV TTVCQALAKDPKLQQGYNAMGFSQGGQFLR AVAQRCPSPPMINLISVGGQHQGVFGLPRCPG ESSHICDFIRKTLNAGAYSKVVQERLVQAEY WHDPIKEDVYRNHSIFLADINQERGINESYKK NLMALKKFVMVKFLNDSIVDPVDSEWFGFY RSGQAKETIPLQETSLYTQDRLGLKEMDNAG QLVFLATEGDHLQLSEEWFYAHIIPFLG [SEQ ID NO:397]	Neuronal ceroid lipofuscinosis 1 (CLN1)	Lysosomal storage disorder
PSAP	5660	0197746	P07602, A0A024QZQ2	MYALFLLASLLGAALAGPVLGLKECTRGSAV WCQNVKTASDCGAVKHCLQTVWNKPTVKS LPCDICKDVVTAAGDMLKDNATEEEILVYLE KTCDWLPKPNMSASCKEIVDSYLPVILDIIKG EMSRPGEVCSALNLCESLQKHLAELNHQKQL ESNKIPELDMTEVVAPFMANIPLLLYPQDGPR SKPQPKDNGDVCQDCIQMVTDIQTAVRTNST FVQALVEHVKEECDRLGPGMADICKNYISQY SEIAIQMMMHMQPKEICALVGFCDEVKEMP MQTLVPAKVASKNVIPALELVEPIKKHEVPA KSDVYCEVCEFLVKEVTKLIDNNKTEKEILDA FDKMCSKLPKSLSEECQEVVDTYGSSILSILLE EVSPELVCSMLHLCSGTRLPALTVHVTQPKD GGFCEVCKKLVGYLDRNLEKNSTKQEILAAL EKGCSFLPDPYQKQCDQFVAEYEPVLIEILVE VMDPSFVCLKIGACPSAHKPLLGTEKCIWGPS YWCQNTETAAQCNAVEHCKRHVWN [SEQ ID NO:398]	Prosaposin deficiency, SapA deficiency (Krabbe variant), SapB deficiency (MLD variant), SapC deficiency (Gaucher variant)	Lysosomal storage disorder
SLC17A5	26503	0119899	Q9NRA2	MRSPVRDLARNDGEESTDRTPLLPGAPRAEA APVCCSARYNLAILAFFGFFIVYALRVNLSVA LVDMVDSNTTLEDNRTSKACPEHSAPIKVHH NQTGKKYQWDAETQGWILGSFFYGYIITQIPG GYVASKIGGKMLLGFGILGTAVLTLFTPIAAD LGVGPLIVLRALEGLGEGVTFPAMHAMWSS WAPPLERSKLLSISYAGAQLGTVISLPLSGIIC YYMNWTYVFYFFGTIGIFWFLLWIWLVSDTP QKHKRISHYEKEYILSSLRNQLSSQKSVPWVP ILKSLPLWAIVVAHFSYNWTFYTLLTLLPTYM KEILRFNVQENGFLSSLPYLGSWLCMILSGQA ADNLRAKWNFSTLCVRRIFSLIGMIGPAVFLV AAGFIGCDYSLAVAFLTISTTLGGFCSSGFSIN HLDIAPSYAGILLGITNTFATIPGMVGPVIAKS LTPDNTVGEWQTVFYIAAAINVFGAIFFTLFA KGEVQNWALNDHHGHRH [SEQ ID NO:399]	Infantile sialic acid storage disease, Salla disease	Lysosomal storage disorder

SMPD1	6609	0166311	P17405, Q59EN6, E9LUE8, Q8IUN0, E9LUE9	MPRYGASLRQSCPRSGREQGQDGTAGAPGLL WMGLVLALALALALALALSDSRVLWAPAEA HPLSPQGHPARLHRIVPRLRDVFGWGNLTCPI CKGLFTAINLGLKKEPNVARVGSVAIKLCNLL KIAPPAVCQSIVHLFEDDMVEVWRRSVLSPSE ACGLLLGSTCGHWDIFSSWNISLPTVPKPPPK PPSPPAPGAPVSRILFLTDLHWDHDYLEGTDP DCADPLCCRRGSGLPPASRPGAGYWGEYSKC DLPLRTLESLLSGLGPAGPFDMVYWTGDIPA HDVWHQTRQDQLRALTTVTALVRKFLGPVP VYPAVGNHESTPVNSFPPPFIEGNHSSRWLYE AMAKAWEPWLPAEALRTLRIGGFYALSPYPG LRLISLNMNFCSRENFWLLINSTDPAGQLQWL VGELQAAEDRGDKVHIIGHIPPGHCLKSWSW NYYRIVARYENTLAAQFFGHTHVDEFEVFYD EETLSRPLAVAFLAPSATTYIGLNPGYRVYQI DGNYSGSSHVVLDHETYILNLTQANIPGAIPH WQLLYRARETYGLPNTLPTAWHNLVYRMRG DMQLFQTFWFLYHKGHPPSEPCGTPCRLATL CAQLSARADSPALCRHLMPDGSLPEAQSLWP RPLFC [SEQ ID NO:400]	Niemann Pick types A and B	Lysosomal storage disorder
SUMF1	285362	0144455	Q8NBK3	MAAPALGLVCGRCPELGLVLLLLLLSLLCGA AGSQEAGTGAGAGSLAGSCGCGTPQRPGAH GSSAAAHRYSREANAPGPVPGERQLAHSKM VPIPAGVFTMGTDDPQIKQDGEAPARRVTIDA FYMDAYEVSNTEFEKFVNSTGYLTEAEKFGD SFVFEGMLSEQVKTNIQQAVAAAPWWLPVK GANWRHPEGPDSTILHRPDHPVLHVSWNDA VAYCTWAGKRLPTEAEWEYSCRGGLHNRLF PWGNKLQPKGQHYANIWQGEFPVTNTGEDG FQGTAPVDAFPPNGYGLYNIVGNAWEWTSD WWTVHHSVEETLNPKGPPSGKDRVKKGGSY MCHRSYCYRYRCAARSQNTPDSSASN LGFRCAADRLPTMD [SEQ ID NO:401]	Multiple sulfatase deficiency	Lysosomal storage disorder
TPP1	1200	0166340	O14773	MGLQACLLGLFALILSGKCSYSPEPDQRRTLP PGWVSLGRADPEEELSLTFALRQQNVERLSE LVQAVSDPSSPQYGKYLTLENVADLVRPSPL TLHTVQKWLLAAGAQKCHSVITQDFLTCWL SIRQAELLLPGAEFHHYVGGPTETHVVRSPHP YQLPQALAPHVDFVGGLHRFPPTSSLRQRPEP QVTGTVGLHLGVTPSVIRKRYNLTSQDVGSG TSNNSQACAQFLEQYFHDSDLAQFMRLFGGN FAHQASVARVVGQQGRGRAGIEASLDVQYL MSAGANISTWVYSSPGRHEG QEPFLQWLMLLSNESALPHVHTVSYGDDEDS LSSAYIQRVNTELMKAAARGLTLLFASGDSG AGCWSVSGRHQFRPTFPASSPYVTTVGGTSF QEPFLITNEIVDYISGGGFSNVFPRPSYQEEAV TKFLSSSPHLPPSSYFNASGRAYPDVAALSDG YWVVSNRVPIPWVSGTSASTPVFGGILSLINE HRILSGRPPLGFLNPRLYQQHGAGLFDVTRGC HESCLDEEVEGQGFCSGPGWDPVTGWGTPNF PALLKTLLNP [SEQ ID NO:402]	Neuronal ceroid lipofuscinosis 2 (CLN2)	Lysosomal storage disorder
AHCY	191	0101444	P23526, Q1RMG2	MSDKLPYKVADIGLAAWGRKALDIAENEMP GLMRMRERYSASKPLKGARIAGCLHMTVET AVLIETLVTLGAEVQWSSCNIFSTQDHAAAAI AKAGIPVYAWKGETDEEYLWCIEQTLYFKDG PLNMILDDGGDLTNLIHTKYPQLLPGIRGISEE TTTGVHNLYKMMANGILKVPAINVNDSVTKS KFDNLYGCRESLIDGIKRATDVMIAGKVAVV	Hypermethioninemia	Aminoacidophaty

				AGYGDVGKGCAQALRGFGARVIITEIDPINAL QAAMEGYEVTTMDEACQEGNIFVTTTGCIDII LGRHFEQMKDDAIVCNIG HFDVEIDVKWLNENAVEKVNIKPQVDRYRL KNGRRIILLAEGRLVNLGCAMGHPSFVMSNS FTNQVMAQIELWTHPDKYPVGVHFLPKKLDE AVAEAHLGKLNVKLTKLTEKQAQYLGMSCD GPFKPDHYRY [SEQ ID NO:403]		
GN MT	27232	0124713	A0A0S2Z 5F2, Q14749, V9HW60	MVDSVYRTRSLGVAAEGLPDQYADGEAARV WQLYIGDTRSRTAEYKAWLLGLLRQHGCQR VLDVACGTGVDSIMLVEEGFSVTSVDASDKM LKYALKERWNRRHEPAFDKWVIEEANWMTL DKDVPQSAEGGFDAVICLGNSFAHLPDCKGD QSEHRLALKNIASMVRAGGLLVIDHRNYDHI LSTGCAPPGKNIYYKSDLTKDVTTSVLIVNNK AHMVTLDYTVQVPGAGQDGSPGLSKFRLSY YPHCLASFTELLQAAFGGKCQHSVLGDFKPY KPGQTYIPCYFIHVLKRTD [SEQ ID NO:404]	Hypermethi oninemia	Aminoaci dophaty
MAT 1A	4143	0151224	Q00266	MNGPVDGLCDHSLSEGVFMFTSESVGEGHPD KICDQISDAVLDAHLKQDPNAKVACETVCKT GMVLLCGEITSMAMVDYQRVVRDTIKHIGY DDSAKGFDFKTCNVLVALEQQSPDIAQCVHL DRNEEDVGAGDQGLMFGYATDETEECMPLTI ILAHKLNARMADLRRSGLLPWLRPDSKTQVT VQYMQDNGAVIPVRIHTIVISVQHNEDITLEE MRRALKEQVIRAVVPAKYLDEDTVYHLQPS GRFVIGGPQGDAGVTGRKIIVDTYGGWGAHG GGAFSGKDYTKVDRSAAYAARWVAKSLVK AGLCRRVLVQVSYAIGVAEPLSISIFTYGTSQ KTERELLDVVHKNFDLRPGVIVRDLDLKKPIY QKTACYGHFGRSEFPWEVPRKLVF [SEQ ID NO:405]	Hypermethi oninemia	Aminoaci dophaty
GCH 1	2643	0131979	A0A024R 642, P30793, Q8IZH9	MEKGPVRAPAEKPRGARCSNGFPERDPPRPG PSRPAEKPPRPEAKSAQPADGWKGERPRSEE DNELNLPNLAAAYSSILSSLGENPQRQGLLKT PWRAASAMQFFTKGYQETISDVLNDAIFDED HDEMVIVKDIDMFSMCEHHLVPFVGKVHIGY LPNKQVLGLSKLARIVEIYSRRLQVQERLTKQ IAVAITEALRPAGVGVVVEATHMCMVMRGV QKMNSKTVTSTMLGVFREDPKTREEFLTLIRS [SEQ ID NO:406]	BH4 cofactor deficiency	Aminoaci dophaty
PCB D1	5092	0166228	P61457	MAGKAHRLSAEERDQLLPNLRAVGWNELEG RDAIFKQFHFKDFNRAFGFMTRVALQAEKLD HHPEWFNVYNKVHITLSTHECAGLSERDINL ASFIEQVAVSMT [SEQ ID NO:407]	BH4 cofactor deficiency	Aminoaci dophaty
PTS	5805	0150787	Q03393	MSTEGGGRRCQAQVSRRISFSASHRLYSKFLS DEENLKLFGKCNNPNGHGHNYKVVVTVHGE IDPATGMVMNLADLKKYMEEAIMQPLDHKN LDMDVPYFADVVSTTENVAVYIWDNLQKVL PVGVLYKVKVYETDNNIVVYKGE [SEQ ID NO:408]	BH4 cofactor deficiency	Aminoaci dophaty
QDP R	5860	0151552	A0A140V KA9, P09417	MAAAAAAGEARRVLVYGGRGALGSRCVQA FRARNWWVASVDVVENEEASASIIVKMTDSF TEQADQVTAEVGKLLGEEKVDAILCVAGGW AGGNAKSKSLFKNCDLMWKQSIWTSTISSHL ATKHLKEGGLLTLAGAKAALDGTPGMIGYG MAKGAVHQLCQSLAGKNSGMPPGAAAIAVL PVTLDTPMNRKSMPEADFSSWTPLEFLVETF HDWITGKNRPSSGSLIQVVTTEGRTELTPAYF [SEQ ID NO:409]	BH4 cofactor deficiency	Aminoaci dophaty

SPR	6697	0116096	P35270	MEGGLGRAVCLLTGASRGFGRTLAPLLASLL SPGSVLVLSARNDEALRQLEAELGAERSGLR VVRVPADLGAEAGLQQLLGALRELPRPKGLQ RLLLINNAGSLGDVSKGFVDLSDSTQVNNYW ALNLTSMLCLTSSVLKAFPDSPGLNRTVVNIS SLCALQPFKGWALYCAGKAARDMLFQVLAL EEPNVRVLNYAPGPLDTDMQQLARETSVDPD MRKGLQELKAKGKLVDCKVSAQKLLSLLEK DEFKSGAHVDFYDK [SEQ ID NO:410]	BH4 cofactor deficiency	Aminoaci dophaty
DNA JC12	56521	0108176	Q6IAH1, Q9UKB3	MDAILNYRSEDTEDYYTLLGCDELSSVEQILA EFKVRALECHPDKHPENPKAVETFQKLQKAK EILTNEESRARYDHWRRSQMSMPFQQWEAL NDSVKTSMHWVVRGKKDLMLEESDKTHTTK MENEECNEQRERKKEELASTAEKTEQKEPKP LEKSVSPQNSDSSGFADVNGWHLRFRWSKD APSELLRKFRNYEI [SEQ ID NO:411]	Phenylalani ne, tyrosine, and tryptophan hydroxylas es heat shock co-chaperone deficiency	Aminoaci dophaty
ALD H4A 1	8659	0159423	P30038, A0A024R AD8	MLLPAPALRRALLSRPWTGAGLRWKHTSSLK VANEPVLAFTQGSPERDALQKALKDLKGRM EAIPCVVGDEEVWTSDVQYQVSPFNHGHKV AKFCYADKSLLNKAIEAALAARKEWDLKPIA DRAQIFLKAADMLSGPRRAEILAKTMVGQGK TVIQAEIDAAAELIDFFRFNAKYAVELEGQQPI SVPPSTNSTVYRGLEGFVAAISPFNFTAIGGNL AGAPALMGNVVLWKPSDTAMLASYAVYRIL REAGLPPNIIQFVPADGPLFGDTVTSSEHLCGI NFTGSVPTFKHLWKQVAQ NLDRFHTFPRLAGECGGKNFHFVHRSADVES VVSGTLRSAFEYGGQKCSACSRLYVPHSLWP QIKGRLLEEHSRIKVGDPAEDFGTFFSAVIDA KSFARIKKWLEHARSSPSLTILAGGKCDDSVG YFVEPCIVESKDPQEPIMKEEIFGPVLSVYVYP DDKYKETLQLVDSTTSYGLTGAVFSQDKDV VQEATKVLRNAAGNFYINDKSTGSIVGQQPF GGARASGTNDKPGGPHYILRWTSPQVIKETH KPLGDWSYAYMQ [SEQ ID NO:412]	Hyperproli nemia	Aminoaci dophaty
PRO DH	5625	0100033	O43272	MALRRALPALRPCIPRFVQLSTAPASREQPAA GPAAVPGGGSATAVRPPVPAVDFGNAQEAY RSRRTWELARSLLVLRLCAWPALLARHEQLL YVSRKLLGQRLFNKLMKMTFYGHFVAGEDQ ESIQPLLRHYRAFGVSAILDYGVEEDLSPEEA EHKEMESCTSAAERDGSGTNKRDKQYQAHR AFGDRRNGVISARTYFYANEAKCDSHMETFL RCIEASGRVSDDGFIAIKLTALGRPQFLLQFSE VLAKWRCFFHQMAVEQGQAGLAAMDTKLE VAVLQESVAKLGIASRAEIEDW FTAETLGVSGTMDLLDWSSLIDSRTKLSKHL VVPNAQTGQLEPLLSRFTEEEELQMTRMLQR MDVLAKKATEMGVRLMVDAEQTYFQPAISR LTLEMQRKFNVEKPLIFNTYQCYLKDAYDNV TLDVELARREGWCFGAKLVRGAYLAQERAR AAEIGYEDPINPTYEATNAMYHRCLDYVLEE LKHNAKAKVMVASHNEDTVRFALRRMEELG LHPADHQVYFGQLLGMCDQISFPLGQAGYPV YKYVPYGPVMEVLPYLSRRALENSSLMKGT HRERQLLWLELLRRLRTGNLFHRPA [SEQ ID NO:413]	Hyperproli nemia	Aminoaci dophaty

HPD	3242	0158104	P32754	MTTYSDKGAKPERGRFLHFHSVTFWVGNAK QAASFYCSKMGFEPLAYRGLETGSREVVSHV IKQGKIVFVLSSALNPWNKEMGDHLVKHGD GVKDIAFEVEDCDYIVQKARERGAKIMREPW VEQDKFGKVKFAVLQTYGDTTHTLVEKMNY IGQFLPGYEAPAFMDPLLPKLPKCSLEMIDHI VGNQPDQEMVSASEWYLKNLQFHRFWSVDD TQVHTEYSSLRSIVVANYEESIKMPINEPAPG KKKSQIQEYVDYNGGAGVQHIALKTEDIITAI RHLRERGLEFLSVPSTYYKQLREKLKTAKIKV KENIDALEELKILVDYDEKGYLLQIFTKPVQD RPTLFLEVIQRHNHQGFGAGNFNSLFKAFEEE QNLRGNLTNMETNGVVPGM [SEQ ID NO:414]	Tyrosinemia type II	Aminoacidophaty
GBA	2629	0177628,02 62446	A0A068F 658, P04062, B7Z6S9	MEFSSPSREECPKPLSRVSIMAGSLTGLLLLQ AVSWASGARPCIPKSFGYSSVVCVCNATYCD SFDPPTFPALGTFSRYESTRSGRRMELSMGPIQ ANHTGTGLLLTLQPEQKFQKVKGFGGAMTD AAALNILALSPPAQNLLLKSYFSEEGIGYNIIR VPMASCDFSIRTYTYADTPDDFQLHNFSLPEE DTKLKIPLIHRALQLAQRPVSLLASPWTSPTW LKTNGAVNGKGSLKGQP GDIYHQTWARYFVKFLDAYAEHKLQFWAVT AENEPSAGLLSGYPFQCLGFTPEHQRDFIARD LGPTLANSTHHNVRLLMLDDQRLLLPHWAK VVLTDPEAAKYVHGIAVHWYLDFLAPAKAT LGETHRLFPNTMLFASEACVGSKFWEQSVRL GSWDRGMQYSHSIITNLLYHVVGWTDWNLA LNPEGGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAV ALMHPDGSAVVVVLNRSSKDVPLTIKDPAVG FLETISPGYSIHTYLWRRQ [SEQ ID NO:415]	Gaucher disease	
HGD	3081	0113924	Q93099, B3KW64	MAELKYISGFGNECSSEDPRCPGSLPEGQNNP QVCPYNLYAEQLSGSAFTCPRSTNKRSWLYR ILPSVSHKPFESIDEGQVTHNWDEVDPDPNQL RWKPFEIPKASQKKVDFVSGLHTLCGAGDIK SNNGLAIHIFLCNTSMENRCFYNSDGDFLIVP QKGNLLIYTEFGKMLVQPNEICVIQRGMRFSI DVFEETRGYILEVYGVHFELPDLGPIGANGLA NPRDFLIPIAWYEDRQVPGGYTVINKYQGKLF AAKQDVSPFNVVAWHGNYTPYKYNLKNFM VINSVAFDHADPSIFTVLTAKSVRPGVAIADF VIFPPRWGVADKTFRPPYYHRNCMSEFMGLI RGHYEAKQGGFLPGGGSLHSTMTPHGPDAD CFEKASKVKLAPERIADGTMAFMFESSLSLA VTKWGLKASRCLDENYHKCWEPLKSHFTPN SRNPAEPN [SEQ ID NO:416]	Alkaptonuria	
AMN	81693	0166126	Q9BXJ7, B3KP64	MGVLGRVLLWLQLCALTQAVSKLWVPNTDF DVAANWSQNRTPCAGGAVEFPADKMVSVLV QEGHAVSDMLLPLDGELVLASGAGFGVSDV GSHLDCGAGEPAVFRDSDRFSWHDPHLWRS GDEAPGLFFVDAERVPCRHDDVFFPPSASFRV GLGPGASPVRVRSISALGRTFTRDEDLAVFLA SRAGRLRFHGPGALSVGPEDCADPSGCVCGN AEAQPWICAALLQPLGGRCPQAACHSALRPQ GQCCDLCGAVVLLTHGPAFDLERYRARILDT FLGLPQYHGLQVAVSKVPRSSRLREADTEIQV VLVENGPETGGAGRLARALLADVAENGEAL GVLEATMRESGAHVWGSSAAGLAGGVAAA VLLALLVLLVAPPLLRRAGRLRWRRHEAAAP	Combined Methylmalonic Acidemia and Homocystinuria	Organic acidemia

				AGAPLGFRNPVFDVTASEELPLPRRLSLVPKA AADSTSHSYFVNPLFAGAEAEA [SEQ ID NO:417]		
CD320	51293	0167775	Q9NPF0	MSGGWMAQVGAWRTGALGLALLLLLGLGL GLEAAASPLSTPTSAQAAGPSSGSCPPTKFQC RTSGLCVPLTWRCDRDLDCSDGSDEEECRIEP CTQKGQCPPPPGLPCPCTGVSDCSGGTDKKL RNCSRLACLAGELRCTLSDDCIPLTWRCDGH PDCPDSSDELGCGTNEILPEGDATTMGPPVTL ESVTSLRNATTMGPPVTLESVPSVGNATSSSA GDQSGSPTAYGVIAAAAVLSASLVTATLLLLS WLRAQERLRPLGLLVAMKESLLLSEQKTSLP [SEQ ID NO:418]	Combined Methylmalonic Acidemia and Homocystinuria	Organic acidemia
CUBN	8029	0107611	O60494	MMNMSLPFLWSLLTLLIFAEVNGEAGELELQ RQKRSINLQQPRMATERGNLVFLTGSAQNIEF RTGSLGKIKLNDEDLSECLHQIQKNKEDIIELK GSAIGLPQNISSQIYQLNSKLVDLERKFQGLQ QTVDKKVCSSNPCQNGGTCLNLHDSFFCICPP QWKGPLCSADVNECEIYSGTPLSCQNGGTCV NTMGSYSCHCPPETYGPQCASKYDDCEGGSV ARCVHGICEDLMREQAGEPKYSCVCDAGWM FSPNSPACTLDRDECSFQPGPCSTLVQCFNTQ GSFYCGACPTGWQGNGYICEDINECEINNGG CSVAPPVECVNTPGSSHCQACPPGYQGDGRV CTLTDICSVSNGGCHPDASCSSTLGSLPLCTCL PGYTGNGYGPNGCVQLSNICLSHPCLNGQCI DTVSGYFCKCDSGWTGVNCTENINECLSNPC LNGGTCVDGVDSFSCECTRLWTGALCQVPQ QVCGESLSGINGSFSYRSPDVGYVHDVNCFW VIKTEMGKVLRITFTFFRLESMDNCPHEFLQV YDGDSSSAFQLGRFCGSSLPHELLSSDNALYF HLYSEHLRNGRGFTVRWETQQPECGGILTGP YGSIKSPGYPGNYPPGRDCVWIVVTSPDLLVT FTFGTLSLEHHDDCNKDYLEIRDGPLYQDPLL GKFCTTFSVPPLQTTGPFARIHFHSDSQISDQG FHITYLTSPSDLRCGGNYTDPEGELFLPELSGP FTHTRQCVYMMKQPQGEQIQINFTHVELQCQ SDSSQNYIEVRDGETLLGKVCGNGTISHIKSIT NSVWIRFKIDASVEKASFRAVYQVACGDELT GEGVIRSPFFPNVYPGERTCRWTIHQPQSQVIL LNFTVFEIGSSAHCETDYVEIGSSSILGSPENK KYCGTDIPSFITSVYNFLYVTFVKSSSTENHGF MAKFSAEDLACGEILTESTGTIQSPGHPNVYP HGINCTWHILVQPNHLIHLMFETFHLEFHYNC TNDYLEVYDTDSETSLGRYCGKSIPPSLTSSG NSL MLVFVTDSDLAYEGFLINYEAISAATACLQD YTDDLGTFTSPNFPNNYPNNWECIYRITVRTG QLIAVHFTNFSLEEAIGNYYTDFLEIRDGGYE KSPLLGIFYGSNLPPTIISHSNKLWLKFKSDQI DTRSGFSAYWDGSSTGCGGNLTTSSGTFISPN YPMPYYHSSECYWWLKSSHGSAFELEFKDFH LEHHPNCTLDYLAVYDGPSSNSHLLTQLCGD EKPPLIRSSGDSMFIKLR TDEGQQGRGFKAEYRQTCENVVIVNQTYGIL ESIGYPNPYSENQHCNWTIRATTGNTVNYTFL AFDLEHHINCSTDYLELYDGPRQMGRYCGVD LPPPGSTTSSKLQVLLLTDGVGRREKGFQMQ WFVYGCGGELSGATGSFSSPGFPNRYPPNKE CIWYIRTDPGSSIQLTIHDFDVEYHSRCNFDVL	Combined Methylmalonic Acidemia and Homocystinuria	Organic acidemia

				EIYGGPDFHSPRIAQLCTQRSPENPMQVSSTG NELAIRFKTDLSINGRGFNASWQAVTGGCGGI FQAPSGEIHSPNYPSPYRSNTDCSWVIRVDRN HRVLLNFTDFDLEPQDSCIMAYDGLSSTMSR LARTCGREQLANPIVSSGNSLFLRFQSGPSRQ NRGFRAQFRQACGGHILTSSFDTVSSPRFPAN YPNNQNCSWIIQAQPPLNHITLSFTHFELERST TCARDFVEILDGGHEDAPLRGRYCGTDMPHP ITSFSSALTLRFVSDSSISAGGFHTTVTASVSA CGGTFYMAEGIFNSPGYPDIYPPNVECVWNIV SSPGNRLQLSFISFQLEDSQDCSRDFVEIREGN ATGHLVGRYCGNSFPLNYSSIVGHTLWVRFIS DGSGSGTGFQATFMKIFGNDNIVGTHGKVAS PFWPENYPHNSNYQWTVNVNASHVVHGRIL EMDIEEIQNCYYDKLRIYDGPSIHARLIGAYC GTQTESFSSTGNSLTFHFYSDSSISGKGFLLEW FAVDAPDGVLPTIAPGACGGFLRTGDAPVFLF SPGWPDSYSNRVDCTWLIQAPDSTVELNILSL DIESHRTCAYDSLVIRDGDNNLAQQLAVLCG REIPGPIRSTGEYMFIRFTSDSSVTRAGFNASF HKSCGGYLHADRGIITSPKYPETYPSNLNCSW HVLVQSGLTIAVHFEQPFQIPNGDSSCNQGDY LVLRNGPDICSPPLGPPGGNGHFCGSHASSTL FTSDNQMFVQFISDHSNEGQGFKIKYEAKSLA CGGNVYIHDADSAGYVTSPNHPHNYPPHADC IWILAAPPETRIQLQFEDRFDIEVTPNCTSNYL ELRDGVDSDAPILSKFCGTSLPSSQWSSGEVM YLRFRSDNSPTHVGFKAKYSIAQCGGRVPGQ SGVVESIGHPTLPYRDNLFCEWHLQGLSGHY LTISFEDFNLQNSSGCEKDFVEIWDNHTSGNIL GRYCGNTIPDSIDTSSNTAVVRFVTDGSVTAS GFRLRFESSMEECGGDLQGSIGTFTSPNYPNP NPHGRICEWRITAPEGRRITLMFNNLRLATHP SCNNEHVIVFNGIRSNSPQLEKLCSSVNVSNEI KSSGNTMKVIFFTDGSRPYGGFTASYTSSEDA VCGGSLPNTPEGNFTSPGYDGVRNYSRNLNC EWTLSNPNQGNSSISIHFEDFYLESHQDCQFD VLEFRVGDADGPLMWRLCGPSKPTLPLVIPY SQVWIHFVTNERVEHIGFHAKYSFTDCGGIQI GDSGVITSPNYPNAYDSLTHCSSLLEAPQGHT ITLTFSDFDIEPHTTCAWDSVTVRNGGSPESPII GQYCGNSNPRTIQSGSNQLVVTFNSDHSLQG GGFYATWNTQTLGCGGIFHSDNGTIRSPHWP QNFPENSRCSWTAITHKSKHLEISFDNNFLIPS GDGQCQNSFVKVWAGTEEVDKALLATGCGN VAPGPVITPSNTFTAVFQSQEAPAQGFSASFV SRCGSNFTGPSGYIISPNYPKQYDNNMNCTYV IEANPLSVVLLTFVSFHLEARSAVTGSCVNDG VHIIRGYSVMSTPFATVCG DEMPAPLTIAGPVLLNFYSNEQITDFGFKFSY RIISCGGVFNFSSGIITSPAYSYADYPNDMHCL YTITVSDDKVIELKFSDFDVVPSTSCSHDYLAI YDGANTSDPLLGKFCGSKRPPNVKSSNNSML LVFKTDSFQTAKGWKMSFRQTLGPQQGCGG YLTGSNNTFASPDSDSNGMYDKNLNCVWIII APVNKVIHLTFNTFALEAASTRQRCLYDYVK LYDGDSENANLAGTFCGSTVPAPFISSGNFLT VQFISDLTLEREGFNATYTIMDMPCGGTYNA TWTPQNISSPNSSDPDVPFSICTWVIDSPPHQQ VKITVWALQLTSQDCTQNYLQLQDSPQGHG		

				NSRFQFCGRNASAVPVFYSSMSTAMVIFKSG VVNRNSRMSFTYQIADCNRDYHKAFGNLRSP GWPDNYDNDKDCTVTLTAPQNHTISLFFHSL GIENSVECRNDFLEVRNGSNSNSPLLGKYCGT LLPNPVFSQNNELYLRFKSDSVTSDRGYEIIW TSSPSGCGGTLYGDRGSFTSPGYPGTYPNNTY CEWVLVAPAGRLVTINFYFISIDDPGDCVQNY LTLYDGPNASSPSSGPYCGGDTSIAPFVASSN QVFIKFHADYARRPSAFRLTWDS[SEQ ID NO:419]		
GIF	2694	0134812	P27352	MAWFALYLLSLLWATAGTSTQTQSSCSVPSA QEPLVNGIQVLMENSVTSSAYPNPSILIAMNL AGAYNLKAQKLLTYQLMSSDNNDLTIGQLG LTIMALTSSCRDPGDKVSILQRQMENWAPSSP NAEASAFYGPSLAILALCQKNSEATLPIAVRF AKTLLANSSPFNVDTGAMATLALTCMYNKIP VGSEEGYRSLFGQVLKDIVEKISMKIKDNGIIG DIYSTGLAMQALSVTPEPSKKEWNCKKTTDM ILNEIKQGKFHNPMSIAQILPSLKGKTYLDVPQ VTCSPDHEVQPTLPSNPGPGPTSASNITVIYTI NNQLRGVELLFNETINVSVKSGSVLLVVLEEA QRKNPMFKFETTMTSWGLVVSSINNIAENVN HKTYWQFLSGVTPLNEGVADYIPFNHEHITA NFTQY [SEQ ID NO:420]	Combined Methylmalonic Acidemia and Homocystinuria	Organic acidemia
TCN1	6947	0134827	P20061	MRQSHQLPLVGLLLFSFIPSQLCEICEVSEENY IRLKPLLNTMIQSNYNRGTSAVNVVLSLKLV GIQIQTLMQKMIQQIKYNVKSRLSDVSSGELA LIILALGVCRNAEENLIYDYHLIDKLENKFQA EIENMEAHNGTPLTNYYQLSLDVLALCLFNG NYSTAEVVNHFTPENKNYYFGSQFSVDTGA MAVLALTCVKKSLINGQIKADEGSLKNISIYT KSLVEKILSEKKENGLIGN TFSTGEAMQALFVSSDYYNENDWNCQQTLN TVLTEISQGAFSNPNAAAQVLPALMGKTFLDI NKDSSCVSASGNFNISADEPITVTPPDSQSYIS VNYSVRINETYFTNVTVLNGSVFLSVMEKAQ KMNDTIFGFTMEERSWGPYITCIQGLCANNN DRTYWELLSGGEPLSQGAGSYVVRNGENLE VRWSKY [SEQ ID NO:421]	Combined Methylmalonic Acidemia and Homocystinuria	Organic acidemia
TCN2	6948	0185339	P20062	MRHLGAFLFLLGVLGALTEMCEIPEMDSHLV EKLGQHLLPWMDRLSLEHLNPSIYVGLRLSSL QAGTKEDLYLHSLKLGYQQCLLGSAFSEDDG DCQGKPSMGQLALYLLALRANCEFVRGHKG DRLVSQLKWFLEDEKRAIGHDHKGHPHTSYY QYGLGILALCLHQKRVHDSVVDKLLYAVEPF HQGHHSVDTAAMAGLAFTCLKRSNFNPGRR QRITMAIRTVREEILKAQTPEGHFGNVYSTPL ALQFLMTSPMRGAELGTACLKARVALLASLQ DGAFQNALMISQLLPVLNHKTYIDLIFPDCLA PRVMLEPAAETIPQTQEIISVTLQVLSLLPPYR QSISVLAGSTVEDVLKKAHELGGFTYETQASL SGPYLTSVMGKAAGEREFWQLLRDPNTPLLQ GIADYRPKDGETIELRLVSW [SEQ ID NO:422]	Combined Methylmalonic Acidemia and Homocystinuria	Organic acidemia
PREPL	9581	0138078	Q4J6C6	MQQKTKLFLQALKYSIPHLGKCMQKQHLNH YNFADHCYNRIKLKKYHLTKCLQNKPKISEL ARNIPSRSFSCKDLQPVKQENEKPLPENMDAF EKVRTKLETQPQEEYEIINVEVKHGGFVYYQ EGCCLVRSKDEEADNDNYEVLFNLEELKLDQ PFIDCIRVAPDEKYVAAKIRTEDSEASTCVIIK LSDQPVMEASFPNVSSFEWVKDEEDEDVLFY	Cystinuria	Aminoacidophaty

				TFQRNLRCHDVYRATFGDNKRNERFYTEKDP SYFVFLYLTKDSRFLTINIMNKTTSEVWLIDG LSPWDPPVLIQKRIHGVLYYVEHRDDELYILT NVGEPTEFKLMRTAADTPAIMNWDLFFTMK RNTKVIDLDMFKDHCVLFLKHSNLLYVNVIG LADDSVRSLKLPPWACGFIMDTNSDPKNCPF QLCSPIRPPKYYTYKFAEGKLFEETGHEDPITK TSRVLRLEAKSKDGKLVPMTVFHKTDSEDLQ KKPLLVHVYGAYGMDLKMNFRPERRVLVDD GWILAYCHVRGGGELGLQWHADGRLTKKLN GLADLEACIKTLHGQGFSQPSLTTLTAFSAGG VLAGALCNSNPELVRAVTLEAPFLDVLNTM MDTTLPLT LEELEEWGNPSSDEKHKNYIKRYCPYQNIKP QHYPSIHITAYENDERVPLKGIVSYTEKLKEAI AEHAKDTGEGYQTPNIILDIQPGGNHVIEDSH KKITAQIKFLYEELGLDSTSVFEDLKKYLKF[S EQ ID NO:423]		
PHG DH	26227	0092621	O43175	MAFANLRKVLISDSLDPCCRKILQDGGLQVV EKQNLSKEELIAELQDCEGLIVRSATKVTADV INAAEKLQVVGRAGTGVDNVDLEAATRKGIL VMNTPNGNSLSAAELTCGMIMCLARQIPQAT ASMKDGKWERKKFMGTELNGKTLGILGLGR IGREVATRMQSFGMKTIGYDPIISPEVSASFGV QQLPLEEIWPLCDFITVHTPLLPSTTGLLNDNT FAQCKKGVRVVNCARGGIVDEGALLRALQS GQCAGAALDVFTEEPPRDRALVDHENVISCP HLGASTKEAQSRCGEEIA VQFVDMVKGKSLTGVVNAQALTSAFSPHTK PWIGLAEALGTLMRAWAGSPKGTIQVITQGT SLKNAGNCLSPAVIVGLLKEASKQADVNLVN AKLLVKEAGLNVTTSHSPAAPGEQGFGECLL AVALAGAPYQAVGLVQGTTPVLQGLNGAVF RPEVPLRRDLPLLLFRTQTSDPAMLPTMIGLL AEAGVRLLSYQTSLVSDGETWHVMGISSLLP SLEAWKQHVTEAFQFHF [SEQ ID NO:424]	Disorders of Serine Biosynthesis	Aminoacidopathy
PSA T1	29968	0135069	A0A024R280, Q9Y617, A0A024R222	MDAPRQVVNFGPGPAKLPHSVLLEIQKELLD YKGVGISVLEMSHRSSDFAKIINNTENLVREL LAVPDNYKVIFLQGGGCGQFSAVPLNLIGLK AGRCADYVVTGAWSAKAAEEAKKFGTINIV HPKLGSYTKIPDPSTWNLNPDASYVYYCANE TVHGVEFDFIPDVKGAVLVCDMSSNFLSKPV DVSKFGVIFAGAQKNVGSAGVTVVIVRDDLL GFALRECPSVLEYKVQAGNSSLYNTPPCFSIY VMGLVLEWIKNNGGAAAMEKLSSIKSQTIYE IIDNSQGFYVCPVEPQNRSKMNIPFRIGNAKG DDALEKRFLDKALELNMLSLKGHRSVGGIRA SLYNAVTIEDVQKLAAFMKKFLEMHQL [SEQ ID NO:425]	Disorders of Serine Biosynthesis	Aminoacidopathy
PSP H	5723	0146733	A0A024R DL3, P78330	MVSHSELRKLFYSADAVCFDVDSTVIREEGID ELAKICGVEDAVSEMTRRAMGGAVPFKAAL TERLALIQPSREQVQRLIAEQPPHLTPGIRELV SRLQERNVQVFLISGGFRSIVEHVASKLNIPAT NVFANRLKFYFNGEYAGFDETQPTAESGGKG KVIKLLKEKFHFKKIIMIGDGATDMEACPPAD AFIGFGGNVIRQQVKDNAKWYITDFVELLGE LEE [SEQ ID NO:426]	Disorders of Serine Biosynthesis	Aminoacidopathy
AMT	275	0145020	A0A024R 2U7, P48728	MQRAVSVVARLGFRLQAFPPALCRPLSCAQE VLRRTPLYDFHLAHGGKMVAFAGWSLPVQY RDSHTDSHLHTRQHCSLFDVSHMLQTKILGS	Glycine Encephalopathy	Aminoacidopathy

				DRVKLMESLVVGDIAELRPNQGTLSLFTNEA GGILDDLIVTNTSEGHLYVVSNAGCWEKDLA LMQDKVRELQNQGRDVGLEVLDNALLALQG PTAAQVLQAGVADDLRKLPFMTSAVMEVFG VSGCRVTRCGYTGEDGVEISVPVAGAVHLAT AILKNPEVKLAGLAARDSLRLEAGLCLYGND IDEHTTPVEGSLSWTLGKRRRAAMDFPGAKV IVPQLKGRVQRRRVGLMCEGAPMRAHSPILN MEGTKIGTVTSGCPSPSLKKNVAMGYVPCEY SRPGTMLLVEVRRKQQMAVVSKMPFVPTNY YTLK [SEQ ID NO:427]		
GCSH	2653	0140905	P23434	MALRVVRSVRALLCTLRAVPSPAAPCPPRPW QLGVGAVRTLRTGPALLSVRKFTEKHEWVTT ENGIGTVGISNFAQEALGDVVYCSLPEVGTKL NKQDEFGALESVKAASELYSPLSGEVTEINEA LAENPGLVNKSCYEDGWLIKMTLSNPSELDE LMSEEAYEKYIKSIEE [SEQ ID NO:428]	Glycine Encephalopathy	Aminoacidopathy
GLDC	2731	0178445	P23378	MQSCARAWGLRLGRGVGGGRRLAGGSGPC WAPRSRDSSSGGGDSAAAGASRLLERLLPRH DDFARRHIGPGDKDQREMLQTLGLASIDELIE KTVPANIRLKRPLKMEDPVCENEILATLHAIS SKNQIWRSYIGMGYYNCSVPQTILRNLLENSG WITQYTPYQPEVSQGRLESLLNYQTMVCDIT GLDMANASLLDEGTAAAEALQLCYRHNKRR KFLVDPRCHPQTIAVVQTRAKYTGVLTELKL PCEMDFSGKDVSGVLFQYPDTEGKVEDFTEL VERAHQSGSLACCATDLLALC ILRPPGEFGVDIALGSSQRFGVPLGYGGPHAA FFAVRESLVRMMPGRMVGVTRDATGKEVYR LALQTREQHIRRDKATSNICTAQALLANMAA MFAIYHGSHGLEHIARRVHNATLILSEGLKRA GHQLQHDLFFDTLKIQCGCSVKEVLGRAAQR QINFRLFEDGTLGISLDETVNEKDLDDLLWIF GCESSAELVAESMGEECRGIPGSVFKRTSPFL THQVFNSYHSETNIVRYMKKLENKDISLVHS MIPLGSCTMKLNSSSELAPITWKEFANIHPFVP LDQAQGYQQLFRELEKDLCELTGYDQVCFQP NSGAQGEYAGLATIRAYLNQKGEGHRTVCLI PKSAHGTNPASAHMAGMKIQPVEVDKYGNI DAVHLKAMVDKHKENLAAIMITYPSTNGVFE ENISDVCDLIHQHGGQVYLDGANMNAQVGIC RPGDFGSDVSHLNLHKTFCIPHGGGGPGMGPI GVKKHLAPFLPNHPVISLKRNEDACPVGTVS AAPWGSSSILPISWAYIKMMGGKGLKQATET AILNANYMAKRLETHYRILFRGARGYVGHEF ILDTRPFKKSANIEAVDVAKRLQDYGFHAPT MSWPVAGTLMVEPTESEDKAELDRFCDAMIS IRQEIADIEEGRIDPRVNPLKMSPHSLTCVTSS HWDRPYSREVAAFPLPFVKPENKFWPTIARID DIYGDQHLVCTCPPMEVYESPFSEQKRASS [SEQ ID NO:429]	Glycine Encephalopathy	Aminoacidopathy
LIAS	11019	0121897	O43766, Q6P5Q6, B4E0L7, A0A024R9W0, A0A1W2PQE9, A0A1X7SBR7	MSLRCGDAARTLGPRVFGRYFCSPVRPLSSLP DKKKELLQNGPDLQDFVSGDLADRSTWDEY KGNLKRQKGERLRLPPWLKTEIPMGKNYNK LKNTLRNLNLHTVCEEARCPNIGECWGGGEY ATATATIMLMGDTCTRGCRFCSVKTARNPPP LDASEPYNTAKAIAEWGLDYVVLTSVDRDD MPDGGAEHIAKTVSYLKERNPKILVECLTPDF RGDLKAIEKVALSGLDVYAHNVETVPELQSK VRDPRANFDQSLRVLKHAKKVQPDVISKTSI	Glycine Encephalopathy	Aminoacidopathy

				MLGLGENDEQVYATMKALREADVDCLTLGQ YMQPTRRHLKVEEYITPEKFKYWEKVGNELG FHYTASGPLVRSSYKAGEFFL KNLVAKRKTKDL [SEQ ID NO:430]		
NFU 1	27247	0169599	Q9UMS0	MAATARRGWGAAAVAAGLRRRFCHMLKNP YTIKKQPLHQFVQRPLFPLPAAFYHPVRYMFI QTQDTPNPNSLKFIPGKPVLETRTMDFPTPAA AFRSPLARQLFRIEGVKSVFFGPDFITVTKENE ELDWNLLKPDIYATIMDFFASGLPLVTEETPS GEAGSEEDDEVVAMIKELLDTRIRPTVQEDG GDVIYKGFEDGIVQLKLQGSCTSCPSSIITLKN GIQNMLQFYIPEVEGVEQVMDDESDEKEANS P [SEQ ID NO:431]	Glycine Encephalop athy	Aminoaci dophaty
SLC 6A9	6536	0196517	P48067, B7Z3W8, B7Z589	MSGGDTRAAIARPRMAAAHGPVAPSSPEQVT LLPVQRSFFLPPFSGATPSTSLAESVLKVWHG AYNSGLLPQLMAQHSLAMAQNGAVPSEATK RDQNLKRGNWGNQIEFVLTSVGYAVGLGNV WRFPYLCYRNGGGAFMFPYFIMLIFCGIPLFF MELSFGQFASQGCLGVWRISPMFKGVGYGM MVVSTYIGIYYNVVICIAFYYFFSSMTHVLPW AYCNNPWNTHDCAGVLDASNLTNGSRPAAL PSNLSHLLNHSLQRTSPSEEYWRLYVLKLSDD IGNFGEVRLPLLGCLGVSWLVVFLCLIRGVKS SGKVVYFTATFPYVVLTILFVRGVTLEGAFDG IMYYLTPQWDKILEAKVWGDAASQIFYSLGC AWGGLITMASYNKFHNNCYRDSVIISITNCAT SVYAGFVIFSILGFMANHLGVDVSRVADHGP GLAFVAYPEALTLLPISPLWSLLFFFMLILLGL GTQFCLLETLVTAIVDEVGNEWILQKKTYVT LGVAVAGFLLGIPLTSQAGIYWLLLMDNYAA SFSLVVISCIMCVAIMYIYGHRNYFQDIQMML GFPPPLFFQICWRFVSPAIIFFILVFTVIQYQPIT YNHYQYPGWAVAIGFLMALSSVLCIPLYAMF RLCRTDGDTLLQRLKNATKPSRDWGPALLEH RTGRYAPTIAPSPEDGFEVQPLHPDKAQIPIVG SNGSSRLQDSRI [SEQ ID NO:432]	Glucose Transporter Type 1 Deficiency	Carbohydr ate disorder
SLC 2A1	6513	0117394	P11166, Q59GX2	MEPSSKKLTGRLMLAVGGAVLGSLQFGYNT GVINAPQKVIEEFYNQTWVHRYGESILPTTLT TLWSLSVAIFSVGGMIGSFSVGLFVNRFGRRN SMLMMNLLAFVSAVLMGFSKLGKSFEMLILG RFIIGVYCGLTTGFVPMYVGEVSPTALRGALG TLHQLGIVVGILIAQVFGLDSIMGNKDLWPLL LSIIFIPALLQCIVLPFCPESPRFLLINRNEENRA KSVLKKLRGTADVTHDLQEMKEESRQMMRE KKVTILELFRSPAYRQPILIAVVLQLSQQLSGI NAVFYYSTSIFEKAGVQQPVYATIGSGIVNTA FTVVSLFVVERAGRRTLHLIGLAGMAGCAIL MTIALALLEQLPWMSYLSIVAIFGFVAFFEVG PGPIPWFIVAELFSQGPRPAAIAVAGFSNWTS NFIVGMCFQYVEQLCGPYVFIIFTVLLVLFFIF TYFKVPETKGRTFDEIASGFRQGGASQSDKTP E ELFHPLGADSQV [SEQ ID NO:433]		
ATP 7A	538	0165240	B4DRW0, Q04656, Q762B6	MDPSMGVNSVTISVEGMTCNSCVWTIEQQIG KVNGVHHIKVSLEEKNATIIYDPKLQTPKTLQ EAIDDMGFDAVIHNPDPLPVLTDTLFLTVTAS LTLPWDHIQSTLLKTKGVTDIKIYPQKRTVAV TIIPSIVNANQIKELVPELSLDTGTLEKKSGAC EDHSMAQAGEVVLKMKVEGMTCHSCTSTIE GKIGKLQGVQRIKVSLDNQEATIVYQPHLISV	ATP7A-Related Disorders Copper Metabolism Disorder	Metal transport disorder

				EEMKKQIEAMGFPAFVKKQPKYLKLGAIDVE RLKNTPVKSSEGSQQRSPSYTNDSTATFIIDG MHCKSCVSNIESTLSALQYVSSIVVSLENRSAI VKYNASSVTPESLRKAIEAVSPGLYRVSITSE VESTSNSPSSSSLQKIPLNVVSQPLTQETVINID GMTCNSCVQSIEGVISKKPGVKSIRVSLANSN GTVEYDPLLTSPETLRGAIEDMGFDATLSDTN EPLVVIAQPSSEMPLLTSTNEFYTKGMTPVQD KEEGKNSSKCYIQVTGMTCASCVANIERNLR REEGIYSILVALMAGKAEVRYNPAVIQPPMIA EFIRELGFGATVIENADEGDGVLELVVRGMT CASCVHKIESSLTKHRGILYCSVALATNKAHI KYDPEIIGPRDIIHTIESLGFEASLVKKDRSASH LDHKREIRQWRRSFLVSLFFCIPVMGLMIYM MVMDHHFATLHHNQNMSKEEMINLHSSMFL ERQILPGLSVMNLLSFLLC VPVQFFGGWYFYIQAYKALKHKTANMDVLI VLATTIAFAYSLIILLVAMYERAKVNPITFFDT PPMLFVFIALGRWLEHIAKGKTSEALAKLISL QATEATIVTLDSDNILLSEEQVDVELVQRGDII KVVPGGKFPVDGRVIEGHSMVDESLITGEAM PVAKKPGSTVIAGSINQNGSLLICATHVGADT TLSQIVKLVEEAQTSKAPIQQFADKLSGYFVP FIVFVSIATLLVWIVIG FLNFEIVETYFPGYNRSISRTETIIRFAFQASITV LCIACPCSLGLATPTAVMVGTGVGAQNGILIK GGEPLEMAHKVKVVVFDKTGTITHGTPVVN QVKVLTESNRISHHKILAIVGTAESNSEHPLGT AITKYCKQELDTETLGTCIDFQVVPGCGISCK VTNIEGLLHKNNWNIEDNNIKNASLVQIDASN EQSSTSSSMIIDAQISNALNAQQYKVLIGNRE WMIRNGLVINNDVN DFMTEHERKGRTAVLVAVDDELCGLIAIADT VKPEAELAIHILKSMGLEVVLMTGDNSKTAR SIASQVGITKVFAEVLPSHKVAKVKQLQEEG KRVAMVGDGINDSPALAMANVGIAIGTGTD VAIEAADVVLIRNDLLDVVASIDLSRKTVKRI RINFVFALIYNLVGIPIAAGVFMPIGLVLQPW MGSAAMAASSVSVVLSSLFLKLYRKPTYESY ELPARSQIGQKSPSEISVHVGIDDTSRNSPKLG LLDRIVNYSRASINSLLSDKRSLNSVVTSEPDK HSLLVGDFREDDDTAL [SEQ ID NO:434]		
AP1 S1	1174	0106367	A0A024Q YT6, P61966	MMRFMLLFSRQGKLRLQKWYLATSDKERKK MVRELMQVVLARKPKMCSFLEWRDLKVVY KRYASLYFCCAIEGQDNELITLELIHRYVELL DKYFGSVCELDIIFNFEKAYFILDEFLMGGDV QDTSKKSVLKAIEQADLLQEEDESPRSVLEEM GLA [SEQ ID NO:435]	Copper Metabolism Disorder	Metal transport disorder
CP	1356	0047457	A5PL27, P00450	MKILILGIFLFLCSTPAWAKEKHYYIGIIETTW DYASDHGEKKLISVDTEHSNIYLQNGPDRIGR LYKKALYLQYTDETFRTTIEKPVWLGFLGPII KAETGDKVYVHLKNLASRPYTFHSHGITYYK EHEGAIYPDNTTDFQRADDKVYPGEQYTYM LLATEEQSPGEGDGNCVTRIYHSHIDAPKDIA SGLIGPLIICKKDSLDKEKEKHIDREFVVMFSV VDENFSWYLEDNIKTYC SEPEKVDKDNEDFQESNRMYSVNGYTFGSLP GLSMCAEDRVKWYLFGMGNEVDVHAAFFH GQALTNKNYRIDTINLFPATLFDAYMVAQNP GEWMLSCQNLNHLKAGLQAFFQVQECNKSS	Copper Metabolism Disorder	Metal transport disorder

				SKDNIRGKHVRHYYIAAEEIIWNYAPSGIDIFT KENLTAPGSDSAVFFEQGTTRIGGSYKKLVY REYTDASFTNRKERGPEEEHLGILGPVIWAEV GDTIRVTFHNKGAYPLSIEPIGVRFNKNNEGT YYSPNYNPQSRSVPPSASHVAPTETFTYEWTV PKEVGPTNADPVCLAKMYY SAVDPTKDIFTGLIGPMKICKKGSLHANGRQK DVDKEFYLFPTVFDENESLLLEDNIRMFTTAP DQVDKEDEDFQESNKMHSMNGFMYGNQPG LTMCKGDSVVWYLFSAGNEADVHGIYFSGN TYLWRGERRDTANLFPQTSLTLHMWPDTEG TFNVECLTTDHYTGGMKQKYTVNQCRRQSE DSTFYLGERTYYIAAVEVEWDYSPQREWEKE LHHLQEQNVSNAFLDKGEFYIGSKYKKVVYR QYTDSTFRVPVERKAEEEHLGILGPQLHADV GDKVKIIFKNMATRPYSIHAHGVQTESSTVTP TLPGETLTYVWKIPERSGAGTEDSACIPWAY YSTVDQVKDLYSGLIGPLIVCRRPYLKVFNPR RKLEFALLFLVFDENESWYLDDNIKTYSDHPE KVNKDDEEFIESNKMHAINGRMFGNLQGLT MHVGDEVNWYLMGMGNEIDLHTVHFHGHS FQYKHRGVYSSDVFDIFPGTYQTLEMFPRTPG IWLLHCHVTDHIHAGMETTYTVLQNEDTKSG [SEQ ID NO:436]		
SLC 33A1	9197	0169359	O00400	MSPTISHKDSSRQRRPGNFSHSLDMKSGPLPP GGWDDSHLDSAGREGDREALLGDTGTGDFL KAPQSFRAELSSILLLLFLYVLQGIPLGLAGSIP LILQSKNVSYTDQAFFSFVFWPFSLKLLWAPL VDAVYVKNFGRRKSWLVPTQYILGLFMIYLS TQVDRLLGNTDDRTPDVIALTVAFFLFEFLAA TQDIAVDGWALTMLSRENVGYASTCNSVGQ TAGYFLGNVLFLALESADFCNKYLRFQPQPR GIVTLSDFLFFWGTVFLITTTLVALLKKENEV SVVKEETQGITDTYKL LFAIIKMPAVLTFCLLILTAKIGFSAADAVTGL KLVEEGVPKEHLALLAVPMVPLQIILPLIISKY TAGPQPLNTFYKAMPYRLLLGLEYALLVWW TPKVEHQGGFPIYYYIVVLLSYALHQVTVYS MYVSIMAFNAKVSDPLIGGTYMTLLNTVSNL GGNWPSTVALWLVDPLTVKECVGASNQNCR TPDAVELCKKLGGSCVTALDGYYVESIICVFI GFGWWFFLGPKFKKLQDEGSSSWKCKRNN[S EQ ID NO:437]	Copper Metabolism Disorder	Metal transport disorder
PEX 7	5191	0112357	O00628, Q6FGN1	MSAVCGGAARMLRTPGRHGYAAEFSPYLPG RLACATAQHYGIAGCGTLLILDPDEAGLRLFR SFDWNDGLFDVTWSENNEHVLITCSGDGSLQ LWDTAKAAGPLQVYKEHAQEVYSVDWSQT RGEQLVVSGSWDQTVKLWDPTVGKSLCTFR GHESIIYSTIWSPHIPGCFASASGDQTLRIWDV KAAGVRIVIPAHQAEILSCDWCKYNENLLVT GAVDCSLRGWDLRNVRQPVFELLGHTYAIRR VKFSPFHASVLASCSYDFTVRFWNFSKPDSLL ETVEHHTEFTCGLDFSLQSPTQVADCSWDETI KIYDPACLTIPA [SEQ ID NO:438]	Adult Refsum Disease Rhizomelic Chondrody splasia Punctata Spectrum	Peroxisomal disorders
PHYH	5264	0107537	O14832	MEQLRAAARLQIVLGHLGRPSAGAVVAHPTS GTISSASFHPQQFQYTLDNNVLTLEQRKFYEE NGFLVIKNLVPDADIQRFRNEFEKICRKEVKP LGLTVMRDVTISKSEYAPSEKMITKVQDFQE DKELFRYCTLPEILKYVECFTGPNIMAMHTM LINKPPDSGKKTSRHPLHQDLHYFPFRPSDLIV	Adult Refsum Disease	Peroxisomal disorders

				CAWTAMEHISRNNGCLVVLPGTHKGSLKPH DYPKWEGGVNKMFHGIQDYEENKARVHLV MEKGDTVFFHPLLIHGSGQNKTQGFRKAISC HFASADCHYIDVKGTSQENIEKEVVGIAHKFF GAENSVNLKDIWMFRARLVKGERTNL [SEQ ID NO:439]		
AGPS	8540	0018510	O00116, B7Z3Q4	MAEAAAAAGGTGLGAGASYGSAADRDRDP DPDRAGRRLRVLSGHLLGRPREALSTNECKA RRAASAATAAPTATPAAQESGTIPKKRQEVM KWNGWGYNDSKFIFNKKGQIELTGKRYPLSG MGLPTFKEWIQNTLGVNVEHKTTSKASLNPS DTPPSVVNEDFLHDLKETNISYSQEADDRVFR AHGHCLHEIFLLREGMFERIPDIVLWPTCHDD VVKIVNLACKYNLCIIPIGGGTSVSYGLMCPA DETRTIISLDTSQMNRILWVDENNLTAHVEAG ITGQELERQLKESGYCTGH EPDSLEFSTVGGWVSTRASGMKKNIYGNIED LVVHIKMVTPRGIIEKSCQGPRMSTGPDIHHFI MGSEGTLGVITEATIKIRPVPEYQKYGSVAFP NFEQGVACLREIAKQRCAPASIRLMDNKQFQ FGHALKPQVSSIFTSFLDGLKKFYITKFKGFDP NQLSVATLLFEGDREKVLQHEKQVYDIAAKF GGLAAGEDNGQRGYLLTYVIAYIRDLALEYY VLGESFETSAPWDRVVDLCRNVKERITRECK EKGVQFAPFSTCRVTQTYDAGACIYFYFAFN YRGISDPLTVFEQTEAAAREEILANGGSLSHH HGVGKLRKQWLKESISDVGFGMLKSVKEYV DPNNIFGNRNLL [SEQ ID NO:440]	Rhizomelic Chondrodysplasia Punctata Spectrum	Peroxisomal disorders
GNPAT	8443	0116906	O15228	MESSSSSNSYFSVGPTSPSAVVLLYSKELKKW DEFEDILEERRHVSDLKFAMKCYTPLVYKGIT PCKPIDIKCSVLNSEEIHYVIKQLSKESLQSVD VLREEVSEILDEMSHKLRLGAIRFCAFTLSKV FKQIFSKVCVNEEGIQKLQRAIQEHPVVLLPS HRSYIDFLMLSFLLYNYDLPVPVIAAGMDFLG MKMVGELLRMSGAFFMRRTFGGNKLYWAV FSEYVKTMLRNGYAPVEFFLEGTRSRSAKTL TPKFGLLNIVMEPFFKREVFDTYLVPISISYDK ILEETLYVYELLGVPKPKESTTGLLKARKILSE NFGSIHVYFGDPVSLRSLAAGRMSRSSYNLVP RYIPQKQSEDMHAFVTEVAYKMELLQIENMV LSPWTLIVAVLLQNRPSMDFDALVEKTLWLK GLTQAFGGFLIWPDNKPAEEVVPASILLHSNI ASLVKDQVILKVDSGDSEVVDGLMLQHITLL MCSAYRNQLLNIFVRPSLVAVALQMTPGFRK EDVYSCFRFLRDVFADEFIFLPGNTLKDFEEG CYLLCKSEAIQVTTKDILVTEKGNTVLEFLVG LFKPFVESYQIICKYLLSEEEDHFSEEQYLAAV RKFTSQLLDQGTSQCYDVLSSDVQKNALAAC VRLGVVEKKKINNNCIFNVNEPATTKLEEML GCKTPIGKPATAKL [SEQ ID NO:441]	Rhizomelic Chondrodysplasia Punctata Spectrum	Peroxisomal disorders
ABCD1	215	0101986	P33897	MPVLSRPRPWRGNTLKRTAVLLALAAYGAH KVYPLVRQCLAPARGLQAPAGEPTQEASGVA AAKAGMNRVFLQRLLWLLRLLFPRVLCRET GLLALHSAALVSRTFLSVYVARLDGRLARCI VRKDPRAFGWQLLQWLLIALPATFVNSAIRY LEGQLALSFRSRLVAHAYRLYFSQQTYYRVS NMDGRLRNPDQSLTEDVVAFAASVAHLYSN LTKPLLDVAVTSYTLLRAARSRGAGTAWPSA IAGLVVFLTANVLRAFSPKFGELVAEEARRK GELRYMHSRVVANSEEIAFYGGHEVELALLQ	X-linked Adrenoleukodystrophy	Peroxisomal disorders

				RSYQDLASQINLILLERLWYVMLEQFLMKYV WSASGLLMVAVPIITATGYSESDAEAVKKAA LEKKEEELVSERTEAFTIARNLLTAAADAIERI MSSYKEVTELAGYTARVHEMFQVFEDVQRC HFKRPRELEDAQAGSGTIGRSGVRVEGPLKIR GQVVDVEQGIICENIPIVTPSGEVVVASLNIRV EEGMHLLITGPNGCGKSSLFRILGGLWPTYGG VLYKPPPQRMFYIPQRPYMSVGSLRDQVIYPD SVEDMQRKGYSEQDLEAILDVVHLHHILQRE GGWEAMCD WKDVLSGGEKQRIGMARMFYHRPKYALLDE CTSAVSIDVEGKIFQAAKDAGIALLSITHRPSL WKYHTHLLQFDGEGGWKFEKLDSAARLSLT EEKQRLEQQLAGIPKMQRRLQELCQILGEAV APAHVPAPSPQGPGGLQGAST [SEQ ID NO:442]		
ACO X1	51	0161533	Q15067	MNPDLRRERDSASFNPELLTHILDGSPEKTRR RREIENMILNDPDFQHEDLNFLTRSQRYEVAV RKSAIMVKKMREFGIADPDEIMWFKKLHLVN FVEPVGLNYSMFIPTLLNQGTTAQKEKWLLS SKGLQIIGTYAQTEMGHGTHLRGLETTATYD PETQEFILNSPTVTSIKWWPGGLGKTSNHAIV LAQLITKGKCYGLHAFIVPIREIGTHKPLPGIT VGDIGPKFGYDEIDNGYLKMDNHRIPRENML MKYAQVKPDGTYVKPLSNKLTYGTMVFVRS FLVGEAARALSKACTIAIRYSAVRHQSEIKPG EPEPQILDFQTQQYKLFPLLATAYAFQFVGAY MKETYHRINEGIGQGDLSELPELHALTAGLK AFTSWTANTGIEACRMACGGHGYSHCSGLPN IYVNFTPSCTFEGENTVMMLQTARFLMKSYD QVHSGKLVCGMVSYLNDLPSQRIQPQQVAV WPTMVDINSPESLTEAYKLRAARLVEIAAKN LQKEVIHRKSKEVAWNLTSVDLVRASEAHCH YVVVKLFSEKLLKIQDKAIQAVLRSLCLLYSL YGISQNAGDFLQGSIMTEPQITQVNQRVKELL TLIRSDAVALVDAFDFQDVTLGSVLGRYDGN VYENLFEWAKNSPLNKAEVHESYKHLKSLQS KL [SEQ ID NO:443]	X-linked Adrenoleukodystrophy	Peroxisomal disorders
PEX 1	5189	0127980	O43933, A0A0C4DG33, B4DER6	MWGSDRLAGAGGGGAAVTVAFTNARDCFL HLPRRLVAQLHLLQNQAIEVVWSHQPAFLSW VEGRHFSDQGENVAEINRQVGQKLGLSNGG QVFLKPCSHVVSCQQVEVEPLSADDWEILEL HAVSLEQHLLDQIRIVFPKAIFPVWVDQQTYI FIQIVALIPAASYGRLETDTKLLIQPKTRRAKE NTFSKADAEYKKLHSYGRDQKGMMKELQTK QLQSNTVGITESNENESEIPVDSSSVASLWTMI GSIFSFQSEKKQETSWGLTEINAFKNMQSKVV PLDNIFRVCKSQPPSIYNASATSVFHKHCAIHV FPWDQEYFDVEPSFTVTYGKLVKLLSPKQQQ SKTKQNVLSPEKEKQMSEPLDQKKIRSDHNE EDEKACVLQVVWNGLEELNNAIKYTKNVEV LHLGKVWIPDDLRKRLNIEMHAVVRITPVEV TPKIPRSLKLQPRENLPKDISEEDIKTVFYSWL QQSTTTMLPLVISEEEFIKLETKDGLKEFSLSI VHSWEKEKDKNIFLLSPNLLQKTTIQVLLDPM VKEEN SEEIDFILPFLKLSSLGGVNSLGVSSLEHITHSL LGRPLSRQLMSLVAGLRNGALLLTGGKGSGK STLAKAICKEAFDKLDAHVERVDCKALRGKR LENIQKTLEVAFSEAVWMQPSVVLLDDLDLI	X-linked Adrenoleukodystrophy	Peroxisomal disorders

				AGLPAVPEHEHSPDAVQSQRLAHALNDMIKE FISMGSLVALIATSQSQQSLHPLLVSAQGVHIF QCVQHIQPPNQEQRCEILCNVIKNKLDCDINK FTDLDLQHVAKETGGFVARDFTVLVDRAIHS RLSRQSISTREKLVLTTLDFQKALRGFLPASLR SVNLHKPRDLGWDKIGGLHEVRQILMDTIQL PAKYPELFANLPIRQRTGILLYGPPGTGKTLL AGVIARESRMNFISVKGPELLSKYIGASEQAV RDIFIRAQAAKPCILFFDEFESIAPRRGHDNTG VTDRVVNQLLTQLDGVEGLQGVYVLAATSR PDLIDPALLRPGRLDKCVYCPPPDQVSRLEIL NVLSDSLPLADDVDLQHVASVTDSFTGADLK ALLYNAQLEALHGMLLSSGLQDGSSSSDSDL SLSSMVFLNHSSGSDDSAGDGECGLDQSLVS LEMSEILPDESKFNMYRLYFGSSYESELGNGT SSDLSSQCLSAPSSMTQDLPGVPGKDQLFSQP PVLRTASQEGCQELTQEQRDQLRADISIIKGR YRSQSGEDESMNQPGPIKTRLAISQSHLMTAL GHTRPSISEDDWKNFAELYESFQNPKRRKNQ SGTMFRPGQKVTLA [SEQ ID NO:444]		
PEX 2	5828	0164751	P28328	MASRKENAKSANRVLRISQLDALELNKALEQ LVWSQFTQCFHGFKPGLLARFEPEVKACLWV FLWRFTIYSKNATVGQSVLNIKYKNDFSPNLR YQPPSKNQKIWYAVCTIGGRWLEERCYDLFR NHHLASFGKVKQCVNFVIGLLKLGGLINFLIF LQRGKFATLTERLLGIHSVFCKPQNICEVGFE YMNRELLWHGFAEFLIFLLPLINVQKLKAKLS SWCIPLTGAPNSDNTLATSGKECALCGEWPT MPHTIGCEHIFCYFCAKSSFLFDVYFTCPKCG TEVHSLQPLKSGIEMSEVNAL [SEQ ID NO:445]	X-linked Adrenoleukodystrophy	Peroxisomal disorders
PEX 3	8504	0034693	P56589	MLRSVWNFLKRHKKKCIFLGTVLGGVYILGK YGQKKIREIQEREAAEYIAQARRQYHFESNQR TCNMTVLSMLPTLREALMQQLNSESLTALLK NRPSNKLEIWEDLKIISFTRSTVAVYSTCMLV VLLRVQLNIIGGYIYLDNAAVGKNGTTILAPP DVQQQYLSSIQHLLGDGLTELITVIKQAVQKV LGSVSLKHSLSLLDLEQKLKEIRNLVEQHKSS SWINKDGSKPLLCHYMMPDEETPLAVQACG LSPRDITTIKLLNETRDMLESPDFSTVLNTCLN RGFSRLLDNMAEFFRPTEQDLQHGNSMNSLS SVSLPLAKIIPIVNGQIHSVCSETPSHFVQDLLT MEQVKDFAANVYEAFSTPQQLEK [SEQ ID NO:446]	X-linked Adrenoleukodystrophy	Peroxisomal disorders
PEX 5	5830	0139197	A0A0S2Z480, P50542, B4DR50, A0A0S2Z4F3, A0A0S2Z4H1, B4E0T2	MAMRELVEAECGGANPLMKLAGHFTQDKA LRQEGLRPGPWPPGAPASEAASKPLGVASED ELVAEFLQDQNAPLVSRAPQTFKMDDLLAE MQQIEQSNFRQAPQRAPGVADLALSENWAQ EFLAAGDAVDVTQDYNETDWSQEFISEVTDP LSVSPARWAEEYLEQSEEKLWLGEPEGTATD RWYDEYHPEEDLQHTASDFVAKVDDPKLAN SEFLKFVRQIGEGQVSLESGAGSGRAQAEQW AAEFIQQQGTSDAWVDQFTRPVNTSALDMEF ERAKSAIESDVDFWDKLQAELEEMAKRDAE AHPWLSDYDDLTSATYDKGYQFEEENPLRD HPQPFEEGLRRLQEGDLPNAVLLFEAAVQQD PKHMEAWQYLGTTQAENEQELLAISALRRCL ELKPDNQTALMALAVSFTNESLQRQACETLR DWLRYTPAYAHLVTPAEEGAGGAGLGPSKRI LGSLLSDSLFLEVKELFLAAVRLDPTSIDPDV	X-linked Adrenoleukodystrophy	Peroxisomal disorders

				QCGLGVLFNLSGEYDKAVDCFTAALSVRPND YLLWNKLGATLANGNQSEEAVAAYRRALEL QPGYIRSRYNLGISCINLGAHREAVEHFLEAL NMQRKSRGPRGEGGAMSENIWSTLRLALSM LGQSDAYGAADARDLSTLLTMFGLPQ [SEQ ID NO:447]		
PEX 6	5190	0124587	A0A024R D09, Q13608	MALAVLRVLEPFPTETPPLAVLLPPGGPWPA AELGLVLALRPAGESPAGPALLVAALEGPDA GTEEQGPGPPQLLVSRALLRLLALGSGAWVR ARAVRRPPALGWALLGTSLGPGLGPRVGPLL VRRGETLPVPGPRVLETRPALQGLLGPGTRLA VTELRGRARLCPESGDSSRPPPPPVVSSFAVS GTVRRLQGVLGGTGDSLGVSRSCLRGLGLFQ GEWVWVAQARESSNTSQPHLARVQVLEPRW DLSDRLGPGSGPLGEPLADGLALVPATLAFNL GCDPLEMGELRIQRYLEGS IAPEDKGSCSLLPGPPFARELHIEIVSSPHYSTN GNYDGVLYRHFQIPRVVQEGDVLCVPTIGQV EILEGSPEKLPRWREMFFKVKKTVGEAPDGP ASAYLADTTHTSLYMVGSTLSPVPWLPSEEST LWSSLSPPGLEALVSELCAVLKPRLQPGGALL TGTSSVLLRGPPGCGKTTVVAAACSHLGLHL LKVPCSSLCAESSGAVETKLQAIFSRARRCRP AVLLLTAVDLLGRDRDGLGEDARVMAVLRH LLLNEDPLNSCPPLMVVATTSRAQDLPADVQ TAFPHELEVPALSEGQRLSILRALTAHLPLGQ EVNLAQLARRCAGFVVGDLYALLTHSSRAA CTRIKNSGLAGGLTEEDEGELCAAGFPLLAED FGQALEQLQTAHSQAVGAPKIPSVSWHDVGG LQEVKKEILETIQLPLEHPELLSLGLRRSGLLL HGPPGTGKTLLAKAVATECSLTFLSVKGPELI NMYVGQSEENVREVFARARAAAPCIIFFDEL DSLAPSRGRSGDSGGVMDRVVSQLLAELDGL HSTQ	X-linked Adrenoleukodystrophy	Peroxisomal disorders
				DVFVIGATNRPDLLDPALLRPGRFDKLVFVG ANEDRASQLRVLSAITRKFKLEPSVSLVNVLD CCPPQLTGADLYSLCSDAMTAALKRRVHDLE EGLEPGSSALMLTMEDLLQAAARLQPSVSEQ ELLRYKRIQRKFAAC [SEQ ID NO:448]		
PEX 10	5192	0157911	A0A024R 068, O60683, A0A024R 0A4	MAPAAASPPEVIRAAQKDEYYRGGLRSAAG GALHSLAGARKWLEWRKEVELLSDVAYFGL TTLAGYQTLGEEYVSIIQVDPSRIHVPSSLRRG VLVTLHAVLPYLLDKALLPLEQELQADPDSG RPLQGSLGPGGRGCSGARRWMRHHTATLTE QQRRALLRAVFVLRQGLACLQRLHVAWFYI HGVFYHLAKRLTGITYLRVRSLPGEDLRARV SYRLLGVISLLHLVLSMGLQLYGFRQRQRAR KEWRLHRGLSHRRASLEERAVSRNPLCTLCL EERRHPTATPCGHLFCWECITAW CSSKAECPLCREKFPPQKLIYLRHYR[SEQ ID NO:449]	X-linked Adrenoleukodystrophy	Peroxisomal disorders
PEX 12	5193	0108733	O00623	MAEHGAHFTAASVADDQPSIFEVVAQDSLMT AVRPALQHVVKVLAESNPTHYGFLWRWFDE IFTLLDLLLQQHYLSRTSASFSENFYGLKRIV MGDTHKSQRLASAGLPKQQLWKSIMFLVLLP YLKVKLEKLVSSLREEDEYSIHPPSSRWKRFY RAFLAAYPFVNMAWEGWFLVQQLRYILGKA QHHSPLLRLAGVQLGRLTVQDIQALEHKPAK ASMMQQPARSVSEKINSALKKAVGGVALSLS TGLSVGVFFLQFLDWWYSSENQETIKSLTALP	X-linked Adrenoleukodystrophy	Peroxisomal disorders

				TPPPPVHLDYNSDSPLLPKMKTVCPLCRKTRV NDTVLATSGYVFCYRCVFHYVRSHQACPITG YPTEVQHLIKLYSPEN [SEQ ID NO:450]		
PEX 13	5194	0162928	Q92968	MASQPPPPPKPWETRRIPGAGPGPGPGPTFQS ADLGPTLMTRPGQPALTRVPPPILPRPSQQTG SSSVNTFRPAYSSFSSGYGAYGNSFYGGYSPY SYGYNGLGYNRLRVDDLPPSRFVQQAEESSR GAFQSIESIVHAFASVSMMMDATFSAVYNSF RAVLDVANHFSRLKIHFTKVFSAFALVRTIRY LYRRLQRMLGLRRGSENEDLWAESEGTVAC LGAEDRAATSAKSWPIFLFFAVILGGPYLIWK LLSTHSDEVTDSINWASGEDDHVVARAEYDF AAVSEEEISFRAGDMLNLALKEQQPKVRGWL LASLDGQTTGLIPANYVKILGKRKGRKTVESS KVSKQQQSFTNPTLTKGATVADSLDEQEAAF ESVFVETNKVPVAPDSIGKDGEKQDL [SEQ ID NO:451]	X-linked Adrenoleukodystrophy	Peroxisomal disorders
PEX 14	5195	0142655	O75381	MASSEQAEQPSQPSSTPGSENVLPREPLIATA VKFLQNSRVRQSPLATRRAFLKKKGLTDEEID MAFQQSGTAADEPSSLGPATQVVPVQPPHLIS QPYSPAGSRWRDYGALAIIMAGIAFGFHQLY KKYLLPLILGGREDRKQLERMEAGLSELSGS VAQTVTQLQTTLASVQELLIQQQQKIQELAH ELAAAKATTSTNWILESQNINELKSEINSLKG LLLNRRQFPPSPSAPKIPSWQIPVKSPSPSSPAA VNHHSSSDISPVSNESTSSSPGKEGHSPEGSTV TYHLLGPQEEGEGVVDVKGQVRMEVQGEEE KREDKEDEEDEEDDDVSHVDEEDCLGVQRE DRRGGDGQINEQVEKLRRPEGASNESERD[SE Q ID NO:452]	X-linked Adrenoleukodystrophy	Peroxisomal disorders
PEX 16	9409	0121680	Q9Y5Y5	MEKLRLLGLRYQEYVTRHPAATAQLETAVR GFSYLLAGRFADSHELSELVYSASNLLVLLND GILRKELRKKLPVSLSQQKLLTWLSVLECVEV FMEMGAAKVWGEVGRWLVIALVQLAKAVL RMLLLLWFKAGLQTSPPIVPLDRETQAQPPD GDHSPGNHEQSYVGKRSNRVVRTLQNTPSLH SRHWGAPQQREGRQQQHHEELSATPTPLGLQ ETIAEFLYIARPLLHLLSLGLWGQRSWKPWLL AGVVDVTSLSLLSDRKGLTRRERRELRRRTIL LLYYLLRSPFYDRFSEARIL FLLQLLADHVPGVGLVTRPLMDYLPTWQKIY FYSWG [SEQ ID NO:453]	X-linked Adrenoleukodystrophy	Peroxisomal disorders
PEX 19	5824	0162735	P40855, A0A0S2Z497	MAAAEEGCSVGAEADRELEELLESALDDFDK AKPSPAPPSTTTAPDASGPQKRSPGDTAKDAL FASQEKFFQELFDSELASQATAEFEKAMKEL AEEEPHLVEQFQKLSEAAGRVGSDMTSQQEF TSCLKETLSGLAKNATDLQNSSMSEEELTKA MEGLGMDEGDGEGNILPIMQSIMQNLLSKDV LYPSLKEITEKYPEWLQSHRESLPPEQFEKYQ EQHSVMCKICEQFEAETPTDSETTQKARFEM VLDLMQQLQDLGHPPKELAGEMPPGLNFDL DALNLSGPPGASGEQCLIM [SEQ ID NO:454]	X-linked Adrenoleukodystrophy	Peroxisomal disorders
PEX 26	55670	0215193	A0A024R100, Q7Z412, A0A0S2Z5M7, Q7Z2D7	MKSDSSTSAAPLRGLGGPLRSSEPVRAVPAR APAVDLLEEAADLLVVHLDFRAALETCERA WQSLANHAVAEEPAGTSLEVKCSLCVVGIQA LAEMDRWQEVLSWVLQYYQVPEKLPPKVLE LCILLYSKMQEPGAVLDVVGAWLQDPANQN LPEYGALAEFHVQRVLLPLGCLSEAEELVVG SAAFGEERRLDVLQAIHTARQQQKQEHSGSE EAQKPNLEGSVSHKFLSLPMLVRQLWDSAVS	X-linked Adrenoleukodystrophy	Peroxisomal disorders

				HFFSLPFKKSLLAALILCLLVVRFDPASPSSLH FLYKLAQLFRWIRKAAFSRLYQ LRIRD [SEQ ID NO:455]		
AM ACR	23600	0242110	Q9UHK6	MALQGISVVELSGLAPGPFCAMVLADFGARV VRVDRPGSRYDVSRLGRGKRSLVLDLKQPRG AAVLRRLCKRSDVLLEPFRRGVMEKLQLGPE ILQRENPRLIYARLSGFGQSGSFCRLAGHDIN YLALSGVLSKIGRSGENPYAPLNLLADFAGG GLMCALGIIMALFDRTRTGKGQVIDANMVEG TAYLSSFLWKTQKLSLWEAPRGQNMLDGGA PFYTTYRTADGEFMAVGAIEPQFYELLIKGLG LKSDELPNQMSMDDWPEMKKKFADVFAEKT KAEWCQIFDGTDACVTPVLTFEEVVHHDHN KERGSFITSEEQDVSPRPAPLLLNTPAIPSFKR DPFIGEHTEEILEEFGFSREEIYQLNSDKIIESN KVKASL [SEQ ID NO:456]	Zellweger Spectrum Disorder	Peroxisomal disorders
ADA	100	0196839	A0A0S2Z381, P00813, F5GWI4	MAQTPAFDKPKVELHVHLDGSIKPETILYYG RRRGIALPANTAEGLLNVIGMDKPLTLPDFLA KFDYYMPAIAGCREAIKRIAYEFVEMKAKEG VVYVEVRYSPHLLANSKVEPIPWNQAEGDLT PDEVVALVGQGLQEGERDFGVKARSILCCMR HQPNWSPKVVELCKKYQQQTVVAIDLAGDE TIPGSSLLPGHVQAYQEAVKSGIHRTVHAGEV GSAEVVKEAVDILKTERLGHGYHTLEDQALY NRLRQENMHFEICPWSSYLTGAWKPDTEHA VIRLKNDQANYSLNTDDPLIF KSTLDTDYQMTKRDMGFTEEEFKRLNINAAK SSFLPEDEKRELLDLLYKAYGMPPSASAGQN L [SEQ ID NO:457]	Purine Metabolism Disorder	Purine Metabolism Disorder
ADSL	158	0239900	P30566, X5D8S6, X5D7W4, A0A1B0GWJ0	MAAGGDHGSPDSYRSPLASRYASPEMCFVFS DRYKFRTWRQLWLWLAEAEQTLGLPITDEQI QEMKSNLENIDFKMAAEEEKRLRHDVMAHV HTFGHCCPKAAGIIHLGATSCYVGDNTDLIIL RNALDLLLPKLARVISRLADFAKERASLPTLG FTHFQPAQLTTVGKRCCLWIQDLCMDLQNLK RVRDDLRFRGVKGTTGTQASFLQLFEGDDHK VEQLDKMVTEKAGFKRAFIITGQTYTRKVDIE VLSVLASLGASVHKICTDIRLLANLKEMEEPF EKQQIGSSAMPYKRNPMRSERCCSLARHLMT LVMDPLQTASVQWFERTLDDSANRRICLAEA FLTADTILNTLQNISEGLVVYPKVIERRIRQEL PFMATENIIMAMVKAGGSRQDCHEKIRVLSQ QAASVVKQEGGDNDLIERIQVDAYFSPIHSQL DHLLDPSSFTGRASQQVQRFLEEEVYPLLKPY ESVMKVKAELCL [SEQ ID NO:458]	Purine Metabolism Disorder	Purine Metabolism Disorder
AMPD1	270	0116748	P23109	MNVRIFYSVSQSPHSLLSLLFYCAILESRISAT MPLFKLPAEEKQIDDAMRNFAEKVFASEVKD EGGRQEISPFDVDEICPISHHEMQAHIFHLETL STSTEARRKKRFQGRKTVNLSIPLSETSSTKLS HIDEYISSSPTYQTVPDFQRVQITGDYASGVT VEDFEIVCKGLYRALCIREKYMQKSFQRFPKT PSKYLRNIDGEAWVANESFYPVFTPPVKKGE DPFRTDNLPENLGYHILKMKDGVVYVYPNEA AVSKDEPKPLPYPNLDTFLDDMNFLLALIAQ GPVKTYTHRRLKFLSSKFQVHQMLNEMDEL KELKNNPHRDFYNCRKVDTHIHAAACMNQK HLLRFIKKSYQIDADRVVYSTKEKNLTLKELF AKLKMHPYDLTVDSLDVHAGRQTFQRFDKF NDKYNPVGASELRDLYLKTDNYINGEYFATII KEVGADLVEAKYQHAEPRLSIYGRSPDEWSK	Purine Metabolism Disorder	Purine Metabolism Disorder

				LSSWFVCNRIHCPNMTWMIQVPRIYDVFRSK NFLPHFGKMLENIFMPVFEATINPQADPELSV FLKHIT GFDSVDDESKHSGHMFSSKSPKPQEWTLEKN PSYTYYAYYMYANIMVLNSLRKERGMNTFL FRPHCGEAGALTHLMTAFMIADDISHGLNLK KSPVLQYLFFLAQIPIAMSPLSNNSLFLEYAKN PFLDFLQKGLMISLSTDDPMQFHFTKEPLMEE YAIAAQVFKLSTCDMCEVARNSVLQCGISHE EKVKFLGDNYLEEGPAGNDIRRTNVAQIRMA YRYETWCYELNLIAEGLKSTE [SEQ ID NO:459]		
GPH N	10243	0171723	Q9NQX3	MATEGMILTNHDHQIRVGVLTVSDSCFRNLA EDRSGINLKDLVQDPSLLGGTISAYKIVPDEIE EIKETLIDWCDEKELNLILTTGGTGFAPRDVT PEATKEVIEREAPGMALAMLMGSLNVTPLG MLSRPVCGIRGKTLIINLPGSKKGSQECFQFIL PALPHAIDLLRDAIVKVKEVHDELEDLPSPPPP LSPPPTTSPHKQTEDKGVQCEEEEEEKKDSGV ASTEDSSSSHITAAAIAAKIPDSIISRGVQVLPR DTASLSTTPSESPRAQATSRLSTASCPTPKVQS RCSSKENILRASHSAVDITKVARRHRMSPFPL TSMDKAFITVLEMTPVLGTEIINYRDGMGRV LAQDVYAKDNLPPFPASVKDGYAVRAADGP GDRFIIGESQAGEQPTQTVMPGQVMRVTTGA PIPCGADAVVQVEDTELIRESDDGTEELEVRIL VQARPGQDIRPIGHDIKRGECVLAKGTHMGP S EIGLLATVGVTEVEVNKFPVVAVMSTGNELL NPEDDLLPGKIRDSNRSTLLATIQEHGYPTINL GIVGDNPDDLLNALNEGISRADVIITSGGVSM GEKDYLKQVLDIDLHAQIHFGRVFMKPGLPT TFATLDIDGVRKIIFALPGNPVSAVVTCNLFV VPALRKMQGILDPRPTIIKARLSCDVKLDPRP EYHRCILTWHHQEPLPWAQSTGNQMSSRLM SMRSANGLLMLPPKTEQYVELHKGEVVDVM VIGRL [SEQ ID NO:460]	Purine Metabolism Disorder	Purine Metabolism Disorder
MO COS	55034	0075643	Q96EN8	MAGAAAESGRELWTFAGSRDPSAPRLAYGY GPGSLRELRAREFSRLAGTVYLDHAGATLFS QSQLESFTSDLMENTYGNPHSQNISSKLTHDT VEQVRYRILAHFHTTAEDYTVIFTAGSTAALK LVAEAFPWVSQGPESSGSRFCYLTDSHTSVV GMRNVTMAINVISTPVRPEDLWSAEERSASA SNPDCQLPHLFCYPAQSNFSGVRYPLSWIEEV KSGRLHPVSTPGKWFVLLDAASYVSTSPLDL SAHQADFVPISFYKIFGFPTGLGALLVHNRAA PLLRKTYFGGGTASAYLAGEDFYIPRQSVAQ RFEDGTISFLDVIALKHGFDTLERLTGGMENI KQHTFTLAQYTYVALSSLQYPNGAPVVRIYS DSEFSSPEVQGPIINFNVLDDKGNIIGYSQVDK MASLYNIHLRTGCFCNTGACQRHLGISNEMV RKHFQAGHVCGDNMDLIDGQPTGSVRISFGY MSTLDDVQAFLRFIIDTRLHSSGDWPVPQAH ADTGETGAPSADSQADVIPAVMGRRSLSPQE DALTGSRVWNNSSTVNAVPVAPPVCDVART QPTPSEKAAGVLEGALGPHVVTNLYLYPIKSC AAFEVTRWPVGNQGLLYDRSWMVVNHNGV CLSQKQEPRLCLIQPFIDLRQRIMVIKAKGME PIEVPLEENSERTQIRQSRVCADRVSTYDCGE KISSWLSTFFGRPCHLIKQSSNSQRNAKKKHG	Purine Metabolism Disorder	Purine Metabolism Disorder

				KDQLPGTMATLSLVNEAQYLLINTSSILELHR QLNTSDENGKEELFSLKDLSLRFRANIIINGKR AFEEEKWDEISIGSLRFQVLGPCHRCQMICID QQTGQRNQHVFQKLSESRETKVNFGMYLMH ASLDLSSPCFLSVGSQVLPVLKENVEGHDLPA SEKHQDVTS [SEQ ID NO:461]		
MOCS1	4337	0124615	A0A024RD17, Q9NZB8	MAARPLSRMLRRLLRSSARSCSSGAPVTQPCP GESARAASEEVSRRRQFLREHAAPFSAFLTDS FGRQHSYLRISLTEKCNLRCQYCMPEEGVPLT PKANLLTTEEILTLARLFVKEGIDKIRLTGGEP LIRPDVVDIVAQLQRLEGLRTIGVTTNGINLA RLLPQLQKAGLSAINISLDTLVPAKFEFIVRRK GFHKVMEGIHKAIELGYNPVKVNCVVMRGL NEDELLDFAALTEGLP LDVRFIEYMPFDGNKWNFKKMVSYKEMLDT VRQQWPELEKVPEEESSTAKAFKIPGFQGQIS FITSMSEHFCGTCNRLRITADGNLKVCLFGNS EVSLRDHLRAGASEQELLRIIGAAVGRKKRQ HAGMFSISQMKNRPMILIELFLMFPNSPPANP SIFSWDPLHVQGLRPRMSFSSQVATLWKGCR VPQTPPLAQQRLGSGSFQRHYTSRADSDANS KCLSPGSWASAAPSGPQLTSEQLTHVDSEGR AAMVDVGRKPDTERVAVASAVVLLGPVAFK LVQQNQLKKGDALVVAQLAG VQAAKVTSQLIPLCHHVALSHIQVQLELDSTR HAVKIQASCRARGPTGVEMEALTSAAVAALT LYDMCKAVSRDIVLEEIKLISKTGGQRGDFHR A [SEQ ID NO:462]	Purine Metabolism Disorder	Purine Metabolism Disorder
PNP	4860	0198805	P00491, V9HWH6	MENGYTYEDYKNTAEWLLSHTKHRPQVAIIC GSGLGGLTDKLTQAQIFDYGEIPNFPRSTVPG HAGRLVFGFLNGRACVMMQGRFHMYEGYP LWKVTFPVRVFHLLGVDTLVVTNAAGGLNP KFEVGDIMLIRDHINLPGFSGQNPLRGPNDER FGDRFPAMSDAYDRTMRQRALSTWKQMGE QRELQEGTYVMVAGPSFETVAECRVLQKLG ADAVGMSTVPEVIVARHCGLRVFGFSLITNK VIMDYESLEKANHEEVLAAGKQAAQKLEQF VSILMASIPLPDKAS [SEQ ID NO:463]	Purine Metabolism Disorder	Purine Metabolism Disorder
XDH	7498	0158125	P47989	MTADKLVFFVNGRKVVEKNADPETTLLAYL RRKLGLSGTKLGCGEGGCGACTVMLSKYDR LQNKIVHFSANACLAPICSLHHVAVTTVEGIG STKTRLHPVQERIAKSHGSQCGFCTPGIVMSM YTLLRNQPEPTMEEIENAFQGNLCRCTGYRPI LQGFRTFARDGGCCGGDGNNPNCCMNQKKD HSVSLSPSLFKPEEFTPLDPTQEPIFPPELLRLK DTPRKQLRFEGERVTWIQASTLKELLDLKAQ HPDAKLVVGNTEIGIEMKFKNMLFPMIVCPA WIPELNSVEHGPDGISFGAACPLSIVEKTLVD AVAKLPAQKTEVFRGVLEQLRWFAGKQVKS VASVGGNIITASPISDLNPVFMASGAKLTLVS RGTRRTVQMDHTFFPGYRKTLLSPEEILLSIEI PYSREGEYFSAFKQASRREDDIAKVTSGMRV LFKPGTTEVQELALCYGGMANRTISALKTTQ RQLSKLWKEELLQDVCAGLAEELHLPPDAPG GMVDFRCTLTLSFFFKFYLTVLQKLGQENLE DKCGKLDPTFASATLLFQKDPPADVQLFQEV PKGQSEEDMVGRPLPHLAADMQASGEAVYC DDIPRYENELSLRLVTSTRAHAKIKSIDTSEAK KVPGFVCFISADDVPGSNITGICNDETVFAKD KVTCVGHIIGAVVADTPEHTQRAAQGVKITY	Purine Metabolism Disorder	Purine Metabolism Disorder

				EELPAIITIEDAIKNNSFYGPELKIEKGDLKKGF SEADNVVSGEIYIGGQEHFYLETHCTIAVPKG EAGEMELFVSTQNTMKTQSFVAKMLGVPAN RIVVRVKRMGGGFGGKETRSTVVSTAVALA AYKTGRPVRCMLDRDEDMLITGGR HPFLARYKVGFMKTGTVVALEVDHFSNVGN TQDLSQSIMERALFHMDNCYKIPNIRGTGRLC KTNLPSNTAFRGFGGPQGMLIAECWMSEVAV TCGMPAEEVRRKNLYKEGDLTHFNQKLEGFT LPRCWEECLASSQYHARKSEVDKFNKENCW KKRGLCIIPTKFGISFTVPFLNQAGALLHVYTD GSVLLTHGGTEMGQGLHTKMVQVASRALKI PTSKIYISETSTNTVPNTSPTAASVSADLNGQA VYAACQTILKRLEPYKKKNPSGSWEDWVTA AYMDTVSLSATGFYRTPNLGYSFETNSGNPF HYFSYGVACSEVEIDCLTGDHKNLRTDIVMD VGSSLNPAIDIGQVEGAFVQGLGLFTLEELHY SPEGSLHTRGPSTYKIPAFGSIPIEFRVSLLRDC PNKKAIYASKAVGEPPLFLAASIFFAIKDAIRA ARAQHTGNNVKELFRLDSPATPEKIRNACVD KFTTLCVTGVPENCKPWSVRV [SEQ ID NO:464]		
SUOX	6821	0139531	A0A024RB79, P51687	MLLLHRAVVLRLQQACRLKSIPSRICIQACST NDSFQPQRPSLTFSGDNSSTQGWRVMGTLLG LGAVLAYQDHRCRAAQESTHIYTKEEVSSHT SPETGIWVTLGSEVFDVTEFVDLHPGGPSKLM LAAGGPLEPFWALYAVHNQSHVRELLAQYKI GELNPEDKVAPTVETSDPYADDPVRHPALKV NSQRPFNAEPPPELLTENYITPNPIFFTRNHLP VPNLDPDTYRLHVVGAPGGQSLSLSLDDLHN FPRYEITVTLQCAGNRRSEMTQVKEVKGLEW RTGAISTARWAGARLCDVLAQAGHQLCETE AHVCFEGLDSDPTGTAYGASIPLARAMDPEA EVLLAYEMNGQPLPRDHGFPVRVVVPGVVG ARHVKWLGRVSVQPEESYSHWQRRDYKGFS PSVDWETVDFDSAPSIQELPVQSAITEPRDGE TVESGEVTIKGYAWSGGGRAVIRVDVSLDGG LTWQVAKLDGEEQRPRKAWAWRLWQLKAP VPAGQKELNIVCKAVDDGYNVQPDTVAPIW NLRGVLSNAWHRVHVYVSP[SEQ ID NO:465]	Purine Metabolism Disorder	Purine Metabolism Disorder
OGDH	4967	0105953	A0A140VJQ5, Q02218, B4E3E9, E9PCR7, E9PDF2	MFHLRTCAAKLRPLTASQTVKTFSQNRPAAA RTFQQIRCYSAPVAAEPFLSGTSSNYVEEMYC AWLENPKSVHKSWDIFFRNTNAGAPPGTAYQ SPLPLSRGSLAAVAHAQSLVEAQPNVDKLVE DHLAVQSLIRAYQIRGHHVAQLDPLGILDAD LDSSVPADIISSTDKLGFYGLDESDLDKVFHLP TTTFIGGQESALPLREIIRRLEMAYCQHIGVEF MFINDLEQCQWIRQKFETPGIMQFTNEEKRTL LARLVRSTRFEEFLQRKWSSEKRFGLEGCEVL IPALKTIIDKSSENGVDYVIMGMPHRGRLNVL ANVIRKELEQIFCQFDSKLEAADEGSGDVKY HLGMYHRRINRVTDRNITLSLVANPSHLEAA DPVVMGKTKAEQFYCGDTEGKKVMSILLHG DAAFAGQGIVYETFHLSDLPSYTTHGTVHVV VNNQIGFTTDPRMARSSPYPTDVARVVNAPIF HVNSDDPEAVMYVCKVAAEWRSTFHKDVV VDLVCYRRNGHNEMDEPMFTQPLMYKQIRK QKPVLQKYAELLVSQGVVNQPEYEEEISKYD KICEEAFARSKDEKILHIKHWLDSPWPGFFTL DGQPRSMSCPSTGLTEDILTHIGNVASSVPVE	2-Ketoglutarate Dehydrogenase Deficiency	PYRUVATE METABOLISM AND TRICARBOXYLIC ACID CYCLE DEFECT

				NFTIHGGLSRILKTRGEMVKNRTVDWALAEY MAFGSLLKEGIHIRLSGQDVERGTFSHRHHVL HDQNVDKRTCIPMNHLWPNQAPYTVCNSSLS EYGVLGFELGFAMASPNALVLWEAQFGDFH NTAQCIIDQFICPGQAKWVRQNGIVLLLPHG MEGMGPEHSSARPERFLQMCNDDPDVLPDL KEANFDINQLYDCNWVVVNCSTPGNFFHVLR RQILLPFRKPLIIFTPKSLLRHPEARSSFDEMLP GTHFQRVIPEDGPAAQNPENVKRLLFCTGKV YYDLTRERKARDMVGQVAITRIEQLSPFPFDL LLKEVQKYPNAELAWCQEEHKNQGYYDYV KPRLRTTISRAKPVWYAGRDPAAAPATGNKK THLTELQRLLDTAFDLDVFKNFS [SEQ ID NO:466]		
SLC 25A19	60386	0125454	Q5JPC1, Q9HC21	MVGYDPKPDGRNNTKFQVAVAGSVSGLVTR ALISPFDVIKIRFQLQHERLSRSDPSAKYHGIL QASRQILQEEGPTAFWKGHVPAQILSIGYGAV QFLSFEMLTELVHRGSVYDAREFSVHFVCGG LAACMATLTVHPVDVLRTRFAAQGEPKVYN TLRHAVGTMYRSEGPQVFYKGLAPTLIAIFPY AGLQFSCYSSLKHLYKWAIPAEGKKNENLQN LLCGSGAGVISKTLTYPLDLFKKRLQVGGFEH ARAAFGQVRRYKGLMDCAKQVLQKEGALG FFKGLSPSLLKAALSTGFMF FSYEFFCNVFHCMNRTASQR [SEQ ID NO:467]	2-Ketoglutarate Dehydrogenase Deficiency	PYRUVATE METABOLISM AND TRICARBOXYLIC ACID CYCLE DEFECT
DHTKD1	55526	0181192	Q96HY7	MASATAAAARRGLGRALPLFWRGYQTERGV YGYRPRKPESREPQGALERPPVDHGLARLVT VYCEHGHKAAKINPLFTGQALLENVPEIQAL VQTLQGPFHTAGLLNMGKEEASLEEVLVYLN QIYCGQISIETSQLQSQDEKDWFAKRFEELQK ETFTTEERKHLSKLMLESQEFDHFLATKFSTV KRYGGEGAESMMGFFHELLKMSAYSGITDVI IGMPHRGRLNLLTGLLQFPPELMFRKMRGLS EFPENFSATGDVLSHLTSSVDLYFGAHHPLHV TMLPNPSHLEAVNPVAVGK TRGRQQSRQDGDYSPDNSAQPGDRVICLQVH GDASFCGQGIVPETFTLSNLPHFRIGGSVHLIV NNQLGYTTPAERGRSSLYCSDIGKLVGCAIIH VNGDSPEEVVRATRLAFEYQRQFRKDVIIDLL CYRQWGHNELDEPFYTNPIMYKIIRARKSIPD TYAEHLIAGGLMTQEEVSEIKSSYYAKLNDH LNNMAHYRPPALNLQAHWQGLAQPEAQITT WSTGVPLDLLRFVGMKSVEVPRELQMHSHL LKTHVQSRMEKMMDGIKLDWATAEALALGS LLAQGFNVRLSGQDVGRGT FSQRHAIVVCQETDDTYIPLNHMDPNQKGFL EVSNSPLSEEAVLGFEYGMSIESPKLLPLWEA QFGDFFNGAQIIFDTFISGGEAKWLLQSGIVIL LPHGYDGAGPDHSSCRIERFLQMCDSAEEGV DGDTVNMFVVHPTTPAQYFHLLRRQMVRNF RKPLIVASPKMLLRLPAAVSTLQEMAPGTTFN PVIGDSSVDPKKVKTLVFCSGKHFYSLVKQR ESLGAKKHDFAIIRVEELCPFPLDSLQQEMSK YKHVKDHIWSQEEPQNMGPWSFVSPRFEKQL ACKLRLVGRPPLPVPAV GIGTVHLHQHEDILAKTFA [SEQ ID NO:468]	2-Ketoglutarate Dehydrogenase Deficiency	PYRUVATE METABOLISM AND TRICARBOXYLIC ACID CYCLE DEFECT
SLC 13A5	284111	0141485	Q68D44, Q86YT5	MASALSYVSKFKSFVILFVTPLLLLPLVILMPA KFVRCAYVIILMAIYWCTEVIPLAVTSLMPVL LFPLFQILDSRQVCVQYMKDTNMLFLGGLIV AVAVERWNLHKRIALRTLLWVGAKPARLML	Citrate Transporter Deficiency	PYRUVATE METABOLISM

				GFMGVTALLSMWISNTATTAMMVPIVEAILQ QMEATSAATEAGLELVDKGKAKELPGSQVIF EGPTLGQQEDQERKRLCKAMTLCICYAASIG GTATLTGTGPNVVLLGQMNELFPDSKDLVNF ASWFAFAFPNMLVMLLFAWLWLQFVYMRF NFKKSWGCGLESKKNEKAALKVLQEEYRKL GPLSFAEINVLICFFLLVILWFSRDPGFMPGWL TVAWVEGETKYVSDATVAIFVATLLFIVPSQ KPKFNFRSQTEEERKTPFYPPPLLDWKVTQEK VPWGIVLLLGGGFALAKGSEASGLSVWMGK QMEPLHAVPPAAITLILSLLVAVFTECTSNVA TTTLFLPIFASMSRSIGLNPLYIMLPCTLSASFA FMLPVATPPNAIVFTYGHLKVADMVKTGVIM NIIGVFCVFLAVNTWGRAIFDLDHFPDWANV THIET [SEQ ID NO:469]		AND TRICAR BOXYLI C ACID CYCLE DEFECT
FH	2271	0091483	A0A0S2Z 4C3, P07954	MYRALRLLARSRPLVRAPAAALASAPGLGGA AVPSFWPPNAARMASQNSFRIEYDTFGELKV PNDKYYGAQTVRSTMNFKIGGVTERMPTPVI KAFGILKRAAAEVNQDYGLDPKIANAIMKAA DEVAEGKLNDHFPLVVWQTGSGTQTNMNVN EVISNRAIEMLGGELGSKIPVHPNDHVNKSQS SNDTFPTAMHIAAAIEVHEVLLPGLQKLHDA LDAKSKEFAQIIKIGRTHTQDAVPLTLGQEFS GYVQQVKYAMTRIKAAMPRIYELAAGGTAV GTGLNTRIGFAEKVAAKVAALTGLPFVTAPN KFEALAAHDALVELSGAMNTTACSLMKIAN DIRFLGSGPRSGLGELILPENEPGSSIMPGKVN PTQCEAMTMVAAQVMGNHVAVTVGGSNGH FELNVFKPMMIKNVLHSARLLGDASVSFTEN CVVGIQANTERINKLMNESLMLVTALNPHIG YDKAAKIAKTAHKNGSTLKETAIELGYLTAE QFDEWVKPKDMLGPK [SEQ ID NO:470]	Fumarase Deficiency	PYRUVA TE METABO LISM AND TRICAR BOXYLI C ACID CYCLE DEFECT
DLA T	1737	0150768	P10515, Q86YI5	MWRVCARRAQNVAPWAGLEARWTALQEVP GTPRVTSRSGPAPARRNSVTTGYGGVRALCG WTPSSGATPRNRLLLQLLGSPGRRYYSLPPHQ KVPLPSLSPTMQAGTIARWEKKEGDKINEGD LIAEVETDKATVGFESLEECYMAKILVAEGTR DVPIGAIICITVGKPEDIEAFKNYTLDSSAAPTP QAAPAPTPAATASPPTPSAQAPGSSYPPHMQV LLPALSPTMTMGTVQRWEKKVGEKLSEGDL LAEIETDKATIGFEVQEEGYLAKILVPEGTRD VPLGTPLCIIVEKEADISAFADYRPTEVTDLKP QVPPPTPPPVAAVPPTPQPLAPTPSAPCPATPA GPKGRVFVSPLAKKLAVEKGIDLTQVKGTGP DGRITKKDIDSFVPSKVAPAPAAVVPPTGPGM APVPTGVFTDIPISNIRRVIAQRLMQSKQTIPH YYLSIDVNMGEVLLVRKELNKILEGRSKISVN DFIIKASALACLKVPEANSSWMDTVIRQNHV VDVSVAVSTPAGLITPIVFNAHIKGVETIAND VVSLATKAREGKLQPHEFQGGTFTISNLGMF GIKNFSAIINPPQACILAIGASEDKLVPADNEK GFDVASMMSVTLSCDHRVVDGAVGAQWLA EFRKYLEKPITMLL [SEQ ID NO:471]	Pyruvate Dehydroge nase Deficiency	PYRUVA TE METABO LISM AND TRICAR BOXYLI
MPC 1	51660	0060762	Q5TI65, Q9Y5U8	MAGALVRKAADYVRSKDFRDYLMSTHFWG PVANWGLPIAAINDMKKSPEIISGRMTFALCC YSLTFMRFAYKVQPRNWLLFACHATNEVAQ LIQGGRLIKHEMTKTASA [SEQ ID NO:472]	Pyruvate Dehydroge nase Deficiency	PYRUVA TE METABO LISM AND TRICAR BOXYLI

						C ACID CYCLE DEFECT
PDHA1	5160	0131828	A0A024RBX9, P08559	MRKMLAAVSRVLSGASQKPASRVLVASRNFANDATFEIKKCDLHRLEEGPPVTTVLTREDGLKYYRMMQTVRRMELKADQLYKQKIIRGFCHLCDGQEACCVGLEAGINPTDHLITAYRAHGFTFTRGLSVREILAELTGRKGGCAKGKGGSMHMYAKNFYGGNGIVGAQVPLGAGIALACKYNGKDEVCLTLYGDGAANQGQIFEAYNMAALWKLPCIFICENNRYGMGTSVERAAASTDYYKRGDFIPGLRVDGMDILCVREATRFAAAYCRSGKGPILMELQTYRYHGHSMSDPGVSYRTREEIQEVRSKSDPIMLLKDRMVNSNLASVEELKEIDVEVRKEIEDAAQFATADPEPPLEELGYHIYSSDPPFEVRGANQWIKFKSVS [SEQ ID NO:473]	Pyruvate Dehydrogenase Deficiency	PYRUVATE METABOLISM AND TRICARBOXYLIC ACID CYCLE DEFECT
PDHB	5162	0168291	P11177	MAAVSGLVRRPLREVSGLLKRRFHWTAPAALQVTVRDAINQGMDEELERDEKVFLLGEEVAQYDGAYKVSRGLWKKYGDKRIIDTPISEMGFAGIAVGAAMAGLRPICEFMTFNFSMQAIDQVINSAAKTYYMSGGLQPVPIVFRGPNGASAGVAAQHSQCFAAWYGHCPGLKVVSPWNSEDAKGLIKSAIRDNNPVVVLENELMYGVPFEFPPEAQSKDFLIPIGKAKIERQGTHITVVSHSRPVGHCLEAAAVLSKEGVECEVINMRTIRPMDMETIEASVMKTNHLVTVEGGWPQFGVGAEICARIMEGPAFNFLDAPAVRVTGADVPMPYAKILEDNSIPQVKDIIFAIKKTLNI [SEQ ID NO:474]	Pyruvate Dehydrogenase Deficiency	PYRUVATE METABOLISM AND TRICARBOXYLIC ACID CYCLE DEFECT
PDHX	8050	0110435	O00330	MAASWRLGCDPRLLRYLVGFPGRRSVGLVKGALGWSVSRGANWRWFHSTQWLRGDPIKILMPSLSPTMEEGNIVKWLKKEGEAVSAGDALCEIETDKAVVTLDASDDGILAKIVVEEGSKNIRLGSLIGLIVEEGEDWKHVEIPKDVGPPPPVSKPSEPRPSPEPQISIPVKKEHIPGTLRFRLSPAARNILEKHSLDASQGTATGPRGIFTKEDALKLVQLKQTGKITESRPTPAPTATPTAPSPLQATAGPSYPRPVIPPVSTPGQPNAVGTFTEIPASNIRRVIAKRLTESKSTVPHAYATADCDLGAVLKVRQDLVKDDIKVSVNDFIIKAAAVTLKQMPDVNVSWDGEGPKQLPFIDISVAVATDKGLLTPIIKDAAAKGIQEIADSVKALSKKARDGKLLPEEYQGGSFSISNLGMFGIDEFTAVINPPQACILAVGRFRPVLKLTEDEEGNAKLQQRQLITVTMSSDSRVVDDELATRFLKSFKANLENPIRLA [SEQ ID NO:475]	Pyruvate Dehydrogenase Deficiency	PYRUVATE METABOLISM AND TRICARBOXYLIC ACID CYCLE DEFECT
PDP1	54704	0164951	Q9P0J1, Q6P1N1, A0A024R9C0	MPAPTQLFFPLIRNCELSRIYGTACYCHHKHLCCSSSYIPQSRLRYTPHPAYATFCRPKENWWQYTQGRRYASTPQKFYLTPPQVNSILKANEYSFKVPEFDGKNVSSILGFDSNQLPANAPIEDRRSAATCLQTRGMLLGVFDGHAGCACSQAVSERLFYYIAVSLLPHETLLEIENAVESGRALLPILQWHKHPNDYFSKEASKLYFNSLRTYWQELIDLNTGESTDIDVKEALINAFKRLDNDISLEAQVGDPNSFLNYLVLRVAFSGATACVAHVDGVDLHVANTGDSRAMLGVQEEDGSWSAVTLSNDHNAQNERELERLKLEHPKSEAKSVVKQDRLLGLLMPFRAFGDVKFKWSIDLQKRVIESGPDQLNDNEYTKFIPPNYHTPPYLTAEPEVTYHRLRPQDKFLVLATDGLWETMHRQDVVRIVGEYL	Pyruvate Dehydrogenase Deficiency	PYRUVATE METABOLISM AND TRICARBOXYLIC ACID CYCLE DEFECT

				TGMHHQQPIAVGGYKVTLGQMHGLLTERRT KMSSVFEDQNAATHLIRHAVGNNEFGTVDHE RLSKMLSLPEELARMYRDDITIIVVQFNSHVV GAYQNQE [SEQ ID NO:476]		
ABC C2	1244	0023839	Q92887	MLEKFCNSTFWNSSFLDSPEADLPLCFEQTVL VWIPLGYLWLLAPWQLLHVYKSRTKRSSTTK LYLAKQVFVGFLLILAAIELALVLTEDSGQAT VPAVRYTNPSLYLGTWLLVLLIQYSRQWCVQ KNSWFLSLFWILSILCGTFQFQTLIRTLLQGDN SNLAYSCLFFISYGFQILILIFSAFSENNESSNN PSSIASFLSSITYSWYDSIILKGYKRPLTLEDV WEVDEEMKTKTLVS KFETHMKRELQKARRALQRRQEKSSQQNSG ARLPGLNKNQSQSQDALVLEDVEKKKKKSG TKKDVPKSWLMKALFKTFYMVLLKSFLLKL VNDIFTFVSPQLLKLLISFASDRDTYLWIGYLC AILLFTAALIQSFCLQCYFQLCFKLGVKVRTAI MASVYKKALTLSNLARKEYTVGETVNLMSV DAQKLMDVTNFMHMLWSSVLQIVLSIFFLW RELGPSVLAGVGVMVLVIPINAILSTKSKTIQV KNMKNKDKRLKIMNEILSGIKILKYFAWEPSF RDQVQNLRKKELKNLLAFS QLQCVVIFVFQLTPVLVSVVTFSVYVLVDSN NILDAQKAFTSITLFNILRFPLSMLPMMISSML QASVSTERLEKYLGGDDLDTSAIRHDCNFDK AMQFSEASFTWEHDSEATVRDVNLDIMAGQ LVAVIGPVGSGKSSLISAMLGEMENVHGHITI KGTTAYVPQQSWIQNGTIKDNILFGTEFNEKR YQQVLEACALLPDLEMLPGGDLAEIGEKGIN LSGGQKQRISLARATYQNLDIYLLDDPLSAVD AHVGKHIFNKVLGPNGLLKGKTRLLVTHSM HFLPQVDEIVVLGNGTIV EKGSYSALLAKKGEFAKNLKTFLRHTGPEEE ATVHDGSEEEDDDYGLISSVEEIPEDAASITM RRENSFRRTLSRSSRSNGRHLKSLRNSLKTRN VNSLKEDEELVKGQKLIKKEFIETGKVKFSIY LEYLQAIGLFSIFFIILAFVMNSVAFIGSNLWLS AWTSDSKIFNSTDYPASQRDMRVGVYGALG LAQGIFVFIAHFWSAFGFVHASNILHKQLLNN ILRAPMRFFDTTPTGRI VNRFAGDISTVDDTLPQSLRSWITCFLGIISTL VMICMATPVFTIIVIPLGIIYVSVQMFYVSTSR QLRRLDSVTRSPIYSHFSETVSGLPVIRAFEHQ QRFLKHNEVRIDTNQKCVFSWITSNRWLAIRL ELVGNLTVFFSALMMVIYRDTLSGDTVGFVL SNALNITQTLNWLVRMTSEIETNIVAVERITE YTKVENEAPWVTDKRPPPDWPSKGKIQFNNY QVRYRPELDLVLRGI TCDIGSMEKIGVVGRTGAGKSSLTNCLFRILE AAGGQIIIDGVDIASIGLHDLREKLTIIPQDPILF SGSLRMNLDPFNNYSDEEIWKALELAHLKSF VASLQLGLSHEVTEAGGNLSIGQRQLLCLGR ALLRKSKILVLDEATAAVDLETDNLIQTTIQN EFAHCTVITIAHRLHTIMDSDKVMVLDNGKII ECGSPEELLQIPGPFYFMAKEAGIENVNSTKF [SEQ ID NO:477]	Dubin-Johnson syndrome	
SLCO1B1	10599	0134538	A0A024RAU7, Q05CV5, Q9Y6L6	MDQNQHLNKTAEAQPSENKKTRYCNGLKMF LAALSLSFIAKTLGAIIMKSSIIHIERRFEISSSL VGFIDGSFEIGNLLVIVFVSYFGSKLHRPKLIGI GCFIMGIGGVLTALPHFFMGYYRYSKETNINS	Rotor Syndrome	

				SENSTSTLSTCLINQILSLNRASPEIVGKGCLK ESGSYMWIYVFMGNMLRGIGETPIVPLGLSYI DDFAKEGHSSLYLGILNAIAMIGPIIGFTLGSL FSKMYVDIGYVDLSTIRITPTDSRWVGAWWL NFLVSGLFSIISSIPFFFLPQTPNKPQKERKASL SLHVLETNDEKDQTANLTNQGKNITKNVTGF FQSFKSILTNPLYVMFVLLTLLQVSSYIGAFTY VFKYVEQQYGQPSSKANILLGVITIPIFASGMF LGGYIIKKFKLNTVGIAKFSCFTAVMSLSFYL LYFFILCENKSVAGLTMTYDGNNPVTSHRDV PLSYCNSDCNCDESQWEPVCGNNGITYISPCL AGCKSSSGNKKPIVFYNCSCLEVTGLQNRNY SAHLGECPRDDACTRKFYFFVAIQVLNLFFSA LGGTSHVMLIVKIVQPELKSLALGFHSMVIRA LGGILAPIYFGALIDTTCIKWSTNNCGTRGSCR TYNSTSFSRVYLGLSSMLRVSSLVLYIILIYAM KKKYQEKDINASENGSVMDEANLESLNKNK HFVPSAGADSETHC [SEQ ID NO:478]		
SLCO1B3	28234	0111700	B3KP78, Q9NPD5	MDQHQHLNKTAESASSEKKKTRRCNGFKMF LAALSFSYIAKALGGIIMKISITQIERRFDISSSL AGLIDGSFEIGNLLVIVFVSYFGSKLHRPKLIGI GCLLMGTGSILTSLPHFFMGYYRYSKETHINP SENSTSSLSTCLINQTLSFNGTSPEIVEKDCVK ESGSHMWIYVFMGNMLRGIGETPIVPLGISYI DDFAKEGHSSLYLGSLNAIGMIGPVIGFALGS LFAKMYVDIGYV DLSTIRITPKDSRWVGAWWLGFLVSGLFSIISS IPFFFLPKNPNKPQKERKISLSLHVLKTNDDRN QTANLTNQGKNVTKNVTGFFQSLKSILTNPL YVIFLLLTLLQVSSFIGSFTYVFKYMEQQYGQ SASHANFLLGIITIPTVATGMFLGGFIIKKFKLS LVGIAKFSFLTSMISFLFQLLYFPLICESKSVAG LTLTYDGNNSVASHVDVPLSYCNSECNCDES QWEPVCGNNGITYLSPCLAGCKSSSGIKKHT VFYNCSCVEVTGLQNRNYSAHLGECPRDNTC TRKFFIYVAIQVINSLFSATGGTTFILLTVKIVQ PELKALAMGFQSMVIRTLGGILAPIYFGALID KTCMKWSTNSCGAQGACRIYNSVFFGRVYL GLSIALRFPALVLYIVFIFAMKKKFQGKDTKA SDNERKVMDEANLEFLNNGEHFVPSAGTDSK TCNLDMQDNAAAN[SEQ ID NO:479]	Rotor Syndrome	
HFE2	148738	0168509	Q6ZVN8, A8K466, A0A024R4F5	MGEPGQSPSPRSSHGSPPTLSTLTLLLLLCGH AHSQCKILRCNAEYVSSTLSLRGGGSSGALRG GGGGGRGGGVGSGGLCRALRSYALCTRRTA RTCRGDLAFHSAVHGIEDLMIQHNCSRQGPT APPPPRGPALPGAGSGLPAPDPCDYEGRFSRL HGRPPGFLHCASFGDPHVRSFHHHFHTCRVQ GAWPLLDNDFLFVQATSSPMALGANATATR KLTIIFKNMQECIDQKVYQAEVDNLPVAFED GSINGGDRPGGSSLSIQTANPGNHVEIQAAYI GTTIIIRQTAGQLSFSIKVAEDVAMAFSAEQDL QLCVGGCPPSQRLSRSERNRRGAITIDTARRL CKEGLPVEDAYFHSCVFDVLISGDPNFTVAA QAALEDARAFLPDLEKLHLFPSDAGVPLSSAT LLAPLLSGLFVLWLCIQ [SEQ ID NO:480]	Hemochromatosis, type 2A	
ADAMTS13	11093	0160323,0281244	Q76LX8	MHQRHPRARCPPLCVAGILACGFLLGCWGPS HFQQSCLQALEPQAVSSYLSPGAPLKGRPPSP GFQRQRQRQRRAAGGILHLELLVAVGPDVFQ AHQEDTERYVLTNLNIGAELLRDPSLGAQFR VHLVKMVILTEPEGAPNITANLTSSLLSVCGW	Congenital thrombotic thrombocytopenic purpura	

				SQTINPEDDTDPGHADLVLYITRFDLELPDGN RQVRGVTQLGGACSPTWSCLITEDTGFDLGV TIAHEIGHSFGLEHDGAPGSGCGPSGHVMAS DGAAPRAGLAWSPCSRRQLLSLLSAGRARCV WDPPRPQPGSAGHPPDAQPGLYYSANEQCRV AFGPKAVACTFAREHLDMCQALSCHTDPLD QSSCSRLLVPLLDGTECGVEKWCSKGRCRSL VELTPIAAVHGRWSSWGPRSPCSRSCGGGVV TRRRQCNNPRPAFGGRACVGADLQAEMCNT QACEKTQLEFMSQQCARTDGQPLRSSPGGAS FYHWGAAVPHSQGDALCRHMCRAIGESFIM KRGDSFLDGTRCMPSGPREDGTLSLCVSGSC RTFGCDGRMDSQQVWDRCQVCGGDNSTCSP RKGSFTAGRAREYVTFLTVTPNLTSVYIANHR PLFTHLAVRIGGRYVVAGKMSISPNTTYPSLL EDGRVEYRVALTEDRLPRLEEIRIWGPLQEDA DIQVYRRYGEEYGNLTRPDITFTYFQPKPRQA WVWAAVRGPCSVSCGAGLRWVNYSCLDQA RKELVETVQCQGSQQPPAWPEACVLEPCPPY WAVGDFGPCSASCGGGLRERPVRCVEAQGS LLKTLPPARCRAGAQQPAVALETCNPQPCPA RWEVSEPSSCTSAGGAGLALENETCVPGADG LEAPVTEGPGSVDEKLPAPEPCVGMSCPPGW GHLDATSAGEKAPSPWGSIRTGAQAAHVWT PAAGSCSVSCGRGLMELRFLCMDSALRVPVQ EELCGLASKPGSRREVCQAVPCPARWQYKLA ACSVSCGRGVVRRILYCARAHGEDDGEEILL DTQCQGLPRPEPQEACSLEPCPPRWKVMSLG PCSASCGLGTARRSVACVQLDQGQDVEVDE AACAALVRPEASVPCLIADCTYRWHVGTWM ECSVSCGDGIQRRRDTCLGPQAQAPVPADFC QHLPKPVTVRGCWAGPCVGQGTPSLVPHEEA AAPGRTTATPAGASLEWSQARGLLFSPAPQP RRLLPGPQENSVQSSACGRQHLEPTGTIDMR GPGQADCAVAIGRPLGEVVTLRVLESSLNCS AGDMLLLWGRLTWRKMCRKLLDMTFSSKT NTLVVRQRCGRPGGGVLLRYGSQLAPETFYR ECDMQLFGPWGEIVSPSLSPATSNAGGCRLFI NVAPHARIAIHALATNMGAGTEGANASYILIR DTHSLRTTAFHGQQVLYWESESSQAEMEFSE GFLKAQASLRGQYWTLQSWVPEMQDPQSW KGKEGT [SEQ ID NO:481]	due to ADAMTS-13 deficiency	
PYGM	5837	0068976	P11217	MSRPLSDQEKRKQISVRGLAGVENVTELKKN FNRHLHFTLVKDRNVATPRDYYFALAHTVR DHLVGRWIRTQQHYYEKDPKRIYYLSLEFYM GRTLQNTMVNLALENACDEATYQLGLDMEE LEEIEEDAGLGNGGLGRLAACFLDSMATLGL AAYGYGIRYEFGIFNQKISGGWQMEEADDW LRYGNPWEKARPEFTLPVHFYGHVEHTSQGA KWVDTQVVLAMPYDTPVPGYRNNVVNTMR LWSAKAPNDFNLKDFNVGGYIQAVLDRNLA ENISRVLYPNDNFFEGKELRLKQEYFVVAATL QDIIRRFKSSKFGCRDPVRTNFDAFPDKVAIQ LNDTHPSLAIPELMRILVDLERM DWDKAWDVTVRTCAYTNHTVLPEALERWP VHLLETLLPRHLQIIYEINQRFLNRVAAAFPG DVDRLRRMSLVEEGAVKRINMAHLCIAGSHA VNGVARIHSEILKKTIFKDFYELEPHKFQNKT NGITPRRWLVLCNPGLAEVIAERIGEDFISDLD QLRKLLSFVDDEAFIRDVAKVKQENKLKFAA	McArdle's Disease	

				YLEREYKVHINPNSLFDIQVKRIHEYKRQLLN CLHVITLYNRIKREPNKFFVPRTVMIGGKAAP GYHMAKMIIRLVTAIGDVVNHDPAVGDRLR VIFLENYRVSLAEKVIPAADLSEQISTAGTEAS GTGNMKFMLNGALTIGTMDGANVEMAEEA GEENFFIFGMRVEDVDKLDQRGYNAQEYYD RIPELRQVIEQLSSGFFSPKQPDLFKDIVNMLM HHDRFKVFADYEDYIKCQEKVSALYKNPRE WTRMVIRNIATSGKFSSDRTIAQYAREIWGVE PSRQRLPAPDEAI[SEQ ID NO:482]		
COL 1A2	1278	0164692	A0A0S2Z 3H5, P08123	MLSFVDTRTLLLLAVTLCLATCQSLQEETVR KGPAGDRGPRGERGPPGPPGRDGEDGPTGPP GPPGPPGPPGLGGNFAAQYDGKGVGLGPGP MGLMGPRGPPGAAGAPGPQGFQGPAGEPGE PGQTGPAGARGPAGPPGKAGEDGHPGKPGRP GERGVVGPQGARGFPGTPGLPGFKGIRGHNG LDGLKGQPGAPGVKGEPGAPGENGTPGQTG ARGLPGERGRVGAPGPAGARGSDGSVGPVGP AGPIGSAGPPGFPGAPGPKGEIGAVGNAGPAG PAGPRGEVGLPGLSGPVGPPGNP GANGLTGAKGAAGLPGVAGAPGLPGPRGIPG PVGAAGATGARGLVGEPGPAGSKGESGNKG EPGSAGPQGPPGPSGEEGKRGPNGEAGSAGPP GPPGLRGSPGSRGLPGADGRAGVMGPPGSRG ASGPAGVRGPNGDAGRPGEPGLMGPRGLPGS PGNIGPAGKEGPVGLPGIDGRPGPIGPAGARG EPGNIGFPGPKGPTGDPGKNGDKGHAGLAGA RGAPGPDGNNGAQGPPGPQGVQGGKGEQGP PGPPGFQGLPGPSGPAGEVGKPGERGLHGEF GLPGPAGPRGERGPPGESGAA GPTGPIGSRGPSGPPGPDGNKGEPGVVGAVG TAGPSGPSGLPGERGAAGIPGGKGEKGEPGLR GEIGNPGRDGARGAPGAVGAPGPAGATGDR GEAGAAGPAGPAGPRGSPGERGEVGPAGPNG FAGPAGAAGQPGAKGERGAKGPKGENGVVG PTGPVGAAGPAGPNGPPGPAGSRGDGGPPGM TGFPGAAGRTGPPGPSGISGPPGPPGPAGKEG LRGPRGDQGPVGRTGEVGAVGPPGFAGEKGP SGEAGTAGPPGTPGPQGLLGAPGILGLPGSRG ERGLPGVAGAVGEPGPLGIAGPPGARGPPGA VGSPGVNGAPGEAGRDGNPGNDGPPGRDGQ PGHKGERGYPGNIGPVGAAGAPGPHGPVGPA GKHGNRGETGPSGPVGPAGAVGPRGPSGPQG IRGDKGEPGEKGPRGLPGLKGHNGLQGLPGI AGHHGDQGAPGSVGPAGPRGPAGPSGPAGK DGRTGHPGTVGPAGIRGPQGHQGPAGPPGPP GPPGPPGVSGGGYDFGYDGDFYRADQPRSAP SLRPKDYEVDATLKSLNNQIETLLTPEGSRKN PARTCRDLRLSHPEWSSGYYWIDPNQGCTMD AIKVYCDFSTGETCIRAQPENIPAKNWYRSSK DKKHVWLGETINAGSQFEYNVEGVTSKEMA TQLAFMRLLANYASQNITYHCKNSIAYMDEE TGNLKKAVILQGSNDVELVAEGNSRFTYTVL VDGCSKKTNEWGKTIIEYKTNKPSRLPFLDIA PLDIGGADQEFFVDIGPVCFK [SEQ ID NO:483]	Ehlers-Danlos syndrome, cardiac valvular type	
TNF RSF 11B	4982	0164761	O00300	MNNLLCCALVFLDISIKWTTQETFPPKYLHYD EETSHQLLCDKCPPGTYLKQHCTAKWKTVC APCPDHYYTDSWHTSDECLYCSPVCKELQYV KQECNRTHNRVCECKEGRYLEIEFCLKHRSC PPGFGVVQAGTPERNTVCKRCPDGFFSNETSS	Juvenile Paget's disease	

				KAPCRKHTNCSVFGLLLTQKGNATHDNICSG NSESTQKCGIDVTLCEEAFFRFAVPTKFTPNW LSVLVDNLPGTKVNAESVERIKRQHSSQEQTF QLLKLWKHQNKDQDIVKKIIQDIDLCENSVQ RHIGHANLTFEQLRSLMESLPGKKVGAEDIEK TIKACKPSDQILKLLSLWRIKNGDQDTLKGL MHALKHSKTYHFPKTVTQSLKKTIRFLHSFT MYKLYQKLFLEMIGNQVQSVKISCL [SEQ ID NO:484]		
TSC 1	7248	0165699	Q86WV8, Q92574, X5D9D2, Q32NF0	MAQQANVGELLAMLDSPMLGVRDDVTAVF KENLNSDRGPMLVNTLVDYYLETSSQPALHI LTTLQEPHDKHLLDRINEYVGKAATRLSILSL LGHVIRLQPSWKHKLSQAPLLPSLLKCLKMD TDVVVLTTGVLVLITMLPMIPQSGKQHLLDFF DIFGRLSSWCLKKPGHVAEVYLVHLHASVYA LFHRLYGMYPCNFVSFLRSHYSMKENLETFE EVVKPMMEHVRIHPELVTGSKDHELDPRRW KRLETHDVVIECAKISLDPTEASYEDGYSVSH QISARFPHRSADVTTSPYADT QNSYGCATSTPYSTSRLMLLNMPGQLPQTLS SPSTRLITEPPQATLWSPSMVCGMTTPPTSPG NVPPDLSHPYSKVFGTTAGGKGTPLGTPATSP PPAPLCHSDDYVHISLPQATVTPPRKEERMDS ARPCLHRQHHLLNDRGSEEPPGSKGSVTLSD LPGFLGDLASEEDSIEKDKEEAAISRELSEITT AEAEPVVPRGGFDSPFYRDSLPGSQRKTHSA ASSSQGASVNPEPLHSSL DKLGPDTPKQAFTPIDLPCGSADESPAGDREC QTSLETSIFTPSPCKIPPPTRVGFGSGQPPPYDH LFEVALPKTAHHFVIRKTEELLKKAKGNTEE DGVPSTSPMEVLDRLIQQGADAHSKELNKLP LPSKSVDWTHFGGSPPSDEIRTLRDQLLLLHN QLLYERFKRQQHALRNRRLLRKVIKAAALEE HNAAMKDQLKLQEKDIQMWKVSLQKEQAR YNQLQEQRDTMVTKLHSQIRQLQHDREEFYN QSQELQTKLEDCRNMIAELRIELKKANNKVC HTELLLSQVSQKLSNSESVQQQMEFLNRQLL VLGEVNELYLEQLQNKHSDTTKEVEMMKAA YRKELEKNRSHVLQQTQRLDTSQKRILELESH LAKKDHLLLEQKKYLEDVKLQARGQLQAAE SRYEAQKRITQVFELEILDLYGRLEKDGLLKK LEEEKAEAAEAAEERLDCCNDGCSDSMVGH NEEASGHNGETKTPRPSSARGSSGSRGGGGSS SSSSELSTPEKPPHQRAGPFSSRWETTMGEAS ASIPTTVGSLPSSKSFLGMKARELFRNKSESQ CDEDGMTSSLSESLKTELGKDLGVEAKIPLNL DGPHPSPPTPDSVGQLHIMDYNETHHEHS [SEQ ID NO:485]	Tuberous sclerosis	
TSC 2	7249	0103197	P49815, X5D7Q2, B3KWH7, Q5HYF7, H3BMQ0, X5D2U8	MAKPTSKDSGLKEKFKILLGLGTPRPNPRSAE GKQTEFIITAEILRELSMECGLNNRIRMIGQIC EVAKTKKFEEHAVEALWKAVADLLQPERPL EARHAVLALLKAIVQGQGERLGVLRALFFKV IKDYPSNEDLHERLEVFKALTDNGRHITYLEE ELADFVLQWMDVGLSSEFLLVLVNLVKFNSC YLDEYIARMVQMICLLCVRTASSVDIEVSLQV LDAVVCYNCLPAESLPLFIVTLCRTINVKELC EPCWKLMRNLLGTHLGHSAIYNMCHLMEDR AYMEDAPLLRGAVFFVGMALWGAHRLYSLR NSPTSVLPSFYQAMACPNEVVSYEIVLSITRLI KKYRKELQVVAWDILLNIIERLLQQLQTLDSP	Tuberous sclerosis	

				ELRTIVHDLLTTVEELCDQNEFHGSQERYFEL VERCADQRPESSLLNLISYRAQSIHPAKDGWI QNLQALMERFFRSESRGAVRIKVLDVLSFVLL INRQFYEEELINSVVISQLSHIPEDKDHQVRKL ATQLLVDLAEGCHTHHFNSLLDIIEKVMARSL SPPPELEERDVAAYSASLEDVKTAVLGLLVIL QTKLYTLPASHATRVYEMLVSHIQLHYKHSY TLPIASSIRLQAFDFLLLLRADSLHRLGLPNKD GVVRFSPYCVCDYMEPERGSEKKTSGPLSPPT GPPGPAPAGPAVRLGSVPYSLLFRVLLQCLKQ ESDWKVLKLVLGRLPESLRYKVLIFTSPCSVD QLCSALCSMLSGPKTLERLRGAPEGFSRTDLH LAVVPVLTALISYHNYL DKTKQREMVYCLEQGLIHRCASQCVVALSIC SVEMPDIIIKALPVLVVKLTHISATASMAVPLL EFLSTLARLPHLYRNFAAEQYASVFAISLPYT NPSKFNQYIVCLAHHVIAMWFIRCRLPFRKDF VPFITKGLRSNVLLSFDDTPEKDSFRARSTSLN ERPKSLRIARPPKQGLNNSPPVKEFKESSAAE AFRCRSISVSEHVVRSRIQTSLTSASLGSADEN SVAQADDSLKNLHL ELTETCLDMMARYVFSNFTAVPKRSPVGEFL LAGGRTKTWLVGNKLVTVTTSVGTGTRSLL GLDSGELQSGPESSSSPGVHVRQTKEAPAKLE SQAGQQVSRGARDRVRSMSGGHGLRVGALD VPASQFLGSATSPGPRTAPAAKPEKASAGTRV PVQEKTNLAAYVPLLTQGWAEILVRRPTGNT SWLMSLENPLSPFSSDINNMPLQELSNALMA AERFKEHRDTALYKSLSVPAASTAKPPPLPRS NTVASFSSLYQSSCQGQLHRSVSWADSAVV MEEGSPGEVPVLVEPPGLEDV EAALGMDRRTDAYSRSSSVSSQEEKSLHAEE LVGRGIPIERVVSSEGGRPSVDLSFQPSQPLSK SSSSPELQTLQDILGDPGDKADVGRLSPEVKA RSQSGTLDGESAAWSASGEDSRGQPEGPLPSS SPRSPSGLRPRGYTISDSAPSRRGKRVERDAL KSRATASNAEKVPGINPSFVFLQLYHSPFFGD ESNKPILLPNESQSFERSVQLLDQIPSYDTHKI AVLYVGEGQSNSELA ILSNEHGSYRYTEFLTGLGRLIELKDCQPDKV YLGGLDVCGEDGQFTYCWHDDIMQAVFHIA TLMPTKDVDKHRCDKKRHLGNDFVSIVYND SGEDFKLGTIKGQFNFVHVIVTPLDYECNLVS LQCRKDMEGLVDTSVAKIVSDRNLPFVARQ MALHANMASQVHHSRSNPTDIYPSKWIARLR HIKRLRQRICEEAAYSNPSLPLVHPPSHSKAPA QTPAEPTPGYEVGQRKRLISSVEDFTEFV[SEQ ID NO:486]		
DHC R7	1717	0172893	A0A024R 5F7, Q9UBM7	MAAKSQPNIPKAKSLDGVTNDRTASQGQWG RAWEVDWFSLASVIFLLLFAPFIVYYFIMACD QYSCALTGPVVDIVTGHARLSDIWAKTPPITR KAAQLYTLWVTFQVLLYTSLPDFCHKFLPGY VGGIQEGAVTPAGVVNKYQINGLQAWLLTH LLWFANAHLLSWFSPTIIFDNWIPLLWCANIL GYAVSTFAMVKGYFFPTSARDCKFTGNFFYN YMMGIEFNPRIGKWFDFKLFFNGRPGIVAWT LINLSFAAKQRELHSHVTNAMVLVNVLQAIY VIDFFWNETWYLKTIDICHD HFGWYLGWGDCVWLPYLYTLQGLYLVYHP VQLSTPHAVGVLLLGLVGYYIFRVANHQKDL	Smith- Lemli- Opitz Syndrome	

				FRRTDGRCLIWGRKPKVIECSYTSADGQRHH SKLLVSGFWGVARHFNYVGDLMGSLAYCLA CGGGHLLPYFYIIYMAILLTHRCLRDEHRCAS KYGRDWERYTAAVPYRLLPGIF [SEQ ID NO:487]		
PGK 1	5230	0102144	P00558, V9HWF4	MSLSNKLTLDKLDVKGKRVVMRVDFNVPM KNNQITNNQRIKAAVPSIKFCLDNGAKSVVL MSHLGRPDGVPMPDKYSLEPVAVELKSLLGK DVLFLKDCVGPEVEKACANPAAGSVILLENL RFHVEEEGKGKDASGNKVKAEPAKIEAFRAS LSKLGDVYVNDAFGTAHRAHSSMVGVNLPQ KAGGFLMKKELNYFAKALESPERPFLAILGG AKVADKIQLINNMLDKVNEMIIGGGMAFTFL KVLNNMEIGTSLFDEEGAKIVKDLMSKAEKN GVKITLPVDFVTADKFDENAKTGQATVASGI PAGWMGLDCGPESSKKYAEAVTRAKQIVWN GPVGVFEWEAFARGTKALMDEVV KATSRGCITIIGGGDTATCCAKWNTEDKVSH VSTGGGASLELLEGKVLPGVDALSNI [SEQ ID NO:488]	D-glycericacidemia	
VLDLR	7436	0147852	P98155, Q5VVF5	MGTSALWALWLLLALCWAPRESGATGTGRK AKCEPSQFQCTNGRCITLLWKCDGDEDCVDG SDEKNCVKKTCAESDFVCNNGQCVPSRWKC DGDPDCEDGSDESPEQCHMRTCRIHEISCGAH STQCIPVSWRCDGENDCDSGEDEENCGNITCS PDEFTCSSGRCISRNFVCNGQDDCSDGSDELD CAPPTCGAHEFQCSTSSCIPISWVCDDDADCS DQSDESLEQCGRQPVIHTKCPASEIQCGSGECI HKKWRCDGDPDCKDGSDEVNCPSRTCRPDQ FECEDGSCIHGSRQCNGI RDCVDGSDEVNCKNVNQCLGPGKFKCRSGE CIDISKVCNQEQDCRDWSDEPLKECHINECLV NNGGCSHICKDLVIGYECDCAAGFELIDRKTC GDIDECQNPGICSQICINLKGGYKCECSRGYQ MDLATGVCKAVGKEPSLIFTNRRDIRKIGLER KEYIQLVEQLRNTVALDADIAAQKLFWADLS QKAIFSASIDDKVGRHVKMIDNVYNPAAIAV DWVYKTIYWTDAASKTISVATLDGTKRKFLF NSDLREPASIAVDPLSGFVYWSDWGEPAKIE KAGMNGFDRRPLVTADIQ WPNGITLDLIKSRLYWLDSKLHMLSSVDLNG QDRRIVLKSLEFLAHPLALTIFEDRVYWIDGE NEAVYGANKFTGSELATLVNNLNDAQDIIVY HELVQPSGKNWCEEDMENGGCEYLCLPAPQI NDHSPKYTCSCPSGYNVEENGRDCQSTATTV TYSETKDTNTTEISATSGLVPGGINVTTAVSE VSVPPKGTSAAWAILPLLLLVMAAVGGYLM WRNWQHKNMKSMNFDNPVYLKTTEEDLSID IGRHSASVGHTYPAISVVSTDDDLA [SEQ ID NO:489]	Dysequilibrium syndrome	
KYNU	8942	0115919	Q16719	MEPSSLELPADTVQRIAAELKCHPTDERVALH LDEEDKLRHFRECFYIPKIQDLPPVDLSLVNK DENAIYFLGNSLGLQPKMVKTYLEEELDKWA KIAAYGHEVGKRPWITGDESIVGLMKDIVGA NEKEIALMNALTVNLHLLMLSFFKPTPKRYKI LLEAKAFPSDHYAIESQLQLHGLNIEESMRMI KPREGEETLRIEDILEVIEKEGDSIAVILFSGVH FYTGQHFNIPAITKAGQAKGCYVGFDLAHAV GNVELYLHDWGVDFACWCSYKYLNAGAGG IAGAFIHEKHAHTIKPALVGWFGHELSTRFKM	Encephalopathy due to hydroxykynureninuria	

				DNKLQLIPGVCGFRISNPPILLVCSLHASLEIFK QATMKALRKKSVLLTGYLEYLIKHNYGKDK AATKKPVVNIITPSHVEERGCQLTITFSVPNKD VFQELEKRGVVCDKRNPNGIRVAPVPLYNSF HDVYKFTNLLTSILDSAETKN [SEQ ID NO:490]		
F5	2153	0198734	P12259	MFPGCPRLWVLVVLGTSWVGWGSQGTEAA QLRQFYVAAQGISWSYRPEPTNSSLNLSVTSF KKIVYREYEPYFKKEKPQSTISGLLGPTLYAE VGDIIKVHFKNKADKPLSIHPQGIRYSKLSEG ASYLDHTFPAEKMDDAVAPGREYTYEWSISE DSGPTHDDPPCLTHIYYSHENLIEDFNSGLIGP LLICKKGTLTEGGTQKTFDKQIVLLFAVFDES KSWSQSSSLMYTVNGYVNGTMPDITVCAHD HISWHLLGMSSGPELFSIHFNGQVLEQNHHK VSAITLVSATSTTANMTVGPEGKWIISSLTPK HLQAGMQAYIDIKNCPKKTRNLKKITREQRR HMKRWEYFIAAEEVIWDYAPVIPANMDKKY RSQHLDNFSNQIGKHYKKVMYTQYEDESFTK HTVNPNMKEDGILGPIIRAQVRDTLKIVFKNM ASRPYSIYPHGVTFSPYEDEVNSSFTSGRNNT MIRAVQPGETYTYKWNILEFDEPTENDAQCL TRPYYSDVDIMRDIASGLIGLLLICKSRSLDRR GIQRAA DIEQQAVFAVFDENKSWYLEDNINKFCENPD EVKRDDPKFYESNIMSTINGYVPESITTLGFCF DDTVQWHFCSVGTQNEILTIHFTGHSFIYGKR HEDTLTLFPMRGESVTVTMDNVGTWMLTSM NSSPRSKKLRLKFRDVKCIPDDDEDSYEIFEPP ESTVMATRKMHDRLEPEDEESDADYDYQNR LAAALGIRSFRNSSLNQEEEEFNLTALALENG TEFVSSNTDIIVGSNYSSPSNISKFTVNNLAEP QKAPSHQQATTAGSPLRHLIGKNSVLNSSTAE HSSPYSEDPIEDPLQPDVTGIRLLSLGAGEFKS QEHAKHKGPKVERDQAAKHRFSWMKLLAH KVGRHLSQDTGSPSGMRPWEDLPSQDTGSPS RMRPWKDPPSDLLLLKQSNSSKILVGRWHLA SEKGSYEIIQDTDEDTAVNNWLISPQNASRA WGESTPLANKPGKQSGHPKFPRVRHKSLQVR QDGGKSRLKKSQFLIKTRKKKKEKHTHHAPL SPRTFHPLRSEAYNTFSERRLKHSLVLHKSNE TSLPT DLNQTLPSMDFGWIASLPDHNQNSSNDTGQA SCPPGLYQTVPPEEHYQTFPIQDPDQMHSTSD PSHRSSSPELSEMLEYDRSHKSFPTDISQMSPS SEHEVWQTVISPDLSQVTLSPELSQTNLSPDLS HTTLSPELIQRNLSPALGQMPISPDLSHTTLSP DLSHTTLSLDLSQTNLSPELSQTNLSPALGQM PLSPDLSHTTLSLDFSQTNLSPELSHMTLSPEL SQTNLSPALGQMP ISPDLSHTTLSLDFSQTNLSPELSQTNLSPALG QMPLSPDPSHTTLSLDLSQTNLSPELSQTNLSP DLSEMPLFADLSQIPLTPDLDQMTLSPDLGET DLSPNFGQMSLSPDLSQVTLSPDISDTTLLPDL SQISPPPDLDQIFYPSESSQSLLLQEFNESFPYP DLGQMPSPSSPTLNDTFLSKEFNPLVIVGLSK DGTDYIEIIPKEEVQSSEDDYAEIDYVPYDDPY KTDVRTNINSSRDPDNIAAWYLRSNNGNRRN YYIAAEEISWDYSEFVQRETDIEDSDDIPEDTT YKKVVFRKYLDSTFTKRDPRGEYEEHLGILG	Factor V deficiency	

				PIIRAEVDDVIQVRFKNLASRPYSLHAHGLSY EKSSEGKTYEDDSPEWFKEDNAVQPNSSYTY VWHATERSGPESPGSACRAWAYYSAVNPEK DIHSGLIGPLLICQKGILHKDSNMPMDMREFV LLFMTFDEKKSWYYEKKSRSSWRLTSSEMK KSHEFHAINGMIYSLPGLKMYEQEWVRLHLL NIGGSQDIHVVHFHGQTLLENGNKQHQLGV WPLLPGSFKTLEMKASKPGWWLLNTEVGEN QRAGMQTPFLIMDRDCRMPMGLSTGIISDSQI KASEFLGYWEPRLARLNNGGSYNAWSVEKL AAEFASKPWIQVDMQKEVIITGIQTQGAKHY LKSCYTTEFYVAYSSNQINWQIFKGNSTRNV MYFNGNSDASTIKENQFDPPIVARYIRISPTRA YNRPTLRLELQGCEVNGCSTPLGMENGKIEN KQITASSFKKSWWGDYWEPFR ARLNAQGRVNAWQAKANNNKQWLEIDLLKI KKITAIITQGCKSLSSEMYVKSYTIHYSEQGVE WKPYRLKSSMVDKIFEGNTNTKGHVKNFFNP PIISRFIRVIPKTWNQSIALRLELFGCDIY [SEQ ID NO:491]		
C3	718	0125730	B4DR57, P01024, V9HWA9	MGPTSGPSLLLLLLTHLPLALGSPMYSIITPNIL RLESEETMVLEAHDAQGDVPVTVTVHDFPG KKLVLSSEKTVLTPATNHMGNVTFTIPANREF KSEKGRNKFVTVQATFGTQVVEKVVLVSLQS GYLFIQTDKTIYTPGSTVLYRIFTVNHKLLPVG RTVMVNIENPEGIPVKQDSLSSQNQLGVLPLS WDIPELVNMGQWKIRAYYENSPQQVFSTEFE VKEYVLPSFEVIVEPTEKFYYIYNEKGLEVTIT ARFLYGKKVEGTAFVIFGIQDGEQRISLPESLK RIPIEDGSGEVVLSRKVLLDGVQNPRAEDLVG KSLYVSATVILHSGSDMVQAERSGIPIVTSPY QIHFTKTPKYFKPGMPFDLMVFVTNPDGSPA YRVPVAVQGEDTVQSLTQGDGVAKLSINTHP SQKPLSITVRTKKQELSEAEQATRTMQALPYS TVGNSNNYLHLSVLRTELRPGETLNVNFLLR MDRAHEAKIRYYTYLIMNKGRLLKAGRQVR EPGQDLVVLPLSITTDFIPSFRLVAYYTLIGAS GQREVVADSVWVDVKDSCVGSLVVKSGQSE DRQPVPGQQMTLKIEGDHGARVVLVAVDKG VFVLNKKNKLTQSKIWDVVEKADIGCTPGSG KDYAGVFSDAGLTFTSSSGQQTAQRAELQCP QPAARRRRSVQLTEKRMDKVGKYPKELRKC CEDGMRENPMRFSCQRRTRFISLGEACKKVF LDCCNYITELRRQHARASHLGLARSNLDEDII AEENIVSRSEFPESWLWNVEDLKEPPKNGIST KLMNIFLKDSITTWEILAVSMSDKKGICVADP FEVTVMQDFFIDLRLPYSVVRNEQVEIRAVLY NYRQNQELKVRVELLHNPAFCSLATTKRRHQ QTVTIPPKSSLSVPYVIVPLKTGLQEVEVKAA VYHHFISDGVRKSLKVVPEGIRMNKTVAVRT LDPERLGREGVQKEDIPPADLSDQVPDTESET RILLQGTPVAQMTEDAVDAERLKHLIVTPSG CGEQNMIGMTPTVIAVHYLDETEQWEKFGLE KRQGALELIKKGYTQQLAFRQPSSAFAAFVK RAPSTWLTA YVVKVFSLAVNLIAIDSQVLCGAVKWLILEK QKPDGVFQEDAPVIHQEMIGGLRNNNEKDM ALTAFVLISLQEAKDICEEQVNSLPGSITKAGD FLEANYMNLQRSYTVAIAGYALAQMGRLKG PLLNKFLTTAKDKNRWEDPGKQLYNVEATS	Atypical hemolytic uremic syndrome with C3 anomaly	

				YALLALLQLKDFDFVPPVVRWLNEQRYYGG GYGSTQATFMVFQALAQYQKDAPDHQELNL DVSLQLPSRSSKITHRIHWESASLLRSEETKEN EGFTVTAEGKGQGTLSVVTMYHAKAKDQLT CNKFDLKVTIKPAPETEKRPQDAKNTMILEIC TRYRGDQDATMSILDISMMTGFAPDTDDLKQ LANGVDRYISKYELDKAFSDRNTLIIYLDKVS HSEDDCLAFKVHQYFNVELIQPGAVKVYAY YNLEESCTRFYHPEKEDGKLNKLCRDELCRC AEENCFIQKSDDKVTLEERLDKACEPGVDYV YKTRLVKVQLSNDFDEYIMAIEQTIKSGSDEV QVGQQRTFISPIKCREALKLEEKKHYLMWGL SSDFWGEKPNLSYIIGKDTWVEHWPEEDECQ DEENQKQCQDLGAFTESMVVFGCPN [SEQ ID NO:492]		
COL 4A1	1282	0187498	A5PKV2, F5H5K0, P02462	MGPRLSVWLLLLPAALLLHEEHSRAAAKGG CAGSGCGKCDCHGVKGQKGERGLPGLQGVI GFPGMQGPEGPQGPPGQKGDTGEPGLPGTKG TRGPPGASGYPGNPGLPGIPGQDGPPGPPGIPG CNGTKGERGPLGPPGLPGFAGNPGPPGLPGM KGDPGEILGHVPGMLLKGERGFPGIPGTPGPP GLPGLQGPVGPPGFTGPPGPPGPPGPPGEKGQ MGLSFQGPKGDKGDQGVSGPPGVPGQAQVQ EKGDFATKGEKGQKGEPGFQGMPGVGEKGE PGKPGPRGKPGKDGDKGEKGSPGFPGEPGYP GLIGRQGPQGEKGEAGPPGPPGIVIGTGPLGE KGERGYPGTPGPRGEPGPKGFPGLPGQPGPPG LPVPGQAGAPGFPGERGEKGDRGFPGTSLPGP SGRDGLPGPPGSPGPPGQPGYTNGIVECQPGP PGDQGPPGIPGQPGFIGEIGEKGQKGESCLICD IDGYRGPPGPQGPPGEIGFPGQPGAKGDRGLP GRDGVAGVPGPQGTPGLIGQPGAKGEPGEFY FDLRLKGDKGDPGFPGQPGMPGRAGSPGRD GHPGLPGPKGSPGSVGLKGERGPPGGVGFPG SRGDTGPPGPPGYGPAGPIGDKGQAGFPGGP GSPGLPGPKGEPGKIVPLPGPPGAEGLPGSPGF PGPQGDRGFPGTPGRPGLPGEKGAVGQPGIGF PGPPGPKGVDGLPGDMGPPGTPGRPGFNGLP GNPGVQGQKGEPGVGLPGLKGLPGLPGIPGT PGEKGSIGVPGVPGEHGAIGPPGLQGIRGEPG PPGLPGSVGSPGVPGIGPPGARGPPGGQGPPG LSGPPGIKGEKGFPGFPGLDMPGPKGDKGAQ GLPGITGQSGLPGLPGQQGAPGIPGFPGSKGE MGVMGTPGQPGSPGPVGAPGLPGEKGDHGF PGSSGPRGDPGLKGDKGDVGLPGKPGSMDK VDMGSMKGQKGDQGEKGQIGPIGEKGSRGD PGTPGVPGKDGQAGQPGQPGPKGDPGISGTP GAPGLPGPKGSVGGMGLPGTPGEKGVPGIPG PQGSPGLPGDKGAKGEKGQAGPPGIGIPGLRG EKGDQGIAGFPGSPGEKGEKGSIGIPGMPGSP GLKGSPGSVGYPGSPGLPGEKGDKGLPGLDG IPGVKGEAGLPGTPGPTGPAGQKGEPGSDGIP GSAGEKGEPGLPGRGFPGFPGAKGDKGSKGE VGFPGLAGSPGIPGSKGEQGFMGPPGPQGQP GLPGSPGHATEGPKGDRGPQGQPGLPGLPGP MGPPGLPGIDGVKGDKGNPGWPGAPGVPGP KGDPGFQGMPGIGGSPGITGSKGDMGPPGVP GFQGPKGLPGLQGIKGDQGDQGVPGAKGLP GPPGPPGPYDIIKGEPGLPGPEGPPGLKGLQGL PGPKGQQGVTGLVGIPGPPGIPGFDGAPGQKG	Autosomal dominant familial hematuria - retinal arteriolar tortuosity - contractures	

				EMGPAGPTGPRGFPGPPGPDGLPGSMGPPGTP SVDHGFLVTRHSQTIDDPQCPSGTKILYHGYS LLYVQGNERAHGQDLGTAGSCLRKFSTMPFL FCNINNVCNFASRNDYSYWLSTPEPMPMSMA PITGENIRPFISRCAVCEAPAMVMAVHSQTIQI PPCPSGWSSLWIGYSFVMHTSAGAEGSGQAL ASPGSCLEEFRSAPFIECHGRGTCNYYANAYS FWLATIERSEMFKKPTPSTLKAGELRTHVSRC QVCMRRT [SEQ ID NO:493]		
CFH	3075	0000971	A0A024R962, P08603, A0A0D9SG88	MRLLAKIICLMLWAICVAEDCNELPPRRNTEI LTGSWSDQTYPEGTQAIYKCRPGYRSLGNVI MVCRKGEWVALNPLRKCQKRPCGHPGDTPF GTFTLTGGNVFEYGVKAVYTCNEGYQLLGEI NYRECDTDGWTNDIPICEVVKCLPVTAPENG KIVSSAMEPDREYHFGQAVRFVCNSGYKIEG DEEMHCSDDGFWSKEKPKCVEISCKSPDVIN GSPISQKIIYKENERFQYKCNMGYEYSERGDA VCTESGWRPLPSCEEKSCDNPYIPNGDYSPLR IKHRTGDEITYQCRNGFYPATRGNTAKCTSTG WIPAPRCTLKPCDYPDIKHGGLYHENMRRPY FPVAVGKYYSYYCDEHFETPSGSYWDHIHCT QDGWSPAVPCLRKCYFPYLENGYNQNYGRK FVQGKSIDVACHPGYALPKAQTTVTCMENG WSPTPRCIRVKTCSKSSIDIENGFISESQYTYA LKEKAKYQCKLGYVTADGETSGSITCGKDG WSAQPTCIKSCDIPVFMNARTKNDFTWFKLN DTLDYECHDGYESNTGSTTGSIVCGYNGWSD LPICYERECELPKIDVHLVPDRKKDQYKVGE VLKFSCKPGFTIVGPNSVQCYHFGLSPDLPICK EQVQSCGPPPELLNGNVKEKTKEEYGHSEVV EYYCNPRFLMKGPNKIQCVDGEWTTLPVCIV EESTCGDIPELEHGWAQLSSPPYYYGDSVEFN CSESFTMIGHRSITCIHGVWTQLPQCVAIDKL KKCKSSNLIILEEHLKNKKEFDHNSNIRYRCR GKEGWIHTVCINGRWDPEVNCSMAQIQLCPP PPQIPNSHNMTTTLNYRDGEKVSVLCQENYLI QEGEEITCKDGRWQSIPLCVEKIPCSQPPQIEH GTINSSRSSQESYAHGTKLSYTCEGGFRISEEN ETTCYMGKWSSPPQCEGLPCKSPPEISHGVVA HMSDSYQYGEEVTYKCFEGFGIDGPAIAKCL GEKWSHPPSCIKTDCLSLPSFENAIPMGEKKD VYKAGEQVTYTCATYYKMDGASNVTCINSR WTGRPTCRDTSCVNPPTVQNAYIVSRQMSKY PSGERVRYQCRSP YEMFGDEEVMCLNGNWTEPPQCKDSTGKCG PPPPIDNGDITSFPLSVYAPASSVEYQCQNLYQ LEGNKRITCRNGQWSEPPKCLHPCVISREIME NYNIALRWTAKQKLYSRTGESVEFVCKRGYR LSSRSHTLRTTCWDGKLEYPTCAKR [SEQ ID NO:494]	Atypical hemolytic uremic syndrome	
SLC 12A2	6558	0064651	P55011, Q53ZR1, B7ZM24	MEPRPTAPSSGAPGLAGVGETPSAAALAAAR VELPGTAVPSVPEDAAPASRDGGGVRDEGPA AAGDGLGRPLGPTPSQSRFQVDLVSENAGRA AAAAAAAAAAAAAAAGAGAGAKQTPADGEA SGESEPAKGSEEAKGRFRVNFVDPAASSSAED SLSDAAGVGVDGPNVSFQNGGDTVLSEGSSL HSGGGGGSGHHQHYYYDTHTNTYYLRTFGH NTMDAVPRIDHYRHTAAQLGEKLLRPSLAEL HDELEKEPFEDGFANGEESTPTRDAVVTYTA ESKGVVKFGWIKGVLVRCMLNIWGVMLFIRL	Bartter syndrome type I (neonatal)	

				SWIVGQAGIGLSVLVIMMATVVTTITGLSTSA IATNGFVRGGGAYYLISRSLGPEFGGAIGLIFA FANAVAVAMYVVGFAETVVELLKEHSILMID EINDIRIIGAITVVILLGISVAGMEWEAKAQIV LLVILLLAIGDFVIGTFIPLESKKPKGFFGYKSE IFNENFGPDFREEETFFSVFAIFFPAATGILAGA NISGDLADPQSAIPKGTLLAILITTLVYVGIAV SV GSCVVRDATGNVNDTIVTELTNCTSAACKLN FDFSSCESSPCSYGLMNNFQVMSMVSGFTPLI SAGIFSATLSSALASLVSAPKIFQALCKDNIYP AFQMFAKGYGKNNEPLRGYILTFLIALGFILIA ELNVIAPIISNFFLASYALINFSVFHASLAKSPG WRPAFKYYNMWISLLGAILCCIVMFVINWW AALLTYVIVLGLYIYVTYKKPDVNWGSSTQA LTYLNALQHSIRLSGVEDHVKNFRPQCLVMT GAPNSRPALLHLVHDFTKNVGLMICGHVHM GPRRQAMKEMSIDQAKYQRWLIKNKMKAFY APVHADDLREGAQYLMQAAGLGRMKPNTL VLGFKKDWLQADMRDVDMYINLFHDAFDIQ YGVVVIRLKEGLDISHLQGQEELLSSQEKSPG TKDVVVSVEYSKKSDLDTSKPLSEKPITHKVE EEDGKTATQPLLKKESKGPIVPLNVADQKLL EASTQFQKKQGKNTIDVWWLFDDGGLTLLIP YLLTTKKKWKDCKIRVFIGGKINRIDHDRRA MATLLSKFRIDFSDIMVLGDINTKPKKENIIAF EEIIEPYRLHEDDKEQDIADKMKEDEPWRITD NELELYKTKTYRQIRLNELLKEHSSTANIIVM SLPVARKGAVSSALYMAWLEALSKDLPPILL VRGNHQSVLTFYS [SEQ ID NO:495]		
GK	2710	0198814	B4DH54, P32189	MAASKKAVLGPLVGAVDQGTSSTRFLVFNSK TAELLSHHQVEIKQEFPREGWVEQDPKEILHS VYECIEKTCEKLGQLNIDISNIKAIGVSNQRET TVVWDKITGEPLYNAVVWLDLRTQSTVESLS KRIPGNNNFVKSKTGLPLSTYFSAVKLRWLL DNVRKVQKAVEEKRALFGTIDSWLIWSLTGG VNGGVHCTDVTNASRTMLFNIHSLEWDKQL CEFFGIPMEILPNVRSSSEIYGLMKISHSVKAG ALEGVPISGCLGDQSAALVGQMCFQIGQAKN TYGTGCFLLCNTGHKCVFSDHGLLTTVAYKL GRDKPVYYALEGSVAIAGAVIRWLRDNLGII KTSEEIEKLAKEVGTSYGCYFVPAFSGLYAPY WEPSARGIICGLTQFTNKCHIAFAALEAVCFQ TREILDAMNRDCGIPLSHLQVDGGMTSNKIL MQLQADILYIPVVKPSMPETTALGAAMAAGA AEGVGVWSLEPEDLSAVTMERFEPQINAEES EIRYSTWKKAVMKSMGWVTTQSPESGDPSIF CSLPLGF FIVSSMVMLIGARYISGIP [SEQ ID NO:496]	Glycerol kinase deficiency	
SFTPC	6440	0168484	A0A0A0MTC9, P11686, A0A0S2Z4Q0, E5RI64	MDVGSKEVLMESPPDYSAAPRGRFGIPCCPV HLKRLLIVVVVVVLIVVVIVGALLMGLHMSQ KHTEMVLEMSIGAPEAQQRLALSEHLVTTAT FSIGSTGLVVYDYQQLLIAYKPAPGTCCYIMK IAPESIPSLEALNRKVHNFQMECSLQAKPAVP TSKLGQAEGRDAGSAPSGGDPAFLGMAVNT LCGEVPLYYI [SEQ ID NO:497]	Chronic respiratory distress with surfactant metabolism deficiency	
CRTAP	10491	0170275	O75718	MEPGRRGAAALLALLCVACALRAGRAQYER YSFRSFPRDELMPLESAYRHALDKYSGEHWA ESVGYLEISLRLHRLLRDSEAFCHRNCSAAPQ	Osteogenesis	

				PEPAAGLASYPELRLFGGLLRRAHCLKRCKQ GLPAFRQSQPSREVLADFQRREPYKFLQFAYF KANNLPKAIAAAHTFLLKHPDDEMMKRNMA YYKSLPGAEDYIKDLETKSYESLFIRAVRAYN GENWRTSITDMELALPDFFKAFYECLAACEG SREIKDFKDFYLSIADHYVEVLECKIQCEENL TPVIGGYPVEKFVATMYHY LQFAYYKLNDLKNAAPCAVSYLLFDQNDKV MQQNLVYYQYHRDTWGLSDEHFQPRPEAVQ FFNVTTLQKELYDFAKENIMDDDEGEVVEYV DDLLELEETS [SEQ ID NO:498]	Imperfecta VII	
P3H 1	64175	0117385	Q32P28	MAVRALKLLTTLLAVVAAASQAEVESEAGW GMVTPDLLFAEGTAAYARGDWPGVVLSMER ALRSRAALRALRLRCRTQCAADFPWELDPD WSPSPAQASGAAALRDLSFFGGLLRRAACLR RCLGPPAAHSLSEEMELEFRKRSPYNYLQVA YFKINKLEKAVAAAHTFFVGNPEHMEMQQN LDYYQTMSGVKEADFKDLETQPHMQEFRLG VRLYSEEQPQEAVPHLEAALQEYFVAYEECR ALCEGPYDYDGYNYLEYNADLFQAITDHYIQ VLNCKQNCVTELASHPSREKPFEDFLPSHYN YLQFAYYNIGNYTQAVECAKTYLLFFPNDEV MNQNLAYYAAMLGEEHTRSIGPRESAKEYR QRSLLEKELLFFAYDVFGIPFVDPDSWTPEEVI PKRLQEKQKSERETAVRISQEIGNLMKEIETL VEEKTKESLDVSRLTREGGPLLYEGISLTMNS KLLNGSQRVVMDGVISDHECQELQRLTNVA ATSGDGYRGQTSPHTPNEKFYGVTVFKALKL GQEGKVPLQSAHLYYNVTEKVRRIMESYFRL DTPLYFSYSHLVCRTAIEEVQAERKDDSHPVH VDNCILNAETLVCVKEPPAYTFRDYSAILYLN GDFDGGNFYFTELDAKTVTAEVQPQCGRAV GFSSGTENPHGVKAVTRGQRCAIALWFTLDP RHSERDRVQADDLVKMLFSPEEMDLSQEQPL DAQQGPPEPAQESLSGSESKPKDEL [SEQ ID NO:499]	Osteogenesis Imperfecta VIII	
COL 7A1	1294	0114270	Q02388, Q59F16	MTLRLLVAALCAGILAEAPRVRAQHRERVTC TRLYAADIVFLLDGSSSIGRSNFREVRSFLEGL VLPFSGAASAQGVRFATVQYSDDPRTEFGLD ALGSGGDVIRAIRELSYKGGNTRTGAAILHVA DHVFLPQLARPGVPKVCILITDGKSQDLVDTA AQRLKGQGVKLFAVGIKNADPEELKRVASQP TSDFFFFVNDFSILRTLLPLVSRRVCTTAGGVP VTRPPDDSTSAPRDLVLSEPSSQSLRVQWTAA SGPVTGYKVQYTPLTGLGQPLPSERQEVNVP AGETSVRLRGLRPLTEYQVTVIALYANSIGEA VSGTARTTALEGPELTIQNTTAHSLLVAWRS VPGATGYRVTWRVLSGGPTQQQELGPGQGS VLLRDLEPGTDYEVTVSTLFGRSVGPATSLM ARTDASVEQTLRPVILGPTSILLSWNLVPEAR GYRLEWRRETGLEPPQKVVLPSDVTRYQLDG LQPGTEYRLTLYTLLEGHEVATPATVVPTGPE LPVSPVTDLQATELPGQRVRVSWSPVPGATQ YRII VRSTQGVERTLVLPGSQTAFDLDDVQAGLSY TVRVSARVGPREGSASVLTVRREPETPLAVPG LRVVVSDATRVRVAWGPVPGASGFRISWSTG SGPESSQTLPPDSTATDITGLQPGTTYQVAVS VLRGREEGPAAVIVARTDPLGPVRTVHVTQA SSSSVTITWTRVPGATGYRVSWHSAHGPEKS	Autosomal recessive dystrophic epidermolysis bullosa	

				QLVSGEATVAELDGLEPDTEYTVHVRAHVA GVDGPPASVVVRTAPEPVGRVSRLQILNASSD VLRITWVGVTGATAYRLAWGRSEGGPMRHQ ILPGNTDSAEIRGLEGGVSY SVRVTALVGDREGTPVSIVVTTPPEAPPALGT LHVVQRGEHSLRLRWEPVPRAQGFLLHWQP EGGQEQSRVLGPELSSYHLDGLEPATQYRVR LSVLGPAGEGPSAEVTARTESPRVPSIELRVV DTSIDSVTLAWTPVSRASSYILSWRPLRGPGQ EVPGSPQTLPGISSSQRVTGLEPGVSYIFSLTP VLDGVRGPEASVTQTPVCPRGLADVVFLPHA TQDNAHRAEATRRVLERLVLALGPLGPQAV QVGLLSYSHRPSPLFPLNGSHDLGIILQRIRDM PYMDPSGNNLGTAVVTAHRYMLAPDAPGRR QHVPGVMVLLVDEPLRGDIFSPIREAQASGLN VVMLGMAGADPEQLRRLAPGMDSVQTFFAV DDGPSLDQAVSGLATALCQASFTTQPRPEPCP VYCPKGQKGEPGEMGLRGQVGPPGDPGLPG RTGAPGPQGPPGSATAKGERGFPGADGRPGS PGRAGNPGTPGAPGLKGSPGLPGPRGDPGER GPRGPKGEPGAPGQVIGGEGPGLPGRKGDPG PSGPPGPRGPLGDPGPRGPPGLPGTAMKGDK GDRGERGPPGPGEGGIAPGEPGLPGLPGSPGP QGPVGPPGKKGEKGDSEDGAPGLPGQPGSPG EQGPRGPPGAIGPKGDRGFPGPLGEAGEKGE RGPPGPAGSRGLPGVAGRPGAKGPEGPPGPT GRQGEKGEPGRPGDPAVVGPAVAGPKGEKG DVGPAGPRGATGVQGERGPPGLVLPGDPGPK GDPGDRGPIGLTGRAGPPGDSGPPGEKGDPG RPGPPGPVGPRGRDGEVGEKGDEGPPGDPGL PGKAGERGLRGAPGVRGPVGEKGDQGDPGE DGRNGSPGSSGPKGDRGEPGPPGPPGRLVDT GPGAREKGEPGDRGQEGPRGPKGDPGLPGAP GERGIEGFRGPPGPQGDPGVRGPAGEKGDRG PPGLDGRSGLDGKPGAAGPSGPNGAAGKAG DPGRDGLPGLRGEQGLPGPSGPPGLPGKPGE DGKPGLNGKNGEPGDPGEDGRKGEKGDSGA SGREGRDGPKGERGAPGILGPQGPPGLPGPVG PPGQGFPGVPGGTGPKGDRGETGSKGEQGLP GERGLRGEPGSVPNVDRLLETAGIKASALREI VETWDESSGSFLPVPERRRGPKGDSGEQGPP GKEGPIGFPGERGLKGDRGDPGPQGPPGLAL GERGPPGPSGLAGEPGKPGIPGLPGRAGGVGE AGRPGERGERGEKGERGEQGRDGPPGLPGTP GPPGPPGPKVSVDEPGPGLSGEQGPPGLKGA KGEPGSNGDQGPKGDRGVPGIKGDRGEPGPR GQDGNPGLPGERGMAGPEGKPGLQGPRGPP GPVGGHGDPGPPGAPGLAGPAGPQGPSGLKG EPGETGPPGRGLTGPTGAVGLPGPPGPSGLVG PQGSPGLPGQVGETGKPGAPGRDGASGKDG DRGSPGVPGSP GLPGPVGPKGEPGPTGAPGQAVVGLPGAKGE KGAPGGLAGDLVGEPGAKGDRGLPGPRGEK GEAGRAGEPGDPGEDGQKGAPGPKGFKGDP GVGVPGSPGPPGPPGVKGDLGLPGLPGAPGV VGFPGQTGPRGEMGQPGPSGERGLAGPPGRE GIPGPLGPPGPPGSVGPPGASGLKGDKGDPGV GLPGPRGERGEPGIRGEDGRPGQEGPRGLTGP PGSRGERGEKGDVGSAGLKGDKGDSAVILGP PGPRGAKGDMGERGPRGLDGDKGPRGDNGD		

				PGDKGSKGEPGDKGSAGLPGLRGLLGPQGQP GAAGIPGDPGSPGKDGVPGIRGEKGDVGFMG PRGLKGERGVKGACGLDGEKGDKGEAGPPG RPGLAGHKGEMGEPGVPGQSGAPGKEGLIGP KGDRGFDGQPGPKGDQGEKGERGTPGIGGFP GPSGNDGSAGPPGPPGSVGPRGPEGLQGQKG ERGPPGERVVGAPGVPGAPGERGEQGRPGPA GPRGEKGEAALTEDDIRGFVRQEMSQHCACQ GQFIASGSRPLPSYAADTAGSQLHAVPVLRVS HAEEEERVPPEDDEYSEYSEYSVEEYQDPEAP WDSDDPCSLPLDEGSCTAYTLRWYHRAVTG STEACHPFVYGGCGGNANRFGTREACERRCP PRVVQSQGTGTAQD [SEQ ID NO:500]		
PKLR	5313	0143627	P30613	MSIQENISSLQLRSWVSKSQRDLAKSILIGAPG GPAGYLRRASVAQLTQELGTAFFQQQQLPAA MADTFLEHLCLLDIDSEPVAARSTSIIATIGPA SRSVERLKEMIKAGMNIARLNFSHGSHEYHA ESIANVREAVESFAGSPLSYRPVAIALDTKGP EIRTGILQGGPESEVELVKGSQVLVTVDPAFR TRGNANTVWVDYPNIVRVVPVGGRIYIDDGL ISLVVQKIGPEGLVTQVENGGVLGSRKGVNL PGAQVDLPGLSEQDVRDLRFGVEHGVDIVFA SFVRKASDVAAVRAALGPEGHGIKIISKIENH EGVKRFDEILEVSDGIMVARGDLGIEIPAEKV FLAQKMMIGRCNLAGKPVVCATQMLESMIT KPRPTRAETSDVANAVLDGADCIMLSGETAK GNFPVEAVKMQHAIAREAEAAVYHRQLFEEL RRAAPLSRDPTEVTAIGAVEAAFKCCAAAIIV LTTTGRSAQLLSRYRPRAAVIAVTRSAQAAR QVHLCRGVFPLLYREPPEAIWADDVDRRVQF GIESG KLRGFLRVGDLVIVVTGWRPGSGYTNIMRVL SIS [SEQ ID NO:501]	Pyruvate Kinase deficiency	
TALDO1	6888	0177156	A0A140VK56, P37837	MSSSPVKRQRMESALDQLKQFTTVVADTGDF HAIDEYKPQDATTNPSLILAAAQMPAYQELV EEAIAYGRKLGGSQEDQIKNAIDKLFVLFGAE ILKKIPGRVSTEVDARLSFDKDAMVARARRLI ELYKEAGISKDRILIKLSSTWEGIQAGKELEEQ HGIHCNMTLLFSFAQAVACAEAGVTLISPFVG RILDWHVANTDKKSYEPLEDPGVKSVTKIYN YYKKFSYKTIVMGASFRNTGEIKALAGCDFL TISPKLLGELLQDNAKLVPVLSAKAAQASDLE KIHLDEKSFRWLHNEDQMAVEKLSDGIRKFA ADAVKLERMLTERMFNAENGK[SEQ ID NO:502]	Transaldolase deficiency	
TF	7018	0091513	A0PJA6, P02787, Q06AH7	MRLAVGALLVCAVLGLCLAVPDKTVRWCA VSEHEATKCQSFRDHMKSVIPSDGPSVACVK KASYLDCIRAIAANEADAVTLDAGLVYDAYL APNNLKPVVAEFYGSKEDPQTFYYAVAVVK KDSGFQMNQLRGKKSCHTGLGRSAGWNIPIG LLYCDLPEPRKPLEKAVANFFSGSCAPCADGT DFPQLCQLCPGCGCSTLNQYFGYSGAFKCLK DGAGDVAFVKHSTIFENLANKADRDQYELLC LDNTRKPVDEYKDCHLAQVPSHTVVARSMG GKEDLIWELLNQAQEHFGKDKSKEFQLFSSP HGKDLLFKDSAHGFLKVPPRMDAKMYLGYE YVTAIRNLREGTCPEAPTDECKP VKWCALSHHERLKCDEWSVNSVGKIECVSA ETTEDCIAKIMNGEADAMSLDGGFVYIAGKC GLVPVLAENYNKSDNCEDTPEAGYFAIAVVK	Atransferrinemia (familial hypotransferrinemia)	

				KSASDLTWDNLKGKKSCHTAVGRTAGWNIP MGLLYNKINHCRFDEFFSEGCAPGSKKDSSLC KLCMGSGLNLCEPNNKEGYYGYTGAFRCLV EKGDVAFVKHQTVPQNTGGKNPDPWAKNLN EKDYELLCLDGTRKPVEEYANCHLARAPNH AVVTRKDKEACVHKILRQQQHLFGSNVTDCS GNFCLFRSETKDLLFRDDTVCLAKLHDRNTY EKYLGEEYVKAVGNLRKCSTSSLLEACTFRR P [SEQ ID NO:503]		
EPC AM	4072	0119888	P16422	MAPPQVLAFGLLLAAATATFAAAQEECVCEN YKLAVNCFVNNNRQCQCTSVGAQNTVICSKL AAKCLVMKAEMNGSKLGRRAKPEGALQNN DGLYDPDCDESGLFKAKQCNGTSMCWCVNT AGVRRTDKDTEITCSERVRTYWIIIELKHKAR EKPYDSKSLRTALQKEITTRYQLDPKFITSILY ENNVITIDLVQNSSQKTQNDVDIADVAYYFE KDVKGESLFHSKKMDLTVNGEQLDLDPGQT LIYYVDEKAPEFSMQGLKAGVIAVIVVVVIAV VAGIVVLVISRKKRMAKYEKA EIKEMGEMHRELNA[SEQ ID NO:504]	Intestinal epithelial dysplasia	
VHL	7428	0134086	A0A024R 2F2, P40337, A0A0S2Z 4K1	MPRRAENWDEAEVGAEEAGVEEYGPEEDGG EESGAEESGPEESGPEELGAEEEMEAGRPRPV LRSVNSREPSQVIFCNRSPRVVLPVWLNFDGE PQPYPTLPPGTGRRIHSYRGHLWLFRDAGTH DGLLVNQTELFVPSLNVDGQPIFANITLPVYT LKERCLQVVRSLVKPENYRRLDIVRSLYEDLE DHPNVQKDLERLTQERIAHQRMGD [SEQ ID NO:505]	Familial erythrocyto sis type 2; von Hippel Lindau disease	
GC	2638	0145321	P02774	MKRVLVLLLAVAFGHALERGRDYEKNKVCK EFSHLGKEDFTSLSLVLYSRKFPSGTFEQVSQ LVKEVVSLTEACCAEGADPDCYDTRTSALSA KSCESNSPFPVHPGTAECCTKEGLERKLCMA ALKHQPQEFPTYVEPTNDEICEAFRKDPKEYA NQFMWEYSTNYGQAPLSLLVSYTKSYLSMV GSCCTSASPTVCFLKERLQLKHLSLLTTLSNR VCSQYAAYGEKKSRLSNLIKLAQKVPTADLE DVLPLAEDITNILSKCCESASEDCMAKELPEH TVKLCDNLSTKNSKFEDCCQEKTAMDVFVCT YFMPAAQLPELPDVELPTNKDVCDPGNTKV MDKYTFELSRRTHLPEVFLSKVLEPTLKSLGE CCDVEDSTTCFNAKGPLLKKELSSFIDKGQEL CADYSENTFTEYKKKLAERLKAKLPDATPTE LAKLVNKHSDFASNCCSINSPPLYCDSEIDAE LKNIL [SEQ ID NO:506]	Vitamin D deficiency	
SER PIN A1	5265	0197249,02 77377	E9KL23, P01009	MPSSVSWGILLLAGLCCLVPVSLAEDPQGDA AQKTDTSHHDQDHPTFNKITPNLAEFAFSLYR QLAHQSNSTNIFFSPVSIATAFAMLSLGTKAD THDEILEGLNFNLTEIPEAQIHEGFQELLRTLN QPDSQLQLTTGNGLFLSEGLKLVDKFLEDVK KLYHSEAFTVNFGDTEEAKKQINDYVEKGTQ GKIVDLVKELDRDTVFALVNYIFFKGKWERP FEVKDTEEEDFHVDQVTTVKVPMMKRLGMF NIQHCKKLSSWVLLMKYLGNATAIFFLPDEG KLQHLENELTHDIITKFLENEDRRSASLHLPK LSITGTYDLKSVLGQLGITKVFSNGADLSGVT EEAPLKLSKAVHKAVLTIDEKGTEAAGAMFL EAIPMSIPPEVKFNKPFVFLMIEQNTKSPLFMG KVVNPTQK [SEQ ID NO:507]	Alpha-1 antitrypsin deficiency	

ABCC6	368	0091262,0275331	O95255	MAAPAEPCAGQGVWNQTEPEPAATSLLSLCF LRTAGVWVPPMYLWVLGPIYLLFIHHHGRGY LRMSPLFKAKMVLGFALIVLCTSSVAVALWK IQQGTPEAPEFLIHPTVWLTTMSFAVFLIHTER KKGVQSSGVLFGYWLLCFVLPATNAAQQAS GAGFQSDPVRHLSTYLCLSLVVAQFVLSCLA DQPPFFPEDPQQSNPCPETGAAFPSKATFWW VSGLVWRGYRRPLRPKDLWSLGRENSSEELV SRLEKEWMRNRSAARRHNKAIAFKRKGGSG MKAPETEPFLRQEGSQWRPLL KAIWQVFHSTFLLGTLSLIISDVFRFTVPKLLS LFLEFIGDPKPPAWKGYLLAVLMFLSACLQTL FEQQNMYRLKVLQMRLRSAITGLVYRKVLA LSSGSRKASAVGDVVNLVSVDVQRLTESVLY LNGLWLPLVWIVVCFVYLWQLLGPSALTAIA VFLSLLPLNFFISKKRNHHQEEQMRQKDSRA RLTSSILRNSKTIKFHGWEGAFLDRVLGIRGQ ELGALRTSGLLFSVSLVSFQVSTFLVALVVFA VHTLVAENAMNAEKAFVTLTVLNILNKAQA FLPFSIHSLVQARVSFDRLVTFLCLEEVDPGV VDSSSSGSAAGKDCITIHSATFAWSQESPPCL HRINLTVPQGCLLAVVGPVGAGKSSLLSALL GELSKVEGFVSIEGAVAYVPQEAWVQNTSVV ENVCFGQELDPPWLERVLEACALQPDVDSFP EGIHTSIGEQGMNLSGGQKQRLSLARAVYRK AAVYLLDDPLAALDAHVGQHVFNQVIGPGG LLQGTTRILVTHALHILPQADWIIVLANGAIAE MGSYQELLQRKGALMCLLDQARQPGDRGEG ETEPGTSTKDPRGTSAGRRPELRRERSIKSVPE KDRTTSEAQTEVPLDDPDRAGWPAGKDSIQY GRVKATVHLAYLRAVGTPLCLYALFLFLCQQ VASFCRGYWLSLWADDPAVGGQQTQAALR GGIFGLLGCLQAIGLFASMAAVLLGGARASR LLFQRLLWDVVRSPISFFERTPIGHLLNRFSKE TDTVDVDIPDKLRSLLMYAFGLLEVSLVVAV ATPLATVAILPLFLLYAGFQSLYVVSSCQLRR LESASYSSVCSHMAETFQGSTVVRAF RTQAPFVAQNNARVDESQRISFPRLVADRWL AANVELLGNGLVFAAATCAVLSKAHLSAGL VGFSVSAALQVTQTLQWVVRNWTDLENSIVS VERMQDYAWTPKEAPWRLPTCAAQPPWPQG GQIEFRDFGLRYRPELPLAVQGVSFKIHAGEK VGIVGRTGAGKSSLASGLLRLQEAAEGGIWID GVPIAHVGLHTLRSRISIIPQDPILFPGSLRMNL DLLQEHSDEAIWAALETVQLKALVASLPGQL QYKCADRGEDLSVGQKQLLCLARALLRKTQI LILDEATAAVDPGTELQM QAMLGSWFAQCTVLLIAHRLRSVMDCARVL VMDKGQVAESGSPAQLLAQKGLFYRLAQES GLV[SEQ ID NO:508]	Pseudoxanthoma elasticum	
F8	2157	0185010	P00451	MQIELSTCFFLCLLRFCFSATRRYYLGAVELS WDYMQSDLGELPVDARFPPRVPKSFPFNTSV VYKKTLFVEFTDHLFNIAKPRPPWMGLLGPTI QAEVYDTVVITLKNMASHPVSLHAVGVSYW KASEGAEYDDQTSQREKEDDKVFPGGSHTY VWQVLKENGPMASDPLCLTYSYLSHVDLVK DLNSGLIGALLVCREGSLAKEKTQTLHKFILL FAVFDEGKSWHSETKNSLMQDRDAASARAW PKMHTVNGYVNRSLPGLIGCHRKSVYWHVI GMGTTPEVHSIFLEGHTFLVRNHRQASLEISPI	Hemophilia A	

				TFLTAQTLLMDLGQFLLFCHISSHQHDGMEA YVKVDSCPEEPQLRMKNNEEAEDYDDDLTD SEMDVVRFDDDNSPSFIQIRSVAKKHPKTWV HYIAAEEEDWDYAPLVLAPDDRSYKSQYLN NGPQRIGRKYKKVRFMAYTDETFKTREAIQH ESGILGPLLYGEVGDTLLIIFKNQASRPYNIYP HGITDVRPLYSRRLPKGVKHLKDFPILPGEIFK YKWTVTVEDGPTKSDPRCLTRYYSSFVNMER DLASGLIGPLLICYKESVDQRGNQIMSDKRNV ILFSVFDENRSWYLTENIQRFLPNPAGVQLED PEFQASNIMHSINGYVFDSLQLSVCLHEVAY WYILSIGAQTDFLSVFFSGYTFKHKMVYEDTL TLFPFSGETVFMSMENPGLWILGCHNSDFRN RGMTALLKVSSCDKNTGDYYEDSYEDISAYL LSKNNAIEPRSFSQNSRHPSTRQKQFNATTIPE NDIEKTDPWFAHRTPMPKIQNVSSSDLLMLL RQSPTPHGLSLSDLQEAKYETFSDDPS PGAIDSNNSLSEMTHFRPQLHHSGDMVFTPES GLQLRLNEKLGTTAATELKKLDFKVSSTSNN LISTIPSDNLAAGTDNTSSLGPPSMPVHYDSQL DTTLFGKKSSPLTESGGPLSLSEENNDSKLLES GLMNSQESSWGKNVSSTESGRLFKGKRAHGP ALLTKDNALFKVSISLLKTNKTSNNSATNRKT HIDGPSLLIENSPSVWQNILESDTEFKKVTPLI HDRMLMDKNATALRLNHMSNKTTSSKNME MVQQKKEGPIPPDAQNPDMSFFKMLFLPESA RWIQRTHGKNSLNSGQGPSPKQLVSLGPEKS VEGQNFLSEKNKVVVGKGEFTKDVGLKEMV FPSSRNLFLTNLDNLHENNTHNQEKKIQEEIE KKETLIQENVVLPQIHTVTGTKNFMKNLFLLS TRQNVEGSYDGAYAPVLQDFRSLNDSTNRTK KHTAHFSKKGEEENLEGLGNQTKQIVEKYAC TTRISPNTSQQNFVTQRSKRALKQFRLPLEET ELEKRIIVDDTSTQWSKNMKHLTPSTLTQIDY NEKE KGAITQSPLSDCLTRSHSIPQANRSPLPIAKVS SFPSIRPIYLTRVLFQDNSSHLPAASYRKKDSG VQESSHFLQGAKKNNLSLAILTLEMTGDQRE VGSLGTSATNSVTYKKVENTVLPKPDLPKTS GKVELLPKVHIYQKDLFPTETSNGSPGHLDLV EGSLLQGTEGAIKWNEANRPGKVPFLRVATE SSAKTPSKLLDPLAWDNHYGTQIPKEEWKSQ EKSPEKTAFKKKDTILSLNACESNHAIAAINE GQNKPEIEVTWAKQGRTERLCSQNPPVLKRH QREITRTTLQSDQEEIDYDDTISVEMKKEDFDI YDEDENQSPRSFQKKTRHYFIAAVERLWDYG MSSSPHVLRNRAQSGSVPQFKKVVFQEFTDG SFTQPLYRGELNEHLGLLGPYIRAEVEDNIMV TFRNQASRPYSFYSSLISYEEDQRQGAEPRKN FVKPNETKTYFWKVQHHMAPTKDEFDCKA WAYFSDVDLEKDVHSGLIGPLLVCHTNTLNP AHGRQVTVQEFALFFTIFDETKSWYFTENME RNCRA PCNIQMEDPTFKENYRFHAINGYIMDTLPGLV MAQDQRIRWYLLSMGSNENIHSIHFSGHVFT VRKKEEYKMALYNLYPGVFETVEMLPSKAGI WRVECLIGEHLHAGMSTLFLVYSNKCQTPLG MASGHIRDFQITASGQYGQWAPKLARLHYSG SINAWSTKEPFSWIKVDLLAPMIIHGIKTQGA RQKFSSLYISQFIIMYSLDGKKWQTYRGNSTG		

				TLMVFFGNVDSSGIKHNIFNPPIIARYIRLHPT HYSIRSTLRMELMGCDLNSCSMPLGMESKAI SDAQITASSYFTNMFATWSPSKARLHLQGRS NAWRPQVNNPKEWLQVDFQKTMKVTGVTT QGVKSLLTSMYVKEFLISSSQDGHQWTLFFQ NGKVKVFQGNQDSFTPVVNSLDPPLLTRYLRI HPQSWVHQIALRMEVLGCEAQDLY [SEQ ID NO:509]		
F9	2158	0101981	P00740	MQRVNMIMAESPGLITICLLGYLLSAECTVFL DHENANKILNRPKRYNSGKLEEFVQGNLERE CMEEKCSFEEAREVFENTERTTEFWKQYVDG DQCESNPCLNGGSCKDDINSYECWCPFGFEG KNCELDVTCNIKNGRCEQFCKNSADNKVVCS CTEGYRLAENQKSCEPAVPFPCGRVSVSQTS KLTRAETVFPDVDYVNSTEAETILDNITQSTQ SFNDFTRVVGGEDAKPGQFPWQVVLNGKVD AFCGGSIVNEKWIVTAAHCVETGVKITVVAG EHNIEETEHTEQKRNVIRII PHHNYNAAINKYNHDIALLELDEPLVLNSYV TPICIADKEYTNIFLKFGSGYVSGWGRVFHKG RSALVLQYLRVPLVDRATCLRSTKFTIYNNM FCAGFHEGGRDSCQGDSGGPHVTEVEGTSFL TGIISWGEECAMKGKYGIYTKVSRYVNWIKE KTKLT [SEQ ID NO:510]	Hemophilia B	
Apo B	338	0084674	P04114	MDPPRPALLALLALPALLLLLLAGARAEEEM LENVSLVCPKDATRFKHLRKYTYNYEAESSS GVPGTADSRSATRINCKVELEVPQLCSFILKTS QCTLKEVYGFNPEGKALLKKTKNSEEFAAA MSRYELKLAIPEGKQVFLYPEKDEPTYILNIK RGIISALLVPPETEEAKQVLFLDTVYGNCSTH FTVKTRKGNVATEISTERDLGQCDRFKPIRTG ISPLALIKGMTRPLSTLIS SSQSCQYTLDAKRKHVAEAICKEQHLFLPFSY KNKYGMVAQVTQTLKLEDTPKINSRFFGEGT KKMGLAFESTKSTSPPKQAEAVLKTLQELKK LTISEQNIQRANLFNKLVTELRGLSDEAVTSL LPQLIEVSSPITLQALVQCGQPQCSTHILQWLK RVHANPLLIDVVTYLVALIPEPSAQQLREIFN MARDQRSRATLYALSHAVNNYHKTNPTGTQ ELLDIANYLMEQIQDDCTGDEDYTYLILRVIG NMGQTMEQLTPELKSSILKCVQSTKPSLMIQK AAIQALRKMEPKDKD QEVLLQTFLDDASPGDKRLAAYLMLMRSPSQ AINKIVQILPWEQNEQVKNFVASHIANILNSEE LDIQDLKKLVKEALKESQLPTVMDFRKFSRN YQLYKSVSLPSLDPASAKIEGNLIFDPNNYLP KESMLKTTLTAFGFASADLIEIGLEGKGFEPTL EALFGKQGFFPDSVNKALYWVNGQVPDGVS KVLVDHFGYTKDDKHEQDMVNGIMLSVEKL IKDLKSKEVPEARAYLRILGEELGFASLHDLQ LLGKLLLMGARTLQGIPQMIGEVIRKGSKNDF FLHYIFMENAFELPTGAGLQLQISSSGVIAPGA KAGVKLEVANMQAELVAKPSVSVEFVTNMG IIIPDFARSGVQMNTNFFHESGLEAHVALKAG KLKFIIPSPKRPVKLLSGGNTLHLVSTTKTEVI PPLIENRQSWSVCKQVFPGLNYCTSGAYSNA SSTDSASYYPLTGDTRLELELRPTGEIEQYSVS ATYELQREDRALVDTLKFVTQAEGAKQTEAT MTFKYNRQSMTLSSEVQIPDFDVDLGTILRVN	Familial hypercholesterolemia	

				DESTEGKTSYRLTLDIQNKKITEVALMGHLSC DTKEERKIKGVISIPRLQAEARSEILAHWSPAK LLLQMDSSATAYGSTVSKRVAWHYDEEKIEF EWNTGTNVDTKKMTSNFPVDLSDYPKSLHM YANRLLDHRVPQTDMTFRHVGSKLIVAMSS WLQKASGSLPYTQTLQDHLNSLKEFNLQNM GLPDFHIPENLFLKSDGRVKYTLNKNSLKIEIP LPFGGKSSRDLKMLETVRTPALHFKSVGFHLP SREFQVPTFTIPKLYQLQVPLLGVLDLSTNVY SNLYNWSASYSGGNTST DHFSLRARYHMKADSVVDLLSYNVQGSGET TYDHKNTFTLSYDGSLRHKFLDSNIKFSHVEK LGNNPVSKGLLIFDASSSWGPQMSASVHLDS KKKQHLFVKEVKIDGQFRVSSFYAKGTYGLS CQRDPNTGRLNGESNLRFNSSYLQGTNQITG RYEDGTLSLTSTSDLQSGIIKNTASLKYENYE LTLKSDTNGKYKNFATSNKMDMTFSKQNAL LRSEYQADYESLRFFSLLSGSLNSHGLELNAD ILGTDKINSGAHKATLRIGQDGISTSATTNLKC SLLVLENELNAELGLSGASMKLTTNGRFREH NAKFSLDGKAALTELSLGSAYQAMILGVDSK NIFNFKVSQEGLKLSNDMMGSYAEMKFDHT NSLNIAGLSLDFSSKLDNIYSSDKFYKQTVNL QLQPYSLVTTLNSDLKYNALDLTNNGKLRLE PLKLHVAGNLKGAYQNNEIKHIYAISSAALSA SYKADTVAKVQGVEFSHRLNTDIAGLASAID MSTNYNSDSLHFSNVFRSVMAPFTMTIDAHT NGNGKLALWGEHTGQLYSKFLLKAEPLAFTF SHDYKGSTSHHLVSRKSISAALEHKVSALLTP AEQTGTWKLKTQFNNNEYSQDLDAYNTKDK IGVELTGRTLADLTLLDSPIKVPLLLSEPINIID ALEMRDAVEKPQEFTIVAFVKYDKNQDVHSI NLPFFETLQEYFERNRQTIIVVLENVQRNLKHI NIDQFVRKYRAALGKLPQQANDYLNSFNWE RQVSHAKEKLTALTKKYRITENDIQIALDDAK INFNEKLSQLQTYMIQFDQYIKDSYDLHDLKI AIANIIDEIIEKLKSLDEHYHIRVNLVKTIHDLH LFIENIDFNKSGSSTASWIQNVDTKYQIRIQIQ EKLQQLKRHIQNIDIQHLAGKLKQHIEAIDVR VLLDQLGTTISFERINDILEHVKHFVINLIGDFE VAEKINAFRAKVHELIERYEVDQQIQVLMDK LVELAHQYKLKETIQKLSNVLQQVKIKDYFE KLVGFIDDAVKKLNELSFKTFIEDVNKFLDML IKKLKSFDYHQFVDETNDKIREVTQRLNGEIQ ALELPQKAEALKLFLEETKATVAVYLESLQD TKITLIINWLQEALSSASLAHMKAKFRETLED TRDRMYQMDIQQELQRYLSLVGQVYSTLVT YISDWWTLAAKNLTDFAEQYSIQDWAKRMK ALVEQGFTVPEIKTILGTMPAFEVSLQALQKA TFQTPDFIVPLTDLRIPSVQINFKDLKNIKIPSR FSTPEFTILNTFHIPSFTIDFVEMKVKIIRTIDQ MLNSELQWPVPDIYLRDLKVEDIPLARITLPD FRLPEIAIPEFIIPTLNLNDFQVPDLHIPEFQLPH ISHTIEVPTFGKLYSILKIQSPLFTLDANADIGN GTTSANEAGIAASITAKGESKLEVLNFDFQAN AQLSNPKINPLALKESVKFSSKYLRTEHGSEM LFFGNAIEGKSNTVASLHTEKNTLELSNGVIV KINNQLTLDSNTKYFHKLNIPKLDFSSQADLR NEIKTLLKAGHIAWTSSGKGSWKWACPRFSD EGTHESQISFTIEGPLTSFGLSNKINSKHLRVN		

				QNLVYESGSLNFSKLEIQSQVDSQHVGHSVLT AKGMALFGEGKAEFTGRHDAHLNGKVIGTL KNSLFFSAQPFEITASTNNEGNLKVRFPLRLT GKIDFLNNYALFLSPSAQQASWQVSARFNQY KYNQNFSAGNNENIMEAHVGINGE ANLDFLNIPLTIPEMRLPYTIITTPPLKDFSLWE KTGLKEFLKTTKQSFDLSVKAQYKKNKHRHS ITNPLAVLCEFISQSIKSFDRHFEKNRNNALDF VTKSYNETKIKFDKYKAEKSHDELPRTFQIPG YTVPVVNVEVSPFTIEMSAFGYVFPKAVSMPS FSILGSDVRVPSYTLILPSLELPVLHVPRNLKL SLPDFKELCTISHIFIPAMGNITYDFSFKSSVIT LNTNAELFNQSDIVAHLLSSSSSVIDALQYKL EGTTRLTRKRGLKLATALSLSNKFVEGSHNST VSLTTKNMEVSVATTTKAQIPILRMNFKQEL NGNTKSKPTVSSSMEFKYDFNSSMLYSTAKG AVDHKLSLESLTSYFSIESSTKGDVKGSVLSR EYSGTIASEANTYLNSKSTRSSVKLQGTSKID DIWNLEVKENFAGEATLQRIYSLWEHSTKNH LQLEGLFFTNGEHTSKATLELSPWQMSALV QVHASQPSSFHDFPDLGQEVALNANTKNQKI RWKNEVRIHSGSFQSQVELSNDQEKAHLDIA GSLEGHLRFLKNIILPVYDKSLWDFLKLDVTT SIGRRQHLRVSTAFVYTKNPNGYSFSIPVKVL ADKFIIPGLKLNDLNSVLVMPTFHVPFTDLQV PSCKLDFREIQIYKKLRTSSFALNLPTLPEVKF PEVDVLTKYSQPEDSLIPFFEITVPESQLTVSQ FTLPKSVSDGIAALDL NAVANKIADFELPTIIVPEQTIEIPSIKFSVPAGI VIPSFQALTARFEVDSPVYNATWSASLKNKA DYVETVLDSTCSSTVQFLEYELNVLGTHKIED GTLASKTKGTFAHRDFSAEYEEDGKYEGLQE WEGKAHLNIKSPAFTDLHLRYQKDKKGISTS AASPAVGTVGMDMDEDDDFSKWNFYYSPQS SPDKKLTIFKTELRVRESDEETQIKVNWEEEA ASGLLTSLKDNVPKATGVLYDYVNKYHWEH TGLTLREVSSKLRRNLQNNAEWVYQGAIRQI DDIDVRFQKAASGTTGT YQEWKDKAQNLYQELLTQEGQASFQGLKDN VFDGLVRVTQEFHMKVKHLIDSLIDFLNFPRF QFPGKPGIYTREELCTMFIREVGTVLSQVYSK VHNGSEILFSYFQDLVITLPFELRKHKLIDVIS MYRELLKDLSKEAQEVFKAIQSLKTTEVLRN LQDLLQFIFQLIEDNIKQLKEMKFTYLINYIQD EINTIFSDYIPYVFKLLKENLCLNLHKFNEFIQ NELQEASQELQQIHQY IMALREEYFDPSIVGWTVKYYELEEKIVSLIK NLLVALKDFHSEYIVSASNFTSQLSSQVEQFL HRNIQEYLSILTDPDGKGKEKIAELSATAQEII KSQAIATKKIISDYHQQFRYKLQDFSDQLSDY YEKFIAESKRLIDLSIQNYHTFLIYITELLKKLQ STTVMNPYMKLAPGELTIIL [SEQ ID NO:511]		
PCSK9	255738	0169174	Q8NBP7	MGTVSSRRSWWPLPLLLLLLLLLGPAGARAQ EDEDGDYEELVLALRSEEDGLAEAPEHGTTA TFHRCAKDPWRLPGTYVVVLKEETHLSQSER TARRLQAQAARRGYLTKILHVFHGLLPGFLV KMSGDLLELALKLPHVDYIEEDSSVFAQSIPW NLERITPPRYRADEYQPPDGGSLVEVYLLDTS IQSDHREIEGRVMVTDFENVPEEDGTRFHRQ ASKCDSHGTHLAGVVSGRDAGVAKGASMRS	Familial hypercholesterolemia	

				LRVLNCQGKGTVSGTLIGLEFIRKSQLVQPVG PLVVLLPLAGGYSRVLNAA CQRLARAGVVLVTAAGNFRDDACLYSPASA PEVITVGATNAQDQPVTLGTLGTNFGRCVDL FAPGEDIIGASSDCSTCFVSQSGTSQAAAHVA GIAAMMLSAEPELTLAELRQRLIHFSAKDVIN EAWFPEDQRVLTPNLVAALPPSTHGAGWQLF CRTVWSAHSGPTRMATAVARCAPDEELLSCS SFSRSGKRRGERMEAQGGKLVCRAHNAFGG EGVYAIARCCLLPQANCSVHTAPPAEASMGT RVHCHQQGHVLTGCSSHWEVEDLGTHKPPV LRPRGQPNQCVGHREASIHASCCHAPGLECK VKEHGIPAPQEQVTVACEEGWTLTGCSALPG TSHVLGAYAVDNTCVVRSRDVSTTGSTSEGA VTAVAICCRSRHLAQASQELQ [SEQ ID NO:512]		
LDL RAP 1	26119	0157978	B3KR97, Q5SW96	MDALKSAGRALIRSPSLAKQSWGGGGRHRK LPENWTDTRETLLEGMLFSLKYLGMTLVEQP KGEELSAAAIKRIVATAKASGKKLQKVTLKV SPRGIILTDNLTNQLIENVSIYRISYCTADKMH DKVFAYIAQSQHNQSLECHAFLCTKRKMAQ AVTLTVAQAFKVAFEFWQVSKEEKEKRDKA SQEGGDVLGARQDCTPSLKSLVATGNLLDLE ETAKAPLSTVSANTTNMDEVPRPQALSGSSV VWELDDGLDEAFSRLAQSRTNPQVLDTGLTA QDMHYAQCLSPVDWDKPDSSGTEQDDLFSF[SEQ ID NO:513]	Familial hypercholesterolemia	
ABC G5	64240	0138075	Q9H222	MGDLSSLTPGGSMGLQVNRGSQSSLEGAPAT APEPHSLGILHASYSVSHRVRPWWDITSCRQQ WTRQILKDVSLYVESGQIMCILGSSGSGKTTL LDAMSGRLGRAGTFLGEVYVNGRALRREQF QDCFSYVLQSDTLLSSLTVRETLHYTALLAIR RGNPGSFQKKVEAVMAELSLSHVADRLIGNY SLGGISTGERRRVSIAAQLLQDPKVMLFDEPT TGLDCMTANQIVVLLVELARRNRIVVLTIHQP RSELFQLFDKIAILSFGELIFCGTPAEMLDFFN DCGYPCPEHSNPFDFYMDLTSVDTQSKEREIE TSKRVQMIESAYKKSAICHKTLKNIERMKHL KTLPMVPFKTKDSPGVFSKLGVLLRRVTRNL VRNKLAVITRLLQNLIMGLFLLFFVLRVRSNV LKGAIQDRVGLLYQFVGATPYTGMLNAVNL FPVLRAVSDQESQDGLYQKWQMMLAYALH VLPFSVVATMIFSSVCYWTLGLHPEVARFGY FSAALLAPHLIGEFLTLVLLGIVQNPNIVNSVV ALLSIAGVLVGSGFLRNIQEMPIPFKIISYFTFQ KYCSEILVVNEFYGLNFTCGSSNVSVTTNPMC AFTQGIQFIEKTCPGATSRFTMNFLILYSFIPAL VILGIVVFKIRDHLISR [SEQ ID NO:514]	Sitosterolemia	
ABC G8	64241	0143921	Q9H221	MAGKAAEERGLPKGATPQDTSGLQDRLFSSE SDNSLYFTYSGQPNTLEVRDLNYQVDLASQV PWFEQLAQFKMPWTSPSCQNSCELGIQNLSF KVRSGQMLAIIGSSGCGRASLLDVITGRGHGG KIKSGQIWINGQPSSPQLVRKCVAHVRQHNQ LLPNLTVRETLAFIAQMRLPRTFSQAQRDKRV EDVIAELRLRQCADTRVGNMYVRGLSGGER RRVSIGVQLLWNPGILILDEPTSGLDSFTAHNL VKTLSRLAKGNRLVLISLHQPRSDIFRLFDLV LLMTSGTPIYLGAAQHMVQYFTAIGYPCPRY SNPADFYVDLTSIDRRSREQELATREKAQSLA ALFLEKVRDLDDFLWKAETKDLDEDTCVESS	Sitosterolemia	

				VTPLDTNCLPSPTKMPGAVQQFTTLIRRQISN DFRDLPTLLIHGAEACLMSMTIGFLYFGHGSI QLSFMDTAALLFMIGALIPFNVILDVISKCYSE RAMLYYELEDGLYTTGPYFFAKILGELPEHC AYIIIYGMPTYWLANLRPGLQPFLLHFLLVWL VVFCCRIMALAAAALLPTFHMASFFSNALYN SFYLAGGFMINLSSLWTVPAWISKVSFLRWC FEGLMKIQFSRRTYKMPLGNLTIAVSGDKILS VMELDSYPLYAIYLIVIGLSGGFMVLYYVSLR FIKQKPSQDW [SEQ ID NO:515]		
LCAT	3931	0213398	A0A140VK24, P04180	MGPPGSPWQWVTLLLGLLLPPAAPFWLLNVL FPPHTTPKAELSNHTRPVILVPGCLGNQLEAK LDKPDVVNWMCYRKTEDFFTIWLDLNMFLP LGVDCWIDNTRVVYNRSSGLVSNAPGVQIRV PGFGKTYSVEYLDSSKLAGYLHTLVQNLVNN GYVRDETVRAAPYDWRLEPGQQEEYYRKLA GLVEEMHAAYGKPVFLIGHSLGCLHLLYFLL RQPQAWKDRFIDGFISLGAPWGGSIKPMLVL ASGDNQGIPIMSSIKLKEEQRITTTSPWMFPSR MAWPEDHVFISTPSFNYTGR DFQRFFADLHFEEGWYMWLQSRDLLAGLPA PGVEVYCLYGVGLPTPRTYIYDHGFPYTDPV GVLYEDGDDTVATRSTELCGLWQGRQPQPV HLLPLHGIQHLNMVFSNLTLEHINAILLGAYR QGPPASPTASPEPPPPE [SEQ ID NO:516]	Lecithin cholesterol acyltransferase deficiency	
SPINK5	11005	0133710	Q9NQ38	MKIATVSVLLPLALCLIQDAASKNEDQEMCH EFQAFMKNGKLFCPQDKKFFQSLDGIMFINK CATCKMILEKEAKSQKRARHLARAPKATAPT ELNCDDFKKGERDGDFICPDYYEAVCGTDGK TYDNRCALCAENAKTGSQIGVKSEGECKSSN PEQDVCSAFRPFVRDGRLGCTRENDPVLGPD GKTHGNKCAMCAELFLKEAENAKREGETRIR RNAEKDFCKEYEKQVRNGRLFCTRESDPVRG PDGRMHGNKCALCAEIFKQRFSEENSKTDQN LGKAEEKTKVKREIVKLCSQYQNQAKNGILF CTRENDPIRGPDGKMHGNLCSMCQAYFQAE NEEKKKAEARARNKRESGKA TSYAELCSEYRKLVRNGKLACTRENDPIQGP DGKVHGNTCSMCEVFFQAEEEEKKKKEGKS RNKRQSKSTASFEELCSEYRKSRKNGRLFCTR ENDPIQGPDGKMHGNTCSMCEAFFQQEERAR AKAKREAAKEICSEFRDQVRNGTLICTREHNP VRGPDGKMHGNKCAMCASVFKLEEEEKKND KEEKGKVEAEKVKREAVQELCSEYRHYVRN GRLPCTRENDPIEGLDGKIHGNTCSMCEAFFQ QEAKEKERAEPRAKVKREAEKETCDEFRRLL QNGKLFCTRENDPVRGPDGKTHGNKCAMCK AVFQKENEERKRKEEEDQRNAAGHGSSGGG GGNTQDECAEYREQMKNGRLS CTRESDPVRDADGKSYNNQCTMCKAKLERE AERKNEYSRSRSNGTGSESGKDTCDEFRSQM KNGKLICTRESDPVRGPDGKTHGNKCTMCKE KLEREAAEKKKKEDEDRSNTGERSNTGERSN DKEDLCREFRSMQRNGKLICTRENNPVRGPY GKMHINKCAMCQSIFDREANERKKKDEEKSS SKPSNNAKDECSEFRNYIRNNELICPRENDPV HGADGKFYTNKCYMCRAVFLTEALERAKLQ EKPSHVRASQEEDSPDSFSSLDSEMCKDYRVL PRIGYLCPKDLKPVCGDDGQTYNNPCMLCHE	Netherton syndrome	

				NLIRQTNTHIRSTGKCEESSTPGTTAASMPPSD E [SEQ ID NO:517]		
GNE	10020	0159921	Q9Y223	MEKNGNNRKLRVCVATCNRADYSKLAPIMF GIKTEPEFFELDVVVLGSHLIDDYGNTYRMIE QDDFDINTRLHTIVRGEDEAAMVESVGLALV KLPDVLNRLKPDIMIVHGDRFDALALATSAA LMNIRILHIEGGEVSGTIDDSIRHAITKLAHYH VCCTRSAEQHLISMCEDHDRILLAGCPSYDKL LSAKNKDYMSIIRMWLGDDVKSKDYIVALQ HPVTTDIKHSIKMFELTLDALISFNKRTLVLFP NIDAGSKEMVRVMRKKGIEHHPNFRAVKHV PFDQFIQLVAHAGCMIGNSSCGVREVGAFGT PVINLGTRQIGRETGENVLHVRDADTQDKILQ ALHLQFGKQYPCSKIYGDGNAVPRILKFLKSI DLQEPLQKKFCFPPVKENISQDIDHILETLSAL AVDLGGTNLRVAIVSMKGEIVKKYTQFNPKT YEERINLILQMCVEAAAEAVKLNCRILGVGIS TGGRVNPREGIVLHSTKLIQEWNSVDLRTPLS DTLHLPVWVDNDGNCAALAERKFGQGKGLE NFVTL ITGTGIGGGIIHQHELIHGSSFCAAELGHLVVS LDGPDCSCGSHGCIEAYASGMALQREAKKLH DEDLLLVEGMSVPKDEAVGALHLIQAAKLG NAKAQSILRTAGTALGLGVVNILHTMNPSLVI LSGVLASHYIHIVKDVIRQQALSSVQDVDVV VSDLVDPALLGAASMVLDYTTRRIY [SEQ ID NO:518]		

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.

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.

Cell-derived fusosomes

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 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 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 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 liver sinusoidal endothelial cell, cholangiocyte, stellate cell, liver-resident antigen-presenting cell (e.g., Kupffer Cell), liver-resident immune lymphocyte (e.g., T cell, B cell, or NK cell), or portal fibroblast.

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 encoding an exogenous agent of Table 5,

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 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 linked to the payload gene, wherein the NTCSRE comprises a non-liver cell-specific miRNA recognition sequence, e.g., a non-liver cell-specific miRNA recognition sequence bound by a miRNA present in a hematopoietic cell or a Plasmacytoid dendritic cell (pDC), e.g., by a miRNA present in a hematopoietic cell or a Plasmacytoid dendritic cell (pDC) at a higher level than in a liver cell, e.g., a non-liver cell-specific miRNA recognition sequence bound by a miRNA of Table 4; 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 hematopoietic cell or a pDC) 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 liver cell, e.g., a liver cell described herein). In some embodiments, the miRNA is not detectably present in a target cell (e.g., a liver cell). In some embodiments, the miRNA is not present in the target cell (e.g., a liver cell).

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 promoter operably linked to the payload gene, wherein the promoter is a liver-specific promoter, e.g., is a promoter specific for a liver sinusoidal endothelial cell, cholangiocyte, stellate cell, liver-resident antigen-presenting cell (e.g., Kupffer Cell), liver-resident immune lymphocyte (e.g., T cell, B cell, or NK cell), or portal fibroblast;

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 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 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 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 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-liver cell-specific miRNA recognition sequence, e.g., a non-liver 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 liver cell-specific regulatory element (e.g., a liver-cell specific promoter) operatively linked to the payload gene, wherein the positive liver 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 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, 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 43;

v) the fusosome fuses at a higher rate with a target cell than with other fusosomes, e.g., by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 20-fold, 50-fold, or 100-fold, e.g., in an assay of Example 43;

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 43;

vii) the fusogen is present at a copy number of at least, or no more than, 10, 50, 100, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000, 100,000,000, 500,000,000, or 1,000,000,000 copies, e.g., as measured by an assay of Example 27;

viii) the fusosome comprises a therapeutic agent at a copy number of at least, or no more than, 10, 50, 100, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000, 100,000,000, 500,000,000, or 1,000,000,000 copies, e.g., as measured by an assay of Example 89;

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

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

xi) the fusosome comprises a proteomic composition similar to that of the source cell, e.g., using an assay of Example 88;

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 41;

xiii) the fusosome comprises a ratio of proteins to nucleic acids (e.g., DNA) that is within 10%, 20%, 30%, 40%, or 50% of the corresponding ratio in the source cell, e.g., as measured using an assay of Example 42;

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 92;

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 61;

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 51;

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 52;

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 54;

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 55;

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 56, 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 56, 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 56,

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 34;

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 39;

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 40;

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 50;

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 65;

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 57;

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 58;

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 59;

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 60, 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 45, 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 49;

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

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

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

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

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

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

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

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 um, 10 um, 20 um, 50 um, or 100 um;

i) 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 +/- 0.05, e.g., as measured by an assay of Example 31;

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

v) the source cell is other than a 293 cell;

vi) the source cell is not transformed or immortalized;

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

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

ix) the fusosome does not comprise Cre or GFP, e.g., EGFP;

x) the fusosome further comprises an exogenous protein other than Cre or GFP, e.g., EGFP

xi) the fusosome further 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

xii) 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 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, 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 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 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.

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.

The present disclosure also provides, in some aspects, a pharmaceutical composition comprising the fusosome composition described herein and pharmaceutically acceptable carrier.

This disclosure also provides, in certain aspects, a method of administering a fusosome 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.

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, 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 fiunction is delivered.

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.

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.

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 90.

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 94;

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.

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.

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 (RELIK) 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, 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 94.

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, 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, 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 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.

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, 500 nm, 1000 nm, 1200 nm, 1400 nm, or 1500 nm;

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

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

ix) 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 exosomes from cell culture supernatants and biological fluids." Curr Protoc Cell Biol. 2006 Apr; Chapter 3:Unit 3.22;

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

xi) the fusosome comprises Phosphatidyl serine (PS) or CD40 ligand or both of PS and CD40 ligand, e.g., when measured in an assay of Example 93;

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 53;

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 90;

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 37;

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 38 or 91, or wherein the average fractional content of Calnexin in the fusosome is less than about $1x10^{-4}$, $1.5x10^{-4}$, $2x10^{-4}$, $2.1x10^{-4}$, $2.2x10^{-4}$, $2.3x10^{-4}$,; $2.4x10^{-4}$, $2.43x10^{-4}$, $2.5x10^{-4}$, $2.6x10^{-4}$, $2.7x10^{-4}$, $2.8x10^{-4}$, $2.9x10^{-4}$, $3x10^{-4}$, $3.5x10^{-4}$, or $4x10^{-4}$, 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 35; 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 93;

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 53;

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 90;

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 37;

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 91, or wherein the average fractional content of Calnexin in the fusosome is less than about $1x10^{-4}$, $1.5x10^{-4}$, $2x10^{-4}$, $2.1x10^{-4}$, $2.2x10^{-4}$, $2.3x10^{-4}$,; $2.4x10^{-4}$, $2.43x10^{-4}$, $2.5x10^{-4}$, $2.6x10^{-4}$, $2.7x10^{-4}$, $2.8x10^{-4}$, $2.9x10^{-4}$, $3x10^{-4}$, $3.5x10^{-4}$, or $4x10^{-4}$, 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 66;

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 46;

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 47, 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 48, e.g., wherein the source cell is a macrophage.

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 cytosolic proteins;

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

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

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

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

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

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, 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 25 and DNA replication is measured by an assay of Example 26;

b) the fusosome is not capable of transcription or has transcriptional activity of less than 1%, 2.5% 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of that of the transcriptional activity of a reference cell, e.g., the source cell, e.g., using an assay of Example 25;

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 26;

d) the fusosome lacks chromatin or has a chromatin content of less than 1%, 2.5% 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the of the chromatin content of a reference cell, e.g., the source cell, e.g., using an assay of Example 33;

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 32;

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 32, 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 33;

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., myelobast 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 x 10^3, 10^4, 5.0 x 10^4, 10^5, 5.0 x 10^5, 10^6, 5.0 x 10^6, 1.0 x 10^7, 5.0 x 10^7, or 1.0 x 10^8, greater than its concentration in the source cell.

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 FGFRL1. 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.

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 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 in size distribution, e.g., based on Example 29. 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 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.

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

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.

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).

In some embodiments, the cargo delivered is determined using an endocytosis inhibition assay, e.g., an assay of Example 81.

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-isopropyl)amiloride (EIPA), e.g., at a concentration of 25 μM), or an actin-independent pathway (e.g., wherein the inhibitor of endocytosis is an inhibitor of actin polymerization is, e.g., Latrunculin B, e.g., at a concentration of 6 μM).

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 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 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%.

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.

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 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 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 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 derivative thereof. In some embodimetns, 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.

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 nucleocapside 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 94.

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 81. 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 μM), or an actin-independent pathway (e.g., wherein the inhibitor of endocytosis is an inhibitor of actin polymerization is, e.g., Latrunculin B, e.g., at a concentration of 6 μM).

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 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 cells, and combinations thereof. Fusosome can be generated from cultured cells from any eukaryotic (e.g., mammalian) organ system, for example, from the cardiovascular system (heart, vasculature); digestive system (esophagus, stomach, liver, gallbladder, pancreas, intestines, colon, rectum and anus); endocrine system (hypothalamus, pituitary gland, pineal body or pineal gland, thyroid, parathyroids, adrenal glands); excretory system (kidneys, ureters, bladder); lymphatic system (lymph, lymph nodes, lymph vessels, tonsils, adenoids, thymus, spleen); integumentary system (skin, hair, nails); muscular system (e.g., skeletal muscle); nervous system

(brain, spinal cord, nerves); reproductive system (ovaries, uterus, mammary glands, testes, vas deferens, seminal vesicles, prostate); respiratory system (pharynx, larynx, trachea, bronchi, lungs, diaphragm); skeletal system (bone, cartilage), and combinations thereof. In embodiments, the cells are from a highly mitotic tissue (e.g., a highly mitotic healthy tissue, such as epithelium, embryonic tissue, bone marrow, intestinal crypts). In embodiments, the tissue sample is a highly metabolic tissue (e.g., skeletal tissue, neural tissue, cardiomyocytes).

In some embodiments, the cells are from a young donor, e.g., a donor 25 years, 20 years, 18 years, 16 years, 12 years, 10 years, 8 years of age, 5 years of age, 1 year of age, or less. In some embodiments, the cells are from fetal tissue.

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

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 72. 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.

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 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 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 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 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 futher comprises collecting the biological sample from the subject. In embodiments, the biological sample includes one or more recipient cells.

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 futher comprises separating recipient cells in the biological sample from unfused fusosomes in the biological sample, e.g., by centrifugation. In some embodiments, the method futher 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 cellsare 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.

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 muscle cell (e.g., skeletal muscle cell), kidney cell, or liver 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 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 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.

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 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, 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.

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.

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 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 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 81.

In some embodiments, an inhibitor of endocytosis (e.g., chloroquine or bafilomycin A1) inhibits inhibits endosomal acidification. In some embodiments, cargo delivery is independent of lysosomal acidification. In some embodiments, an inhibitor of endocytosis (e.g., Dynasore) inhibits dynamin. In some embodiments, cargo delivery is independent of dynamin activity.

In some embodiments (e.g., embodiments for specific delivery of cargo to a target cell versus a non-target cell), cargo delivery is assayed using one or more of (e.g., all of) the following steps: (a) placing 30,000 HEK-293T target cells that over-express CD8a and CD8b into a first well of a 96-well plate and placing 30,000 HEK-293T non-target cells that do not over-express CD8a and CD8b into a second well of a 96-well plate, (b) culturing the cells for four hours in DMEM media at 37°C and 5% CO2, (c) contacting the target cells with 10 ug of fusosomes that comprise cargo, (d) incubating the target cells and fusosomes for 24 hrs at 37°C and 5% 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 72.

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 43 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 43. In some embodiments, the fusosome fuses with target cells at a rate such that an agent in the fusosome is delivered to at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, of target cells after 24, 48, or 72 hours, e.g., in an assay of Example 54. In embodiments, the amount of targeted fusion is about 30%-70%, 35%-65%, 40%-60%, 45%-55%, or 45%-50%, e.g., about 48.8% e.g., in an assay of Example 43. 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 44.

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 27. 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 95 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 95. 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 89. 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 89. 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 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, 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 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 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, 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 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 - 0.0000001, 0.0000001 - 0.000001, 0.000001 - 0.00001, 0.00001 - 0.0001, 0.0001 - 0.001, 0.001 - 0.01, 0.01 - 0.1, or 0.1 – 1 mg fusogen per mg of total protein in fusosome. In some embodiments, the fusosome comprises 0.00000001 mg fusogen to 5 mg fusogen per mg of lipid in fusosome, e.g., 0.00000001 - 0.0000001, 0.0000001 - 0.000001, 0.000001 - 0.00001, 0.00001 - 0.0001, 0.0001 - 0.001, 0.001 - 0.01, 0.01 - 0.1, 0.1 – 1, or 1-5 mg fusogen per mg of lipid in fusosome.

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 89. 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) about1-30%, 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 essay is an assay of Example 95.

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 87.

In some embodiments, the number of protein species present in both of (e.g., shared between) the fusosomes are 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 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 embodiments, the mass spectrometry assay is an assay of Example 88.

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 90.

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 um. 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 um. In some embodiments, the fusosomes have (or is identified as having) an average diameter of at least 1, 2, 3, 4, or 5 um.

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 97.

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 97.

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). 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 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 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 (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).

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 87. 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 bring) enriched and/or depleted for specific lipids relative to the parental cell, e.g., as determined according to the method described in Example 97.

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 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% 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 : 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%, 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 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

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 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 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 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 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 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 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 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: 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 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 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 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: 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 88. 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 88. 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 98. 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 41. 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 μmol/g, e.g., in an assay of Example 84. 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 84.

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 42. 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 42. 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 85. 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 85.

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 92. 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 μmol/mg, e.g., by an assay of Example 86. 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 92. In embodiments, the fusosome comprises (or is identified as comprising) a greater lipid:DNA ratio than the parental cell. In 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 61. 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 61. In 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 80. 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 51. 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 73. 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 73. 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 52. 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 74. 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

74. 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 75. 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 54. 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 54. In some embodiments, the fusosome comprises (or is identified as comprising) a citrate synthase activity that is about 1E-2 - 2 E-2, 1.3E-2 - 1.8E-2, 1.4E-2 - 1.7E-2, 1.5E-2 - 1.6E-2, or 1.57E-2 umol/ug fusosome/min, e.g., by an assay of Example 76. 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 55. 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 55. 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 pmol/min/20µg fusosome, e.g., by an assay of Example 77. 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 pmol/min/20µg fusosome, e.g., by an assay of Example 77. 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 pmol/min/20µg fusosome, e.g., by an assay of Example 77. 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 77. In embodiments, the fusosome has (or is identified as having) a higher maxaimal 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 77. 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 56, 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 56, 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 56. 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 78. 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 78.

In some embodiments, 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 34. 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 34. In some embodiments, the 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 67.

In some embodiments, 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 39. 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

39. 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 soluble:insoluble protein mass ratio is determined by an assay of Example 71. 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 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 40. In some embodiments, 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 50. In some embodiments, the fusosome targets a tissue, e.g., liver, lungs, heart, spleen, pancreas, gastrointestinal tract, kidney, testes, ovaries, brain, reproductive organs, central nervous system, peripheral nervous system, skeletal muscle, endothelium, inner ear, 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 65. 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 57. 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%, 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 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%, 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 58. 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 58. 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% 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 59. 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 82. 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 82. In some embodiments, 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 60, 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. 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 79. 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 45, e.g., wherein the source 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 46. 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%, 80%, or 90% (e.g., up to 100%) compared to a reference cell, e.g., a macrophage, e.g., using an assay of Example 48. 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 mouse embryonic fibroblast, e.g., using an assay of Example 49. 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 49.

In some embodiments, the fusosome is not capable of transcription or has transcriptional activity of less than 1%, 2.5% 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of that of the transcriptional activity of a reference cell, e.g., the source cell, e.g., using an assay of Example 25. 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 26. In some embodiments, the fusosome lacks chromatin or has a chromatin content of less than 1%, 2.5% 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the of the chromatin content of a reference cell, e.g., the source cell, e.g., using an assay of Example 33.

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 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 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 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-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 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 100.

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 fusosomes 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 101.

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 100. In 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 100.

In some embodiments, a fusosome does not substantially induce complement activity, e.g., as measured according to the method described in Example 101. 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 gial cell, a bone marrow stromal cell, a pancreatic progenitor cell, an endothelial progenitor cell, a blast cell), or an

immortalized cell (e.g., HeLa, HEK293, HFF-1, MRC-5, WI-38, IMR 90, IMR 91, PER.C6, HT-1080, or BJ cell). 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 99. 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 95. 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 96. 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 99.

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 63. In some embodiments, at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% of fusosomes in a fusosome composition that enter a target cell enter via a non-endocytic pathway, e.g., the fusosomes enter the target cell via fusion with the cell surface. In some embodiments, the level of a therapeutic agent delivered via a non-endocytic pathway

for a given fusosome is 0.1-0.95, 0.1-0.2, 0.2-0.3, 0.3-0.4, 0.4-0.5, 0.5-0.6, 0.6-0.7, 0.7-0.8, 0.8-0.9, 0.9-

0.95, or at least at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or

greater than a chloroquine treated reference cell, e.g., using an assay of Example 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 the cytoplasm (e.g., do not enter an endosome or lysosome). In some embodiments, less than 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, 5%, 4%, 3%, 2%, or 1% of fusosomes in a fusosome composition that enter a target cell enter an endosome or lysosome. In some embodiments, the fusosome enters the target cell by a non-endocytic pathway, e.g., wherein the level of therapeutic agent

delivered is at least 90%, 95%, 98%, or 99% that of a chloroquine treated reference cell, e.g., using an assay of Example 63. 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 64. In an embodiment, a fusosome delivers an agent to a target cell via macropinocytosis. In an embodiment, the level of agent delivered via macropinocytosis is in the range of 0.01-0.6, or at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater than EIPA treated target cells contacted with similar fusosomes, e.g., as measured in an assay of Example 64. In an embodiment, a fusosome delivers an agent to a target cell via an actin-mediated pathway. In an embodiment, the level of agent delivered via an actin-mediated pathway will be in the range of 0.01-0.6, or at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater than Latrunculin B treated target cells contacted with similar fusosomes, e.g., as measured in an assay of Example 64.

In some embodiments, the fusosome has a density of <1, 1-1.1, 1.05-1.15, 1.1-1.2, 1.15-1.25, 1.2-1.3, 1.25-1.35, or >1.35 g/ml, e.g., by an assay of Example 31.

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%, 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 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 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 28. 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%, 40%, 50%, 60%, 70%, 80%, 90%, of that of the source cell, e.g., as measured by an assay of Example 28. 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 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 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 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 28. 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 28. In 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 70. 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 70. In 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 70. 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 70. In embodiments, the fusosome has a volume, or the 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 70. 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 30. In 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., ±20%) e.g., as measured by an assay of Example 30. 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 30. 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., ±20%), e.g., as measured by an assay of Example 30. 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 69.

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 83. 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 83. 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 83. 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 $1x10^{-4}$, $1.5x10^{-4}$, $2x10^{-4}$, $2.1x10^{-4}$, $2.2x10^{-4}$, $2.3x10^{-4}$,; $2.4x10^{-4}$, $2.43x10^{-4}$, $2.5x10^{-4}$, $2.6x10^{-4}$, $2.7x10^{-4}$, $2.8x10^{-4}$, $2.9x10^{-4}$, $3x10^{-4}$, $3.5x10^{-4}$, or $4x10^{-4}$. 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 (Ptdlns), 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-betacyclodextrane (mβ-CD) is used to enrich or deplete lipids (see, e.g., Kainu et al, Journal of Lipid Research, 51(12): 3533-3541, 2010).

Pharmaceutical compositions and methods of making them

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 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.

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 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 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 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, 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 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

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.

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, 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 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 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 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 biding 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 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 trifluororacetic 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 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 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 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 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 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 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 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 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 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 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 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 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/cm^3). 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/cm^3. 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 occurrs 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 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 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

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.

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, 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, 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, Endoplasmin, DnaJ homolog subfamily A member 1, Heat shock 70 kDa protein 1L, T-complex protein 1 e subunit, GCN1-like protein 1, Serotransferrin, Fructose bisphosphate 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, Coatomer 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.

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 embodiments, 90% of the retroviral vectors fall within 10% of the median diameter of the retrovirus.

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, 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. In some embodiments, the fusosome, e.g. retroviral vectors or particles, contains nucleic acid sequences encoding an exogenous agent for treating the disease or condition in the subject. For example, the exogenous agent is one that is specific for or can be used to treat a genetic deficiency, e.g. a genetic deficiency listed in Table 5, and the fusosome is administered to a subject for treating the genetic deficiency in the subject.

Thus, also provided are methods of administering and uses, such as therapeutic and prophylactic uses, of the provided fusosomes, including the provided retroviral vectors and particles, such as lentiviral vectors and particles, and/or compositions comprising the same. Such methods and uses include therapeutic methods and uses, for example, involving administration of the fusosomes, including retroviral vectors or particles, such as lentiviral vectors or particles, or compositions containing the same, to a subject having a disease, condition, or disorder for delivery of an exogenous agent for treatment of the disease, condition or disorder. In some embodiments, the fusosome (e.g. retroviral vector or particle, such as

lentiviral vector or particle) is administered in an effective amount or dose to effect treatment of the disease, condition or disorder. Provided herein are uses of any of the provided fusosomes (e.g. retroviral vector or particle, such as lentiviral vector or particle) in such methods and treatments, and in the preparation of a medicament in order to carry out such therapeutic methods. In some embodiments, the methods are carried out by administering the fusosomes (e.g. retroviral vector or particle, such as lentiviral vector or particle), or compositions comprising the same, to the subject having, having had, or suspected of having the disease or condition or disorder. In some embodiments, the methods thereby treat the disease or condition or disorder in the subject. Also provided herein are use of any of the compositions, such as pharmaceutical compositions provided herein, for the treatment of a disease, condition or disorder associated with a particular gene or protein targeted by or provided by the exogenous agent.

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.

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 (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 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 inhibits membrane fusion. For example, Suppressyn is a human protein that inhibits cell-cell fusion (Sugimoto et al., "A novel human endogenous retroviral protein inhibits cell-cell fusion" Scientific Reports 3:1462 DOI: 10.1038/srep01462). Thus, in some embodiments, the fusosome is co-administered with an inhibitor of sypressyn, e.g., a siRNA or inhibitory antibody.

Compositions described herein may also be used to similarly modulate the cell or tissue function or physiology of a variety of other organisms including but not limited to: farm or working animals (horses, cows, pigs, chickens etc.), pet or zoo animals (cats, dogs, lizards, birds, lions, tigers and bears etc.), aquaculture animals (fish, crabs, shrimp, oysters etc.), plants species (trees, crops, ornamentals flowers etc), fermentation species (saccharomyces etc.). Fusosome compositions described herein can be made from such non-human sources and administered to a non-human target cell or tissue or subject.

Fusosome compositions can be autologous, allogeneic or xenogeneic to the target.

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 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, azathriopine, 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-TNFa), 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.

In some embodiments, the fusosome composition is used in combination with a gene therapy, e.g., a gene therapy that delivers a transgene of interest to a tissue of interest. In some embodiments, the tissue of interest is liver. In some embodiments, the tissue of interest is not liver. In some embodiments, the fusosome composition targets the same transgene of interest for expression in liver. Without wishing to be bound by theory, expressing the transgene of interest in hepatocytes may favor systemtic induction of regulatory T cells that are specific to the transgene, thereby achieving immune tolerance to the transgene.

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.

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 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

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 containing 5 μM EDTA and mixed immediately to prevent clotting. The tubes are kept on ice and red blood cells are removed using a buffered ammonium chloride (ACK) solution. Cells are stained with a murine 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 #: 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.

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

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.

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.

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.

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.

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 μM EDTA and mixed immediately to prevent clotting. The tubes are kept on ice and red blood cells are removed using a buffered ammonium chloride (ACK) solution. Cells are stained with a murine 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). 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.

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.

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.

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.

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.

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).

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), 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 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 ^{63}Eu-labelled 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 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 (1x 10^2 -1x 10^8) of retroviral vectors with 1% triton (sigma-aldrich) for an appropriate amount of time. Spontaneous release of europium by target retroviral vector is measured by incubation of labeled target retroviral vector without effector cells. Percentage leakage is then calculated as: (spontaneous release/maximum release) x100%. 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 analsysis 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 CSFE.

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. Profectin (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 CSFE.

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).

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.

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 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 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 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 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).

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 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 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.

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 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 development of anti-retroviral vector antibodies, e.g., antibodies that recognize retroviral vectors.

In an embodiment, antibodies that recognize retroviral vectors can bind in a manner that can limit retroviral vector activity or longevity and mediate complement degradation.

In this Example, serum inactivation is examined after one or more administrations of retroviral vectors. Retroviral vectors are produced by any one of the previous Examples. In this example, retroviral are created from: 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). In some embodiments, retroviral vectors are derived from cells that are expressing other immunoregulatory proteins.

Serum is drawn from different cohorts: mice injected systemically and/or locally with 1, 2, 3, 5, or 10 injections of vehicle (retroviral vector naïve group), HEK293-HLA-G retroviral vector, or 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 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 exposed to serum and incubated with target cells as described in Example 11 above.

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 mice treated with HEK293-HLA-G retroviral vectors, indicating that there is not serum inactivation of retroviral vectors or an adaptive immune response. In some embodiments, the percent of cells which receive the exogenous agent will not be different between retroviral vector samples that have been incubated from mice treated 1, 2, 3, 5 or 10 times with HEK293-HLA-G retroviral vectors, indicating that there is not serum inactivation of retroviral vectors or an adaptive immune response. 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 mice treated with vehicle and from mice treated with

HEK293-HLA-G retroviral vectors, indicating that there is not serum inactivation of retroviral vectors or an adaptive immune response. In some embodiments, the percent of cells which receive the exogenous agent will be less for retroviral vectors derived from HEK293 than for HEK293-HLA-G retroviral vectors indicating that there is not serum inactivation of HEK293-HLA-G retroviral vectors or an adaptive immune response.

Example 13: Measuring pre-existing IgG and IgM antibodies reactive against retroviral vectors

This Example describes quantification of pre-existing anti- retroviral vector antibody titers measured using flow cytometry.

In some embodiments, a measure of immunogenicity for a retroviral vector is antibody responses. Antibodies that recognize retroviral vector can bind in a manner that can limit retroviral vector activity or longevity. In an embodiment, some recipients of a retroviral vector described herein will have pre-existing antibodies which bind to and recognize retroviral vector.

In this Example, anti- retroviral vector antibody titers are tested using retroviral vector produced using a xenogeneic source cell. In this Example, a retroviral vector naïve mouse is assessed for the presence of anti- retroviral vector antibodies. Notably, the methods described herein may be equally applicable to humans, rats, monkeys with optimization to the protocol.

The negative control is mouse serum which has been depleted of IgM and IgG, and the positive control is serum derived from a mouse that has received multiple injections of retroviral vector generated from a xenogeneic source cell.

To assess the presence of pre-existing antibodies which bind to retroviral vector, sera from retroviral vector -naïve mice is first decomplemented by heating to 56°C for 30 min and subsequently diluted by 33% in PBS containing 3% FCS and 0.1% NaN3. Equal amounts of sera and retroviral vector ($1x10^2$ - $1x10^8$ retroviral vectors per mL) suspensions are incubated for 30 min at 4°C and washed with PBS through a calf-serum cushion.

IgM xenoreactive antibodies are stained by incubation of the retroviral vector with PE-conjugated goat antibodies specific for the Fc portion of mouse IgM (BD Bioscience) at 4°C for 45 min. Notably, anti-mouse IgG1 or IgG2 secondary antibodies may also be used. Retroviral vector from all groups are washed twice with PBS containing 2% FCS and then analyzed on a

FACS system (BD Biosciences). Fluorescence data are collected by use of logarithmic amplification and expressed as mean fluorescent intensity.

In an embodiment, the negative control serum will show negligible fluorescence comparable to the no serum or secondary alone controls. In an embodiment, the positive control will show more fluorescence than the negative control, and more than the no serum or secondary alone controls. In an embodiment, in cases where immunogenicity occurs, serum from retroviral vector -naïve mice will show more fluorescence than the negative control. In an embodiment, in cases where immunogenicity does not occur, serum from retroviral vector -naïve mice will show similar fluorescence compared to the negative control.

Example 14: Measuring IgG and IgM antibody responses after multiple administrations of retroviral vectors

This Example describes quantification of the humoral response of a modified retroviral vector following multiple administrations of the modified retroviral vector. 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) humoral response following multiple (e.g., more than one, e.g., 2 or more), administrations of the modified retroviral vector.

In some embodiments, a measure of immunogenicity for a retroviral vector is the antibody responses. In an embodiment, repeated injections of a retroviral vector can lead to the development of anti-retroviral vector antibodies, e.g., antibodies that recognize retroviral vector. In an embodiment, antibodies that recognize retroviral vector can bind in a manner that can limit retroviral vector activity or longevity.

In this Example, anti- retroviral vector antibody titers are examined after one or more administrations of retroviral vector. Retroviral vector is produced by any one of the previous Examples. In this example, retroviral are created from: cells that are not transfected with an immunomodulatory protein (NMCs), 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). In some embodiments, retroviral vectors are derived from cells that are expressing other immunoregulatory proteins.

Serum is drawn from different cohorts: mice injected systemically and/or locally with 1, 2, 3, 5, 10 injections of vehicle (retroviral vector naïve group), NMC retroviral vector, NMC-HLA-G retroviral vector, or NMC-empty vectors retroviral vector.

To assess the presence and abundance of anti- retroviral vector antibodies, sera from the mice is first decomplemented by heating to 56°C for 30 min and subsequently diluted by 33% in PBS with 3% FCS and 0.1% NaN3. Equal amounts of sera and retroviral vector ($1x10^2$ - $1x10^8$ retroviral vector per mL) are incubated for 30 min at 4°C and washed with PBS through a calf-serum cushion.

Retroviral vector reactive IgM antibodies are stained by incubation of the retroviral vector with PE-conjugated goat antibodies specific for the Fc portion of mouse IgM (BD Bioscience) at 4°C for 45 min. Notably, anti-mouse IgG1 or IgG2 secondary antibodies may also be used. Retroviral vector from all groups are washed twice with PBS containing 2% FCS and then analyzed on a FACS system (BD Biosciences). Fluorescence data are collected by use of logarithmic amplification and expressed as mean fluorescent intensity.

In an embodiment, NMC-HLA-G retroviral vectors will have decreased anti-viral IgM (or IgG1/2) antibody titers (as measured by fluorescence intensity on FACS) after injections, as compared to NMC retroviral vectors or NMC-empty retroviral vectors.

Example 15: Measuring IgG and IgM titers antibody responses to retroviral vector recipient cells

This Example describes quantification of antibody titers against recipient cells (cells that have fused with retroviral vectors) using flow cytometry. In some embodiments, a measure of the immunogenicity of recipient cells is the antibody response. Antibodies that recognize recipient cells can bind in a manner that can limit cell activity or longevity. In an embodiment, recipient cells will not be targeted by an antibody response, or an antibody response will be below a reference level.

In this Example, anti-recipient cell antibody titers in a subject (e.g., human, rat, or monkey) are tested. In addition, the protocol may be adapted to any cell type for which suitable surface markers exist. In this example, the target recipient cell is a CD3+ cell.

Mice are treated with retroviral vectors produced via any of the methods described in this application or with PBS (negative control) daily for 5 days. 28 days following the final treatment, peripheral blood is collected from mice that received retroviral vectors and mice that received PBS treatment. Blood is collected into 1ml PBS containing 5 μM EDTA and mixed immediately to prevent clotting. The tubes are kept on ice and red blood cells are removed using a buffered ammonium chloride (ACK) solution. Cells are stained with a murine CD3-FITC antibody (Thermo Fisher Catalog #:11-0032-82), at 4°C for 30 minutes in the dark, after being blocked with bovine serum albumin for 10 minutes. After being washed two times with PBS, cells are analyzed on a LSR II (BD Biosciences, San Jose, CA) with 488nm laser excitation and emission collected at 530+/-30nmrunning the FACSDiva™ software (BD Biosciences, San Jose, CA). CD3+ cells are sorted.

The sorted CD3+ cells are then stained with IgM antibodies by incubation of the reaction mixture with PE-conjugated goat antibodies specific for the Fc portion of mouse IgM (BD Bioscience) at 4°C for 45 min. Notably, anti-mouse IgG1 or IgG2 secondary antibodies may also be used. Cells from all groups are washed twice with PBS containing 2% FCS and then analyzed on a FACS system (BD Biosciences). Fluorescence data are collected by use of logarithmic amplification and expressed as mean fluorescent intensity. The mean fluorescence intensity is calculated for the sorted CD3 cells from mice treated with retroviral vectors and the mice treated with PBS.

A low mean fluorescence intensity is indicative of a low humoral response against the recipient cells. Mice treated with PBS are expected to have low mean fluorescence intensity. In an embodiment, the mean fluorescence intensity will be similar for recipient cells from mice treated with retroviral vectors and mice treated with PBS.

Example 16: Measuring phagocytic response to retroviral vector recipient cells

This Example describes quantification of macrophage response against recipient cells with a phagocytosis assay.

In some embodiments, a measure of the immunogenicity of recipient cells is the macrophage response. Macrophages engage in phagocytosis, engulfing cells and enabling the

sequestration and destruction of foreign invaders, like bacteria or dead cells. In some embodiments, phagocytosis of recipient cells by macrophages would reduce their activity.

In an embodiment, recipient cells are not targeted by macrophages. In this Example, the macrophage response against recipient cells in a subject is tested. In addition, the protocol may be adapted to any cell type for which suitable surface markers exist. In this example, the target recipient cell is a CD3+ cell.

Mice are treated with retroviral vectors produced via any of the methods described in this application or with PBS (negative control) daily for 5 days. 28 days following the final treatment, peripheral blood is collected from mice that received retroviral vectors and mice that received PBS treatment. Blood is collected into 1ml PBS containing 5 μM EDTA and mixed immediately to prevent clotting. The tubes are kept on ice and red blood cells are removed using a buffered ammonium chloride (ACK) solution.

Cells are stained with a murine CD3-FITC antibody (Thermo Fisher Catalog #:11-0032-82), at 4°C for 30 minutes in the dark, after being blocked with bovine serum albumin for 10 minutes. After being washed two times with PBS, cells are analyzed on a LSR II (BD Biosciences, San Jose, CA) with 488nm laser excitation and emission collected at 530+/-30nmrunning the FACSDiva™ software (BD Biosciences, San Jose, CA.). CD3+ cells are then sorted.

A phagocytosis assay is run to assess macrophage mediated immune clearance 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 sorted and FITC-stained CD3+ cells derived from mice that received retroviral vectors and PBS are added to the macrophages as indicated in the protocol, and are incubated for 2h, e.g., as described in the Vybrant™ Phagocytosis Assay Kit product information insert (Molecular Probes, revised 18-March-2001, found at tools.thermofisher.com/content/sfs/manuals/mp06694.pdf).

After 2h, the dish is gently washed and intracellular fluorescence is examined. To identify macrophages, cells are first incubated with Fc-receptor blocking antibody (eBioscence cat. no. 14-0161-86, clone 93) for 15 min on ice to block the binding of labeled mAbs to Fc receptors, which are abundantly expressed on macrophages. Following this step anti-F4/80-PE

(ThermoFisher cat. No. 12-4801-82, clone BM8) and anti-CD11b-PerCP-Cy5.5 (BD Biosciences cat. No. 550993, clone M1/70) conjugated antibodies are added to stain macrophage surface antigens. Cells are incubated for 30 min in the dark at 4C followed by centrifugation and washing in PBS. The cells are then resuspended in PBS. Flow cytometry of samples is then performed and macrophages are identified via positive fluorescence signal for F4/80-PE and CD11b-PerCP-Cy5.5 using 533nm and 647nm laser excitation, respectively. After gating for macrophages, intracellular fluorescence emitted by engulfed recipient cells is assessed by 488nm laser 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.

A low phagocytic index is indicative of low phagocytosis and targeting by macrophages. Mice treated with PBS are expected to have a low phagocytic index. In an embodiment, the phagocytic index will be similar for recipient cells derived from mice treated with retroviral vectors and mice treated with PBS.

Example 17: Measuring PBMC response to retroviral vector recipient cells

This Example describes quantification of a PBMC response against recipient cells with a cell lysis assay.

In some embodiments, a measure of the immunogenicity of recipient cells is the PBMC response. In an embodiment, cytotoxicity mediated cell lysis of recipient cells by PBMCs is a measure of immunogenicity, as lysis will reduce, e.g., inhibit or stop, the activity of a retroviral vector.

In an embodiment, recipient cells do not elicit a PBMC response. In this Example, the PBMC response against recipient cells in a subject is tested.

In addition, the protocol may be adapted to any cell type for which suitable surface markers exist. In this example, the target recipient cell is a CD3+ cell.

Mice are treated with retroviral vector produced via any of the methods described in this application or with PBS (negative control) daily for 5 days. 28 days following the final 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 μM EDTA and mixed immediately to prevent clotting. The tubes are kept on ice and red blood cells are removed using a buffered ammonium chloride (ACK) solution. Cells are stained with a murine CD3:APC-Cy7 antibody (Biolgend Catalog #: 100330) or an isotype control APC-Cy7 (IC:APC-Cy7) antibody (Biolgend Catalog #: 400230) at 4°C for 30 minutes in the dark, after being Fc blocked (Biolgend Catalog #: 101319) in cell staining buffer (Biolgend Catalog #: 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 labelled cells and then APC-Cy7 postive cells are sorted and collected. Sorted CD3+ cells are then labelled with either CellMask™ Green Plasma membrane Stain (CMG, ThermoFisher Catalog #: C37608) or DMSO as the negative control.

7 days prior to the isolation of CD3+ cells from the mice treated with retroviral vector or PBS, PBMCs are isolated from mice treated with retroviral vector or PBS according to the methods in Crop et al. Cell transplantation (20):1547-1559; 2011 and simulated in the presence of IL-2 recombinant mouse protein (R&D Systems Catalog #: 402-ML-020) and CD3/CD28 beads (ThermoFisher Catalog #: 11456D) in a round bottom 96 well plate for 7 days at 37C. At day 7, the stimulated PBMCs are co-incubated with CD3+/CMG+ or CD3+/DMSO control cells for 1, 2, 3, 4, 5, 6, 8, 10, 15, 20, 24, 48 hours at a plating ratio of PBMC:CD3+/CMG+ or PBMC: CD3+/DMSO control cells ranging from 1000:1-1:1 and 1:1.25-1:1000. As a negative control a set of wells would receive CD3+/CMG+ and CD3+/DMSO control cells only, no PBMCs. After incubation, the plates are centrifuged and processed so that they are labelled with either murine CD3:APC-Cy7 antibody or an IC:APC-Cy7 antibody as per above. After being washed two times with PBS, cells are re-suspended in PBS and analyzed on a FACS Aria (APC-Cy7: 640nm laser excitation/emission collected at 780 -/+ 60 nm and CMG 561 nm laser excitation/emission collected at 585 -/+ 16 nm) running the FACSDiva™ software (BD Biosciences, San Jose, CA). The FSC/SSC event data would then be used initially to set the gate for events labelled “cells”. This “cells” gate would be then used to display events to set the PMT voltage for the the 640nm and 561 nm laser analyzing samples labelled with IC:APC-Cy7/DMSO only. This sample would also be used to set the gates for negative cells for both

APC-Cy7 and CMG. The CD3+/CMG+ cells that did not receive any PBMCs would then used to set the positive gates for CD3+ and CMG+ cells.

The data is analyzed by looking at the percentage of CD3+/CMG+ cells in the population of total cells. When comparing treatment groups, a relatively lower percentage of CD3+/CMG+ cells at any given assay ratio of PBMC:CD3+/CMG+ cells is indicative of recipient cell lysis. In an embodiment, the percent of CD3+/CMG+ will be similar for recipient cells derived from mice treated with retroviral vector and mice treated with PBS.

Example 18: Measuring NK cell response to retroviral vector recipient cells

This Example describes quantification of a natural killer cell response against recipient cells with a cell lysis assay.

In some embodiments, a measure of the immunogenicity of recipient cells is the natural killer cell response. In an embodiment, cytotoxicity mediated cell lysis of recipient cells by natural killer cells is a measure of immunogenicity, as lysis will reduce, e.g., inhibit or stop, the activity of a retroviral vector.

In an embodiment, recipient cells do not elicit a natural killer cell response. In this Example, the natural killer response against recipient cells in a subject is tested. In addition, the protocol may be adapted to any cell type for which suitable surface markers exist. In this example, the target recipient cell is a CD3+ cell.

Mice are treated with retroviral vector, a blood sample is drawn, and CD3+ cells are sorted as described above in Example 17. NK cells are isolated, cultured with the CD3+ cells, and analyzed by FACS according to the protocol described above in Example 17 except that NK cells are used in place of the PBMC cells used in Example 17.

The data is analyzed by looking at the percentage of CD3+/CMG+ cells in the population of total cells. When comparing treatment groups, a relatively lower percentage of CD3+/CMG+ cells at any given assay ratio of NK cells:CD3+/CMG+ cells is indicative of recipient cell lysis. In an embodiment, the percent of CD3+/CMG+ will be similar for recipient cells derived from mice treated with retroviral vector and mice treated with PBS.

Example 19: Measuring CD8 T cell response to retroviral vector recipient cells

This Example describes quantification of a CD8+ T cell response against recipient cells (cells that have fused with retroviral vectors) with a cell lysis assay.

In some embodiments, a measure of the immunogenicity of recipient cells is the CD8+ T cell response. In an embodiment, cytotoxicity mediated cell lysis of recipient cells by CD8+ T cells is a measure of immunogenicity, as lysis will reduce, e.g., inhibit or stop, the activity of a retroviral vector.

In an embodiment, recipient cells do not elicit a CD8+ T cell response. In this Example, the CD8+ T cell response against recipient cells in a subject is tested. In addition, the protocol may be adapted to any cell type for which suitable surface markers exist. In this example, the target recipient cell is a CD3+ cell.

Mice are treated with retroviral vector, a blood sample is drawn, and CD3+ cells are sorted as described above in Example 17. CD8+ T cells are isolated, cultured with the CD3+ cells, and analyzed by FACS according to the protocol described above in Example 17 except that CD8+ T cells are used in place of the PBMC cells used in Example 17.

The data is analyzed by looking at the percentage of CD3+/CMG+ cells in the population of total cells. When comparing treatment groups, a relatively lower percentage of CD3+/CMG+ cells at any given assay ratio of CD8+ cells:CD3+/CMG+ cells is indicative of recipient cell lysis. In an embodiment, the percent of CD3+/CMG+ will be similar for recipient cells derived from mice treated with retroviral vectors and mice treated with PBS.

Example 20: Measuring Liver Specific Promoter Activity

This Example describes the measurement of the activity of a liver specific promoter (a positive TCSRE) in hepatocytes compared to non-target cells. In this example, the non-target cells are CD11c+ cells.

CD11c+ cells are collected from mice as described in Li et al., *Journal of Immunology* 2008, 2483-2493. Hepatocytes are derived from mice as described in Li et al., *Methods in Molecular Biology* 2010, 185-196.

The two cell types are cultured separately and treated with a retroviral vector produced as described herein. The retroviral vector is pseudotyped with a VSV-G and codes for

tdtomato fluorescent protein reporter under the control of a liver specific promoter, e.g., a liver specific promoter of Table 3.

Two days after transduction, gene expression in the cells is measured via flow cytometry and the average vector copy number in the cells is measured with quantitative PCR. The median tdtomato gene expression per cell in the cell population is normalized to the population vector copy number.

In some embodiments, the population of hepatocytes will have a greater ratio of tdtomato expression to vector copy number than the population of CD11c+ cells. This will demonstrate that the liver specific promoter is more active in liver cells.

Example 21: Measuring Change in Expression from Restrictive microRNA

This Example describes the measurement of the activity of a hematopoietic cell-restrictive microRNA (a NTCSRE) in hepatocytes compared to hematopoietic cells. In this example, the hematopoietic cells are CD11c+ cells.

CD11c+ cells are collected from mice as described in Li et al., *Journal of Immunology* 2008, 2483-2493. Hepatocytes are derived from mice as described in Li et al., *Methods in Molecular Biology* 2010, 185-196.

The two cell types are cultured separately and treated with a retroviral vector produced as described herein. The retroviral vector is pseudotyped with a VSV-G and codes for tdtomato fluorescent protein reporter under the control of a ubiquitously active promoter and a hematopoietic cell-restrictive microRNA, e.g., a microRNA of Table 4.

Two days after transduction, gene expression in the cells is measured via flow cytometry and the average vector copy number in the cells is measured with quantitative PCR. The median tdtomato gene expression per cell in the cell population is normalized to the population vector copy number.

In some embodiments, the population of hepatocytes will have a greater ratio of tdtomato expression to vector copy number than the population of CD11c+ cells. This will demonstrate that the hematopoietic cell restrictive microRNA decreases expression in CD11c+ cells.

Example 22: Treatment with tdTomato with VSV-G, hepatocyte specific promoter, and hematopoietic restrictive microRNA

This Example describes quantification of the expression of an exogenous agent in target and non-target recipient cells by exogenous agent expression in single cells.

In an embodiment, treated mice will have similar exogenous agent expression in non-target cells as those from untreated mice. In an embodiment, treated mice will have a similar percent of non-target cells that express the exogenous agent as those from untreated mice. In an embodiment, treated mice will have greater exogenous protein agent expression in target cells than those from untreated mice. In an embodiment, treated mice will have a greater percent of target cells that express the exogenous protein agent than those from untreated mice.

In this example, the non-target cell is a CD11c+ cell. In this example, the target cell is a hepatocyte. 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.

Mice are treated with retroviral vector pseudotyped with VSV-G and carrying a tdtomato fluorescent protein agent under the control of a hepatocyte-specific promoter (a positive TCSRE) and hematopoietic cell restrictive microRNA sequence (a NTCSRE). The retroviral vector is produced via any of the methods described herein. Negative control mice are treated with PBS.

28 days following treatment, mice are sacrificed and CD11c+ cells are collected from mice as described in Li et al., *Journal of Immunology* 2008, 2483-2493. Hepatocytes are derived from mice as described in Li et al., *Methods in Molecular Biology* 2010, 185-196. Tdtomato expression in the isolated cells is assayed via flow cytometry on a FACS Aria (BD Biosciences, San Jose, CA.) running the FACSDiva™ software (BD Biosciences, San Jose, CA). A negative gate for tdtomato expression is set with hepatocytes and CD11c+ cells isolated from mice treated with vehicle and with a 552 nm laser excitation and an emission collected at 585 -/+ 42 nm.

The percent of CD11c+ cells that are tdtomato positive is measured. In some embodiments, the percent of CD11c+ cells that are tdtomato positive will be 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 will be similar in cells from treated and untreated mice. The percent of hepatocytes that are tdtomato positive is measured. In some embodiments, the percent of hepatocytes that are tdtomato positive will be greater in cells from treated than untreated mice. The median tdtomato fluorescence level is measured in hepatocytes. In some embodiments, the median tdtomato fluorescence level in hepatocytes will be greater in cells from treated than untreated mice. In some embodiments, the percent of hepatocytes that are tdtomato positive will be greater than the percent of CD11c+ that are tdtomato positive in cells from treated mice. In some embodiments, the median tdtomato fluorescence level of hepatocytes will be greater than that of CD11c+ in cells from treated mice.

Example 23: Treatment for Ornithine Transcarbomylase Deficiency with VSV-G pseudotype *in vitro*

This example describes delivery of a therapeutic transgene to cells *in vitro*. In this example, the therapeutic transgene is *ornithine transcarbomylase* (*otc*).

Hepatocytes are derived from spf^{ash} mice. The hepatocytes are isolated as described in Li et al., *Methods in Molecular Biology* 2010, 185-196. The hepatocytes are transduced with a retroviral vector produced as described herein or with PBS. The retroviral vector is pseudotyped with VSV-G and carries the *otc* gene under the control of a hepatocyte-specific promoter (a positive TCSRE) and hematopoietic cell restrictive microRNA sequence (a negative TCSRE).

Following sufficient time for OTC expression, the cells are prepared for imaging. The cells are fixed, permeabilized, blocked, and immunostained with an anti-OTC antibody (for example, abcam catalog number ab91418). Following immunostaining, the cells are counterstained with a secondary antibody conjugated to Alexa Fluor 488 (for example, abcam catalog number ab150077). The cells are imaged on a Zeiss LSM 710 confocal microscope with a 63x oil immersion objective while maintained at 37C and 5% CO2. Alexa Fluor is subjected to 488nm laser excitation and emission captured at 510±15nm. The Alexa Flour average intensity

per cell is calculated to determine the level of OTC expression per cell. For each group at least 30-40 cells are imaged and analyzed.

In some embodiments, the level of OTC expression per cell will be higher in hepatocytes treated with the retroviral vector encoding the OTC gene than in hepatocytes treated with PBS.

Example 24: Treatment for Ornithine Transcarbomylase Deficiency with VSV-G pseudotyped retrovirus *in vivo*

This example describes delivery of a therapeutic transgene to cells *in vivo*. In this example, the therapeutic transgene is *ornithine transcarbomylase* (*otc*).

spf^{ash} mice are treated with retroviral vector pseudotyped with VSV-G and carrying the *otc* gene agent under the control of a hepatocyte-specific promoter (a positive TCSRE) and hematopoietic cell restrictive microRNA sequence (a NTCSRE). The retroviral vector is produced via any of the methods described in this application. Negative control mice are treated with PBS.

28 days following treatment, hepatocytes are obtained from mice treated with retrovirus or PBS and stained for OTC expression as described in previous examples. In some embodiments, the level of OTC expression per cell will be higher in hepatocytes derived from mice treated with the retroviral vector encoding the OTC gene than in hepatocytes treated with PBS.

In a separate group of mice, 28 days after treatment with retrovirus or PBS the mice are fed a 1-week course of a high-protein diet. Blood ammonia levels and urinary orotic acid are measured. In some embodiments, the level of both blood ammonia and/or urinary orotic acid will be lower in mice treated with retrovirus than mice treated with PBS. Mice are maintained on the high-protein diet for another 28 days. In some embodiments, more mice in the group treated with retroviral vector will survive the entire 28-day high-protein diet period than mice in the group treated with PBS. In some embodiments, mice in the group treated with retroviral vector will have a significantly longer survival time than mice in the group treated with PBS.

Example 25: Lack of transcriptional activity in fusosomes

This Example quantifies transcriptional activity in fusosomes compared to parent cells, e.g., source cells, used for fusosome generation. In an embodiment, transcriptional activity will be low or absent in fusosomes compared to the parent cells, e.g., source cells.

Fusosomes are a chassis for the delivery of therapeutic agent. Therapeutic agents, such as miRNA, mRNAs, proteins and/or organelles that can be delivered to cells or local tissue environments with high efficiency could be used to modulate pathways that are not normally active or active at pathological low or high levels in recipient tissue. In an embodiment, the observation that fusosomes are not capable of transcription, or that fusosomes have transcriptional activity of less than their parent cell, will demonstrate that removal of nuclear material has sufficiently occurred.

Fusosomes are prepared by any one of the methods described in previous Examples. A sufficient number of fusosomes and parent cells used to generate the fusosomes are then plated into a 6 well low-attachment multiwell plate in DMEM containing 20% Fetal Bovine Serum, 1x Penicillin/Streptomycin and the fluorescent-taggable alkyne–nucleoside EU for 1hr at 37°C and 5% CO2. For negative controls, a sufficient number of fusosomes and parent cells are also plated in multiwell plate in DMEM containing 20% Fetal Bovine Serum, 1x Penicillin/Streptomycin but with no alkyne–nucleoside EU.

After the 1 hour incubation the samples are processed following the manufacturer's instructions for an imaging kit (ThermoFisher Scientific). The cell and fusosome samples including the negative controls are washed thrice with 1xPBS buffer and resuspended in 1xPBS buffer and analyzed by flow cytometry (Becton Dickinson, San Jose, CA, USA) using a 488nm argon laser for excitation, and the 530+/-30nm emission. BD FACSDiva software was used for acquisition and analysis. The light scatter channels are set on linear gains, and the fluorescence channels on a logarithmic scale, with a minimum of 10,000 cells analyzed in each condition.

In an embodiment, transcriptional activity as measured by 530+/-30nm emission in the negative controls will be null due to the omission of the alkyne–nucleoside EU. In some embodiments, the fusosomes will have less than about 70%, 60%, 50%, 40%, 30%, 20%, 10%, 5%, 4%, 3%, 2%, 1% or less transcriptional activity than the parental cells.

See also, Proc Natl Acad Sci U S A, 2008, Oct 14;105(41):15779-84. doi: 10.1073/pnas.0808480105. Epub 2008 Oct 7.

Example 26: Lack of DNA replication or replication activity

This Example quantifies DNA replication in fusosomes. In an embodiment, fusosomes will replicate DNA at a low rate compared to cells.

Fusosomes are prepared by any one of the methods described in previous Examples. Fusosome and parental cell DNA replication activity is assessed by incorporation of a fluorescent-taggable nucleotide (ThermoFisher Scientific # C10632). Fusosomes and an equivalent number of cells are incubated with EdU at a final concentration of 10 μM for 2hr, after preparation of an EdU stock solution with in dimethylsulfoxide. The samples are then fixed for 15 min using 3.7% PFA, washed with 1xPBS buffer, pH 7.4 and permeabilized for 15 min in 0.5% detergent solution in 1xPBS buffer, pH 7.4.

After permeabilization, fusosomes and cells in suspension in PBS buffer containing 0.5% detergent are washed with 1xPBS buffer, pH 7.4 and incubated for 30 min at 21°C in reaction cocktail, 1xPBS buffer, CuSO4 (Component F), azide-fluor 488, 1x reaction buffer additive.

A negative control for fusosome and cell DNA replication activity is made with samples treated the same as above but with no azide-fluor 488 in the 1x reaction cocktail.

The cell and fusosome samples are then washed and resuspended in 1xPBS buffer and analyzed by flow cytometry. Flow cytometry is done with a FACS cytometer (Becton Dickinson, San Jose, CA, USA) with 488nm argon laser excitation, and a 530+/-30nm emission spectrum is collected. FACS analysis software is used for acquisition and analysis. The light scatter channels are set on linear gains, and the fluorescence channels on a logarithmic scale, with a minimum of 10,000 cells analyzed in each condition. The relative DNA replication activity is calculated based on the median intensity of azide-fluor 488 in each sample. All events are captured in the forward and side scatter channels (alternatively, a gate can be applied to select only the fusosome population). The normalized fluorescence intensity value for the fusosomes is determined by subtracting from the median fluorescence intensity value of the fusosome the median fluorescence intensity value of the respective negative control sample. Then the normalized

relative DNA replication activity for the fusosomes samples is normalized to the respective nucleated cell samples in order to generate quantitative measurements for DNA replication activity.

In an embodiment, fusosomes have less DNA replication activity than parental cells. See, also, Salic, 2415–2420, doi: 10.1073/pnas.0712168105.

Example 27: Quantification of fusogens

This example describes quantification of the absolute number of fusogens per fusosome.

A fusosome composition is produced by any one of the methods described in the previous Examples, except the fusosome is engineered as described in a previous Example to express a fusogen (VSV-G) tagged with GFP. In addition, a negative control fusosome is engineered with no fusogen (VSV-G) or GFP present.

The fusosomes with the GFP-tagged fusogen and the negative control(s) are then assayed for the absolute number of fusogens as follows. Commercially acquired recombinant GFP is serially diluted to generate a calibration curve of protein concentration. The GFP fluorescence of the calibration curve and a sample of fusosomes of known quantity is then measured in a fluorimeter using a GFP light cube (469/35 excitation filter and a 525/39 emission filter) to calculate the average molar concentration of GFP molecules in the fusosome preparation. The molar concentration is then converted to the number of GFP molecules and divided by the number of fusosomes per sample to achieve an average number of GFP-tagged fusogen molecules per fusosome and thus provides a relative estimate of the number of fusogens per fusosome.

In an embodiment, GFP fluorescence will be higher in the fusosomes with GFP tag as compared to the negative controls, where no fusogen or GFP is present. In an embodiment, GFP fluorescence is relative to the number of fusogen molecules present.

Alternatively, individual fusosomes are isolated using a single cell prep system (Fluidigm) per manufacturer's instructions, and qRT-PCR is performed using a commercially available probeset (Taqman) and master mix designed to quantify fusogen or GFP cDNA levels based upon the C_t value. A RNA standard of the same sequence as the cloned fragment of the fusogen gene or the GFP gene is generated by synthesis (Amsbio) and then added to single cell

prep system qRT-PCR experimental reaction in serial dilutions to establish a standard curve of C_t vs concentration of fusogen or GFP RNA.

The C_t value from fusosomes is compared to the standard curve to determine the amount of fusogen or GFP RNA per fusosome.

In an embodiment, fusogen and GFP RNA will be higher in the fusosomes with engineered to express the fusogens as compared to the negative controls, where no fusogen or GFP is present.

Fusogens may further be quantified in the lipid bilayer by analyzing the lipid bilayer structure as previously described and quantifying fusogens in the lipid bilayer by LC-MS as described in other Examples herein.

Example 28: Measuring the average size of fusosomes

This Example describes measurement of the average size of fusosomes.

Fusosomes are prepared by any one of the methods described in previous Examples. The fusosomes measured to determine the average size using commercially available systems (iZON Science). The system is used with software according to manufacturer's instructions and a nanopore designed to analyze particles within the 40 nm to 10 µm size range. Fusosomes and parental cells are resuspended in phosphate-buffered saline (PBS) to a final concentration range of 0.01-0.1 µg protein/mL. Other instrument settings are adjusted as indicated in the following table:

Table 6: Fusosome measurement parameters and settings

Measurement Parameter	Setting
Pressure	6
Nanopore type	NP300
Calibration sample	CPC400_6P
Gold standard analysis	no
Capture assistant	none

All fusosomes are analyzed within 2 hours of isolation. In an embodiment, the fusosomes will have a size within about 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or greater than the parental cells.

Example 29: Measuring the average size distribution of fusosomes

This Example describes measurement of the size distribution of fusosomes.

Fusosomes are generated by any one of the methods described in previous Examples, and are tested to determine the average size of particles using a commercially available system, such as described in a previous Example. In an embodiment, size thresholds for 10%, 50%, and 90% of the fusosomes centered around the median are compared to parental cells to assess fusosome size distribution.

In an embodiment, the fusosomes will have less than about 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, 5%, or less of the parental cell's variability in size distribution within 10%, 50%, or 90% of the sample.

Example 30: Average volume of fusosomes

This example describes measurement of the average volume of fusosomes. Without wishing to be bound by theory, varying the size (e.g., volume) of fusosomes can make them versatile for distinct cargo loading, therapeutic design or application.

Fusosomes are prepared as described in previous Examples. The positive control is HEK293 cells or polystyrene beads with a known size. The negative control is HEK293 cells that are passed through a 36 gauge needle approximately 50 times.

Analysis with a transmission electron microscope, as described in a previous Example, is used to determine the size of the fusosomes. The diameter of the fusosome is measured and volume is then calculated.

In an embodiment, fusosomes will have an average size of approximately 50nm or greater in diameter.

Example 31: Average density of fusosomes

Fusosome density is measured via a continuous sucrose gradient centrifugation assay as described in Théry et al., Curr Protoc Cell Biol. 2006 Apr; Chapter 3:Unit 3.22. Fusosomes are obtained as described in previous Examples.

First, a sucrose gradient is prepared. A 2 M and a 0.25 sucrose solution are generated by mixing 4 ml HEPES/sucrose stock solution and 1 ml HEPES stock solution or 0.5 ml HEPES/sucrose stock solution and 4.5 ml HEPES stock solution, respectively. These two fractions are loaded into the gradient maker with all shutters closed, the 2 M sucrose solution in the proximal compartment with a magnetic stir bar, and the 0.25 M sucrose solution in the distal compartment. The gradient maker is placed on a magnetic stir plate, the shutter between proximal and distal compartments is opened and the magnetic stir plate is turned on. HEPES stock solution is made as follows: 2.4 g N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES; 20mMfinal), 300 H2O, adjust pH to 7.4 with 10 N NaOH and finally adjust volume to 500 ml with H2O. HEPES/sucrose stock solution is made as follows: 2.4 g hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES; 20 mM final), 428 g protease-free sucrose (ICN; 2.5 M final), 150 ml H2O, adjust pH to 7.4 with 10 N NaOH and finally adjust volume to 500 ml with H2O.

The fusosomes are resuspended in 2 ml of HEPES/sucrose stock solution and are poured on the bottom of an SW 41 centrifuge tube. The outer tubing is placed in the SW 41 tube, just above the 2 ml of fusosomes. The outer shutter is opened, and a continuous 2 M (bottom) to 0.25 M (top) sucrose gradient is slowly poured on top of the fusosomes. The SW 41 tube is lowered as the gradient is poured, so that the tubing is always slightly above the top of the liquid.

All tubes with gradients are balanced with each other, or with other tubes having the same weight of sucrose solutions. The gradients are centrifuged overnight (≥14 hr) at 210,000 x g, 4°C, in the SW 41 swinging-bucket rotor with the brake set on low.

With a micropipettor, eleven 1-ml fractions, from top to bottom, are collected and placed in a 3-ml tube for the TLA-100.3 rotor. The samples are set aside and, in separate wells of a 96-well plate, 50 µl of each fraction is used to measure the refractive index. The plate is covered with adhesive foil to prevent evaporation and stored for no more than 1 hour at room temperature. A

refractometer is used to measure the refractive index (hence the sucrose concentration, and the density) of 10 to 20 µl of each fraction from the material saved in the 96-well plate.

A table for converting the refractive index into g/ml is available in the ultracentrifugation catalog downloadable from the Beckman website.

Each fraction is then prepared for protein content analysis. Two milliliters of 20 mM HEPES, pH 7.4, is added to each 1-ml gradient fraction, and mixed by pipetting up and down two to three times. One side of each tube is marked with a permanent marker, and the tubes are placed marked side up in a TLA-100.3 rotor.

The 3 ml-tubes with diluted fractions are centrifuged for 1 hr at 110,000 x g, 4◦C. The TLA-100.3 rotor holds six tubes, so two centrifugations for each gradient is performed with the other tubes kept at 4°C until they can be centrifuged.

The supernatant is aspirated from each of the 3-ml tubes, leaving a drop on top of the pellet. The pellet most probably is not visible, but its location can be inferred from the mark on the tube. The invisible pellet is resuspended and transferred to microcentrifuge tubes. Half of each resuspended fraction is used for protein contentment analysis by bicinchoninic acid assay, described in another Example. This provides a distribution across the various gradient fractions of the fusosome preparation. This distribution is used to determine the average density of the fusosomes. The second half volume fraction is stored at -80°C and used for other purposes (e.g. functional analysis, or further purification by immunoisolation) once protein analysis has revealed the fusosome distribution across fractions.

In an embodiment, using this assay, the average density of the fusosomes will be 1.25 g/ml +/- 0.05 standard deviation. In an embodiment, the average density of the fusosomes will be in the range of 1-1.1, 1.05-1.15, 1.1-1.2, 1.15-1.25, 1.2-1.3, or 1.25-1.35. In an embodiment, the average density of the fusosomes will be less than 1 or more than 1.35.

Example 32: Measuring nuclear envelope content

This Example describes a measurement of the nuclear envelope content in enucleated fusosomes. The nuclear envelope isolates DNA from the cytoplasm of the cell.

In an embodiment, a purified fusosome composition comprises a mammalian cell, such as HEK-293Ts (293 [HEK-293] (ATCC® CRL-1573™), that has been enucleated as described

herein. This Example describes the quantification of different nuclear membrane proteins as a proxy to measure the amount of intact nuclear membrane that remains after fusosome generation.

In this Example, $10x10^6$ HEK-293Ts and the equivalent amount of fusosomes prepared from $10x10^6$ HEK-293Ts are fixed for 15 min using 3.7% PFA, washed with 1xPBS buffer, pH 7.4 and permeabilized simultaneously, and then blocked for 15 min using 1xPBS buffer containing 1% Bovine Serum Albumin and 0.5% Triton® X-100, pH 7.4. After permeabilization, fusosomes and cells are incubated for 12 hours at 4°C with different primary antibodies, e.g. (anti-RanGAP1 antibody [EPR3295] (Abcam - ab92360), anti-NUP98 antibody [EPR6678] - nuclear pore marker (Abcam - ab124980), anti-nuclear pore complex proteins antibody [Mab414] - (Abcam- ab24609), anti-importin 7 antibody (Abcam - ab213670), at manufacturer suggested concentrations diluted in 1xPBS buffer containing 1% bovine serum albumin and 0.5% Triton® X-100, pH 7.4. Fusosomes and cells are then washed with 1xPBS buffer, pH 7.4, and incubated for 2hr at 21°C with an appropriate fluorescent secondary antibody that detects the previous specified primary antibody at manufacturer suggested concentrations diluted in 1xPBS buffer containing 1% bovine serum albumin and 0.5% detergent, pH 7.4. Fusosomes and cells are then washed with 1xPBS buffer, re-suspended in 300µL of 1xPBS buffer, pH 7.4 containing 1 µg/ml Hoechst 33342, filtered through a 20 µm FACS tube and analyzed by flow cytometry.

Negative controls are generated using the same staining procedure but with no primary antibody added. Flow cytometry is performed on a FACS cytometer (Becton Dickinson, San Jose, CA, USA) with 488nm argon laser excitation, and a 530+/-30nm emission spectrum is collected. FACS acquisition software is used for acquisition and analysis. The light scatter channels are set on linear gains, and the fluorescence channels on a logarithmic scale, with a minimum of 10,000 cells analyzed in each condition. The relative intact nuclear membrane content is calculated based on the median intensity of fluorescence in each sample. All events are captured in the forward and side scatter channels.

The normalized fluorescence intensity value for the fusosomes is determined by subtracting from the median fluorescence intensity value of the fusosome the median fluorescence intensity value of the respective negative control sample. Then the normalized

fluorescence for the fusosomes samples is normalized to the respective nucleated cell samples in order to generate quantitative measurements of intact nuclear membrane content.

In an embodiment, enucleated fusosomes will comprise less than 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% fluorescence intensity or nuclear envelope content compared to the nucleated parental cells.

Example 33: Measuring chromatin levels

This Example describes measurement of chromatin in enucleated fusosomes.

DNA can be condensed into chromatin to allow it to fit inside the nucleus. In an embodiment, a purified fusosome composition as produced by any one of the methods described herein will comprise low levels of chromatin.

Enucleated fusosomes prepared by any of the methods previously described and positive control cells (e.g., parental cells) are assayed for chromatin content using an ELISA with antibodies that are specific to histone protein H3 or histone protein H4. Histones are the chief protein component of chromatin, with H3 and H4 the predominant histone proteins.

Histones are extracted from the fusosome preparation and cell preparation using a commercial kit (e.g. Abcam Histone Extraction Kit (ab113476)) or other methods known in the art. These aliquots are stored at -80 C until use. A serial dilution of standard is prepared by diluting purified histone protein (either H3 or H4) from 1 to 50 ng/µl in a solution of the assay buffer. The assay buffer may be derived from a kit supplied by a manufacturer (e.g. Abcam Histone H4 Total Quantification Kit (ab156909) or Abcam Histone H3 total Quantification Kit (ab115091)). The assay buffer is added to each well of a 48- or 96-well plate, which is coated with an anti-histone H3 or anti-H4 antibody and sample or standard control is added to the well to bring the total volume of each well to 50 µl. The plate is then covered and incubated at 37 degrees for 90 to 120 minutes.

After incubation, any histone bound to the anti-histone antibody attached to the plate is prepared for detection. The supernatant is aspirated and the plate is washed with 150 µl of wash buffer. The capture buffer, which includes an anti-histone H3 or anti-H4 capture antibody, is then added to the plate in a volume of 50 µl and at a concentration of 1 µg/mL. The plate is then incubated at room temperature on an orbital shaker for 60 minutes.

Next, the plate is aspirated and washed 6 times using wash buffer. Signal reporter molecule activatable by the capture antibody is then added to each well. The plate is covered and incubated at room temperature for 30 minutes. The plate is then aspirated and washed 4 times using wash buffer. The reaction is stopped by adding stop solution. The absorbance of each well in the plate is read at 450 nm, and the concentration of histones in each sample is calculated according to the standard curve of absorbance at 450 nm vs. concentration of histone in standard samples.

In an embodiment, fusosome samples will comprise less than 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% the histone concentration of the nucleated parental cells.

Example 34: Measuring miRNA content in fusosomes

This example describes quantification of microRNAs (miRNAs) in fusosomes. In an embodiment, a fusosome comprises miRNAs.

MiRNAs are regulatory elements that, among other activities, control the rate by which messenger RNAs (mRNAs) are translated into proteins. In an embodiment, fusosomes carrying miRNA may be used to deliver the miRNA to target sites.

Fusosomes are prepared by any one of the methods described in previous Examples. RNA from fusosomes or parental cells is prepared as described previously. At least one miRNA gene is selected from the Sanger Center miRNA Registry at www.sanger.ac.uk/Software/Rfam/mirna/index.shtml. miRNA is prepared as described in Chen *et al*, *Nucleic Acids Research*, 33(20), 2005. All TaqMan miRNA assays are available through Thermo Fisher (A25576, Waltham, MA).

qPCR is carried out according to manufacturer's specifications on miRNA cDNA, and C_T values are generated and analyzed using a real-time PCR system as described herein.

In an embodiment, the miRNA content of fusosomes will be at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or greater than that of their parental cells.

Example 35: Quantifying expression of an endogenous RNA or synthetic RNA in fusosomes

This example describes quantification of levels of endogenous RNA with altered expression, or a synthetic RNA that is expressed in a fusosome.

The fusosome or parental cell is engineered to alter the expression of an endogenous or synthetic RNA that mediates a cellular function to the fusosomes.

Transposase vectors (System Biosciences, Inc.) includes the open reading frame of the Puromycin resistance gene together with an open reading frame of a cloned fragment of a protein agent. The vectors are electroporated into 293Ts using an electroporator (Amaxa) and a 293T cell line specific nuclear transfection kit (Lonza).

Following selection with puromycin for 3-5 days in DMEM containing 20% Fetal Bovine Serum and 1x Penicillin/Streptomycin, fusosomes are prepared from the stably expressing cell line by any one of the methods described in previous Examples.

Individual fusosomes are isolated and protein agent or RNA per fusosome is quantified as described in a previous Example.

In an embodiment, the fusosomes will have at least 1, 2, 3, 4, 5, 10, 20, 50, 100, 500, 10^3, 5.0 x 10^3, 10^4, 5.0 x 10^4, 10^5, 5.0 x 10^5, 10^6, 5.0 x 10^6, or more of the RNA per fusosome.

Example 36: Measuring proteomic composition in fusosomes

This Example describes quantification of the protein composition of fusosomes. In an embodiment, the protein composition of fusosomes will be similar to the cells that they are derived from.

Fusosomes are prepared by any one of the methods described in previous Examples. Fusosomes are resuspended in lysis buffer (7M Urea, 2M Thiourea, 4% (w/v) Chaps in 50 mM Tris pH 8.0) and incubated for 15 minutes at room temperature with occasional vortexing. Mixtures are then lysed by sonication for 5 minutes in an ice bath and spun down for 5 minutes at 13,000 RPM. Protein content is determined by a colorimetric assay (Pierce) and protein of each sample is transferred to a new tube and the volume is equalized with 50 mM Tris pH 8.

Proteins are reduced for 15 minutes at 65 Celsius with 10 mM DTT and alkylated with 15 mM iodoacetamide for 30 minutes at room temperature in the dark. Proteins are precipitated with gradual addition of 6 volumes of cold (-20 Celsius) acetone and incubated overnight at -80

Celsius. Protein pellets are washed 3 times with cold (-20 Celsius) methanol. Proteins are resuspended in 50 mM Tris pH 8.3.

Next, trypsin/lysC is added to the proteins for the first 4h of digestion at 37 Celsius with agitation. Samples are diluted with 50mM Tris pH 8 and 0.1% sodium deoxycholate is added with more trypsin/lysC for digestion overnight at 37 Celsius with agitation. Digestion is stopped and sodium deoxycholate is removed by the addition of 2% v/v formic acid. Samples are vortexed and cleared by centrifugation for 1 minute at 13,000 RPM. Peptides are purified by reversed phase solid phase extraction (SPE) and dried down. Samples are reconstituted in 20 µl of 3% DMSO, 0.2% formic acid in water and analyzed by LC-MS.

To have quantitative measurements, a protein standard is also run on the instrument. Standard peptides (Pierce, equimolar, LC-MS grade, #88342) are diluted to 4, 8, 20, 40 and 100 fmol/ul and are analyzed by LC-MS/MS. The average AUC (area under the curve) of the 5 best peptides per protein (3 MS/MS transition/peptide) is calculated for each concentration to generate a standard curve.

Acquisition is performed with a high resolution mass spectrometer (ABSciex, Foster City, CA, USA) equipped with an electrospray interface with a 25 µm iD capillary and coupled with micro-ultrahigh performance liquid chromatography (µUHPLC) (Eksigent, Redwood City, CA, USA). Analysis software is used to control the instrument and for data processing and acquisition. The source voltage is set to 5.2 kV and maintained at 225°C, curtain gas is set at 27 psi, gas one at 12 psi and gas two at 10 psi. Acquisition is performed in Information Dependent Acquisition (IDA) mode for the protein database and in SWATH acquisition mode for the samples. Separation is performed on a reversed phase column 0.3 µm i.d., 2.7 µm particles, 150mm long (Advance Materials Technology, Wilmington, DE) which is maintained at 60°C. Samples are injected by loop overfilling into a 5µL loop. For the 120 minute (samples) LC gradient, the mobile phase includes the following: solvent A (0.2% v/v formic acid and 3% DMSO v/v in water) and solvent B (0.2% v/v formic acid and 3% DMSO in EtOH) at a flow rate of 3 µL/min.

For the absolute quantification of the proteins, a standard curve (5 points, R2>0.99) is generated using the sum of the AUC of the 5 best peptides (3 MS/MS ion per peptide) per protein. To generate a database for the analysis of the samples, the DIAUmpire algorithm is run

on each of the 12 samples and combined with the output MGF files into one database. This database is used with software (ABSciex) to quantify the proteins in each of the samples, using 5 transition/peptide and 5 peptide/protein maximum. A peptide is considered as adequately measured if the score computed is superior to 1.5 or had a FDR < 1%. The sum of the AUC of each of the adequately measured peptides is mapped on the standard curve, and is reported as fmol.

The resulting protein quantification data is then analyzed to determine protein levels and proportions of known classes of proteins as follows: enzymes are identified as proteins that are annotated with an Enzyme Commission (EC) number; ER associated proteins are identified as proteins that had a Gene Ontology (GO; http://www.geneontology.org) cellular compartment classification of ER and not mitochondria; exosome associated proteins are identified as proteins that have a Gene Ontology cellular compartment classification of exosomes and not mitochondria; and mitochondrial proteins are identified as proteins that are identified as mitochondrial in the MitoCarta database (Calvo et al., NAR 2015l doi:10.1093/nar/gkv1003). The molar ratios of each of these categories are determined as the sum of the molar quantities of all the proteins in each class divided by the sum of the molar quantities of all identified proteins in each sample.

Fusosome proteomic composition is compared to parental cell proteomic composition. In an embodiment, a similar proteomic compositions between fusosomes and parental cells will be observed when >50% of the identified proteins are present in the fusosome, and of those identified proteins the level is >25% of the corresponding protein level in the parental cell.

Example 37: Measuring GAPDH in fusosomes

This assay describes quantification of the level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) in the fusosomes, and the relative level of GAPDH in the fusosomes compared to the parental cells.

GAPDH is measured in the parental cells and the fusosomes using a standard commercially available ELISA for GAPDH (ab176642, Abcam) per the manufacturer's directions.

Total protein levels are similarly measured via bicinchoninic acid assay as previously described in the same volume of sample used to measure GAPDH. In embodiments, using this assay, the level of GAPDH per total protein in the fusosomes will be <100ng GAPDH / µg total protein. Similarly, in embodiments, the decrease in GAPDH levels relative to total protein from the parental cells to the fusosomes will be greater than a 10% decrease.

In an embodiment, GAPDH content in the preparation in ng GAPDH / µg total protein will be less than 500, less than 250, less than 100, less than 50, less than 20, less than 10, less than 5, or less than1.

In an embodiment, the decrease in GAPDH per total protein in ng/µg from the parent cell to the preparation will be more than 1%, more than 2.5%, more than 5%, more than 10%, more than 15%, more than 20%, more than 30%, more than 40%, more than 50%, more than 60%, more than 70%, more than 80%, or more than 90%.

Example 38: Measuring calnexin in fusosomes

This assay describes quantification of the level of calnexin (CNX) in the fusosomes, and the relative level of CNX in the fusosomes compared to the parental cells.

Calnexin is measured in the starting cells and the preparation using a standard commercially available ELISA for calnexin (MBS721668, MyBioSource) per the manufacturer's directions.

Total protein levels are similarly measured via bicinchoninic acid assay as previously described in the same volume of sample used to measure calnexin. In embodiments, using this assay, the level of calnexin per total protein in the fusosomes will be <100ng calnexin / µg total protein. Similarly, in embodiments, the increase in calnexin levels relative to total protein from the parental cell to the fusosomes will be greater than a 10% increase.

In an embodiment, calnexin content in the preparation in ng calnexin / µg total protein will be less than 500, 250, 100, 50, 20, 10, 5, or 1.

In an embodiment, the decrease in calnexin per total protein in ng/µg from the parent cell to the preparation will be more than 1%, 2.5%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%.

Example 39: Comparison of soluble to insoluble protein mass

This Example describes quantification of the soluble:insoluble ratio of protein mass in fusosomes. In an embodiment, the soluble:insoluble ratio of protein mass in fusosomes will be similar to nucleated cells.

Fusosomes are prepared by any one of the methods described in previous Examples. The fusosome preparation is tested to determine the soluble : insoluble protein ratio using a standard bicinchoninic acid assay (BCA) (e.g. using the commercially available Pierce™ BCA Protein Assay Kit, Thermo Fischer product# 23225). Soluble protein samples are prepared by suspending the prepared fusosomes or parental cells at a concentration of 1x10^7 cells or fusosomes/mL in PBS and centrifuging at 1600g to pellet the fusosomes or cells. The supernatant is collected as the soluble protein fraction.

The fusosomes or cells in the pellet are lysed by vigorous pipetting and vortexing in PBS with 2% Triton-X-100. The lysed fraction represents the insoluble protein fraction.

A standard curve is generated using the supplied BSA, from 0 to 20 µg of BSA per well (in triplicate). The fusosome or cell preparation is diluted such that the quantity measured is within the range of the standards. The fusosome preparation is analyzed in triplicate and the mean value is used. The soluble protein concentration is divided by the insoluble protein concentration to yield the soluble:insoluble protein ratio.

In an embodiment, the fusosome soluble:insoluble protein ratio will be within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or greater compared to the parental cells.

Example 40: Measuring LPS in fusosomes

This example describes quantification of levels of lipopolysaccharides (LPS) in fusosomes as compared to parental cells. In an embodiment, fusosomes will have lower levels of LPS compared to parental cells.

LPS are a component of bacterial membranes and potent inducer of innate immune responses.

The LPS measurements are based on mass spectrometry as described in the previous Examples.

In an embodiment, less than 5%, 1%, 0.5%, 0.01%, 0.005%, 0.0001%, 0.00001% or less of the lipid content of fusosomes will be LPS.

Example 41: Ratio of lipids to proteins in fusosomes

This Example describes quantification of the ratio of lipid mass to protein mass in fusosomes. In an embodiment, fusosomes will have a ratio of lipid mass to protein mass that is similar to nucleated cells.

Total lipid content is calculated as the sum of the molar content of all lipids identified in the lipidomics data set outlined in a previous Example. Total protein content of the fusosomes is measured via bicinchoninic acid assay as described herein.

Alternatively, the ratio of lipids to proteins can be described as a ratio of a particular lipid species to a specific protein. The particular lipid species is selected from the lipidomics data produced in a previous Example. The specific protein is selected from the proteomics data produced in a previous Example. Different combinations of selected lipid species and proteins are used to define specific lipid:protein ratios.

Example 42: Ratio of proteins to DNA in fusosomes

This Example describes quantification of the ratio of protein mass to DNA mass in fusosomes. In an embodiment, fusosomes will have a ratio of protein mass to DNA mass that is much greater than cells.

Total protein content of the fusosomes and cells is measured as described in in a previous Example. The DNA mass of fusosomes and cells is measured as described in a previous Example. The ratio of proteins to total nucleic acids is then determined by dividing the total protein content by the total DNA content to yield a ratio within a given range for a typical fusosome preparation.

Alternatively, the ratio of proteins to nucleic acids is determined by defining nucleic acid levels as the level of a specific house-keeping gene, such as GAPDH, using semi-quantitative real-time PCR (RT-PCR).

The ratio of proteins to GAPDH nucleic acids is then determined by dividing the total protein content by the total GAPDH DNA content to define a specific range of protein:nucleic acid ratio for a typical fusosome preparation.

Example 43: Measuring fusion with a target cell

This example describes quantification of fusosome fusion with a target cell compared to a non-target cell.

In an embodiment, fusosome fusion with a target cell allows the cell-specific delivery of a cargo, carried within the lumen of the fusosome, to the cytosol of the recipient cell. Fusosomes produced by the herein described methods are assayed for fusion rate with a target cell as follows.

In this example, the fusosome comprises a HEK293T cell expressing Myomaker on its plasma membrane. In addition, the fusosome expresses mTagBFP2 fluorescent protein and Cre recombinase. The target cell is a myoblast cell, which expresses both Myomaker and Myomixer, and the non-target cell is a fibroblast cell, which expresses neither Myomaker nor Myomixer. A Myomaker-expressing fusosome is predicted to fuse with the target cell that expresses both Myomaker and Myomixer but not the non-target cell (Quinn et al., 2017, Nature Communications, 8, 15665. doi.org/10.1038/ncomms15665) (Millay et al., 2013, Nature, 499(7458), 301–305. doi.org/10.1038/nature12343). Both the target and non-target cell types are isolated from mice and stably-express "LoxP-stop-Loxp-tdTomato" cassette under a CMV promoter, which upon recombination by Cre turns on tdTomato expression, indicating fusion.

The target or non-target recipient cells are plated into a black, clear-bottom 96-well plate. Both target and non-target cells are plated for the different fusion groups. Next, 24 hours after plating the recipient cells, the fusosomes expressing Cre recombinase protein and Myomaker are applied to the target or non-target recipient cells in DMEM media. The dose of fusosomes is correlated to the number of recipient cells plated in the well. After applying the fusosomes, the cell plate is centrifuged at 400g for 5 minutes to help initiate contact between the fusosomes and the recipient cells.

Starting at four hours after fusosome application, the cell wells are imaged to positively identify RFP-positive cells versus GFP-positive cells in the field or well.

In this example, cell plates are imaged using an automated microscope (www.biotek.com/products/imaging-microscopy-automated-cell-imagers/lionheart-fx-automated-live-cell-imager/). The total cell population in a given well is determined by first staining the cells with Hoechst 33342 in DMEM media for 10 minutes. Hoechst 33342 stains cell nuclei by intercalating into DNA and therefore is used to identify individual cells. After staining, the Hoechst media is replaced with regular DMEM media.

The Hoechst is imaged using the 405 nm LED and DAPI filter cube. GFP is imaged using the 465 nm LED and GFP filter cube, while RFP is imaged using 523 nm LED and RFP filter cube. Images of target and non-target cell wells are acquired by first establishing the LED intensity and integration times on a positive-control well; i.e., recipient cells treated with adenovirus coding for Cre recombinase instead of fusosomes.

Acquisition settings are set so that RFP and GFP intensities are at the maximum pixel intensity values but not saturated. The wells of interest are then imaged using the established settings. Wells are imaged every 4 hours to acquire time-course data for rates of fusion activity.

Analysis of GFP and RFP-positive wells is performed with software provided with the fluorescent microscope or other software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, rsb.info.nih.gov/ij/, 1997–2007).

The images are pre-processed using a rolling ball background subtraction algorithm with a 60 μm width. The total cell mask is set on the Hoechst-positive cells. Cells with Hoechst intensity significantly above background intensities are thresholded and areas too small or large to be Hoechst-positive cells are excluded.

Within the total cell mask, GFP and RFP-positive cells are identified by again thresholding for cells significantly above background and extending the Hoechst (nuclei) masks for the entire cell area to include the entire GFP and RFP cellular fluorescence. The number of RFP-positive cells identified in control wells containing target or non-target recipient cells is used to subtract from the number of RFP-positive cells in the wells containing fusosome (to subtract for non-specific Loxp recombination). The number of RFP-positive cells (fused recipient cells) is then divided by the sum of the GFP-positive cells (recipient cells that have not fused) and RFP-positive cells at each time point to quantify the rate of fusosome fusion within the recipient cell population. The rate is normalized to the given dose of fusosome applied to the

recipient cells. For rates of targeted fusion (fusosome fusion to targeted cells), the rate of fusion to the non-target cell is subtracted from the rate of fusion to the target cell in order to quantify rates of targeted fusion.

In an embodiment, the average rate of fusion for the fusosomes with the target cells will be in the range of 0.01-4.0 RFP/GFP cells per hour for target cell fusion or at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater than non-target recipient cells with fusosomes. In an embodiment, groups with no fusosome applied will show a background rate of <0.01 RFP/GFP cells per hour.

Example 44: In vitro fusion to deliver a membrane protein

This example describes fusosome fusion with a cell *in vitro*. In an embodiment, fusosome fusion with a cell in vitro results in delivery of an active membrane protein to the recipient cell.

In this example, the fusosomes are generated from a HEK293T cell expressing the Sendai virus HVJ-E protein (Tanaka et al., 2015, Gene Therapy, 22(October 2014), 1–8. doi.org/10.1038/gt.2014.12). In an embodiment, the fusosomes are generated to express the membrane protein, GLUT4, which is found primarily in muscle and fat tissues and is responsible for the insulin-regulated transport of glucose into cells. Fusosomes with and without GLUT4 are prepared from HEK293T cells as described by any of the methods described in a previous Example.

Muscles cells, such as, C2C12 cells, are then treated with fusosomes expressing GLUT4, fusosomes that do not express GLUT4, PBS (negative control), or insulin (positive control). The activity of GLUT4 on C2C12 cells is measured by the uptake of the fluorescent 2-deoxyglucose analog, 2-[N-(7-nitrobenz-2-oxa-1,3-diaxol-4-yl)amino]-2-deoxyglucose (2-NBDG). The fluorescence of C2C12 cells is assessed via microscopy using methods described in previous Examples.

In an embodiment, C2C12 cells that are treated with fusosomes that express GLUT4 and insulin are expected to demonstrate increased fluorescence compared to C2C12 cells treated with PBS or fusosomes not expressing GLUT4.

See, also, Yang et al., *Advanced Materials* 29, 1605604, 2017.

Example 45: Measuring extravasation from blood vessels

This Example describes quantification of fusosome extravasation across an endothelial monolayer as tested with an in vitro microfluidic system (J.S Joen et al. 2013, journals.plos.org/plosone/article?id=10.1371/journal.pone.0056910).

Cells extravasate from the vasculature into surrounding tissue. Without wishing to be bound by theory, extravasation is one way for fusosomes to reach extravascular tissues.

The system includes three independently addressable media channels, separated by chambers into which an ECM-mimicking gel can be injected. In brief, the microfluidics system has molded PDMS (poly-dimethyl siloxane; Silgard 184; Dow Chemical, MI) through which access ports are bored and bonded to a cover glass to form microfluidic channels. Channel cross-sectional dimensions are 1 mm (width) by 120 μm (height). To enhance matrix adhesion, the PDMS channels are coated with a PDL (poly-D-lysine hydrobromide; 1 mg/ml; Sigma-Aldrich, St. Louis, MO) solution.

Next, collagen type I (BD Biosciences, San Jose, CA, USA) solution (2.0 mg/ml) with phosphate-buffered saline (PBS; Gibco) and NaOH is injected into the gel regions of the device via four separate filling ports and incubated for 30 min to form a hydrogel. When the gel is polymerized, endothelial cell medium (acquired from suppliers such as Lonza or Sigma) is immediately pipetted into the channels to prevent dehydration of the gel. Upon aspirating the medium, diluted hydrogel (BD science) solution (3.0 mg/ml) is introduced into the cell channel and the excess hydrogel solution is washed away using cold medium.

Endothelial cells are introduced into the middle channel and allowed to settle to form an endothelium. Two days after endothelial cell seeding, fusosomes or macrophage cells (positive control) are introduced into the same channel where endothelial cells had formed a complete monolayer. The fusosomes are introduced so they adhere to and transmigrate across the monolayer into the gel region. Cultures are kept in a humidified incubator at 37°C and 5% CO_2. A GFP-expressing version of the fusosome is used to enable live-cell imaging via fluorescent microscopy. On the following day, cells are fixed and stained for nuclei using DAPI staining in the chamber, and multiple regions of interest are imaged using confocal microscope to determine how many fusosomes passed through the endothelial monolayer.

In an embodiment, DAPI staining will indicate that fusosomes and positive control cells are able to pass through the endothelial barrier after seeding.

Example 46: Measuring chemotactic cell mobility

This Example describes quantification of fusosome chemotaxis. Cells can move towards or away from a chemical gradient via chemotaxis. In an embodiment, chemotaxis will allow fusosomes to home to a site of injury, or track a pathogen. A purified fusosome composition as produced by any one of the methods described in previous Examples is assayed for its chemotactic abilities as follows.

A sufficient number of fusosomes or macrophage cells (positive control) are loaded in a micro-slide well according to the manufacturer's provided protocol in DMEM media (ibidi.com/img/cms/products/labware/channel_slides/S_8032X_Chemotaxis/IN_8032X_Chemotaxis.pdf). Fusosomes are left at 37°C and 5% CO2 for 1h to attach. Following cell attachment, DMEM (negative control) or DMEM containing MCP1 chemoattractant is loaded into adjacent reservoirs of the central channel and the fusosomes are imaged continuously for 2 hours using a Zeiss inverted widefield microscope. Images are analyzed using ImageJ software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/, 1997–2007). Migration co-ordination data for each observed fusosome or cell is acquired with the manual tracking plugin (Fabrice Cordelières, Institut Curie, Orsay, France). Chemotaxis plots and migration velocities is determined with the Chemotaxis and Migration Tool (ibidi).

In an embodiment, the average accumulated distance and migration velocity of fusosomes will be within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or greater than the response of the positive control cells to chemokine. The response of cells to a chemokine is described, e.g., in Howard E. Gendelman et al., *Journal of Neuroimmune Pharmacology*, 4(1): 47-59, 2009.

Example 47: Measuring homing potential

This Example describes homing of fusosomes to a site of injury. Cells can migrate from a distal site and/or accumulate at a specific site, e.g., home to a site. Typically, the site is a site

of injury. In an embodiment, fusosomes will home to, e.g., migrate to or accumulate at, a site of injury.

Eight week old C57BL/6J mice (Jackson Laboratories) are dosed with notexin (NTX) (Accurate Chemical & Scientific Corp), a myotoxin, in sterile saline by intramuscular (IM) injection using a 30G needle into the right tibialis anterior (TA) muscle at a concentration of 2 µg/mL. The skin over the tibialis anterior (TA) muscle is prepared by depilating the area using a chemical hair remover for 45 seconds, followed by 3 rinses with water. This concentration is chosen to ensure maximum degeneration of the myofibers, as well as minimal damage to their satellite cells, the motor axons and the blood vessels.

On day 1 after NTX injection, mice receive an IV injection of fusosomes or cells that express firefly luciferase. Fusosomes are produced from cells that stably express firefly luciferase by any one of the methods described in previous Examples. A bioluminescent imaging system (Perkin Elmer) is used to obtain whole animal images of bioluminescence at 0, 1, 3, 7, 21, and 28 post injection.

Five minutes before imaging, mice receive an intraperitoneal injection of bioluminescent substrate (Perkin Elmer) at a dose of 150mg/kg in order to visualize luciferase. The imaging system is calibrated to compensate for all device settings. The bioluminescent signal is measured using Radiance Photons, with Total Flux used as a measured value. The region of interest (ROI) is generated by surrounding the signal of the ROI in order to give a value in photons/second. An ROI is assessed on both the TA muscle treated with NTX and on the contralateral TA muscle, and the ratio of photons/second between NTX-treated and NTX-untreated TA muscles is calculated as a measure of homing to the NTX-treated muscle.

In an embodiment, the ratio of photons/second between NTX-treated and NTX-untreated TA muscles in fusosomes and cells will be greater than 1 indicating site specific accumulation of luciferase-expressing fusosomes at the injury.

See, for example, Plant et al., *Muscle Nerve* 34(5)L 577-85, 2006.

Example 48: Measuring phagocytic activity

This Example demonstrates phagocytic activity of fusosomes. In an embodiment, fusosomes have phagocytic activity, e.g., are capable of phagocytosis. Cells engage in

phagocytosis, engulfing particles, enabling the sequestration and destruction of foreign invaders, like bacteria or dead cells.

A purified fusosome composition as produced by any one of the methods described in previous Examples comprising a fusosome from a mammalian macrophage having partial or complete nuclear inactivation was capable of phagocytosis assayed via pathogen bioparticles. This estimation was made by using a fluorescent phagocytosis assay according to the following protocol.

Macrophages (positive control) and fusosomes were plated immediately after harvest in separate confocal glass bottom dishes. The macrophages and fusosomes were incubated in DMEM+10%FBS+1%P/S for 1h to attach. Fluorescein-labeled *E. coli* K12 and non-fluorescein-labeled *Escherichia coli* K-12 (negative control) were added to the macrophages/fusosomes as indicated in the manufacturer's protocol, and were incubated for 2h, tools.thermofisher.com/content/sfs/manuals/mp06694.pdf. After 2h, free fluorescent particles were quenched by adding Trypan blue. Intracellular fluorescence emitted by engulfed particles was imaged by confocal microscopy at 488 excitation. The number of phagocytotic positive fusosome were quantified using image J software.

The average number of phagocytotic fusosomes was at least 30% 2h after bioparticle introduction, and was greater than 30% in the positive control macrophages.

Example 49: Measuring potential for protein secretion

This Example describes quantification of secretion by fusosomes. In an embodiment, fusosomes will be capable of secretion, e.g., protein secretion. Cells can dispose or discharge of material via secretion. In an embodiment, fusosomes will chemically interact and communicate in their environment via secretion.

The capacity of fusosomes to secrete a protein at a given rate is determined using the Gaussia luciferase flash assay from ThermoFisher Scientific (catalog #16158). Mouse embryonic fibroblast cells (positive control) or fusosomes as produced by any one of the methods described in previous Examples are incubated in growth media and samples of the media are collected every 15 minutes by first pelleting the fusosomes at 1600g for 5min and then collecting the

supernatant. The collected samples are pipetted into a clear-bottom 96-well plate. A working solution of assay buffer is then prepared according to the manufacturer's instructions.

Briefly, colenterazine, a luciferin or light-emitting molecule, is mixed with flash assay buffer and the mixture is pipetted into each well of the 96 well plate containing samples. Negative control wells that lack cells or fusosomes include growth media or assay buffer to determine background Gaussia luciferase signal. In addition, a standard curve of purified Gaussia luciferase (Athena Enzyme Systems, catalog #0308) is prepared in order to convert the luminescence signal to molecules of Gaussia luciferase secretion per hour.

The plate is assayed for luminescence, using 500 msec integration. Background Gaussia luciferase signal is subtracted from all samples and then a linear best-fit curve is calculated for the Gaussia luciferase standard curve. If sample readings do not fit within the standard curve, they are diluted appropriately and re-assayed. Using this assay, the capacity for fusosomes to secrete Gaussia luciferase at a rate (molecules/hour) within a given range is determined.

In an embodiment, fusosomes will be capable of secreting proteins at a rate that is 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or greater than the positive control cells.

Example 50: Measuring signal transduction potential

This Example describes quantification of signal transduction in fusosomes. In an embodiment, fusosomes are capable of signal transduction. Cells can send and receive molecular signals from the extracellular environment through signaling cascades, such as phosphorylation, in a process known as signal transduction. A purified fusosome composition as produced by any one of the methods described in previous Examples comprising a fusosome from a mammalian cell having partial or complete nuclear inactivation is capable of signal transduction induced by insulin. Signal transduction induced by insulin is assessed by measuring AKT phosphorylation levels, a key pathway in the insulin receptor signaling cascade, and glucose uptake in response to insulin.

To measure AKT phosphorylation, cells, e.g., Mouse Embryonic Fibroblasts (MEFs) (positive control), and fusosomes are plated in 48-well plates and left for 2 hours in a humidified incubator at 37°C and 5% CO_2. Following cell adherence, insulin (e.g. at 10 nM), or a negative

control solution without insulin, is add to the well containing cells or fusosomes for 30 min. After 30 minutes, protein lysate is made from the fusosomes or cells, and phospho-AKT levels are measured by western blotting in insulin stimulated and control unstimulated samples.

Glucose uptake in response to insulin or negative control solution is measured as it is explained in the glucose uptake section by using labeled glucose (2-NBDG). (S. Galic et al., *Molecular Cell Biology* 25(2): 819-829, 2005).

In an embodiment, fusosomes will enhance AKT phosphorylation and glucose uptake in response to insulin over the negative controls by at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or greater.

Example 51: Measuring ability to transport glucose across cell membrane

This Example describes quantification of the levels of a 2-NBDG (2-(*N*-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)Amino)-2-Deoxyglucose) a fluorescent glucose analog that can be used to monitor glucose uptake in live cells, and thus measure active transport across the lipid bilayer. In an embodiment, this assay can be used to measure the level of glucose uptake and active transport across the lipid bilayer of the fusosome.

A fusosome composition is produced by any one of the methods described in previous Examples. A sufficient number of fusosomes are then incubated in DMEM with no glucose, 20% Fetal Bovine Serum and 1x Penicillin/Streptomycin for 2hr at 37°C and 5% CO_2. After a 2hr glucose starvation period, the medium is changed such that it includes DMEM with no glucose, 20% Fetal Bovine Serum, 1x Penicillin/Streptomycin and 20 uM 2-NBDG (ThermoFisher) and incubated for an additional 2hr at 37°C and 5% CO_2.

Negative control fusosomes are treated the same, except an equal amount of DMSO is added in place of 2-NBDG.

The fusosomes are then washed thrice with 1xPBS and re-suspended in an appropriate buffer, and transferred to a 96 well imaging plate. 2-NBDG fluorescence is then measured in a fluorimeter using a GFP light cube (469/35 excitation filter and a 525/39 emission filter) to quantify the amount of 2-NBDG that has been transported across the fusosome membrane and accumulated in the fusosome in the 1hr loading period.

In an embodiment, 2-NBDG fluorescence will be higher in the fusosome with 2-NBDG treatment as compared to the negative (DMSO) control. Fluorescence measure with a 525/39 emission filter will correlate with to the number of 2-NBDG molecules present.

Example 52: Measuring esterase activity in the cytosol

This Example describes quantification of esterase activity, as a surrogate for metabolic activity, in fusosomes. The cytosolic esterase activity in fusosomes is determined by quantitative assessment of calcein-AM staining (Bratosin et al., Cytometry 66(1): 78-84, 2005).

The membrane-permeable dye, calcein-AM (Molecular Probes, Eugene OR USA), is prepared as a stock solution of 10 mM in dimethylsulfoxide and as a working solution of 100 mM in PBS buffer, pH 7.4. Fusosomes as produced by any one of the methods described in previous Examples or positive control parental Mouse Embryonic Fibroblast cells are suspended in PBS buffer and incubated for 30 minutes with calcein-AM working solution (final concentration in calcein-AM: 5 mM) at 37°C in the dark and then diluted in PBS buffer for immediate flow cytometric analysis of calcein fluorescence retention.

Fusosomes and control parental Mouse Embryonic Fibroblast cells are experimental permeabilized as a negative control for zero esterase activity with saponin as described in (Jacob et al., *Cytometry* 12(6): 550-558, 1991). Fusosomes and cells are incubated for 15 min in 1% saponin solution in PBS buffer, pH 7.4, containing 0.05% sodium azide. Due to the reversible nature of plasma membrane permeabilization, saponin is included in all buffers used for further staining and washing steps. After saponin permeabilization, fusosomes and cells are suspended in PBS buffer containing 0.1% saponin and 0.05% sodium azide and incubated (37C in the dark for 45 min) with calcein-AM to a final concentration of 5 mM, washed three times with the same PBS buffer containing 0.1% saponin and 0.05% sodium azide, and analyzed by flow cytometry. Flow cytometric analyses are performed on a FACS cytometer (Becton Dickinson, San Jose, CA, USA) with 488nm argon laser excitation and emission is collected at 530+/-30nm. FACS software is used for acquisition and analysis. The light scatter channels are set on linear gains, and the fluorescence channels are set on a logarithmic scale, with a minimum of 10,000 cells analyzed in each condition. Relative esterase activities are calculated based on the intensity of calcein-AM in each sample. All events are captured in the forward and side scatter channels

(alternatively, a gate can be applied to select only the fusosome population). The fluorescence intensity (FI) value for the fusosomes is determined by subtracting the FI value of the respective negative control saponin-treated sample. The normalized esterase activity for the fusosomes samples are normalized to the respective positive control cell samples in order to generate quantitative measurements for cytosolic esterase activities.

In an embodiment, a fusosome preparation will have within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or greater esterase activity compared to the positive control cell.

See also, Bratosin D, Mitrofan L, Palii C, Estaquier J, Montreuil J. Novel fluorescence assay using calcein-AM for the determination of human erythrocyte viability and aging. Cytometry A. 2005 Jul;66(1):78-84; and Jacob BC, Favre M, Bensa JC. Membrane cell permeabilisation with saponin and multiparametric analysis by flow cytometry. Cytometry 1991;12:550–558.

Example 53: Measuring acetylcholinesterase activity in fusosomes

Acetylcholinesterase activity is measured using a kit (MAK119, SIGMA) that follows a procedure described previously (Ellman, et al., Biochem. Pharmacol. 7, 88, 1961) and following the manufacturer's recommendations.

Briefly, fusosomes are suspended in 1.25 mM acetylthiocholine in PBS, pH 8, mixed with 0.1 mM 5,5-dithio-bis(2-nitrobenzoic acid) in PBS, pH 7. The incubation is performed at room temperature but the fusosomes and the substrate solution are pre-warmed at 37 °C for 10 min before starting the optical density readings.

Changes in absorption are monitored at 450 nm for 10 min with a plate reader spectrophotometer (ELX808, BIO-TEK instruments, Winooski, VT, USA). Separately, a sample is used for determining the protein content of the fusosomes via bicinchoninic acid assay for normalization. Using this assay, the fusosomes are determined to have <100 AChE activity units/μg of protein.

In an embodiment, AChE activity units/μg of protein values will be less than 0.001, 0.01, 0.1, 1, 10, 100, or 1000.

Example 54: Measuring metabolic activity level

This Example describes quantification of the measurement of citrate synthase activity in fusosomes.

Citrate synthase is an enzyme within the tricarboxylic acid (TCA) cycle that catalyzes the reaction between oxaloacetate (OAA) and acetyl-CoA to generate citrate. Upon hydrolysis of acetyl-CoA, there is a release of CoA with a thiol group (CoA-SH). The thiol group reacts with a chemical reagent, 5,5-Dithiobis-(2-nitrobenzoic acid) (DTNB), to form 5-thio-2-nitrobenzoic acid (TNB), which is a yellow product that can be measured spectrophotometrically at 412 nm (Green 2008). Commercially-available kits, such as the Abcam Human Citrate Synthase Activity Assay Kit (Product #ab119692) provide all the necessary reagents to perform this measurement.

The assay is performed as per the manufacturer's recommendations. Fusosome sample lysates are prepared by collecting the fusosomes as produced by any one of the methods described in previous Examples and solubilizing them in Extraction Buffer (Abcam) for 20 minutes on ice. Supernatants are collected after centrifugation and protein content is assessed by bicinchoninic acid assay (BCA, ThermoFisher Scientific) and the preparation remains on ice until the following quantification protocol is initiated.

Briefly, fusosome lysate samples are diluted in 1X Incubation buffer (Abcam) in the provided microplate wells, with one set of wells receiving only 1X Incubation buffer. The plate is sealed and incubated for 4 hours at room temperature with shaking at 300rpm. The buffer is then aspirated from the wells and 1X Wash buffer is added. This washing step is repeated once more. Then, 1X Activity solution is added to each well, and the plate is analyzed on a microplate reader by measuring absorbance at 412nm every 20 seconds for 30 minutes, with shaking between readings.

Background values (wells with only 1X Incubation buffer) are subtracted from all wells, and the citrate synthase activity is expressed as the change in absorbance per minute per μg of fusosome lysate sample loaded (ΔmOD@412nm/min/ug protein). Only the linear portion from 100-400 seconds of the kinetic measurement is used to calculate the activity.

In an embodiment, a fusosome preparation will have within 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or greater synthase activity compared to the control cell.

See, for example, Green HJ et al. Metabolic, enzymatic, and transporter response in human muscle during three consecutive days of exercise and recovery. Am J Physiol Regul Integr Comp Physiol 295: R1238-R1250, 2008.

Example 55: Measuring respiration levels

This Example describes quantification of the measurement of respiration level in fusosomes. Respiration level in cells can be a measure of oxygen consumption, which powers metabolism. Fusosome respiration is measured for oxygen consumption rates by a Seahorse extracellular flux analyzer (Agilent) (Zhang 2012).

Fusosomes as produced by any one of the methods described in previous Examples or cells are seeded in a 96-well Seahorse microplate (Agilent). The microplate is centrifuged briefly to pellet the fusosomes and cells at the bottom of the wells. Oxygen consumption assays are initiated by removing growth medium, replacing with a low-buffered DMEM minimal medium containing 25mM glucose and 2mM glutamine (Agilent) and incubating the microplate at 37°C for 60 minutes to allow for temperature and pH equilibrium.

The microplate is then assayed in an extracellular flux analyzer (Agilent) that measures changes in extracellular oxygen and pH in the media immediately surrounding adherent fusosomes and cells. After obtaining steady state oxygen consumption (basal respiration rate) and extracellular acidification rates, oligomycin (5μM), which inhibits ATP synthase, and proton ionophore FCCP (carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone; 2μM), which uncouples mitochondria, are added to each well in the microplate to obtain values for maximal oxygen consumption rates.

Finally, 5 μM antimycin A (inhibitor of mitochondria complex III) is added to confirm that respiration changes are due mainly to mitochondrial respiration. The minimum rate of oxygen consumption after antimycin A addition is subtracted from all oxygen consumption measurements to remove the non-mitochondrial respiration component. Cell samples that do not appropriately respond to oligomycin (at least a 25% decrease in oxygen consumption rate from

basal) or FCCP (at least a 50% increase in oxygen consumption rate after oligomycin) are excluded from the analysis. Fusosomes respiration level is then measured as pmol O2/min/1e4 fusosomes.

This respiration level is then normalized to the respective cell respiration level. In an embodiment, fusosomes will have at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or greater respiration level compared to the respective cell samples.

See, for example, Zhang J, Nuebel E, Wisidagama DRR, et al. Measuring energy metabolism in cultured cells, including human pluripotent stem cells and differentiated cells. Nature protocols. 2012;7(6):10.1038/nprot.2012.048. doi:10.1038/nprot.2012.048.

Example 56: Measuring phosphatidylserine levels of fusosomes

This Example describes quantification of the level of annexin-V binding to the surface of fusosomes.

Dying cells can display phosphatidylserine on the cell surface which is a marker of apoptosis in the programmed cell death pathway. Annexin-V binds to phosphatidylserine, and thus, annexin-V binding is a proxy for viability in cells.

Fusosomes were produced as described herein. For detection of apoptosis signals, fusosomes or positive control cells were stained with 5% annexin V fluor 594 (A13203, Thermo Fisher, Waltham, MA). Each group (detailed in the table below) included an experimental arm that was treated with an apoptosis-inducer, menadione. Menadione was added at 100 μM menadione for 4 h. All samples were run on a flow cytometer (Thermo Fisher, Waltham, MA) and fluorescence intensity was measured with the YL1 laser at a wavelength of 561 nm and an emission filter of 585/16 nm. The presence of extracellular phophatidyl serine was quantified by comparing fluorescence intensity of annexin V in all groups.

The negative control unstained fusosomes were not positive for annexin V staining.

In an embodiment, fusosomes were capable of upregulating phosphatidylserine display on the cell surface in response to menadione, indicating that non-menadione stimulated fusosomes are not undergoing apoptosis. In an embodiment, positive control cells that were stimulated with menadione demonstrated higher-levels of annexin V staining than fusosomes not stimulated with menadione.

Table 7: Annexin V staining parameter

Experimental Arm	Mean Fluorescence Intensity of Annexin V Signal (and standard deviation)
Unstained Fusosomes (negative control)	941 (937)
Stained Fusosomes	11257 (15826)
Stained Fusosomes + Menadione	18733 (17146)
Stained Macrophages + Menadione (positive control)	14301 (18142)

Example 57: Measuring juxtacrine-signaling levels

This Example describes quantification of juxtacrine-signaling in fusosomes.

Cells can form cell-contact dependent signaling via juxtacrine signaling. In an embodiment, presence of juxtacrine signaling in fusosomes will demonstrate that fusosomes can stimulate, repress, and generally communicate with cells in their immediate vicinity.

Fusosomes produced by any one of the methods described in previous Examples from mammalian bone marrow stromal cells (BMSCs) having partial or complete nuclear inactivation trigger IL-6 secretion via juxtacrine signaling in macrophages. Primary macrophages and BMSCs are co-cultured. Bone marrow-derived macrophages are seeded first into 6-well plates, and incubated for 24h, then primary mouse BMSC-derived fusosomes or BMSC cells (positive control parental cells) are placed on the macrophages in a DMEM medium with 10% FBS. The supernatant is collected at different time points (2, 4, 6, 24 hours) and analyzed for IL-6 secretion by ELISA assay. (Chang J. et al., 2015).

In an embodiment, the level of juxtacrine signaling induced by BMSC fusosomes is measured by an increase in macrophage-secreted IL-6 levels in the media. In an embodiment, the level of juxtacrine signaling will be at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or greater than the levels induced by the positive control bone marrow stromal cells (BMSCs).

Example 58: Measuring paracrine-signaling levels

This Example describes quantification of paracrine signaling in fusosomes.

Cells can communicate with other cells in the local microenvironment via paracrine signaling. In an embodiment, fusosomes will be capable of paracrine signaling, e.g., to communicate with cells in their local environment. In an embodiment, the ability of fusosomes to trigger Ca^{2+} signaling in endothelial cells via paracrine-derived secretion with the following protocol will measure Ca^{2+} signaling via the calcium indicator, fluo-4 AM.

To prepare the experimental plate, murine pulmonary microvascular endothelial cells (MPMVECs) are plated on a 0.2% gelatin coated 25mm glass bottom confocal dish (80% confluence). MPMVECs are incubated at room temperature for 30 min in ECM containing 2% BSA and 0.003% pluronic acid with 5 μM fluo-4 AM (Invitrogen) final concentration to allow loading of fluo-4 AM. After loading, MPMVECs are washed with experimental imaging solution (ECM containing 0.25% BSA) containing sulfinpyrazone to minimize dye loss. After loading fluo-4, 500μl of pre-warmed experimental imaging solution is added to the plate, and the plate is imaged by a Zeiss confocal imaging system.

In a separate tube, freshly isolated murine macrophages are either treated with 1μg/ml LPS in culture media (DMEM+10% FBS) or not treated with LPS (negative control). After stimulation, fusosomes are generated from macrophages by any one of the methods described in previous Examples.

Fusosomes or parental macrophages (positive control) are then labeled with cell tracker red, CMTPX (Invitrogen), in ECM containing 2%BSA and 0.003% pluronic acid. Fusosomes and macrophages are then washed and resuspended in experimental imaging solution. Labeled fusosomes and macrophages are added onto the fluo-4 AM loaded MPMVECs in the confocal plate.

Green and red fluorescence signal is recorded every 3s for 10-20 min using Zeiss confocal imaging system with argon ion laser source with excitation at 488 and 561 nm for fluo-4 AM and cell tracker red fluorescence respectively. Fluo-4 fluorescence intensity changes are analyzed using imaging software (Mallilankaraman, K. et al., *J Vis Exp.* (58): 3511, 2011). The level of Fluo-4 intensity measured in negative control fusosome and cell groups is subtracted from LPS-stimulated fusosome and cell groups.

In an embodiment, fusosomes, e.g., activated fusosomes, will induce an increase in Fluo-4 fluorescence intensity that is at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or greater than the positive control cell groups.

Example 59: Measuring ability to polymerize actin for mobility

This Example describes quantification of cytoskeletal components, such as actin, in fusosomes. In an embodiment, fusosomes comprise cytoskeletal components such as actin, and are capable of actin polymerization.

Cells use actin, which is a cytoskeletal component, for motility and other cytoplasmic processes. The cytoskeleton is essential to creating motility driven forces and coordinating the process of movement

C2C12 cells were enucleated as described herein. Fusosomes obtained from the 12.5% and 15% Ficoll layers were pooled and labeled 'Light', while fusosomes from the 16 – 17% layers were pooled and labeled 'Medium'. Fusosomes or cells (parental C2C12 cells, positive control) were resuspended in DMEM + Glutamax + 10% Fetal Bovine Serum (FBS), plated in 24-well ultra-low attachment plates (#3473, Corning Inc, Corning, NY) and incubated at 37 °C + 5% CO_2. Samples were taken periodically (5.25 hr, 8.75 hr, 26.5 hr) and stained with 165 μM rhodamine phalloidin (negative control was not stained) and measured on a flow cytometer (#A24858, Thermo Fisher, Waltham, MA) with a FC laser YL1 (561 nm with 585/16 filter) to measure F-actin cytoskeleton content. The fluorescence intensity of rhodamine phalloidin in fusosomes was measured along with unstained fusosomes and stained parental C2C12 cells.

Fusosome fluorescence intensity was greater (FIG. 1) than the negative control at all timepoints, and fusosomes were capable of polymerizing actin at a similar rate to the parental C2C12 cells.

Additional cytoskeletal components, such as those listed in the table below, are measured via a commercially available ELISA systems (Cell Signaling Technology and MyBioSource), according to manufacturer's instructions.

Table 8: Cytoskeletal components

Cytoskeletal protein measured	Commercial Kit Type	Kit ID
Actin	Path Scan Total B-Actin Sandwich ELISA Kit	Cell Signaling, 7880
Arp2/3	Human Actin Related protein 2/3 complex subunit(APRC2) ELISA KIT	MyBioSource, MBS7224740
Formin	**Formin Binding Protein 1 (FNBP1), ELISA Kit**	MyBioSource, MBS9308864
Coronin	Human Coronin 1A ELISA Kit	MyBioSource, MBS073640
Dystrophin	Human dystrophin ELISA Kit	MyBioSource MBS722223
Keratin	Human Keratin 5 ELISA Kit	MyBioSource, MBS081200
Myosin	Human Myosin IG (MYO1G) ELISA Kit	MyBioSource, MBS9312965
Tubulin	Human Tubulin Beta 3 ELISA Kit	MyBioSource, MBS097321

Then 100uL of appropriately-diluted lysate is added to the appropriate well from the microwell strips. The microwells are sealed with tape and incubated for 2 hrs at 37C. After incubation, the sealing tape is removed and the contents are discarded. Each microwell is washed four times with 200uL of 1X Wash Buffer. After each individual wash, plates are struck onto an absorbent cloth so that the residual wash solution is removed from each well. However, wells are not completely dry at any time during the experiment.

Next, 100ul of the reconstituted Detection Antibody (green) is added each individual well, except for negative control wells. Then wells are sealed and incubated for 1 hour at 37°C. The washing procedure is repeated after incubation is complete. 100uL of reconstituted HRP-Linked secondary antibody (red) is added to each of the wells. The wells are sealed with tape and incubated for 30 minutes at 37°C. The sealing tape is then removed and the washing procedure is repeated. 100uL of TMB Substrate is then added to each well. The wells are sealed with tape, then incubated for 10 minutes at 37°C. Once this final incubation is complete, 100uL of STOP solution is added to each of the wells and the plate is shaken gently for several seconds.

Spectrophotometric analysis of the assay is conducted within 30 minutes of adding the STOP solution. The underside of the wells is wiped with lint-free tissue and then absorbance is

read at 450nm. In an embodiment, fusosome samples that have been stained with the detection antibody will absorb more light at 450 nm that negative control fusosome samples, and absorb less light than cell samples that have been stained with the detection antibody.

Example 60: Measuring average membrane potential

This Example describes quantification of the mitochondrial membrane potential of fusosomes. In an embodiment, fusosomes comprising a mitochondrial membrane will maintain mitochondrial membrane potential.

Mitochondrial metabolic activity can be measured by mitochondrial membrane potential. The membrane potential of the fusosome preparation is quantified using a commercially available dye, TMRE, for assessing mitochondrial membrane potential (TMRE: tetramethyl rhodamine, ethyl ester, perchlorate, Abcam, Cat# T669).

Fusosomes are generated by any one of the methods described in previous Examples. Fusosomes or parental cells are diluted in growth medium (phenol-red free DMEM with 10% fetal bovine serum) in 6 aliquots (untreated and FCCP-treated triplicates). One aliquot of the samples is incubated with FCCP, an uncoupler that eliminates mitochondrial membrane potential and prevents TMRE staining. For FCCP-treated samples, 2μM FCCP is added to the samples and incubated for 5 minutes prior to analysis. Fusosomes and parental cells are then stained with 30nM TMRE. For each sample, an unstained (no TMRE) sample is also prepared in parallel. Samples are incubated at 37°C for 30 minutes. The samples are then analyzed on a flow cytometer with 488nm argon laser, and excitation and emission is collected at 530+/-30nm.

Membrane potential values (in millivolts, mV) are calculated based on the intensity of TMRE. All events are captured in the forward and side scatter channels (alternatively, a gate can be applied to exclude small debris). The fluorescence intensity (FI) value for both the untreated and FCCP-treated samples are normalized by subtracting the geometric mean of the fluorescence intensity of the unstained sample from the geometric mean of the untreated and FCCP-treated sample. The membrane potential state for each preparation is calculated using the normalized fluorescent intensity values with a modified Nernst equation (see below) that can be used to determine mitochondrial membrane potential of the fusosomes or cells based on TMRE fluorescence (as TMRE accumulates in mitochondria in a Nernstian fashion).

Fusosome or cell membrane potential is calculated with the following formula: (mV) = -61.5 * log(FIuntreated-normalized/FIFCCP-treated-normalized). In an embodiment, using this assay on fusosome preparations from C2C12 mouse myoblast cells, the membrane potential state of the fusosome preparation will be within about 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or greater than the parental cells. In an embodiment, the range of membrane potential is about -20 to -150mV.

Example 61: Measuring persistence half-life in a subject

This Example describes the measurement of fusosome half-life.

Fusosomes are derived from cells that express Gaussia luciferase produced by any one of the methods described in previous Examples, and pure, 1:2, 1:5, and 1:10 dilutions in buffered solution are made. A buffered solution lacking fusosomes is used as a negative control.

Each dose is administered to three eight week old male C57BL/6J mice (Jackson Laboratories) intravenously. Blood is collected from the retro-orbital vein at 1, 2, 3, 4, 5, 6, 12, 24, 48, and 72 hours after intravenous administration of the fusosomes. The animals are sacrificed at the end of the experiment by CO_2 inhalation.

Blood is centrifuged for 20 min at room temperature. The serum samples are immediately frozen at –80°C until bioanalysis. Then, each blood sample is used to carry out a Gaussia luciferase activity assay after mixing the samples with Gaussia luciferase substrate (Nanolight, Pinetop, AZ). Briefly, colenterazine, a luciferin or light-emitting molecule, is mixed with flash assay buffer and the mixture is pipetted into wells containing blood samples in a 96 well plate. Negative control wells that lack blood contain assay buffer to determine background Gaussia luciferase signal.

In addition, a standard curve of positive-control purified Gaussia luciferase (Athena Enzyme Systems, catalog #0308) is prepared in order to convert the luminescence signal to molecules of Gaussia luciferase secretion per hour. The plate is assayed for luminescence, using 500 msec integration. Background Gaussia luciferase signal is subtracted from all samples and then a linear best-fit curve is calculated for the Gaussia luciferase standard curve. If sample readings do not fit within the standard curve, they are diluted appropriately and re-assayed. The luciferase signal from samples taken at 1, 2, 3, 4, 5, 6, 12, 24, 48, and 72 hours is interpolated to

the standard curve. The elimination rate constant k_e (h^{-1}) is calculated using the following equation of a one-compartment model: $C(t) = C_0 \, x \, e^{-kext}$, in which $C(t)$ (ng/mL) is the concentration of fusosomes at time t (h) and C_0 the concentration of fusosomes at time = 0 (ng/mL). The elimination half-life $t_{1/2,e}$ (h) is calculated as $\ln(2)/k_e$.

In an embodiment, fusosomes will have a half-life of at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or greater than the negative control cells.

Example 62: Delivery of fusosomes via non-endocytic pathway

This example describes quantification of fusosome delivery of Cre to a recipient cell via a non-endocytic pathway.

In an embodiment, fusosomes will deliver agents via a fusosome-mediated, non-endocytic pathway. Without wishing to be bound by theory, delivery of an agent, e.g., Cre, which is carried within the lumen of the fusosomes, directly to the cytosol of the recipient cells without any requirement for endocytosis-mediated uptake of the fusosomes, will occur through a fusosome-mediated, non-endocytic pathway delivery.

In this example, the fusosome comprises a HEK293T cell expressing the Sendai virus H and F protein on its plasma membrane (Tanaka et al., 2015, Gene Therapy, 22(October 2014), 1–8. https://doi.org/10.1038/gt.2014.123). In addition, the fusosome expresses mTagBFP2 fluorescent protein and Cre recombinase. The target cell is a RPMI8226 cell which stably-expresses "LoxP-GFP-stop-LoxP-RFP" cassette under a CMV promoter, which upon recombination by Cre switches from GFP to RFP expression, indicating fusion and Cre, as a marker, delivery.

Fusosomes produced by the herein described methods are assayed for delivery of Cre via a non-endocytic pathway as follows. The recipient cells are plated into a black, clear-bottom 96-well plate. Next, 24 hours after plating the recipient cells, the fusosomes expressing Cre recombinase protein and possessing the particular fusogen protein are applied to the recipient cells in DMEM media. To determine the level of Cre delivery via a non-endocytic pathway, a parallel group of recipient cells receiving fusosomes is treated with an inhibitor of endosomal acidification, chloroquine (30 µg/mL). The dose of fusosomes is correlated to the number of recipient cells plated in the well. After applying the fusosomes, the cell plate is centrifuged at

400g for 5 minutes to help initiate contact between the fusosomes and the recipient cells. The cells are then incubated for 16 hours and agent delivery, Cre, is assessed via imaging.

The cells are imaged to positively identify RFP-positive cells versus GFP-positive cells in the field or well. In this example cell plates are imaged using an automated fluorescence microscope. The total cell population in a given well is determined by first staining the cells with Hoechst 33342 in DMEM media for 10 minutes. Hoechst 33342 stains cell nuclei by intercalating into DNA and therefore is used to identify individual cells. After staining, the Hoechst media is replaced with regular DMEM media.

The Hoechst is imaged using the 405 nm LED and DAPI filter cube. GFP is imaged using the 465 nm LED and GFP filter cube, while RFP is imaged using 523 nm LED and RFP filter cube. Images of the different cell groups are acquired by first establishing the LED intensity and integration times on a positive-control well; i.e., recipient cells treated with adenovirus coding for Cre recombinase instead of fusosomes.

Acquisition settings are set so that RFP and GFP intensities are at the maximum pixel intensity values but not saturated. The wells of interest are then imaged using the established settings.

Analysis of GFP and RFP-positive wells is performed with software provided with the fluorescence microscope or other software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, 1997–2007). The images are pre-processed using a rolling ball background subtraction algorithm with a 60 μm width. The total cell mask is set on the Hoechst-positive cells. Cells with Hoechst intensity significantly above background intensities are used to set a threshold, and areas too small or large to be Hoechst-positive cells are excluded.

Within the total cell mask, GFP and RFP-positive cells are identified by again setting a threshold for cells significantly above background and extending the Hoechst (nuclei) masks for the entire cell area to include the entire GFP and RFP cellular fluorescence.

The number of RFP-positive cells identified in control wells containing recipient cells is used to subtract from the number of RFP-positive cells in the wells containing fusosomes (to subtract for non-specific Loxp recombination). The number of RFP-positive cells (recipient cells that received Cre) is then divided by the sum of GFP-positive cells (recipient cells that have not received Cre) and RFP-positive cells to quantify the fraction of fusosome Cre delivered to the

recipient cell population. The level is normalized to the given dose of fusosomes applied to the recipient cells. To calculate the value of fusosome Cre delivered via a non-endocytic pathway, the level of fusosome Cre delivery in the presence of chloroquine (FusL+CQ) is determined as well as the level of fusosome Cre delivery in the absence of chloroquine (FusL-CQ). To determine the normalized value of fusosome Cre delivered via a non-endocytic pathway, the following equation is used: [(FusL-CQ)-(FusL+CQ)]/(FusL-CQ).

In an embodiment, the average level of fusosome Cre delivered via a non-endocytic pathway for a given fusosome will be in the range of 0.1-0.95, or at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater than chloroquine treated recipient cells.

Example 63: Delivery of fusosomes via endocytic pathway

This example describes fusosome delivery of Cre to a recipient cell via an endocytic pathway.

In an embodiment, fusosomes will deliver agents via a fusosome-mediated, endocytic pathway. Without wishing to be bound by theory, delivery of an agent, e.g., a cargo, carried in the lumen of the fusosomes, to the recipient cells with the route of uptake being endocytosis-dependent will occur through a fusosome-mediated, endocytic pathway delivery.

In this example the fusosome comprises microvesicles that were produced by extruding a HEK293T cell expressing a fusogen protein on its plasma membrane through a 2 μm filter (Lin et al., 2016, Biomedical Microdevices, 18(3). doi.org/10.1007/s10544-016-0066-y)(Riedel, Kondor-Koch, & Garoff, 1984, The EMBO Journal, 3(7), 1477–83. Retrieved from www.ncbi.nlm.nih.gov/pubmed/6086326). In addition, the fusosome expresses mTagBFP2 fluorescent protein and Cre recombinase. The target cell is a PC3 cell which stably-expresses "LoxP-GFP-stop-LoxP-RFP" cassette under a CMV promoter, which upon recombination by Cre switches from GFP to RFP expression, indicating fusion and Cre, as a marker, delivery.

Fusosomes produced by the herein described methods are assayed for delivery of Cre via an endocytic pathway as follows. The recipient cells are plated into a cell culture multi-well plate compatible with the imaging system to be used (in this example cells are plated in a black, clear-bottom 96-well plate). Next, 24 hours after plating the recipient cells, the fusosomes expressing

Cre recombinase protein and possessing the particular fusogen protein are applied to the recipient cells in DMEM media. To determine the level of Cre delivery via an endocytic pathway, a parallel group of recipient cells receiving fusosomes is treated with an inhibitor of endosomal acidification, chloroquine (30 μg/mL). The dose of fusosomes is correlated to the number of recipient cells plated in the well. After applying the fusosomes, the cell plate is centrifuged at 400g for 5 minutes to help initiate contact between the fusosomes and the recipient cells. The cells are then incubated for 16 hours and agent delivery, Cre, is assessed via imaging.

The cells are imaged to positively identify RFP-positive cells versus GFP-positive cells in the field or well. In this example cell plates are imaged using an automated fluorescent microscope. The total cell population in a given well is determined by first staining the cells with Hoechst 33342 in DMEM media for 10 minutes. Hoechst 33342 stains cell nuclei by intercalating into DNA and therefore is used to identify individual cells. After staining the Hoechst media is replaced with regular DMEM media.

The Hoechst is imaged using the 405 nm LED and DAPI filter cube. GFP is imaged using the 465 nm LED and GFP filter cube, while RFP is imaged using 523 nm LED and RFP filter cube. Images of the different cell groups are acquired by first establishing the LED intensity and integration times on a positive-control well; i.e., recipient cells treated with adenovirus coding for Cre recombinase instead of fusosomes.

Acquisition settings are set so that RFP and GFP intensities are at the maximum pixel intensity values but not saturated. The wells of interest are then imaged using the established settings.

Analysis of GFP and RFP-positive wells is performed with software provided with the fluorescent microscope or other software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, 1997–2007). The images are pre-processed using a rolling ball background subtraction algorithm with a 60 μm width. The total cell mask is set on the Hoechst-positive cells. Cells with Hoechst intensity significantly above background intensities are thresholded and areas too small or large to be Hoechst-positive cells are excluded.

Within the total cell mask, GFP and RFP-positive cells are identified by again thresholding for cells significantly above background and extending the Hoechst (nuclei) masks for the entire cell area to include the entire GFP and RFP cellular fluorescence.

The number of RFP-positive cells identified in control wells containing recipient cells is used to subtract from the number of RFP-positive cells in the wells containing fusosomes (to subtract for non-specific Loxp recombination). The number of RFP-positive cells (recipient cells that received Cre) is then divided by the sum of the GFP-positive cells (recipient cells that have not received Cre) and RFP-positive cells to quantify the fraction of fusosome Cre delivered to the recipient cell population. The level is normalized to the given dose of fusosomes applied to the recipient cells. To calculate the value of fusosome Cre delivered via an endocytic pathway, the level of fusosome Cre delivery in the presence of chloroquine (FusL+CQ) is determined as well as the level of fusosome Cre delivery in the absence of chloroquine (FusL-CQ). To determine the normalized value of fusosome Cre delivered via an endocytic pathway, the following equation is used: (FusL+CQ)/(FusL-CQ).

In an embodiment, the average level of fusosome Cre delivered via an endocytic pathway for a given fusosome will be in the range of 0.01-0.6, or at least 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater than chloroquine treated recipient cells.

Example 64: Delivery of fusosomes via a dynamin mediated pathway, a macropinocytosis pathway, or an actin mediated pathway

This example describes fusosome delivery of Cre to a recipient cell via a dynamin mediated pathway. A fusosome comprising a microvesicle may be produced as described in the preceding example. Fusosomes are assayed for delivery of Cre via a dynamin-mediated pathway according to the preceding example, except that a group of recipient cells receiving fusosomes is treated with an inhibitor of dynamin, Dynasore (120 μM). To calculate the value of fusosome Cre delivered via a dynamin-mediated pathway, the level of fusosome Cre delivery in the presence of Dynasore (FusL+DS) is determined as well as the level of fusosome Cre delivery in the absence of Dynasore (FusL-DS). The normalized value of fusosome Cre delivered may be calculated as described in the preceding example.

This example also describes delivery of Cre to a recipient cell via macropinocytosis. A fusosome comprising a microvesicle may be produced as described in the preceding example. Fusosomes are assayed for delivery of Cre via macropinocytosis according to the preceding example, except that a group of recipient cells receiving fusosomes is treated with an inhibitor of

macropinocytosis, 5-(*N*-ethyl-*N*-isopropyl)amiloride (EIPA) (25 μM). To calculate the value of fusosome Cre delivered via macropinocytosis, the level of fusosome Cre delivery in the presence of EIPA (FusL+EPIA) is determined as well as the level of fusosome Cre delivery in the absence of EPIA (FusL-EIPA). The normalized value of fusosome Cre delivered may be calculated as described in the preceding example.

This example also describes fusosome delivery of Cre to a recipient cell via an actin mediated pathway. A fusosome comprising a microvesicle may be produced as described in the preceding example. Fusosomes are assayed for delivery of Cre via macropinocytosis according to the preceding example, except that a group of recipient cells receiving fusosomes is treated with an inhibitor of actin polymerization, Latrunculin B (6 μM). To calculate the value of fusosome Cre delivered via an actin-mediated pathway, the level of fusosome Cre delivery in the presence of Latrunculin B (FusL+ LatB) is determined as well as the level of fusosome Cre delivery in the absence of Latrunculin B (FusL- LatB). The normalized value of fusosome Cre delivered may be calculated as described in the preceding example.

Example 65: In vivo delivery of protein

This example describes the delivery of therapeutic agents to the eye by fusosomes.

Fusosomes are derived from hematopoietic stem and progenitor cells using any of the methods described in previous Examples and are loaded with a protein that is deficient in a mouse knock-out.

Fusosomes are injected subretinally into the right eye of a mouse that is deficient for the protein and vehicle control is injected into the left eye of the mice. A subset of the mice is euthanized when they reach 2 months of age.

Histology and H&E staining of the harvested retinal tissue is conducted to count the number of cells rescued in each retina of the mice (described in Sanges et al., The Journal of Clinical Investigation, 126(8): 3104-3116, 2016).

The level of the injected protein is measured in retinas harvested from mice euthanized at 2 months of age via a western blot with an antibody specific to the PDE6B protein.

In an embodiment, the left eyes of mice, which are administered fusosomes, will have an increased number of nuclei present in the outer nuclear level of the retina compared to the right

eyes of mice, which are treated with vehicle. The increased protein is suggestive of complementation of the mutated PBE6B protein.

Example 66: Assessment of teratoma formation after administration of fusosome

This Example describes the absence of teratoma formation with a fusosome. In an embodiment, a fusosome will not result in teratoma formation when administered to a subject.

The fusosomes are produced by any one of the methods described in a previous Example. Fusosomes, tumor cells (positive control) or vehicle (negative control) are subcutaneously injected in PBS into the left flank of mice (12-20 weeks old). Teratoma, e.g., tumor, growth is analyzed 2-3 times a week by determination of tumor volume by caliper measurements for eight weeks after fusosome, tumor cell, or vehicle injection.

In an embodiment, mice administered fusosomes or vehicle will not have a measurable tumor formation, e.g., teratoma, via caliper measurements. In an embodiment, positive control animals treated with tumor cells will demonstrate an appreciable tumor, e.g., teratoma, size as measured by calipers over the eight weeks of observation.

Example 67: Measuring total RNA in a fusosome and source cell

This Example describes a method to quantify the amount of RNA in a fusosome relative to a source cell. In an embodiment, a fusosome will have similar RNA levels to the source cell. In this assay, RNA levels are determined by measuring total RNA.

Fusosomes are prepared by any one of the methods described in previous Examples. Preparations of the same mass as measured by protein of fusosomes and source cells are used to isolate total RNA (e.g., using a kit such as Qiagen RNeasy catalog #74104), followed by determination of RNA concentration using standard spectroscopic methods to assess light absorbance by RNA (e.g. with Thermo Scientific NanoDrop).

In an embodiment, the concentration of RNA in fusosomes will be 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% of that of source cells per mass of protein.

Example 68: Isolating fusogenic microvesicles freely released from cells

This example describes the isolation of fusogenic microvesicles freely released from cells. Fusogenic microvesicles were isolated as follows. 9.2 x 10^6 HEK-293T (ATCC, Cat# CRL-3216) were reverse transfected using Xfect transfection reagent (Takara, Cat# 631317) with 10 μg of the pcDNA3.1 expression plasmid containing the open reading frame for VSVg and 15ug of the pcDNA3.1 expression plasmid containing the open reading frame for bacteriophage P1 Cre Recombinase with a SV40 Nuclear localization sequence in 7.5mL of complete media (Dulbecco's Modified Eagle Medium (DMEM) supplemented with GlutaMAX (ThermoFisher), 10% fetal calf serum (ThermoFisher), and penicillin/streptomycin antibiotics (ThermoFisher)) in a 100mm collagen coated dish (Corning). Twelve hours after seeding, an additional 7.5mL of complete medium was carefully added. The cells were separated from culture media by centrifugation at 200 *x g* for 10 minutes. Supernatants were collected and centrifuged sequentially twice at 500 x g for 10 minutes, once at 2,000 x g for 15 minutes, once at 10,000 x g for 30 min, and once at 70,000 x g for 60 minutes. Freely released fusosomes were pelleted during the final centrifugation step, resuspended in PBS and repelleted at 70,000 x g. The final pellet was resuspended in PBS.

See also, Wubbolts R *et al.* Proteomic and Biochemical Analyses of Human B Cell-derived Exosomes: Potential Implications for their Function and Multivesicular Body Formation. *J. Biol. Chem.* 278:10963-10972 2003.

Example 69: Measuring the average size distribution of fusosomes

This Example describes measurement of the size distribution of fusosomes.

Fusosomes were prepared as described herein by transient transfection of HEK293T with VSV-G, enucleation and subsequent fractionation with Ficoll. The fusosomes were measured to determine the size distribution using the method of Example 28, as shown in FIG. 3. It is contemplated that the fusosomes can have less than about 50%, 40%, 30%, 20%, 10%, 5%, or less of the parental cell's variability in size distribution within 90% of the sample. It is contemplated that the fusosomes can have 58% less of the parental cell's variability in size distribution within 90% of the sample.

Example 70: Average volume of fusosomes

This example describes measurement of the average volume of fusosomes. Varying the size (e.g., volume) of fusosomes can make them versatile for distinct cargo loading, therapeutic design or application.

Fusosomes were prepared as described herein by transient transfection of HEK293T with VSV-G, enucleation and subsequent fractionation with Ficoll. The positive control was HEK293T cells.

Analysis with a combination of NTA and confocal microscopy as described in Example 28 was used to determine the size of the fusosomes. The diameter of the fusosomes were measured and the volume calculated, as shown in FIG. 4. It is contemplated that fusosomes can have an average size of greater than 50 nm in diameter. It is contemplated that fusosomes can have an average size of 129 nm in diameter.

Example 71: Comparison of soluble to insoluble protein mass

This Example describes quantification of the soluble:insoluble ratio of protein mass in fusosomes. The soluble:insoluble ratio of protein mass in fusosomes can, in some instances, be similar to that of nucleated cells.

Fusosomes were prepared as described herein by transient transfection of HEK293T with VSV-G, enucleation and subsequent fractionation with Ficoll. The fusosome preparation was tested to determine the soluble:insoluble protein ratio using a standard bicinchoninic acid assay (BCA) (Pierce™ BCA Protein Assay Kit, Thermo Fischer product# 23225). Soluble protein samples were prepared by suspending the prepared fusosomes or parental cells at a concentration of $1x10^7$ cells or ~1 mg/mL total fusosomes in PBS and centrifuging at 1,500 x g to pellet the cells or 16,000 x g to pellet the fusosomes. The supernatant was collected as the soluble protein fraction.

The fusosomes or cells were then resuspended in PBS. This suspension represents the insoluble protein fraction.

A standard curve was generated using the supplied BSA, from 0 to 15 µg of BSA per well (in duplicate). The fusosome or cell preparation was diluted such that the quantity measured is within the range of the standards. The fusosome preparation was analyzed in duplicate and the

mean value was used. The soluble protein concentration was divided by the insoluble protein concentration to yield the soluble:insoluble protein ratio (FIG. 5).

Example 72: Measuring fusion with a target cell

Fusosomes derived from HEK-293T cells expressing the engineered hemagglutinin glycoprotein of measles virus (MvH) and the fusion protein (F) on the cell surface and containing Cre recombinase protein were generated, as described herein. The MvH was engineered so that its natural receptor binding is ablated and target cell specificity is provided through a single-chain anbitody (scFv) that recognizes the cell surface antigen, in this case the scFv is designed to target CD8, a co-receptor for the T cell receptor. A control fusosome was used which was derived from HEK-293T cells expressing the fusogen VSV-G on its surface and containing Cre recombinase protein. The target cell was a HEK-293T cell engineered to express a "Loxp-GFP-stop-Loxp-RFP" cassette under CMV promoter, as well as engineered to over-express the co-receptors CD8a and CD8b. The non-target cell was the same HEK-293T cell expressing "Loxp-GFP-stop-Loxp-RFP" cassette but without CD8a/b over-expression. The target or non-target recipient cells were plated 30,000 cells/well into a black, clear-bottom 96-well plate and cultured in DMEM media with 10% fetal bovine serum at 37ºC and 5% CO_2. Four to six hours after plating the recipient cells, the fusosomes expressing Cre recombinase protein and MvH+F were applied to the target or non-target recipient cells in DMEM media. Recipient cells were treated with 10 μg of fusosomes and incubated for 24 hours at 37°C and 5% CO_2.

Cell plates were imaged using an automated microscope (www.biotek.com/products/imaging-microscopy-automated-cell-imagers/lionheart-fx-automated-live-cell-imager/). The total cell population in a given well was determined by staining the cells with Hoechst 33342 in DMEM media for 10 minutes. Hoechst 33342 stains cell nuclei by intercalating into DNA and therefore is used to identify individual cells. The Hoechst was imaged using the 405 nm LED and DAPI filter cube. GFP was imaged using the 465 nm LED and GFP filter cube, while RFP was imaged using 523 nm LED and RFP filter cube. Images of target and non-target cell wells were acquired by first establishing the LED intensity and integration times on a positive-control well; i.e., recipient cells treated with adenovirus coding for Cre recombinase instead of fusosomes.

Acquisition settings were set so that Hoescht, RFP, and GFP intensities are at the maximum pixel intensity values but not saturated. The wells of interest were then imaged using the established settings. Focus was set on each well by autofocusing on the Hoescht channel and then using the established focal plane for the GFP and RFP channels. Analysis of GFP and RFP-positive cells was performed with Gen5 software provided with automated fluorescent microscope (https://www.biotek.com/products/software-robotics-software/gen5-microplate-reader-and-imager-software/).

The images were pre-processed using a rolling ball background subtraction algorithm with a 60 μm width. Cells with GFP intensity significantly above background intensities were thresholded and areas too small or large to be GFP-positive cells were excluded. The same analysis steps were applied to the RFP channel. The number of RFP-positive cells (recipient cells receiving Cre) was then divided by the sum of the GFP-positive cells (recipient cells that did not show delivery) and RFP-positive cells to quantify the percent RFP conversion, which describes the amount of fusosome fusion within the target and non-target recipient cell population. For amounts of targeted fusion (fusosome fusion to targeted recipient cells), the percent RFP conversion value is normalized to the percentage of recipient cells that are target recipient cells (i.e., expressing CD8), which was assessed by staining with anti-CD8 antibody conjugated to phycoerythrin (PE) and analyzed by flow cytometry. Finally, the absolute amount of targeted fusion was determined by subtracting the amount of non-target cell fusion from the target cell fusion amount (any value <0 was considered to be 0).

With this assay, the fusosome derived from a HEK-293T cell expressing the engineered MvH(CD8)+F on its surface and containing Cre recombinase protein showed a percentage RFP conversion of 25.2 +/- 6.4% when the recipient cell was the target HEK-293T cell expressing the "Loxp-GFP-stop-Loxp-RFP" cassette, and 51.1% of these recipient cells were observed to be CD8-positive. From these results, the normalized percentage RFP conversion or amount of targeted fusion was determined to be 49.3 +/- 12.7% for targeted fusion. The same fusosome showed a percentage RFP conversion of 0.5 +/- 0.1% when the recipient was the non-target HEK-293T cell expressing "Loxp-GFP-stop-Loxp-RFP" but with no expression of CD8. Based on the above, the absolute amount of targeted fusion for the MvH(CD8)+F fusosome determined

to be 48.8% and the absolute amount of targeted fusion for the control VSV-G fusosome was determined to be 0% (FIG. 6).

Example 73: Measuring ability to transport glucose across cell membrane

Fusosomes from HEK-293T cells expressing the envelope glycoprotein G from vesicular stomatitis virus (VSV-G) on the cell surface and expressing Cre recombinase protein were generated according by the standard procedure of ultracentrifugation through a Ficoll gradient to obtain small particle fusosomes as described herein. To measure the ability of the fusosomes to transport glucose across the cell membrane, the levels of a 2-NBDG (2-(*N*-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)Amino)-2-Deoxyglucose) fluorescent glucose analog, that can be used to monitor glucose uptake in live cells, was quantified to assess active transport across the lipid bilayer. A commercially-available kit from Biovision Inc. (Cat #K682) was used for the assay according to manufacturer's instructions.

Briefly, the fusosome sample was measured for total protein content by bicinchoninic acid assay (BCA, ThermoFisher, Cat #23225) according to manufacturer's instructions. Next 40ug of fusosome total protein was pelleted by centrifugation at 3000g for 5 minutes in a table-top centrifuge, followed by resuspension in 400uL of DMEM supplemented with 0.5% fetal bovine serum. This was done in duplicate for each sample, and one of the duplicates was treated with 4uL of phloretin (provided with the kit), a natural phenol that inhibits glucose uptake, as a control for glucose uptake inhibition. The samples were then incubated for 1 hour at room temperature. After the incubation, the fusosome sample was pelleted and resuspended in 400uL of glucose uptake mix prepared previously (see Table 12 below for formulation). Samples pre-treated with phloretin were resuspended in glucose uptake mix with phloretin; samples not pre-treated were resuspended in glucose uptake mix with 20uL of PBS instead of phloretin. Also a parallel set of fusosome samples were resuspended in DMEM media with 0.5% FBS only as a negative control for flow cytometry analysis.

Table 12: Glucose uptake mix formulation

Reagent	Volume (uL)
DMEM media with 0.5% FBS	1880
2-NBDG reagent	20

Glucose Uptake Enhancer	100
Optional: Phloretin	20

The samples were then incubated at 37°C with 5% CO_2 for 30 minutes. After the incubation cells were pelleted, washed once with 1mL of 1X Analysis Buffer (provided with kit), pelleted again, and resuspended in 400uL of 1X Analysis Buffer.

The samples were then measured for 2-NBDG uptake by flow cytometry analysis using an Invitrogen Attune NxT acoustic focusing cytometer. 2-NBDG was excited with a 488nm laser and emission captured at 513±26nm. Forward and side scatter gating was initially used to capture fusosome-sized events and discard small debris. Events positive for 2-NBDG were determined by gating at the minimum level for which the 2-NBDG negative control sample showed <0.5% of events positive for 2-NBDG staining. The gated cells positive for 2-NBDG fluorescence were then assessed for the mean fluorescence intensity (F.I.) of 2-NBDG in order to calculate a value for glucose uptake for the fusosomes with and without phloretin treatment.

With this assay, the fusosome derived from a HEK-293T cell expressing the VSV-G and Cre showed a 2-NBDG mean F.I. of 631.0 +/- 1.4 without phloretin treatment and a mean F.I. of 565.5 +/- 4.9 with phloretin treatment (FIG. 7).

Example 74: Measuring esterase activity in the cytosol

Fusosomes from C2C12 cells were generated according to the standard procedure of ultracentrifugation through a Ficoll gradient to obtain small particle fusosomes as described herein. To measure the esterase activity in the cytosol of the fusosomes, samples were stained with Calcein AM (BD Pharmigen, Cat #564061), a fluorescein derivative and nonfluorescent vital dye that passively crosses the cell membrane of viable cells and is converted by cytosolic esterases into green fluorescent calcein, which is retained by cells with intact membranes and inactive multidrug resistance protein.

Briefly, the fusosome sample was measured for total protein content by bicinchoninic acid assay (BCA, ThermoFisher, Cat #23225) according to manufacturer's instructions. Next 20ug of fusosome total protein was pelleted by centrifugation at 3000g for 5 minutes in a table-top centrifuge, followed by resuspension in 400uL of DMEM supplemented with 0.5% fetal bovine serum. The membrane-permeable dye, calcein-AM was prepared as a stock solution of 10

mM in dimethylsulfoxide and as a working solution of 1 mM in PBS buffer, pH 7.4. VSV-G fusosomes were stained with 1 μM solution of calcein-AM diluted in DMEM media. Samples were incubated at 37°C in the dark for 30 minutes and then pelleted by centrifugation. After washing twice with PBS buffer, fusosomes were resuspended in PBS and analyzed by flow cytometry.

The samples were measured for calcein fluorescence retention using an Invitrogen Attune NxT acoustic focusing cytometer. Calcein AM was excited with a 488nm laser and emission captured at 513±26nm. Forward and side scatter gating was initially used to capture fusosome-sized events and discard small debris. Events positive for calcein were determined by gating at the minimum level for which the calcein negative control sample showed <0.5% of events positive for calcein staining. The gated cells positive for calcein fluorescence were then assessed for the mean fluorescence intensity (F.I.) of calcein in order to calculate a value for esterase activity in the cytosol of fusosomes.

With this assay the fusosome derived from a C2C12 cell showed an esterase activity (mean calcein F.I.) of 631.0 +/- 1.4 (FIG. 8).

Example 75: Measuring acetylcholinesterase activity in fusosomes

Fusosomes from HEK-293T cells expressing the placental cell-cell fusion protein syncytin-1 (Syn1) on the cell surface and expressing Cre recombinase protein were generated as described herein. Acetylcholinesterase activity was measured using the FluoroCet Quantitation Kit (System Biosciences, Cat #FCET96A-1) following the manufacturer's recommendations.

Briefly, fusosomes were pelleted via ultracentrifugation at 120,000 g for 90 minutes and resuspended carefully in phosphate-buffered saline (PBS). Next fusosomes were quantified for total protein content by bicinchoninic acid assay (BCA, ThermoFisher, Cat #23225) according to manufacturer's instructions. After BCA quantification of protein concentration, 1000ng of total fusosome protein was diluted with PBS to a volume of 60uL, followed by addition of 60uL of Lysis Buffer to lyse the particles. After a 30 minute incubation on ice the samples were ready to run in the FluoroCet assay.

In duplicate wells of a 96-well plate, 50uL of lysed fusosome sample was mixed with 50uL of Working stock of Buffer A and 50uL of Working stock of Buffer B. In parallel, a

standard curve was prepared by pipetting 2uL of the provided standard in 126uL of 1X Reaction buffer. This standard solution was then serial diluted 5X to make a six-point standard curve consisting of 2.0E+08, 1.0E+08, 5.0E+07, 2.5E+07, 1.25E+07, and 6.25E+06 exosome equivalents of acetylcholinesterase activity. 50uL of each standard was then mixed with 50uL of Working stock of Buffer A and 50uL of Working stock of Buffer B in duplicate wells of the 96-well plate. 50uL of 1X Reaction buffer was used as a blank. The plate was mixed by tapping the sides followed by incubation in the dark for 20 minutes at room temperature. The plate was then measured immediately using a fluorescence plate reader set at Excitation: 530-570nm and Emission: 590- 600nm. The plate was shaken for 30 sec before reading.

The relative fluorescence units (RFU) were then plotted against the known exosome equivalents of acetylcholinesterase activity after subtracting the RFU values from the blank wells. A linear regression line was then calculated and the equation used to determine the acetylcholinesterase activity (in exosome equivalents) for the fusosome samples from the measured RFU values. The measured acetylcholinesterase activity for Syn1 fusosomes are shown in Table 13:

Table 13: Acetylcholinesterase activity in fusosomes and control particles

Sample	**Acetylcholinesterase activity (exosome equivalents)**
Syn1 fusosomes	6.83E+05 +/- 2.21E+05

Example 76: Measuring metabolic activity level

Fusosomes from HEK-293T cells expressing the envelope glycoprotein G from vesicular stomatitis virus (VSV-G) on the cell surface and expressing Cre recombinase protein were generated as described herein. To determine the metabolic activity level of the fusosome preparation, citrate synthase activity was assessed using a commercially available kit from Sigma (Cat #CS0720) which provides all of the necessary reagents. Citrate synthase is an enzyme within the tricarboxylic acid (TCA) cycle that catalyzes the reaction between oxaloacetate (OAA) and acetyl-CoA to generate citrate. Upon hydrolysis of acetyl-CoA, there is a release of CoA with a thiol group (CoA-SH). The thiol group reacts with a chemical reagent, 5,5-Dithiobis-(2-nitrobenzoic acid) (DTNB), to form 5-thio-2-nitrobenzoic acid (TNB), which has a yellow product that can be measured spectrophotometrically at 412 nm.

The assay was performed as per the manufacturer's recommendations. Briefly, fusosome sample was measured for total protein content by bicinchoninic acid assay (BCA, ThermoFisher, Cat #23225) according to manufacturer's instructions. Next 400ug of fusosome total protein was pelleted by centrifugation at 3000g for 5 minutes in a table-top centrifuge. The fusosomes were washed once by pelleting again and resuspending in ice-cold PBS. Fusosomes were pelleted again and supernatant was removed. The pellet was lysed in 100uL of CellLytic M buffer with 1X protease inhibitors. After mixing by pipetting, the lysed sample was incubated for 15 minutes at room temperature to complete lysis. The sample was then centrifuged at 12,000g for 10 minutes and the supernatant was transferred to a new microcentrifuge tube and stored at -80°C until the subsequent assay was performed.

To initiate the citrate synthase activity assay, all assay solutions were warmed to room temperature prior to using. The lysed fusosome sample was mixed with assay solutions according to Table 14 below:

Table 14: Reaction Scheme for Citrate Synthase Activity Measurement in 96 Well Plate

Sample	Assay buffer	30mM Acetyl CoA solution	10mM DTNB solution	10mM OAA solution (added last)
4uL	182uL	2uL	2uL	10uL

The volumes in Table 14 represent volumes for a single well of a 96-well plate. Samples were measured in duplicates. All components of the reaction were mixed and pipetted into a single well of a 96-well plate. The absorbance at 412nm was then analyzed on a microplate reader for 1.5 minutes to measure the baseline reaction. Next, 10uL of the 10mM OAA solution was added to each well to initiate the reaction. The plate was shaken for 10 seconds in the microplate reader before reading the absorbance at 412nm for 1.5 minutes with a measurement every 10 seconds.

To calculate the citrate synthase activity, the absorbance at 412nm was plotted against time for each reaction. The change in absorbance per minute was calculated for the linear range of the plot for before (endogenous activity) and after (total activity) OAA addition. The net citrate synthase activity was then calculated by subtracting the endogenous activity from the total activity for the sample. This value was then used to calculate the citrate synthase activity based

on the equation and constant values provided by the manufacturer. The measured citrate synthase activity for the VSV-G fusosomes was 1.57E-02 +/- 1.86E-03 umol/ug fusosome/min.

Example 77: Measuring respiration levels

Fusosomes from HEK-293T cells expressing the envelope glycoprotein G from vesicular stomatitis virus (VSV-G) on the cell surface were generated according by the standard procedure of ultracentrifugation through a Ficoll gradient to obtain small particle fusosomes as described herein. Respiration level in the fusosome preparation were determined by measuring mitochondrial oxygen consumption rates by a Seahorse extracellular flux analyzer (Agilent).

Briefly, the fusosome sample was measured for total protein content by bicinchoninic acid assay (BCA, ThermoFisher, Cat #23225) according to manufacturer's instructions. Next 20 µg of fusosome total protein was pelleted by centrifugation at 3000g for 5 minutes in a table-top centrifuge, followed by resuspension (in quadruplicates) in 150 µL of XF Assay media (Agilent Cat # 103575-100) supplemented with 25 mM glucose and 2 mM glutamine (pH 7.4). The resuspended samples were then added to one well of a 96-well Seahorse plate (Agilent).

Oxygen consumption assays were initiated by incubating the 96-well Seahorse plate with samples at 37°C for 60 minutes to allow temperature and pH to reach equilibrium. The microplate was then assayed in the XF96 Extracellular Flux Analyzer (Agilent) to measure extracellular flux changes of oxygen and pH in the media immediately surrounding the fusosomes. After obtaining steady state oxygen consumption and extracellular acidification rates, oligomycin (5µM), which inhibits ATP synthase, and proton ionophore FCCP (carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone; 2µM), which uncouples mitochondria, were injected sequentially through reagent delivery chambers for each well in the microplate to obtain values for maximal oxygen consumption rates. Finally, 5 µM antimycin A (inhibitor of mitochondrial complex III) was injected to confirm that respiration changes were due mainly to mitochondrial respiration. The rates of antimycin A respiration were subtracted from the other three respiration rates in order to determine the basal, uncoupled (oligomycin-resistant), and maximal (FCCP-induced) mitochondrial respiration rates.

Using this assay it was determined that donor VSV-G fusosomes showed basal, uncoupled, and maximal oxygen consumption (respiration) rates according to Table 15 below.

Table 15: Respiration rates of VSV-G fusosomes

Respiration state	**Mitochondrial oxygen consumption (respiration) rate (pmol/min/20µg fusosome) AVG ± SEM**
Basal	11.3 ± 3.0
Uncoupled	10.1 ± 2.3
Maximal	20.0 ± 1.9

Example 78: Measuring phosphatidylserine levels of fusosomes

Fusosomes from HEK-293T cells expressing the envelope glycoprotein G from vesicular stomatitis virus (VSV-G) on the cell surface and expressing Cre recombinase protein were generated according by the standard procedure of ultracentrifugation through a Ficoll gradient to obtain small particle fusosomes as described herein. To measure the phosphatidylserine levels of the fusosomes, annexin V staining was performed using a commercially available annexin V conjugated with Alexa Fluor 647 dye (Cat #A23204) according to the manufacturer's instructions. Annexin V is a cellular protein that can bind phosphatidylserine when it is exposed on the outer leaflet of the plasma membrane; thus, the readout of annexin V binding to a sample can provide an assessment of phosphatidylserine levels in the sample.

Briefly, the fusosome sample was measured for total protein content by bicinchoninic acid assay (BCA, ThermoFisher, Cat #23225) according to manufacturer's instructions. Next 40 µg of fusosome total protein was pelleted by centrifugation (in sample triplicates) at 3000g for 5 minutes in a table-top centrifuge, followed by resuspension in 400uL of DMEM supplemented with 2% fetal bovine serum. One sample was treated with 40 µM antimycin A. The samples were then incubated for 1 hour at 37C. After the incubation samples were then pelleted by centrifugation again and resuspended in 100 µL annexin-binding buffer (ABB; 10 mM HEPES, 140 mM NaCl, 2.5 mM $CaCl_2$, pH 7.4). Next 5 µL of annexin V conjugated with Alexa Fluor 647 was added to each sample (except for the negative control with no annexin V staining). The samples were incubated for 15 minutes at room temperature followed by addition of 400 µL ABB.

The samples were then measured for annexin V staining by flow cytometry analysis using an Invitrogen Attune NxT acoustic focusing cytometer. Annexin V conjugated with Alexa

Fluor 647 was excited with a 638 nm laser and emission captured at 670 ± 14 nm. Forward and side scatter gating was initially used to capture fusosome-sized events and discard small debris. Events positive for Alexa Fluor 647 (annexin V) staining were determined by gating at the minimum level for which the unstained, annexin V-negative control sample showed <0.5% of events positive for Alexa Fluor 647 staining. The gated events positive for Alexa Fluor 647 staining were then assessed for the percentage of annexin V-positive events of the total parent population (fusosome-sized events in the forward/side scatter gate) and this value was used as the quantification of phosphatidylserine levels in the fusosome sample.

With this assay the fusosome derived from a HEK-293T cell expressing the VSV-G and Cre showed a % annexin V-positive fusosomes of 63.3 ± 2.3% without antimycin A treatment and percentage of annexin V-positive fusosomes of 67.6 ± 5.7% with antimycin A treatment.

Example 79: Measuring average mitochondrial membrane potential

Fusosomes from HEK-293T cells expressing the envelope glycoprotein G from vesicular stomatitis virus (VSV-G) on the cell surface and expressing Cre recombinase protein were generated according by the standard procedure of ultracentrifugation through a Ficoll gradient to obtain small particle fusosomes as described herein. To measure the average mitochondrial membrane potential levels of the fusosomes, a commercially available dye that is mitochondrial membrane potential sensitive, tetramethyl rhodamine, ethyl ester, perchlorate (TMRE; Abcam, Cat# T669) was used for assessing mitochondrial membrane potential. To normalize TMRE fluorescence intensity (FI) to the amount of mitochondria in the sample, MitoTracker Green FM dye (MTG; ThermoFisher, Cat #M7514) was used to co-stain samples in order to normalize TMRE FI to the MTG FI and thus to the amount of mitochondria in the sample. In addition, carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP; Sigma Cat #C2920) was used to treat a parallel set of samples in order to fully depolarize the mitochondrial membrane potential and thus allow quantification of mitochondrial membrane potential in millivolts based on the decrease in TMRE FI.

Briefly, the fusosome sample was measured for total protein content by bicinchoninic acid assay (BCA, ThermoFisher, Cat #23225) according to manufacturer's instructions. Next 40 µg of fusosome total protein was pelleted by centrifugation (in sample quadruplicates for

untreated and FCCP-treated duplicates) at 3000g for 5 minutes in a table-top centrifuge, followed by resuspension in 100uL of DMEM supplemented with 2% fetal bovine serum and containing TMRE and MTG dyes at a final concentration of 30 nM and 200 nM, respectively. A parallel set of fusosome samples was left unstained as a negative control. The samples were incubated at for 45 minutes at 37ºC. After incubation, samples were pelleted by centrifugation and resuspended in 400 µL of phenol red-free DMEM media containing 30 nm TMRE. One set of duplicates was treated with 20 µM FCCP for 5 minutes before assessment by flow cytometry.

The samples were then measured for annexin V staining by flow cytometry analysis using an Invitrogen Attune NxT acoustic focusing cytometer. MTG was excited with a 488 nm laser and emission captured at 530 ± 30 nm. TMRE was excited with a 561 nm laser and emission captured at 585 ± 16 nm. Forward and side scatter gating was initially used to capture fusosome-sized events and discard small debris. Events positive for MTG and TMRE staining were determined by gating at the minimum level for which the unstained control sample showed <0.5% of events positive for MTG or TMRE staining. The gated events positive for MTG and TMRE staining were then assessed for the mean FI of MTG and TMRE.

Membrane potential values (in millivolts, mV) are calculated based on the intensity of TMRE after normalizing TMRE FI values to MTG FI values. This TMRE/MTG ratio value allows for normalization TMRE intensity to the amount of mitochondria in the sample. The TMRE/MTG ratio value for both the untreated and FCCP-treated samples are calculated and used to determine the membrane potential in millivolts using a modified Nernst equation (see below) that can determine mitochondrial membrane potential based on TMRE fluorescence (as TMRE accumulates in mitochondria in a Nernstian fashion). Fusosome membrane potential is calculated with the following formula: (mV) = -61.5 * log(FI(untreated)/FI(FCCP-treated)). Using this equation, the calculated mitochondrial membrane potential of the VSV-G fusosome sample was -29.6 ± 1.5 millivolts.

Example 80: Measuring targeting potential in a subject (BiVs- Cre Gesicles)

This example assesses the ability of a fusosome to target a specific body site. Fusosomes were derived using methods as described herein and were loaded with cre-recombinase protein.

Two doses of fusosomes (1x and 3x) were delivered into Loxp Luciferase (Jackson Laboratory, 005125) mice were injected intravenously (I.V.) via tail vein. Mice were placed underneath a heat lamp (utilizing a 250W(infrared) heat lamp bulb) for ~5 minutes (or until mice begin to groom their whiskers excessively) to dilate the tail vein. Mice were placed on a restrainer and tail was wiped down with 70% ethanol to better visualize the vein.

Using a tuberculin syringe, 200 µL of fusosome 1x solution (8.5e8±1.4e8 particles/ µL, mean(SEM)) or 3x solution (2.55e9±1.4e8 particles/ µL, mean(SEM)) was injected IV. Upon completion of injection, the syringe was removed, and pressure was applied to the injection site.

After fusion, CRE protein translocated to the nucleus to carry out recombination, which resulted in the constitutive expression of luciferase. Three days post-treatment, the ventral region of subjects was prepared by depilating the area (Nair Hair Remover cream for 45 seconds, followed by cleaning the area with 70% ethanol). Subjects were then treated with D-luciferin (Perkin Elmer, 150 mg/kg) via intraperitoneal administration. This enabled the detection of luciferase expression via in vivo bioluminescent imaging. The animal was placed into an *in vivo* bioluminescent imaging chamber (Perkin Elmer) which houses a cone anesthetizer (isoflurane) to prevent animal motion. Photon collection was carried out between 3-15 minutes post-injection to observe the maximum bioluminescent signal due to D-luciferin pharmacokinetic clearance. Maximum radiance was recorded in photons/sec/cm2/radians. Total flux, which integrates the radiance over the area, was quantified using a region of interest (ROI) tool within the Living Image Software (Perkin Elmer) and reported in photons/sec.

Evidence of protein (Cre recombinase) delivery by fusosomes was detected by bioluminescent imaging in the recipient tissue of the animal, as shown in FIGS. 9A-9B. Signal was seen primarily in the spleen and liver, with the 3x group showing the highest signal.

Following whole body imaging, mice were cervically dislocated and liver, heart, lungs, kidney, small intestines, pancreas, and spleen were collected and imaged within 5 minutes of euthanasia. Evidence of protein (Cre recombinase) delivery to the liver and spleen by fusosomes was detected by bioluminescent imaging in the extracted recipient tissue of the animals. This can be seen in FIGS. 10A-10B. Signal was highest in spleen and the lowest in heart, with the 3x group showing the highest significant signal ($p=0.0004$ as compared to heart).

Example 81: Delivery of fusosomes via a pathway that is independent of lysosome acidification

Often, entry of complex biological cargo into target cells is accomplished by endocytosis. Endocytosis requires the cargo to enter an endosome, which matures into an acidified lysosome. Disadvantageously, cargo that enters a cell through endocytosis may become trapped in an endosome or lysosome and be unable to reach the cytoplasm. The cargo may also be damaged by acidic conditions in the lysosome. Some viruses are capable of non-endocytic entry into target cells; however this process is incompletely understood. This example demonstrates that a viral fusogen can be isolated from the rest of the virus and confer non-endocytic entry on a fusosome that lacks other viral proteins.

Fusosomes from HEK-293T cells expressing the Nipah virus receptor-binding G protein and fusion F protein (NivG+F) on the cell surface and expressing Cre recombinase protein were generated according by the standard procedure of ultracentrifugation through a Ficoll gradient to obtain small particle fusosomes, as described herein. To demonstrate delivery of the fusosome to a recipient cell via a non-endocytic pathway, the NivG+F fusosomes were used to treat recipient HEK-293T cells engineered to express a "Loxp-GFP-stop-Loxp-RFP" cassette under CMV promoter. NivF protein is a pH-independent envelope glycoprotein that has been shown to not require environmental acidification for activation and subsequent fusion activity (Tamin, 2002).

The recipient cells were plated 30,000 cells/well into a black, clear-bottom 96-well plate. Four to six hours after plating the recipient cells, the NivG+F fusosomes expressing Cre recombinase protein were applied to the target or non-target recipient cells in DMEM media. The fusosome sample was first measured for total protein content by bicinchoninic acid assay (BCA, ThermoFisher, Cat #23225) according to manufacturer's instructions. Recipient cells were treated with 10 µg of fusosomes and incubated for 24 hrs at 37ºC and 5% CO2. To demonstrate that Cre delivery via NivG+F fusosomes was through a non-endocytic pathway, a parallel wells of recipient cells receiving NivG+F fusosome treatment were co-treated with an inhibitor of endosome/lysosome acidification, bafilomycin A1 (Baf; 100 nM; Sigma, Cat #B1793).

Cell plates were imaged using an automated microscope (www.biotek.com/products/imaging-microscopy-automated-cell-imagers/lionheart-fx-automated-live-cell-imager/). The total cell population in a given well was determined by

staining the cells with Hoechst 33342 in DMEM media for 10 minutes. Hoechst 33342 stains cell nuclei by intercalating into DNA and was therefore used to identify individual cells. Hoechst staining was imaged using the 405 nm LED and DAPI filter cube. GFP was imaged using the 465 nm LED and GFP filter cube, while RFP was imaged using the 523 nm LED and RFP filter cube. Images of target and non-target cell wells were acquired by first establishing the LED intensity and integration times on a positive control well containing recipient cells treated with adenovirus coding for Cre recombinase instead of fusosomes.

Acquisition settings were set so that Hoescht, RFP, and GFP intensities were at the maximum pixel intensity values but not saturated. The wells of interest were then imaged using the established settings. Focus was set on each well by autofocusing on the Hoescht channel and then using the established focal plane for the GFP and RFP channels. Analysis of GFP and RFP-positive cells was performed with Gen5 software provided with automated fluorescent microscope (https://www.biotek.com/products/software-robotics-software/gen5-microplate-reader-and-imager-software/).

The images were pre-processed using a rolling ball background subtraction algorithm with a 60 μm width. Cells with GFP intensity significantly above background intensities were thresholded and areas too small or large to be GFP-positive cells were excluded. The same analysis steps were applied to the RFP channel. The number of RFP-positive cells (recipient cells receiving Cre) was then divided by the sum of the GFP-positive cells (recipient cells that did not show delivery) and RFP-positive cells to quantify the percentage RFP conversion, which indicates the amount of fusosome fusion with the recipient cells.

With this assay, the fusosome derived from a HEK-293T cell expressing NivG+F on its surface and containing Cre recombinase protein showed significant delivery via a lysosome-independent pathway, which is consistent with entry via a non-endocytic pathway, as evidenced by a significant delivery of Cre cargo by NivG+F fusosomes even when recipient cells were co-treated with Baf to inhibit endocytosis-mediated uptake (FIG. 11). In this case, the inhibition of cargo delivery by Baf co-treatment was 23.4%.

Example 82: Measuring ability to polymerize actin for mobility

Fusosomes were generated by the standard procedure of harvesting and preparing fusosomes produced from HEK-293T cells expressing the envelope glycoprotein G from vesicular stomatitis virus (VSV-G) on the cell surface, as described herein. Control particles (non-fusogenic fusosomes) were produced from HEK-293T cells reverse transiently transfected with pcDNA3.1 empty vector. Fusosomes and parental cells were then assayed for their ability to polymerize actin (over time) using a rhodamine phalloidin-flow cytometry assay and Tubulin ELISA. Briefly, approximately $1x10^6$ fusosomes corresponding to 60 µL of a standard VSV-G fusosome preparation and $1x10^5$ parent cells used to generate the fusosomes were plated in 1 mL of complete media in a 96 well low-attachment multi-well plate in complete and incubated at 37°C and 5% CO_2. Samples were taken periodically, at 3 hr, 5 hr and 24 hr post plating. Samples were centrifuged at 21,000xg for 10mins, re-suspended in 200uL 4% (v/v) PFA in phosphate buffered saline for 10mins, washed with 1mL of phosphate buffered saline, centrifuged at 21,000xg for 10mins, washed again and stored at 4°C until further use.

For rhoamine-phalloidin staining, samples were centrifuged at 21,000xg for 10mins, and incubated in 100uL of 0.1% (v/v) Triton X-100 in phosphate buffered saline for 20 mins. Following the 20-min incubation, an additional 100uL of 0.1% (v/v) Triton X-100 in phosphate buffered saline containing 165 µM rhodamine-phalloidin was added to the sample and pipette mixed, negative control received and additional 100uL of 100uL of 0.1% (v/v) Triton X-100 in phosphate buffered saline only. Samples were incubated for 45 mins before being washed with 1mL of phosphate buffered saline, centrifuged at 21,000xg for 10mins, washed again and re-suspended in 300uL of phosphate buffered saline and analyzed by flow cytometry (Attune, ThermoFisher) using a 561 nm laser for excitation, and 585 +/- 16nm filter emission, as shown in the table below:

Flow cytometer settings

Dye	Attune Laser/Filter	Laser Wavelength	Emission Filter (nm)
AF47	YL1	585	585/16

Attune NxT software was used for acquisition and FlowJo used analysis. For data acquisition the FSC and SSC channels were set on linear axis to determine a population representative of the cells or fusosomes. This population was then gated and events only inside this gate were used to display events in the 585 +/- 16nm emission channel on a logarithmic scale. A minimum of 10,000 events within the cells or fusosomes gate was collected for in each condition. For data analysis, the FSC and SSC channels were set on linear axis to determine a population representative of the cells or fusosomes. This population was then gated and events only inside this gate were used to display events in the 585 +/- 16nm emission channel on a logarithmic scale. The negative control 585 +/- 16nm emission was used to determine where to place the gate on the histogram such that it was less the gate include less than 1% positive. Using analysis criteria listed above parent cells demonstrated 19.9%, 24.8% and 82.5% rhodamine-phalloidin positive events, at the 3hr, 5hr and 24hr time-points, respectively. The fusosomes were 44.6%, 41.9% and 34.9% rhodamine-phalloidin at the 3hr, 5hr and 24hr time-points, respectively (FIG. 2). This example demonstrates that fusosomes do not increase in amount of actin over time, whereas the parent cells do.

Example 83: Measuring GAPDH in fusosomes

This example describes quantification of the level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) in the fusosomes, and the relative level of GAPDH in the fusosomes compared to the parental cells. Fusosomes were prepared as described in Examples 68 and 87.

GAPDH was measured in the parental cells and the fusosomes using a standard commercially available ELISA for GAPDH (ab176642, Abcam) per the manufacturer's directions. Total protein levels were similarly measured via bicinchoninic acid assay. Measured GAPDH and protein levels are shown in the table below:

	[Protein] (mg/mL)	[GAPDH] (ng/mL)	GAPDH:Protein (µg/g)
Fusosomes	0.82	37.2	45.3
Cells	0.45	50.4	112.0

GAPDH: Total protein ratios are also shown in FIG. 12.

Example 84: Ratio of lipids to proteins in fusosomes

This Example describes quantification of the ratio of lipid mass to protein mass in fusosomes. It is contemplated that fusosomes can have a ratio of lipid mass to protein mass that is similar to that of nucleated cells. Fusosomes and parental cells were prepared as described herein in Examples 68 and 87.

The lipid content was calculated using choline-containing phospholipids as a subset of total lipids using a commercially available phospholipid assay kit (MAK122 Sigma St. Louis, MO) according to manufacturer's instructions. Total protein content of the fusosomes was measured via bicinchoninic acid assay as described herein. Measured phospholipid levels, protein levels, and the ratio of phospholipids to protein are shown in FIG. 13 and the table below:

	Phospholipids (μM)	Protein (g/L)	Phospholipids:Protein (μmol/g)
Fusosomes	115.6	0.82	141.0
Cells	47.9	0.45	106.4

Example 85: Ratio of proteins to DNA in fusosomes

This Example describes quantification of the ratio of protein mass to DNA mass in fusosomes. It is contemplated that fusosomes can have a ratio of protein mass to DNA mass that is much greater than that of cells. Fusosomes were prepared as described in Examples 68 and 87.

Total protein content of the fusosomes and cells was measured via bicinchoninic acid as described herein. The DNA mass of fusosomes and cells were measured by absorption at 280 nm after extraction of total DNA using a commercially available isolation kit (#69504 Qiagen Hilden, Germany) according to the manufacturer's instructions. The ratio of proteins to total nucleic acids was determined by dividing the total protein content by the total DNA content to yield a ratio within a given range for a typical fusosome preparation. Measured protein levels, DNA levels, and the ratio of protein to DNA are shown in FIG. 14 and the table below:

	[Protein] (mg/mL)	[DNA] (ng/μL)	Protein:DNA (g/g)
Fusosomes	0.82	29.5	27.8
Cells	0.45	15.9	28.3

Example 86: Ratio of lipids to DNA in fusosomes

This Example describes quantification of the ratio of lipids to DNA in fusosomes compared to parental cells. In an embodiment, fusosomes will have a greater ratio of lipids to DNA compared to parental cells. Fusosomes were prepared as described previously in Examples 68 and 87.

This ratio is defined as the lipid content outlined in Example 41, and nucleic acid content is determined as described in Example 42. Measured lipid levels, DNA levels, and the ratio of lipid to DNA are shown in FIG. 15 and the table below:

	[Lipids] (μM)	[DNA] (ng/μL)	Lipids:DNA (μmol/mg)
Fusosomes	115.6	29.5	3.92
Cells	47.9	15.9	3.01

Example 87: Measuring lipid composition in fusosomes

This Example describes quantification of the lipid composition of fusosomes. It is contemplated that the lipid composition of fusosomes can be similar to the cells from which they are derived. Lipid composition affects important biophysical parameters of fusosomes and cells, such as size, electrostatic interactions, and colloidal behavior.

The lipid measurements were based on mass spectrometry. Fusosomes were prepared as described herein by transient transfection of VSV-G and GFP in 10 cm dishes, followed by filtration and ultracentrifugation of the conditioned media 48 h after transfection to obtain fusosomes. Transfected cells were harvested in parallel to the conditioned media and submitted for analysis. Exosomes were also harvested from cells that were not transfected with VSV-G or GFP.

Mass spectrometry-based lipid analysis was performed by Lipotype GmbH (Dresden, Germany) as described (Sampaio et al. 2011). Lipids were extracted using a two-step chloroform/methanol procedure (Ejsing et al. 2009). Samples were spiked with internal lipid standard mixture containing: cardiolipin 16:1/15:0/15:0/15:0 (CL), ceramide 18:1;2/17:0 (Cer), diacylglycerol 17:0/17:0 (DAG), hexosylceramide 18:1;2/12:0 (HexCer), lyso-phosphatidate 17:0 (LPA), lyso-phosphatidylcholine 12:0 (LPC), lyso-phosphatidylethanolamine 17:1 (LPE), lyso-phosphatidylglycerol 17:1 (LPG), lyso-phosphatidylinositol 17:1 (LPI), lyso-

phosphatidylserine 17:1 (LPS), phosphatidate 17:0/17:0 (PA), phosphatidylcholine 17:0/17:0 (PC), phosphatidylethanolamine 17:0/17:0 (PE), phosphatidylglycerol 17:0/17:0 (PG), phosphatidylinositol 16:0/16:0 (PI), phosphatidylserine 17:0/17:0 (PS), cholesterol ester 20:0 (CE), sphingomyelin 18:1;2/12:0;0 (SM), triacylglycerol 17:0/17:0/17:0 (TAG) and cholesterol D6 (Chol).

After extraction, the organic phase was transferred to an infusion plate and dried in a speed vacuum concentrator. 1st step dry extract was re-suspended in 7.5 mM ammonium acetate in chloroform/methanol/propanol (1:2:4, V:V:V) and 2nd step dry extract in 33% ethanol solution of methylamine in chloroform/methanol (0.003:5:1; V:V:V). All liquid handling steps were performed using Hamilton Robotics STARlet robotic platform with the Anti Droplet Control feature for organic solvents pipetting.

Samples were analyzed by direct infusion on a QExactive mass spectrometer (Thermo Scientific) equipped with a TriVersa NanoMate ion source (Advion Biosciences). Samples were analyzed in both positive and negative ion modes with a resolution of $R_{m/z=200}$=280000 for MS and $R_{m/z=200}$=17500 for MSMS experiments, in a single acquisition. MSMS was triggered by an inclusion list encompassing corresponding MS mass ranges scanned in 1 Da increments (Surma et al. 2015). Both MS and MSMS data were combined to monitor CE, DAG and TAG ions as ammonium adducts; PC, PC O-, as acetate adducts; and CL, PA, PE, PE O-, PG, PI and PS as deprotonated anions. MS only was used to monitor LPA, LPE, LPE O-, LPI and LPS as deprotonated anions; Cer, HexCer, SM, LPC and LPC O- as acetate adducts and cholesterol as ammonium adduct of an acetylated derivative (Liebisch et al. 2006).

Data were analyzed with in-house developed lipid identification software based on LipidXplorer (Herzog et al. 2011; Herzog et al. 2012). Data post-processing and normalization were performed using an in-house developed data management system. Only lipid identifications with a signal-to-noise ratio >5, and a signal intensity 5-fold higher than in corresponding blank samples were considered for further data analysis.

Fusosome lipid composition was compared to lipid compositions of parental cells, with undetected lipid species assigned a value of zero. The lipid species identified in fusosomes and parental cells are shown in the table below:

	Total Lipid Species Identified	Shared Lipid Species (identified in both parental cells and fusomes)	Shared Lipid Species with 25% of parental expression in fusosomes	Fraction of Shared Lipid Species to Total Lipids
Fusosomes	679	569	548	0.700
Parental Cells	783			

It is contemplated that fusosomes and parental cells can have a similar lipid composition if ≥70% of the lipid species identified in any replicate sample of the parental cells are present in any replicate sample of the fusosomes, and of those identified lipids, the average level in the fusosome can be >25% of the corresponding average lipid species level in the parental cell.

Example 88: Measuring proteomic composition in fusosomes

This Example describes quantification of the protein composition of fusosomes. It is contemplated that the protein composition of fusosomes can be similar to the parental cells from which they are derived.

Fusosomes and parental cells were prepared as described herein by the method of Examples 68 and 87.

Each sample was resuspended in lysis buffer (6 M urea, 2 M thiourea, 4% CHAPS, 50 mM Tris pH 8.0), sonicated on an ice bath and ran through a small gauge syringe. Proteins were reduced with 10 mM DTT for 15 minutes at 65°C and alkylated with 15 mM iodoacetamide (IAA) for 30 minutes in the dark at room temperature. Excess IAA was quenched with an additional 10 mM DTT. Proteins were then precipitated with the addition of 8 volumes of ice cold acetone + 1 volume of ice cold methanol and placed at -80°C overnight. The precipitated proteins were pelleted by centrifugation. Remaining lysis buffer was washed with 200 µl of ice cold methanol 3 times. Proteins were resuspended in 0.75 M urea + 50 mM Tris pH 8.0 + 1 µg Trypsin/LysC and pre-digested for 4 hours at 37°C with agitation. An additional 1 µg of trypsin/LysC was added to the proteins and the digestion was continued overnight. Peptides were purified by reversed phase SPE and analyzed by LC-MS.

A replicate sample for each condition was lysed and combined in one tube. This pool was then either subjected to the same preparation protocol as the samples and analyzed by LC-MS in information dependent acquisition or separated on a gel as described below.

A total of 100 µg of pooled proteins was placed in 2x Laemmli loading buffer and separated on a 12.5% SDS PAGE. Proteins were briefly stained with Coomassie blue and the protein lanes were separated into 12 fractions. Each fraction was then dehydrated with 50% acetonitrile and rehydrated with 10 mM DTT for the reduction. Gel pieces were placed at 65°C for 15 minutes and alkylated for 30 minutes at room temperature with 15 mM IAA in the dark. Gels were further dehydrated with 50% acetonitrile and rehydrated in 50 mM Tris pH 8 with 1 µg of trypsin/LysC overnight at 37°C. Peptides were extracted from the gel by dehydration and sonication. Peptides were purified by reversed phase SPE and analyzed by LC-MS/MS (1xIDA per fraction).

Acquisition was performed with an ABSciex TripleTOF 5600 (ABSciex, Foster City, CA, USA) equipped with an electrospray interface with a 25 µm iD capillary and coupled to an Eksigent µUHPLC (Eksigent, Redwood City, CA, USA). Analyst TF 1.7 software was used to control the instrument and for data processing and acquisition. Acquisition was performed in Information Dependent Acquisition (IDA) mode for the 12 fractions from the gel or the unfractionated pool. The samples were analyzed in SWATH acquisition mode. For the IDA mode, the source voltage was set to 5.2 kV and maintained at 225°C, curtain gas was set at 27 psi, gas one at 12 psi and gas two at 10 psi. For the SWATH mode, the source voltage was set to 5.5 kV and maintained at 225°C, curtain gas was set at 25 psi, gas one at 16 psi and gas two at 15 psi. Separation was performed on a reversed phase HALO C18-ES column 0.3 mm i.d., 2.7 µm particles, 150 mm long (Advance Materials Technology, Wilmington, DE) which was maintained at 60°C. Samples were injected by loop overfilling into a 5 µL loop. For the 60 minutes LC gradient, the mobile phase consisted of the following solvent A (0.2% v/v formic acid and 3% DMSO v/v in water) and solvent B (0.2% v/v formic acid and 3% DMSO in EtOH) at a flow rate of 3 µL/min.

To generate the ion library for the analysis of the samples, the ProteinPilot software was run on the wiff files that were generated by the IDA runs. This database was used in the Peakview software (ABSciex) to quantify the proteins in each of the samples, using 3 transition/peptide and 15 peptide/protein. To maximize the number of quantified proteins, the samples were quantified on a publicly available human SWATH database (Atlas) with the same parameters. A peptide was considered as adequately measured if the score computed by

Peakview was superior to 1.5 and had an FDR < 1%. The quantification from each of the database was combined into one final quantification using the protein name from both databases. A correction factor was computed for every sample by taking into account the total signal of every protein in that sample when compared to the average of the total signal for every sample.

The fusosome proteomic composition was compared to the parental cell proteomic composition. A similar proteomic composition between fusosomes and parental cells was observed when >33% of the identified proteins were present in the fusosome, and of those identified proteins, the level was >25% of the corresponding protein level in the parental cell, as shown in the table below.

	Total Proteins Identified	Shared proteins (identified in both parental cells and fusomes)	Shared proteins with 25% of parental expression in fusosomes	Fraction of shared proteins to total proteins
Fusosomes	1926	1487	957	0.333
Cells	2870			

Example 89: Quantifying an endogenous or synthetic protein level per fusosome

This example describes quantification of an endogenous or synthetic protein cargo in fusosomes. Fusosomes can, in some instances, comprise an endogenous or synthetic protein cargo. The fusosome or parental cell described in this Example was engineered to alter the expression of an endogenous protein or express a synthetic cargo that mediates a therapeutic or novel cellular function.

Fusosomes and parental cells expressing GFP were prepared as described herein by the method of Examples 68 and 87. Quantification of GFP in fusosomes was accomplished using a commercially available ELISA kit (ab171581 Abcam Cambridge, United Kingdom) according to the manufacturer's instructions. Fusosome quantification was performed by Nanoparticle Tracking Analysis using a NanoSight NS300 (Malvern Instruments, Malvern, Worcestershire, United Kingdom). Results are shown in the table below.

	Concentration (#/mL)
GFP Protein	4.41×10^{13}
Fusosomes	2.66×10^{11}
GFP:Fusosome	165.8

It is contemplated that the fusosomes can have at least 1, 2, 3, 4, 5, 10, 20, 50, 100, or more protein agent molecules per fusosome. In an embodiment, the fusosomes will have 166 protein agent molecules per fusosome.

Example 90: Measuring markers of exosomal proteins in fusosomes

This assay describes quantification of the proportion of proteins that are known to be specific markers of exosomes.

Fusosomes were prepared as described herein by the method of Examples 68 and 87. Exosomes were prepared as described herein for fusosomes by the method of Examples 68 and 87with the exception that the parental cells were not transfected with VSV-G or GFP. Protein quantification by mass spectrometry for fusosomes and exosomes was performed as described herein in Example 36.

The resulting protein quantification data was analyzed to determine protein levels and proportions of the known exosomal marker CD63. Average log intensities per group were calculated by adding 1 to intensity values from mass spectrometry, transforming by log10, and computing the mean across replicates. The results are shown in FIG. 16.

Example 91: Measuring calnexin in fusosomes

This assay describes quantification of the level of calnexin (CNX) in the fusosomes, and the relative level of CNX in the fusosomes compared to the parental cells.

Fusosomes and parental cells were prepared as described herein in Examples 68 and 87. Calnexin and total protein was measured using mass spectrometry conducted according to the method of Example 36. The calnexin signal intensity determined for parental cells and fusosomes is shown in FIG. 17.

In embodiments, using this assay, the average fractional content (calculated as described herein in Example 36) of CNX in the fusosomes will be $< 2.43 \times 10^{-4}$.

In an embodiment, the decrease in calnexin per total protein in ng/µg from the parent cell to the preparation will be more than 88%.

Example 92: Ratio of lipids to DNA in fusosomes

This Example describes quantification of the ratio of lipids to DNA in fusosomes compared to parental cells. In an embodiment, fusosomes will have a greater ratio of lipids to DNA compared to parental cells. Fusosomes were prepared as described previously in Examples 68 and 87.

This ratio is defined as the lipid content outlined in Example 41, and nucleic acid content is determined as described in Example 42. As shown in FIG. 18 and in the table below, fusosomes were found to exhibit a greater lipid:DNA ratio than parental cells.

	[Lipids] (μM)	[DNA] (ng/μL)	Lipids:DNA (μmol/mg)
Fusosomes	115.6	29.5	3.92
Cells	47.9	15.9	3.01

Example 93: Analyzing surface markers on fusosomes

This assay describes identification of surface markers on the fusosomes.

Fusosomes were prepared as described herein in Examples 68 and 87. Phosphatidylserine was measured by mass spectrometry as described herein in Examples 68 and 87. The quantity of phosphatidylserine relative to total lipids in fusosomes was determined to be 121% greater than the quantity of phosphatidylserine relative to total lipid in parental cells, as shown in the table below.

	Phosphatidylserine (molar %)	Phosphatidylserine Percent change
Fusosomes	14.6	121%
Parental Cells	6.6	

Example 94: Analysis of viral capsid proteins in fusosomes

In this example, the makeup of the sample preparation was analyzed and the proportion of proteins that are derived from viral capsid sources was assessed.

Fusosomes were prepared as described herein by the method of Examples 68 and 87. Protein quantification by mass spectrometry for fusosomes was performed as described herein in Example 36. The fractional content of the viral capsid proteins was calculated as described herein in Example 36, averaged over fusosome samples, and expressed as a percent.

Using this approach, the sample was found to contain 0.05% viral capsid protein, as shown in the table below. The only viral capsid protein detected was Complex of Rabbit Endogenous Lentivirus (RELIK) Capsid with Cyclophilin A (PDB 2XGY|B).

	Raw MS Intensity	Viral:Total Protein (%)
Viral Capsid Proteins	5.10×10^5	0.05
Total Proteins	9.46×10^8	

Example 95: Quantification of fusogen protein ratios in fusosomes

This example describes quantification of the ratio of fusogen protein to total protein or other proteins of interest in fusosomes. Other proteins of interest may include, but are not limited to, EGFP, CD63, ARRDC1, GAPDH, Calnexin (CNX), and TSG101. Fusosomes were prepared as described herein by the method of Examples 68 and 87. Protein quantification by mass spectrometry for fusosomes was performed as described herein in Example 36. The quantification of all proteins was calculated as described herein in Example 36, averaged over fusosome samples, and expressed as a fraction.

As shown in the table below, the fusogen was found to have a ratio to EGFP of 156.9, a ratio to CD63 of 2912.0, a ratio to ARRDC1 of 664.9, a ratio to GAPDH of 69.0, a ratio to CNX of 558.4, and a ratio to TSG101 of 3064.1.

Proteins	Raw MS Intensity	Fusogen:Protein(s) Ratio
VSV-G	1.29×10^8	N/A
Total Proteins	9.46×10^8	0.136
EGFP	8.22×10^5	156.9
CD63	4.43×10^4	2912.0
ARRDC1	1.94×10^5	664.9
GAPDH	1.87×10^6	69.0
CNX	2.31×10^5	558.4
TSG101	4.21×10^4	3064.1

Example 96: Quantification of endogenous and synthetic protein ratios in fusosomes

This example describes the quantification of an endogenous or synthetic protein cargo relative to total protein or other proteins of interest in fusosomes. Other proteins of interest may include, but are not limited to, VSV-G, CD63, ARRDC1, GAPDH, Calnexin (CNX), or TSG101. Fusosomes were prepared as described herein by the method of Examples 68 and 87. Protein quantification by mass spectrometry for fusosomes was performed as described herein in

Example 36. The quantification of all proteins was calculated as described herein in Example 36, averaged over fusosome samples, and expressed as a fraction.

As shown in the table below, the synthetic protein cargo was found to have a ratio to VSV-G of 6.37×10^{-3}, a ratio to CD63 of 18.6, a ratio to ARRDC1 of 4.24, a ratio to GAPDH of 0.44, a ratio to CNX of 3.56, and a ratio to TSG101 of 19.52.

Proteins	Raw MS Intensity	Protein Cargo:Protein(s) Ratio
EGFP	8.22×10^{5}	N/A
Total Proteins	9.46×10^{8}	8.69×10^{-4}
VSV-G	1.29×10^{8}	6.37×10^{-3}
CD63	4.43×10^{4}	18.6
ARRDC1	1.94×10^{5}	4.24
GAPDH	1.87×10^{6}	0.44
CNX	2.31×10^{5}	3.56
TSG101	4.21×10^{4}	19.52

Example 97: Enriched lipid composition in fusosomes

This Example describes quantification of the lipid composition of fusosomes, parental cells, and exosomes. It is contemplated that the lipid composition of fusosomes can be enriched and/or depleted for specific lipids relative to the cells from which they are derived. Lipid composition affects important biophysical parameters of fusosomes and cells, such as size, electrostatic interactions, and colloidal behavior.

The lipid composition was measured as described in Examples 68 and 87. Fusosomes were prepared as described herein by transient transfection of VSV-G and GFP in 10 cm dishes, followed by filtration and ultracentrifugation of the conditioned media 48 hours after transfection to obtain fusosomes. Transfected cells were harvested in parallel to the conditioned media and submitted for analysis. Exosomes were prepared as described herein for fusosomes with the exception that the parental cells were not transfected with VSV-G or GFP.

The lipid composition for fusosomes, exosomes, and parental cells is shown in FIGS. 19A-19B. Compared to parental cells, fusosomes were enriched for cholesteryl ester, free cholesterol, ether-linked lyso-phosphatidylethanolamine, lyso-phosphatidylserine, phosphatidate, ether-linked phosphatidylethanolamine, phosphatidylserine, and sphingomyelin. Compared to parental cells, fusosomes are depleted for ceramide, cardiolipin, lyso-phosphatidylcholine, lyso-phosphatidylethanolamine, lyso-phosphatidylglycerol, lyso-phosphatidylinositol, ether-linked

phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, and triacylglycerol. Compared to exosomes, fusosomes were enriched for cholesteryl ester, ceramide, diacylglycerol, lyso-phosphatidate, and phosphatidylethanolamine, triacylglycerol. Compared to exosomes, fusosomes are depleted for free cholesterol, hexosyl ceramide, lyso-phosphatidylcholine, ether-linked lyso-phosphatidylcholine, lyso-phosphatidylethanolamine, ether-linked lyso-phosphatidylethanolamine, and lyso-phosphatidylserine,

Example 98: Measuring compartment-specific proteomic content of fusosomes

This Example describes quantification of the proportion of proteins that are known to be derived from specific cellular compartments in fusosomes, fusosome parental cells, and exosomes.

Fusosomes and parental cells were prepared as described herein by the method of Examples 68 and 87. Exosomes were prepared as described herein for fusosomes by the method of Examples 68 and 87with the exception that the parental cells were not transfected with VSV-G or GFP. Protein quantification by mass spectrometry for fusosomes and exosomes was performed as described herein in Example 36. The resulting protein quantification data was analyzed to determine protein levels and proportions of known exosomal, , endoplasmic reticulum, ribosome, nuclear, and mitochondrial proteins as annotated by Gene Ontology Cellular Compartment annotation terms (exosome: GO:0070062, endoplasmic reticulum: GO:0005783, ribosome: GO:0005840, GO:0022625, GO:0022626, GO:0022627, GO:0044391, GO:0042788, GO:0000313) with evidence code IDA (inferred by direct assay). The fraction of compartment-specific proteins relative to total protein in each sample was determined for fusosome samples, exosome samples, and parental cells.

As shown in FIG. 20, fusosomes were found to be depleted with endoplasmic reticulum protein compared to parental cells and exosomes. Fusosomes were also found to be depleted for exosomal protein compared to exosomes. Fusosomes were depleted for mitochondrial protein compared to parental cells. Fusosomes were enriched for nuclear protein compared to parental cells. Fusosomes were enriched for ribosomal proteins compared to parental cells and exosomes.

Example 99: Measuring TSG101 and ARRDC1 content in fusosomes

This Example describes quantification of the proportion of proteins that are known to be important in fusosome release from cells.

Fusosomes and parental cells were prepared as described herein by the method of Examples 68 and 87. Exosomes were prepared as described herein for fusosomes by the method of Examples 68 and 87with the exception that the parental cells were not transfected with VSV-G or GFP. Protein quantification by mass spectrometry for fusosomes and exosomes was performed as described herein in Example 36. The resulting protein quantification data was analyzed to determine protein levels and proportions of the protein TSG101 and ARRDC1. Average log intensities per group were calculated by adding 1 to intensity values from mass spectrometry, transforming by log10, and computing the mean across replicates. The percentage of total protein content of TSG101 or ARRDC1 in fusosomes relative to exosomes or parental cells was determined as the average log intensity of TSG101 or ARRDC1 for each sample, divided by the sum of intensities of all proteins in the same sample, averaged over replicates and expressed as a percent.

As shown in FIG. 21, ARRDC1 was found to be present at greater levels as a percentage of total protein content in fusosomes than in parental cells or exosomes. The level of ARRDC1 as a percentage of total protein content was at least 0.02% in fusosomes. TSG101 was found to be present at greater levels as a percentage of total protein content in fusosomes than in parental cells or exosomes. The level of TSG101 as a percentage of total protein content was at least 0.004% in fusosomes.

Example 100: Measuring serum inactivation of fusosomes after multiple administrations

This Example describes quantification of serum inactivation of fusosomes using an *in vitro* delivery assay following multiple administrations of the fusosome. It is contemplated that a modified fusosome, e.g., modified by a method described herein, can have 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. In some instances, a fusosome described herein will not be inactivated by serum following multiple administrations.

A measure of immunogenicity for fusosomes is serum inactivation. In an embodiment, repeated injections of a fusosome can lead to the development of anti-fusosome antibodies, e.g., antibodies that recognize fusosomes. In an embodiment, antibodies that recognize fusosomes can bind in a manner that can limit fusosome activity or longevity and mediate complement degradation.

In this Example, serum inactivation is examined after one or more administrations of fusosomes. Fusosomes are produced by any one of the previous Examples. In this example, fusosomes are generated from: HEK293 cells modified with a lentiviral-mediated expression of HLA-G (hereafter HEK293-HLA-G), and HEK293 modified with a lentiviral-mediated expression of an empty vector (hereafter HEK293). In some embodiments, fusosomes are derived from cells that are expressing other immunoregulatory proteins.

Serum is drawn from the different cohorts: mice injected systemically and/or locally with 1, 2, 3, 5, 10 injections of vehicle (Fusosome naïve group), HEK293-HLA-G fusosomes, or HEK293 fusosomes. 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 fusosomes are tested for the dose at which 50% of cells in a recipient population receive the payload in the fusosomes. The fusosomes may be produced via any of the other examples described herein and may contain any of the payloads described herein. Many methods for assaying fusosome delivery of a payload to recipient cells are also described herein. In this particular example, the payload is Cre protein and the recipient cells are RPMI8226 cell which stably-expresses "LoxP-GFP-stop-LoxP-RFP" cassette under a CMV promoter, which upon recombination by Cre switches from GFP to RFP expression, indicating fusion and Cre, as a marker, of delivery. The identified dose at which 50% of the recipient cells are RFP positive is used for further experiments. In other embodiments, the identified dose at which 50% of the recipient cells receive the payload is used for further experiments.

To assess serum inactivation of fusosomes, fusosomes are diluted 1:5 into 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 fusosomes should be present at the previously identified dose at which 50% of the recipient cells have received the payload (e.g. are RFP positive). It is contemplated that the identified dose at which 50% of recipient cells receive the payload may be similar across fusosomes.

Fusosomes that have been exposed to serum are then incubated with recipient cells. The percent of cells which receive the payload, and thus are RFP positive, is calculated. The percent of cells which receive the payload may not be different between fusosome samples that have been incubated with serum and heat-inactivated serum from mice treated with HEK293-HLA-G fusosomes, indicating that there is not serum inactivation of fusosomes or an adaptive immune response. The percent of cells that receive the payload may not be different between fusosome samples that have been incubated from mice treated 1, 2, 3, 5 or 10 times with HEK293-HLA-G fusosomes, which would indicate that there was not serum inactivation of fusosomes or an adaptive immune response. In some instances, the percent of cells which receive the payload is not different between fusosome samples that have been incubated with serum from mice treated with vehicle and from mice treated with HEK293-HLA-G fusosomes, indicating that there is not serum inactivation of fusosomes or an adaptive immune response. In some instances, the percent of cells which receive the payload is less for fusosomes derived with HEK293 than for HEK293-HLA-G fusosomes, indicating that there is not serum inactivation of HEK293-HLA-G fusosomes or an adaptive immune response.

Example 101: Measuring complement targeting of fusosomes

This Example describes quantification of complement activity against fusosomes using an *in vitro* assay. It is contemplated that a modified fusosome described herein can induce reduced complement activity compared to a corresponding unmodified fusosome.

In this Example, serum from a mouse is assessed for complement activity against a fusosome. The example measures the level of complement C3a, which is a central node in all complement pathways. Notably, the methods described herein may be equally applicable to humans, rats, monkeys with optimization to the protocol.

In this Example, fusosomes are produced by any one of the previous Examples. Fusosomes are generated from HEK293 cells modified with a lentiviral-mediated expression of a complement regulatory protein DAF (HEK293-DAF fusosomes) or HEK 293 cells not expressing a complementary regulatory protein (HEK293 fusosomes). Other complement regulatory proteins may also 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. Profectin (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 fusosomes, or mice that are administered HEK293 fusosomes. 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 fusosomes are tested for the dose at which 50% of cells in a recipient population receive the payload in the fusosomes. The fusosomes may be produced via any of the other examples described herein and may contain any of the payloads described herein. Many methods for assaying fusosome delivery of a payload to recipient cells are also described herein. In this particular example, the payload is Cre protein and the recipient cells are RPMI8226 cell which stably-expresses "LoxP-GFP-stop-LoxP-RFP" cassette under a CMV promoter, which upon recombination by Cre switches from GFP to RFP expression, indicating fusion and Cre, as a marker, of delivery. The identified dose at which 50% of the recipient cells are RFP positive is used for further experiments. In other embodiments, the identified dose at which 50% of the recipient cells receive the payload is used for further experiments. In preferred embodiments, the identified dose at which 50% of recipient cells receive the payload is similar across fusosomes.

Two-fold dilutions of the fusosomes starting at the dose of fusosomes at which 50% of the recipient cells receive the payload in phosphate-buffered saline (PBS, pH 7.4) are mixed with a 1:10 dilution of the sera from mice treated with the same fusosomes 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 fusosomes at which 200 pg/ml of C3a is present is compared across sera isolated from mice.

In some instances, the dose of fusosomes at which 200 pg/ml of C3a is present is greater for HEK293-DAF fusosomes incubated with HEK-293 DAF mouse sera than for HEK293 fusosomes incubated with HEK293 mouse sera, indicating that complement activity targeting fusosomes is greater in mice treated with HEK293 fusosomes than HEK293-DAF fusosomes. In some instances, the dose of fusosomes at which 200 pg/ml of C3a is present is greater for HEK293-DAF fusosomes incubated with naive mouse sera than for HEK293 fusosomes incubated with naive mouse sera, indicating that complement activity targeting fusosomes is greater in mice treated with HEK293 fusosomes than HEK293-DAF fusosomes.

Example 102: Assessment of Specificity of Transgene Expression Using Tissue-specific Promoters and miRNA Mediated Gene Silencing.

This Example describes quantification of an exogenous agent in target human hepatoma cell line (HepG2) and compared to non-target (non-hepatic) cell lines. Cell lines were transduced with lentiviruses (LV) containing positive TCSREs (e.g. tissue-specific promoter) or a combination of positive TCSREs and NTCSREs (e.g. miRNA-mediated gene silencing with a tissue-specific miRNA recognition sequence). Target and non-target cells lines were transduced with generated lentiviral particles containing the positive and negative regulatory elements and the effect of transgene expression in the cells lines was assessed.

A. Effect of miRNA-Mediated Gene Regulation on Specificity of Transgene Expression.

In addition to hepatocytes, major cell populations that line liver sinusoids include endothelial cells and Kupffer cells (resident macrophages derived from hematopoietic lineage), which express mir-126-3p and mir-142-3p, respectively. These miRNAs are not substantially expressed in hepatocytes. Lentiviral vectors were constructed to contain an enhanced green fluorescent protein (eGFP) expression cassette under the control of the constitutively active promoter phosphoglycerate kinase (hPGK, positive TCSRE; see e.g. Table 3, with or without

four tandem copies each of sequences complementary to mir-142-3p (e.g. Table 4) and miR-126-3p (e.g. Table4) as an NTCSRE. Lentiviral vector (LV) constructs with the NTCSRE are designated hPGK-eGFP+miRT and constructs without the NTCSRE are designated hPGK-eGFP.

Lentiviruses (LVs) generated from these hPGK-eGFP+miRT and hPGK-eGFP vectors, respectively, were used to transduce a target human hepatoma cell line (HepG2) or human embryonic kidney cell line (293LX), human T-cell line of hematopoietic origin (Molt4.8) and endothelial cell line derived from mouse brain (bEND.3). Seven days post-transduction, GFP expression was measured by flow cytometry.

As shown in **FIG. 22A**, 18-30% of all cell types transduced with hPGK-eGFP LV expressed GFP. Following transduction of LVs containing mirT sequences (hPGK-eGFP+miRT) in non-target cells, only 0.6% GFP expression was observed in Molt4.8 cells (express mir-142-3p) and no expression was observed in bEND.3 cells (express mir-126-3p). Mild reduction of GFP expression was observed in 293LX cells, which may have very low level expression of one or both of these miRNAs. No effect on GFP expression was observed in HepG2 target cells that had been transduced with LVs containing mirT sequences (hPGK-eGFP+miRT). These results demonstrate that incorporation of miRT sequences in lentiviral vectors resulted in at least a 50-fold reduction in transgene expression in cells of the hematopoietic and endothelial lineages, while maintaining robust expression in hepatic cells.

B. Combined Effect of miRNA-Mediated Gene Regulation and Tissue-Specific Promoters on Transgene Expression.

Additional lentiviral vectors (LVs) were generated substantially as described above, but with either an eGFP or with a transgene encoding the enzyme phenylalanine ammonia lyase (*PAL*) (see e.g. Table 5) with an N-terminal flag tag, under the control of a hepatocyte-specific human (ApoE.HCR-hAAT (hApoE) promoter (e.g. Table 3) as a tissue-specific regulatory promoter as a positive TCSREs or a constitutively active Spleen-focus-forming Virus (SFFV) promoter (e.g. Table 3). Expression of PAL in liver cells using the provided nucleic acid constructs is representative of expression of a desired exogenous agent in target cells. For example, endogenous PAH deficiency in human liver cells can result in toxic accumulation of

Phe in the blood leading to phenylketonuria (PKU), a clinical condition characterized by severe neurological disorders and stunted growth. In some aspects, early administration of PAL to PKU patients has been shown to successfully decrease blood Phe levels and alleviate symptoms.

As shown in **FIG. 22B**, transduction with LVs containing hPGK-eGFP or LVs containing mirT sequences and GFP under the control of the hepatocyte specific promoter (hApoE-eGFP+miRT), resulted in >250-fold repression of GFP expression in 293LX cells, with no substantial effect in HepG2 cells, as measured by flow cytometry.

HepG2 and 293LX cells were transduced with LVs containing the PAL transgene under the control of the SFFV promoter (SFFV-PAL), or LVs containing PAL transgene along with mirT sequences under the control of the hApoE promoter (hApoE-PAL+miRT). PAL catalyzes the conversion of phenylalanine (Phe) to ammonia and cinnamic acid, and has been used in enzyme replacement therapy in patients with an inborn deficiency of phenylalanine hydroxylase (PAH). Specificity of PAL transgene expression was measured by reduction in Phe levels in culture supernatant (SN) relative to fresh medium. As shown in **FIG. 22**C, expression from a constitutive promoter (SFFV) resulted in substantial reduction in Phe levels in SN collected from both cell types. However, the hApoE-PAL+miRT construct led to substantial Phe reduction only in HepG2 cells; Phe levels in SN collected from 293LX cells transduced with the hApoE-PAL+miRT LVs were indistinguishable from the untransduced controls. This result is consistent with high expression of the exemplary transgene PAL in HepG2 target cells when transduced with LV constructs containing a postive TSCRE and a NTSCRE but not in non-target 293LX cells.

Additional vectors were produced using: i) the ApoE.HCR-hAAT promoter, which is a chimeric promoter formed by the hepatic control region of the human Apolipoprotein E gene and the human alpha-antitrypsin promoter (Miao et al, Mol Ther, 2000; PMID: 10933977), ii) the Enhanced Transthyretin (ET) promoter, a synthetic promoter generated by the random assembly of hepatocyte-specific transcription factor binding sites to the transthyretin promoter (Vigna et al, MolTher, 2005; PMID: 15851015 and Brown et al, Blood, 2007; PMID: 17726165), and iii) the TBG promoter, which is a hybrid promoter based on the human thyroid hormone-binding globulin and α1-Microglobulin/Bikunin enhancer (Yan et al, Gene, 2012; PMID: 22820390). These three promoters were cloned into a lentiviral backbone plasmid (pSF) upstream of the

eGFP gene. These constructs, along with a ubiquitous SFFV cassette, were packaged into VSVg-pseudotyped LVs, and transduced into HepG2 (a human hepatocyte cell line), primary human hepatocytes, and SupT1 (a human T-cell line, to assess specificity). Image analyses of the transduced cells demonstrated that the three hepatocyte-specific promoters produced potent and specific expression in both hepatocyte cell lines, but not in non-hepatocyte cells (see table below). Whereas ApoE and TBG did not mediate any detectable expression on T cells, one of the promoters, ET, still produced low levels of GFP in the T-cell line. In order to further increase hepatocyte specificity, we included 4x tandem target sites for miR142-3p (a miRNA expressed in cells of hematopoietic origin) (Brown et al, Blood, 2007; PMID: 17726165), as well as for miR126-3p (a miRNA expressed in most endothelial cells) (Chiriaco et al, MolTher, 2014; PMID: 24869932) downstream of the expressed sequence. Image analysis of hepatocytic and non-hepatocytic cell lines transduced with these new constructs clearly demonstrated that the addition of miRNA target sites strongly suppress the expression of the transgene in immune cells, leading to complete suppression of GFP expression in non-hepatocyte cells, while maintaining significant expression in both primary and immortalized human hepatocytes.

	Primary human Hepatocytes	HepG2	SupT1
pSF-SFFV-eGFP	++++	++++	+++++
pSF-SFFV-eGFP+1xmiRT	+++	++++	++
pSF-SFFV-eGFP+4xmiRT	+++	++	+/-
pSF-ApoE-eGFP	++	++	-
pSF-ET-eGFP	+++	++++	+/-
pSF-TBG-eGFP	++	+++	-
pSF-ApoE-eGFP+4xmiRT	++	+	-
pSF-ET-eGFP+4xmiRT	++	++	-
pSF-TBG-eGFP+4xmiRT	+	++	-

C. Conclusion

Together, these data are supportive of the finding that the use of a tissue-specific promoter in conjunction with miRNA target sites can impart substantial specificity to transgene expression.

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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 liver cell-specific regulatory element operatively linked to the payload gene, wherein the positive liver cell-specific regulatory element increases expression of the payload gene in a liver cell relative to an otherwise similar fusosome lacking the positive liver cell-specific regulatory element.

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-liver cell relative to an otherwise similar fusosome lacking the NTCSRE.

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 an Apoa2, Cyp3a4, LP1B, MIR122, hemopexin, SERPINA1, or HLP 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 is a non-liver cell-specific miRNA recognition sequence, a non-liver cell-specific protease recognition site, a non-liver cell-specific ubiquitin ligase site, a non-liver cell-specific transcriptional repression site, or a non-liver cell-specific epigenetic repression site, and the NTCSRE decreases expression of the payload gene in a non-liver cell or tissue relative to an otherwise similar fusosome lacking the NTCSRE.

5. The fusosome of claim 4, wherein the nucleic acid further comprises a positive liver cell-specific regulatory element operatively linked to the payload gene, wherein the positive liver cell-specific regulatory element increases expression of the payload gene in a liver cell relative to an otherwise similar fusosome lacking the positive liver cell-specific regulatory element.

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 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 genetic deficiency; 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 6-9, 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 liver cell-specific regulatory element operatively linked to the payload gene, wherein the positive liver cell-specific regulatory element increases expression of the payload gene in a liver cell relative to an otherwise similar fusosome lacking the positive liver cell-specific regulatory element.

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-liver cell or tissue relative to an otherwise similar fusosome lacking the NTCSRE.

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 the payload gene is selected from among OTC, CPS1, NAGS, BCKDHA, BCKDHB, DBT, DLD, MUT, MMAA, MMAB, MMACHC, MMADHC, MCEE, PCCA, PCCB, UGT1A1, ASS1, PAH, PAL, ATP8B1, ABCB11, ABCB4, TJP2, IVD, GCDH, ETFA, ETFB, ETFDH, ASL, D2HGDH, HMGCL,

MCCC1, MCCC2, ABCD4, HCFC1, LNBRD1, ARG1, SLC25A15, SLC25A13, ALAD, CPOX, HMBS, PPOX, BTD, HLCS, PC, SLC7A7, CPT2, ACADM, ACADS, ACADVL, AGL, G6PC, GBE1, PHKA1, PHKA2, PHKB, PHKG2, SLC37A4, PMM2, CBS, FAH, TAT, GALT, GALK1, GALE, G6PD, SLC3A1, SLC7A9, MTHFR, MTR, MTRR, ATP7B, HPRT1, HJV, HAMP, JAG1, TTR, AGXT, LIPA, SERPING1, HSD17B4, UROD, HFE, LPL,GRHPR, HOGA1, LDLR, ACAD8, ACADSB, ACAT1, ACSF3, ASPA, AUH, DNAJC19, ETHE1, FBP1, FTCD, GSS, HIBCH, IDH2, L2HGDH, MLYCD, OPA3, OPLAH, OXCT1, POLG, PPM1K, SERAC1, SLC25A1, SUCLA2, SUCLG1, TAZ, AGK, CLPB, TMEM70, ALDH18A1, OAT, CA5A, GLUD1, GLUL, UMPS, SLC22A5, CPT1A, HADHA, HADH, SLC52A1, SLC52A2, SLC52A3, HADHB, GYS2, PYGL, SLC2A2, ALG1, ALG2, ALG3, ALG6, ALG8, ALG9, ALG11, ALG12, ALG13, ATP6V0A2, B3GLCT, CHST14, COG1, COG2, COG4, COG5, COG6, COG7, COG8, DOLK, DHDDS, DPAGT1, DPM1, DPM2, DPM3, G6PC3, GFPT1, GMPPA, GMPPB, MAGT1, MAN1B1, MGAT2, MOGS, MPDU1, MPI, NGLY1, PGM1, PGM3, RFT1, SEC23B, SLC35A1, SLC35A2, SLC35C1, SSR4, SRD5A3, TMEM165, TRIP11, TUSC3, ALG14, B4GALT1, DDOST, NUS1, RPN2, SEC23A, SLC35A3, ST3GAL3, STT3A, STT3B, AGA, ARSA, ARSB, ASAH1, ATP13A2, CLN3, CLN5, CLN6, CLN8, CTNS, CTSA, CTSD, CTSF, CTSK, DNAJC5, FUCA1, GAA, GALC, GALNS, GLA, GLB1, GM2A, GNPTAB, GNPTG, GNS, GRN, GUSB, HEXA, HEXB, HGSNAT, HYAL1, IDS, IDUA, KCTD7, LAMP2, MAN2B1, MANBA, MCOLN1, MFSD8, NAGA, NAGLU, NEU1 NPC1, NPC2, SGSH, PPT1, PSAP, SLC17A5, SMPD1, SUMF1, TPP1, AHCY, GNMT, MAT1A, GCH1, PCBD1, PTS, QDPR, SPR, DNAJC12, ALDH4A1, PRODH, HPD, GBA, HGD, AMN, CD320, CUBN, GIF, TCN1, TCN2, PREPL, PHGDH, PSAT1, PSPH, AMT,GCSH, GLDC, LIAS, NFU1, SLC6A9, SLC2A1, ATP7A, AP1S1, CP, SLC33A1, PEX7 PHYH, AGPS, GNPAT, ABCD1, ACOX1, PEX1, PEX2, PEX3, PEX5, PEX6, PEX10, PEX12, PEX13, PEX14, PEX16, PEX19, PEX26, AMACR, ADA, ADSL, AMPD1, GPHN, MOCOS, MOCS1, PNP, XDH, SUOX, OGDH, SLC25A19, DHTKD1, SLC13A5, FH, DLAT, MPC1, PDHA1, PDHB, PDHX, PDP1, ABCC2, SLCO1B1, SLCO1B3, HFE2, ADAMTS13, PYGM , COL1A2, TNFRSF11B, TSC1, TSC2, DHCR7, PGK1, VLDLR, KYNU, F5, C3, COL4A1, CFH, SLC12A2, GK, SFTPC, CRTAP, P3H1, COL7A1, PKLR, TALDO1, TF, EPCAM, VHL, GC, SERPINA1, ABCC6, F8, F9, ApoB, PCSK9, LDLRAP1,ABCG5, ABCG8, LCAT, SPINK5

and GNE.

19. The fusosome of any of claims 1-18, wherein the payload gene encodes an exogenous agent comprising the sequence set forth in any one of SEQ ID NOS: 161-518, 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: 161-518.

20. The fusosome of any of claims 1-19, wherein one or more of:

i) the fusosome fuses at a higher rate with a liver cell than with a non-liver 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 a liver 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 liver 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 liver cells after 24, 48, or 72 hours;

iv) the fusosome delivers the nucleic acid to a liver cell at a higher rate than to a non-liver 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 a liver 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 a liver 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.

21. The fusosome of any of claims 1-20, wherein the payload gene encoding an exogenous agent is chosen from: OTC, CPS1, NAGS, BCKDHA, BCKDHB, DBT, DLD, MUT, MMAA, MMAB, MMACHC, MMADHC, MCEE, PCCA, PCCB, UGT1A1, ASS1, PAH, ATP8B1, ABCB11, ABCB4, TJP2, IVD, GCDH, ETFA, ETFB, ETFDH, ASL, D2HGDH, HMGCL, MCCC1, MCCC2, ABCD4, HCFC1, LMBRD1, ARG1, SLC25A15, SLC25A13, ALAD, CPOX, HMBS, PPOX, BTD, HLCS, PC, SLC7A7, CPT2, ACADM, ACADS, ACADVL, AGL, G6PC, GBE1, PHKA1, PHKA2, PHKB, PHKG2, SLC37A4, PMM2, CBS, FAH, TAT, GALT, GALK1, GALE, G6PD, SLC3A1, SLC7A9, MTHFR, MTR, MTRR, ATP7B, HPRT1, HJV, HAMP, JAG1, TTR, AGXT, LIPA, SERPING1, HSD17B4, UROD, HFE, LPL, GRHPR, HOGA1, or LDLR.

22. The fusosome of any of claims 1-21, wherein the fusogen is a viral envelope protein.

23. The fusosome of any of claims 1-22, wherein the fusogen comprises VSV-G.

24. The fusosome of any of claims 1-21, 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.

25. The fusosome of any of claims 1-21 and 24, 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: 1-132.

26. The fusosome of claim 25, wherein the wild-type paramyxovirus is a Nipah virus, optionally wherein the Nipah virus is a henipavirus.

27. The fusosome of any of claims 1-26, wherein the fusogen is re-targeted for delivery to a liver cell.

28. The fusosome of any claims 1, 2, 5, 6, 7 and 14-27, wherein the positive liver-specific regulatory element comprises a liver-specific promoter, a liver-specific enhancer, a liver-specific splice site, a liver-specific site extending half-life of an RNA or protein, a liver-specific mRNA nuclear export promoting site, a liver-specific translational enhancing site, or a liver-specific post-translational modification site.

29. The fusosome of claim 28, wherein the positive liver-specific regulatory element comprises a hepatocyte-specific promoter.

30. The fusosome of claim 28 or 29, wherein the positive liver-specific regulatory element comprises a promoter selected from an enhanced transthyretin (ET), Alb, Apoa2, Cyp3a4, LP1B, MIR122, hemopexin, SERPINA1, or HLP promoter.

31. The fusosome of claim 3 or claim 30, wherein the promoter has the sequence set forth in any of SEQ ID NO: 133-136, or 519-525 or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

32. The fusosome of claim 28 or claim 29, wherein the positive liver-specific regulatory element comprises a ApoE.HCR-hAAT promoter, optionally wherein the promoter comprises the sequence set forth in SEQ ID NO:133, or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the sequence set forth in SEQ ID NO:133.

33. The fusosome of any of claims 2, 6, 7 and 15-32, wherein the NTCSRE comprises a non-liver cell-specific miRNA recognition sequence, non-liver cell-specific protease

recognition site, non-liver cell-specific ubiquitin ligase site, non-liver cell-specific transcriptional repression site, or non-liver cell-specific epigenetic repression site.

34. The fusosome of claim 33, wherein the NTCSRE comprises a non-liver cell-specific miRNA recognition sequence and the miRNA recognition sequence is able to be bound by one or more of miR-142, mir-181a-2, mir-181b-1, mir-181c, mir-181a-1, mir-181b-2, mir-181d, miR-223, or miR-126.

35. The fusosome of claim 33 or claim 34, 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.

36. The fusosome of any of claims 1-35, wherein the nucleic acid comprises one or more insulator elements.

37. The fusosome of claim 36, 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.

38. The fusosome of any of claims 1-37, wherein the fusosome is a retroviral vector particle.

39. The fusosome of any of claims 1-38, wherein the nucleic acid is capable of integrating into the genome of a liver cell.

40. The fusosome of any of claims 1-39, wherein the liver cell is chosen from a hepatocyte, liver sinusoidal endothelial cell, cholangiocyte, stellate cell, liver-resident antigen-presenting cell, liver-resident immune lymphocyte, or portal fibroblast.

41. A pharmaceutical composition comprising the fusosome of any of claims 1-40, and a pharmaceutically acceptable carrier, diluent, or excipient.

42. A method of delivering an exogenous agent to a subject comprising administering to the subject the fusosome of any of claims 1-40 or the pharmaceutical composition of claim 41, thereby delivering the exogenous agent to the subject.

43. A method of modulating a function, in a subject, liver or liver cell, comprising contacting the liver or the liver cell of the subject with the fusosome of any of claims 1-40 or the pharmaceutical composition of claim 41.

44. The method of claim 43, wherein the target tissue or the target cell is present in the subject.

45. The method of claims 43 or claim 44, wherein the contacting is carried out by administering the fusosome to the subject.

46. A method of treating a genetic deficiency in a subject comprising administering to the subject the fusosome of any of claims 1-40 or the pharmaceutical composition of claim 41.

47. The method of claim 46, wherein the genetic deficiency is a genetic deficiency able to be treated by the payload gene encoding the exogenous agent.

48. The method of any of claims 42-47, wherein the subject is a human subject.

49. A fusosome of any of claims 1-40 or pharmaceutical composition of claim 41 for use in treating a subject with a genetic deficiency.

50. Use of a fusosome of any of claims 1-40 or pharmaceutical composition of claim 41 for manufacture of a medicament for use in treating a subject with a genetic deficiency.

51. The fusosome or pharamaceutical composition for use of claim 49 or the use of claim 50 wherein the fusosome comprises a payload gene encoding an exogenous agent for treating the genetic deficiency.

52. A method of making the fusosome of any of claims 1-40, 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.

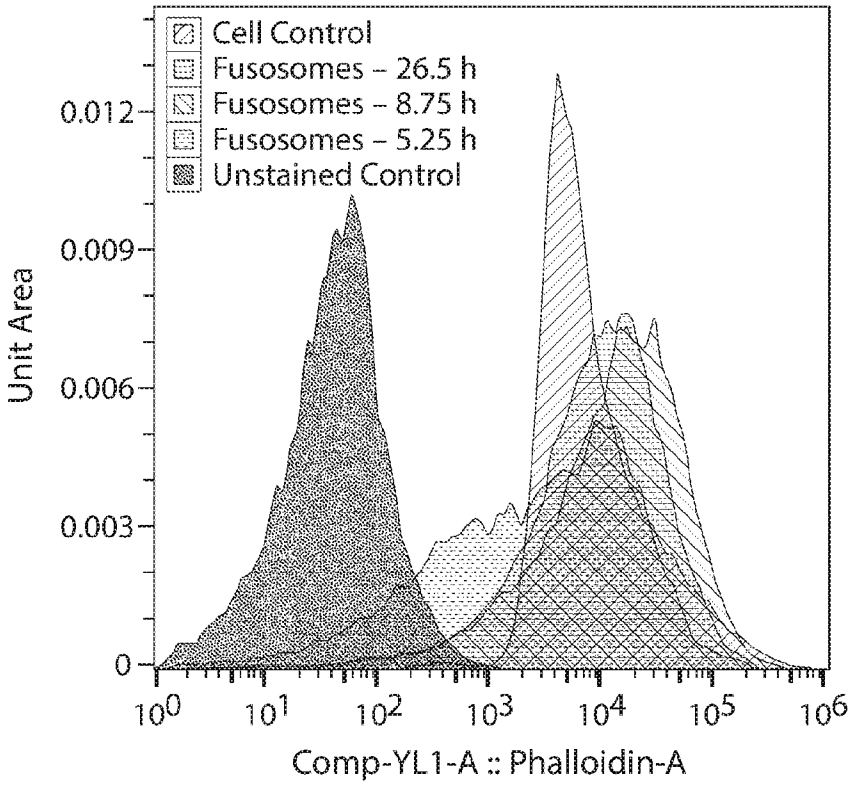

Cell Control
Fusosomes – 26.5 h
Fusosomes – 8.75 h
Fusosomes – 5.25 h
Unstained Control
Unit Area
0.012
0.009
0.006
0.003
0
10^0
10^1
10^2
10^3
10^4
10^5
10^6
Comp-YL1-A :: Phalloidin-A

Fig. 1

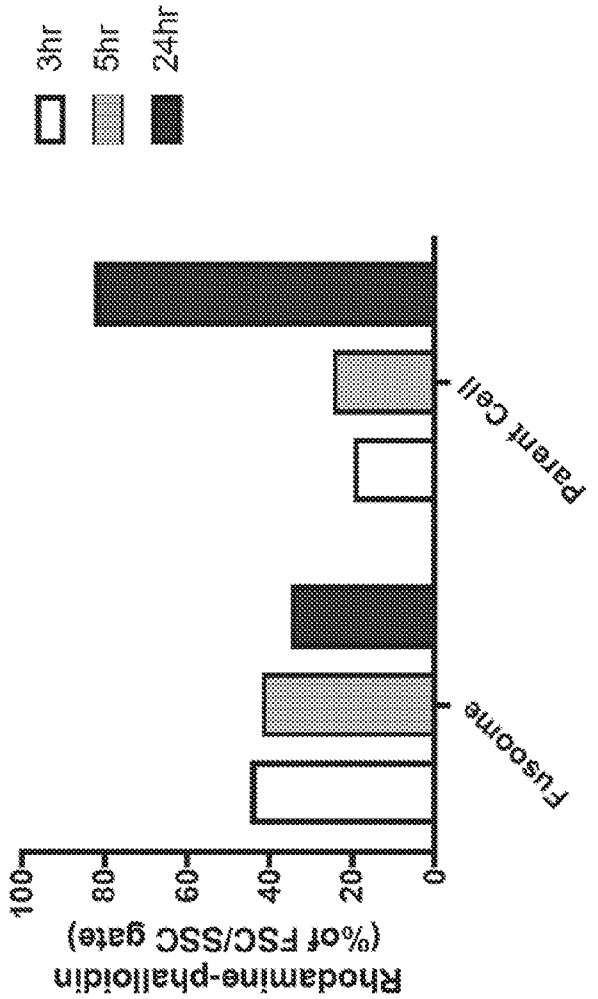

3hr
5hr
24hr
Rhodamine-phalloidin
(%of FSC/SSC gate)
100
80
60
40
20
0
Fusogenic
Parent Cell

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) Insoluble	Soluble	Ratio
Cells	2.12	0.06	0.028
Fusosomes	1.28	0.72	0.563

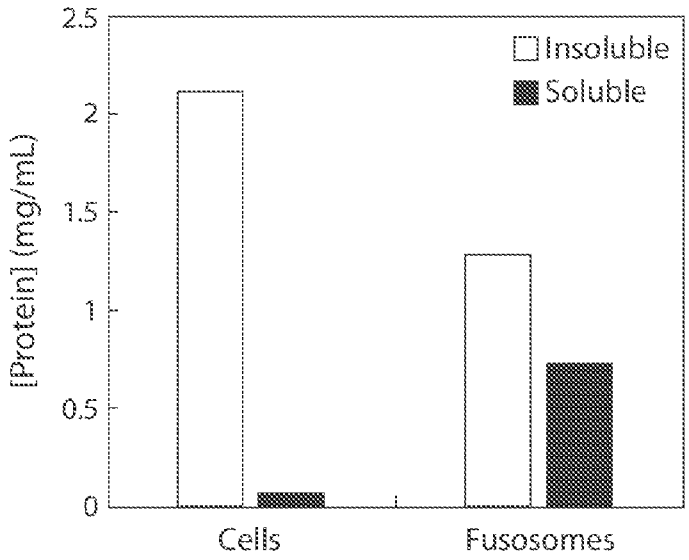
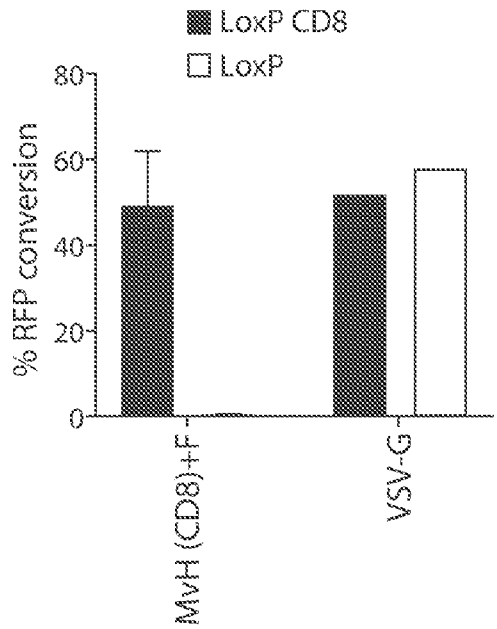


Fig. 5



Fusosome	Absolute amount of targeted fusion
MvH(CD8)+F	48.8%
VSV-G	0%

Fig. 6

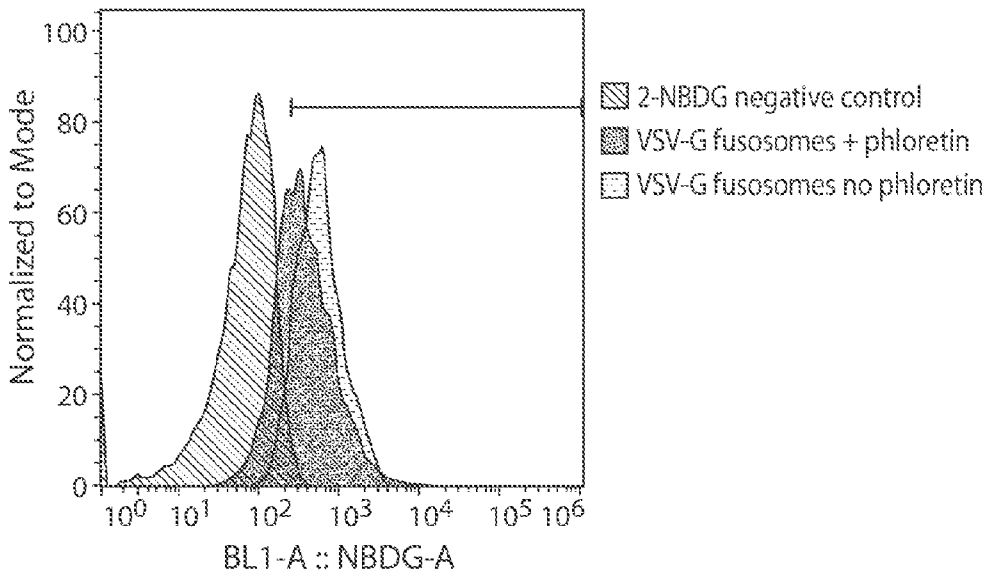

100
80
60
40
20
0
Normalized to Mode
10^0 10^1 10^2 10^3 10^4 10^5 10^6
BL1-A :: NBDG-A
2-NBDG negative control
VSV-G fusosomes + phloretin
VSV-G fusosomes no phloretin

Fig. 7

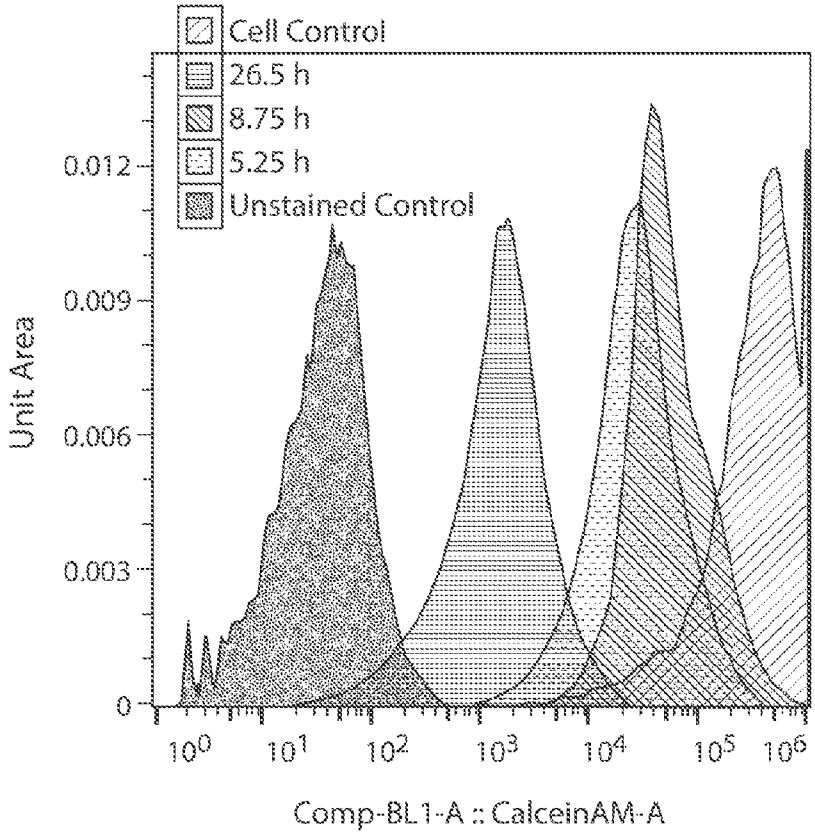

Cell Control
26.5 h
8.75 h
5.25 h
Unstained Control
Unit Area
0.012
0.009
0.006
0.003
0
10^0
10^1
10^2
10^3
10^4
10^5
10^6
Comp-BL1-A :: CalceinAM-A

Fig. 8

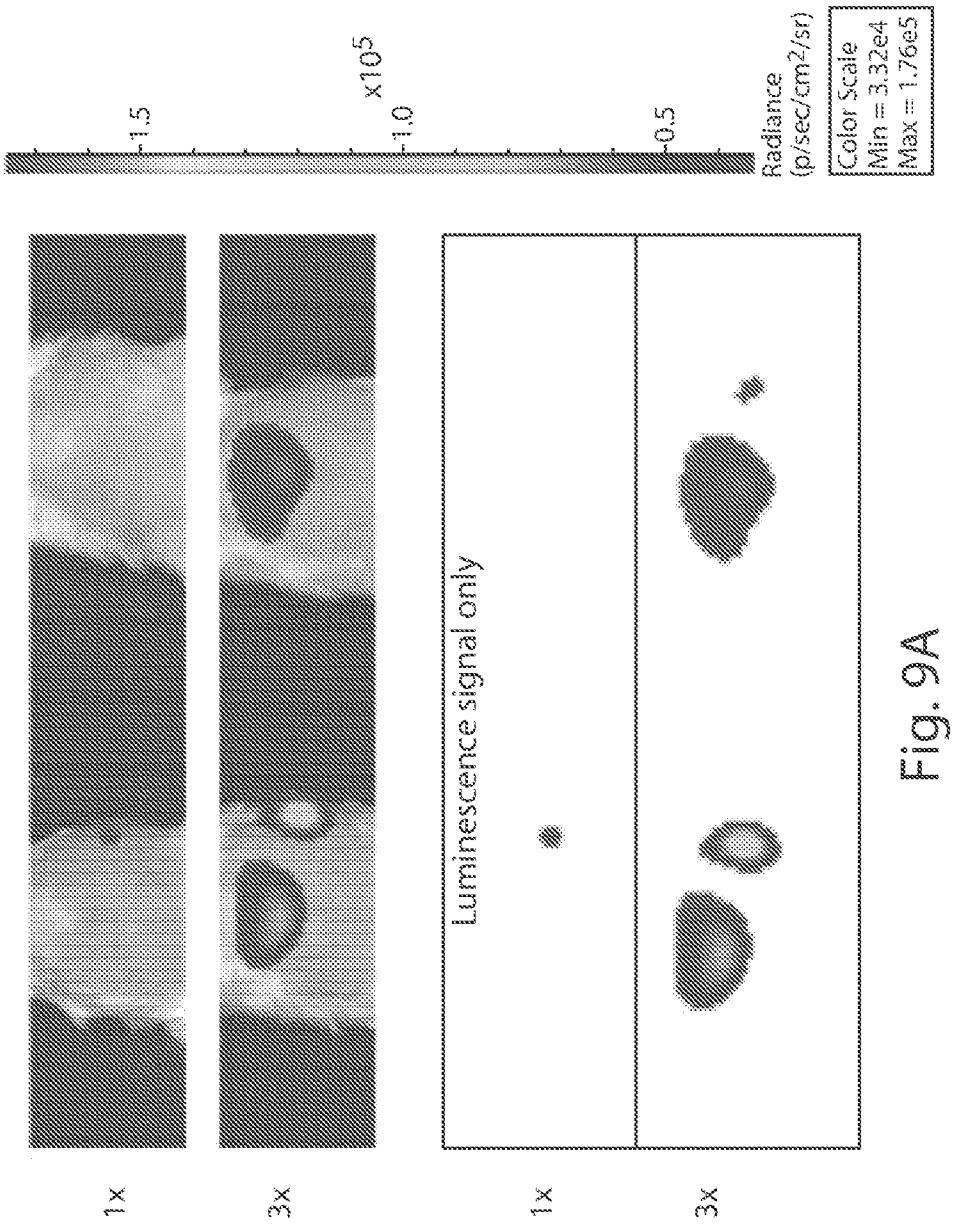

1.5
x10^5
1.0
0.5
Radiance (p/sec/cm²/sr)
Color Scale
Min = 3.32e4
Max = 1.76e5
Luminescence signal only
1x
3x
1x
3x

Fig. 9A

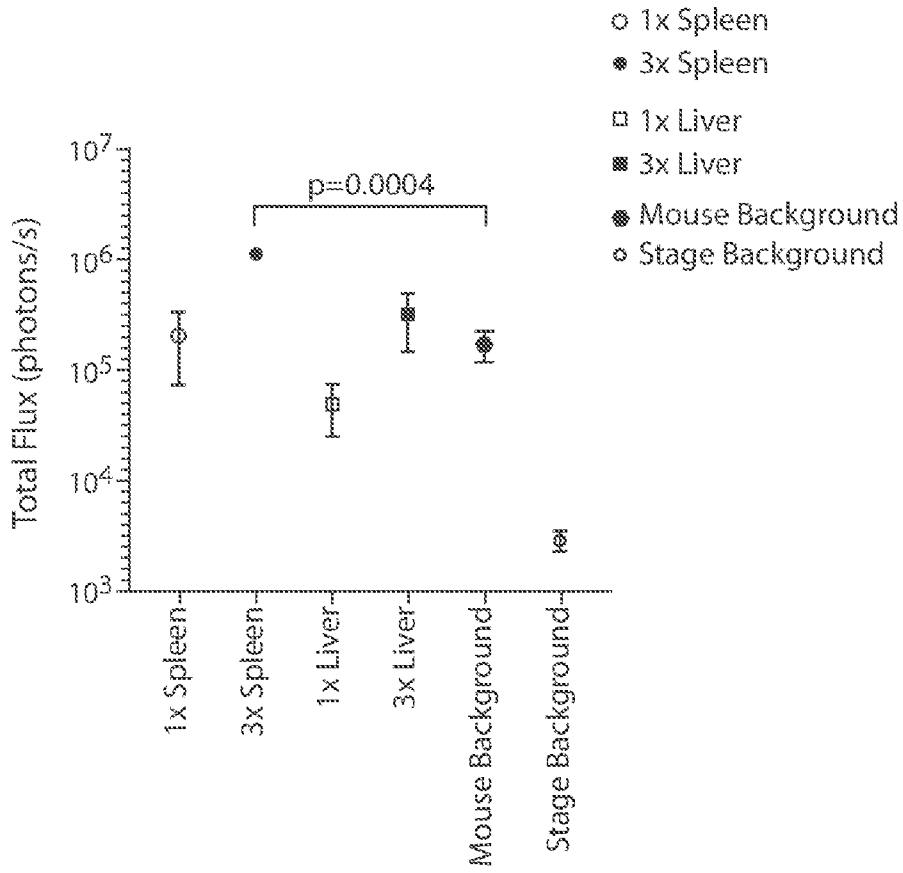

1x Spleen
3x Spleen
1x Liver
3x Liver
Mouse Background
Stage Background
p=0.0004
Total Flux (photons/s)
10^7
10^6
10^5
10^4
10^3
1x Spleen
3x Spleen
1x Liver
3x Liver
Mouse Background
Stage Background

Fig. 9B

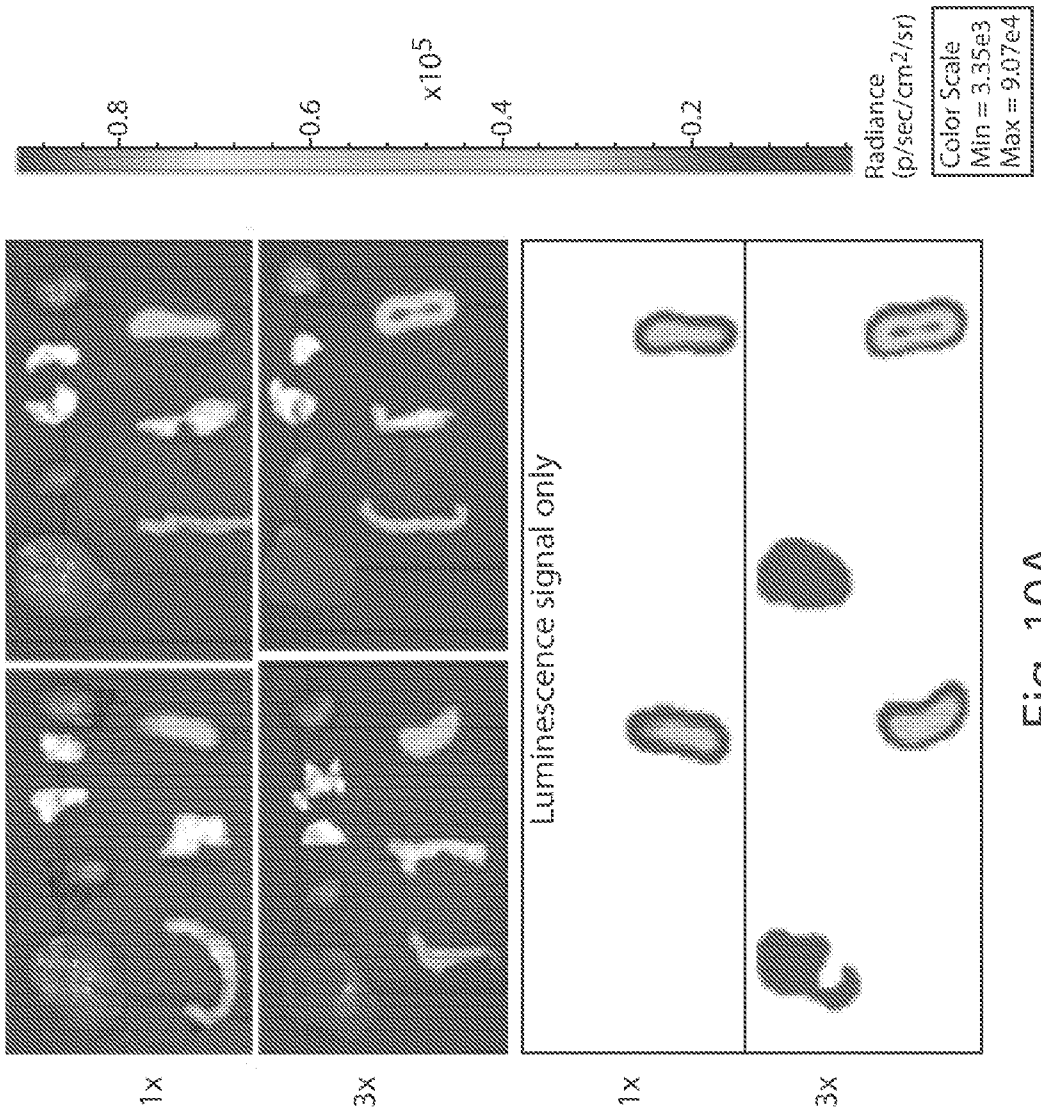

-0.8
-0.6
x10^5
-0.4
-0.2
Radiance (p/sec/cm²/sr)
Color Scale
Min = 3.35e3
Max = 9.07e4
Luminescence signal only
1x
3x
1x
3x

Fig. 10A

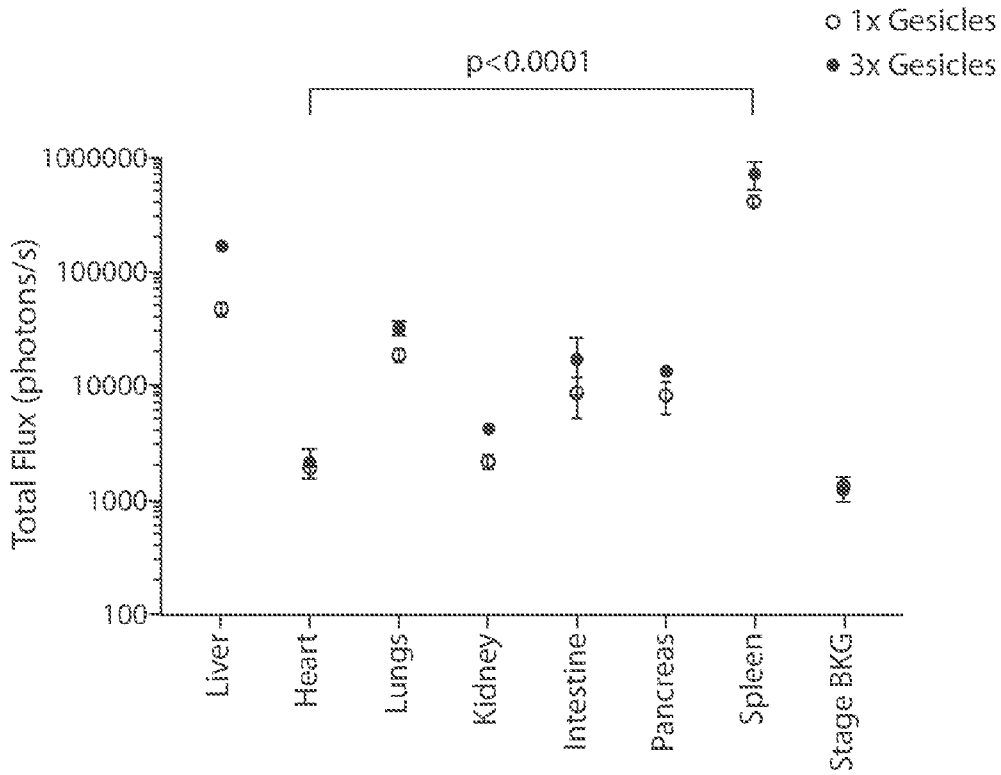

1x Gesicles
3x Gesicles
p<0.0001
Total Flux (photons/s)
1000000
100000
10000
1000
100
Liver
Heart
Lungs
Kidney
Intestine
Pancreas
Spleen
Stage BKG

Fig. 10B

Group	% RFP conversion (± SD)
Recipient + no fusosome	0.4 ± 0.2%
Recipient + NivG+F fusosome	88.9 ± 3.4%
Recipient + NivG+F fusosome + Baf	68.1 ± 2.7%

Fig. 11

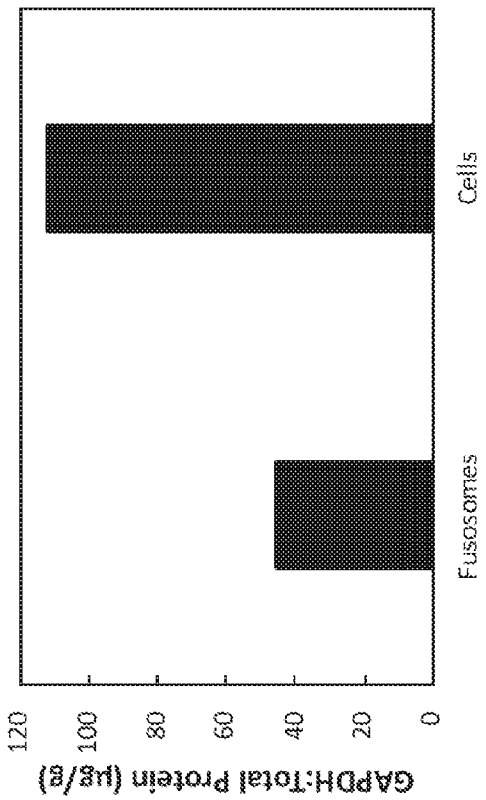


Fig. 12

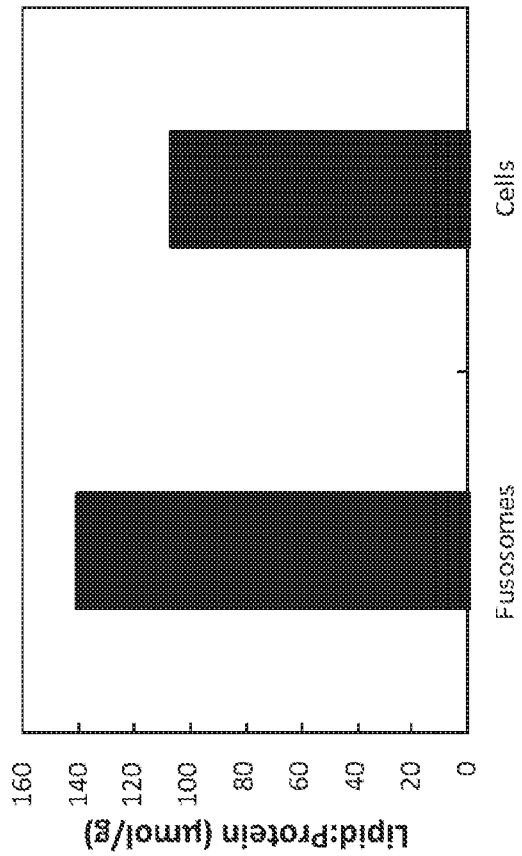

Lipid:Protein (µmol/g)
160
140
120
100
80
60
40
20
0
Fusosomes
Cells

Fig. 13

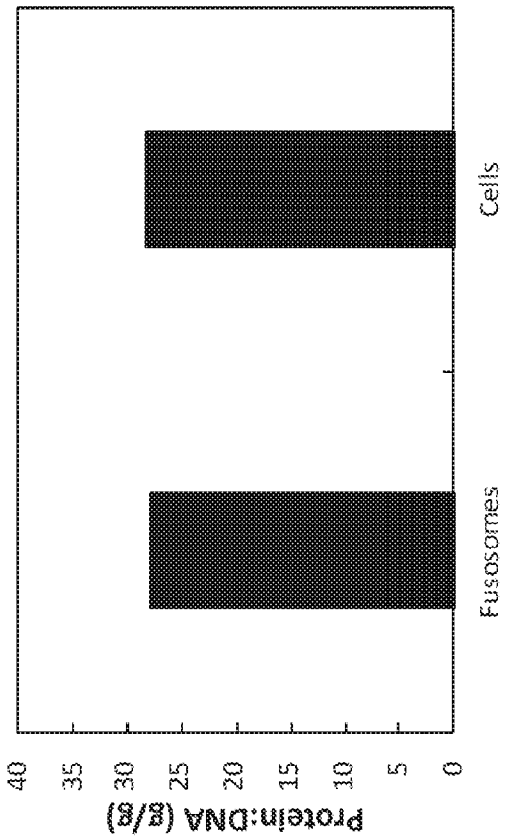


Fig. 14

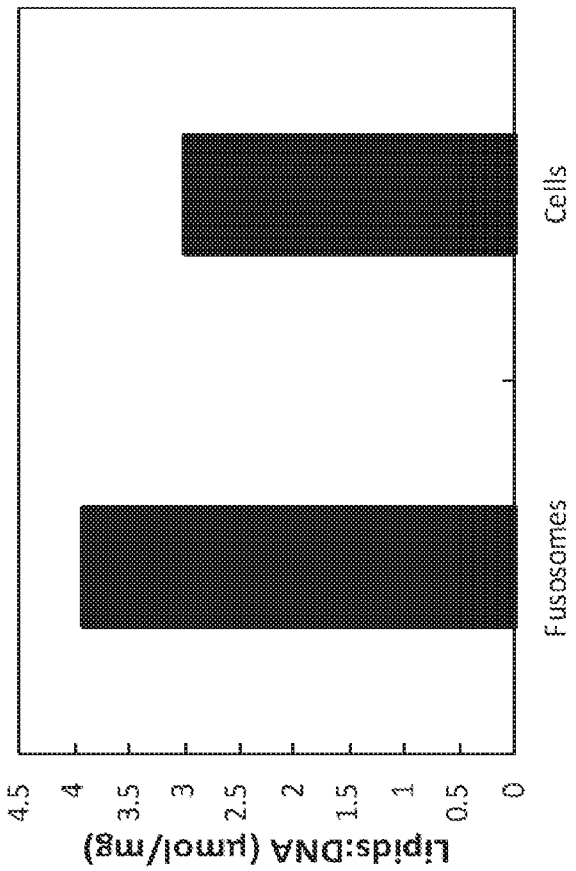


Fig. 15

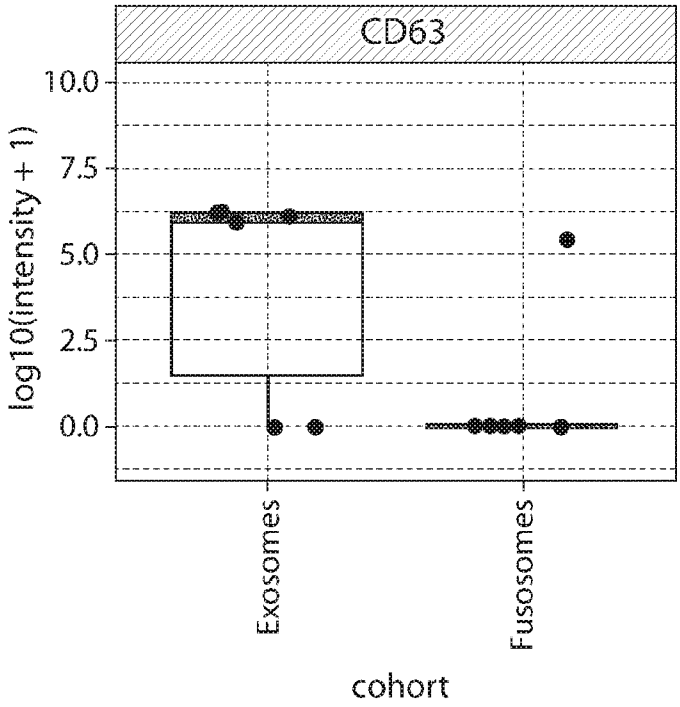

CD63
10.0
7.5
5.0
2.5
0.0
log10(intensity + 1)
Exosomes
Fusosomes
cohort

Fig. 16

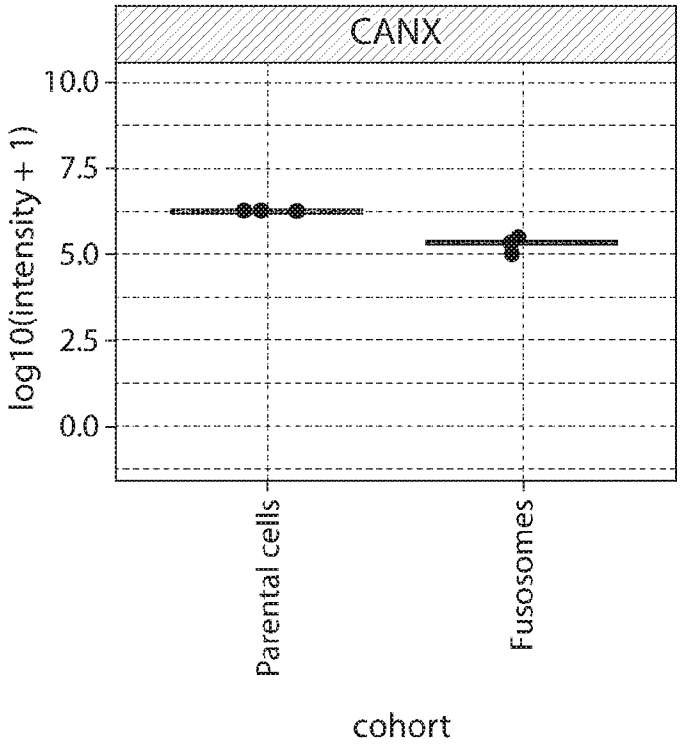

CANX
10.0
7.5
5.0
2.5
0.0
log10(intensity + 1)
Parental cells
Fusosomes
cohort

Fig. 17

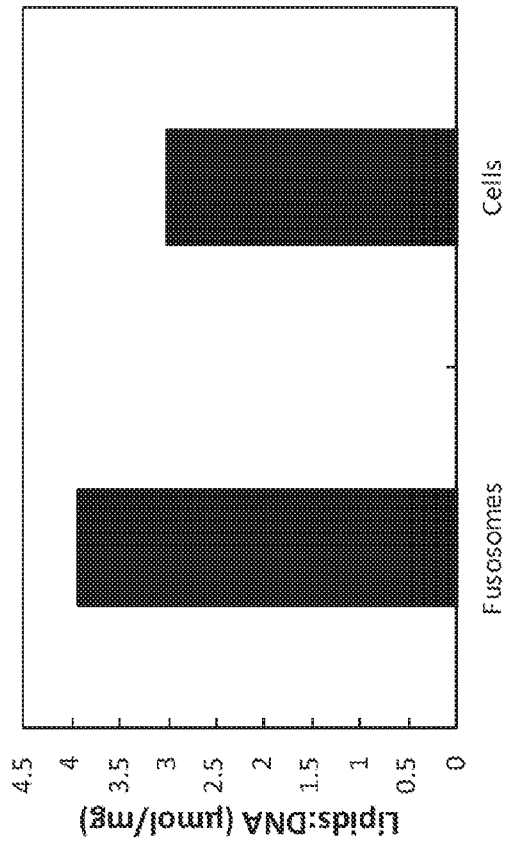


Fig. 18

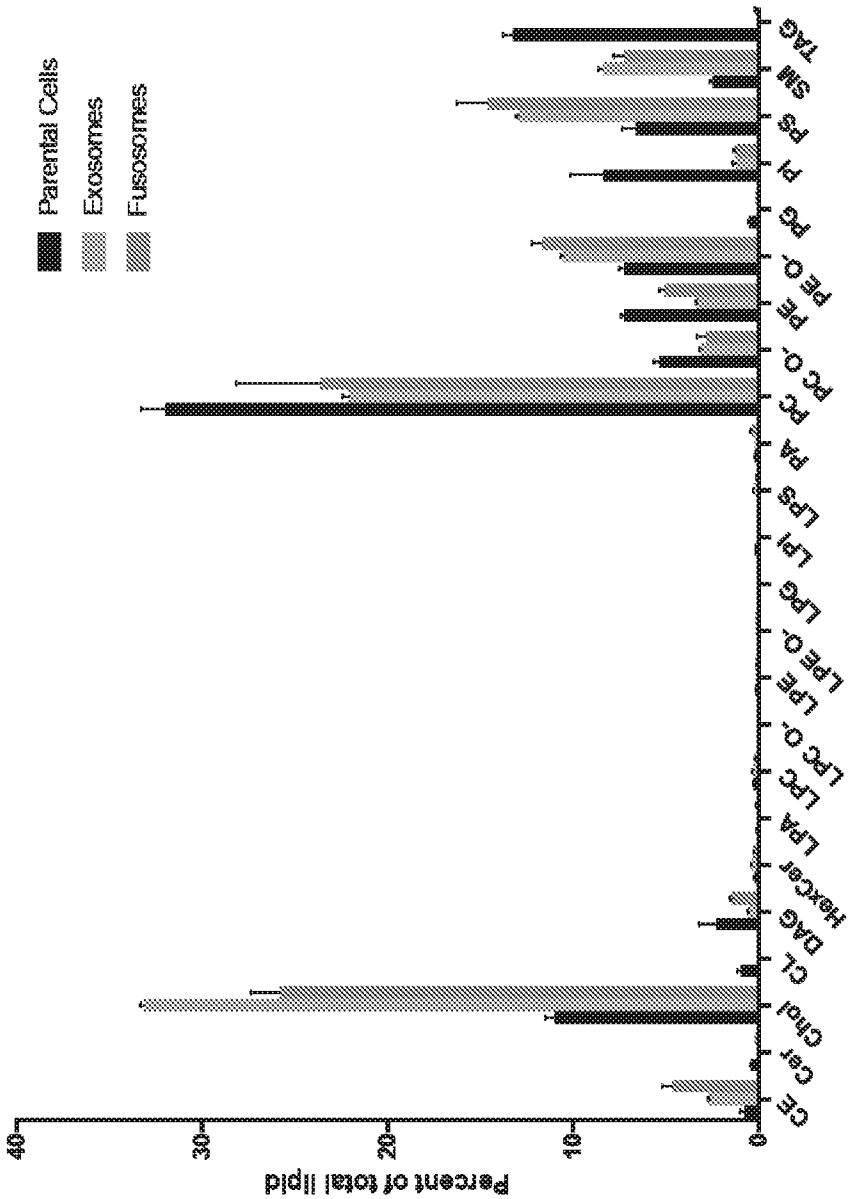

Parental Cells
Exosomes
Fusosomes
Percent of total lipid
0
10
20
30
40
CE
Cer
Chol
CL
DAG
HexCer
LPA
LPC
LPC O-
LPE
LPE O-
LPG
LPI
LPS
PA
PC
PC O-
PE
PE O-
PG
PI
PS
SM
TAG

Fig. 19A

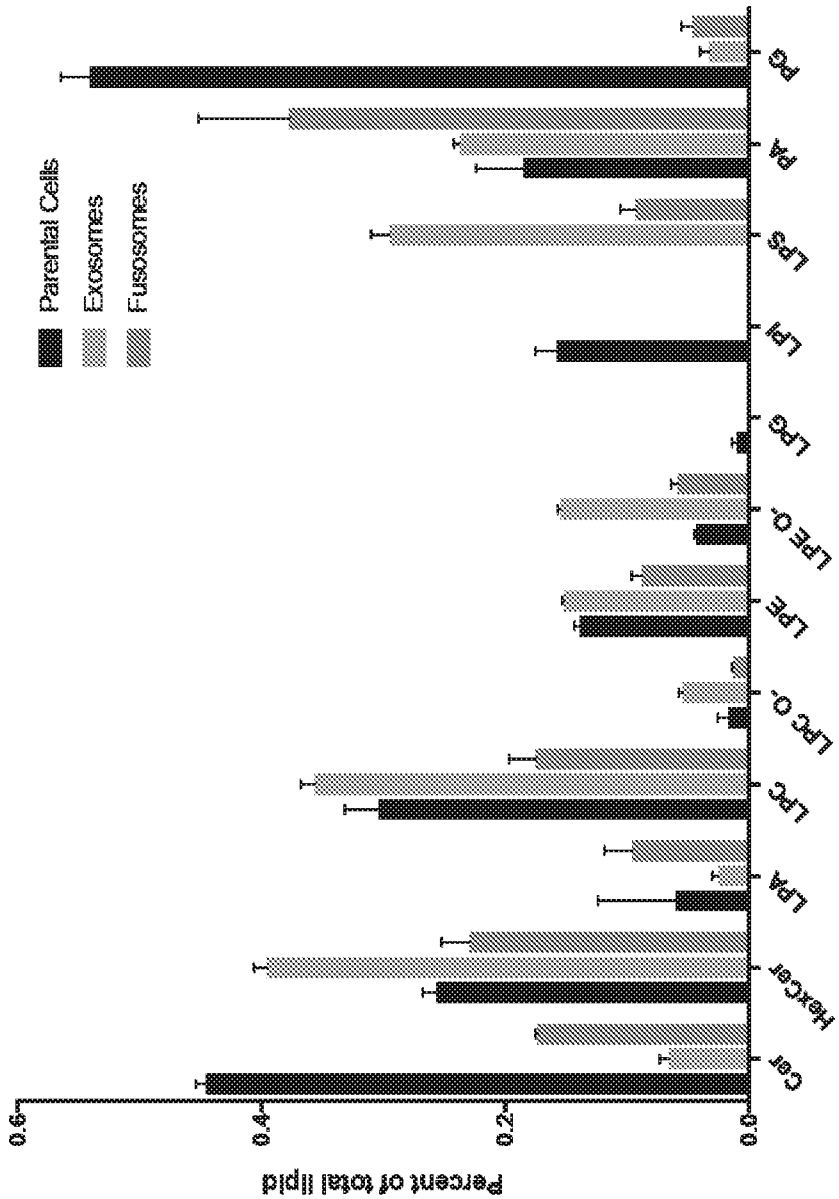

Parental Cells
Exosomes
Fusosomes
Percent of total lipid
0.0
0.2
0.4
0.6
Cer
HexCer
LPA
LPC
LPC O-
LPE
LPE O-
LPG
LPI
LPS
PA
PG

Fig. 19B

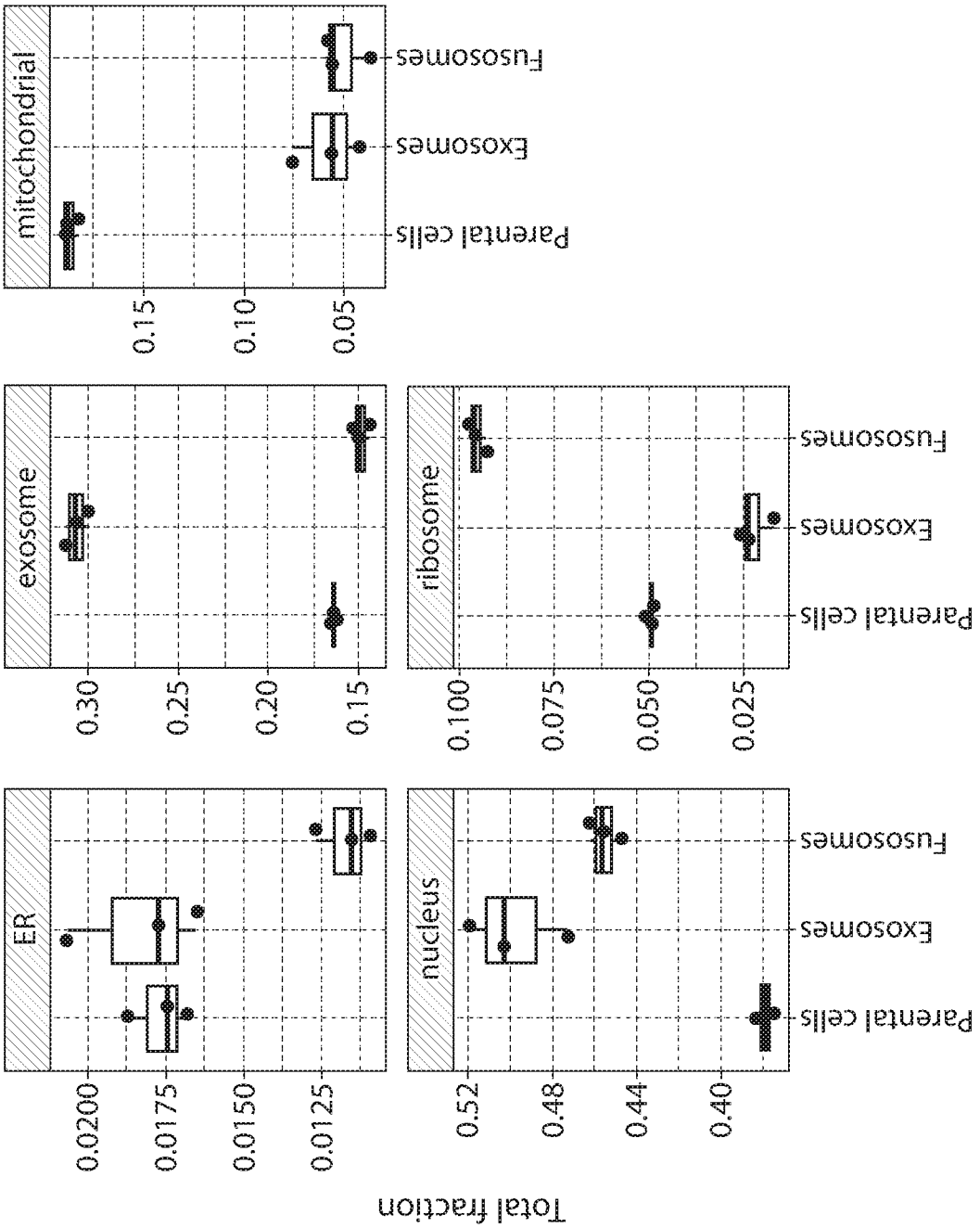

Total fraction
ER
0.0200
0.0175
0.0150
0.0125
nucleus
0.52
0.48
0.44
0.40
exosome
0.30
0.25
0.20
0.15
ribosome
0.100
0.075
0.050
0.025
mitochondrial
0.15
0.10
0.05
Parental cells
Exosomes
Fusosomes

Fig. 20

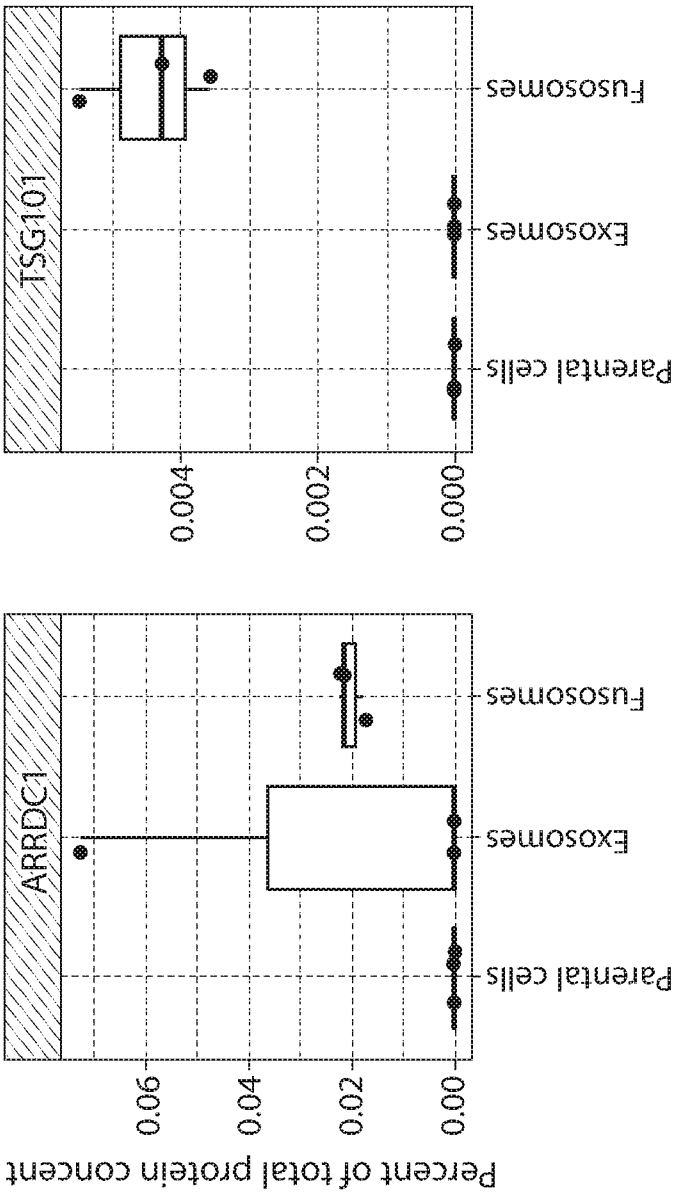

Percent of total protein concent
0.06
0.04
0.02
0.00
ARRDC1
Parental cells
Exosomes
Fusosomes
0.004
0.002
0.000
TSG101
Parental cells
Exosomes
Fusosomes

Fig. 21

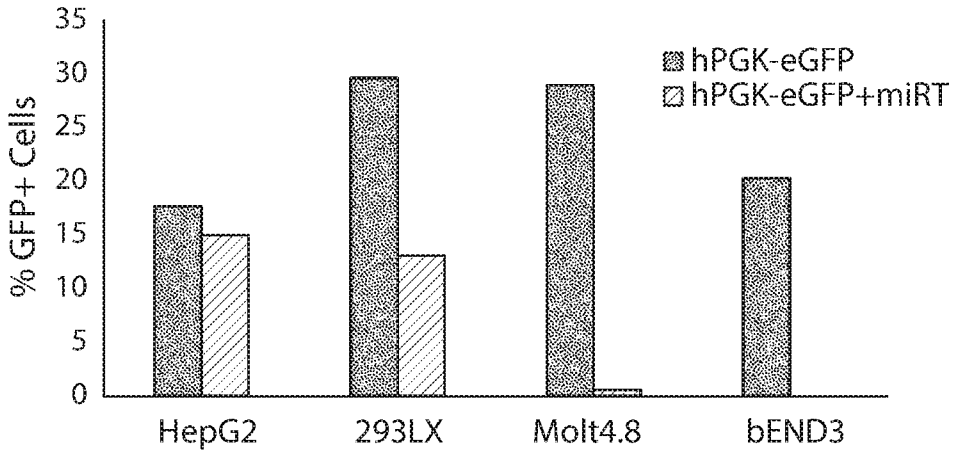


Fig. 22A

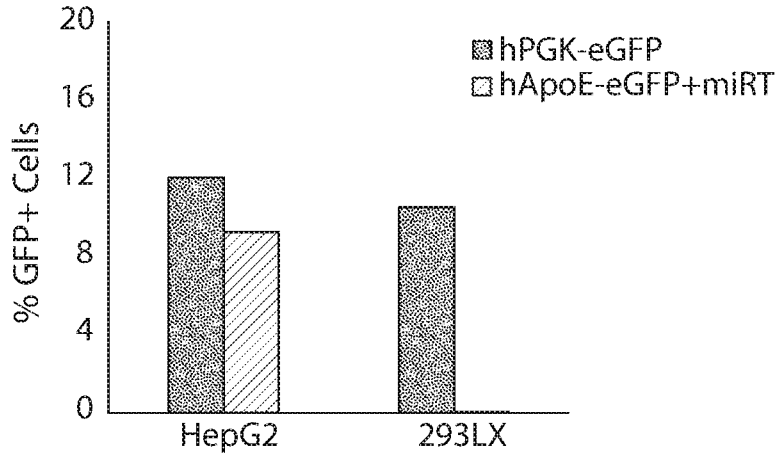


Fig. 22B

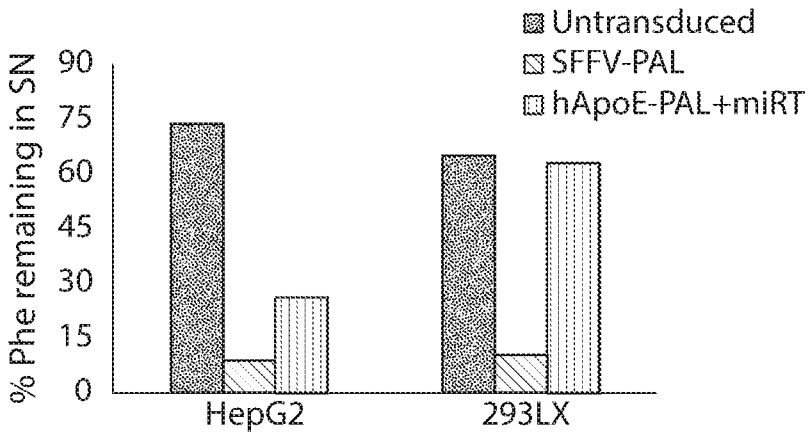


Fig. 22C

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/040978

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/12 C12N7/00 C07K14/005 C12N15/88
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K C12N

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MOHAMMAD KHALID ZAKARIA ET AL: "Combination of hepatocyte specific delivery and transformation dependent expression of shRNA inducing transcriptional gene silencing of c-Myc promoter in hepatocellular carcinoma cells", BMC CANCER, BIOMED CENTRAL, LONDON, GB, vol. 14, no. 1, 10 August 2014 (2014-08-10), page 582, XP021195683, ISSN: 1471-2407, DOI: 10.1186/1471-2407-14-582 p. 2 col. 2, 3rd -penultimate , p. 3 col. 1, 2nd , p. 3 col. 2, 1st , p. 6 col. 2, 3rd , p. 8 col. 1, 1st , p. 9 col. 1, 1st , p. 14 col. 1, 2nd -last , p. 14 col. 2, 3rd , p. 2 col. 1, , p. 9 col. 2, 3rd ; -/--	1-3,6,7, 9-40, 42-52

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 September 2019	05/12/2019

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Landré, Julien

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/040978

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	figure Cover figure -----	
X	WO 01/74861 A2 (MAYO FOUNDATION [US]; VILE RICHARD G [US] ET AL.) 11 October 2001 (2001-10-11) p. 1 li. 24-27, p. 2 li. 11-13p. 20 li. 18-p. 21 li. 12, p. 31 li. 10-15, p. 36 li. 25-p. 37 li. 3, p. 2 li. 1-6, p. 2 li. 16, p. 4 li. 3, p. 2 li. 17-p. 3 li. 6, p. 4 li. 4-23, p. 24 li. 21-24, p. 25 li. 5-14, p. 44 li. 13-15, p. 3 li. 8-9, p. 30 li. 1-4, p. 40 li. 25-p. 41 li. 2, p. 52 li. 1-4; figures 3,6 -----	1-3,6,7, 9-52
Y	WO 2009/130208 A1 (VIB VZW [BE]; LIFE SCIENCES RES PARTNERS VZW [BE] ET AL.) 29 October 2009 (2009-10-29) p. 2, last , p. 3, 2nd , p. 12, last , p. 15, last , p. 17, 2nd , p. 20, 4th , p. 22, 3rd ; sequence HC070343 -----	16-19, 21,23, 26,27, 31-37,46
Y	WO 2008/115199 A2 (UNIV NORTH CAROLINA [US]; JOHNSTON ROBERT E [US] ET AL.) 25 September 2008 (2008-09-25) p. 28, 2nd , p. 36, 3rd -p. 37, 1st , p. 46, last -p. 47, 1st , p. 51, 3rd , p. 36, 3rd -----	16-19, 21,23, 26,27, 31-37,46
L	WO 2018/208728 A1 (FLAGSHIP PIONEERING INC [US]) 15 November 2018 (2018-11-15) the whole document -----	1-52

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/040978

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

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

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

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

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1, 2(completely); 3, 6, 7, 9-52(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1, 2(completely); 3, 6, 7, 9-52(partially)

   A fusosome comprising a lipid bilayer comprising a fusogene and a nucleic acid comprising a heterologous gene and a liver specific regulatory element.

   ---

2. claims: 4, 5(completely); 3, 6, 7, 9-52(partially)

   A fusosome comprising a lipid bilayer comprising a fusogene and a nucleic acid comprising a heterologous gene and any other regulatory elements. This invention may also lack unity.

   ---

3. claims: 8(completely); 9-27, 36-52(partially)

   A fusosome comprising a lipid bilayer comprising a fusogene and a nucleic acid comprising a heterologous gene. This invention may also lack unity.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2019/040978

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0174861 A2	11-10-2001	AU 5113801 A	15-10-2001
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		WO 2018208728 A1	15-11-2018