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 comprises or encodes a protein having a nuclear localization signal (NLS).

2. The fusosome of claim 1, wherein the nuclear protein payload agent comprises 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 protein payload agent or a protein encoded therein becomes present in the nucleus of the target cell at a level at least 10%, 20%, 50%, 100%, 2-fold, 5-fold, 10-fold, 20-fold, 50-fold, or 100-fold higher than the level of the nuclear protein payload agent in the cytoplasm of the target cell;

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

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

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

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

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

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

7. The fusosome of any of claims 1-4, wherein the nuclear protein payload agent comprises or 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 comprises or 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 (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 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 comprises 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 comprises 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 protein payload agent is or comprises a protein selected from fibrillarin; KRI1 homolog; nucleolar protein 11; upstream binding transcription factor; EBNA1 binding protein 2; coilin; promyelocytic leukemia; chromatin target of PRMT1; TERF1 interacting nuclear factor 2; ABL proto-oncogene 1, non-receptor tyrosine kinase; lamin B1; centromere protein B; H1 histone family member 0; kinetochore scaffold 1; RB binding protein 7, chromatin remodeling factor; signal transducer and activator of transcription 3; forkhead box P3; hepatocyte nuclear factor 4 alpha; runt related transcription factor 1; lymphoid enhancer binding factor 1; forkhead box P1; cut like homeobox 2; POU class 2 homeobox 1; SIX homeobox 3; RELA proto-oncogene, NF-kB subunit; SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b,

member 1; chromodomain helicase DNA binding protein 7; chromatin accessibility complex subunit 1; APOBEC1 complementation factor; bromodomain containing 4; Cbp/p300 interacting transactivator with Glu/Asp rich carboxy-terminal domain 1; lysine acetyltransferase 2A; histone acetyltransferase 1; TATA-box binding protein associated factor 5 like; lysine acetyltransferase 5; sirtuin 1; sirtuin 2; histone deacetylase 11; histone deacetylase 3; histone deacetylase 6; ASH1 like histone lysine methyltransferase; DOT1 like histone lysine methyltransferase; nuclear receptor binding SET domain protein 1; SET nuclear proto-oncogene; lysine methyltransferase 2A; lysine demethylase 2A; lysine demethylase 1A; lysine demethylase 3A; lysine demethylase 4B; lysine demethylase 5C; DNA methyltransferase 1; DNA methyltransferase 3 alpha; adenosine deaminase 2; adenosine deaminase, RNA specific; apolipoprotein B mRNA editing enzyme catalytic subunit 1; spliceosome associated factor 3, U4/U6 recycling protein; cyclin dependent kinase 19; cyclin L1; pre-mRNA processing factor 31; splicing factor 3a subunit 3; mediator of DNA damage checkpoint 1; ATM serine/threonine kinase; poly(ADP-ribose) polymerase 1; DNA topoisomerase I; or DNA ligase 4.

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 of claims 1-19, wherein the source cell is allogeneic, e.g., obtained from a different organism of the same species as the target cell.

21. The fusosome of any of claims 1-19, wherein the source cell is autologous, e.g., obtained from the same organism as the target cell.

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

23. The fusosome of any of claims 1-22, 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.

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

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

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

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

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

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

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

31. The fusosome of any one of claims 1-29, wherein the target cell is a primary cell isolated from an organism.

32. The fusosome of any one of claims 1-31, 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).

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

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

i) providing a plurality of fusosomes according to any of claims 1-32; and

ii) formulating the plurality of fusosomes, fusosome composition, or pharmaceutical composition e.g., as a fusosome drug product suitable for administration to a subject.

35. Use of a fusosome composition comprising a plurality of fusosomes according to any of claims 1-32, in the manufacture of a pharmaceutical composition for delivery of a nuclear payload agent to the nucleus of a target cell of a subject a.

36. The method of claim 35, wherein the subject is a human subject.