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 payload agent comprises or encodes a protein having a nuclear localization signal (NLS) which is the same as the NLS in a naturally-occurring counterpart of the protein.~~

~~3. The fusosome of claim 1, wherein the nuclear protein payload agent comprises or encodes a protein having a NLS which is not comprised by a naturally-occurring counterpart of the protein.~~

2~~4~~. The fusosome of claim 1~~any of claims 1-~~3, wherein the nuclear protein payload agent comprises nuclear localization signal set forth in any one of SEQ ID NOS: 128-507 and 605-626.

3~~5~~. The fusosome of claim 1 or claim 2~~any of claims 1-4~~, wherein:

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

(ii) when a population of target cells is contacted with a plurality of the fusosomes, the nuclear protein payload agent or a protein encoded therein becomes enriched in the

nucleus of at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of cells in the population of target cells;

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

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

46. The fusosome of any of claims 1-35, 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.

57. The fusosome of any of claims 1-46, 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.

68. The fusosome of any of claims 1-57, 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.

79. The fusosome of any of claims 1-46, wherein the nuclear protein payload agent comprises or encodes a fusion protein comprising an effector domain and a localization domain.

8~~10~~. The fusosome of claim 7~~9~~, wherein the localization domain comprises a TAL domain, a Zinc Finger domain, a Cas9 domain, or a meganuclease domain.

9~~11~~. The fusosome of claim 7~~9~~ or claim 8~~10~~, wherein the effector domain comprises a nuclease, recombinase, integrase, base editor (deaminase), transcription factor, or epigenetic modifier.

10~~12~~. The fusosome of any of claims 1-~~4~~6, wherein the nuclear payload agent comprises or encodes a gene editing protein or nucleic acid, optionally Cas9 or a guide RNA.

11~~13~~. The fusosome of any of claims 1-~~4~~6, wherein the nuclear payload agent comprises a functional RNA.

12~~14~~. The fusosome of any of claims 1-~~4~~6, wherein the nuclear payload agent comprises (i) a nucleic acid, and (ii) a protein that promotes nuclear import of the nucleic acid.

13~~15~~. The fusosome of claim 12~~14~~, wherein the nucleic acid comprises a binding site, and the protein that promotes nuclear import binds to the binding site.

14~~16~~. The fusosome of claim 13~~15~~, wherein the binding site comprises a TetR binding site and the protein comprises TetR.

15~~17~~. The fusosome of any of claims 1-~~4~~6, 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~~18~~. The fusosome of any of claims 1-~~4~~6, wherein the nuclear payload agent comprises a synthetic epigenetic modifier that is a fusion protein comprising (i) a DNA binding domain and (ii) an epigenetic modifier domain.

1719. The fusosome of any of claims 1-46, wherein the nuclear protein payload agent is or comprises a protein selected from fibrillarin; KRI1 homolog; nucleolar protein 11; upstream binding transcription factor; EBNA1 binding protein 2; coilin; promyelocytic leukemia; chromatin target of PRMT1; TERF1 interacting nuclear factor 2; ABL proto-oncogene 1, non-receptor tyrosine kinase; lamin B1; centromere protein B; H1 histone family member 0; kinetochore scaffold 1; RB binding protein 7, chromatin remodeling factor; signal transducer and activator of transcription 3; forkhead box P3; hepatocyte nuclear factor 4 alpha; runt related transcription factor 1; lymphoid enhancer binding factor 1; forkhead box P1; cut like homeobox 2; POU class 2 homeobox 1; SIX homeobox 3; RELA proto-oncogene, NF-kB subunit; SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1; chromodomain helicase DNA binding protein 7; chromatin accessibility complex subunit 1; APOBEC1 complementation factor; bromodomain containing 4; Cbp/p300 interacting transactivator with Glu/Asp rich carboxy-terminal domain 1; lysine acetyltransferase 2A; histone acetyltransferase 1; TATA-box binding protein associated factor 5 like; lysine acetyltransferase 5; sirtuin 1; sirtuin 2; histone deacetylase 11; histone deacetylase 3; histone deacetylase 6; ASH1 like histone lysine methyltransferase; DOT1 like histone lysine methyltransferase; nuclear receptor binding SET domain protein 1; SET nuclear proto-oncogene; lysine methyltransferase 2A; lysine demethylase 2A; lysine demethylase 1A; lysine demethylase 3A; lysine demethylase 4B; lysine demethylase 5C; DNA methyltransferase 1; DNA methyltransferase 3 alpha; adenosine deaminase 2; adenosine deaminase, RNA specific; apolipoprotein B mRNA editing enzyme catalytic subunit 1; spliceosome associated factor 3, U4/U6 recycling protein; cyclin dependent kinase 19; cyclin L1; pre-mRNA processing factor 31; splicing factor 3a subunit 3; mediator of DNA damage checkpoint 1; ATM serine/threonine kinase; poly(ADP-ribose) polymerase 1; DNA topoisomerase I; or DNA ligase 4.

~~20. A fusosome comprising:~~

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

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

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

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

21. The fusosome of claim 20, wherein the organellar payload agent comprises or encodes a protein having a signal sequence which is the same as the signal sequence in a naturally-occurring counterpart of the protein.

22. The fusosome of claim 20, wherein the organellar payload agent comprises or encodes a protein having a signal sequence which is not comprised by a naturally-occurring counterpart of the protein.

23. A fusosome comprising:

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

(b) a lumen surrounded by the lipid bilayer;

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

(d) an organellar payload agent that comprises or encodes a heterologous signal sequence to the organellar payload agent that is sufficient to enrich the organellar payload agent in a cytoplasmic organelle of a target cell.

24. The fusosome of claim 23, wherein the cytoplasmic organelle is chosen from a mitochondrion, Golgi apparatus, lysosome, endoplasmic reticulum, vacuole, endosome, acrosome, autophagosome, centriole, glycosome, glyoxysome, hydrogenosome, melanosome,

~~mitosome, cnidocyst, peroxisome, proteasome, vesicle, or stress granule.~~

~~25. The fusosome of any of claims 20-24, wherein the heterologous signal sequence is set forth in any one of SEQ ID NOS: 39-127 and 605-626.~~

~~26. The fusosome of any of claims 20-25, wherein, when a target cell is contacted with a plurality of the fusosomes, the organellar payload agent becomes enriched in one or more of the Golgi apparatus, endoplasmic reticulum, vacuole, acrosome, autophagosome, centriole, glycosome, glyoxysome, hydrogenosome, melanosome, mitosome, cnidocyst, peroxisome, proteasome, vesicle, or stress granule of the target cell relative to the cyosol or plasma membrane of the target cell.~~

~~27. The fusosome of any of claims 20-26, wherein the cytoplasmic organelle is a secretory pathway organelle and the heterologous signal sequence is set forth in any one of SEQ ID NOS: 108-120.~~

~~28. The fusosome of any of claims 20-26, wherein the cytoplasmic organelle is a endoplasmic reticulum and the heterologous signal sequence is set forth in any one of SEQ ID NOS: 72-93 and 121-127.~~

~~29. The fusosome of any of claims 20-26, wherein the cytoplasmic organelle is a Golgi apparatus and the heterologous signal sequence is set forth in any one of SEQ ID NOS: 94-107 and 609-610.~~

~~30. The fusosome of any of claims 23-25, wherein, when a target cell is contacted with a plurality of the fusosomes, the encoded polypeptide becomes enriched in the lysosome and/or endosome, relative to the cytosol or plasma membrane of the target cell.~~

~~31. The fusosome of any of claims 23-25 and 30, wherein the cytoplasmic organelle is a lysosome and the heterologous signal sequence is set forth in any one of SEQ ID NOS: 39-~~

71 and 605-608.

32. The fusosome of any of claims 23-25, wherein the organellar payload agent becomes enriched in mitochondria of the target cell at a level at least 10%, 20%, 50%, 100%, 2-fold, 5-fold, 10-fold, 20-fold, 50-fold, or 100-fold higher than the level of the organellar payload agent in the cytoplasm or the target cell plasma membrane.

33. The fusosome of any of claims 20-32, wherein the organellar payload agent comprises or encodes a polypeptide chosen from a mitochondrial membrane protein, a outer mitochondrial membrane protein, an inner mitochondrial membrane protein, a mitochondrial inner boundary membrane protein, a mitochondrial cristal membrane protein, a mitochondrial DNA protein, a mitochondrial intermembrane space protein, a mitochondrial matrix protein, a mitochondrial matrix granule protein, a mitochondrial cristae protein, a mitochondrial ribosome protein, a mitochondrial intracristal protein, a mitochondrial peripheral space protein, a peroxisomal membrane protein, a peroxisomal crystalloid core protein, a peroxisomal matrix protein, a peroxin.

34. The fusosome of any of claims 20-33, wherein the organellar payload agent comprises or encodes a polypeptide chosen from:

i) a metabolic protein;

ii) a protease;

iii) a chaperone;

iv) a protein transport protein;

v) a mitochondrial ribosome protein;

vi) a mitochondrial transcription protein; or

vii) a mitochondrial replication protein.

35. The fusosome of any of claims 20-34, wherein the organellar payload agent comprises a protein selected from B-cell receptor-associated protein 31; calnexin; KDEL endoplasmic reticulum protein retention receptor 3; reticulon 4; Sec61 translocon alpha 1

~~subunit; Sarcoplasmic/endoplasmic reticulum calcium ATPase 1; Derlin-2; Stress-associated endoplasmic reticulum protein 2; Reticulocalbin-2; Atlastin-3; cytochrome c, somatic; translocase of inner mitochondrial membrane 44; translocase of outer mitochondrial membrane 40; voltage dependent anion channel 2; coenzyme Q8B; Acetyl-CoA acetyltransferase, mitochondrial; Citrate synthase, mitochondrial; Cytochrome c oxidase subunit 7A-related protein, mitochondrial; Fumarate hydratase, mitochondrial; NADPH:adrenodoxin oxidoreductase, mitochondrial; Induced myeloid leukemia cell differentiation protein Mcl-1; NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 13; Transcription factor A, mitochondrial; fatty acyl-CoA reductase 1; acetyl-CoA acyltransferase 1; ATP binding cassette subfamily D member 3; hydroxysteroid 17-beta dehydrogenase 4; peroxisomal biogenesis factor 14; Non-specific lipid-transfer protein; Peroxisomal biogenesis factor 19; Enoyl-CoA delta isomerase 2; Acyl-CoA-binding domain-containing protein 5; golgin A1; golgi integral membrane protein 4; golgi associated PDZ and coiled-coil motif containing; trans-golgi network vesicle protein 23 homolog C; zinc finger DHHC-type containing 7; Coatomer subunit delta; BSD domain-containing protein 1; Platelet glycoprotein 4; Conserved oligomeric Golgi complex subunit 7; Coatomer subunit epsilon; Protein FAM3C; Alpha-(1,6)-fucosyltransferase; General vesicular transport factor p115; Transmembrane protein 165; Vacuolar protein sorting-associated protein 54; Protein YIPF3; Fas associated factor family member 2; lysophosphatidylcholine acyltransferase 2; NAD(P) dependent steroid dehydrogenase-like; perilipin 3; patatin like phospholipase domain containing 2; cadherin 4; catenin beta 1; epidermal growth factor receptor; ezrin; syntaxin 4; ATP-binding cassette sub-family G member 2; Caskin-2; Junction plakoglobin; Neural cell adhesion molecule 1; Pannexin-1; Solute carrier family 2, facilitated glucose transporter member 1; Stomatin-like protein 3; FUS RNA binding protein; TAR DNA binding protein; TATA-box binding protein associated factor 15; EWS RNA binding protein 1; DEAD-box helicase 4; TAR DNA-binding protein 43; Heterogeneous nuclear ribonucleoprotein A1; Heterogeneous nuclear ribonucleoproteins A2/B1; Nucleolysin TIA-1 isoform p40; centrosomal protein 164; doublecortin domain containing 2; keratin 8; myosin heavy chain 10; actin bet; Coiled-coil domain-containing protein 14; Keratin, type I cytoskeletal 18; Pericentrin; Tubulin alpha-1A chain; or Actin, alpha skeletal muscle.~~

~~36. The fusosome of any of claims 20-34, wherein the organellar payload agent comprises or encodes an antibody molecule, e.g., a Fab, an scFV, an scFab, a sdAb, a duobody, a minibody, a nanobody, a diabody, a zybody, a camelid antibody, a BiTE, a quadroma, or a bsDb.~~

~~37. The fusosome of any of claims 20-34, wherein the organellar payload agent comprises a functional RNA.~~

~~38. The fusosome of any of claims 20-37, wherein, when a population of target cells is contacted with a plurality of the fusosomes, the organellar payload agent becomes enriched in the cytoplasmic organelle of at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of cells in the population of target cells.~~

18~~39~~. The fusosome of any of claims 1-17~~38~~, wherein the source cell is a primary cell, a cultured cell, an immortalized cell, or a cell line , optionally wherein the cell line is a myelobast cell line, optionally C2C12.

19~~40~~. The fusosome of any of claims 1-18~~39~~, wherein the source cell is an endothelial cell, a fibroblast, a blood cell (e.g., a macrophage, a neutrophil, a granulocyte, a leukocyte), a stem cell (e.g., a mesenchymal stem cell, an umbilical cord stem cell, bone marrow stem cell, a hematopoietic stem cell, an induced pluripotent stem cell e.g., an induced pluripotent stem cell derived from a subject's cells), an embryonic stem cell (e.g., a stem cell from embryonic yolk sac, placenta, umbilical cord, fetal skin, adolescent skin, blood, bone marrow, adipose tissue, erythropoietic tissue, hematopoietic tissue), a myoblast, a parenchymal cell (e.g., hepatocyte), an alveolar cell, a neuron (e.g., a retinal neuronal cell) a precursor cell (e.g., a retinal precursor cell, a myeloblast, myeloid precursor cells, a thymocyte, a meiocyte, a megakaryoblast, a promegakaryoblast, a melanoblast, a lymphoblast, a bone marrow precursor cell, a normoblast, or an angioblast), a progenitor cell (e.g., a cardiac progenitor cell, a satellite cell, a radial gial cell, a bone marrow stromal cell, a pancreatic progenitor cell, an endothelial progenitor cell, a blast cell), or an immortalized cell (e.g., HeLa, HEK293, HFF-1, MRC-5, WI-38, IMR 90, IMR 91, PER.C6, HT-1080, or BJ cell).

2041. The fusosome of any of claims 1-1940, wherein the source cell is allogeneic, e.g., obtained from a different organism of the same species as the target cell.

2142. The fusosome of any of claims 1-1940, wherein the source cell is autologous, e.g., obtained from the same organism as the target cell.

2243. The fusosome of any of claims 1-2142, wherein the source cell is selected from a white blood cell or a stem cell.

2344. The fusosome of any of claims 1-2243, 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.

2445. The fusosome of any of claims 1-2344, wherein the fusosome is from a source cell having a modified genome that exhibits reduced immunogenicity, optionally by genome editing to remove MHC complexes.

2546. The fusosome of any of claims 1-2445 wherein the fusosome has a diameter that is less than about 0.01% or 1%, of that of the source cell.

2647. The fusosome of any of claims 1-2546, wherein the fusogen is a mammalian fusogen or a viral fusogen.

2748. The fusosome of any of claims 1-2647, wherein the fusogen is active at a pH of 6-8.

2849. The fusosome of any of claims 1-27, wherein the fusosome comprises a targeting domain which localizes the fusosome to a target cell.

2950. The fusosome of claim 2849, wherein the targeting domain interacts with a target cell moiety on the target cell.

3051. The fusosome of any one of claims 1-2950, wherein the target cell is in an organism.

3152. The fusosome of any one of claims 1-2950, wherein the target cell is a primary cell isolated from an organism.

3253. The fusosome of any one of claims 1-3152, 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).

3354. A pharmaceutical composition comprising a fusosome of any of claims 1-3253.

3455. A method of manufacturing a fusosome composition, comprising:

i) providing a plurality of fusosomes according to any of claims 1-3253; 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.

3556. ~~A method of administering a fusosome composition to a subject, comprising administering to the subject a~~ Use of a fusosome composition comprising a plurality of fusosomes according to any of claims 1-3253, in the manufacture of a pharmaceutical composition for delivery of a nuclear payload agent to the nucleus of a target cell of a subject~~thereby~~ a~~dministering the fusosome composition to the subject~~.

~~57. A method of delivering a protein membrane payload to a subject, comprising administering to the subject a fusosome composition comprising a plurality of fusosomes according to any of claims 1-53, wherein the fusosome composition is administered in an amount and/or time such that the protein payload gene is delivered.~~

~~58. A method of treating a disease or disorder in a patient comprising administering to the subject a plurality of fusosomes according to any of claims 1-53, wherein the fusosome composition is administered in an amount and/or time such that the disease or disorder is treated.~~

~~59. The method of claim 58, wherein the disease or disorder is selected from cancer, autoimmune disorder, or infectious disease.~~

3660. The method of claim 35~~any of claims 56-59~~, wherein the subject is a human subject.