CLAIMS

What is claimed is:

1. A fusosome comprising:

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

(b) a lumen surrounded by the lipid bilayer;

(c) a fusogen that is exogenous or overexpressed relative to the source cell, wherein the fusogen is disposed in the lipid bilayer, and wherein the fusogen is other than VSV-G; and

(d) a nuclear payload agent for delivery to the nucleus of a target cell, wherein the nuclear payload agent encodes a protein having a nuclear localization signal (NLS), and

wherein the source cell is allogeneic, e.g., obtained from a different organism of the same species as the target.

2. The fusosome of claim 1, wherein the nuclear payload agent encodes nuclear localization signal set forth in any one of SEQ ID NOS: 128-507 and 605-626.

3. The fusosome of claim 1 or claim 2, wherein:

(i) when a target cell is contacted with a plurality of the fusosomes, the nuclear payload agent or a protein encoded therein becomes present in the nucleus of the target cell at a level at least 10%, 20%, 50%, 100%, 2-fold, 5-fold, 10-fold, 20-fold, 50-fold, or 100-fold higher than the level of the nuclear payload agent in the cytoplasm of the target cell;

(ii) when a population of target cells is contacted with a plurality of the fusosomes, the nuclear payload agent or a protein encoded therein becomes enriched in the nucleus of at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of cells in the population of target cells;

(iii) when a population of target cells is contacted with a plurality of the fusosomes, the nuclear payload agent or a protein encoded therein becomes present in a sub-population, which is the recipient cell population, of the population of target cells, wherein the nuclear payload agent or protein encoded therein becomes enriched in the nucleus in at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of cells in the recipient cell population; and/or

(iv) at least 1, 2, 5, 10, 20, 50, 100, 200, 500, or 1,000 copies of the nuclear payload agent, or a protein encoded by a nucleic acid nuclear payload agent, are present in the nucleus in a target cell.

4. The fusosome of any of claims 1-3, wherein the nuclear payload agent encodes one or more of a transcriptional activator, transcriptional repressor, epigenetic modifier, histone acetyltransferase, histone deacetylase, histone methyltransferase, DNA methyltransferase, a DNA nickase, a site-specific DNA editing enzyme, optionally a deaminase, DNA transposase, DNA integrase, an RNA editor, an RNA splicing factor, or a PIWI protein.

5. The fusosome of any of claims 1-4, wherein the nuclear payload agent encodes: a transcription factor, a nuclease, a recombinase, an epigenetic factor, a post-transcriptional RNA modification factor, a non-coding RNA or ribonucleic protein, or a structural protein.

6. The fusosome of any of claims 1-5, wherein the nuclear payload agent encodes an antibody molecule, e.g., a Fab, an scFv, an scFab, a sdAb, a duobody, a minibody, a nanobody, a diabody, a zybody, a camelid antibody, a BiTE, a quadroma, or a bsDb.

7. The fusosome of any of claims 1-4, wherein the nuclear payload agent encodes a fusion protein comprising an effector domain and a localization domain.

8. The fusosome of claim 7, wherein the localization domain comprises a TAL domain, a Zinc Finger domain, a Cas9 domain, or a meganuclease domain.

9. The fusosome of claim 7 or claim 8, wherein the effector domain comprises a nuclease, recombinase, integrase, base editor (deaminase), transcription factor, or epigenetic modifier.

10. The fusosome of any of claims 1-4, wherein the nuclear payload agent encodes a gene editing protein or nucleic acid, optionally Cas9 or a guide RNA.

11. The fusosome of any of claims 1-4, wherein the nuclear payload agent comprises a functional RNA.

12. The fusosome of any of claims 1-4, wherein the nuclear payload agent comprises (i) a nucleic acid, and further comprises (ii) a protein that promotes nuclear import of the nucleic acid.

13. The fusosome of claim 12, wherein the nucleic acid comprises a binding site, and the protein comprises a protein that promotes nuclear import binds to the binding site.

14. The fusosome of claim 13, wherein the binding site comprises a TetR binding site and the protein comprises TetR.

15. The fusosome of any of claims 1-4, wherein the nuclear payload agent encodes a synthetic transcription factor that is a fusion protein comprising (i) a DNA binding domain, optionally wherein the DNA binding domain is a TAL domain, ZF domain, or Cas9 domain and (ii) a transcriptional activator domain or transcriptional repressor domain.

16. The fusosome of any of claims 1-4, wherein the nuclear payload agent encodes a synthetic epigenetic modifier that is a fusion protein comprising (i) a DNA binding domain and (ii) an epigenetic modifier domain.

17. The fusosome of any of claims 1-4, wherein the nuclear payload agent encodes a protein selected from fibrillarin; KRI1 homolog; nucleolar protein 11; upstream binding transcription factor; EBNA1 binding protein 2; coilin; promyelocytic leukemia; chromatin target of PRMT1; TERF1 interacting nuclear factor 2; ABL proto-oncogene 1, non-receptor tyrosine kinase; lamin B1; centromere protein B; H1 histone family member 0; kinetochore scaffold 1; RB binding protein 7, chromatin remodeling factor; signal transducer and activator of transcription 3; forkhead box P3; hepatocyte nuclear factor 4 alpha; runt related transcription factor 1; lymphoid enhancer binding factor 1; forkhead box P1; cut like homeobox 2; POU class 2 homeobox 1; SIX homeobox 3; RELA proto-oncogene, NF-kB subunit; SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1; chromodomain helicase DNA binding protein 7; chromatin accessibility complex subunit 1; APOBEC1 complementation factor; bromodomain containing 4; Cbp/p300 interacting transactivator with Glu/Asp rich carboxy-terminal domain 1; lysine acetyltransferase 2A; histone acetyltransferase 1; TATA-box binding protein associated factor 5 like; lysine acetyltransferase

5; sirtuin 1; sirtuin 2; histone deacetylase 11; histone deacetylase 3; histone deacetylase 6; ASH1 like histone lysine methyltransferase; DOT1 like histone lysine methyltransferase; nuclear receptor binding SET domain protein 1; SET nuclear proto-oncogene; lysine methyltransferase 2A; lysine demethylase 2A; lysine demethylase 1A; lysine demethylase 3A; lysine demethylase 4B; lysine demethylase 5C; DNA methyltransferase 1; DNA methyltransferase 3 alpha; adenosine deaminase 2; adenosine deaminase, RNA specific; apolipoprotein B mRNA editing enzyme catalytic subunit 1; spliceosome associated factor 3, U4/U6 recycling protein; cyclin dependent kinase 19; cyclin L1; pre-mRNA processing factor 31; splicing factor 3a subunit 3; mediator of DNA damage checkpoint 1; ATM serine/threonine kinase; poly(ADP-ribose) polymerase 1; DNA topoisomerase I; or DNA ligase 4.

18. The fusosome of any of claims 1-17, wherein the source cell is a primary cell, a cultured cell, an immortalized cell, or a cell line, optionally wherein the cell line is a myelobast cell line, optionally C2C12.

19. The fusosome of any of claims 1-18, wherein the source cell is an endothelial cell, a fibroblast, a blood cell (e.g., a macrophage, a neutrophil, a granulocyte, a leukocyte), a stem cell (e.g., a mesenchymal stem cell, an umbilical cord stem cell, bone marrow stem cell, a hematopoietic stem cell, an induced pluripotent stem cell e.g., an induced pluripotent stem cell derived from a subject's cells), an embryonic stem cell (e.g., a stem cell from embryonic yolk sac, placenta, umbilical cord, fetal skin, adolescent skin, blood, bone marrow, adipose tissue, erythropoietic tissue, hematopoietic tissue), a myoblast, a parenchymal cell (e.g., hepatocyte), an alveolar cell, a neuron (e.g., a retinal neuronal cell) a precursor cell (e.g., a retinal precursor cell, a myeloblast, myeloid precursor cells, a thymocyte, a meiocyte, a megakaryoblast, a promegakaryoblast, a melanoblast, a lymphoblast, a bone marrow precursor cell, a normoblast, or an angioblast), a progenitor cell (e.g., a cardiac progenitor cell, a satellite cell, a radial gial cell, a bone marrow stromal cell, a pancreatic progenitor cell, an endothelial progenitor cell, a blast cell), or an immortalized cell (e.g., HeLa, HEK293, HFF-1, MRC-5, WI-38, IMR 90, IMR 91, PER.C6, HT-1080, or BJ cell).

20. The fusosome of any one of claims 1-19, wherein the nuclear payload agent encodes a protein having a NLS which is not comprised by a naturally occurring counterpart of

the protein.

21. The fusosome of any of claims 1-20, wherein the source cell is selected from a white blood cell or a stem cell.

22. The fusosome of any of claims 1-21, wherein the source cell is selected from a neutrophil, a lymphocyte (e.g., a T cell, a B cell, a natural killer cell), a macrophage, a granulocyte, a mesenchymal stem cell, a bone marrow stem cell, an induced pluripotent stem cell, an embryonic stem cell, or a myeloblast.

23. The fusosome of any of claims 1-22, wherein the fusosome is from a source cell having a modified genome that exhibits reduced immunogenicity, optionally by genome editing to remove MHC complexes.

24. The fusosome of any of claims 1-23 wherein the fusosome has a diameter that is less than about 0.01% or 1%, of that of the source cell.

25. The fusosome of any of claims 1-24, wherein the fusogen is a mammalian fusogen or a viral fusogen.

26. The fusosome of any of claims 1-25, wherein the fusogen is active at a pH of 6-8.

27. The fusosome of any of claims 1-26, wherein the fusosome comprises a targeting domain which localizes the fusosome to a target cell.

28. The fusosome of claim 27, wherein the targeting domain interacts with a target cell moiety on the target cell.

29. The fusosome of any one of claims 1-28, wherein the target cell is in an organism.

30. The fusosome of any one of claims 1-28, wherein the target cell is a primary cell isolated from an organism.

31. The fusosome of any one of claims 1-30, wherein the target cell is selected from an endothelial cell, a fibroblast, a blood cell (e.g., a macrophage, a neutrophil, a granulocyte, a leukocyte), a stem cell (e.g., a mesenchymal stem cell, an umbilical cord stem cell, bone marrow stem cell, a hematopoietic stem cell, an induced pluripotent stem cell e.g., an induced pluripotent stem cell derived from a subject's cells), an embryonic stem cell (e.g., a stem cell from embryonic yolk sac, placenta, umbilical cord, fetal skin, adolescent skin, blood, bone marrow, adipose tissue, erythropoietic tissue, hematopoietic tissue), a myoblast, a parenchymal cell (e.g., hepatocyte), an alveolar cell, a neuron (e.g., a retinal neuronal cell) a precursor cell (e.g., a retinal precursor cell, a myeloblast, myeloid precursor cells, a thymocyte, a meiocyte, a megakaryoblast, a promegakaryoblast, a melanoblast, a lymphoblast, a bone marrow precursor cell, a normoblast, or an angioblast), a progenitor cell (e.g., a cardiac progenitor cell, a satellite cell, a radial gial cell, a bone marrow stromal cell, a pancreatic progenitor cell, an endothelial progenitor cell, a blast cell), or an immortalized cell (e.g., HeLa, HEK293, HFF-1, MRC-5, WI-38, IMR 90, IMR 91, PER.C6, HT-1080, or BJ cell).

32. A pharmaceutical composition comprising a fusosome of any of claims 1-31.

33. The fusosome of any of claims 1-31 for use in therapy.

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