

## LIPID PARTICLES FOR DELIVERING A PAYLOAD

### CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority benefit under 35 U.S.C. § 119(e) to U.S. Provisional Patent Application Nos. 63/619,395 filed January 10, 2024 and 63/511,130 filed June 29, 2023, the disclosure of which is each incorporated herein by reference in its entirety.

### FIELD OF THE INVENTION

[0002] The disclosure relates to lipid particles, pharmaceutical compositions comprising the lipid particles described herein, and methods of use comprising administering the lipid particles described herein.

### BACKGROUND

[0003] Although lipid nanoparticles have been developed to deliver a payload to cells, there remains a need in the art for improved lipid particle compositions that are suitable for therapeutic use. Disclosed herein are lipid particles designed to deliver a payload (e.g., a nucleic acid) that are well-tolerated and provide an adequate therapeutic response.

### SUMMARY OF THE INVENTION

[0004] The present disclosure is based, in part, on lipid particles comprising one or more ionizable lipids, one or more neutral lipids, one or more sterols, one or more charged lipids, and one or more stealth lipids.

[0005] Lipid particles comprising cholesteryl hemisuccinate ("CHEMS") and a payload and optionally one or more of:

- a. one or more ionizable lipids;
- b. one or more neutral lipids;
- c. one or more sterols; and
- d. one or more stealth lipids

are contemplated herein.

[0006] Methods of targeting a payload to a spleen of a subject *in vivo* comprising combining a lipid particle or a composition comprising the payload encapsulated within a lipid particle or a composition comprising CHEMS in a molar amount of at least 11% are also contemplated herein. In some aspects, the lipid particle or composition further comprises one or more ionizable

lipids, one or more neutral lipids, one or more sterols, one or more stealth lipids, or any combination thereof. In some aspects, the lipid particle delivers the payload to the spleen.

**[0007]** In some aspects, the one or more ionizable lipids in the lipid particles described herein are one or more selected from the group consisting of DLin-MC3-DMA (“MC3”), DLin-KC2-DMA (“KC2”), ssPalmO-Phe (“SS-OP”), C12-200, SM-102,  $\alpha$ -D-tocopherolsuccinoyl (“SS-EC”), ALC-0315 (“ALC”), Tri-N-tridecyl 3-(ethyl(methyl)amino)propanoate (“304-O13”), tetrakis(2-(octyldisulfaneyl)ethyl) 3,3',3'',3'''-(((methylazanediy)bis(propane-3,1-diyl))bis(azanetriyl))tetrapropionate (“306-O12B”), 9-[4-(dimethylamino)-1-oxobutoxy]-heptadecanedioic acid, 1,17-di-(2Z)-2-nonen-1-yl ester (“L319”), 9,12-octadecadienoic acid, (9Z,12Z)-1,1',1'',1'''-[(3,6-dioxo-2,5-piperazinediy)bis(4,1-butanediylnitrilodi-4,1-butanediyl)] ester (“OF-C4-Deg-Lin”), 2-(dioctylamino)ethyl nonyl hydrogen phosphate (“9A1P9”), 5-(((3-(dibutylamino)propyl)amino)methyl)-6-hydroxyundecane-1,11-diyl (9Z,9'Z,12Z,12'Z)-bis(octadeca-9,12-dienoate) (“IR-117-17”), 9Z,12Z-octadecadienoic acid, 3-[4,4-bis(octyloxy)-1-oxobutoxy]-2-[[[3-(diethylamino)propoxy]carbonyl]oxy]methyl]propyl ester (“LP-01”) and 4A3-SC8. In some aspects, the one or more ionizable lipids in the lipid particles described herein are one or more selected from the group consisting of DLin-MC3-DMA (“MC3”), DLin-KC2-DMA (“KC2”), ssPalmO-Phe (“SS-OP”), C12-200, SM-102,  $\alpha$ -D-tocopherolsuccinoyl (“SS-EC”), and ALC-0315 (“ALC”). In some aspects, only one ionizable lipid (e.g., SS-OP) is in the lipid particle.

**[0008]** In some aspects, the lipid particles described herein comprises one or more neutral lipids. In some aspects, the one or more neutral lipids in the lipid particle are in a molar amount of at least 0.5%. In some aspects, the one or more neutral lipids in the lipid particles are one or more phosphatidylcholine (“PC”), phosphatidylethanolamine (“PE”), and combination thereof. In some aspects, the one or more neutral lipids in the lipid particles described herein are one or more selected from the group consisting of 1,2-diastearoyl-sn-glycero-3-phosphocholine (“DSPC”), 1,2-dioleoyl-sn-glycero-3-phosphocholine (“DOPC”), 1,2-dioctadecanoyl-sn-glycerol (“DSDG”), 1,2-dioleoyl-sn-glycerol, 1,2-di-(9Z-octadecenoyl)-sn-glycerol (“DODG”), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (“DOPE”), diacylphosphatidylcholine, diacylphosphatidylethanolamine, ceramide, sphingomyelin, cephalin, cerebrosides, diacylglycerols dipalmitoylphosphatidylcholine (“DPPC”), palmitoyl-oleoyl-phosphatidylcholine (“POPC”), palmitoyl-oleoyl-phosphatidylethanolamine (“POPE”), palmitoyl-oleoyl-phosphatidylglycerol (POPG), dipalmitoyl-phosphatidylethanolamine (“DPPE”), dimyristoyl-

phosphatidylethanolamine (“DMPE”), distearoyl-phosphatidylethanolamine (“DSPE”), monomethyl-phosphatidylethanolamine, dimethyl-phosphatidylethanolamine, dielaidoyl-phosphatidylethanolamine (“DEPE”), stearyl-oleoyl-phosphatidylethanolamine (“SOPE”), and egg phosphatidylcholine (“EPC”). In some aspects, only one neutral lipid (e.g., DOPE, DODG) is in the lipid particles described herein.

**[0009]** In some aspects, the lipid particles described herein comprises one or more sterols. In some aspects, the one or more sterols in the lipid particles described herein are in a molar amount of at least 5%. In some aspects, the one or more sterols in the lipid particles described herein are cholesterol,  $\beta$ -sitosterol, stigmasterol, campesterol, fucosterol, brassicasterol, ergosterol, 9,11-dehydroergosterol, daucosterol,  $\beta$ -sitosterol acetate, and other C-24 alkyl derivatives, and combinations thereof. In some aspects, the one or more sterols in the lipid particles described herein are cholesterol,  $\beta$ -sitosterol, and combinations thereof. In some aspects, the lipid particles described herein, only one sterol (e.g., cholesterol) is in the lipid particle.

**[0010]** In some aspects, the lipid particles described herein comprise one or more stealth lipids. In some aspects, the one or more stealth lipids in the lipid particles described herein are in a molar amount of at least 0.1%. In some aspects, the one or more stealth lipids in the lipid particles described herein are one or more PEG terminated lipids, one or more polysarcosine derivatives, or combinations thereof. In some aspects, only one polyethylene glycol (“PEG”) terminated lipid (DMG-PEG2000, DMPE-PEG2000, PEG2000-PE) is in the lipid particles described herein.

**[0011]** Lipid particles comprising:

- a. one or more ionizable lipids in a molar amount of about 20% to about 70%;
- b. one or more neutral lipids in a molar amount of about 2.5% to about 25%;
- c. one or more sterols in a molar amount of about 20% to about 50%;
- d. one or more charged lipids in a molar amount of about 10% or less than 10%; and
- e. one or more stealth lipids in a molar amount of about 0.25% to about 3%.

are additionally contemplated herein.

**[0012]** Methods of targeting a payload to a tissue other than a spleen of a subject in vivo comprising combining a lipid particle or a composition comprising the payload encapsulated within a lipid particle or a composition comprising one or more ionizable lipids, one or more neutral lipids, one or more sterols, one or more stealth lipids, and/or one or more charged lipids,

wherein the lipid particle or composition (a) does not comprise cholesteryl hemisuccinate (“CHEMS”) or (b) comprises a molar amount of about 10% or less than 10% of CHEMS.

**[0013]** In some aspects, the one or more charged lipids in the lipid particles described herein are selected from the group consisting of cholesteryl hemisuccinate (“CHEMS”), 1,2-distearoyl-3-trimethylammonium-propane (“DSTAP”), 18:1 1,2-dioleoyl-3-trimethylammonium-propane (“DOTAP”), 1,2-dioleoyl-sn-glycero-3-phosphate (“18:1 PA”), 18:1 1-stearoyl-2-oleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (“18:1 PG”), phosphatidylglycerols, cardiolipins, diacylphosphatidylserines, diacylphosphatidic acids, N-dodecanoyl phosphatidylethanolamines, N-succinyl phosphatidylethanolamines, N-glutarylphosphatidylethanolamines, lysylphosphatidylglycerols, palmitoyloleyolphosphatidylglycerol (“POPG”), 1,2-dimyristoyl-3-trimethylammonium-propane (“DMTAP”), 1,2-dipalmitoyl-3-trimethylammonium-propane (“DPTAP”), palmitoyloleyoyl-3-trimethylammonium-propane (“POTAP”), 1,2-dioleoyl-3-dimethylammonium-propane (“DODAP”), palmitoyloleyoyl-3-dimethylammonium-propane (“PODAP”), 1,2-dimyristoyl-3-dimethylammonium-propane (“DMDAP”), 1,2-dipalmitoyl-3-dimethylammonium-propane (“DPDAP”), 1,2-distearoyl-3-dimethylammonium-propane (“DSDAP”), 1,2-dioleoyl-3-dimethylhydroxyethyl-ammonium-propane (“DODMHEAP”) (also known as DORI), palmitoyloleyoyl-3-dimethylhydroxyethyl-ammonium-propane (“PODMHEAP”) (also known as PORI), 1,2-dimyristoyl-3-dimethylhydroxyethyl-ammonium-propane (“DMDMHEAP”) (also known as DMRI), 1,2-dipalmitoyl-3-dimethylhydroxyethyl-ammonium-pPropane (“DPDMHEAP”) (also known as DPRI), 1,2-distearoyl-3-dimethylhydroxyethyl-ammonium-propane (“DSDMHEAP”) (also known as DSRI), 1,2-diioleoyl-3-methylhydroxyethylammonium-propane (“DOMDHEAP”), palmitoyloleyoyl-3-methylhydroxyethylammonium-propane (“POMDHEAP”), 1,2-dimyristoyl-3-methyldihydroxyethylammonium-propane (“DMMDHEAP”), 1,2-dipalmitoyl-3-methyldihydroxyethylammonium-propane (“DPMDHEAP”), 1,2-distearoyl-3-methyldihydroxyethylammonium-propane (“DSMDHEAP”), 1,2-dioleoyl-3-methyldihydroxyethylammonium-propane (“DOMHEAP”), palmitoyloleyoyl-3-methylhydroxyethylammonium-propane (“POMHEAP”), 1,2-dimyristoyl-3-methylhydroxyethylammonium-propane (“DMMHEAP”), 1,2-dipalmitoyl-3-methylhydroxyethylammonium-propane (“DPMHEAP”), 1,2-distearoyl-3-methylhydroxyethylammonium-propane (“DSMHEAP”), 1,2-dioleoyl-3-dihydroxyethylammonium-propane (“DODHEAP”), palmitoyloleyoyl-3-dihydroxyethylammonium-propane (“PODHEAP”), 1,2-dimyristoyl-3-



dihydroxyethylammonium-propane ("DMDHEAP"), 1,2-dipalmitoyl-3-dihydroxyethylammonium-propane ("DPDHEAP"), 1,2-distearoyl-3-dihydroxyethylammonium-propane ("DSDHEAP"), dimethyldioctadecylammonium bromide ("DDAB"), dioleyldimethylammonium chloride ("DODAC"), 1,2-dioleoyl-sn-glycero-3-ethylphosphocholine ("DOEPC"), 1,2-dimyristoyl-sn-glycero-3-ethylphosphocholine ("DMEPC"), 1,2-dipalmitoyl-sn-glycero-3-ethylphosphocholine ("DPEPC"), 1,2-distearoyl-sn-glycero-3-ethylphosphocholine ("DSEPC"), palmitoyloleoyl-sn-glycero-3-ethylphosphocholine ("POEPC"), 1,2-dioleoyl-3-dimethyl-hydroxyethyl ammonium propane ("DORIE"), 1,2-dimyristyl-3-dimethyl-hydroxyethyl ammonium propane ("DMRIE"), 1,2-dioleoyl-3-methyl-(methoxycarbonyl-ethyl)ammonium-propane ("DOMCAP"), 1,2-dioleoyl-3-methyl-(methoxycarbonylmethyl)ammonium-propane ("DOMGME"), 1,2-dioleoyl-3-N-pyrrolidine-propane ("DOP5P"), 1,2-dioleoyl-3-N-pyrridinium-propane, bromide salt ("DOP6P"), 3b-[N-(N9,N9-dimethylaminoethane)carbamoyl] cholesterol ("DC-Chol"), 3b-[N-(N9,N9-trimethylaminoethane) carbamoyl] cholesterol ("TC-Chol"), 3b(N-(N,N'-Dimethylaminoethan)-carbamoyl)cholesterol ("DAC-Chol"), cholesteryl-oxycarbonyl-methyl-trimethylammonium chloride ("Chol-Betaine"), N-methyl{4-N-amino[(3'-β-cholesteryl) carbamoyl]}piperazine ("N-methyl-PipChol"), cetyltrimethylammonium bromide ("CTAB"), N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethyl ammonium chloride ("DOTMA"), 4-(2-aminoethyl)-morpholino-cholesterolhemisuccinate: ("MoChol"), histaminyl-Cholesterolhemisuccinate ("HisChol"), cholesterol-(3-imidazol-1-yl propyl)carbamate ("Chim"), (N-2-propylamino[(3'-β-cholesteryl) carbamoyl]}morpholine ("MoC3Chol"), [(3-morpholine-4-yl-propylcarbamoyl)-methyl]-carbamic acid cholesteryl ester ("Chol-C3N-Mo3"), ("Chol-C3N-Mo2"), [(2-morpholine-4-yl-ethylcarbamoyl)methyl]-carbamic acid cholesteryl ester ("Chol-C4N-Mo2"), [1-methyl-2-(2-morpholine-4-yl-ethylcarbamoyl)-propyl]-carbamic acid cholesteryl ester ("Chol-DMC3N-Mo2"), 2-(2-morpholine-4-yl-ethylcarbamoyl)-cyclohexane carboxylic acid cholesteryl ester ("CholC4Hex-Mo2"), 4-(2-aminoethyl)-morpholino-cholesterol-2,3-dimethylhemisuccinate ("DmC4Mo2"), 4-(2-aminoethyl)-morpholino-cholesterol-2,2-dimethylhemimalonate ("DmC3Mo2"), 4-(2-aminoethyl)-morpholino-cholesterol-hemimalonate ("C3Mo2"), 4-(2-aminopropyl)-morpholino-cholesterol-hemimalonate ("C3Mo3"), 4-(2-aminoethyl)-morpholino-cholesterol-hemiglutarate ("C5Mo2"), 4-(2-aminoethyl)-morpholino-cholesterol-hemiadipate ("C6Mo2"), 4-(2-aminoethyl)-morpholino-cholesterol-hemiadipate ("C8Mo2"), 4-(2-aminobutyl)-morpholino-cholesterol-hemisuccinate ("C4Mo4"), 4{N-2-ethylamino[(3'-β-cholesteryl) carbamoyl]}piperazine ("PipC2Chol"), {N-2-ethylamino[(3'-β-cholesteryl)

carbamoyl]]morpholine ("MoC2Chol"), {N-2-ethylamino[(3'-β-cholesteryl)  
 carbamoyl]]pyrrolidine ("PyrroC2Chol"), {N-2-propylamino[(3'-β-cholesteryl)  
 carbamoyl]]imidazole ("ImC3Chol"), {N-2-ethylamino[(3'-β-cholesteryl) carbamoyl]]pyridine  
 ("PyC2Chol"), 1,2-dioleoyl-3-N-morpholine-propane ("MoDO"), 1,2-dipalmitoyl-3-N-  
 morpholine-propane ("MoDP"), 4,(2,3-bis-acyloxy-propyl)-1-methyl-1H-imidazole ("DOIM")  
 (also known as DPIM). diacylglycerolhemisuccinate, e.g. dioctadecylamido-glycylspermine  
 ("DOGS"), dimyristoylglycerolhemisuccinate ("DMGS") (also known as DMG-Succ), 1-  
 palmitoyl-2-oleoylglycerolhemisuccinate ("POGS") (also known as POG-Succ),  
 dipalmitoylglycerolhemisuccinate ("DPGS") (also known as POG-Succ),  
 distearoylglycerolhemisuccinate ("DSGS") (also known as DSG-Succ),  
 diacylglycerolhemimalonate, e.g. dioleoylglycerolhemimalonate ("DOGM"),  
 dimyristoylglycerolhemimalonate ("DMGM"), diacylglycerolhemiglutarate, e.g.  
 dioleoylglycerolhemiglutarate ("DOGG"), dimyristoylglycerolhemiglutarate ("DMGG"),  
 diacylglycerolhemiadipate, e.g. dioleoylglycerolhemiadipate ("DOGA"),  
 dimyristoylglycerolhemiadipate ("DMGA"), diacylglycerolhemicyclohexane-1,4-dicarboxylic  
 acid, e.g. dioleoylglycerolhemicyclohexane-1,4-dicarboxylic acid ("DO-cHA"),  
 dimyristoylglycerolhemicyclohexane-1,4-dicarboxylic acid ("DM-cHA"), (2,3-Diacyl-  
 propyl)amino}-oxoalkanoic acid, e.g. 4-{(2,3-dioleoyl-propyl)amino}-4-oxobutanoic acid  
 ("DOAS"), 3-{(2,3-dioleoyl-propyl)amino}-3-oxopropanoic acid ("DOAM"), 5-{(2,3-dioleoyl-  
 propyl)amino}-5-oxopentanoic acid ("DOAG"), 6-{(2,3-dioleoyl-propyl)amino}-6-oxohexanoic  
 acid ("DOAA"), 4-{(2,3-dimyristoyl-propyl)amino}-4-oxobutanoic acid ("DMAS"), 3-{(2,3-  
 dimyristoyl-propyl)amino}-3-oxopropanoic acid ("DMAM"), 5-{(2,3-dimyristoyl-  
 propyl)amino}-5-oxopentanoic acid ("DMAG"), 6-{(2,3-dimyristoyl-propyl)amino}-6-  
 oxohexanoic acid ("DMAA"), diacyl-alkanoic acid, e.g. 2,3-dioleoyl-propanoic acid ("DOP"),  
 3,4-dioleoyl-butanoic acid ("DOB"), 5,6-dioleoyl-hexanoic acid ("DOS"), 4,5-dioleoyl-pentanoic  
 acid ("DOM"), 6,7-dioleoyl-heptanoic acid ("DOG"), 7,8-dioleoyl-octanoic acid ("DOA"), 2,3-  
 dimyristoyl-propanoic acid ("DMP"), 3,4-dioleoyl-butanoic acid ("DOB"), 5,6-dimyristoyl-  
 hexanoic acid ("DMS"), 4,5-dimyristoyl-pentanoic acid ("DMM"), 6,7-dimyristoyl-heptanoic  
 acid ("DMG"), 7,8-dimyristoyl-octanoic acid ("DMA"), cholesteryloxycarbonylaminocarboxylic  
 acid, e.g. cholesterolhemidodecane dicarboxylic acid ("Chol-C12"), 12-  
 cholesteryloxycarbonylaminododecanoic acid ("CholC13N"), fatty acids, e.g. oleic acid, myristic  
 acid, palmitic acid, stearic acid, nervonic acid, behenic acid, dioleoylphosphatidic acid  
 ("DOPA"), 1,2-dimyristoyl-sn-glycero-3-phosphate ("DMPA"), 1,2-dipalmitoyl-sn-glycero-3-

phosphate ("DPPA"), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphate ("POPA"), 1,2-Distearoyl-sn-glycero-3-phosphate ("DSPA"), cholesterol sulphate ("Chol-SO<sub>4</sub>"), dioleoylphosphatidylglycerol ("DOPG"), 1,2-dimyristoyl-sn-glycero-3-phospho-(1'-rac-glycerol), ("DMPG"), dipalmitoylphosphatidylglycerol ("DPPG"), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol ("POPG"), 1,2-distearoyl-sn-glycero-3-phospho-rac-glycerol ("DSPG"), dioleoylphosphatidylserine ("DOPS"), 1,2-dimyristoyl-sn-glycero-3-phospho-L-serine ("DMPS"), dipalmitoylphosphatidylserine ("DPPS"), 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine ("POPS"), 1,2-distearoyl-sn-glycero-3-phospho-L-serine ("DSPS"), or cetylphosphate. In some aspects, the one or more charged lipids in the lipid particles described herein are selected from the group consisting of cholesteryl hemisuccinate ("CHEMS"), 1,2-distearoyl-3-trimethylammonium-propane ("DSTAP"), 18:1 1,2-dioleoyl-3-trimethylammonium-propane ("DOTAP"), 1,2-dioleoyl-sn-glycero-3-phosphate ("18:1 PA"), 18:1 1-stearoyl-2-oleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) ("18:1 PG"), phosphatidylglycerols, cardiolipins, diacylphosphatidylserines, diacylphosphatidic acids, N-dodecanoyl phosphatidylethanolamines, N-succinyl phosphatidylethanolamines, N-glutarylphosphatidylethanolamines, lysylphosphatidylglycerols, and palmitoyl-oleoylphosphatidylglycerol ("POPG"). In some aspects, only one charged lipid (e.g., CHEMS) is in the lipid particles described herein. In some aspects, the one or more charged lipids (e.g., CHEMS) in the lipid particles described herein are in a molar of about 10% or less than 10%. In some aspects, the lipid particles described herein do not comprise a charged lipid (e.g., CHEMS).

**[0014]** The lipid particles described herein can comprise a payload. In some aspects, the payload comprises a biologically active molecule. In some aspects, the biologically active molecule is a small molecule, a nucleic acid, an aptamer, or any combination thereof. In some aspects, the biologically active molecule comprises a nucleic acid. In some aspects, the nucleic acid comprises a small interfering ribonucleic acid (siRNA), a short hairpin RNA (shRNA), a micro-ribonucleic acid (miRNA), a primary micro-ribonucleic acid (pri-miRNA), a long non-coding RNA (lncRNA), a messenger ribonucleic acid (mRNA), a clustered regularly interspaced short palindromic repeats (CRISPR) related nucleic acid, a CRISPR-RNA (crRNA), a single guide ribonucleic acid (sgRNA), a trans-activating CRISPR ribonucleic acid (tracrRNA), a plasmid deoxyribonucleic acid (pDNA), a transfer ribonucleic acid (tRNA), an antisense oligonucleotide (ASO), an antisense ribonucleic acid (RNA), a guide ribonucleic acid, deoxyribonucleic acid (DNA), a double stranded deoxyribonucleic acid (dsDNA), a single stranded deoxyribonucleic acid (ssDNA), a single stranded ribonucleic acid (ssRNA), a double

stranded ribonucleic acid (dsRNA), a CRISPR-associated (Cas) protein, or combinations thereof. In some aspects, the nucleic acid in the lipid particles described herein comprises mRNA. In some aspects, the nucleic acid in the lipid particles described herein encodes a peptide having therapeutic activity. In some aspects, the peptide comprises an epitope amino acid sequence. In some aspects, the peptide induces immune tolerance to cells.

**[0015]** In some aspects, the lipid particles described herein, when administered to a subject in need thereof, the subject exhibits one or more of: (i) an increase in Treg cells, (ii) reduction in effector T effector cells, (iii) reduction in B-cells, (iv) reduction in cytokine production, (v) reduction in activated B cells; (vi) reduction in activated effector T cells; or (vii) any combination thereof.

**[0016]** In some aspects, the lipid particles described herein, comprise one or more ionizable lipids comprising  $\alpha$ -D-tocopherolsuccinoyl ("SS-EC"). In some aspects, a subject administered the lipid particles comprising SS-EC described herein exhibits (i) higher levels of B-cells, (ii) higher levels of T cells, and (iii) higher levels of  $\text{INF}\alpha$  as compared with a subject administered a lipid particle containing MC-3 as the ionizable lipid.

**[0017]** Pharmaceutical compositions comprising the lipid particles described herein and one or more pharmaceutically acceptable carriers are contemplated. In some aspects, one of the pharmaceutically acceptable carrier is sucrose. In some aspects, one of the pharmaceutically acceptable carrier is saline. In some aspects, the saline is buffered with tris.

**[0018]** Methods of targeting a payload to the pancreas of a subject in need thereof comprising intraperitoneally administering the lipid particles described herein to a subject are contemplated.

**[0019]** Methods of targeting a payload to a lymph node of a subject in need thereof comprising subcutaneously administering the lipid particles described herein to the subject are also contemplated.

**[0020]** Methods for treating a disease or disorder in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising the lipid particles described herein are additionally contemplated. In some aspects, the disease is an autoimmune disease. In some aspects, the autoimmune disease is selected from the group consisting of autoimmune hepatitis, inflammatory bowel disease (including Crohn's disease and ulcerative colitis), nonalcoholic fatty pancreas disease, scleroderma, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, celiac disease, Type 1 diabetes,

Guillain-Barré syndrome, Hashimoto's thyroiditis, polymyalgia rheumatic, alopecia areata, fibrosis, psoriasis, pemphigus vulgaris, vitiligo, ankylosing spondylitis, juvenile idiopathic arthritis, psoriatic arthritis, mixed connective tissue disease, neuromyelitis optica, latent autoimmune diabetes in adults ("LADA"), autoimmune thyroid disease, Grave's disease, Addison's disease, autoimmune atrophic gastritis, pernicious anemia, atopic dermatitis, bullous pemphigoid, myasthenia gravis, poly/dermatomyositis), rheumatic fever, primary sclerosing cholangitis, autoimmune uveitis and Behcet's disease), diseases that affect the blood or bone marrow (e.g., autoimmune haemolytic anemia, idiopathic thrombocytopenic purpura, idiopathic leucopenia, Goodpasture's syndrome, autoimmune nephritis, glomerulonephritis, Wegener's granulomatosis, chronic inflammatory demyelinating polyradiculoneuropathy, Sjogren's syndrome, primary biliary cholangitis, Parkinson's disorder, and antiphospholipid syndrome. In some aspects, the autoimmune disease is autoimmune hepatitis, Type 1 diabetes or multiple sclerosis. In some aspects, the disease or disorder is an infectious disorder or cancer.

**[0021]** Methods of preventing or delaying the onset or recurrence of a disease or disorder in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising the lipid particles described herein are further contemplated.

**[0022]** Methods for restoring immune homeostasis in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising the lipid particles described herein are contemplated.

**[0023]** Methods for limiting or reducing immunogenicity responses in a subject in need thereof comprising administering to the subject in need thereof a therapeutically effective amount of a pharmaceutical composition comprising lipid particles described herein are also contemplated.

**[0024]** Methods for limiting or reducing inflammatory responses in a subject in need thereof comprising administering to the subject in need thereof a therapeutically effective amount of a composition of lipid particles described herein are additionally contemplated.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

**[0025]** **FIG. 1** shows an exemplary process for preparing lipid particles provided herein. Exemplary components and reagents are non-limiting and are solely representative. Alternative components or reagents or additional or fewer components or reagents can be used in the process.

[0026] **FIGs. 2A-C** show the Bioluminescence Imaging (“BLI”) values of luciferase in mice administered Lipid Particle Formulation Nos. 1, 3-6, and 53-55 (in phosphate buffered saline (“PBS”)) and PBS control each of which contains mRNA encoding luciferase. **FIG. 2A** shows the BLI values of luciferase in the spleen of mice. **FIG. 2B** shows the BLI values of luciferase in the liver of mice. **FIG. 2C** is a graph of the spleen to liver ratio of BLI values for luciferase.

[0027] **FIGs. 3A-3C** show the BLI values of luciferase in mice administered Lipid Particle Formulation Nos. 1, 30, 31, 2, 32, and 33 (in PBS) and PBS control each of which contains mRNA encoding luciferase in mice. **FIG. 3A** shows the BLI values of luciferase in the spleen of mice. **FIG. 3B** shows the BLI values of luciferase in the liver of mice. **FIG. 3C** is a graph of the spleen to liver ratio of BLI values for luciferase.

[0028] **FIGs. 4A-4C** show the BLI values of luciferase in mice administered Lipid Particle Formulation Nos. 13, 1, and 43-48 (in PBS) and PBS control. **FIG. 4A** shows the BLI values of luciferase in the liver of mice. **FIG. 4B** shows the BLI values of luciferase in the spleen of mice. **FIG. 4C** is a graph of the spleen to liver ratio of BLI values for luciferase.

[0029] **FIGs. 5A-5C** show the BLI values of luciferase in mice administered Lipid Particle Formulation Nos. 15, 16, 20-22, 13, 23, and 24 (in PBS), and PBS control each of which includes mRNA encoding luciferase. **FIG. 5A** shows the BLI values of luciferase in the spleen of mice. **FIG. 5B** shows the BLI values of luciferase in the liver of mice. **FIG. 5C** is a graph of the spleen to liver ratio of BLI values for luciferase.

[0030] **FIG. 6** shows the BLI values of luciferase in the spleen of mice administered Lipid Particle Formulation Nos. 16, 25-29, 14, and 1 (in PBS) and PBS control, each of which includes mRNA encoding luciferase.

[0031] **FIGs. 7A-7C** show the BLI values of luciferase in mice administered Lipid Particle Formulation Nos. 13, 1, and 37-42 and PBS control each of which include mRNA encoding luciferase. **FIG. 7A** shows the BLI values of luciferase in the liver of mice. **FIG. 7B** shows the BLI values of luciferase in the spleen of mice. **FIG. 7C** is a graph of the spleen to liver ratio of BLI values for luciferase.

[0032] **FIGs. 8A-8C** show the BLI values of luciferase in mice administered Lipid Particle Formulations Nos. 16, 25-29, 14, and 1 (in PBS) and PBS control each of which includes mRNA encoding luciferase. **FIG. 8A** shows the BLI values of luciferase in the liver of mice. **FIG. 8B**

shows the BLI values of luciferase in the spleen of mice. **FIG. 8C** is a graph of the spleen to liver ratio of BLI values for luciferase.

**[0033]** **FIGs. 9A-9C** show BLI values of luciferase in mice administered Lipid Particle Formulation Nos. 14 (in PBS) (intravenously administered), 1 (intravenously administered as Tris buffered solution), 1 (in PBS) (after being frozen for 1 month), and 7 (in PBS), each of which includes mRNA encoding luciferase. **FIG. 9A** shows the BLI values of luciferase in the spleen of mice. **FIG. 9B** shows the BLI values of luciferase in the liver of mice. **FIG. 9C** is a graph of the spleen to liver ratio of BLI values for luciferase.

**[0034]** **FIGs. 10A** and **10B** show spleen BLI values (**FIG. 10A**) and spleen to liver BLI values (**FIG. 10B**) for luciferase in mice after intravenous administration of Lipid Particle Formulation Nos. 34, 35, and 1 (in PBS), each of which includes mRNA encoding luciferase.

**[0035]** **FIGs. 11A-11C** show spleen BLI values (**FIG. 11A**), liver BLI values (**FIG. 11B**), and spleen to liver ratio (**FIG. 11C**) in mice after administration of Lipid Particle Formulation No. 1 including mRNA encoding luciferase at different time points following manufacture: fresh at 4 °C, after 1 month storage at 4 °C, freshly frozen at -80 °C (frozen for two weeks), after two freeze-thaw cycles at -80 °C, after 1 month storage at -80 °C, or freshly made. A “fresh” formulation means it was typically formulated the day before injection and stored at 4 °C overnight.

**[0036]** **FIGs. 12A-12C** show  $\text{INF}\alpha$  (**FIG. 12A**), CD69+ B-cell levels (**FIG. 12B**), and CD69+ T-cell levels (**FIG. 12C**) in mice after administration of PBS control, R848 (resiquimod), Lipid Particle Formulation No. 79 (in PBS), and Lipid Particle Formulation No. 80 (in PBS).

**[0037]** **FIG. 13** shows levels of T regulatory cells as a percentage of MOG-specific CD4+ splenocytes in mice after administration of Lipid Particle Formulation No. 1 (in PBS) with or without mRNA encoding myelin oligodendrocyte glycoprotein (“MOG”) epitope, IL-10, TGF- $\beta$ , and/or PD-L1.

**[0038]** **FIG. 14** shows FR4+ CD73+ anergic T cells as a percentage of conventional CD4+ splenocytes in mice after administration of Lipid Particle Formulation No. 1 with or without mRNA encoding MOG epitope, IL-10, TGF- $\beta$ , and/or PD-L1 (in PBS).

**[0039]** **FIGs. 15A-15E** show images of tissues in mice in which Lipid Particle Formulations delivered mRNA encoding luciferase to liver, spleen, and/or pancreas. **FIG. 15A** shows Lipid Particle Formulation No. 14 (in Tris-buffered saline (“TBS”)) delivered mRNA encoding

luciferase to liver and spleen when intravenously administered to mice. **FIG. 15B** shows Lipid Particle Formulation No. 14 (in TBS) delivered mRNA encoding luciferase to pancreas and spleen when intraperitoneally administered to mice. **FIG. 15C** shows Lipid Particle Formulation No. 1 (in TBS) after being frozen for two weeks delivered mRNA encoding luciferase to spleen when intravenously administered to mice. **FIG. 15D** shows Lipid Particle Formulation No. 1 (in TBS) delivered mRNA to spleen when intravenously administered to mice. **FIG. 15E** shows Lipid Particle Formulation No. 1 (in TBS) delivered mRNA to pancreas and spleen when intraperitoneally administered to mice.

**[0040]** **FIG. 16** shows whole body images of mice 16 hours, 24 hours, or 48 hours after subcutaneous administration of Lipid Particle Formulation Nos. 13, 14, and 1 (in PBS) delivered mRNA encoding luciferase. The regions of interest (“ROIs”) on images show the subcutaneous injection site and liver.

**[0041]** **FIGs. 17A-17C** show the impact on T cells in mice administered Lipid Particle Formulation No. 1 delivering mRNA encoding MOG epitope with and without IL-10. **FIG. 17A** provides an overview of the experiment. **FIG. 17B** shows the number of epitope-specific Foxp3+ regulatory T cells following administration of the Lipid Particle Formulation No. 1. **FIG. 17C** shows the number of epitope-specific CD4+, CD44hi, Foxp3- conventional T cells following administration of the Lipid Particle Formulation No. 1.

**[0042]** **FIGs. 18A-18B** show the *in vivo* efficacy of an mRNA tolerizing vaccine in an Experimental Autoimmune Encephalomyelitis (EAE) mouse model of multiple sclerosis. **FIG. 18A** provides the EAE score following administration of Lipid Particle Formulation No. 15 in a prophylactic study, as well as serum IL-17 levels. **FIG. 18B** provides the EAE score following administration of Lipid Particle Formulation No. 15 in a therapeutic study.

**[0043]** **FIGs. 19A-19B** show the *in vivo* efficacy of an mRNA tolerizing vaccine in a Nonobese Diabetic (“NOD”) mouse model of Type 1 Diabetes. **FIG. 19A** provides the blood glucose levels (mg/dL) in the mice following administration of Lipid Particle Formulation No. 1 containing KIF1a, tol105 (9-epitopes) and IL-10 mRNA. **FIG. 19B** provides the percent normal glucose in the NOD mice following administration of Lipid Particle Formulation No. 1 containing KIF1a mRNA, tol105 (9-epitopes) and IL-10.

**[0044]** **FIGs. 20A-20C** show the pKa determination of Lipid Particle Formulation Nos. 1 (**FIG. 20A**), 13 (**FIG. 20B**), and 14 (**FIG. 20C**).



[0045] FIG. 21 shows that for Lipid Particle Formulation No. 1, the relative concentration % (fLUC mRNA) remained above 85% for 4 months. For Lipid Particle Formulation Nos. 13 and 14, the relative concentration (%) decreased below 85% after 6 and 11 weeks respectively after storage at 2-8°C.

[0046] FIGs. 22A-22B show the capillary gel electrophoresis ("CE") analysis of Lipid Particle Formulation Nos. 1, 13, and 14 and fLuc mRNA at T=0 and T=16 weeks of storage at 2-8°C.

[0047] FIGs. 23A-23B provide results from a CE analysis. FIG. 23A provides a CE electropherogram for three different mRNA constructs encoding MOG epitope, IL-10, and ovalbumin ("OVA") epitope loaded in Lipid Particle Formulation No. 1. FIG. 23B provides the CE gel image for the three different mRNA constructs loaded in Formulation 1.

[0048] FIG. 24 provides IL-1 $\beta$  levels of Lipid Particle Formulation No. 1 and lipid particle formulations containing an ionizable lipid (SS-OP, MC-3, or SM-102), DSPC, cholesterol, and DMG-PEG2000 in a 50/10/38.5/1.5 molar ratio (mol% of each lipid in the formulation). None of the formulations generated any significant levels of IL-1 $\beta$  relative to the media alone (no LNP) group. This shows that the production and clean-up processes employed for mRNA and formulations generate a drug product that does not induce production of pro-inflammatory cytokines like IL-1 $\beta$  and does not stimulate the innate immune system. The positive control nigericin generated significantly high levels of IL-1 $\beta$  after treatment.

[0049] FIGs. 25A-25E shows the delivery efficacy in human peripheral blood mononuclear cells (PBMCs) and PBMC subsets *in vitro* (dendritic cells ("DCs") (FIG. 25A); monocytes (FIG. 25B); B cells (FIG. 25C); CD8 (FIG. 25D); and CD4 (FIG. 25E) of Lipid Particle Formulation Nos. 13, 14, and 1 and four-component formulations containing an ionizable lipid (DLin-MC3-DMA, SM-102, or SS-OP), DSPC, cholesterol, and DMG-PEG2k in a 50/10/38.5/1.5 molar ratio (mol% of each lipid in the formulation). Lipid Particle Formulation No. 1 showed a significantly higher delivery efficacy in all of the human PBMC subsets versus the other tested Lipid Particle Formulations.

[0050] FIG. 26 provides a comparison of Lipid Particle Formulation Nos. 13, 14, and 1 and four-component formulations containing an ionizable lipid (DLin-MC3-DMA or SM-102), DSPC, cholesterol, and DMG-PEG2k in a 50/10/38.5/1.5 molar ratio (mol% of each lipid in the

formulation). Lipid Particle Formulation No. 1 showed a significantly higher delivery efficacy in human PBMCs than the other tested Lipid Particle Formulations.

**[0051]** FIG. 27 provides the relative luminescence units (“RLU”) observed in human PBMCs after treatment of Lipid Particle Formulation No. 1 at various mRNA concentrations (firefly luciferase mRNA). A dose-response was observed with increasing concentrations of mRNA.

**[0052]** FIGs. 28A-28B show levels of T regulatory cells in mice following administration of Lipid Particle Formulation No. 1 encapsulating mouse serum albumin (naïve), MOG<sub>35-55</sub>, IL-10, or both MOG<sub>35-55</sub> and IL-10. FIG. 28A reports the number of MOG-specific FoxP3 positive Tregs observed. FIG. 28B reports the percentage of MOG-specific FoxP3 positive Tregs. Co-encapsulation of epitope (MOG<sub>35-55</sub>) and immunomodulatory (IL-10) induced significantly higher levels of MOG-specific Tregs compared to the epitope or immunomodulatory. An additive effect of administering the two mRNAs together was evident from the data.

**[0053]** FIG. 29 reports the IL-10 concentration in mice that were treated with Lipid Particle Formulation No. 1 encapsulating mouse serum albumin (“MSA”) MOG<sub>35-55</sub>, IL-10, or both MSA or MOG<sub>35-55</sub> and IL-10. Naïve mice were not treated with Lipid Particle Formulation No. 1. A clear dose response was observed for IL-10 concentrations in plasma, with higher IL-10 mRNA doses generating significantly higher plasma IL-10. Lipid Formulation No. 1 maintained the mRNA delivery efficacy through the duration of the study, with Day 8 and Day 18 IL-10 levels showing no differences.

**[0054]** FIGs. 30A-30D provide the particle size (FIG. 30A), polydispersity index (“PDI”) (FIG. 30B), mRNA encapsulation (FIG. 30C), BLI (FIG. 30D) for Lipid Particle Formulation No. 1 containing firefly luciferase mRNA after storage at -80°C until the designated time point. Lipid Particle Formulation No. 1 stock retained its original critical quality attributes (size, PDI, and mRNA encapsulation) as well as functional delivery upon long term storage -80°C for 6 months. The spleen specificity is also maintained.

**[0055]** FIGs. 31A-31C show liver BLI values (FIG. 31A), spleen BLI values (FIG. 31B), and spleen to liver ratio (FIG. 31C) in mice after administration of Lipid Particle Formulation No. 1 including mRNA encoding luciferase at different time points following manufacture: fresh at 4 °C, frozen at -80 °C (frozen for two weeks), after 1 month storage at -80 °C, after 3 months storage at -80 °C, after 6 months storage at -80 °C.

[0056] FIGs. 32A-32F show characteristics of Lipid Particle Formulation No. 1 T=0 at 4 °C and after 1 month at 4 °C compared with a second freshly made Lipid Particle Formulation No. 1. These characteristics are particle size (FIG. 32A), polydispersity index (FIG. 32B), % mRNA encapsulation (FIG. 32C), spleen BLI (FIG. 32D), liver BLI (FIG. 32E), and spleen to liver ratio (FIG. 32F).

[0057] FIGs 33A-33C provide the whole body images of mice administered Lipid Particle Formulation No. 1 T=0 at 4 °C and after 1 month at 4 °C compared with a second freshly made Lipid Particle Formulation No. 1.

[0058] FIGs. 34A-34B provide the whole body images of mice administered Lipid Particle Formulation Nos. 78, 7, 11, 12 (FIG. 34A), 77, 75, 76, and 7-10 (FIG. 34B) as well as the average spleen BLI, average liver BLI, and the spleen to liver ratio.

[0059] FIGs. 35A-35C show Lipid Formulation No. 1 inhibits disease activity and reduces monocyte infiltration when administered either intravenously or subcutaneously in mice. FIG. 35A show both i.v. and s.c. administration inhibit paralysis in an EAE mouse model. FIG. 35B compares the number of mononuclear cells of mice administered mouse serum albumin (naïve), control mRNA, and MOG<sub>35-55</sub> and IL-10 in Lipid Particle Formulation No. 1 administered *via* i.v. or s.c. FIG. 35C reports percentage of MOG-specific CD4<sup>+</sup> splenocytes that are Tregs in mice after administration of mice administered mouse serum albumin (naïve), control mRNA, and MOG<sub>35-55</sub> and IL-10 in Lipid Particle Formulation No. 1 administered *via* i.v. or s.c.

## DETAILED DESCRIPTION

[0060] The present disclosure is based, in part, a method of targeting a payload to a tissue (e.g., spleen, pancreas, lymph nodes, or liver) of a subject in need thereof, comprising combining the payload with one or more neutral lipids, one or more ionizable lipids (e.g., cholesteryl hemisuccinate (e.g., cholesteryl hemisuccinate (“CHEMS”)), one or more sterols, one or more stealth lipids, or any combination thereof. The lipid particles, or a composition comprising a payload encapsulated within the lipid particles described herein, are useful for treating a disease or disorder in a subject, e.g., an autoimmune disease, infectious disease, or cancer.

### I. DEFINITIONS

[0061] To facilitate an understanding of the present disclosure, a number of terms and phrases are defined below.

**[0062]** As used herein, the terms “a” and “an” mean “one or more” and include the plural unless the context is inappropriate.

**[0063]** Where the use of the term “about” is before a quantitative value, the present disclosure also includes the specific quantitative value itself, unless specifically stated otherwise. As used herein, the term “about” refers to a  $\pm 10\%$  variation from the nominal value unless otherwise indicated or inferred.

**[0064]** As used herein, the term “and/or” in connection with two or more recited objects includes individually each of the recited objects and the various combinations of two or more of the recited objects, unless otherwise understood from the context and use.

**[0065]** As used herein, the term “nucleic acid” as used herein refers to a polymer containing at least two deoxyribonucleotides or ribonucleotides in either single- or double-stranded form and includes DNA and RNA. Examples of nucleic acids include small interfering ribonucleic acid (siRNA), a short hairpin RNA (shRNA), a micro-ribonucleic acid (miRNA), a primary micro-ribonucleic acid (pri-miRNA), a long non-coding RNA (lncRNA), a messenger ribonucleic acid (mRNA), a clustered regularly interspaced short palindromic repeats (CRISPR) related nucleic acid, a CRISPR-RNA (crRNA), a single guide ribonucleic acid (sgRNA), a trans-activating CRISPR ribonucleic acid (tracrRNA), a plasmid deoxyribonucleic acid (pDNA), a transfer ribonucleic acid (tRNA), an antisense oligonucleotide (ASO), an antisense ribonucleic acid (RNA), a guide ribonucleic acid, deoxyribonucleic acid (DNA), a double stranded deoxyribonucleic acid (dsDNA), a single stranded deoxyribonucleic acid (ssDNA), a single stranded ribonucleic acid (ssRNA), a double stranded ribonucleic acid (dsRNA), a CRISPR-associated (Cas) protein, or combinations thereof.

**[0066]** Nucleic acids include nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, and which have similar binding properties as the reference nucleic acid. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2'-O-methyl ribonucleotides, and peptide-nucleic acids (PNAs). Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated. Specifically,

degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer *et al.*, *Nucleic Acid Res.*, 19:5081 (1991); Ohtsuka *et al.*, *J. Biol. Chem.*, 260:2605-2608 (1985); Rossolini *et al.*, *Mol. Cell. Probes*, 8:91-98 (1994)).

**[0067]** As used herein, the term “effective amount” refers to the amount of a payload (*e.g.*, a compound of the present disclosure) sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or dosages and is not intended to be limited to a particular formulation or administration route.

**[0068]** As used herein, percent “identity” between a polypeptide sequence and a reference sequence is defined as the percentage of amino acid residues in the polypeptide sequence that are identical to the amino acid residues in the reference sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Similarly, percent “identity” between a nucleic acid sequence and a reference sequence is defined as the percentage of nucleotides in the nucleic acid sequence that are identical to the nucleotides in the reference sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent sequence identity (*e.g.*, amino acid sequence identity or nucleic acid sequence identity) can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, MEGALIGN (DNASTAR), CLUSTALW, CLUSTAL OMEGA, or MUSCLE software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

**[0069]** The use of the term “include,” “includes,” “including,” “have,” “has,” “having,” “contain,” “contains,” or “containing,” including grammatical equivalents thereof, should be understood generally as open-ended and non-limiting, for example, not excluding additional unrecited elements or steps, unless otherwise specifically stated or understood from the context.

**[0070]** As used herein, the term “pharmaceutical composition” refers to the combination of an active agent with a carrier, inert or active, making the composition especially suitable for diagnostic or therapeutic use *in vivo* or *ex vivo*.

**[0071]** As used herein, the term “pharmaceutically acceptable carrier” refers to any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, emulsions

(e.g., such as an oil/water or water/oil emulsions), and various types of wetting agents. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants, *see e.g.*, Adejare, *Remington, The Science and Practice of Pharmacy* (23rd ed. 2020).

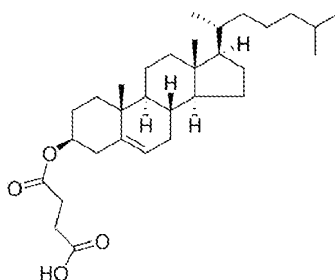
**[0072]** As used herein, the terms “subject” and “patient” are used interchangeably and refer to an organism to be treated by the methods and compositions described herein. Such organisms preferably include, but are not limited to, mammals (e.g., murines, simians, equines, bovines, porcines, canines, felines, and the like), and more preferably include humans.

**[0073]** As used herein, the term “treating” includes any effect, e.g., lessening, reducing, modulating, ameliorating or eliminating, that results in the improvement of the condition, disease, disorder, and the like, or ameliorating a symptom thereof.

**[0074]** As used herein, the term “lipid” refers to a group of organic compounds that include, but are not limited to, esters of fatty acids and are characterized by being insoluble in water, but soluble in many organic solvents.

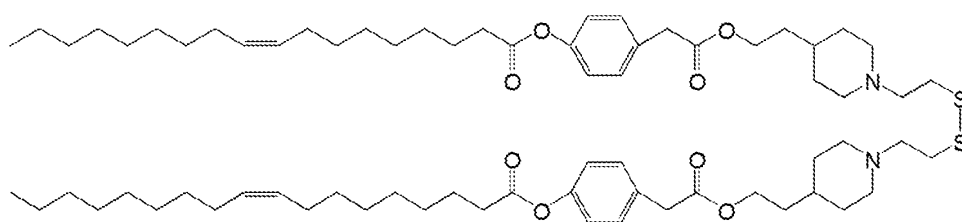
**[0075]** As used herein, the term “lipid particle” refers to a lipid formulation that can be used to deliver an active agent or therapeutic agent, such as a nucleic acid (e.g., an interfering RNA), to a target site of interest, e.g., spleen.

**[0076]** As used herein, the term “cholesteryl hemisuccinate” (“CHEMS”) refers to an acidic cholesterol ester having the following structure:



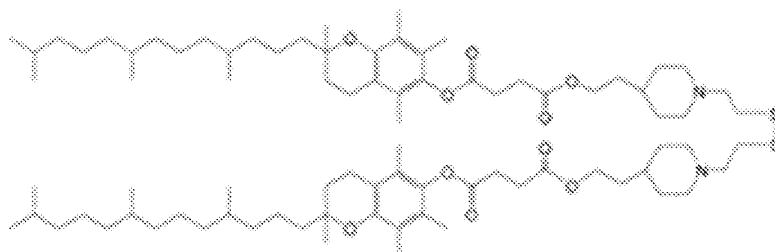
CHEMS is also known as 3 $\beta$ -hydroxy-5-cholestene 3-hemisuccinate, 5-cholesten-3 $\beta$ -ol, 3-hemisuccinate, and cholesteryl hydrogen succinate.

**[0077]** As used herein, the term “SS-OP” refers to an ionizable lipid having the following structure:



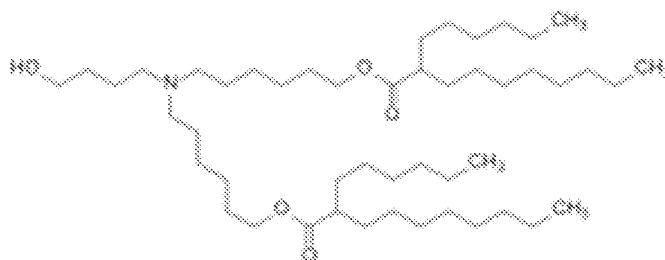
SS-OP is commercially available from NOF America Corporation (Coatsome SS-OP). SS-OP is also known as ssPalmO-Phe and Bis{2-(4-2-[4-cis-9-octadecenoyloxy]phenylacetoxylethyl}piperidinylethyl]disulfide.

**[0078]** As used herein the term “SS-EC” refers to an ionizable lipid having the following structure:



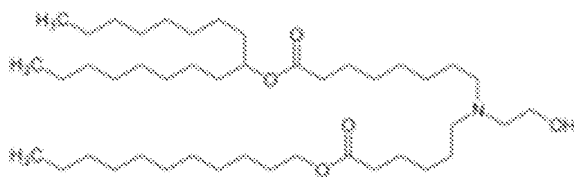
SS-EC is commercially available from NOF America Corporation (Coatsome SS-EC). SS-EC was formerly known as SS-33/4PE-15. SS-EC is also known as  $\alpha$ -D-tocopheryl succinoyl.

**[0079]** As used herein, the term “ALC-0315” refers to an ionizable lipid having the following structure:



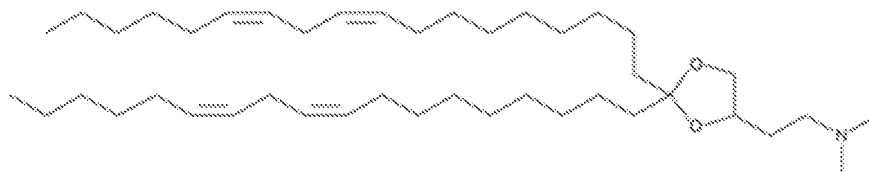
ALC-0315 is also known as [(4-hydroxybutyl)azanediyl]di(hexane-6,1-diyl) bis(2-hexyldecanoate) and 6-[6-(2-hexyldecanoyloxy)hexyl-(4-hydroxybutyl)amino]hexyl 2-hexyldecanoate.

**[0080]** As used herein, the term “SM-102” refers to an ionizable lipid having the following structure:



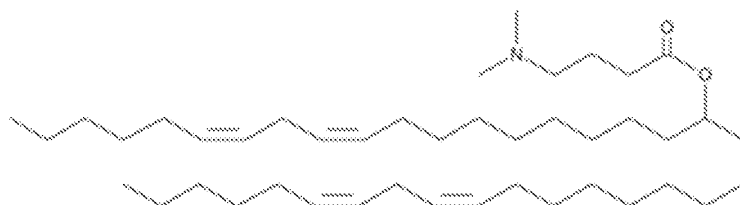
SM-102 is also known as 9-heptadecanyl 8-[(2-hydroxyethyl)[6-oxo-6-(undecyloxy)hexyl]amino]octanoate, 1-octylnonyl 8-[(2-hydroxyethyl)[6-oxo-6-(undecyloxy)hexyl]amino]octanoate, and heptadecan-9-yl 8-[2-hydroxyethyl-(6-oxo-6-undecyloxyhexyl)amino]octanoate.

**[0081]** As used herein, the term “DLin-KC2-DMA” (“KC2”) refers to an ionizable lipid having the following structure;



KC2 is also known as N,N-dimethyl-2,2-di-(9Z,12Z)-9,12-octadecadien-1-yl-1,3-dioxolane-4-ethanamine and 2-[2,2-bis[(9Z,12Z)-octadeca-9,12-dienyl]-1,3-dioxolan-4-yl]-N,N-dimethylethanamine.

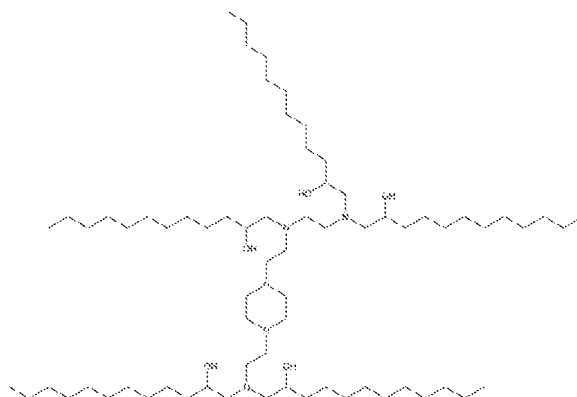
**[0082]** As used herein, the term “DLin-MC3-DMA” (“MC3”) refers to an ionizable lipid having the following structure:



MC3 is also known as 4-(dimethylamino)-butanoic acid, (10Z,13Z)-1-(9Z,12Z)-9,12-octadecadien-1-yl-10,13-nonadecadien-1-yl ester and (6Z,9Z,28Z,31Z)-Heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate.

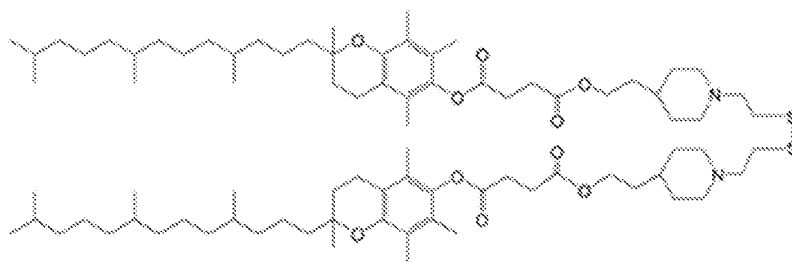
**[0083]** As used herein, the term “C12-200” refers to an ionizable lipid having the following structure:





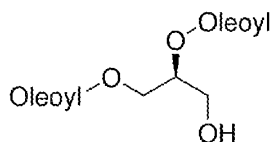
C12-200 is also known as 1-[2-[bis(2-hydroxydodecyl)amino]ethyl]-[2-[4-[2-[bis(2-hydroxydodecyl)amino]ethyl]piperazin-1-yl]ethyl]amino]dodecan-2-ol.

**[0084]** As used herein, the term “SS-EC” refers to an ionizable lipid having the following structure:



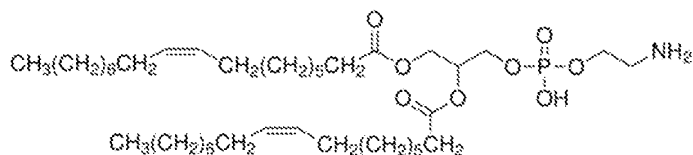
SS-EC is an  $\alpha$ -D-tocopherolsuccinoyl and is also known as Bis{2-[4-( $\alpha$ -D-tocopherolhemisuccinateethyl)piperidyl]ethyl} disulfide.

**[0085]** As used herein, the term “DODG” refers to a neutral lipid having the following structure:



DODG is also known as 1-2-di-(9Z-octadecenoyl)-sn-glycerol, [(2S)-3-hydroxy-2-[(Z)-octadec-9-enoyl]oxypropyl] (Z)-octadec-9-enoate.

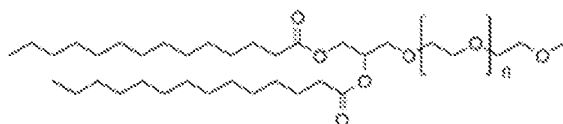
**[0086]** As used herein, the term “DOPE” refers to a neutral lipid having the following structure:



DOPE is also known as 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine.

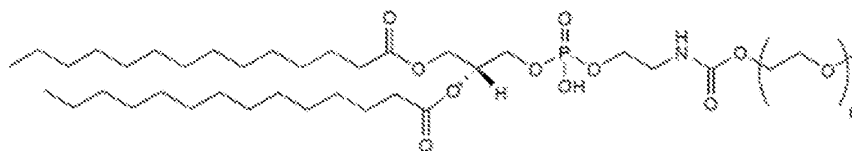
**[0087]** As used herein, the term “stealth lipid” refers to lipids when used in lipid particles that reduce recognition of the mononuclear phagocyte system, preventing protein adsorption, and leading to extended circulation time. An example of a stealth lipid is a PEG terminated lipid.

**[0088]** As used herein, the term “DMG-PEG2000” refers to a PEG terminated lipid having the following structure:



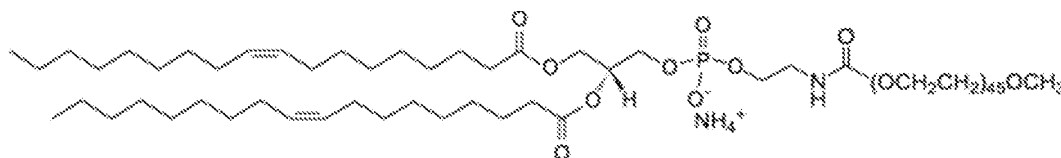
DMG-PEG2000 is also known as 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000.

**[0089]** As used herein, the term “DMPE-PEG2000” refers to a PEG terminated lipid having the following structure:



DMPE-PEG is also known as 3-[hydroxy-[2-[2-(2-methoxyethoxy)ethylamino]ethoxy]phosphoryl]oxy-2-tetradecanoyloxypropyl] tetradecanoate.

**[0090]** As used herein, the term “18:1 PEG2000-PE” refers to a PEG terminated lipid having the following structure:



18:1 PEG2000-PE is also known as 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000].

## II. LIPID PARTICLES

[0091] The present disclosure provides lipid particles comprising one or more ionizable lipids, one or more neutral lipids, one or more sterols, one or more charged lipids, and/or one or more polyethylene glycol (“PEG”) terminated lipids.

[0092] Lipid particles comprising cholesteryl hemisuccinate (“CHEMS”) and a payload and optionally one or more of:

- a. one or more ionizable lipids;
- b. one or more neutral lipids;
- c. one or more sterols; and
- d. one or more stealth lipids

are contemplated herein. Methods of targeting a payload to a spleen of a subject *in vivo* comprising combining a lipid particle or a composition comprising the payload encapsulated within a lipid particle or a composition comprising CHEMS in a molar amount of at least 11% are also contemplated herein.

[0093] Lipid particles comprising:

- a. one or more ionizable lipids in a molar amount of about 20% to about 70%;
- b. one or more neutral lipids in a molar amount of about 2.5% to about 25%;
- c. one or more sterols in a molar amount of about 20% to about 50%;
- d. one or more charged lipids in a molar amount of about 10% or less than 10%; and
- e. one or more stealth lipids in a molar amount of about 0.25% to about 3%.

are additionally contemplated herein. Methods of targeting a payload to a tissue other than a spleen of a subject *in vivo* comprising combining a lipid particle or a composition comprising the payload encapsulated within a lipid particle or a composition comprising one or more ionizable lipids, one or more neutral lipids, one or more sterols, one or more stealth lipids, and/or one or more charged lipids, wherein the lipid particle or composition does not comprise cholesteryl hemisuccinate (“CHEMS”) or comprises a molar amount of about 10% or less than 10% of CHEMS are also contemplated herein.

[0094] In some aspects, the desired tissue of a subject is a spleen. In some aspects, the desired tissue of a subject is a liver. In some aspects, the desired tissue of a subject is a lymph node. In some aspects, the desired tissue of a subject is a pancreas. In some aspects, the desired

tissue of a subject is brain, muscle, or lung. In some aspects, the desired tissue contains one or more tumors. In some aspects, the one or more tumors is malignant.

**a. COMPONENTS OF LIPID PARTICLES**

**i. IONIZABLE LIPIDS**

**[0095]** The lipid particles described herein can comprise one or more ionizable lipids. An ionizable lipid is one that can be readily protonated, e.g., an amine-containing lipid.

**[0096]** The one or more ionizable lipids in the lipid particles described herein are in a molar amount of at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 11%, at least 12%, at least 13%, at least 14%, at least 15%, at least 16%, at least 17%, at least 18%, at least 19%, or at least 20%. The one or more ionizable lipids in the lipid particles described herein are in a molar amount of about 20% to about 70%, about 20% to about 65%, about 20% to about 60%, about 20% to about 55%, about 20% to about 50%, about 20% to about 45%, about 20% to about 40%, about 20% to about 35%, about 20% to about 30%, about 20% to about 25%, about 25% to about 70%, about 25% to about 65%, about 25% to about 60%, about 25% to about 55%, about 25% to about 50%, about 25% to about 45%, about 25% to about 40%, about 25% to about 35%, about 25% to about 30%, about 30% to about 70%, about 30% to about 65%, about 30% to about 60%, about 30% to about 55%, about 30% to about 50%, about 30% to about 45%, about 30% to about 40%, about 30% to about 35%, about 35% to about 70%, about 35% to about 65%, about 35% to about 60%, about 35% to about 55%, about 35% to about 50%, about 35% to about 45%, about 35% to about 40%, about 40% to about 70%, about 40% to about 65%, about 40% to about 60%, about 40% to about 55%, about 40% to about 50%, about 40% to about 45%, about 45% to about 70%, about 45% to about 65%, about 45% to about 60%, about 45% to about 55%, about 45% to about 50%, about 50% to about 70%, about 50% to about 65%, about 50% to about 60%, about 55% to about 70%, about 55% to about 65%, about 60% to about 70%, about 60% to about 65%, or about 65% to about 70%. In some aspects, the one or more ionizable lipids in the lipid particles described herein are in a molar amount of about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, or about 70%. In some aspects, the one or more ionizable lipids in the lipid particles described herein is present in a molar amount of about 20%. In some aspects, the one or more ionizable lipids in the lipid particles described herein is present in a molar amount of about 30%. In some aspects, the one or more ionizable lipids in the lipid particles described herein are in a molar amount of about 40%. In some aspects, the one or more ionizable lipids in the lipid

particles described herein are in a molar amount of about 50%. In some aspects, the one or more ionizable lipids in the lipid particles described herein are in a molar amount of about 60%. In some aspects, the one or more ionizable lipids in the lipid particles described herein are in a molar amount of about 65%. In some aspects, the one or more ionizable lipids in the lipid particles described herein are in a molar amount of about 70%.

**[0097]** The one or more ionizable lipids in the lipid particles described herein are one or more selected from the group consisting of DLin-MC3-DMA (“MC3”), DLin-KC2-DMA (“KC2”), ssPalmO-Phe (“SS-OP”), C12-200, SM-102,  $\alpha$ -D-tocopherolsuccinoyl (“SS-EC”), ALC-0315 (“ALC”), Tri-N-tridecyl 3-(ethyl(methyl)amino)propanoate (“304-O13”), tetrakis(2-(octyldisulfaneyl)ethyl) 3,3',3'',3'''-(((methylazanediyl))bis(propane-3,1-diyl))bis(azanetriyl))tetrapropionate (“306-O12B”), 9-[4-(dimethylamino)-1-oxobutoxy]-heptadecanedioic acid, 1,17-di-(2Z)-2-nonen-1-yl ester (“L319”), 9,12-octadecadienoic acid, (9Z,12Z)-1,1',1'',1'''-[(3,6-dioxo-2,5-piperazinediyl)bis(4,1-butanediyl)nitrilodi-4,1-butanediyl] ester (“OF-C4-Deg-Lin”), 2-(dioctylamino)ethyl nonyl hydrogen phosphate (“9A1P9”), 5-(((3-(dibutylamino)propyl)amino)methyl)-6-hydroxyundecane-1,11-diyl (9Z,9'Z,12Z,12'Z)-bis(octadeca-9,12-dienoate) (“IR-117-17”), 9Z,12Z-octadecadienoic acid, 3-[4,4-bis(octyloxy)-1-oxobutoxy]-2-[[[3-(diethylamino)propoxy]carbonyl]oxy]methyl]propyl ester (“LP-01”) and 4A3-SC8. In some aspects, the one or more ionizable lipids in the lipid particles described herein are one or more selected from the group consisting of DLin-MC3-DMA (“MC3”), DLin-KC2-DMA (“KC2”), ssPalmO-Phe (“SS-OP”), C12-200, SM-102,  $\alpha$ -D-tocopherolsuccinoyl (“SS-EC”), and ALC-0315 (“ALC”). In some aspects, the lipid particles described herein contains only one ionizable lipid.

**[0098]** The choice of the one or more ionizable lipids is dependent upon the tissue to which the payload is to be delivered or use of the lipid particles described herein. For example, if the lipid particle is to deliver a payload to a subject's spleen (e.g., for a tolerizing vaccine), the lipid particles can comprise certain ionizable lipids in the lipid particles described herein, such as SS-OP, KC2, and combinations thereof. Also, if the lipid particle is to deliver a payload to a subject's liver (e.g., for a cancer or infectious agent vaccine), the lipid particles can comprise certain ionizable lipids in the lipid particles described herein, such as MC3, KC2, and combinations thereof.

**[0099]** Additionally, in some aspects, the lipid particles described herein, when administered to a subject in need thereof, reduces immunogenicity (e.g., increasing Treg cells, reducing

effector T cells, reducing B-cells, reducing cytokine production, reducing activated B cells, and/or reducing activated effector T cells) in the subject. In some aspects, the reduction in immunogenicity is compared with that of a reference lipid particle. In some aspects, the reduction in immunogenicity in a subject is compared with the subject prior to administration of the lipid particles described herein. In some aspects, the reference lipid particle contains MC-3 and the one or more ionizable lipids in the lipid particle comprises SS-OP. In some aspects, the reference lipid particle contains KC-2 and the one or more ionizable lipids in the lipid particle comprises SS-OP. In some aspects, the reference lipid particle contains SM-102 and the one or more ionizable lipids in the lipid particle comprises SS-OP.

**[0100]** In some aspects, the lipid particle, when administered to a subject in need thereof, increases immunogenicity (e.g., decreasing Treg cells, increasing effector T cells, increasing B-cells, increasing cytokine production, increasing activated B cells, and/or increasing activated effector T cells) in the subject. In some aspects, the increase in immunogenicity is compared with that of a reference lipid particle or the subject prior to administration of the lipid particles described herein. In some aspects, the reference lipid particle contains MC-3 and the one or more ionizable lipids in the lipid particle comprises SS-EC. In some aspects, the reference lipid particle contains KC-2 and the one or more ionizable lipids in the lipid particle comprises SS-EC. In some aspects, the reference lipid particle contains SM-102 and the one or more ionizable lipids in the lipid particle comprises SS-EC.

**[0101]** In some aspects, the only ionizable lipid in the lipid particles described herein is SS-OP. In some aspects, the only ionizable lipid in the lipid particles described herein is KC2. In some aspects, the only ionizable lipid in the lipid particles described herein is MC3. In some aspects, the only ionizable lipid in the lipid particles described herein is SM-102.

**[0102]** In some aspects, the SS-OP in the lipid particles described herein is in a molar amount of at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 11%, at least 12%, at least 13%, at least 14%, at least 15%, at least 16%, at least 17%, at least 18%, at least 19%, or at least 20%. In some aspects, the SS-OP in the lipid particles described herein is in a molar amount of about 20% to about 70%, about 20% to about 65%, about 20% to about 60%, about 20% to about 55%, about 20% to about 50%, about 20% to about 45%, about 20% to about 40%, about 20% to about 35%, about 20% to about 30%, about 20% to about 25%, about 25% to about 70%, about 25% to about 65%, about 25% to about 60%, about 25% to about 55%, about 25% to about 50%, about 25% to about 45%, about 25% to about 40%, about 25% to about 35%,

about 25% to about 30%, about 30% to about 70%, about 30% to about 65%, about 30% to about 60%, about 30% to about 55%, about 30% to about 50%, about 30% to about 45%, about 30% to about 40%, about 30% to about 35%, about 35% to about 70%, about 35% to about 65%, about 35% to about 60%, about 35% to about 55%, about 35% to about 50%, about 35% to about 45%, about 35% to about 40%, about 40% to about 70%, about 40% to about 65%, about 40% to about 60%, about 40% to about 55%, about 40% to about 50%, about 40% to about 45%, about 45% to about 70%, about 45% to about 65%, about 45% to about 60%, about 45% to about 55%, about 45% to about 50%, about 50% to about 70%, about 50% to about 65%, about 50% to about 60%, about 55% to about 70%, about 55% to about 65%, about 55% to about 60%, about 60% to about 70%, about 60% to about 65%, or about 65% to about 70%. In some aspects, the SS-OP in the lipid particles described herein are in a molar amount of about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, or about 70%. In some aspects, the SS-OP in the lipid particles described herein is present in a molar amount of about 20%. In some aspects, the SS-OP in the lipid particles described herein is present in a molar amount of about 30%. In some aspects, the SS-OP in the lipid particles described herein are in a molar amount of about 40%. In some aspects, the SS-OP in the lipid particles described herein are in a molar amount of about 50%. In some aspects, the SS-OP in the lipid particles described herein are in a molar amount of about 60%. In some aspects, the SS-OP in the lipid particles described herein are in a molar amount of about 65%. In some aspects, the SS-OP in the lipid particles described herein are in a molar amount of about 70%.

**[0103]** In some aspects, the KC2 in the lipid particles described herein is in a molar amount of at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 11%, at least 12%, at least 13%, at least 14%, at least 15%, at least 16%, at least 17%, at least 18%, at least 19%, or at least 20%. In some aspects, the KC2 in the lipid particles described herein is in a molar amount of about 20% to about 70%, about 20% to about 65%, about 20% to about 60%, about 20% to about 55%, about 20% to about 50%, about 20% to about 45%, about 20% to about 40%, about 20% to about 35%, about 20% to about 30%, about 20% to about 25%, about 25% to about 70%, about 25% to about 65%, about 25% to about 60%, about 25% to about 55%, about 25% to about 50%, about 25% to about 45%, about 25% to about 40%, about 25% to about 35%, about 25% to about 30%, about 30% to about 70%, about 30% to about 65%, about 30% to about 60%, about 30% to about 55%, about 30% to about 50%, about 30% to about 45%, about 30% to about 40%, about 30% to about 35%, about 35% to about 70%, about 35% to about 65%, about 35% to about 60%, about 35% to about 55%, about 35% to about 50%, about 35% to about 45%,

about 35% to about 40%, about 40% to about 70%, about 40% to about 65%, about 40% to about 60%, about 40% to about 55%, about 40% to about 50%, about 40% to about 45%, about 45% to about 70%, about 45% to about 65%, about 45% to about 60%, about 45% to about 55%, about 45% to about 50%, about 50% to about 70%, about 50% to about 65%, about 50% to about 60%, about 55% to about 70%, about 55% to about 65%, about 55% to about 60%, about 60% to about 70%, about 60% to about 65%, or about 65% to about 70%. In some aspects, the KC2 in the lipid particles described herein are in a molar amount of about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, or about 70%. In some aspects, the KC2 in the lipid particles described herein is present in a molar amount of about 20%. In some aspects, the KC2 in the lipid particles described herein is present in a molar amount of about 30%. In some aspects, the KC2 in the lipid particles described herein are in a molar amount of about 40%. In some aspects, the KC2 in the lipid particles described herein are in a molar amount of about 50%. In some aspects, the KC2 in the lipid particles described herein are in a molar amount of about 60%. In some aspects, the KC2 in the lipid particles described herein are in a molar amount of about 65%. In some aspects, the KC2 in the lipid particles described herein are in a molar amount of about 70%.

**[0104]** In some aspects, the MC3 in the lipid particles described herein is in a molar amount of at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 11%, at least 12%, at least 13%, at least 14%, at least 15%, at least 16%, at least 17%, at least 18%, at least 19%, or at least 20%. In some aspects, the MC3 in the lipid particles described herein is in a molar amount of about 20% to about 70%, about 20% to about 65%, about 20% to about 60%, about 20% to about 55%, about 20% to about 50%, about 20% to about 45%, about 20% to about 40%, about 20% to about 35%, about 20% to about 30%, about 20% to about 25%, about 25% to about 70%, about 25% to about 65%, about 25% to about 60%, about 25% to about 55%, about 25% to about 50%, about 25% to about 45%, about 25% to about 40%, about 25% to about 35%, about 25% to about 30%, about 30% to about 70%, about 30% to about 65%, about 30% to about 60%, about 30% to about 55%, about 30% to about 50%, about 30% to about 45%, about 30% to about 40%, about 30% to about 35%, about 35% to about 70%, about 35% to about 65%, about 35% to about 60%, about 35% to about 55%, about 35% to about 50%, about 35% to about 45%, about 35% to about 40%, about 40% to about 70%, about 40% to about 65%, about 40% to about 60%, about 40% to about 55%, about 40% to about 50%, about 40% to about 45%, about 45% to about 70%, about 45% to about 65%, about 45% to about 60%, about 45% to about 55%, about 45% to about 50%, about 50% to about 70%, about 50% to about 65%, about 50% to about 60%,



about 55% to about 70%, about 55% to about 65%, about 55% to about 60%, about 60% to about 70%, about 60% to about 65%, or about 65% to about 70%. In some aspects, the MC3 in the lipid particles described herein are in a molar amount of about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, or about 70%. In some aspects, the MC3 in the lipid particles described herein is present in a molar amount of about 20%. In some aspects, the MC3 in the lipid particles described herein is present in a molar amount of about 30%. In some aspects, the MC3 in the lipid particles described herein are in a molar amount of about 40%. In some aspects, the MC3 in the lipid particles described herein are in a molar amount of about 50%. In some aspects, the MC3 in the lipid particles described herein are in a molar amount of about 60%. In some aspects, the MC3 in the lipid particles described herein are in a molar amount of about 65%. In some aspects, the MC3 in the lipid particles described herein are in a molar amount of about 70%.

**[0105]** In some aspects, the SM-102 in the lipid particles described herein is in a molar amount of at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 11%, at least 12%, at least 13%, at least 14%, at least 15%, at least 16%, at least 17%, at least 18%, at least 19%, or at least 20%. In some aspects, the SM-102 in the lipid particles described herein is in a molar amount of about 20% to about 70%, about 20% to about 65%, about 20% to about 60%, about 20% to about 55%, about 20% to about 50%, about 20% to about 45%, about 20% to about 40%, about 20% to about 35%, about 20% to about 30%, about 20% to about 25%, about 25% to about 70%, about 25% to about 65%, about 25% to about 60%, about 25% to about 55%, about 25% to about 50%, about 25% to about 45%, about 25% to about 40%, about 25% to about 35%, about 25% to about 30%, about 30% to about 70%, about 30% to about 65%, about 30% to about 60%, about 30% to about 55%, about 30% to about 50%, about 30% to about 45%, about 30% to about 40%, about 30% to about 35%, about 35% to about 70%, about 35% to about 65%, about 35% to about 60%, about 35% to about 55%, about 35% to about 50%, about 35% to about 45%, about 35% to about 40%, about 40% to about 70%, about 40% to about 65%, about 40% to about 60%, about 40% to about 55%, about 40% to about 50%, about 40% to about 45%, about 45% to about 70%, about 45% to about 65%, about 45% to about 60%, about 45% to about 55%, about 45% to about 50%, about 50% to about 70%, about 50% to about 65%, about 50% to about 60%, about 55% to about 70%, about 55% to about 65%, about 55% to about 60%, about 60% to about 70%, about 60% to about 65%, or about 65% to about 70%. In some aspects, the SM-102 in the lipid particles described herein are in a molar amount of about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%,

or about 70%. In some aspects, the SM-102 in the lipid particles described herein is present in a molar amount of about 20%. In some aspects, the SM-102 in the lipid particles described herein is present in a molar amount of about 30%. In some aspects, the SM-102 in the lipid particles described herein are in a molar amount of about 40%. In some aspects, the SM-102 in the lipid particles described herein are in a molar amount of about 50%. In some aspects, the SM-102 in the lipid particles described herein are in a molar amount of about 60%. In some aspects, the SM-102 in the lipid particles described herein are in a molar amount of about 65%. In some aspects, the SM-102 in the lipid particles described herein are in a molar amount of about 70%.

## ii. NEUTRAL LIPIDS

**[0106]** The lipid particles disclosed herein can comprise one or more neutral lipids. A neutral lipid is a lipid without a charge or is a zwitterion, i.e., a molecule having both a positive and a negative charge yielding an overall neutral molecule.

**[0107]** In some aspects, the one or more neutral lipids in the lipid particles described herein are in a molar amount of at least 0.5%, at least 0.6%, at least 0.7%, at least 0.8%, at least 0.9%, at least 1.1%, at least 1.2%, at least 1.3%, at least 1.4%, at least 1.5%, at least 1.6%, at least 1.7%, at least 1.8%, at least 1.9%, at least 2%, at least 2.1%, at least 2.2%, at least 2.3%, at least 2.4%, at least 2.5%, at least 5%, at least 7.5%, at least 10%, at least 12.5%, at least 15%, at least 17.5%, at least 20%, or at least 22.5%. In some aspects, the one or more neutral lipids in the lipid particles described herein are in a molar amount of about 2.5% to about 25%, about 2.5% to about 20%, about 2.5% to about 17.5%, about 2.5% to about 15%, about 2.5% to about 12.5%, about 2.5% to about 10%, about 2.5% to about 7.5%, about 2.5% to about 5%, about 5% to about 25%, about 5% to about 20%, about 5% to about 17.5%, about 5% to about 15%, about 5% to about 12.5%, about 5% to about 10%, about 5% to about 7.5%, about 7.5% to about 25%, about 7.5% to about 20%, about 7.5% to about 17.5%, about 7.5% to about 15%, about 7.5% to about 12.5%, about 7.5% to about 10%, about 10% to about 25%, about 10% to about 20%, about 10% to about 17.5%, about 10% to about 15%, about 10% to about 12.5%, about 12.5% to about 25%, about 12.5% to about 20%, about 12.5% to about 17.5%, or about 12.5% to about 15%. In some aspects, the one or more neutral lipids in the lipid particles described herein are in a molar amount of about 5%, about 7.5%, about 10%, about 12.5%, about 15%, about 17.5%, about 20%, about 22.5%, or about 25%. In some aspects, the one or more neutral lipids in the lipid particles described herein are in a molar amount of about 5% to about 20%. In some aspects, the one or more neutral lipids in the lipid particles described herein are in a molar amount of about 7.5% to

about 17.5%. In some aspects, the one or more neutral lipids in the lipid particles described herein are in a molar amount of about 2.5%. In some aspects, the one or more neutral lipids in the lipid particles described herein are in a molar amount of about 5%. In some aspects, the one or more neutral lipids in the lipid particles described herein are in a molar amount of about 7.5%. In some aspects, the one or more neutral lipids in the lipid particle are in a molar amount of about 10%. In some aspects, the one or more neutral lipids in the lipid particles described herein are in a molar amount of about 12.5%. In some aspects, the one or more neutral lipids in the lipid particles described herein are in a molar amount of about 15%. In some aspects, the one or more neutral lipids in the lipid particles described herein are in a molar amount of about 17.5%. In some aspects, the one or more neutral lipids in the particles described herein are in a molar amount of about 20%. In some aspects, the one or more neutral lipids in the lipid particles described herein are in a molar amount of about 25%.

**[0108]** In some aspects, the lipid particles described herein do not contain a neutral lipid.

**[0109]** In some aspects, the lipid particles described herein comprise one or more neutral lipids. In some aspects, the one or more neutral lipids in the lipid particles are one or more phosphatidylcholine ("PC"), phosphatidylethanolamine ("PE"), and combination thereof. The one or more neutral lipids in the lipid particles described herein can be one or more selected from the group consisting of 1,2-diastearoyl-sn-glycero-3-phosphocholine ("DSPC"), 1,2-dioleoyl-sn-glycero-3-phosphocholine ("DOPC"), 1,2- dioctadecanoyl-sn-glycerol ("DSDG"), 1,2-dioleoyl-sn-glycerol, 1,2-di-(9Z-octadecenoyl)-sn-glycerol ("DODG"), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine ("DOPE"), diacylphosphatidylcholine, diacylphosphatidylethanolamine, ceramide, sphingomyelin, cephalin, cerebrosides, diacylglycerols dipalmitoylphosphatidylcholine ("DPPC"), palmitoyl-oleoyl-phosphatidylcholine ("POPC"), palmitoyl-oleoyl-phosphatidylethanolamine ("POPE"), palmitoyl-oleoyl-phosphatidylglycerol (POPG), dipalmitoyl-phosphatidylethanolamine ("DPPE"), dimyristoyl-phosphatidylethanolamine ("DMPE"), distearoyl-phosphatidylethanolamine ("DSPE"), monomethyl-phosphatidylethanolamine, dimethyl-phosphatidylethanolamine, dielaidoyl-phosphatidylethanolamine ("DEPE"), stearyl-oleoyl-phosphatidylethanolamine ("SOPE"), and egg phosphatidylcholine ("EPC"). In some aspects, the one or more neutral lipids in the lipid particles described herein are DOPE, DODG, or a combination thereof. In some aspects, the lipid particles described herein contain only one neutral lipid.

**[0110]** In some aspects, the only neutral lipid in the lipid particles described herein is DOPE. In some aspects, the DOPE is in a molar amount of at least 0.5%, at least 0.6%, at least 0.7%, at least 0.8%, at least 0.9%, at least 1.1%, at least 1.2%, at least 1.3%, at least 1.4%, at least 1.5%, at least 1.6%, at least 1.7%, at least 1.8%, at least 1.9%, at least 2%, at least 2.1%, at least 2.2%, at least 2.3%, at least 2.4%, at least 2.5%, at least 5%, at least 7.5%, at least 10%, at least 12.5%, at least 15%, at least 17.5%, at least 20%, or at least 22.5%. In some aspects, the DOPE is in a molar amount of about 2.5% to about 25%, about 2.5% to about 20%, about 2.5% to about 17.5%, about 2.5% to about 15%, about 2.5% to about 12.5%, about 2.5% to about 10%, about 2.5% to about 7.5%, about 2.5% to about 5%, about 5% to about 25%, about 5% to about 20%, about 5% to about 17.5%, about 5% to about 15%, about 5% to about 12.5%, about 5% to about 10%, about 5% to about 7.5%, about 7.5% to about 25%, about 7.5% to about 20%, about 7.5% to about 17.5%, about 7.5% to about 15%, about 7.5% to about 12.5%, about 7.5% to about 10%, about 10% to about 25%, about 10% to about 20%, about 10% to about 17.5%, about 10% to about 15%, about 10% to about 12.5%, about 12.5% to about 25%, about 12.5% to about 20%, about 12.5% to about 17.5%, or about 12.5% to about 15%. In some aspects, the DOPE is in a molar amount of about 5%, about 7.5%, about 10%, about 12.5%, about 15%, about 17.5%, about 20%, about 22.5%, or about 25%. In some aspects, the DOPE is in a molar amount of about 5% to about 20%. In some aspects, the DOPE is in a molar amount of about 7.5% to about 17.5%. In some aspects, the DOPE is in a molar amount of about 2.5%. In some aspects, the DOPE is in a molar amount of about 5%. In some aspects, the DOPE is in a molar amount of about 7.5%. In some aspects, the DOPE is in a molar amount of about 10%. In some aspects, the DOPE is in a molar amount of about 12.5%. In some aspects, the DOPE is in a molar amount of about 15%. In some aspects, the DOPE is in a molar amount of about 17.5%. In some aspects, the DOPE is in a molar amount of about 20%. In some aspects, the DOPE is in a molar amount of about 25%.

**[0111]** In some aspects, the only neutral lipid in the lipid particles described herein is DODG. In some aspects, the DODG is in a molar amount of at least 0.5%, at least 0.6%, at least 0.7%, at least 0.8%, at least 0.9%, at least 1.1%, at least 1.2%, at least 1.3%, at least 1.4%, at least 1.5%, at least 1.6%, at least 1.7%, at least 1.8%, at least 1.9%, at least 2%, at least 2.1%, at least 2.2%, at least 2.3%, at least 2.4%, at least 2.5%, at least 5%, at least 7.5%, at least 10%, at least 12.5%, at least 15%, at least 17.5%, at least 20%, or at least 22.5%. In some aspects, the DODG is in a molar amount of about 2.5% to about 25%, about 2.5% to about 20%, about 2.5% to about 17.5%, about 2.5% to about 15%, about 2.5% to about 12.5%, about 2.5% to about 10%, about

2.5% to about 7.5%, about 2.5% to about 5%, about 5% to about 25%, about 5% to about 20%, about 5% to about 17.5%, about 5% to about 15%, about 5% to about 12.5%, about 5% to about 10%, about 5% to about 7.5%, about 7.5% to about 25%, about 7.5% to about 20%, about 7.5% to about 17.5%, about 7.5% to about 15%, about 7.5% to about 12.5%, about 7.5% to about 10%, about 10% to about 25%, about 10% to about 20%, about 10% to about 17.5%, about 10% to about 15%, about 10% to about 12.5%, about 12.5% to about 25%, about 12.5% to about 20%, about 12.5% to about 17.5%, or about 12.5% to about 15%. In some aspects, the DODG is in a molar amount of about 5%, about 7.5%, about 10%, about 12.5%, about 15%, about 17.5%, about 20%, about 22.5%, or about 25%. In some aspects, the DODG is in a molar amount of about 5% to about 20%. In some aspects, the DODG is in a molar amount of about 7.5% to about 17.5%. In some aspects, the DODG is in a molar amount of about 2.5%. In some aspects, the DODG is in a molar amount of about 5%. In some aspects, the DODG is in a molar amount of about 7.5. In some aspects, the DODG is in a molar amount of about 10%. In some aspects, the DODG is in a molar amount of about 12.5%. In some aspects, the DODG is in a molar amount of about 15%. In some aspects, the DODG is in a molar amount of about 17.5%. In some aspects, the DODG is in a molar amount of about 20%. In some aspects, the DODG is in a molar amount of about 25%.

### iii. STEROLS

**[0112]** The lipid particles disclosed herein can comprise one or more sterols. In some aspects, the one or more sterols in the lipid particles described herein is in a molar amount of at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 11%, at least 12%, at least 13%, at least 14%, at least 15%, at least 16%, at least 17%, at least 18%, at least 19%, or at least 20%. In some aspects, the one or more sterols in the lipid particles described herein is in a molar amount of about 20% to about 50%. In some aspects, the one or more sterols in the lipid particle are in a molar amount of about 20% to about 50%, about 20% to about 45%, about 20% to about 40%, about 20% to about 35%, about 20% to about 30%, about 20% to about 25%, about 25% to about 50%, about 25% to about 45%, about 25% to about 40%, about 25% to about 35%, about 25% to about 30%, about 30% to about 50%, about 30% to about 45%, about 30% to about 40%, about 30% to about 35%, about 35% to about 50%, about 35% to about 45%, about 35% to about 40%, about 40% to about 50%, or about 40% to about 45%. In some aspects, the one or more sterols in the lipid particles described herein is in a molar amount of about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, or about 50%. In some aspects, the one or more

sterols in the lipid particles described herein is in a molar amount of about 20% to about 40%. In some aspects, the one or more sterols in the lipid particles described herein is in a molar amount of about 20% to about 30%. In some aspects, the one or more sterols in the lipid particles described herein is in a molar amount of about 30% to about 40%. In some aspects, the one or more sterols in the lipid particle are in a molar amount of about 20%. In some aspects, the one or more sterols in the lipid particles described herein is in a molar amount of about 28.5%. In some aspects, the one or more sterols in the lipid particles described herein is in a molar amount of about 30%. In some aspects, the one or more sterols in the lipid particles described herein is in a molar amount of about 38.5%. In some aspects, the one or more sterols in the lipid particles described herein is in a molar amount of about 40%.

**[0113]** In some aspects, the one or more sterols in the lipid particles described herein are cholesterol,  $\beta$ -sitosterol, stigmasterol, campesterol, fucosterol, brassicasterol, ergosterol, 9,11-dehydroergosterol, daucosterol,  $\beta$ -sitosterol acetate, and other C-24 alkyl derivatives, or combinations thereof. In some aspects, the sterol is cholesterol,  $\beta$ -sitosterol, and combinations thereof. In some aspects, only one sterol is in the lipid particle. In some aspects, the one sterol in the lipid particle comprises cholesterol.

**[0114]** In some aspects, the cholesterol in the lipid particles described herein is in a molar amount of at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 11%, at least 12%, at least 13%, at least 14%, at least 15%, at least 16%, at least 17%, at least 18%, at least 19%, or at least 20%. In some aspects, the cholesterol in the lipid particles described herein is in a molar amount of about 20% to about 50%. In some aspects, the cholesterol in the lipid particle is in a molar amount of about 20% to about 50%, about 20% to about 45%, about 20% to about 40%, about 20% to about 35%, about 20% to about 30%, about 20% to about 25%, about 25% to about 50%, about 25% to about 45%, about 25% to about 40%, about 25% to about 35%, about 25% to about 30%, about 30% to about 50%, about 30% to about 45%, about 30% to about 40%, about 30% to about 35%, about 35% to about 50%, about 35% to about 45%, about 35% to about 40%, about 40% to about 50%, or about 40% to about 45%. In some aspects, the cholesterol in the lipid particles described herein is in a molar amount of about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, or about 50%. In some aspects, the cholesterol in the lipid particles described herein is in a molar amount of about 20% to about 40%. In some aspects, the cholesterol in the lipid particles described herein is in a molar amount of about 20% to about 30%. In some aspects, the cholesterol in the lipid particles described

herein is in a molar amount of about 30% to about 40%. In some aspects, the cholesterol in the lipid particle is in a molar amount of about 20%. In some aspects, the cholesterol in the lipid particles described herein is in a molar amount of about 28.5%. In some aspects, the cholesterol in the lipid particles described herein is in a molar amount of about 30%. In some aspects, the cholesterol in the lipid particles described herein is in a molar amount of about 38.5%. In some aspects, the cholesterol in the lipid particles described herein is in a molar amount of about 40%.

#### iv. CHARGED LIPIDS

[0115] The lipid particles disclosed herein can comprise one or more charged lipids. In some aspects, the one or more charged lipids are in a molar amount of about 10% or less than 10%. A charged lipid is an anionic lipid *i.e.*, one that has more negative charges than positive charges in an aqueous medium, or a cationic lipid, *i.e.*, one that has more positive charges than negative charges in an aqueous medium.

[0116] In some aspects, the one or more charged lipids in the lipid particles disclosed herein are in a molar amount of 10%. In some aspects, the one or more charged lipids in the lipid particles disclosed herein are in a molar amount of less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, less than 4%, less than 3%, less than 2%, less than 1%, or less than 0.1%. In some aspects, the one or more charged lipids in the lipid particles disclosed herein are in a molar amount of about 1% to about 10%, about 1% to about 9%, about 1% to about 8%, about 1% to about 7%, about 1% to about 6%, about 1% to about 5%, about 1% to about 4%, about 1% to about 3%, about 1% to about 2%, about 2% to about 10%, about 2% to about 9%, about 2% to about 8%, about 2% to about 7%, about 2% to about 6%, about 2% to about 5%, about 2% to about 4%, about 2% to about 3%, about 3% to about 10%, about 3% to about 9%, about 3% to about 8%, about 3% to about 7%, about 3% to about 6%, about 3% to about 5%, about 3% to about 4%, about 4% to about 10%, about 4% to about 9%, about 4% to about 8%, about 4% to about 7%, about 4% to about 6%, about 4% to about 5%, about 5% to about 10%, about 5% to about 9%, about 5% to about 8%, about 5% to about 7%, or about 5% to about 6%. In some aspects, the one or more charged lipids in the lipid particles disclosed herein are in a molar amount of about 0.1%, about 0.25%, about 0.5%, about 0.7%, about 1%, about 1.25%, about 1.5%, about 1.75%, about 2%, about 2.25%, about 2.5%, about 2.75%, about 3%, about 3.25%, about 3.5%, about 3.75%, about 4%, about 4.25%, about 4.5%, about 4.75%, about 5%, about 5.25%, about 5.5%, about 5.75%, about 6%, about 6.25%, about 6.5%, about 6.75%,

about 7%, about 7.25%, about 7.5%, about 7.75%, about 8%, about 8.25%, about 8.5%, about 8.75%, about 9%, about 9.25%, about 9.5%, about 9.75%, or about 10%.

[0117] In some aspects, the one or more charged lipids in the lipid particle is selected from the group consisting of cholesteryl hemisuccinate ("CHEMS"), 1,2-distearoyl-3-trimethylammonium-propane ("DSTAP"), 18:1 1,2-dioleoyl-3-trimethylammonium-propane ("DOTAP"), 1,2-dioleoyl-sn-glycero-3-phosphate ("18:1 PA"), 18:1 1-stearoyl-2-oleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) ("18:1 PG"), phosphatidylglycerols, cardiolipins, diacylphosphatidylserines, diacylphosphatidic acids, N-dodecanoyl phosphatidylethanolamines, N-succinyl phosphatidylethanolamines, N-glutarylphosphatidylethanolamines, lysylphosphatidylglycerols, palmitoyloleyolphosphatidylglycerol ("POPG"), 1,2-dimyristoyl-3-trimethylammonium-propane ("DMTAP"), 1,2-dipalmitoyl-3-trimethylammonium-propane ("DPTAP"), palmitoyloleyoyl-3-trimethylammonium-propane ("POTAP"), 1,2-dioleoyl-3-dimethylammonium-propane ("DODAP"), palmitoyloleyoyl-3-dimethylammonium-propane ("PODAP"), 1,2-dimyristoyl-3-dimethylammonium-propane ("DMDAP"), 1,2-dipalmitoyl-3-dimethylammonium-propane ("DPDAP"), 1,2-distearoyl-3-dimethylammonium-propane ("DSDAP"), 1,2-dioleoyl-3-dimethylhydroxyethyl-ammonium-propane ("DODMHEAP") (also known as DORI), palmitoyloleyoyl-3-dimethylhydroxyethyl-ammonium-propane ("PODMHEAP") (also known as PORI), 1,2-dimyristoyl-3-dimethylhydroxyethyl-ammonium-propane ("DMDMHEAP") (also known as DMRI), 1,2-dipalmitoyl-3-dimethylhydroxyethyl-ammonium-pPropane ("DPDMHEAP") (also known as DPRI), 1,2-distearoyl-3-dimethylhydroxyethyl-ammonium-propane ("DSDMHEAP") (also known as DSRI), 1,2-diioleoyl-3-methylhydroxyethylammonium-propane ("DOMDHEAP"), palmitoyloleyoyl-3-methylhydroxyethylammonium-propane ("POMDHEAP"), 1,2-dimyristoyl-3-methyldihydroxyethylammonium-propane ("DMMDHEAP"), 1,2-dipalmitoyl-3-methyldihydroxyethylammonium-propane ("DPMDHEAP"), 1,2-distearoyl-3-methyldihydroxyethylammonium-propane ("DSMDHEAP"), 1,2-dioleoyl-3-methyldihydroxyethylammonium-propane ("DOMHEAP"), palmitoyloleyoyl-3-methylhydroxyethylammonium-propane ("POMHEAP"), 1,2-dimyristoyl-3-methylhydroxyethylammonium-propane ("DMMHEAP"), 1,2-dipalmitoyl-3-methylhydroxyethylammonium-propane ("DPMHEAP"), 1,2-distearoyl-3-methylhydroxyethylammonium-propane ("DSMHEAP"), 1,2-dioleoyl-3-dihydroxyethylammonium-propane ("DODHEAP"), palmitoyloleyoyl-3-dihydroxyethylammonium-propane ("PODHEAP"), 1,2-dimyristoyl-3-



dihydroxyethylammonium-propane ("DMDHEAP"), 1,2-dipalmitoyl-3-dihydroxyethylammonium-propane ("DPDHEAP"), 1,2-distearoyl-3-dihydroxyethylammonium-propane ("DSDHEAP"), dimethyldioctadecylammonium bromide ("DDAB"), dioleyldimethylammonium chloride ("DODAC"), 1,2-dioleoyl-sn-glycero-3-ethylphosphocholine ("DOEPC"), 1,2-dimyristoyl-sn-glycero-3-ethylphosphocholine ("DMEPC"), 1,2-dipalmitoyl-sn-glycero-3-ethylphosphocholine ("DPEPC"), 1,2-distearoyl-sn-glycero-3-ethylphosphocholine ("DSEPC"), palmitoyloleoyl-sn-glycero-3-ethylphosphocholine ("POEPC"), 1,2-dioleoyl-3-dimethyl-hydroxyethyl ammonium propane ("DORIE"), 1,2-dimyristyl-3-dimethyl-hydroxyethyl ammonium propane ("DMRIE"), 1,2-dioleoyl-3-methyl-(methoxycarbonyl-ethyl)ammonium-propane ("DOMCAP"), 1,2-dioleoyl-3-methyl-(methoxycarbonylmethyl)ammonium-propane ("DOMGME"), 1,2-dioleoyl-3-N-pyrrolidine-propane ("DOP5P"), 1,2-dioleoyl-3-N-pyrridinium-propane, bromide salt ("DOP6P"), 3b-[N-(N9,N9-dimethylaminoethane)carbamoyl] cholesterol ("DC-Chol"), 3b-[N-(N9,N9-trimethylaminoethane) carbamoyl] cholesterol ("TC-Chol"), 3b(N-(N,N'-Dimethylaminoethan)-carbamoyl)cholesterol ("DAC-Chol"), cholesteryl-oxycarbonyl-methyl-trimethylammonium chloride ("Chol-Betaine"), N-methyl{4-N-amino[(3'-β-cholesteryl) carbamoyl]}piperazine ("N-methyl-PipChol"), cetyltrimethylammonium bromide ("CTAB"), N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethyl ammonium chloride ("DOTMA"), 4-(2-aminoethyl)-morpholino-cholesterolhemisuccinate: ("MoChol"), histaminyl-Cholesterolhemisuccinate ("HisChol"), cholesterol-(3-imidazol-1-yl propyl)carbamate ("Chim"), (N-2-propylamino[(3'-β-cholesteryl) carbamoyl]}morpholine ("MoC3Chol"), [(3-morpholine-4-yl-propylcarbamoyl)-methyl]-carbamic acid cholesteryl ester ("Chol-C3N-Mo3"), ("Chol-C3N-Mo2"), [(2-morpholine-4-yl-ethylcarbamoyl)methyl]-carbamic acid cholesteryl ester ("Chol-C4N-Mo2"), [1-methyl-2-(2-morpholine-4-yl-ethylcarbamoyl)-propyl]-carbamic acid cholesteryl ester ("Chol-DMC3N-Mo2"), 2-(2-morpholine-4-yl-ethylcarbamoyl)-cyclohexane carboxylic acid cholesteryl ester ("CholC4Hex-Mo2"), 4-(2-aminoethyl)-morpholino-cholesterol-2,3-dimethylhemisuccinate ("DmC4Mo2"), 4-(2-aminoethyl)-morpholino-cholesterol-2,2-dimethylhemimalonate ("DmC3Mo2"), 4-(2-aminoethyl)-morpholino-cholesterol-hemimalonate ("C3Mo2"), 4-(2-aminopropyl)-morpholino-cholesterol-hemimalonate ("C3Mo3"), 4-(2-aminoethyl)-morpholino-cholesterol-hemiglutarate ("C5Mo2"), 4-(2-aminoethyl)-morpholino-cholesterol-hemiadipate ("C6Mo2"), 4-(2-aminoethyl)-morpholino-cholesterol-hemiadipate ("C8Mo2"), 4-(2-aminobutyl)-morpholino-cholesterol-hemisuccinate ("C4Mo4"), 4{N-2-ethylamino[(3'-β-cholesteryl) carbamoyl]}piperazine ("PipC2Chol"), {N-2-ethylamino[(3'-β-cholesteryl)

carbamoyl]]morpholine ("MoC2Chol"), {N-2-ethylamino[(3'-β-cholesteryl)  
 carbamoyl]]pyrrolidine ("PyrroC2Chol"), {N-2-propylamino[(3'-β-cholesteryl)  
 carbamoyl]]imidazole ("ImC3Chol"), {N-2-ethylamino[(3'-β-cholesteryl) carbamoyl]]pyridine  
 ("PyC2Chol"), 1,2-dioleoyl-3-N-morpholine-propane ("MoDO"), 1,2-dipalmitoyl-3-N-  
 morpholine-propane ("MoDP"), 4,(2,3-bis-acyloxy-propyl)-1-methyl-1H-imidazole ("DOIM")  
 (also known as DPIM). diacylglycerolhemisuccinate, e.g. dioctadecylamido-glycylspermine  
 ("DOGS"), dimyristoylglycerolhemisuccinate ("DMGS") (also known as DMG-Succ), 1-  
 palmitoyl-2-oleoylglycerolhemisuccinate ("POGS") (also known as POG-Succ),  
 dipalmitoylglycerolhemisuccinate ("DPGS") (also known as POG-Succ),  
 distearoylglycerolhemisuccinate ("DSGS") (also known as DSG-Succ),  
 diacylglycerolhemimalonate, e.g. dioleoylglycerolhemimalonate ("DOGM"),  
 dimyristoylglycerolhemimalonate ("DMGM"), diacylglycerolhemiglutarate, e.g.  
 dioleoylglycerolhemiglutarate ("DOGG"), dimyristoylglycerolhemiglutarate ("DMGG"),  
 diacylglycerolhemiadipate, e.g. dioleoylglycerolhemiadipate ("DOGA"),  
 dimyristoylglycerolhemiadipate ("DMGA"), diacylglycerolhemicyclohexane-1,4-dicarboxylic  
 acid, e.g. dioleoylglycerolhemicyclohexane-1,4-dicarboxylic acid ("DO-cHA"),  
 dimyristoylglycerolhemicyclohexane-1,4-dicarboxylic acid ("DM-cHA"), (2,3-Diacyl-  
 propyl)amino}-oxoalkanoic acid, e.g. 4-{(2,3-dioleoyl-propyl)amino}-4-oxobutanoic acid  
 ("DOAS"), 3-{(2,3-dioleoyl-propyl)amino}-3-oxopropanoic acid ("DOAM"), 5-{(2,3-dioleoyl-  
 propyl)amino}-5-oxopentanoic acid ("DOAG"), 6-{(2,3-dioleoyl-propyl)amino}-6-oxohexanoic  
 acid ("DOAA"), 4-{(2,3-dimyristoyl-propyl)amino}-4-oxobutanoic acid ("DMAS"), 3-{(2,3-  
 dimyristoyl-propyl)amino}-3-oxopropanoic acid ("DMAM"), 5-{(2,3-dimyristoyl-  
 propyl)amino}-5-oxopentanoic acid ("DMAG"), 6-{(2,3-dimyristoyl-propyl)amino}-6-  
 oxohexanoic acid ("DMAA"), diacyl-alkanoic acid, e.g. 2,3-dioleoyl-propanoic acid ("DOP"),  
 3,4-dioleoyl-butanoic acid ("DOB"), 5,6-dioleoyl-hexanoic acid ("DOS"), 4,5-dioleoyl-pentanoic  
 acid ("DOM"), 6,7-dioleoyl-heptanoic acid ("DOG"), 7,8-dioleoyl-octanoic acid ("DOA"), 2,3-  
 dimyristoyl-propanoic acid ("DMP"), 3,4-dioleoyl-butanoic acid ("DOB"), 5,6-dimyristoyl-  
 hexanoic acid ("DMS"), 4,5-dimyristoyl-pentanoic acid ("DMM"), 6,7-dimyristoyl-heptanoic  
 acid ("DMG"), 7,8-dimyristoyl-octanoic acid ("DMA"), cholesteryloxycarbonylaminocarboxylic  
 acid, e.g. cholesterolhemidodecane dicarboxylic acid ("Chol-C12"), 12-  
 cholesteryloxycarbonylaminododecanoic acid ("CholC13N"), fatty acids, e.g. oleic acid, myristic  
 acid, palmitic acid, stearic acid, nervonic acid, behenic acid, dioleoylphosphatidic acid  
 ("DOPA"), 1,2-dimyristoyl-sn-glycero-3-phosphate ("DMPA"), 1,2-dipalmitoyl-sn-glycero-3-

phosphate ("DPPA"), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphate ("POPA"), 1,2-Distearoyl-sn-glycero-3-phosphate ("DSPA"), cholesterol sulphate ("Chol-SO<sub>4</sub>"), dioleoylphosphatidylglycerol ("DOPG"), 1,2-dimyristoyl-sn-glycero-3-phospho-(1'-rac-glycerol), ("DMPG"), dipalmitoylphosphatidylglycerol ("DPPG"), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol ("POPG"), 1,2-distearoyl-sn-glycero-3-phospho-rac-glycerol ("DSPG"), dioleoylphosphatidylserine ("DOPS"), 1,2-dimyristoyl-sn-glycero-3-phospho-L-serine ("DMPS"), dipalmitoylphosphatidylserine ("DPPS"), 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine ("POPS"), 1,2-distearoyl-sn-glycero-3-phospho-L-serine ("DSPS"), or cetylphosphate. In some aspects, the one or more charged lipids in the lipid particle is selected from the group consisting of cholesteryl hemisuccinate ("CHEMS"), 1,2-distearoyl-3-trimethylammonium-propane ("DSTAP"), 18:1 1,2-dioleoyl-3-trimethylammonium-propane ("DOTAP"), 1,2-dioleoyl-sn-glycero-3-phosphate ("18:1 PA"), 18:1 1-stearoyl-2-oleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) ("18:1 PG"), phosphatidylglycerols, cardiolipins, diacylphosphatidylserines, diacylphosphatidic acids, N-dodecanoyl phosphatidylethanolamines, N-succinyl phosphatidylethanolamines, N-glutarylphosphatidylethanolamines, lysylphosphatidylglycerols, and palmitoyloleoylphosphatidylglycerol ("POPG"). In some aspects, the lipid particles described herein contain only one charged lipid. In some aspects, the only charged lipid is CHEMS.

**[0118]** In some aspects, the lipid particles disclosed herein contain CHEMS. In some aspects, the CHEMS in the lipid particles disclosed herein are in a molar amount of 10%. In some aspects, the CHEMS in the lipid particles disclosed herein are in a molar amount of less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, less than 4%, less than 3%, less than 2%, less than 1%, or less than 0.1%. In some aspects, the CHEMS in the lipid particles disclosed herein are in a molar amount of about 1% to about 10%, about 1% to about 9%, about 1% to about 8%, about 1% to about 7%, about 1% to about 6%, about 1% to about 5%, about 1% to about 4%, about 1% to about 3%, about 1% to about 2%, about 2% to about 10%, about 2% to about 9%, about 2% to about 8%, about 2% to about 7%, about 2% to about 6%, about 2% to about 5%, about 2% to about 4%, about 2% to about 3%, about 3% to about 10%, about 3% to about 9%, about 3% to about 8%, about 3% to about 7%, about 3% to about 6%, about 3% to about 5%, about 3% to about 4%, about 4% to about 10%, about 4% to about 9%, about 4% to about 8%, about 4% to about 7%, about 4% to about 6%, about 4% to about 5%, about 5% to about 10%, about 5% to about 9%, about 5% to about 8%, about 5% to about 7%, or about 5% to about 6%. In some aspects, the CHEMS in the lipid particles disclosed herein

are in a molar amount of about 0.1%, about 0.25%, about 0.5%, about 0.7%, about 1%, about 1.25%, about 1.5%, about 1.75%, about 2%, about 2.25%, about 2.5%, about 2.75%, about 3%, about 3.25%, about 3.5%, about 3.75%, about 4%, about 4.25%, about 4.5%, about 4.75%, about 5%, about 5.25%, about 5.5%, about 5.75%, about 6%, about 6.25%, about 6.5%, about 6.75%, about 7%, about 7.25%, about 7.5%, about 7.75%, about 8%, about 8.25%, about 8.5%, about 8.75%, about 9%, about 9.25%, about 9.5%, about 9.75%, or about 10%. In some aspects, the lipid particles described herein do not contain a charged lipid. In some aspects, the lipid particles disclosed herein do not contain CHEMS.

**[0119]** In some aspects, the CHEMS is included in the lipid particles disclosed herein in a molar amount that is sufficient to deliver a payload delivered by the lipid particles to a spleen, e.g., at least 11%, at least 12%, at least 13%, at least 14%, at least 15%, at least 16%, at least 17%, at least 18%, at least 19%, or at least 20%. In some aspects, the CHEMS in the lipid particles disclosed herein that deliver a payload to a spleen is in a molar amount of about 11% to about 45%, about 11% to about 40%, about 11% to about 35%, about 11% to about 30%, about 11% to about 25%, about 11% to about 20%, about 11% to about 15%, about 15% to about 45%, about 15% to about 40%, about 15% to about 35%, about 15% to about 30%, about 15% to about 25%, about 15% to about 20%, about 20% to about 45%, about 20% to about 40%, about 20% to about 35%, about 20% to about 30%, about 20% to about 25%, about 25% to about 45%, about 25% to about 40%, about 25% to about 35%, about 25% to about 30%, about 30% to about 45%, about 30% to about 40%, about 30% to about 35%, about 35% to about 45%, about 35% to about 40%, or about 40% to about 45%. In some aspects, the CHEMS in the lipid particles disclosed herein that deliver a payload to a spleen is in a molar amount of about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 25%, about 30%, about 35%, about 40%, or about 45%. In some aspects, the lipid particles that deliver a payload to a spleen contain CHEMS in a molar amount of about 11% to about 20%, about 20% to about 30%, or about 11% to about 30%. In some aspects, the lipid particles contain CHEMS in a molar amount of about 11% to about 20%. In some aspects, the lipid particles contain CHEMS in a molar amount of about 11%. In some aspects, the lipid particles contain CHEMS in a molar amount of about 15%. In some aspects, the lipid particles contain CHEMS in a molar amount of about 20%. In some aspects, the lipid particles contain CHEMS in a molar amount of about 25%.

**v. STEALTH LIPIDS**

[0120] The lipid particles disclosed herein can comprise one or more stealth lipids. In some aspects, the one or more stealth lipids in the lipid particles described herein are in a molar amount of at least 0.1%, at least 0.15%, at least 0.2%, or at least 0.25%. In some aspects, the one or more stealth lipids in the lipid particles described herein are in a molar amount of about 0.1% to about 3%, about 0.1% to 2.75%, about 0.1% to about 2.5%, about 0.1% to about 2.25%, about 0.1% to about 2%, about 0.1% to about 1.75%, about 0.1% to about 1.5%, about 0.1% to about 1.25%, about 0.1% to about 1%, about 0.1% to about 0.75%, about 0.1% to about 0.5%, about 0.1% to about 0.25%, about 0.25% to about 3%, about 0.25% to about 2.75%, about 0.25% to about 2.5%, about 0.25% to about 2.25%, about 0.25% to about 2%, about 0.25% to about 1.75%, about 0.25% to about 1.5%, about 0.25% to about 1.25%, about 0.25% to about 1%, about 0.25% to about 0.75%, about 0.25% to about 0.5%, about 0.5% to about 3%, about 0.5% to about 2.75%, about 0.5% to about 2.5%, about 0.5% to about 2.25%, about 0.5% to about 2%, about 0.5% to about 1.75%, about 0.5% to about 1.5%, about 0.5% to about 1.25%, about 0.5% to about 1%, about 0.5% to about 0.75%, about 0.75% to about 3%, about 0.75% to about 2.75%, about 0.75% to about 2.5%, about 0.75% to about 2%, about 0.75% to about 1.75%, about 0.75% to about 1.5%, about 0.75% to about 1.25%, about 0.75% to about 1%, about 1% to about 3%, about 1% to about 2.75%, about 1% to about 2.5%, about 1% to about 2.25%, about 1% to about 2%, about 1% to about 1.75%, about 1% to about 1.5%, about 1% to about 1.25%, about 1% to about 1.25%, about 1.25% to about 3%, about 1.25% to about 2.75%, about 1.25% to about 2.5%, about 1.25% to about 2.25%, about 1.25% to about 2%, about 1.25% to about 1.75%, about 1.25% to about 1.5%, about 1.5% to about 3%, about 1.5% to about 2.75%, about 1.5% to about 2.5%, about 1.5% to about 2.25%, about 1.5% to about 2%, about 1.5% to about 1.75%, about 1.75% to about 3%, about 1.75% to about 2.75%, about 1.75% to about 2.5%, about 1.75% to about 2.25%, about 1.75% to about 2%, about 2% to about 3%, about 2% to about 2.75%, about 2% to about 2.5%, about 2% to about 2.25%, about 2.25% to about 3%, about 2.25% to about 2.75%, about 2.25% to about 2.5%, about 2.5% to about 3%, about 2.5% to about 2.75%, about 2.75% to about 3%. In some aspects, the one or more stealth lipids in the lipid particles described herein are in a molar amount of about 1% to about 3%. In some aspects, the one or more stealth lipids in the lipid particles described herein are in a molar amount of about 0.5%, about 1%, about 1.5%, about 2%, about 2.5%, or about 3%. In some aspects, the one or more stealth lipids in the lipid particles described herein are in a molar amount of about 1%. In some aspects, the one or more stealth lipids in the lipid particles described herein are in a molar amount

of about 1.5%. In some aspects, the one or more stealth lipids in the lipid particles described herein are in a molar amount of about 2%. In some aspects, the one or more stealth lipids in the lipid particles described herein are in a molar amount of about 2.5%.

**[0121]** In some aspects, the one or more stealth lipids in the lipid particles disclosed herein comprise one or more PEG terminated lipids, one or more polysarcosine derivatives, or combinations thereof. In some aspects, only one or more PEG terminated lipids are present. PEG terminated lipids are a class of polyethylene ("PEG") derivatives attached to a lipid moiety.

**[0122]** In some aspects, the one or more PEG terminated lipids in the lipid particles described herein are in a molar amount of at least 0.1%, at least 0.15%, at least 0.2%, or at least 0.25%. In some aspects, the one or more PEG terminated lipids in the lipid particles described herein are in a molar amount of about 0.1% to about 3%, about 0.1% to about 2.75%, about 0.1% to about 2.5%, about 0.1% to about 2.25%, about 0.1% to about 2%, about 0.1% to about 1.75%, about 0.1% to about 1.5%, about 0.1% to about 1.25%, about 0.1% to about 1%, about 0.1% to about 0.75%, about 0.1% to about 0.5%, about 0.1% to about 0.25%, about 0.25% to about 3%, about 0.25% to about 2.75%, about 0.25% to about 2.5%, about 0.25% to about 2.25%, about 0.25% to about 2%, about 0.25% to about 1.75%, about 0.25% to about 1.5%, about 0.25% to about 1.25%, about 0.25% to about 1%, about 0.25% to about 0.75%, about 0.25% to about 0.5%, about 0.5% to about 3%, about 0.5% to about 2.75%, about 0.5% to about 2.5%, about 0.5% to about 2.25%, about 0.5% to about 2%, about 0.5% to about 1.75%, about 0.5% to about 1.5%, about 0.5% to about 1.25%, about 0.5% to about 1%, about 0.5% to about 0.75%, about 0.75% to about 3%, about 0.75% to about 2.75%, about 0.75% to about 2.5%, about 0.75% to about 2%, about 0.75% to about 1.75%, about 0.75% to about 1.5%, about 0.75% to about 1.25%, about 0.75% to about 1%, about 1% to about 3%, about 1% to about 2.75%, about 1% to about 2.5%, about 1% to about 2.25%, about 1% to about 2%, about 1% to about 1.75%, about 1% to about 1.5%, about 1% to about 1.25%, about 1.25% to about 3%, about 1.25% to about 2.75%, about 1.25% to about 2.5%, about 1.25% to about 2.25%, about 1.25% to about 2%, about 1.25% to about 1.75%, about 1.25% to about 1.5%, about 1.5% to about 3%, about 1.5% to about 2.75%, about 1.5% to about 2.5%, about 1.5% to about 2.25%, about 1.5% to about 2%, about 1.5% to about 1.75%, about 1.75% to about 3%, about 1.75% to about 2.75%, about 1.75% to about 2.5%, about 1.75% to about 2.25%, about 1.75% to about 2%, about 2% to about 3%, about 2% to about 2.75%, about 2% to about 2.5%, about 2% to about 2.25%, about 2.25% to about 3%, about 2.25% to about 2.75%, about 2.25% to about 2.5%, about 2.5% to about 3%,

about 2.5% to about 2.75%, about 2.75% to about 3%. In some aspects, the one or more PEG terminated lipids in the lipid particles described herein are in a molar amount of about 1% to about 3%. In some aspects, the one or more PEG terminated lipids in the lipid particles described herein are in a molar amount of about 0.5%, about 1%, about 1.5%, about 2%, about 2.5%, or about 3%. In some aspects, the one or more PEG terminated lipids in the lipid particles described herein are in a molar amount of about 1%. In some aspects, the one or more PEG terminated lipids in the lipid particles described herein are in a molar amount of about 1.5%. In some aspects, the one or more PEG terminated lipids in the lipid particles described herein are in a molar amount of about 2%. In some aspects, the one or more PEG terminated lipids in the lipid particles described herein are in a molar amount of about 2.5%.

**[0123]** The one or more PEG terminated lipids in the lipid particle can be one or more selected from the group consisting of 1,2-dimyristoyl-rac-glycerol-3-methoxypolyethylene glycol-2000 ("DMG-PEG2000"), distearoyl-rac-glycerol-PEG2K ("DSG-PEG2k"), [(2R)-2,3-di(octadecanoyloxy)propyl] 2-(2-methoxyethoxycarbonylamino)ethyl phosphate ("C18-mPEG2000"), [3-[3-(2-methoxyethoxy)propylcarbamoyloxy]-2-tetradecanoyloxypropyl] tetradecanoate ("PEG2000-c-DMG"), 3-[hydroxy-[2-[2-(2-methoxyethoxy)ethylamino]ethoxy]phosphoryl]oxy-2-tetradecanoyloxypropyl] tetradecanoate ("DMPE-PEG2000"), 1,2-distearoyl-sn-glycerol-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] ("18:1 PEG2000-PE"), 1,2-distearoyl-sn-glycerol-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)-2000] ("DSPE-PEG2000-COOH"), and Bis(1,2-distearoyl-sn-glycerol-3-phosphoethanolamine)-N-[(polyethylene glycol)-2000] ("Bis-DSPE-PEG2000"). In some aspects, the one or more PEG terminated lipids in the lipid particles described herein is DMG-PEG2000, DMPE-PEG2000, or a combination thereof. In some aspects, the lipid particles described herein contains only one PEG terminated lipid.

**[0124]** In some aspects, the only PEG terminated lipid in the lipid particles described herein is DMG-PEG2000. In some aspects, the DMG-PEG2000 is in a molar amount of at least 0.1%, at least 0.15%, at least 0.2%, or at least 0.25%. In some aspects, the DMG-PEG2000 is in a molar amount of about 0.1% to about 3%, about 0.1% to 2.75%, about 0.1% to about 2.5%, about 0.1% to about 2.25%, about 0.1% to about 2%, about 0.1% to about 1.75%, about 0.1% to about 1.5%, about 0.1% to about 1.25%, about 0.1% to about 1%, about 0.1% to about 0.75%, about 0.1% to about 0.5%, about 0.1% to about 0.25%, about 0.25% to about 3%, about 0.25% to about 2.75%, about 0.25% to about 2.5%, about 0.25% to about 2.25%, about 0.25% to about 2%, about 0.25%

to about 1.75%, about 0.25% to about 1.5%, about 0.25% to about 1.25%, about 0.25% to about 1%, about 0.25% to about 0.75%, about 0.25% to about 0.5%, about 0.5% to about 3%, about 0.5% to about 2.75%, about 0.5% to about 2.5%, about 0.5% to about 2.25%, about 0.5% to about 2%, about 0.5% to about 1.75%, about 0.5% to about 1.5%, about 0.5% to about 1.25%, about 0.5% to about 1%, about 0.5% to about 0.75%, about 0.75% to about 3%, about 0.75% to about 2.75%, about 0.75% to about 2.5%, about 0.75% to about 2%, about 0.75% to about 1.75%, about 0.75% to about 1.5%, about 0.75% to about 1.25%, about 0.75% to about 1%, about 1% to about 3%, about 1% to about 2.75%, about 1% to about 2.5%, about 1% to about 2.25%, about 1% to about 2%, about 1% to about 1.75%, about 1% to about 1.5%, about 1% to about 1.25%, about 1% to about 1.25%, about 1.25% to about 3%, about 1.25% to about 2.75%, about 1.25% to about 2.5%, about 1.25% to about 2.25%, about 1.25% to about 2%, about 1.25% to about 1.75%, about 1.25% to about 1.5%, about 1.5% to about 3%, about 1.5% to about 2.75%, about 1.5% to about 2.5%, about 1.5% to about 2.25%, about 1.5% to about 2%, about 1.5% to about 1.75%, about 1.75% to about 3%, about 1.75% to about 2.75%, about 1.75% to about 2.5%, about 1.75% to about 2.25%, about 1.75% to about 2%, about 2% to about 3%, about 2% to about 2.75%, about 2% to about 2.5%, about 2% to about 2.25%, about 2.25% to about 3%, about 2.25% to about 2.75%, about 2.25% to about 2.5%, about 2.5% to about 3%, about 2.5% to about 2.75%, about 2.75% to about 3%. In some aspects, the DMG-PEG2000 is in a molar amount of about 1% to about 3%. In some aspects, the DMG-PEG2000 is in a molar amount of about 0.5%, about 1%, about 1.5%, about 2%, about 2.5%, or about 3%. In some aspects, the DMG-PEG2000 is in a molar amount of about 1%. In some aspects, the DMG-PEG2000 is in a molar amount of about 1.5%. In some aspects, the DMG-PEG2000 is in a molar amount of about 2%. In some aspects, the DMG-PEG2000 is in a molar amount of about 2.5%.

**[0125]** In some aspects, the only PEG terminated lipid in the lipid particles described herein is DMPE-PEG2000. In some aspects, the DMPE-PEG2000 is in a molar amount of at least 0.1%, at least 0.15%, at least 0.2%, or at least 0.25%. In some aspects, the DMPE-PEG2000 is in a molar amount of about 0.1% to about 3%, about 0.1% to 2.75%, about 0.1% to about 2.5%, about 0.1% to about 2.25%, about 0.1% to about 2%, about 0.1% to about 1.75%, about 0.1% to about 1.5%, about 0.1% to about 1.25%, about 0.1% to about 1%, about 0.1% to about 0.75%, about 0.1% to about 0.5%, about 0.1% to about 0.25%, about 0.25% to about 3%, about 0.25% to about 2.75%, about 0.25% to about 2.5%, about 0.25% to about 2.25%, about 0.25% to about 2%, about 0.25% to about 1.75%, about 0.25% to about 1.5%, about 0.25% to about 1.25%, about 0.25% to about 1%, about 0.25% to about 0.75%, about 0.25% to about 0.5%, about 0.5% to



about 3%, about 0.5% to about 2.75%, about 0.5% to about 2.5%, about 0.5% to about 2.25%, about 0.5% to about 2%, about 0.5% to about 1.75%, about 0.5% to about 1.5%, about 0.5% to about 1.25%, about 0.5% to about 1%, about 0.5% to about 0.75%, about 0.75% to about 3%, about 0.75% to about 2.75%, about 0.75% to about 2.5%, about 0.75% to about 2%, about 0.75% to about 1.75%, about 0.75% to about 1.5%, about 0.75% to about 1.25%, about 0.75% to about 1%, about 1% to about 3%, about 1% to about 2.75%, about 1% to about 2.5%, about 1% to about 2.25%, about 1% to about 2%, about 1% to about 1.75%, about 1% to about 1.5%, about 1% to about 1.25%, about 1% to about 1.25%, about 1.25% to about 3%, about 1.25% to about 2.75%, about 1.25% to about 2.5%, about 1.25% to about 2.25%, about 1.25% to about 2%, about 1.25% to about 1.75%, about 1.25% to about 1.5%, about 1.5% to about 3%, about 1.5% to about 2.75%, about 1.5% to about 2.5%, about 1.5% to about 2.25%, about 1.5% to about 2%, about 1.5% to about 1.75%, about 1.75% to about 3%, about 1.75% to about 2.75%, about 1.75% to about 2.5%, about 1.75% to about 2.25%, about 1.75% to about 2%, about 2% to about 3%, about 2% to about 2.75%, about 2% to about 2.5%, about 2% to about 2.25%, about 2.25% to about 3%, about 2.25% to about 2.75%, about 2.25% to about 2.5%, about 2.5% to about 3%, about 2.5% to about 2.75%, about 2.75% to about 3%. In some aspects, the DMPE-PEG2000 is in a molar amount of about 0.5%, about 1%, about 1.5%, about 2%, about 2.5%, or about 3%. In some aspects, the DMPE-PEG2000 is in a molar amount of about 1%. In some aspects, the DMPE-PEG2000 is in a molar amount of about 1.5%. In some aspects, the DMPE-PEG2000 is in a molar amount of about 2%. In some aspects, the DMPE-PEG2000 is in a molar amount of about 2.5%.

**[0126]** In some aspects, the only PEG terminated lipid in the lipid particles described herein is 18:1 PEG2000-PE. In some aspects, the 18:1 PEG2000-PE is in a molar amount of at least 0.1%, at least 0.15%, at least 0.2%, or at least 0.25%. In some aspects, the 18:1 PEG2000-PE is in a molar amount of about 0.25% to about 3%, about 0.5% to about 3%, about 1% to about 3%, about 1.5% to about 3%, about 2% to about 3%, about 2.5% to about 3%, about 0.25% to about 2.5%, about 0.5% to about 2.5%, about 1% to about 2.5%, about 1.5% to about 2.5%, about 2% to about 2.5%, about 0.25% to about 2%, about 0.5% to about 2%, about 1% to about 2%, about 1.5% to about 2%, about 0.25% to about 1.5%, about 0.5% to about 1.5%, or about 1% to about 1.5%. In some aspects, the 18:1 PEG2000-PE is in a molar amount of about 1% to about 3%. In some aspects, the 18:1 PEG2000-PE is in a molar amount of about 0.5%, about 1%, about 1.5%, about 2%, about 2.5%, or about 3%. In some aspects, the 18:1 PEG2000-PE is in a molar amount of about 1%. In some aspects, the 18:1 PEG2000-PE is in a molar amount of about 1.5%. In

some aspects, the 18:1 PEG2000-PE is in a molar amount of about 2%. In some aspects, the 18:1 PEG2000-PE is in a molar amount of about 2.5% molar amount of about 0.1% to about 3%, about 0.1% to 2.75%, about 0.1% to about 2.5%, about 0.1% to about 2.25%, about 0.1% to about 2%, about 0.1% to about 1.75%, about 0.1% to about 1.5%, about 0.1% to about 1.25%, about 0.1% to about 1%, about 0.1% to about 0.75%, about 0.1% to about 0.5%, about 0.1% to about 0.25%, about 0.25% to about 3%, about 0.25% to about 2.75%, about 0.25% to about 2.5%, about 0.25% to about 2.25%, about 0.25% to about 2%, about 0.25% to about 1.75%, about 0.25% to about 1.5%, about 0.25% to about 1.25%, about 0.25% to about 1%, about 0.25% to about 0.75%, about 0.25% to about 0.5%, about 0.5% to about 3%, about 0.5% to about 2.75%, about 0.5% to about 2.5%, about 0.5% to about 2.25%, about 0.5% to about 2%, about 0.5% to about 1.75%, about 0.5% to about 1.5%, about 0.5% to about 1.25%, about 0.5% to about 1%, about 0.5% to about 0.75%, about 0.75% to about 3%, about 0.75% to about 2.75%, about 0.75% to about 2.5%, about 0.75% to about 2%, about 0.75% to about 1.75%, about 0.75% to about 1.5%, about 0.75% to about 1.25%, about 0.75% to about 1%, about 1% to about 3%, about 1% to about 2.75%, about 1% to about 2.5%, about 1% to about 2.25%, about 1% to about 2%, about 1% to about 1.75%, about 1% to about 1.5%, about 1% to about 1.25%, about 1% to about 1.25%, about 1.25% to about 3%, about 1.25% to about 2.75%, about 1.25% to about 2.5%, about 1.25% to about 2.25%, about 1.25% to about 2%, about 1.25% to about 1.75%, about 1.25% to about 1.5%, about 1.5% to about 3%, about 1.5% to about 2.75%, about 1.5% to about 2.5%, about 1.5% to about 2.25%, about 1.5% to about 2%, about 1.5% to about 1.75%, about 1.75% to about 3%, about 1.75% to about 2.75%, about 1.75% to about 2.5%, about 1.75% to about 2.25%, about 1.75% to about 2%, about 2% to about 3%, about 2% to about 2.75%, about 2% to about 2.5%, about 2% to about 2.25%, about 2.25% to about 3%, about 2.25% to about 2.75%, about 2.25% to about 2.5%, about 2.5% to about 3%, about 2.5% to about 2.75%, about 2.75% to about 3%.

**[0127]** In some aspects, the one or more stealth lipids in the lipid particles disclosed herein comprise one or more polysarcosine derivatives, or combinations thereof. In some aspects, the one or more polysarcosine derivatives in the lipid particle are one or more selected from the group consisting of N-tetradecyl-polysarcosine-25 (“N-tetradecyl-pSar25”), N-hexadecyl-polysarcosine-25 (“N-hexadecyl-pSar25”), N-octadecyl-polysarcosine-25, N-dodecyl-polysarcosine-25 (“N-octadecyl-pSar25”), 1,2-dimyristoyl-sn-glycero-3-succinyl-N-polysarcosine-25 (“DMG-pSar25”), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-polysarcosine-25 (ammonium salt) (“18:1 PE (DOPE), pSar25”), N,N-ditetradecylamine-N-

succinyl[methyl(polysarcosine)45] (“N-TETAMINE-pSar45”), N,N-ditetradecylamine-N-succinyl[methyl(polysarcosine)35] (“N-TETAMINE-pSar35”), N,N-ditetradecyl-polysarcosine-25 (“N-TETAMINE-pSar25”), N-TETAMINE-pSar45-maleimide, or N-TETAMINE-PEOZ-40. In some aspects, only one polysarcosin derivative is in the lipid particle.

#### **b. EXEMPLARY LIPID PARTICLES**

**[0128]** In some aspects, the lipid particles described herein comprise about 25% to about 45% of SS-OP, about 5% to about 25% of DOPE, about 15% to about 35% of cholesterol, about 10% to about 30% CHEMS, and about 0.5% to about 2.5% of DMG-PEG2000 (in molar amounts). In some aspects, the lipid particles described herein comprise about 35% of SS-OP, about 15% of DOPE, about 28.5% of cholesterol, about 20% CHEMS, and about 1.5% of DMG-PEG2000 (in molar amounts).

**[0129]** In some aspects, the lipid particles described herein comprise about 25% to about 45% of SS-OP, about 5% to about 25% of DODG, about 15% to about 35% of cholesterol, about 10% to about 30% CHEMS, and about 0.5% to about 2.5% of DMPE-PEG2000 (in molar amounts). In some aspects, the lipid particles described herein comprise about 35% of SS-OP, about 15% of DODG, about 28.5% of cholesterol, about 20% CHEMS, and about 1.5% of DMPE-PEG2000 (in molar amounts).

**[0130]** In some aspects, the lipid particles described herein comprise about 25% to about 45% of SS-OP, about 5% to about 25% of DOPE, about 15% to about 35% of cholesterol, about 10% to about 30% CHEMS, and about 0.5% to about 2.5% of DMPE-PEG2000 (in molar amounts). In some aspects, the lipid particles described herein comprise about 35% of SS-OP, about 15% of DOPE, about 28.5% of cholesterol, about 20% CHEMS, and about 1.5% of DMPE-PEG2000 (in molar amounts).

**[0131]** In some aspects, the lipid particles described herein comprise about 25% to about 45% of SS-OP, about 5% to about 25% of DODG, about 15% to about 35% of cholesterol, about 10% to about 30% CHEMS, and about 0.5% to about 2.5% of DMG-PEG2000 (in molar amounts). In some aspects, the lipid particle comprises about 35% of SS-OP, about 15% of DODG, about 28.5% of cholesterol, about 20% CHEMS, and about 1.5% of DMG-PEG2000 (in molar amounts).

**[0132]** In some aspects, the lipid particles described herein comprise about 25% to about 45% of SS-OP, about 5% to about 25% of DODG, about 15% to about 35% of cholesterol, about

10% to about 30% CHEMS, and about 0.5% to about 2.5% of 18:1 PEG2000-PE (in molar amounts). In some aspects, the lipid particles described herein comprise about 35% of SS-OP, about 15% of DODG, about 28.5% of cholesterol, about 20% CHEMS, and about 1.5% of 18:1 PEG2000-PE (in molar amounts).

**[0133]** In some aspects, the lipid particles described herein comprise about 25% to about 45% of SS-OP, about 5% to about 25% of DOPE, about 15% to about 35% of cholesterol, about 10% to about 30% CHEMS, and about 0.5% to about 2.5% of 18:1 PEG2000-PE (in molar amounts). In some aspects, the lipid particles described herein comprise about 35% of SS-OP, about 15% of DOPE, about 28.5% of cholesterol, about 20% CHEMS, and about 1.5% of 18:1 PEG2000-PE (in molar amounts).

**[0134]** In some aspects, the lipid particles described herein comprise about 30% to about 50% of SS-OP, about 15% to about 55% of cholesterol, about 10% to about 30% CHEMS, and about 0.5% to about 2.5% of DMG-PEG2000 (in molar amounts). In some aspects, the lipid particle comprises about 40% of SS-OP, about 38.5% of cholesterol, about 20% CHEMS, and about 1.5% of DMG-PEG2000 (in molar amounts).

**[0135]** In some aspects, the lipid particles described herein comprise about 20% to about 45% of SS-OP, about 15% to about 55% of cholesterol, about 15% to about 35% CHEMS, and about 0.5% to about 2.5% of DMG-PEG2000 (in molar amounts). In some aspects, the lipid particles described herein comprise about 35% of SS-OP, about 38.5% of cholesterol, about 25% CHEMS, and about 1.5% of DMG-PEG2000 (in molar amounts).

**[0136]** In some aspects, the lipid particles described herein comprise about 20% to about 40% of SS-OP, about 15% to about 55% of cholesterol, about 20% to about 40% CHEMS, and about 0.5% to about 2.5% of DMG-PEG2000 (in molar amounts). In some aspects, the lipid particles described herein comprise about 30% of SS-OP, about 38.5% of cholesterol, about 30% CHEMS, and about 1.5% of DMG-PEG2000 (in molar amounts).

**[0137]** In some aspects, the lipid particles described herein comprise about 15% to about 35% of SS-OP, about 15% to about 55% of cholesterol, about 25% to about 45% CHEMS, and about 0.5% to about 2.5% of DMG-PEG2000 (in molar amounts). In some aspects, the lipid particles described herein comprise about 25% of SS-OP, about 38.5% of cholesterol, about 35% CHEMS, and about 1.5% of DMG-PEG2000 (in molar amounts).

**[0138]** In some aspects, the lipid particles described herein comprise about 30% to about 50% of SS-OP, about 15% to about 55% of cholesterol, about 15% to about 35% CHEMS, and about 0.5% to about 2.5% of DMG-PEG2000 (in molar amounts). In some aspects, the lipid particles described herein comprise about 40% of SS-OP, about 33.5% of cholesterol, about 25% CHEMS, and about 1.5% of DMG-PEG2000 (in molar amounts). In some aspects, the lipid particles described herein comprise about 30% to about 50% of SS-OP, about 15% to about 55% of cholesterol, about 20% to about 40% CHEMS, and about 0.5% to about 2.5% of DMG-PEG2000 (in molar amounts). In some aspects, the lipid particles described herein comprise about 40% of SS-OP, about 28.5% of cholesterol, about 30% CHEMS, and about 1.5% of DMG-PEG2000 (in molar amounts).

**[0139]** In some aspects, the lipid particles described herein comprise about 40% to about 60% of SS-OP, about 1% to about 20% of DODG, about 25% to about 45% of cholesterol, about 1% to about 10% of CHEMS, and about 0.5% to about 2.5% of DMG-PEG2000 (in molar amounts). In some aspects, the lipid particles described herein comprise about 50% of SS-OP, about 10% of DODG, about 38.5% of cholesterol, about 10% of CHEMS, and about 1.5% of DMG-PEG2000 (in molar amounts).

**[0140]** In some aspects, the lipid particles described herein comprise about 40% to about 60% of SS-OP, about 1% to about 20% of DODG, about 25% to about 45% of cholesterol, and about 0.5% to about 2.5% of DMG-PEG2000 (in molar amounts). In some aspects, the lipid particles described herein comprise about 50% of SS-OP, about 10% of DODG, about 38.5% of cholesterol, and about 1.5% of DMG-PEG2000 (in molar amounts).

**[0141]** In some aspects, the lipid particles described herein comprise about 40% to about 60% of SS-OP, about 1% to about 20% of DODG, about 25% to about 45% of cholesterol, and about 0.5% to about 2.5% of DMG-PEG2000 (in molar amounts). In some aspects, the lipid particles described herein comprise about 50% of SS-OP, about 10% of DODG, about 38.5% of cholesterol, and about 1.5% of DMG-PEG2000 (in molar amounts).

**[0142]** The lipid particles described herein can be contained in a composition. The composition comprises lipid particles having an average particle size of less than 150 nm, less than 140 nm, less than 130 nm, less than 120 nm, less than 110 nm, less than 100 nm, less than 90 nm, less than 85 nm, less than 80 nm, or less than 75 nm. In some aspects, the composition contains lipid particles having an average particle size of about 50 nm to about 150 nm, about 50 nm, to about 140 nm, about 50 nm to about 140 nm, about 50 nm to about 130 nm, about 50 nm

to about 120 nm, about 50 nm to about 110 nm, about 50 nm to about 100 nm, about 50 nm to about 90 nm, about 50 nm to about 80 nm, about 60 nm to about 150 nm, about 60 nm, to about 140 nm, about 60 nm to about 140 nm, about 60 nm to about 130 nm, about 60 nm to about 120 nm, about 60 nm to about 110 nm, about 60 nm to about 100 nm, about 60 nm to about 90 nm, about 70 nm to about 150 nm, about 70 nm, to about 140 nm, about 70 nm to about 140 nm, about 70 nm to about 130 nm, about 70 nm to about 120 nm, about 70 nm to about 110 nm, or about 70 nm to about 100 nm. In some aspects, the size of the lipid particles is determined by dynamic light scattering using the NanoBrook Omni instrument from Brookhaven Instruments.

**[0143]** The composition comprises lipid particles having an encapsulation efficiency of less than 99.9%, less than 99.5%, less than 99%, less than 98.5%, less than 98%, less than 97.5%, less than 97%, less than 96.5%, less than 96%, less than 95.5%, or less than 95%. In some aspects, the encapsulation efficiency is about 75% to about 99.9%, about 80% to about 99.9%, about 85% to about 99.9%, about 90% to about 99.9%, about 75% to about 95%, about 80% to about 95%, about 85% to about 95%, or about 75% to about 90%. In some aspects, the encapsulation efficiency is about 75%, about 76%, about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99%. In some aspects, the encapsulation efficiency is determined by a QuantiT Ribogreen assay. In some aspects, the encapsulation efficiency is determined by LC-MS.

### **III. PAYLOADS**

**[0144]** The lipid particles described herein can comprise a payload. The payload can be a biologically active molecule. In some aspects, the biologically active molecule comprises a nucleic acid, a small molecule, peptide, protein, or any combination thereof

**[0145]** In some aspects, the lipid particles described herein comprise a nucleic acid. Examples of nucleic acids include, but are not limited to, small interfering ribonucleic acid (siRNA), a short hairpin RNA (shRNA), a micro-ribonucleic acid (miRNA), a primary micro-ribonucleic acid (pri-miRNA), a long non-coding RNA (lncRNA), a messenger ribonucleic acid (mRNA), a clustered regularly interspaced short palindromic repeats (CRISPR) related nucleic acid, a CRISPR-RNA (crRNA), a single guide ribonucleic acid (sgRNA), a trans-activating CRISPR ribonucleic acid (tracrRNA), a plasmid deoxyribonucleic acid (pDNA), a transfer ribonucleic

acid (tRNA), an antisense oligonucleotide (ASO), an antisense ribonucleic acid (RNA), a guide ribonucleic acid, deoxyribonucleic acid (DNA), a double stranded deoxyribonucleic acid (dsDNA), a single stranded deoxyribonucleic acid (ssDNA), a single stranded ribonucleic acid (ssRNA), a double stranded ribonucleic acid (dsRNA), a CRISPR-associated (Cas) protein, or combinations thereof.

**[0146]** In some aspects, the lipid particles described herein comprise a nucleic acid that is an mRNA. In some aspects, the nucleic acid encodes a peptide having therapeutic activity. The peptide having therapeutic activity can comprise an epitope amino acid sequence. The peptide can also induce immune tolerance to cells in a subject, e.g., a human. The lipid particles or compositions described herein deliver the payload, e.g., nucleic acid to the target organ of interest of a subject *in vivo*. In some aspects, the lipid particles or compositions described herein deliver the payload, e.g., nucleic acid, to a target organ of interest of a subject *in vivo* that is the spleen. In some aspects, the ratio of the payload delivered to the spleen versus liver is greater than 1.1. In some aspects, the ratio of the payload delivered to the spleen versus liver is about 1.1 to about 12, about 1.1 to about 11, about 1.1 to about 10, about 1.1 to about 9, about 1.1 to about 8, about 1.1 to about 7, about 1.1 to about 6, about 1.1 to about 5, about 1.1 to about 4, about 1.5 to about 12, about 1.5 to about 11, about 1.5 to about 10, about 1.5 to about 9, about 1.5 to about 8, about 1.5 to about 7, about 1.5 to about 6, about 1.5 to about 5, about 1.5 to about 4, about 2 to about 12, about 2 to about 11, about 2 to about 10, about 2 to about 9, about 2 to about 8, about 2 to about 7, about 2 to about 6, about 2 to about 5, about 2 to about 4, about 2.5 to about 12, about 2.5 to about 11, about 2.5 to about 10, about 2.5 to about 9, about 2.5 to about 8, about 2.5 to about 7, about 2.5 to about 6, about 2.5 to about 5, about 2.5 to about 4, about 3 to about 12, about 3 to about 11, about 3 to about 10, about 3 to about 9, about 3 to about 8, about 3 to about 7, about 3 to about 6, about 3 to about 5, about 3 to about 4, about 3.5 to about 12, about 3.5 to about 11, about 3.5 to about 10, about 3.5 to about 9, about 3.5 to about 8, about 3.5 to about 7, about 3.5 to about 6, about 3.5 to about 5, about 4 to about 12, about 4 to about 11, about 4 to about 10, about 4 to about 9, about 4 to about 8, about 4 to about 7, about 4 to about 6, about 4 to about 5, about 5 to about 12, about 5 to about 11, about 5 to about 10, about 5 to about 9, 5 to about 8, about 5 to about 7, about 5 to about 6, about 6 to about 12, about 6 to about 11, about 6 to about 10, about 6 to about 9, about 6 to about 8, about 6 to about 7, about 7 to about 12, about 7 to about 11, about 7 to about 10, about 7 to about 9, about 7 to about 8, about 8 to about 12,

about 8 to about 11, about 8 to about 10, about 8 to about 9, about 9 to about 12, about 9 to about 11, about 9 to about 10, about 10 to about 12, or about 10 to about 11.

**[0147]** The ratio of the payload to the spleen versus liver is determined by comparing the BLI values for luciferase mRNA payload in the spleen versus the BLI values in the liver. In some aspects, the BLI values in the spleen and the liver are determined by imaging the organ of interest. In some aspects, the BLI values in the spleen and liver are determined by imaging the organ of interest using an imaging system. In some aspects, a software is used to determine the BLI values from the images generated by the imaging system. In some aspects, the BLI values in the spleen and liver are determined by imaging the organ of interest using a LICOR Pearl imaging system. In some aspects, the software used to determine the BLI values is software provided by LICOR. In some aspects, the software used to determine the BLI values is the Image Studio software from LICOR. In some aspects, the software used to determine the BLI values is the Image Studio software from LICOR version 5.2.5.

**[0148]** In some aspects, the ratio of the payload to the spleen versus liver is determined by homogenizing the organ of the subject (e.g., mouse) and quantifying the luciferase signal using a plate based luciferase assay.

**[0149]** In some aspects, the lipid particles or compositions described herein deliver the payload, e.g., nucleic acid, to a target organ of interest of a subject *in vivo* that is not the spleen. In some aspects, the lipid particles or compositions described herein deliver the payload, e.g., nucleic acid, to a liver of a subject *in vivo*. In some aspects, the lipid particles or compositions described herein deliver the payload, e.g., nucleic acid, to a pancreas of a subject *in vivo*. In some aspects, the lipid particles or compositions described herein deliver the payload, e.g., nucleic acid, to the lymph nodes of a subject *in vivo*. In some aspects, the lipid particles or composition described herein deliver the payload, e.g., nucleic acid, to a brain of a subject *in vivo*. In some aspects, the lipid particles or compositions described herein deliver the payload, e.g., a nucleic acid, to muscle cell of a subject *in vivo*. In some aspects, the lipid particles or composition described herein deliver the payload, e.g., nucleic acid, to a lung of a subject *in vivo*.

**[0150]** In some aspects, the ratio of the payload delivered to the liver versus spleen is greater than 1.1. In some aspects, the ratio of the payload delivered to the liver versus spleen is about 1.1 to about 12, about 1.1 to about 11, about 1.1 to about 10, about 1.1 to about 9, about 1.1 to about 8, about 1.1 to about 7, about 1.1 to about 6, about 1.1 to about 5, about 1.1 to about 4, about 1.5 to about 12, about 1.5 to about 11, about 1.5 to about 10, about 1.5 to about 9, about 1.5 to about



8, about 1.5 to about 7, about 1.5 to about 6, about 1.5 to about 5, about 1.5 to about 4, about 2 to about 12, about 2 to about 11, about 2 to about 10, about 2 to about 9, about 2 to about 8, about 2 to about 7, about 2 to about 6, about 2 to about 5, about 2 to about 4, about 2.5 to about 12, about 2.5 to about 11, about 2.5 to about 10, about 2.5 to about 9, about 2.5 to about 8, about 2.5 to about 7, about 2.5 to about 6, about 2.5 to about 5, about 2.5 to about 4, about 3 to about 12, about 3 to about 11, about 3 to about 10, about 3 to about 9, about 3 to about 8, about 3 to about 7, about 3 to about 6, about 3 to about 5, about 3 to about 4, about 3.5 to about 12, about 3.5 to about 11, about 3.5 to about 10, about 3.5 to about 9, about 3.5 to about 8, about 3.5 to about 7, about 3.5 to about 6, about 3.5 to about 5, about 4 to about 12, about 4 to about 11, about 4 to about 10, about 4 to about 9, about 4 to about 8, about 4 to about 7, about 4 to about 6, about 4 to about 5, about 5 to about 12, about 5 to about 11, about 5 to about 10, about 5 to about 9, about 5 to about 8, about 5 to about 7, about 5 to about 6, about 6 to about 12, about 6 to about 11, about 6 to about 10, about 6 to about 9, about 6 to about 8, about 6 to about 7, about 7 to about 12, about 7 to about 11, about 7 to about 10, about 7 to about 9, about 7 to about 8, about 8 to about 12, about 8 to about 11, about 8 to about 10, about 8 to about 9, about 9 to about 12, about 9 to about 11, about 9 to about 10, about 10 to about 12, or about 10 to about 11.

**[0151]** The ratio of the payload to the liver versus spleen is determined by comparing the BLI values in the spleen versus the BLI values in the liver. In some aspects, the BLI values in the spleen and the liver are determined by imaging the organ of interest.

**[0152]** In some aspects, the BLI values in the spleen and liver are determined by imaging the organ of interest using an imaging system. In some aspects, a software is used to determine the BLI values from the images generated by the imaging system. In some aspects, the BLI values in the spleen and liver are determined by imaging the organ of interest using a LICOR Pearl imaging system. In some aspects, the software used to determine the BLI values is software provided by LICOR. In some aspects, the software used to determine the BLI values is the Image Studio software from LICOR. In some aspects, the software used to determine the BLI values is the Image Studio software from LICOR version 5.2.5.

**[0153]** In some aspects, the ratio of the payload to the spleen versus liver is determined by homogenizing the organ of the subject (e.g., mouse) and quantifying the luciferase signal using a plate based luciferase assay.

#### IV. PHARMACEUTICAL COMPOSITIONS

[0154] The present disclosure provides pharmaceutical compositions (*e.g.*, tolerogenic compositions) comprising one or more lipid particles described herein comprising one or more payloads (*e.g.*, nucleic acids encoding one or more peptides or epitopes). In some aspects, the peptides or epitopes constitute a fragment of a complete antigen protein. Such pharmaceutical compositions are also known as minigene vaccines.

[0155] In some aspects, at least one nucleic acid included in the lipid particles disclosed herein encodes a plurality of different peptides. The nucleic acid can be engineered to insert protease cleavage sites and/or ribosomal skipping elements between the peptides, such that the encoded peptides are processed to produce separate peptides. Such polynucleotide constructs, also called “strings,” are useful as pharmaceutical compositions (see, *e.g.*, Velders *et al.*, *J. Immunol.* (2001) 166:5366-73; Schubert *et al.*, *Genome Medicine* (2016) 8:9). In some aspects, the pharmaceutical compositions comprise the lipid particles described herein comprising one or more polynucleotide constructs that encode one or more peptides with one or more protease cleavage sites and/or ribosomal skipping elements between the peptides. Both coding and non-coding strands can be incorporated into the nucleic acid included in the lipid particles disclosed herein. In some aspects, the string refers to a polynucleotide chain that encodes a plurality of the peptides in tandem. In some aspects, the string encodes 2 to 100, 2 to 90, 2 to 80, 2 to 70, 2 to 60, 2 to 50, 2 to 40, 2 to 30, 2 to 20, 2 to 10, 10 to 100, 10 to 90, 10 to 80, 10 to 70, 10 to 60, 10 to 50, 10 to 40, 10 to 30, 10 to 20, 20 to 100, 20 to 90, 20 to 80, 20 to 70, 20 to 60, 20 to 50, 20 to 40, 20 to 30, 50 to 100, 50 to 90, 50 to 80, 50 to 70, or 50 to 60 peptides. In some aspects, the peptides are arranged on a string to maximize recognition by a certain HLA. In some aspects, the epitope-coding sequences in a string construct are flanked by one or more sequences selected for better cleavability for peptide presentation by MHCs, better expression, and/or improved translation in a cell in a subject. The flanking sequences can comprise ribosomal skipping elements such as a T2A, P2A, F2A, or E2A sequence. In some aspects, the one or more cleavage sequences are selected from the group consisting of FRAC, KRCF, KKRY, ARMA, RRSF, MRAC, KMCQ, ARCA, KKQG, YRSY, SFMN, FKAQ, KRNG, YNSF, KKNG, RRRG, KRYS, and ARYA. In some aspects, the amino acid sequences encoded by a string construct further comprises a signal protein sequence. In some aspects, the string constructs may be mRNA. In some aspects, the lipid particles described herein may comprise one or more mRNA string constructs.

[0156] In some aspects, the pharmaceutical composition comprises lipid particles comprising a nucleic acid that comprises RNA encoding for one or more antigen peptides and/or T cell epitopes. In some aspects, the RNA is a messenger RNA (mRNA). The RNA can optionally further comprises one or more chemical modifications. For example, the RNA can comprise a five-prime (5') and/or a three-prime (3') untranslated region (UTR) and can be further stabilized by 7-methylguanosine (m7G) 5' cap and 3' poly (A) tails respectively, and/or a suitable Poly(A) sequence. In some aspects, the mRNA comprises modified nucleosides, for example Pseudouridine ( $\Psi$ ), 1-methylpseudouridine (m1 $\Psi$ ), and 5-methylcytidine (m5C). In some aspects, the RNA comprises a self-amplifying RNA (saRNA), which further encodes an RNA-dependent RNA polymerase that can be translated *in situ*, thereby amplifying the peptide- or antigen-encoding sequence(s) *in situ* (see, *e.g.*, Bloom *et al.*, *Gene Therapy* (2021) 28:117–29). An overview of mRNA compositions such as mRNA cancer vaccines is provided, for example, in Miao, L. *et al.*, *Molecular Cancer* (2021) 20:41.

[0157] In some aspects, the pharmaceutical composition comprises lipid particles described herein comprising both an antigen peptide and a nucleic acid coding for the peptide. In some aspects, the antigen peptide and the nucleic acid are linked.

[0158] In some aspects, the lipid particles deliver the payload (*e.g.*, nucleic acid(s)) to a target cell (*e.g.*, tolerogenic target cell). The target cell, after internalizing the peptide(s), can present the peptide(s) on the cell surface with a cognate MHC, thereby to stimulate tolerogenic T cell responses. The lipid particles described herein deliver nucleic acids to target cell (*e.g.*, tolerogenic target cell).

[0159] In some aspects, the nucleic acid in the lipid particles described herein further comprises an immunomodulator. An immunomodulator can be an immunosuppressor, which reduces effector cell (*e.g.*, cytotoxic T cell) activity, or an immunostimulator, which promotes T cell anergy or exhaustion (*e.g.*, described in Kwong *et al.*, *Immunology & Cell Biology* (2021) 99: 486–495, and Linsley *et al.*, *Curr Opin Endocrinol Diabetes Obes* (2019) 26:213–218). The immunomodulator, when delivered to or expressed by the target cell, may induce or stabilize a tolerogenic, anergic, or exhausted status of the target cell or a cell in the vicinity. In some aspects, the immunomodulator comprises an immunomodulatory cytokine, for example, an immunomodulatory cytokine selected from IL-2, IL-10, TGF- $\beta$ , IL-37, IL-27, IL-35, Vasoactive Intestinal Peptide (VIP), and variants thereof. In some aspects, the immunomodulatory cytokine is a mutant IL-2 that preferentially activates the IL-2R $\alpha\beta\gamma$  receptor complex relative to the IL-

2R $\beta$  $\gamma$  receptor complex (see, *e.g.*, Ghelani *et al.*, *Front. Immunol.* (2020) 11:1106; Khoryati *et al.*, *Sci Immunol.* (2020) 5(50):eaba5264). In some aspects, the immunomodulator comprises a nucleic acid encoding an immunomodulatory cytokine. In some aspects, the immunomodulator comprises a nucleic acid encoding an intracellular or transmembrane immunomodulatory protein, for example, an immunomodulatory protein selected from PD-L1, PD-L2, ICOS ligand, ILT3, ILT4, BTLA, Fas, CD39, and indoleamine 2,3-dioxygenase 1 (IDO1), heme oxygenase 1, HLA-G, CD95L, galectin-1, and DC-SIGN. In some aspects, the immunomodulator comprises an immunomodulatory compound, for example, an immunomodulatory compound selected from vitamin A, vitamin D (*e.g.*, 1 $\alpha$ ,25-dihydroxyvitamin D3, a.k.a. calcitriol), adenosine, kynurenine, retinoic acid, rapamycin, dexamethasone, corticosteroids, and a AhR ligand such as 2-(1' H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE). Depending on the form of the immunomodulator and delivery agent, the immunomodulator can be encapsulated or otherwise contained in the lipid particle, or covalently or non-covalently attached to an outer or inner surface of the lipid particle.

**[0160]** Pharmaceutical compositions comprising a lipid particle described herein and one or more pharmaceutically acceptable carriers are contemplated. In some aspects, the pharmaceutically acceptable carrier be one which has no detrimental side effects or toxicity under the conditions of use. In some aspects, a pharmaceutical composition is sterile and produced according to GMP guidelines. The one or more pharmaceutically acceptable carriers contained in the pharmaceutical compositions described herein include, but are not limited to, lactose, dextrose, sucrose, sorbitol, mannitol, starch, rubber arable, potassium phosphate, alginate, gelatin, potassium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrups, methylcellulose, methylhydroxy benzoate, propylhydroxy benzoate, talc, magnesium stearate, and mineral oils. The pharmaceutical compositions described herein can further include a lubricant, a humectant, a sweetener, a flavoring agent, an emulsifier, a suspending agent, and a preservative.

**[0161]** In some aspects, the pharmaceutical composition is in the form of a lyophilized formulation or an aqueous solution. The pharmaceutical composition can be in dosages suspended in any appropriate pharmaceutical vehicle or carrier in sufficient volume to carry the dosage.

**[0162]** The pharmaceutical compositions comprising such adjuvants, carriers, and/or excipients can be formulated by well-known conventional methods. These pharmaceutical compositions can be administered to the subject at a suitable dose.

**[0163]** In some aspects, the pharmaceutical composition is in the form of a lyophilized formulation or an aqueous solution. The pharmaceutical composition can be in dosages suspended in any appropriate pharmaceutical carrier in sufficient volume to carry the dosage. Generally, the final volume, including carriers, adjuvants, and the like, typically will be at least 0.5 mL. The upper limit is governed by the practicality of the amount to be administered, generally in the range of 0.5 mL to about 4.0 mL, such as 0.5 mL to about 2.0 mL. In some aspects, the pharmaceutical composition is administered to a patient enterally or parenterally. In some aspects, the pharmaceutical compositions are administered to a patient by intravenous, intramuscular, intratumoral, intradermal, intrajejunal, intraileal, intracolonic, or intrarectal administration.

**[0164]** In some aspects, the lipid particles and the pharmaceutical compositions disclosed herein are stable after storage at -80 °C. In some aspects, the lipid particles and the pharmaceutical compositions disclosed herein are stable after storage at -80 °C for at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 1 year, at least 2 years, at least 3 years, at least 4 years, or at least 5 years.

**[0165]** In some aspects, the lipid particles and the pharmaceutical compositions disclosed herein are stable after storage at 4 °C. In some aspects, the lipid particles and the pharmaceutical compositions disclosed herein are stable after storage at 4 °C for at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 1 year, at least 2 years, at least 3 years, at least 4 years, or at least 5 years.

**[0166]** In some aspects, the lipid particles and the pharmaceutical compositions disclosed herein are stable after storage at room temperature. In some aspects, the lipid particles and the pharmaceutical compositions disclosed herein are stable after storage at room temperature for at

least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 1 year, at least 2 years, at least 3 years, at least 4 years, or at least 5 years.

**[0167]** In some aspects, the lipid particles and the pharmaceutical compositions disclosed herein are stored at -80 °C, 4 °C, or at room temperature as a lyophilized formulation.

**[0168]** In some aspects, the lyophilized formulation is reconstituted with an aqueous solution, e.g., normal saline or buffered formulation (e.g., Tris buffered) to make a reconstituted formulation. In some aspects, the reconstituted formulation is stored at 4 °C or at room temperature. In some aspects, the reconstituted formulation is stable for at least 10 minutes, at least 15 minutes, at least 20 minutes, at least 25 minutes, at least 30 minutes, at least 35 minutes, at least 40 minutes, at least 45 minutes, at least 50 minutes, at least 55 minutes, at least 1 hour, at least 2 hours, at least 3 hours, at least 4 hours, at least 5 hours, at least 6 hours, at least 7 hours, at least 8 hours, at least 9 hours, at least 10 hours, at least 11 hours, at least 12 hours, at least 13 hours, at least 14 hours, at least 15 hours, at least 16 hours, at least 17 hours, at least 18 hours, at least 19 hours, at least 20 hours, at least 21 hours, at least 22 hours, at least 23 hours, at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 1 year, at least 2 years, at least 3 years, at least 4 years, or at least 5 years.

**[0169]** In some aspects, the reconstituted formulation is stable for at least 10 minutes, at least 15 minutes, at least 20 minutes, at least 25 minutes, at least 30 minutes, at least 35 minutes, at least 40 minutes, at least 45 minutes, at least 50 minutes, at least 55 minutes, at least 1 hour, at least 2 hours, at least 3 hours, at least 4 hours, at least 5 hours, at least 6 hours, at least 7 hours, at least 8 hours, at least 9 hours, at least 10 hours, at least 11 hours, at least 12 hours, at least 13 hours, at least 14 hours, at least 15 hours, at least 16 hours, at least 17 hours, at least 18 hours, at least 19 hours, at least 20 hours, at least 21 hours, at least 22 hours, at least 23 hours, at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9

months, at least 10 months, at least 11 months, at least 1 year, at least 2 years, at least 3 years, at least 4 years, or at least 5 years after storage at 4 °C.

**[0170]** In some aspects, the reconstituted formulation is stable for at least 10 minutes, at least 15 minutes, at least 20 minutes, at least 25 minutes, at least 30 minutes, at least 35 minutes, at least 40 minutes, at least 45 minutes, at least 50 minutes, at least 55 minutes, at least 1 hour, at least 2 hours, at least 3 hours, at least 4 hours, at least 5 hours, at least 6 hours, at least 7 hours, at least 8 hours, at least 9 hours, at least 10 hours, at least 11 hours, at least 12 hours, at least 13 hours, at least 14 hours, at least 15 hours, at least 16 hours, at least 17 hours, at least 18 hours, at least 19 hours, at least 20 hours, at least 21 hours, at least 22 hours, at least 23 hours, at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 1 year, at least 2 years, at least 3 years, at least 4 years, or at least 5 years after storage at room temperature.

**[0171]** In some aspects, the lipid particles and the pharmaceutical compositions disclosed herein are stored at -80 °C, 4 °C, or at room temperature as an aqueous solution formulation, e.g., normal saline or buffered formulation (e.g., Tris buffered). In some aspects, the aqueous solution formulation is stable for at least 10 minutes, at least 15 minutes, at least 20 minutes, at least 25 minutes, at least 30 minutes, at least 35 minutes, at least 40 minutes, at least 45 minutes, at least 50 minutes, at least 55 minutes, at least 1 hour, at least 2 hours, at least 3 hours, at least 4 hours, at least 5 hours, at least 6 hours, at least 7 hours, at least 8 hours, at least 9 hours, at least 10 hours, at least 11 hours, at least 12 hours, at least 13 hours, at least 14 hours, at least 15 hours, at least 16 hours, at least 17 hours, at least 18 hours, at least 19 hours, at least 20 hours, at least 21 hours, at least 22 hours, at least 23 hours, at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 1 year, at least 2 years, at least 3 years, at least 4 years, or at least 5 years.

**[0172]** In some aspects, the aqueous solution formulation is stable for at least 10 minutes, at least 15 minutes, at least 20 minutes, at least 25 minutes, at least 30 minutes, at least 35 minutes, at least 40 minutes, at least 45 minutes, at least 50 minutes, at least 55 minutes, at least 1 hour, at least 2 hours, at least 3 hours, at least 4 hours, at least 5 hours, at least 6 hours, at least 7 hours, at

least 8 hours, at least 9 hours, at least 10 hours, at least 11 hours, at least 12 hours, at least 13 hours, at least 14 hours, at least 15 hours, at least 16 hours, at least 17 hours, at least 18 hours, at least 19 hours, at least 20 hours, at least 21 hours, at least 22 hours, at least 23 hours, at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 1 year, at least 2 years, at least 3 years, at least 4 years, or at least 5 years after storage at -80 °C

**[0173]** In some aspects, the aqueous solution formulation is stable for at least 10 minutes, at least 15 minutes, at least 20 minutes, at least 25 minutes, at least 30 minutes, at least 35 minutes, at least 40 minutes, at least 45 minutes, at least 50 minutes, at least 55 minutes, at least 1 hour, at least 2 hours, at least 3 hours, at least 4 hours, at least 5 hours, at least 6 hours, at least 7 hours, at least 8 hours, at least 9 hours, at least 10 hours, at least 11 hours, at least 12 hours, at least 13 hours, at least 14 hours, at least 15 hours, at least 16 hours, at least 17 hours, at least 18 hours, at least 19 hours, at least 20 hours, at least 21 hours, at least 22 hours, at least 23 hours, at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 1 year, at least 2 years, at least 3 years, at least 4 years, or at least 5 years after storage at 4 °C.

**[0174]** In some aspects, the aqueous solution formulation is stable for at least 10 minutes, at least 15 minutes, at least 20 minutes, at least 25 minutes, at least 30 minutes, at least 35 minutes, at least 40 minutes, at least 45 minutes, at least 50 minutes, at least 55 minutes, at least 1 hour, at least 2 hours, at least 3 hours, at least 4 hours, at least 5 hours, at least 6 hours, at least 7 hours, at least 8 hours, at least 9 hours, at least 10 hours, at least 11 hours, at least 12 hours, at least 13 hours, at least 14 hours, at least 15 hours, at least 16 hours, at least 17 hours, at least 18 hours, at least 19 hours, at least 20 hours, at least 21 hours, at least 22 hours, at least 23 hours, at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 1 year, at least 2 years, at least 3 years, at least 4 years, or at least 5 years after storage at room temperature.



**[0175]** In some aspects, the lipid particles and the pharmaceutical compositions disclosed herein are administered to a subject intranasally, intracranially, intrathecally, intradermally, intratracheally, transdermally, intravenously, intraperitoneally, by injection, by infusion, intramuscularly, or subcutaneously to the subject, e.g., a human. In some aspects, the lipid particles and the pharmaceutical compositions disclosed herein are administered to a subject intravenously, intraperitoneally, by injection, by infusion, intramuscularly, or subcutaneously to the subject, e.g., a human.

**[0176]** Methods of targeting a payload to the pancreas of a subject in need thereof comprising intraperitoneally administering the lipid particles or the pharmaceutical compositions disclosed herein to a subject are contemplated.

**[0177]** Methods of targeting a payload to a lymph node of a subject in need thereof comprising subcutaneously administering the lipid particles or the pharmaceutical compositions disclosed herein to a subject are also contemplated.

**[0178]** Methods of targeting a payload to one or more tumors of a subject in need thereof comprising injecting the lipid particles or the pharmaceutical compositions disclosed herein to a subject are contemplated.

**[0179]** Methods of preventing or delaying the onset or recurrence of a disease or disorder in a subject in need thereof comprising administering a therapeutically effective amount of lipid particles or the pharmaceutical compositions disclosed herein to the subject are also contemplated.

**[0180]** Methods of restoring immune homeostasis in a subject in need thereof comprising administering a therapeutically effective amount of lipid particles or the pharmaceutical compositions disclosed herein to the subject are contemplated. In some aspects, the subject exhibits increased percentage of MOG-specific CD4<sup>+</sup> splenocytes.

**[0181]** Methods of limiting or reducing immunogenicity responses in a subject in need thereof comprising administering a therapeutically effective amount of lipid particles or the pharmaceutical compositions disclosed herein are also contemplated. In some aspects, the subject exhibits increased FR4<sup>+</sup> CD73<sup>+</sup> anergic T cells as a percentage of conventional CD4<sup>+</sup> splenocytes.

**[0182]** Methods of limiting or reducing inflammatory responses in a subject in need thereof comprising administering a therapeutically effective amount of lipid particles or the

pharmaceutical compositions disclosed herein are further contemplated. In some aspects, the subject exhibits one or more of: (i) an increase in Treg cells, (ii) reduction in effector T cells, (iii) reduction in B-cells, (iv) reduction in cytokine production, (v) reduction in activated B cells; (vi) reduction in activated effector T cells; or (vii) any combination thereof.

**[0183]** The lipid particles and the pharmaceutical compositions disclosed herein are useful in treating an autoimmune disorder. Accordingly, the present disclosure provides methods of treating an autoimmune disorder comprising administering a therapeutically effective amount of lipid particles or a pharmaceutical composition disclosed herein to a subject in need thereof. In some aspects, the autoimmune disorder is selected from the group consisting of autoimmune hepatitis, inflammatory bowel disease (including Crohn's disease and ulcerative colitis), nonalcoholic fatty pancreas disease, scleroderma, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, celiac disease, Type 1 diabetes, Guillain-Barré syndrome, Hashimoto's thyroiditis, polymyalgia rheumatica, alopecia areata, fibrosis, psoriasis, pemphigus vulgaris, vitiligo, ankylosing spondylitis, juvenile idiopathic arthritis, psoriatic arthritis, mixed connective tissue disease, neuromyelitis optica, latent autoimmune diabetes in adults ("LADA"), autoimmune thyroid disease, Grave's disease, Addison's disease, autoimmune atrophic gastritis, pernicious anemia, atopic dermatitis, bullous pemphigoid, myasthenia gravis, poly/dermatomyositis), rheumatic fever, primary sclerosing cholangitis, autoimmune uveitis and Behcet's disease), diseases that affect the blood or bone marrow (e.g., autoimmune haemolytic anemia, idiopathic thrombocytopenic purpura, idiopathic leucopenia, Goodpasture's syndrome, autoimmune nephritis, glomerulonephritis, Wegener's granulomatosis, chronic inflammatory demyelinating polyradiculoneuropathy, Sjogren's syndrome, primary biliary cholangitis, Parkinson's disorder, and antiphospholipid syndrome. In some aspects, the autoimmune disorder is autoimmune hepatitis, Type 1 diabetes, or multiple sclerosis.

**[0184]** The lipid particles and the pharmaceutical compositions disclosed herein are also useful in treating a disease that is cancer. Accordingly, the present disclosure provides methods of treating a cancer comprising administering a therapeutically effective amount of lipid particles or a pharmaceutical composition disclosed herein to a subject in need thereof. In some aspects, the cancer is selected from the group consisting of breast cancer, pancreatic cancer, colorectal cancer, endometrial cancer, lung cancer, bladder cancer, kidney cancer, and thyroid cancer.

[0185] The lipid particles and the pharmaceutical compositions disclosed herein are also useful in treating a disease that is an infectious disease. Accordingly, the present disclosure provides methods of treating an infectious disease comprising administering a therapeutically effective amount of lipid particles or a pharmaceutical composition disclosed herein to a subject in need thereof. In some aspects, the present disclosure provides methods of prophylactically preventing an infectious disease comprising administering a therapeutically effective amount of lipid particles or a pharmaceutical composition disclosed herein to a subject in need thereof. In some aspects, the infectious disease is COVID, influenza, monkey pox, Middle East Respiratory Syndrome ("MERS"), Severe Acute Respiratory Syndrome ("SARS"), Human Immunodeficiency Virus ("HIV"), Cytomegalovirus ("CMV"), Epstein-Barr virus ("EBV"), Human Papillomavirus ("HPV"), hepatitis B virus ("HBV"), Herpes simplex virus ("HSV"), Ebola, Lyme disease, bird flu, or rabies.

[0186] In some aspects, the subject is a mammal. In some aspects, the mammal is a human.

[0187] In some aspects, the pharmaceutical compositions comprising the lipid particles described herein can be administered in individual injections as a desired dose depending upon the intended use. The pharmaceutical compositions comprising the lipid particles described herein can be delivered in a desired dosing regimen, for example, administered once, weekly, monthly, or yearly. In some aspects, the pharmaceutical compositions comprising the lipid particles described herein can be administered as a booster inoculation.

## V. METHODS OF MAKING

[0188] Methods of making lipid particles and pharmaceutical compositions described herein are contemplated. Specifically, the methods comprise (a) dissolving one or more lipids (e.g., CHEMS, one or more ionizable lipids, one or more neutral lipids, cholesterol, and/or one or more PEG terminated lipids) in a solvent; (b) adding a payload to the lipid mixture; and (c) mixing.

[0189] In some aspects, the solvent used to dissolve the lipids is an organic solvent. In some aspects, the solvent used to dissolve the lipids is a mixture of organic solvents. Additionally, the solvent can be a mixture of one or more organic solvents and water. In some aspects, the organic solvent is ethanol or butanol (e.g., tert-butanol). In some aspects, absolute ethanol (100%) a 95% ethanol/water mixture, or 90% tert-butanol/water mixture is used.

[0190] In some aspects, the payload is a nucleic acid as described above. In some aspects, the nucleic acid is included in a buffer. Examples of suitable buffers include, citrate, acetate,

phosphate, and 2-(N-morpholino)ethanesulfonate (MES). In some aspects, the buffer has a pH of less than the pKa of the protonatable lipid in the lipid mixture. In some aspects, the pH of the buffer is about 2 to about 8, about 2 to about 7.5, about 2 to about 7, about 2 to about 6.5, about 2 to about 6, about 2 to about 5.5, about 2 to about 5, about 2 to about 4.5, about 2 to about 4, about 2 to about 3.5, about 2 to about 3, about 2 to about 2.5, about 2.5 to about 8, about 2.5 to about 7.5, about 2.5 to about 7, about 2.5 to about 6.5, about 2.5 to about 6, about 2.5 to about 5.5, about 2.5 to about 5, about 2.5 to about 4.5, about 2.5 to about 4, about 2.5 to about 3.5, about 2.5 to about 3, about 3 to about 8, about 3 to about 7.5, about 3 to about 7, about 3 to about 6.5, about 3 to about 6, about 3 to about 5.5, about 3 to about 5, about 3 to about 4.5, about 3 to about 4, about 3 to about 3.5, about 4 to about 8, about 4 to about 7.5, about 4 to about 7, about 4 to about 6.5, about 4 to about 6, about 4 to about 5.5, about 4 to about 5, about 4 to about 4.5, about 4.5 to about 8, about 4.5 to about 7.5, about 4.5 to about 7, about 4.5 to about 6.5, about 4.5 to about 6, about 4.5 to about 5.5, about 4.5 to about 5, about 5 to about 8, about 5 to about 7.5, about 5 to about 7, about 5 to about 6.5, about 5 to about 6, about 5 to about 5.5, about 5.5 to about 8, about 5.5 to about 7.5, about 5.5 to about 7, about 5.5 to about 6.5, about 5.5 to about 6, about 6 to about 8, about 6 to about 7.5, about 6 to about 7, about 6 to about 6.5, about 6.5 to about 8, about 6.5 to about 7.5, about 6.5 to about 7, about 7 to about 8, or about 7.5 to about 8. In some aspects, the pH of the buffer is about 2, about 2.1, about 2.2, about 2.3, about 2.4, about 2.5, about 2.6, about 2.7, about 2.8, about 2.9, about 3, about 3.1, about 3.2, about 3.3, about 3.4, about 3.5, about 3.6, about 3.7, about 3.8, about 3.9, about 4, about 4.1, about 4.2, about 4.3, about 4.4, about 4.5, about 4.6, about 4.7, about 4.8, about 4.9, about 5, about 5.1, about 5.2, about 5.3, about 5.4, about 5.5, about 5.6, about 5.7, about 5.8, about 5.9, about 6, about 6.1, about 6.2, about 6.3, about 6.4, about 6.5, about 6.6, about 6.7, about 6.8, about 6.9, about 7, about 7.1, about 7.2, about 7.3, about 7.4, about 7.5, about 7.6, about 7.7, about 7.8, about 7.9, or about 8.

**[0191]** In some aspects, the buffer is citrate. In some aspects, the pH of the citrate buffer is about 2 to about 8, about 2 to about 7.5, about 2 to about 7, about 2 to about 6.5, about 2 to about 6, about 2 to about 5.5, about 2 to about 5, about 2 to about 4.5, about 2 to about 4, about 2 to about 3.5, about 2 to about 3, about 2 to about 2.5, about 2.5 to about 8, about 2.5 to about 7.5, about 2.5 to about 7, about 2.5 to about 6.5, about 2.5 to about 6, about 2.5 to about 5.5, about 2.5 to about 5, about 2.5 to about 4.5, about 2.5 to about 4, about 2.5 to about 3.5, about 2.5 to about 3, about 3 to about 8, about 3 to about 7.5, about 3 to about 7, about 3 to about 6.5, about 3 to about 6, about 3 to about 5.5, about 3 to about 5, about 3 to about 4.5, about 3 to about 4, about 3 to about 3.5, about 4 to about 8, about 4 to about 7.5, about 4 to about 7, about 4 to about 6.5,

about 4 to about 6, about 4 to about 5.5, about 4 to about 5, about 4 to about 4.5, about 4.5 to about 8, about 4.5 to about 7.5, about 4.5 to about 7, about 4.5 to about 6.5, about 4.5 to about 6, about 4.5 to about 5.5, about 4.5 to about 5, about 5 to about 8, about 5 to about 7.5, about 5 to about 7, about 5 to about 6.5, about 5 to about 6, about 5 to about 5.5, about 5.5 to about 8, about 5.5 to about 7.5, about 5.5 to about 7, about 5.5 to about 6.5, about 5.5 to about 6, about 6 to about 8, about 6 to about 7.5, about 6 to about 7, about 6 to about 6.5, about 6.5 to about 8, about 6.5 to about 7.5, about 6.5 to about 7, about 7 to about 8, or about 7.5 to about 8. In some aspects, the pH of the citrate buffer is about 2, about 2.1, about 2.2, about 2.3, about 2.4, about 2.5, about 2.6, about 2.7, about 2.8, about 2.9, about 3, about 3.1, about 3.2, about 3.3, about 3.4, about 3.5, about 3.6, about 3.7, about 3.8, about 3.9, about 4, about 4.1, about 4.2, about 4.3, about 4.4, about 4.5, about 4.6, about 4.7, about 4.8, about 4.9, about 5, about 5.1, about 5.2, about 5.3, about 5.4, about 5.5, about 5.6, about 5.7, about 5.8, about 5.9, about 6, about 6.1, about 6.2, about 6.3, about 6.4, about 6.5, about 6.6, about 6.7, about 6.8, about 6.9, about 7, about 7.1, about 7.2, about 7.3, about 7.4, about 7.5, about 7.6, about 7.7, about 7.8, about 7.9, or about 8. In some aspects, the buffer is citrate having a pH of about 4.

## EXAMPLES

**[0192]** The invention now being generally described, will be more readily understood by reference to the following examples, which are included merely for purposes of illustration of certain aspects and embodiments of the present invention, and is not intended to limit the invention.

### **Example 1. Process-flow for lipid nanoparticles**

**[0193]** A general process that can be used to manufacture lipid particles described herein is provided as **FIG. 1**. Specifically, the desired lipids (e.g., CHEMS, one or more ionizable lipids, one or more neutral lipids, cholesterol, and/or one or more stealth lipids (e.g., PEG terminated lipids) are dissolved in a solvent (e.g., ethanol). mRNA in citrate buffer (pH 4.0) can be added to the lipid solution and mixed (e.g., using T-junction mixer or microfluidic mixing) to produce the lipid particles. The particles can then be stabilized by diluting with an in-line buffer and two-step pH adjustment (e.g., 20 mM citrate, pH 6.0 followed by 1 M Tris HCl, pH 8.0). The lipid particles can then be dialyzed against Tris buffered saline, pH 7.4, PBS, or HEPES pH 7.4. The lipid particles can then be concentrated (e.g., using 100 kDa concentrators). The lipid particles

can then be subjected to sterile filtration through 0.22 µm polyethersulfone (PES) syringe filters to yield the final lipid particle formulation.

**[0194]** The lipid particles can then be characterized for particle size and size-distribution using e.g., using dynamic light scattering (DLS), surface charge (zeta potential) using e.g., phase-analysis light scattering (PALS), mRNA encapsulation efficiency using e.g., Ribogreen assay or LC-MS, integrity of mRNA in LNPs using capillary electrophoresis and endotoxin burden using e.g., a LAL cartridge-based test (Endosafe® LAL cartridge from Charles River labs).

#### **Example 2. Lipid Particles**

**[0195]** Lipid particles having the components set forth in **Tables 1** and **2** were prepared consistently with the method provided in **Example 1**. The formulations set forth in **Table 3** were prepared on a small-scale

Table 1. Lipid Particles

| Lipid Particle Formulation No. | Composition (molar ratios) mol%   | Molar ratios (mol% of each lipid in the formulation) | Anionic lipid | mol % of anionic lipid | CHEMS % if used | Organ Uptake | Size (nm)    | %encap      |
|--------------------------------|-----------------------------------|------------------------------------------------------|---------------|------------------------|-----------------|--------------|--------------|-------------|
| 1                              | SS-OP/DODG/CHEMS/CHOL/DMG-PEG2k   | 35/15/20/28.5/1.5                                    | CHEMS         | 20                     | 20              | Spleen       | 85.45 ± 3.66 | 88.21± 3.59 |
| 2                              | SS-OP/DOPE/CHEMS/CHOL/DMG-PEG 2k  | 35/15/20/28.5/1.5                                    | CHEMS         | 20                     | 20              | Spleen       | 82.82 ± 1.4  | 91.99       |
| 3                              | SS-OP/DODG/Chol/DMPE-PEG2k/CHEMS  | 35/15/28.5/1.5/20                                    | CHEMS         | 20                     | 20              | Spleen       | 84.75 ± 2.82 | 87.49       |
| 4                              | SS-OP/DOPE/Chol/DMPE-PEG2k/CHEMS  | 35/15/28.5/1.5/20                                    | CHEMS         | 20                     | 20              | Spleen       | 80.86 ± 2.17 | 92.51       |
| 5                              | SS-OP/DODG/Chol/18:1 PEG-PE/CHEMS | 35/15/28.5/1.5/20                                    | CHEMS         | 20                     | 20              | Spleen       | 78.38 ± 0.89 | 84.12       |
| 6                              | SS-OP/DOPE/Chol/18:1 PEG-PE/CHEMS | 35/15/28.5/1.5/20                                    | CHEMS         | 20                     | 20              | Spleen       | 75.42 ± 2.08 | 92.38       |
| 7                              | SS-OP/Chol/DMG-PEG2k/CHEMS        | 40/38.5/1.5/20                                       | CHEMS         | 20                     | 20              | Spleen       | 95.3 ± 1.1   | 85.3        |
| 8                              | SS-OP/Chol/DMG-PEG2k/CHEMS        | 35/38.5/1.5/25                                       | CHEMS         | 25                     | 25              | Spleen       | 89.93 ±2.05  | 89.35       |
| 9                              | SS-OP/Chol/DMG-PEG2k/CHEMS        | 30/38.5/1.5/30                                       | CHEMS         | 30                     | 30              | Spleen       | 89.50 ±1.90  | 84          |
| 10                             | SS-OP/Chol/DMG-PEG2k/CHEMS        | 25/38.5/1.5/35                                       | CHEMS         | 35                     | 35              | Spleen       | 97.92 ±0.63  | 73.07       |

| Lipid Particle Formulation No. | Composition (molar ratios) mol%   | Molar ratios (mol% of each lipid in the formulation) | Anionic lipid | mol % of anionic lipid | CHEMS % if used | Organ Uptake     | Size (nm)     | %encap |
|--------------------------------|-----------------------------------|------------------------------------------------------|---------------|------------------------|-----------------|------------------|---------------|--------|
| 11                             | SS-OP/Chol/DMG-PEG2k/CHEMS        | 40/33.5/1.5/25                                       | CHEMS         | 25                     | 25              | Spleen           | 93.41 ±0.96   | 85.42  |
| 12                             | SS-OP/Chol/DMG-PEG2k/CHEMS        | 40/28.5/1.5/30                                       | CHEMS         | 30                     | 30              | Spleen           | 88.46 ±1.64   | 85.39  |
| 13                             | SS-OP/DODG/Chol/DMG-PEG2k         | 50/10/38.5/1.5                                       |               |                        |                 | Liver            | 122.24 ± 4.28 | 89.04  |
| 14                             | SS-OP/DODG/CHEMS/CHOL/DMG-PEG2k   | 50/10/10/28.5/1.5                                    | CHEMS         | 10                     | 10              | Spleen and liver | 98.55 ± 1.74  | 85.10  |
| 15                             | SS-OP/DODG/Chol/DMG-PEG2k/18:1 PA | 34.6/6.9/27/1.5/30                                   | 18:1 PA       |                        | 0               | Liver and Spleen | 100.24 ± 1.70 | 76.10  |
| 16                             | SS-OP/DODG/Chol/CHEM S/DMG-PEG2k  | 34.6/6.9/27/30/1.5                                   | CHEMS         | 30                     | 30              | Spleen           | 98.69 ± 2.89  | 87.80  |
| 17                             | SS-OP/DODG/Chol/18:1P G/DMG-PEG2k | 34.6/6.9/27/30/1.5                                   | 18:1 PG       | 30                     | 0               | Liver and spleen | 97.12 ± 4.14  | 74.60  |
| 18                             | SS-OP/DOPE/Chol/CHEM S/DMG-PEG2k  | 45/15/28.5/10/1.5                                    | CHEMS         | 28.5                   | 28.5            | Liver and spleen | 95.56 ± 1.74  | 75.40  |
| 19                             | MC3/DODG/Chol/DM G-PEK2k          | 50/10/38.5/1.5                                       |               |                        |                 | Liver            | 113.40 ± 2.32 | 76.30  |
| 20                             | SS-OP/DODG/Chol/DMG-PEG2k         | 40/20/38.5/1.5                                       |               |                        |                 | Liver            | 112.93 ± 2.66 | 80.16  |



| Lipid Particle Formulation No. | Composition (molar ratios) mol%      | Molar ratios (mol% of each lipid in the formulation) | Anionic lipid | mol % of anionic lipid | CHEMS % if used | Organ Uptake                           | Size (nm)     | %encap |
|--------------------------------|--------------------------------------|------------------------------------------------------|---------------|------------------------|-----------------|----------------------------------------|---------------|--------|
| 21                             | SS-<br>OP/DODG/Chol/DMG-PEG2k        | 45/15/38.5/1.5                                       |               |                        |                 | Liver                                  | 121.15 ± 2.25 | 77.04  |
| 22                             | SS-<br>OP/DODG/Chol/DMG-PEG2k        | 48/12/38.5/1.5                                       |               |                        |                 | Liver                                  | 122.50 ± 2.73 | 86.71  |
| 23                             | SS-<br>OP/DOPE/Chol/DMG-PEG2k/CHEMS  | 34.6/6.9/27/1.5/30                                   | CHEMS         | 30                     | 30              | Spleen                                 | 91.06 ± 3.09  | 89.63  |
| 24                             | SS-<br>OP/DSPC/Chol/DMG-PEG2k/CHEMS  | 34.6/6.9/27/1.5/30                                   | CHEMS         | 30                     | 30              | Spleen                                 | 82.83 ± 1.58  | 91.18  |
| 25                             | SS-<br>OP/DODG/CHEMS/CH OL/DMG-PEG2k | 30/10/40/18.5/1.5                                    | CHEMS         | 40                     | 40              | Spleen                                 | 87.08 ± 2.23  | 70.69  |
| 26                             | SS-<br>OP/DODG/CHEMS/CH OL/DMG-PEG2k | 50/8/25/15.5/1.5                                     | CHEMS         | 25                     | 25              | Spleen                                 | 90.86 ± 3.49  | 84.12  |
| 27                             | SS-<br>OP/DODG/CHEMS/CH OL/DMG-PEG2k | 45/7/15/31.5/1.5                                     | CHEMS         | 15                     | 15              | Spleen primarily and some liver uptake | 90.58 ± 2.63  | 84.19  |
| 28                             | SS-<br>OP/DODG/CHEMS/CH OL/DMG-PEG2k | 40/10/10/38.5/1.5                                    | CHEMS         | 10                     | 10              | Spleen and liver                       | 95.17 ± 1.3   | 89.63  |
| 29                             | SS-<br>OP/DODG/CHEMS/CH OL/DMG-PEG2k | 45/15/10/28.5/1.5                                    | CHEMS         | 10                     | 10              | Spleen and liver                       | 102.89 ± 1.0  | 87.29  |

| Lipid Particle Formulation No. | Composition (molar ratios) mol%    | Molar ratios (mol% of each lipid in the formulation) | Anionic lipid | mol % of anionic lipid | CHEMS % if used | Organ Uptake           | Size (nm)    | %encap |
|--------------------------------|------------------------------------|------------------------------------------------------|---------------|------------------------|-----------------|------------------------|--------------|--------|
| 30                             | SS-OP/DODG/CHEMS/CHOL/DMG-PEG2k    | 35/15/25/23.5/1.5                                    | CHEMS         | 25                     | 25              | Spleen                 | 86.36 ± 1.26 | 84.65  |
| 31                             | SS-OP/DODG/CHEMS/CHOL/DMG-PEG2k    | 35/20/20/23.5/1.5                                    | CHEMS         | 20                     | 20              | Poor uptake overall    | 89.76 ± 2.35 | 86.32  |
| 32                             | SS-OP/DOPE/CHEMS/CHOL/DMG-PEG 2k   | 40/20/20/18.5/1.5                                    | CHEMS         | 20                     | 20              | Spleen                 | 91.78 ± 1.91 | 84.44  |
| 33                             | SS-OP/DOPE/CHEMS/CHOL/DMG-PEG 2k   | 34.5/17/21.5/25.5/1.5                                | CHEMS         | 21.5                   | 21.5            | Spleen                 | 85.97 ± 2.32 | 89.46  |
| 34                             | MC3/DODG/Chol/DMG-PEG2k/CHEMS      | 35/15/28.5/1.5/20                                    | CHEMS         | 20                     | 20              | Spleen                 | 88.58 ± 1.52 | 70.28  |
| 35                             | SM-102/DODG/Chol/DMG-PEG2k/CHEMS   | 35/15/28.5/1.5/20                                    | CHEMS         | 20                     | 20              | Spleen                 | 93.78 ± 2.72 | 72.21  |
| 36                             | ALC-0315/DODG/Chol/DMG-PEG2k/CHEMS | 35/15/28.5/1.5/20                                    | CHEMS         | 20                     | 20              | did not encapsulate    | 89.48 ± 3.42 | N/A    |
| 37                             | SS-OP/DOPC/Chol/DMG-PEG2k/CHEMS    | 35/15/28.5/1.5/20                                    | CHEMS         | 20                     | 20              | very low spleen uptake | 83.04 ± 1.77 | 85.14  |
| 38                             | SS-OP/DOCP/Chol/DMG-PEG2k/CHEMS    | 35/15/28.5/1.5/20                                    | CHEMS         | 20                     | 20              | very low spleen uptake | 85.24 ± 2.58 | 79.26  |

| Lipid Particle Formulation No. | Composition (molar ratios) mol%  | Molar ratios (mol% of each lipid in the formulation) | Anionic lipid | mol % of anionic lipid | CHEMS % if used | Organ Uptake                | Size (nm)     | %encap |
|--------------------------------|----------------------------------|------------------------------------------------------|---------------|------------------------|-----------------|-----------------------------|---------------|--------|
| 39                             | SS-OP/DOCPe/Chol/DMG-PEG2k/CHEMS | 35/15/28.5/1.5/20                                    | CHEMS         | 20                     | 20              | low spleen uptake           | 81.13 ± 1.52  | 87.38  |
| 40                             | SS-OP/DOPC/Chol/DMG-PEG2k        | 50/10/38.5/1.5                                       |               |                        |                 | spleen and liver            | 96.29 ± 1.11  | 84.25  |
| 41                             | SS-OP/DOCP/Chol/DMG-PEG2k        | 50/10/38.5/1.5                                       |               |                        |                 | spleen and low liver uptake | 99.84 ± 1.60  | 83.28  |
| 42                             | SS-OP/DOCPe/Chol/DMG-PEG2k       | 50/10/38.5/1.5                                       |               |                        |                 | spleen and low liver uptake | 91.58 ± 2.06  | 90.88  |
| 43                             | SS-OP/DODG/DMG-PEG2k/CHEMS       | 50/10/1.5/38.5                                       | CHEMS         | 38.5                   | 38.5            | low spleen uptake           | 89.98 ± 1.86  | 75.63  |
| 44                             | SS-OP/DODG/Chol/DMPE-PEG2k       | 50/10/1.5/38.5                                       |               |                        |                 | Liver                       | 120.18 ± 1.97 | 77.32  |
| 45                             | SS-OP/DODG/Chol/DSPE-PEG-COOH    | 50/10/38.5/1.5                                       |               |                        |                 | Liver                       | 127.83 ± 3.28 | 82.03  |
| 46                             | SS-OP/DODG/Chol/DMG-PEG2k/CHEMS  | 55/10/23.5/1.5/10                                    | CHEMS         | 10                     | 10              | Liver and Spleen            | 116.19 ± 2.95 | 74.16  |
| 47                             | SS-OP/DODG/Chol/DMG-PEG2k        | 55/10/33.5/1.5                                       |               |                        |                 | Liver                       | 140.06 ± 1.94 | 56.64  |

| Lipid Particle Formulation No. | Composition (molar ratios) mol%         | Molar ratios (mol% of each lipid in the formulation) | Anionic lipid | mol % of anionic lipid | CHEMS % if used | Organ Uptake                | Size (nm)     | %encap |
|--------------------------------|-----------------------------------------|------------------------------------------------------|---------------|------------------------|-----------------|-----------------------------|---------------|--------|
| 48                             | SS-<br>OP/DODG/Chol/DMG-PEG2k           | 60/10/28/2                                           |               |                        |                 | Liver                       | 137.45 ±2.21  | 48.17  |
| 49                             | SS-<br>OP/DODG/Chol/DMPE-PEG2k/CHEMS    | 50/10/28.5/1.5/10                                    | CHEMS         | 10                     | 10              | Liver and Spleen            | 104.77 ±2.15  | 78.32  |
| 50                             | SS-<br>OP/DODG/Chol/18:1 PEG2k-PE       | 50/10/38.5/1.5                                       |               |                        |                 | Liver                       | 118.26 ±2.42  | 81.63  |
| 51                             | SS-<br>OP/DODG/Chol/18:1 PEG2k-PE/CHEMS | 50/10/28.5/1.5/10                                    | CHEMS         | 10                     | 10              | spleen and low liver uptake | 95.96 ±2.10   | 89.79  |
| 52                             | SS-<br>OP/DODG/Chol/DMG-PEG2k           | 60/10/28.5/1.5                                       |               |                        |                 | Liver                       | 155.86 ±2.41  | 56.32  |
| 53                             | SS-<br>OP/DODG/Chol/DMG-PEG2k/CHEMS     | 35/15/29/1/20                                        | CHEMS         | 20                     | 20              | Spleen                      | 103.81 ± 1.47 | 90.03  |
| 54                             | SS-<br>OP/DODG/Chol/DMG-PEG2k/CHEMS     | 35/10/33.5/1.5/20                                    | CHEMS         | 20                     | 20              | Spleen                      | 88.35 ± 2.37  | 91.15  |
| 55                             | SS-<br>OP/DODG/Chol/DMG-PEG2k/CHEMS     | 35/10/28.5/1.5/25                                    | CHEMS         | 25                     | 25              | Spleen                      | 84.65 ± 1.82  | 83.86  |
| 56                             | SS-<br>OP/DOPE/Chol/DMG-PEG2k/CHEMS     | 35/10/33.5/1.5/20                                    | CHEMS         | 20                     | 20              | Spleen                      | 90.25 ± 1.70  | 90.07  |

| Lipid Particle Formulation No. | Composition (molar ratios) mol%         | Molar ratios (mol% of each lipid in the formulation) | Anionic lipid | mol % of anionic lipid | CHEMS % if used | Organ Uptake     | Size (nm)     | %encap |
|--------------------------------|-----------------------------------------|------------------------------------------------------|---------------|------------------------|-----------------|------------------|---------------|--------|
| 57                             | SS-OP/DOPE/Chol/DMPE-PEG2k/CHEMS        | 35/10/33.5/1.5/20                                    | CHEMS         | 20                     | 20              | Spleen           | 86.05 ± 2.15  | 92.67  |
| 58                             | SS-OP/DODG/Chol/DMPE-PEG2k/CHEMS        | 45/10/18.5/1.5/25                                    | CHEMS         | 25                     | 25              | Spleen           | 89.24 ± 2.10  | 77.59  |
| 59                             | SS-OP/DOPE/Chol/DMPE-PEG2k/CHEMS        | 45/10/18.5/1.5/25                                    | CHEMS         | 25                     | 25              | spleen and liver | 95.48 ± 1.95  | 86.33  |
| 60                             | SS-OP/DODG/Chol/DMG-PEG2k/CHEMS         | 45/10/18.5/1.5/25                                    | CHEMS         | 25                     | 25              | spleen and liver | 102.69 ± 1.74 | 82.36  |
| 61                             | SS-OP/DODG/Chol/DMG-PEG2k/CHEMS         | 35/8/30.5/1.5/25                                     | CHEMS         | 25                     | 25              | Spleen           | 96.71 ± 2.20  | 87.48  |
| 62                             | SS-OP/DOPE/Chol/DMPE-PEG2k/CHEMS        | 35/10/28.5/1.5/25                                    | CHEMS         | 25                     | 25              | Spleen           | 98.89 ± 2.26  | 66.21  |
| 63                             | SS-OP/DODG/β-sitosterol/CHEMS/DMG-PEG2k | 35/15/28.5/20/1.5                                    | CHEMS         | 20                     | 20              | Spleen           | 84.23 ± 0.66  | 85.59  |
| 64                             | SS-OP/DOPE/Chol/DMG-PEG2k/CHEMS         | 33.5/15/30/1.5/20                                    | CHEMS         | 20                     | 20              | Spleen           | 84.95 ± 2.34  | 90.38  |
| 65                             | SS-OP/DOPE/β-sitosterol/DMG-PEG2k/CHEMS | 33.5/15/30/1.5/20                                    | CHEMS         | 20                     | 20              | spleen           | 79.47 ± 0.50  | 93.24  |

| Lipid Particle Formulation No. | Composition (molar ratios) mol%                | Molar ratios (mol% of each lipid in the formulation) | Anionic lipid | mol % of anionic lipid | CHEMS % if used | Organ Uptake      | Size (nm)         | %encap |
|--------------------------------|------------------------------------------------|------------------------------------------------------|---------------|------------------------|-----------------|-------------------|-------------------|--------|
| 66                             | SS-OP/DODG/ $\beta$ -sitosterol/DMGPEG2k/CHEMS | 33.5/15/30/1.5/20                                    | CHEMS         | 20                     | 20              | Spleen            | 97.56 $\pm$ 1.66  | 74.13  |
| 67                             | SS-OP/DODG/Chol/DMPE-PEG/CHEMS                 | 33.5/15/30/1.5/20                                    | CHEMS         | 20                     | 20              | spleen            | 86.42 $\pm$ 1.91  | 84.08  |
| 68                             | SS-OP/DODG/Chol/DMG-PEG2k/CHEMS                | 33.5/15/30/1.5/20                                    | CHEMS         | 20                     | 20              | Spleen            | 89.55 $\pm$ 1.44  | 87.43  |
| 69                             | SS-OP/DSPC/Chol/DMG-PEG2k/CHEMS                | 35/15/28.5/1.5/20                                    | CHEMS         | 20                     | 20              | Spleen            | 79.86 $\pm$ 1.10  | 93.41  |
| 70                             | SS-OP/DODG/Chol/DMG-PEG2k/CHEMS                | 30/10/38.5/1.5/20                                    | CHEMS         | 20                     | 20              | Spleen            | 92.60 $\pm$ 1.29  | 87.03  |
| 71                             | SS-OP/DODG/Chol/DMG-PEG2k/CHEMS                | 35/5/38.5/1.5/20                                     | CHEMS         | 20                     | 20              | Spleen            | 96.62 $\pm$ 2.84  | 89.93  |
| 72                             | SS-OP/DOPE/Chol/DMG-PEG2k/CHEMS                | 30/10/38.5/1.5/20                                    | CHEMS         | 20                     | 20              | Spleen            | 87.25 $\pm$ 3.03  | 92.69  |
| 73                             | SS-OP/DOPE/Chol/DMG-PEG2k/CHEMS                | 35/5/38.5/1.5/20                                     | CHEMS         | 20                     | 20              | Spleen            | 112.88 $\pm$ 2.60 | 81.48  |
| 74                             | SS-EC/DOPC/Chol/DSG-PEG2k                      | 60/10/30/3                                           |               |                        |                 | Negligible spleen | 104.5 $\pm$ 2.89  | 44.52  |

| Lipid Particle Formulation No. | Composition (molar ratios) mol% | Molar ratios (mol% of each lipid in the formulation) | Anionic lipid | mol % of anionic lipid | CHEMS % if used | Organ Uptake     | Size (nm)  | %encap |
|--------------------------------|---------------------------------|------------------------------------------------------|---------------|------------------------|-----------------|------------------|------------|--------|
| 75                             | SS-OP/Chol/DMG-PEG2k/CHEMS      | 50/38.5/1.5/10                                       | CHEMS         | 10                     | 10              | Spleen and liver | 92.86±1.95 | 82.56  |
| 76                             | SS-OP/Chol/DMG-PEG2k/CHEMS      | 45/38.5/1.5/15                                       | CHEMS         | 10                     | 10              | Spleen and liver | 94.08±0.86 | 77.72  |
| 77                             | SS-OP/Chol/DMG-PEG2k/CHEMS      | 55/38.5/1.5/5                                        | CHEMS         | 5                      | 5               | Spleen and liver | 94.21±1.27 | 60.12  |
| 78                             | SS-OP/Chol/DMG-PEG2k/CHEMS      | 40/43.5/1.5/15                                       | CHEMS         | 15                     | 15              | Spleen and liver | 90.94±1.72 | 85.40  |

Table 2.

| Lipid Particle Formulation No. | Composition (molar ratios) mol%   | Molar ratios (mol% of each lipid in the formulation) | Anionic lipid | mol % of anionic lipid | CHEMS % if used | Organ Uptake     | Size (nm)   | %encap |
|--------------------------------|-----------------------------------|------------------------------------------------------|---------------|------------------------|-----------------|------------------|-------------|--------|
| 79                             | MC3/DSPC/Chol/DMG-PEG2k           | 50/10/38/2                                           |               |                        |                 | Liver            | 76.1±0.5    | 88.5   |
| 80                             | SS-OP/DSPC/Chol/DMG-PEG2k         | 50/10/38/2                                           |               |                        |                 | Liver            | 93.94±3.44  | 85.97  |
| 81                             | SS-OP/DSPC/Chol/DMG-PEG2k/18:1 PA | 34.6/6.9/26.5/2/30                                   | 18:1 PA       | 30                     |                 | Liver and Spleen | 98.07±2.10  | 77.7   |
| 82                             | MC3/DSDG/Chol/DMG-PEG2k           | 50/10/38/2                                           |               |                        |                 | Liver            | 141.65±3.81 | 80.5   |
| 83                             | SS-OP/DSDG/Chol/DMG-PEG2k         | 50/10/38/2                                           |               |                        |                 | Liver            | 119.80±3.07 | 76.6   |
| 84                             | MC3/DSPC/Chol/DMG-PEG2k/CHEMS     | 34.6/6.9/26.5/2/30                                   | CHEMS         | 30                     | 30              |                  | 78.64±0.68  | 79.5   |
| 85                             | SS-OP/DSDG/Chol/DMG-PEG2k/CHEMS   | 34.6/6.9/26.5/2/30                                   | CHEMS         | 30                     | 30              |                  | 148.43±1.38 | 70.1   |
| 86                             | SS-OP/DSPC/Chol/DSG-PEG2k         | 50/10/35/5                                           |               |                        |                 |                  | 80.74±1.87  | 65.86  |



| Lipid Particle Formulation No. | Composition (molar ratios) mol%           | Molar ratios (mol% of each lipid in the formulation) | Anionic lipid | mol % of anionic lipid | CHEMS % if used | Organ Uptake | Size (nm)   | %encap |
|--------------------------------|-------------------------------------------|------------------------------------------------------|---------------|------------------------|-----------------|--------------|-------------|--------|
| 87                             | SS-OP/DSPC/Chol/MPEG2k-C18                | 50/10/38/2                                           |               |                        |                 |              | 111.72±1.08 | 88.39  |
| 88                             | SS-OP/DSDG/Chol/DMG-PEG2k                 | 50/10/38/2                                           |               |                        |                 |              | 119.80±3.07 | 76.62  |
| 89                             | SS-OP/DSDG/Chol/MPEG2k-C18                | 50/10/38/2                                           |               |                        |                 |              | 137.23±4.82 | 66.47  |
| 90                             | SS-OP/DSPC/Chol/DMG-PEG2k/18:1 PA low PEG | 34.6/6.9/26.5/1.1/30                                 | 18:1 PA       | 30                     |                 |              | 140.02±3.21 | 76.20  |
| 91                             | SS-OP/DSDG/Chol/DMG-PEG2k/18:1 PA         | 34.6/6.9/26.5/2/30                                   | 18:1 PA       | 30                     |                 |              | 112.96±2.40 | 79.08  |
| 92                             | SS-OP/DSPC/Chol/DSG-PEG2k                 | 50/10/35/5                                           |               |                        |                 |              | 82.9±1.32   | 64.3   |
| 93                             | SS-OP/DSDG/Chol/MPEG2k-C18                | 50/10/38.5/1.5                                       |               |                        |                 |              | 123.01±1.43 | 65     |

| Lipid Particle Formulation No. | Composition (molar ratios) mol%                | Molar ratios (mol% of each lipid in the formulation) | Anionic lipid | mol % of anionic lipid | CHEMS % if used | Organ Uptake | Size (nm)   | %encap |
|--------------------------------|------------------------------------------------|------------------------------------------------------|---------------|------------------------|-----------------|--------------|-------------|--------|
| 94                             | SS-OP/DSDG/Chol/MPEG 2k-C18/CHEMS              | 34.6/6.9/27/1.5/30                                   | CHEMS         | 30                     | 30              |              | 132.40±3.13 | 73.6   |
| 95                             | SS-OP/DSDG/Chol/MPEG 2k-C18/18:1 PA            | 34.6/6.9/27/1.5/30                                   | 18:1 PA       | 30                     |                 |              | 147.62±3.27 | 70.9   |
| 96                             | SM-102/DSDG/Chol/MPEG 2k-C18                   | 50/10/38.5/1.5                                       |               |                        |                 |              | 123.17±2.25 | 74.3   |
| 97                             | SM-102/DSDG/Chol/MPEG 2k-C18 (10:1) lipid:mRNA | 50/10/38.5/1.5                                       |               |                        |                 |              | 126.60±2.24 | 57.4   |
| 98                             | SM-102/DSDG/Chol/MPEG 2k-C18                   | 50/10/38.5/1.5                                       |               |                        |                 |              | 124.19±2.36 | 89.2   |
| 99                             | SM-102/DODG/Chol/MPEG 2k-C18                   | 50/10/38.5/1.5                                       |               |                        |                 |              | 128.24±3.44 | 67.3   |
| 100                            | SM-102/DSDG/Chol/MPEG 2k-C18                   | 50/10/38.5/1.5                                       |               |                        |                 |              | 125.60±4.37 | 86.7   |

| Lipid Particle Formulation No. | Composition (molar ratios) mol%   | Molar ratios (mol% of each lipid in the formulation) | Anionic lipid | mol % of anionic lipid | CHEMS % if used | Organ Uptake     | Size (nm)   | %encap |
|--------------------------------|-----------------------------------|------------------------------------------------------|---------------|------------------------|-----------------|------------------|-------------|--------|
| 101                            | SM-102/DSDG/Chol/MPEG 2k-C16      | 50/10/38.5/1.5                                       |               |                        |                 |                  | 105.76±1.86 | 69.9   |
| 102                            | SS-OP/DSPC/Chol/MPEG2 k-C18       | 50/10/38.5/1.5                                       |               |                        |                 |                  | 117.28±2.42 | 93.06  |
| 103                            | SS-OP/DODG/Chol/MPEG 2k-C18       | 50/10/38.5/1.5                                       |               |                        |                 |                  | 121.49±4.52 | 79.9   |
| 104                            | SS-OP/DODG/Chol/DMG-PEG2k/18:1 PA | 34.6/6.9/27/1.5/30                                   | 18:1 PA       | 30                     |                 | Liver and spleen | 102.93±1.37 | 80.7   |
| 105                            | SS-OP/DODG/Chol/DMG-PEG2k         | 50/10/38/2                                           |               |                        |                 | Liver            | 116.65±1.35 | 76.7   |
| 106                            | SS-OP/DODG/Chol/MPEG 2k-C18       | 50/10/38/2                                           |               |                        |                 | Liver            | 107.55±1.70 | 85.6   |

Table 3

| Lipid Particle Formulation No. | Composition                       | Molar ratios (mol%) | Size       | PDI          | Zeta potential | %encapsulation |
|--------------------------------|-----------------------------------|---------------------|------------|--------------|----------------|----------------|
| 80                             | SS-OP/DSPC/Chol/DMG-PEG2k         | 50/10/38/2          | 89.8± 3.9  | 0.157± 0.033 | -3.5±7.8       | 95.2           |
| 107                            | SS-OP/DOPE/Chol/DMG-PEG2k         | 50/10/38/2          | 80.1± 3.5  | 0.160± 0.049 | 1.2± 6.5       | 94.2           |
| 83                             | SS-OP/DSDG/Chol/DMG-PEG2k         | 50/10/38/2          | 85.5± 1.7  | 0.173± 0.021 | -3.3± 5.3      | 93.2           |
| 108                            | SS-OP/DSPC/Chol/DMG-PEG2k/CHEMS   | 30/10/38/2/20       | 77.0± 2.4  | 0.204± 0.012 | -10.8± 0.5     | 94             |
| 109                            | SS-OP/DOPE/Chol/DMG-PEG2k/CHEMS   | 30/10/38/2/20       | 74.6± 2.2  | 0.189± 0.021 | -4.7± 1.1      | 92.6           |
| 110                            | SS-OP/DSDG/Chol/DMG-PEG2k/CHEMS   | 30/10/38/2/20       | 81.3± 4.3  | 0.183± 0.036 | -8.3± 1.5      | 93.4           |
| 111                            | SS-OP/DOPC/CHOL/DMG-PEG2k         | 50/10/38/2          | 95.8±2.3   | 0.159±0.033  | -7.5±3.12      | 92.4           |
| 112                            | SSOP/DOPC/CHOL/DMGPEG2k/CHEMS     | 30/10/38/2/20       | 84.7±1.1   | 0.193±0.024  | -8.9±3.2       | 91.1           |
| 81                             | SS-OP/DSPC/CHOL/18:1 PA/DMG-PEG2k | 34.6/6.9/26.5/30/2  | 117.4± 2.5 | 0.125± 0.064 | -8.5± 2.2      | 88.3           |
| 82                             | SS-OP/DOPE/CHOL/18:1 PA/DMG-PEG2k | 34.6/6.9/26.5/30/2  | 108.7±2.5  | 0.162±0.043  | -7.3±1.9       | 84.8           |
| 91                             | SS-OP/DSDG/CHOL/18:1 PA/DMG-PEG2k | 34.6/6.9/26.5/30/2  | 102.6±1.8  | 0.193± 0.035 | -8.8±2.4       | 94.3           |

|     |                                         |                        |             |              |                |      |
|-----|-----------------------------------------|------------------------|-------------|--------------|----------------|------|
| 113 | SS-OP/DOPC/CHOL/18:1<br>PA/DMG-PEG2k    | 34.6/6.9/26.5/30/<br>2 | 99.6± 4.2   | 0.211± 0.027 | -10.9±<br>2.6  | 85.4 |
| 114 | SS-<br>OP/DSPC/CHOL/CHEMS/DMG<br>-PEG2k | 34.6/6.9/26.5/30/<br>2 | 81.8± 2.1   | 0.202±0.025  | 0.5±1.54       | 89.9 |
| 115 | SS-<br>OP/DOPE/CHOL/CHEMS/DMG<br>-PEG2k | 34.6/6.9/26.5/30/<br>2 | 74.7±0.70   | 0.148±0.068  | 0.6±1.40       | 94   |
| 85  | SS-<br>OP/DSDG/CHOL/CHEMS/DMG<br>-PEG2k | 34.6/6.9/26.5/30/<br>2 | 75.8±1.4    | 0.153±0.055  | 0.1±0.9        | 96.2 |
| 116 | SS-<br>OP/DOPC/CHOL/CHEMS/DMG<br>-PEG2k | 34.6/6.9/26.5/30/<br>2 | 82.8±2.6    | 0.148±0.032  | -1.9±1.3       | 92.6 |
| 117 | SS-OP/DSPC/CHOL/DMG-<br>PEG2k/18:1 PA   | 30/8/30/2/30           | 97.95±2.43  | 0.117±0.055  | -<br>0.42±0.61 | 89.5 |
| 118 | SS-OP/DSPC/CHOL/DMG-<br>PEG2k/18:1 PA   | 25/8/35/2/30           | 97.29±1.91  | 0.104±0.052  | -<br>0.77±2.28 | 78.7 |
| 119 | SS-OP/DSPC/CHOL/DMG-<br>PEG2k/18:1 PA   | 20/10/38/2/30          | 79.31±0.91  | 0.139±0.043  | 0.01±0.75      | 70.1 |
| 120 | SS-OP/DSPC/CHOL/DMG-<br>PEG2k/18:1 PA   | 25/8/30/2/35           | 105.63±1.49 | 0.150±0.025  | 0.38±9.63      | 40.3 |
| 121 | SS-OP/DSPC/CHOL/DMG-<br>PEG2k/18:1 PA   | 30/7/26/2/35           | 93.27±7.39  | 0.196±0.120  | -<br>1.49±2.64 | 39.7 |
| 122 | SS-OP/DSPC/CHOL/DMG-<br>PEG2k/18:1 PA   | 28/7/30/2/33           | 72.16±0.59  | 0.158±0.039  | -<br>0.08±0.95 | <1%  |

|     |                                   |                    |             |              |                |       |
|-----|-----------------------------------|--------------------|-------------|--------------|----------------|-------|
| 123 | SS-OP/DSPC/CHOL/DMG-PEG2k/18:1 PA | 30/10/38/2/20      | 78.24±4.10  | 0.154±0.070  | -<br>1.57±1.51 | 95.1  |
| 124 | SS-OP/DSPC/CHOL/DMG-PEG2k/18:1 PA | 27/8/30/2/33       | 85.08±3.98  | 0.241±0.022  | 0.06±1.78      | <1%   |
| 89  | SS-OP/DSDG/Chol/MPEG-C18          | 50/10/38/2         | 108.75±2.49 | 0.168±0.048  | 0.81±0.49      | 82.8  |
| 105 | SS-OP/DODG/Chol/DMG-PEG2k         | 50/10/38/2         | 100.65±1.04 | 0.102±0.049  | -<br>0.12±0.29 | 89.8  |
| 87  | SS-OP/DSPC/Chol/DSG-PEG2k         | 50/10/35/5         | 63.37±0.87  | 0.164±0.026  | 0.21±1.51      | 93.3  |
| 125 | SS-OP/DSPC/Chol/DMG-PEG2k/18:1 PG | 30/10/38/2/20      | 93.68±1.98  | 0.110±0.039  | -<br>0.26±0.35 | 88.8  |
| 91  | SS-OP/DSPC/Chol/DMG-PEG2k/18:1 PG | 34.6/6.9/26.5/2/30 | 105.54±1.90 | 0.095±0.036  | 0.46±2.33      | 83.5  |
| 41  | SS-OP/DOCP/Chol/DMG-PEG2k         | 50/10/38.5/1.5     | 126.93±1.95 | 0.269±0.017  | -<br>1.35±3.30 | 85.5  |
| 42  | SS-OP/DOCPe/Chol/DMG-PEG2k        | 50/10/38.5/1.5     | 140.05±6.22 | 0.239±0.027  | 1.02±0.54      | 92.7  |
| 126 | SS-OP/DODG/Chol/Bis DSPE-PEG2k    | 50/10/38.5/1.5     | 125.86±4.88 | 0.162±0.046  | 1.68±6.99      | 85.3  |
| 50  | SS-OP/DODG/Chol/18:1 PEG2k PE     | 50/10/38.5/1.5     | 123.08±3.08 | 0.131±0.043  | 0.08±0.75      | 89.6  |
| 127 | SS-OP/DODG/Chol/DMPE-PEG2k        | 50/10/38.5/1.5     | 116.95±3.82 | 0.104±0.063  | 0.57±0.89      | 89.2  |
| 45  | SS-OP/DODG/Chol/DSPE-PEG2k-COOH   | 50/10/38.5/1.5     | 120.13±7.52 | 0.117±0.057  | -<br>0.22±0.64 | 86.5  |
| 128 | KC2/DSPC/Chol/DMG-PEG2k           | 50/10/38/2         | 84.3± 1.5   | 0.132± 0.040 | -<br>0.20±0.88 | 87.72 |

|     |                                 |                        |           |              |                     |       |
|-----|---------------------------------|------------------------|-----------|--------------|---------------------|-------|
| 129 | KC2/DOPE/Chol/DMG-PEG2k         | 50/10/38/2             | 85.7±1.8  | 0.142±0.020  | 0.01±0.46           | 92.15 |
| 130 | KC2/DSDG/Chol/DMG-PEG2k         | 50/10/38/2             | 87.6±2.1  | 0.115±0.024  | -<br>1.37±4.84      | 90.52 |
| 131 | KC2/DOPC/Chol/DMG-PEG2k         | 50/10/38/2             | 93.6± 1.9 | 0.092±0.032  | -<br>0.62±1.60      | 93.13 |
| 132 | KC2/DSPC/Chol/DMG-PEG2k/CHEMS   | 30/10/38/2/20          | 80.3± 4.2 | 0.223±0.014  | -<br>1.1±1.1.7<br>5 | 84.36 |
| 133 | KC2/DOPE/Chol/DMG-PEG2k/CHEMS   | 30/10/38/2/20          | 86.8± 3.7 | 0.170± 0.029 | -<br>0.99±1.12      | 84.04 |
| 134 | KC2/DSDG/Chol/DMG-PEG2k/CHEMS   | 30/10/38/2/20          | 86.2±5.2  | 0.207±0.019  | 1.56±0.46           | 92.09 |
| 135 | KC2/DOPC/Chol/DMG-PEG2k/CHEMS   | 30/10/38/2/20          | 90.9±1.4  | 0.138±0.066  | 0.24±0.72           | 88.09 |
| 136 | KC2/DSPC/Chol/DMG-PEG2k/18:1 PA | 34.6/6.9/26.5/2/3<br>0 | 135.3±3.4 | 0.189±0.020  | -<br>0.56±0.60      | 11.97 |
| 137 | KC2/DOPE/Chol/DMG-PEG2k/18:1PA  | 34.6/6.9/26.5/2/3<br>0 | 135.1±4.2 | 0.123±0.057  | 0.54±1.04           | 9.23  |
| 138 | KC2/DSDG/Chol/DMG-PEG2k/18:1 PA | 34.6/6.9/26.5/2/3<br>0 | 136.5±2.8 | 0.135±0.017  | 1.24±1.29           | 8.98  |
| 139 | KC2/DOPC/Chol/DMG-PEG2k/18:1 PA | 34.6/6.9/26.5/2/3<br>0 | 135.2±5.9 | 0.134±0.058  | -<br>0.17±1.65      | 6.23  |
| 140 | MC3/DSPC/Chol/CHEMS/DMG-PEG2k   | 30/10/37/21/2          | 73.9± 3.1 | 0.225± 0.019 | -11.5±<br>2.4       | 86    |
| 84  | MC3/DSPC/Chol/DMG-PEG2k/CHEMS   | 34.6/6.9/26.5/2/3<br>0 | 80.3±1.0  | 0.199±0.030  | 0.73±0.84           | 86.5  |

|     |                                         |                        |             |             |                |       |
|-----|-----------------------------------------|------------------------|-------------|-------------|----------------|-------|
| 141 | MC3/DOPE/Chol/DMG-PEG2k/CHEMS           | 34.6/6.9/26.5/2/3<br>0 | 78.8±1.4    | 0.124±0.042 | -<br>0.51±0.29 | 84.7  |
| 142 | MC3/DSDG/Chol/DMG-PEG2k/CHEMS           | 34.6/6.9/26.5/2/3<br>0 | 77.0±2.7    | 0.155±0.043 | -<br>0.25±0.75 | 91.3  |
| 143 | MC3/DOPE/Chol/DMG-PEG2k/CHEMS           | 34.6/6.9/26.5/2/3<br>0 | 79.9±1.9    | 0.157±0.035 | 0.13±0.75      | 85.9  |
| 144 | MC3/DSPC/Chol/DMG-PEG2k/CHEMS           | 30/10/38/2/20          | 81.1±2.2    | 0.225±0.013 | 0.18±0.45      | 86.2  |
| 145 | MC3/DOPE/Chol/DMG-PEG2k/CHEMS           | 30/10/38/2/20          | 87.3±1.2    | 0.147±0.056 | 0.33±2.41      | 86.9  |
| 146 | MC3/DSDG/Chol/DMG-PEG2k/CHEMS           | 30/10/38/2/20          | 79.4±2.2    | 0.197±0.031 | -0.04±<br>1.30 | 86.5  |
| 147 | MC3/DOPC/Chol/DMG-PEG2k/CHEMS           | 30/10/38/2/20          | 87.5±3.0    | 0.187±0.020 | -<br>0.23±0.97 | 88.7  |
| 82  | MC3/DSDG/Chol/DMG-PEG2k                 | 50/10/38/2             | 94.04±4.69  | 0.106±0.034 | -<br>1.50±1.44 | 89.48 |
| 148 | MC3/DSPC/Chol/DSG-PEG2k                 | 50/10/35/5             | 60.59±1.97  | 0.173±0.036 | 0.64±1.03      | 89.25 |
| 149 | MC3/DSPC/Chol/DMG-PEG2k/18:1 PG         | 30/10/38/2/20          | 98.53±1.87  | 0.167±0.024 | -<br>1.30±1.96 | 84.56 |
| 150 | MC3/DSPC/Chol/DMG-PEG2k/18:1 PG         | 34.6/6.9/26.5/2/3<br>0 | 118.37±2.67 | 0.124±0.027 | 1.27±0.51      | 72.00 |
| 151 | MC3/DOPE/Chol/DMG-PEG2k<br>(50/10/38/2) | 50/10/38/2             | 73.35±1.36  | 0.133±0.014 | 0.08±1.32      | 93.3  |
| 152 | MC3/DSDG/Chol/C18-MPEG<br>(50/10/38/2)  | 50/10/38/2             | 119.30±1.11 | 0.169±0.021 | 0.74±0.42      | 89.48 |



|     |                                         |                        |            |             |   |           |       |
|-----|-----------------------------------------|------------------------|------------|-------------|---|-----------|-------|
| 153 | MC3/DODG/Chol/DMG-PEG2k<br>(50/10/38/2) | 50/10/38/2             | 85.84±1.99 | 0.129±0.041 | - | 0.32±1.15 | 89.82 |
| 79  | MC3/DSPC/Chol/DMG-PEG2k<br>(50/10/38/2) | 50/10/38/2             | 78.27±2.11 | 0.143±0.041 |   | 0.92±1.33 | 91.48 |
| 154 | SM-102/DSPC/Chol/DMG-<br>PEG2k          | 50/10/38/2             | 84.3±1.5   | 0.211±0.030 |   | -0.2±0.9  | 92.7  |
| 155 | SM-102/DSDG/Chol/DMG-<br>PEG2k          | 50/10/38/2             | 83.4±1.2   | 0.116±0.023 |   | -1.1±0.6  | 90.6  |
| 156 | SM-102/DOPE/Chol/DMG-<br>PEG2k          | 50/10/38/2             | 75.8±2.1   | 0.104±0.056 |   | -1.1±2.3  | 98    |
| 157 | SM-102/DODG/Chol/DMG-<br>PEG2k          | 50/10/38/2             | 96.0±1.6   | 0.154±0.038 |   | 1.4±1.5   | 89.7  |
| 158 | SM-102/DSPC/Chol/DMG-<br>PEG2k/18:1 PA  | 34.6/6.9/26.5/2/3<br>0 | 145.4±5.7  | 0.241±0.020 |   | 0.4±1.9   | <1%   |
| 159 | SM-102/DSDG/Chol/DMG-<br>PEG2k/18:1 PA  | 34.6/6.9/26.5/2/3<br>0 | 126.3±2.7  | 0.232±0.022 |   | 0.4±1.9   | <1%   |
| 160 | SM-102/DSDG/Chol/s.c PEG                | 50/10/38/2             | 132.9±2.5  | 0.204±0.025 |   | -0.1±1.5  | 92.39 |
| 161 | SM-102/DSPC/Chol/s.c PEG                | 50/10/38/2             | 100.2±2.8  | 0.195±0.034 |   | -0.7±3.6  | 90.57 |
| 162 | SM-102/DOPE/Chol/s.c PEG                | 50/10/38.5/1.5         | 136.8±2.8  | 0.184±0.027 |   | -0.01±1.3 | 96.4  |
| 163 | SM-102/DODGChol/s.c PEG                 | 50/10/38.5/1.5         | 132.4±1.9  | 0.100±0.063 |   | 0.12±1.0  | 92.4  |
| 164 | SM-102/DSDG/Chol/s.c. PEG               | 50/10/38.5/1.5         | 123.4±3.6  | 0.083±0.045 |   | 0.9±0.9   | 97.3  |
| 165 | SM-102/DODG/Chol/DMG-<br>PEG2k/18:1 PA  | 34.6/6.9/27/1.5/3<br>0 | 134.0±1.6  | 0.180±0.018 |   | -0.03±0.5 | <1%   |
| 166 | SM-102/DSDG/Chol/s.c.<br>PEG/CHEMS      | 34.6/6.9/27/1.5/3<br>0 | 117.3±2.5  | 0.124±9.031 |   | -0.3±0.5  | 69.27 |

|     |                                       |                        |             |             |                |       |
|-----|---------------------------------------|------------------------|-------------|-------------|----------------|-------|
| 167 | SM-102/DSDG/Chol/C16 s.c<br>PEG       | 50/10/38.5/1.5         | 131.0±3.9   | 0.186±0.029 | -0.8±0.83      | 94    |
| 168 | ALC/DSPC/Chol/DMG-PEG2k               | 50/10/38/2             | 81.26±1.72  | 0.229±0.014 | -<br>0.09±1.38 | 89.8  |
| 169 | ALC/DSDG/Chol/DMG-PEG2k               | 50/10/38/2             | 115.11±2.21 | 0.204±0.022 | 1.33±1.28      | 79.3  |
| 170 | ALC/DODG/Chol/DMG-PEG2k               | 50/10/38/2             | 81.82±2.51  | 0.087±0.065 | 0.69±2.67      | 81    |
| 171 | ALC/DSPC/Chol/MPEG2k-C18              | 50/10/38/2             | 86.28±2.24  | 0.169±0.036 | 0.79±5.59      | 92.24 |
| 172 | ALC/DSDG/Chol/MPEG2k-C18              | 50/10/38/2             | 167.25±4.09 | 0.139±0.051 | 0.21±0.96      | <1%   |
| 173 | ALC/DSPC/Chol/DMG-<br>PEG2k/18:1 PA   | 34.6/6.9/26.5/2/3<br>0 | 152.32±4.02 | 0.283±0.029 | 1.96±2.72      | <1%   |
| 174 | ALC/DSDG/Chol/DMG-<br>PEG2k/18:1 PA   | 34.6/6.9/26.5/2/3<br>0 | 153.02±4.28 | 0.237±0.024 | 0.85±0.96      | <1%   |
| 175 | C12-200/DSPC/Chol/DMG-<br>PEG2k       | 50/10/38/2             | 123.75±5.50 | 0.157±0.038 | 0.70±2.39      | 87.79 |
| 176 | C12-200/DOPE/Chol/DMG-<br>PEG2k       | 50/10/38/3             | 85.77±1.18  | 0.063±0.020 | 0.88±2.14      | 73.27 |
| 177 | C12-200/DSDG/Chol/DMG-<br>PEG2k       | 50/10/38/4             | 91.00±3.30  | 0.114±0.038 | -<br>0.15±1.52 | <1%   |
| 178 | C12-200/DOPC/Chol/DMG-<br>PEG2k       | 50/10/38/5             | 94.63±2.75  | 0.116±0.033 | -<br>0.09±2.52 | 90.06 |
| 179 | C12-200/DSPC/Chol/DMG-<br>PEG2k/CHEMS | 30/10/38/2/20          | 74.49±5.81  | 0.165±0.052 | -<br>0.66±1.14 | 74.45 |
| 180 | C12-200/DOPE/Chol/DMG-<br>PEG2k/CHEMS | 30/10/38/2/21          | 79.20±1.35  | 0.113±0.027 | 0.31±1.32      | 62.77 |

|     |                                         |                        |            |             |                |       |
|-----|-----------------------------------------|------------------------|------------|-------------|----------------|-------|
| 181 | C12-200/DSDG/Chol/DMG-<br>PEG2k/CHEMS   | 30/10/38/2/22          | 84.58±2.29 | 0.147±0.028 | 0.58±1.74      | 30.9  |
| 182 | C12-200/DOPC/Chol/DMG-<br>PEG2k/CHEMS   | 30/10/38/2/23          | 92.08±3.89 | 0.161±0.030 | -<br>0.81±1.44 | 75.11 |
| 183 | C12-200/DSPC/Chol/DMG-<br>PEG2k/18:1 PA | 34.6/6.9/26.5/2/3<br>0 | 75.47±0.81 | 0.124±0.049 | 0.42±1.05      | <1%   |
| 184 | C12-200/DOPE/Chol/DMG-<br>PEG2k/18:1 PA | 34.6/6.9/26.5/2/3<br>0 | 69.80±1.02 | 0.122±0.023 | 0.78±0.93      | <1%   |
| 185 | C12-200/DSDG/Chol/DMG-<br>PEG2k/18:1 PA | 34.6/6.9/26.5/2/3<br>0 | 70.96±1.02 | 0.080±0.048 | -<br>0.42±0.73 | <1%   |
| 186 | C12-200/DOPC/Chol/DMG-<br>PEG2k/18:1 PA | 34.6/6.9/26.5/2/3<br>0 | 75.69±1.76 | 0.086±0.033 | 1.32±1.32      | <1%   |

**Example 3. BLI Protocol**

[0196] 6-8 week old C57BL/6J mice were used for this study. Prior to injections with the particular Lipid Particle Formulation to be tested, the mouse fur was clipped along the left lateral aspect of the abdomen up to the spine to highlight both splenic and liver expression fields. The mice were injected intravenously via tail vein with 100  $\mu$ L of the Lipid Particle Formulation to be tested. The Lipid Particle Formulation to be tested contains an equivalent to 20  $\mu$ g firefly luciferase mRNA. After 6 h, the mice were administered 200  $\mu$ L D-luciferin (3 mg/mouse) via intraperitoneal injection (i.p), anaesthetized using isoflurane and imaged within 10 min using a LICOR Pearl imager. Images were obtained in right lateral recumbency under 3% isoflurane for anaesthetic maintenance. LICOR settings: BLI and White channels only, 85um resolution, focus at 0. The BLI values were determined using the Image Studio software, version 5.2.5 from LICOR. Regions of interest were drawn around organs (e.g., spleen or liver) using software tools and the corresponding BLI signal was calculated by the software after subtracting background values. Data were reported as spleen BLI values, liver BLI values and spleen to liver ratio (i.e. the ratio obtained by dividing the BLI value in spleen by the BLI value in the liver of the same animal).

**Example 4. DOPE v. DODG in Lipid Particles**

[0197] The spleen to liver ratio of each of Lipid Particle Formulation Nos. 1, 3-6, and 53-55 (in PBS) and a PBS control were determined using the BLI protocol described in **Example 3**. The results are provided in **FIGs. 2A-2C**.

**Example 5. Effects of CHEMS on Spleen Targeting**

[0198] The spleen to liver ratio of each of Lipid Particle Formulation Nos. 1, 2, and 30-33 (in PBS) and PBS control were determined using the BLI protocol described in **Example 3**. The results are provided in **FIGs. 3A-3C**.

[0199] The spleen to liver ratio of each of Lipid Particle Formulation Nos. 1, 13, and 43-48 (in PBS) and PBS control were determined using the BLI protocol described in **Example 3**. The results are provided in **FIGs. 4A-4C**.

[0200] Additionally, the spleen to liver ratio of each of Lipid Particle Formulation Nos. 13, 15, 16, and 20-24 (in PBS) and PBS control were determined using the BLI protocol described in **Example 3**. The results are provided in **FIGs. 5A-5C**.

[0201] BLI values for each of Lipid Particle Formulation Nos. 1, 14, 16, and 25-29 (in PBS) and PBS in the spleen of mice are provided in **FIG. 6**. The spleen to liver ratio of Lipid Particle Formulation Nos. 1, 16, and 23-26 (in PBS) in mice are provided as **Table 4**. The BLI values were determined using the protocol described in **Example 3**.

**Table 4. Spleen to Liver Ratios**

| Lipid Particle Formulation No. | Helper lipid | Spleen BLI | S/L ratio |
|--------------------------------|--------------|------------|-----------|
| 1*                             | DODG         | 16.62      | 7.49      |
| 21                             | DOPE         | 14.36      | 5.78      |
| 37                             | DOPC         | 1.87       | 1.44      |
| 38                             | DOCP         | 0.78       | 0.08      |
| 39                             | DOCPe        | 4.54       | 4.96      |
| 69                             | DSPC         | 0.42       | 2.37      |

**Example 6. Effect of neutral lipids**

[0202] The spleen to liver ratio of each of Lipid Particle Formulation Nos. 1, 13, and 37-42 (in PBS) and PBS control were determined using the protocol described in **Example 3**. The results are provided in **FIGs. 7A-7C**. Using the same lipid molar ratios, the choice of neutral lipid impacts spleen signal. DODG and DOPE target the payload to the spleen better than DOPC, DSPC, or DOCP.

**Example 7. Dual-targeted formulations (spleen and liver)**

[0203] The spleen to liver ratio of each of Lipid Particle Formulation Nos. 1, 14, 16, and 25-29 (in PBS) and PBS control were determined using the protocol described in **Example 3**. The results are provided in **FIGs. 8A-8C**.

**Example 8. Four-Component System Targeting Spleen**

[0204] The BLI values for spleen in mice were determined for Lipid Particle Formulation Nos. 7 (four-component) and Lipid Particle Formulation Nos. 14 (intravenous administration), 1 (pharmaceutical composition containing Tris intravenously administered), and 1 (lipid particles frozen) each of which contains five components and is reported in **FIGs. 9A-9C**. The BLI values were determined using the imaging protocol and software as described in **Example 3**.

#### **Example 9. Intraperitoneal delivery**

[0205] 6-8 week old C57BL/6J mice were injected intravenously or intraperitoneally with 100  $\mu$ L Lipid Particle Formulation No. 1 or 14 (in TBS) containing firefly luciferase mRNA equivalent to 20  $\mu$ g. Six hours after the injections, the mice were injected with 200  $\mu$ L of D-luciferin (3 mg/mouse) administered i.p. After 2 min to allow for the distribution of luciferin, mice were put in a CO<sub>2</sub> chamber and euthanized. After necropsy, the organs of interest (spleen, liver, and pancreas) were excised and placed individually into wells of a 24 well-plate. Enough luciferin (300  $\mu$ g/mL) was added to cover the tissues (~0.5 mL/well). The organs were transferred to solution basins, with divisions between chambers to avoid spillover of luminescence from larger organs. Additional luciferin solution was added to the chambers during imaging if needed. The organs were imaged using the LICOR Pearl imager. Images of individual organs were analyzed using the Image studio software (version 5.2.5) from LICOR. Regions of interest were drawn around individual organs using software tools. Organs isolated from a PBS injected mouse were used as background control. The resulting images from the software and BLI values were reported for organs. The results are provided in **FIGs. 15A-15E**.

[0206] As shown in **FIGs. 15B** and **15E**, the luciferase was detected in the pancreas after Lipid Particle Formulation Nos. 1 and 14 were each intraperitoneally administered. However, as shown in **FIGs. 15A, 15C, and 15D**, the luciferase was detected in the liver and/or spleen when the same Lipid Particle Formulations were intravenously administered.

#### **Example 10. Subcutaneous delivery of Lipid Particle Formulation Nos. 1, 13, and 14**

[0207] 6-8 week old C57BL/6J mice were used for this study. Prior to injections with the Lipid Particle Formulation No. 1, 13, or 14 (in PBS), the mouse fur was clipped along the left lateral aspect of the abdomen up to the spine to highlight both splenic and liver expression fields. The mice were injected subcutaneously in the left flank with 100  $\mu$ L of Lipid Particle Formulation No. 1, 13, or 14 (in PBS) containing an equivalent to 20  $\mu$ g firefly luciferase mRNA. After 16 h, 24 h and 48 h respectively, the mice were administered 200  $\mu$ L D-luciferin (3

mg/mouse) via intraperitoneal injection (i.p), anaesthetized using isofluorane and imaged within 10 min using a LICOR Pearl imager. Images were obtained in right lateral recumbency under 3% isofluorane for anaesthetic maintenance. LICOR settings: BLI and White channels only, 85um resolution, focus at 0. Lymph nodes (“LN”) were imaged only for the 24 h time point. For LN imaging, mice were euthanized using CO<sub>2</sub>, inguinal lymph nodes were isolated, placed in weigh boats and imaged using the LICOR Pearl imaging system. The BLI values were determined using the Image Studio software, version 5.2.5 from LICOR. For whole body images of animals or for isolated inguinal LN, regions of interest (“ROIs”) were drawn around organs (e.g. liver, site of injection or LN) using software tools and the corresponding BLI signal was calculated by the software after subtracting background values. Data were reported as spleen BLI values, liver BLI values and spleen to liver ratio for whole body imaging (i.e. the ratio obtained by dividing the BLI value in spleen by the BLI value in the liver of the same animal). For LN images, the BLI values for dissected LN and the underlying s.c tissue were reported for each formulation. The images are provided in **FIG. 16**.

**[0208]** As shown in **FIG. 16**, upon subcutaneous administration to mice, the luciferase from Lipid Particle Formulation No. 13 shows accumulation in the liver for at least 48 hours. In contrast, the luciferase from Lipid Particle Formulation No. 14 has a low liver signal up to 24 hours. The majority of the luciferase from Lipid Particle Formulation Nos. 1, 13, and 14 was retained at the site of injection and taken up into the lymph nodes. The luciferase from Lipid Particle Formulation No. 1 accumulated to a greater extent in the lymph nodes with very negligible subcutaneous signal.

#### **Example 11. Spleen targeting using different ionizable lipids**

**[0209]** BLI values in the spleen and liver of mice for luciferase delivered by Lipid Particle Formulation Nos. 1, 34, and 35 were determined using the protocol of **Example 3**. The results are provided in **FIGs. 10A-10B**.

#### **Example 12. Four-Component Lipid Particles**

**[0210]** The average spleen BLI value, average liver BLI value, and spleen to liver ratios in mice for Lipid Particle Formulation Nos. 7-10 and 76-77 (in PBS) were determined using the protocol provided in **Example 3**. The results are reported in **Table 5** below. Each of the formulations contain 38.5% molar weight percent cholesterol and 1.5% molar weight percent of DMG-PEG2000.

**Table 5**

|                             | Lipid Particle Formulation No. 77 | Lipid Particle Formulation No. 75 | Lipid Particle Formulation No. 76 | Lipid Particle Formulation No. 7 | Lipid Particle Formulation No. 8 | Lipid Particle Formulation No. 9 | Lipid Particle Formulation No. 10 |
|-----------------------------|-----------------------------------|-----------------------------------|-----------------------------------|----------------------------------|----------------------------------|----------------------------------|-----------------------------------|
| SS-OP/CHEMS Ratio (molar %) | 55/5                              | 50/10                             | 45/15                             | 40/20                            | 35/25                            | 30/30                            | 25/35                             |
| Avg Spleen BLI              | 15.3                              | 14.2                              | 20.35                             | 9.9                              | 6.88                             | 2.97                             | 2.68                              |
| Avg Liver BLI               | 130                               | 70.11                             | 41.13                             | 4.35                             | 1.18                             | 0.37                             | 0.38                              |
| Spleen to liver ratio       | 0.117                             | 0.202                             | 0.4985                            | 2.28                             | 5.83                             | 8.02                             | 7.05                              |

[0211] Whole body scans of the mice are shown in **FIG. 34B**.

#### **Example 13. Four-Component Lipid Particles**

[0212] The average spleen BLI value, average liver BLI value, and spleen to liver ratios for the luciferase delivered Lipid Particle Formulation Nos. 7, 11, 12, and 78 (in PBS) were determined using the protocol described in **Example 3**. The results are reported in **Table 6** below. Each of the formulations contained 40% molar ratio SS-OP and 1.5% molar ratio of DMG-PEG2000.

**Table 6**

|                                   | Lipid Particle Formulation No. 78 | Lipid Particle Formulation No. 7 | Lipid Particle Formulation No. 11 | Lipid Particle Formulation No. 12 |
|-----------------------------------|-----------------------------------|----------------------------------|-----------------------------------|-----------------------------------|
| Cholesterol/CHEMS Ratio (molar %) | 43.5/15                           | 38.5/20                          | 33.25/25                          | 28.5/30                           |
| Avg Spleen BLI                    | 8.17                              | 9.9                              | 5.03                              | 4.16                              |
| Avg Liver BLI                     | 11.33                             | 4.35                             | 0.78                              | 0.59                              |
| Spleen to liver ratio             | 0.78                              | 2.28                             | 5.61                              | 6.14                              |



[0213] Whole body scans of the mice are shown in **FIG. 34A**.

#### **Example 14. Stability**

[0214] The BLI values for spleen (**FIG. 11A**), liver (**FIG. 11B**), and spleen to liver ratios (**FIG. 11C**) in mice were determined for Lipid Particle Formulation No. 1 (at different time points following manufacture: fresh at 4 °C, after 1 month storage at 4 °C, freshly frozen at -80 °C (frozen for two weeks), after two freeze-thaw cycles at -80 °C, after 1 month storage at -80 °C, or freshly made. A “fresh” formulation means it was typically formulated the day before injection and stored at 4 °C overnight. The BLI values were determined using the protocol described in **Example 3**.

#### **Example 15. Immunogenicity Assay**

[0215] The goal of the study was to determine the immunogenicity of mRNA encapsulated in Lipid Particle Formulation of interest. The mRNA was purified via oligo-dT column or RP-HPLC to remove dsRNA.

[0216] 6–8-week-old C57BL/6 mice were injected intravenously via the tail vein with Lipid Particle Formulation No. 79 (containing MC3) or 80 (containing SS-OP). R848 (Resiquimod, Invivogen) was injected as a control for B and T cell activation. 6 hours post-injection, the mice were sacrificed and blood was collected via cardiac puncture into tubes containing an anticoagulant. Plasma was separated by centrifuging the blood at 3000 rpm for 10 min at 4°C. Plasma was analyzed for INF- $\alpha$  using an ELISA kit (mouse Quantikine IFN- $\alpha$  ELISA, R&D Systems). Spleens were processed with a GentleMACS in RPMI medium to obtain splenocytes. The splenocytes ( $1 \times 10^6$  cells/well) were then blocked with FcBlock (BioLegend) and stained for CD45 (all immune cells), CD3 (T cells), CD19 (B cells), and CD69 (activation marker) all from BioLegend (all at 1:100) for 1 hr at 4 °C in MACS buffer (PBS, 0.05% BSA, 2mM EDTA). Cells were washed with MACS buffer and flow cytometry was then performed on the BD FACSCelesta. Activated B cells were gated as CD45+ CD19+ CD69+ CD19-, and activated T cells were gated as CD45+ CD3+ CD69+. Percentages in the charts indicate the percentage of CD69+ cells of the parent gate (either B or T cells).

[0217] IFN- $\alpha$  is a marker of innate immune cell activation of TLR3 by dsRNA. CD69 is a marker of activated B and T cells. R848 is a small molecule TLR7/8 agonist that induces inflammation in C57BL/6 mice and can activate B and T cells but does not induce IFN- $\alpha$  production. The results are provided in **FIGs. 12A-12C**.

[0218] Neither MC3 nor SS-OP ionizable lipids alone (no luciferase mRNA) induced any IFN- $\alpha$  or lead to the activation of B or T cells. Lipid Particle Formulations encapsulating OdT purified fLuc mRNA, which has been demonstrated to contain some residual dsRNA, potentially induced IFN- $\alpha$  as well as the activation of CD69+ B and T cells. Lipid Particle Formulation No. 80 (containing SS-OP) which contained the same amount of fLuc mRNA as the Lipid Particle Formulation No. 79 (containing MC3) induced significantly less IFN- $\alpha$  and B and T cell activation. HPLC purification of the mRNA did not activate B or T cells and there was no detectable amount of IFN- $\alpha$  produced, indicating that HPLC is a superior method for removal of dsRNA.

#### **Example 16. Induction of Tregs and anergic T-cells**

[0219] C57BL/6J female mice were injected intravenously with 100  $\mu$ L Formulation 1 via the tail vein. The following groups were injected on days 0, 3, 7 and 10: MOG (20  $\mu$ g), MOG+IL-10 (20+20)  $\mu$ g, MOG+TGF  $\beta$  (20+20)  $\mu$ g, MOG+PD-L1 (20+20)  $\mu$ g, MOG+IL-10+PD-L1 (20+20+20)  $\mu$ g or MOG+ TGF  $\beta$ + PD-L1 (20+20+20)  $\mu$ g.

[0220] The C57BL/6J female mice were euthanized using CO<sub>2</sub>, three days after the final dose on day 13. The spleens were harvested and weighed and placed in gentleMACS tubes containing 5 mL RPMI on ice. The spleens were homogenized for 1 min in the gentleMACS dissociator, filtered via 40  $\mu$ m filter caps into 50 mL conical tubes and washed with MACS buffer. Cells were pelleted after spinning for 5 min at 500 xg and subjected to RBC lysis using ACK lysis buffer for 2-3 min at room temperature. The cells were washed with PBS (10 mL) and pelleted again. Cells were finally resuspended in 5 mL MACS and counted using Cellaca MX high-throughput cell counter after 1:1 dilution with 25  $\mu$ L AOPI. For flow cytometric staining, 4 million cells were placed into 1.5 mL eppendorf tubes and topped up to 1 mL with MACS buffer; then, cells were spun down and resuspended in 0.4 mL MACS buffer prior to plating of 2e6 cells/well in a 96 well VB plate by transfer of 0.2 mL. For FMOs, 0.2 mL from various groups were pooled and distributed to 9 separate wells at 0.2 mL. Flow staining for tetramer and the Treg panel was performed. Briefly, cells were stained for viability with Zombie Aqua and Fc block for 15 min at RT in 200  $\mu$ L PBS. Then, after pelleting and removal of supernatant, cells were stained for 1 hour at room temperature in 50  $\mu$ L MACS containing 10  $\mu$ L of MOG:IA-b tetramer-PE (MBL International) for 2e6 total cells. After 1 hour, surface staining antibodies were added in each well at 50  $\mu$ L/well to top up to 100  $\mu$ L and cells were left to stain for 20 min more at RT. After pelleting and removal of supernatants, cells were fixed with Foxp3 transcription factor

fixation/permeabilization buffer for 20 min at RT and finally stored in 200  $\mu$ L MACS buffer O/N. On the following day, cells were pelleted and resuspended in 80  $\mu$ L permeabilization/wash (P/W) buffer per well containing 1  $\mu$ L Fc block and 1  $\mu$ L mouse serum for 30 min. Then, Foxp3 antibody was added directly to wells in 20  $\mu$ L and left to stain for 30 min at R.T. in 100  $\mu$ L total. Cells were washed with 100  $\mu$ L P/W buffer and resuspended in 100  $\mu$ L of MACS buffer. Then, cells received 20  $\mu$ L of MACS buffer containing 10k counting beads per well. Cells were run on the BD Celesta and the program was set to record 1 million live cells per sample, however, the range of live cells collected was between 100-300k cells. Final analyses were performed using FlowJo and excel.

**[0221]** The results from the assay are provided in **FIGs. 13** and **14**. Results from a similar study are provided in **FIGs. 17A-17C**. The figures show that Lipid Particle Formulation No. 1 is capable of delivering and expressing the mRNAs encoding epitopes or epitopes together with IL-10 in the target tissue, i.e., spleen.

**Example 17. *In vivo* Efficacy in EAE Mouse Model of Multiple Sclerosis**

**[0222]** C57BL/6 mice (10-13 weeks old) were anaesthetized using isoflurane on day 0. EAE was induced in these mice by subcutaneous (s.c) injection of 200  $\mu$ L total of an emulsion of MOG<sub>35-55</sub> in complete Freund's adjuvant (MOG<sub>35-55</sub>/CFA) (#EK-2110, Hooke laboratories) containing around 200 ug MOG<sub>35-55</sub>. Two injections of 100  $\mu$ L each were administered on the hind flanks, just adjacent to the spinal column. The animals were placed back in their cages for 3 h and injected intraperitoneally (i.p.) with 100  $\mu$ L PBS containing 150 ng pertussis toxin (PTX) per mouse.

**[0223]** On day 1, all animals were again injected intraperitoneally (i.p.) with 100  $\mu$ L PBS containing 150 ng PTX/mouse. Lipid Particle Formulation No. 15 containing 20  $\mu$ g MOG<sub>35-55</sub> or an irrelevant mRNA were administered intravenously via tail vein injection on day 7 and day 10 (prophylactic study) or on day 12 and day 15 (therapeutic study). Control mice were injected with MOG<sub>35-55</sub>/CFA and PTX but no Lipid Particle Formulation No. 15. Animals were monitored for their body weights and scored for signs of EAE induction daily from day 10 onwards using the EAE scoring rubric in **Table 7** below. The study was terminated on day 20 and spleens were harvested from mice on day 21.

**Table 7. EAE scoring**

| Score | Clinical Observations                                                          |
|-------|--------------------------------------------------------------------------------|
| 0.5   | Distal tip of tail limp                                                        |
| 1     | Limp tail                                                                      |
| 1.5   | Limp tail and weakness in one leg                                              |
| 2     | Weakness with both hind limbs                                                  |
| 2.5   | Weak hind limbs                                                                |
| 3     | One hind limb paralyzed                                                        |
| 3.5   | Both hind limbs paralyzed<br>Hind end limbs of animal splayed out and dragging |
| 4     | Both hind limbs paralyzed with weak front limbs                                |
| 4.5   | Both hind limbs paralyzed, and one front limb paralyzed or non-functional      |
| 5     | Both hind limbs and front limbs paralyzed or moribund                          |

[0224] Results from the study are provided in **FIGs. 18A-18B**. The figures show that the formulation is capable of delivering and expressing mRNA encoding the MOG antigen to the spleen and spinal cords of the subject mice.

[0225] Additionally, serum IL-17 levels were measured in the inducible EAE mice following treatment with either Lipid Particle Formulation No. 15 encoding MOG peptide or Lipid Particle Formulation No. 15 encoding irrelevant mRNA. A significant reduction in IL-17 levels was observed in the EAE mice treated with a formulation containing mRNA that encodes the MOG peptide compared to the EAE mice treated with formulation containing mRNA that encodes irrelevant mRNA (**FIG. 18A**).

#### **Restimulation assay (cytokine analysis):**

[0226] C57BL/6J female mice were euthanized using CO<sub>2</sub> after the study was terminated. The spleens were harvested, homogenized and after removal of RBCs using ACK lysis, splenocytes were counted and resuspended in RPMI media containing 10% FBS. Bulk splenocytes were plated at 200,000 cells per 96-well plate in duplicates. Cells were stimulated with 10 µg/ml MOG peptide or unstimulated (no-peptide) for 48 h. Supernatants were collected from the plates and run on Magpix using a custom 7-plex (IL-2, IL-4, IL-6, IL-10, IFN $\gamma$ , TNF $\alpha$ , and IL-17a) Luminex kit from R&D systems. The data were analyzed using two-way ANOVA with Tukey's post hoc tests for multiple comparisons.

**Example 18. *In vivo* Efficacy in NOD Mouse Model of Type 1 Diabetes**

[0227] NOD mice were immunized intravenously once a week for five weeks starting when the mice were 6 weeks of age. The tolerizing vaccine used in this experiment used Lipid Particle Formulation No. 1 as follows:

- Formulation 1: Lipid Particle Formulation No. 15 with tol105 mRNA and KIF1a mRNA (20 µg of each, 40 µg total)
- Formulation 2: Lipid Particle Formulation No. 15 with tol105 mRNA, KIF1a mRNA, and IL-10HA mRNA (20 µg of each, 60 µg total)
- Formulation 3: Lipid Particle Formulation No. 15 with no mRNA, dosed at a lipid equivalence to the 60 µg dose of formulation 2.

[0228] Tol105 is a construct made using literature epitopes described in Podojil, JR, *et al.*, “Tolerogenic Immune-Modifying Nanoparticles Encapsulating Multiple Recombinant Pancreatic  $\beta$  Cell Proteins Prevent Onset and Progression of Type 1 Diabetes in Nonobese Diabetic Mice,” *J Immunol.* (Aug 1 2022);209(3):465-75. doi: 10.4049/jimmunol.2200208. Epub 2022 Jun 20. PMID: 35725270; PMCID: PMC9339508.

[0229] The KIF1a mRNA consists of two overlapping DECODE class I epitopes with flanks. The sequences for all of the epitopes in tol105 and the Kif1a sequences are listed in Table 8 below.

**Table 8**

| Mouse Peptide Antigens   | Sequence                 |
|--------------------------|--------------------------|
| Insulin A14-20           | YQLENYC                  |
| IGRP (206-214 )          | VYLKTNVFL                |
| Chromogranin A (359-372) | SRMDQLAKELTAEK           |
| Insulin B9-23            | SHLVEALYLVCGERG          |
| GAD65 (286-300)          | KGAAALGIGTDSVI           |
| GAD65 (514-524)          | RTLEDNEERMS              |
| HSP60 (437-460)          | VLGGGCALLRCIPALDSLKPANED |
| ZnT8 (345-359)           | LTIQIESAADQDPSC          |

|                |                              |
|----------------|------------------------------|
| IGRP(123-145)  | WYVMVTAALSYTISRMEESSVTL      |
| Kif1a (DECODE) | SLVGRAFVYLSNLLYPVPLVHRVAIVSE |

**[0230]** Following all five immunizations, the mice were monitored for blood glucose levels once a week. Hyperglycemia was indicated when blood glucose levels exceeded 200mg/dL glucose and diabetes was indicated when blood glucose levels exceeded 400mg/dL. Diabetic mice were euthanized and their spleens and pancreas collected. All remaining mice on study were euthanized when the mice were 30 weeks of age and spleen and pancreas collected. Results from the study are provided in **FIGs. 19A-19B**. The results again show that the formulation is capable of delivering and expressing mRNAs encoding T1D epitopes to the target tissue, i.e., spleen.

**Example 19. Determining pKa of Lipid Particle Formulation using the (2-(p-toluidino)-6-naphthalene sulfonic acid) (“TNS”) assay**

**[0231]** TNS, a non-fluorescent dye in aqueous solutions, exhibits strong fluorescence by binding to cationic lipids or polymers. At low pH values, the interaction between TNS and cationic lipid increases and leads to a steady increase in fluorescence until it reaches a plateau. The pH value at the half maximum value of fluorescence represents the pKa of a Lipid Particle Formulation Nos. 1 (**FIG. 20A**), 13 (**FIG. 20B**), and 14 (**FIG. 20C**).

**[0232]** A master buffer stock was made using 20 mM sodium phosphate, 25 mM sodium citrate, 20 mM ammonium acetate, and 150 mM sodium chloride. pH buffers from 2.5 to 11.0 in increments of 0.5 units were prepared by adjusting the master buffer with concentrated NaOH and HCl. 250  $\mu$ L of buffer at each pH was first added to a 96 well black plate in triplicate. Next, 5  $\mu$ L of the Lipid Particle Formulation diluted to 0.1 mg/mL were added to each of the buffer containing wells. Finally, TNS was added at a final concentration of 6 $\mu$ M from a 0.16mM stock in DMSO (10  $\mu$ L), the wells were mixed well and the plate was set aside for 5 min. The TNS fluorescence was read at 322<sub>Ex</sub>/431<sub>Em</sub> using the Spectramax ID3 plate reader. A graph of normalized fluorescence of TNS vs. pH was plotted. The pKa of the particular Lipid Particle Formulation which is defined as the pH value at the half maximum value of TNS fluorescence was determined from the graph (**FIGs. 20A-20C**).

**[0233]** The pKa of Formulation Nos. 1, 13, and 14 are each in the ranges reported for commercially approved lipid nanoparticles.

**Example 20. mRNA integrity**

[0234] To determine mRNA integrity, mRNA is first released from the mRNA-Lipid Particle Formulation. In this study, Lipid Particle Formulation Nos. 1, 13, and 14 were used.

[0235] For capillary gel electrophoresis (“CE”) analysis under denaturing conditions, 10  $\mu$ L of the respective mRNA-Lipid Particle Formulation sample was mixed with 20  $\mu$ L of 0.3% Triton X-100 in CE-grade water, 50  $\mu$ L of formamide solution (SLS) and 10  $\mu$ L of CE-grade water. Samples were incubated at room temperature for 20 minutes and then heated for 10 minutes at 70 °C using a thermal cycler. Next, samples were chilled on ice for at least 5 minutes and transferred to the multi-capillary sample plate that was pre-filled with RNA dilution buffer. An RNA ladder was included on the plate. The plate was run on an Agilent 5200 fragment analyzer. The relative concentration (%) was determined from the RFU values. Reduction in the relative concentration (%) of mRNA for each sample at time T was compared to the original sample at time T=0 and data was obtained for up to 16 weeks. A cut off of 85% was used as the minimum value for integrity measurements.

[0236] The results are provided in **FIGs. 21** and **FIGs. 22A-22B**. For Lipid Particle Formulation No. 1, the mRNA integrity was retained for up to 4 months at 2-8 °C; for Lipid Particle Formulation Nos. 13 and 14, the relative concentration % values dropped after 6 and 11 weeks, respectively.

**Example 21. Multiple mRNAs can be encapsulated into the Lipid Particle Formulations**

[0237] Lipid Particle Formulation No. 1 was used as a representative example. Lipid Particle Formulation No. 1 was formulated using three mRNAs (MOG<sub>35-55</sub>, OVA and IL-10). Each of the three mRNAs was added at 20  $\mu$ g in the mRNA mix for a final combined concentration of 0.5 mg/mL. After formulating, the lipid particles were lysed and analyzed using capillary gel electrophoresis to look at the integrity of Lipid Particle encapsulated mRNA (method detailed in **Example 20**). The relative concentration (%) of each mRNA was determined from the CE electropherogram.

[0238] As shown in **FIGs. 23A** and **23B**, it was clear that the Lipid Particle Formulations described herein (*e.g.*, Lipid Particle Formulation No. 1) can encapsulate at least three different mRNA payloads at a time.

**Example 22. The Lipid Particle Formulations do not generate IL-1 $\beta$  in human PBMCs *in vitro***

[0239] mRNA Lipid Particle Formulations were formulated using the T-mixing method as described in **FIG. 1**. Lipid Particle Formulation No. 1 and base formulations (**ionizable lipid**/DSPC/Cholesterol/DMG-PEG2000, 50/10/38.5/1.5) with different ionizable lipids namely, SS-OP, MC-3 and SM-102 were prepared using HPLC purified mRNA. All formulations were tested for endotoxin before treating cells.

[0240] Fresh human PBMCs were seeded in U-bottom 96 well plates at  $2 \times 10^5$  cells/well in RPMI with 2% human AB serum. The cells were primed for 2 h with LPS (1  $\mu$ g/ml) or R848 (0.5  $\mu$ g/ml). Following priming, cells were treated with the various mRNA Lipid Particle Formulations at an mRNA dose of 30  $\mu$ g/mL for 17 h or with nigericin, the positive control at a dose of 10  $\mu$ g/mL for 1 h. After 17h, the plates were centrifuged for 5 min at 300 x g, supernatants were transferred to fresh plates, and IL-1 $\beta$  levels were analyzed using human IL-1 $\beta$ /IL-1F2 quantikine ELISA kit from R&D systems.

[0241] As shown in **FIG. 24**, none of the Lipid Particle Formulations generated any significant levels of IL-1 $\beta$  relative to the non-treated (media alone) group. This shows that the production and clean-up processes employed for mRNA and Lipid Particle Formulations generate a drug product that does not induce production of pro-inflammatory cytokines like IL-1 $\beta$  and does not stimulate the innate immune system. The positive control nigericin generated significantly high levels of IL-1 $\beta$  after treatment.

**Example 23. Lipid Particle Formulation delivery efficacy in human PBMC subsets**

[0242] Fresh human PBMCs were used to isolate PBMC subsets (DCs, monocytes, CD4 T-cells, CD8 T-cells, and B cells). StemCell kits (positive or negative selection) were used per manufacturer's instructions to isolate the individual cell subsets. About  $2 \times 10^5$  cells/well/100  $\mu$ L were seeded in the respective cell culture medium for each of these cell types. Cells were treated with 100  $\mu$ L of a particular Lipid Particle Formulation (3 wells/treatment), equivalent to an mRNA concentration of 20  $\mu$ g/ml (firefly luciferase mRNA, 4  $\mu$ g/well) for 17 h. Lipid Particle Formulation Nos. 13, 14, and 1 were compared to comparator 4-component formulations (**ionizable lipid**/DSPC/Cholesterol/DMG-PEG2k, 50/10/38.5/1.5) using either Dlin-MC3-DMA, SM-102 or SS-OP as the ionizable lipids. At the end of the incubation, plates were spun



for 5 min at 300 x g and supernatants were removed. The cells were then resuspended in 100 µL fresh culture medium and 100 µL steady-glo luciferin substrate was added to cells and mixed well. After 5 min to allow for cell lysis, 150 µL of the lysate was transferred to white opaque plates and the luciferase signal was read using the luminescence protocol in a Spectramax iD3 plate reader. The relative luminescence units (“RLU”) were plotted for each of the treatments. Higher signal indicated greater delivery efficacy.

**[0243]** As shown in **FIGs. 25A-25E**, Lipid Particle Formulation No. 1 showed a significantly higher delivery efficacy in all of the PBMC subsets versus the other tested Lipid Particle formulations.

#### **Example 24. Lipid Particle Formulation delivery efficacy in human PBMCs**

**[0244]** Human PBMCs were seeded at  $2 \times 10^5$  cells/well/100 µL in RPMI with 2% human AB serum. Cells were treated with 100 µL Lipid Particle Formulation (3 wells/treatment), equivalent to an mRNA concentration of 20 µg/mL (firefly luciferase mRNA, 4 µg/well) for 17 h. Lipid Particle Formulation Nos. 13, 14, and 1 were compared to comparator 4-component formulations (**ionizable lipid**/DSPC/Cholesterol/DMG-PEG2k, 50/10/38.5/1.5) using either Dlin-MC3-DMA or SM-102 as the ionizable lipids. At the end of the incubation, the plates were spun for 5 min at 300 x g and the supernatants were removed. The cells were then resuspended in 100 µL fresh RPMI with 2% human AB serum. 100 µL steady-glo luciferin substrate was added to the cells and mixed well. After 5 min to allow for cell lysis, 150 µL of the lysate was transferred to white opaque plates and the luciferase signal was read using the luminescence protocol in a Spectramax iD3 plate reader. The relative luminescence units (“RLU”) were plotted for each of the treatments. Higher signal indicated greater delivery efficacy.

**[0245]** As shown in **FIG. 26**, Lipid Particle Formulation No. 1 showed a significantly higher delivery efficacy than the other tested Lipid Particle Formulations

#### **Example 25. mRNA-Lipid Particle Formulation dose response in human PBMCs *in vitro***

**[0246]** Human PBMCs were seeded at  $2 \times 10^5$  cells/well/100 µL in RPMI with 2% human AB serum. Cells were treated with 100 µL Lipid Particle Formulation No. 1 (3 wells/treatment) containing various mRNA concentrations (firefly luciferase mRNA) for 17h. A final mRNA concentration range of 0.25 to 30 µg/mL (0.05 to 6 µg mRNA/well) was evaluated. At the end of the incubation, plates were spun for 5 min at 300 xg and supernatants were removed. The cells

were then resuspended in 100  $\mu$ L fresh RPMI with 2% human AB serum and 100  $\mu$ L steady-glo luciferin substrate was added to cells and mixed well. After 5 min to allow for cell lysis, 150  $\mu$ L of the lysate was transferred to white opaque plates and the luciferase signal was read using the luminescence protocol in a Spectramax iD3 plate reader. The relative luminescence units (“RLU”) were plotted for each of the treatments. Higher signal indicated greater delivery efficacy.

[0247] As shown in **FIG. 27**, clear dose-response was observed with increasing concentrations of mRNA. It is also clear that Lipid Particle Formulation No. 1 improved the delivery efficacy.

**Example 26. Induction of antigen-specific Tregs *in vivo* by Lipid Particle Formulations co-encapsulating epitope and immunomodulatory**

[0248] C57BL/6J female mice were immunized with 100  $\mu$ g MOG<sub>35-55</sub>/CFA emulsion subcutaneously. They were treated with Lipid Particle Formulation No. 1 encapsulating an irrelevant mRNA (mouse serum albumin (“MSA”)), MOG<sub>35-55</sub>, IL-10, or both MOG<sub>35-55</sub> and IL-10. Mice were treated with four doses of Lipid Particle Formulation No. 1 administered twice weekly starting on day 7 post-immunization (days 7, 10, 14 and 17).

[0249] The mice were euthanized using CO<sub>2</sub>, three days after the final dose on day 17. The spleens were harvested, weighed and placed in gentleMACS tubes containing 5 mL RPMI on ice. The spleens were homogenized for 1 min in the gentleMACS dissociator, filtered via 40  $\mu$ m filter caps into 50 mL conical tubes and washed with MACS buffer. Cells were pelleted after spinning for 5 min at 500 x g and subjected to RBC lysis using ACK lysis buffer for 2-3 min at room temperature. The cells were washed with PBS (10 mL) and pelleted again. The cells were finally resuspended in 5 mL MACS and counted using Cellaca MX high-throughput cell counter after 1:1 dilution with 25  $\mu$ L AOPI. For flow cytometric staining, 4 million cells were placed into 1.5 mL eppendorf tubes and topped up to 1 mL with MACS buffer. Then cells were spun down and resuspended in 0.4 mL MACS buffer prior to plating of  $2 \times 10^6$  cells/well in a 96 well VB plate by transfer of 0.2 mL. For FMOs, 0.2 mL from various groups were pooled and distributed to 9 separate wells at 0.2 mL.

[0250] Flow staining for tetramer and the Treg panel was performed. Briefly, cells were stained for viability with Zombie Aqua and Fc block for 15 min at R.T. in 200  $\mu$ L PBS. Then, after pelleting and removal of supernatant, cells were stained for 1 hour at room temperature in

50  $\mu$ L MACS containing 10  $\mu$ L of MOG:IA-b tetramer-PE (MBL International) for 2e6 total cells. After 1 hour, surface staining antibodies were added in each well at 50  $\mu$ L/well to top up to 100  $\mu$ L and cells were left to stain for 20 min more at R.T. After pelleting and removal of supernatants, cells were fixed with Foxp3 transcription factor fixation/permeabilization buffer for 20 min at R.T. and finally stored in 200  $\mu$ L MACS buffer O/N.

**[0251]** On the following day, cells were pelleted and resuspended in 80  $\mu$ L permeabilization/wash (P/W) buffer per well containing 1  $\mu$ L Fc block and 1  $\mu$ L mouse serum for 30 min. Then Foxp3 antibody was added directly to wells in 20  $\mu$ L and left to stain for 30 min at R.T. in 100  $\mu$ L total. Cells were washed with 100  $\mu$ L P/W buffer and resuspended in 100  $\mu$ L of MACS buffer. Then cells received 20  $\mu$ L of MACS buffer containing 10k counting beads per well. Cells were run on the BD Celesta and the program was set to record 1 million live cells per sample. The range of live cells collected was between 100-300k cells. Final analyses were performed using FlowJo and excel.

**[0252]** As shown in **FIGs. 28A-28B**, co-encapsulation of epitope (MOG<sub>35-55</sub>) and immunomodulator (IL-10) induced significantly higher levels of MOG-specific Tregs compared to the epitope or immunomodulator. An additive effect of administering the two mRNAs together was evident from the data.

**Example 27. IL-10 was detected in mouse plasma on day 8 and 18, 24h post injections**

**[0253]** C57BL/6J female mice were immunized with 100  $\mu$ g MOG<sub>35-55</sub>/CFA emulsion subcutaneously. They were treated with Lipid Particle Formulation No. 1 encapsulating an irrelevant mRNA (mouse serum albumin ("MSA")), MOG<sub>35-55</sub>, IL-10, or both MOG<sub>35-55</sub> and IL-10.

**[0254]** Mice were treated with four doses of the Lipid Particle Formulation No. 1 administered twice weekly starting on day 7 post-immunization (days 7, 10, 14 and 17). On day 8 and day 18, 24 h after the 1<sup>st</sup> and 4<sup>th</sup> doses respectively, mice were bled via retro-orbital sinus and blood was collected into tubes containing EDTA. Plasma was isolated from blood and stored at -80 °C until analysis. To detect IL-10 levels, the plasma samples were diluted 2-fold and analyzed using the mouse IL-10 Quantikine ELISA kit from R&D systems per the manufacturer's instructions.

**[0255]** As shown in **FIG. 29**, a clear dose response was observed for IL-10 concentrations in plasma with higher IL-10 mRNA doses generating significantly higher plasma IL-10. Lipid

Particle Formulation No. 1 maintained the mRNA delivery efficacy through the duration of the study, with Day 8 and Day18 IL-10 levels showing no differences.

**Example 28. Lipid Particle Formulation stock stored at -80°C for up to 6 months retains original critical quality attributes and functional mRNA delivery *in vivo***

[0256] For long term storage stability studies, multiple batches of Lipid Particle Formulation No. 1 containing firefly luciferase mRNA were formulated. Before filtration, sucrose was spiked into the formulation from an 80% w/v sucrose stock in Tris-buffered saline for a final sucrose concentration of 7% w/v for each batch. The formulations were then stored at -80°C until the designated time point.

[0257] At designated time points 1 month, 3 months and 6 months, each Lipid Particle Formulation No. 1 was batch was analyzed for particle size, PDI, and mRNA encapsulation. The functional delivery of mRNA was evaluated by injecting the formulations into 6-8 week old C57BL/6J mice (20 µg) and comparing the BLI signal of a freshly made batch of Lipid Particle Formulation No. 1 with that of the formulation stored at -80°C.

[0258] Prior to injections, mouse fur was clipped along the left lateral aspect of the abdomen up to the spine in order to highlight both splenic and liver expression fields. Mice were injected intravenously via tail vein with 100 µL Lipid Particle Formulation No. 1 containing equivalent to 20 µg firefly luciferase mRNA. After 6 h, mice were administered 200 µL D-luciferin (3 mg/mouse) *via* intraperitoneal injection (i.p), anaesthetized using isofluorane and imaged within 10 min using a LICOR Pearl imager. Images were obtained in right lateral recumbency under 3% isofluorane for anaesthetic maintenance. LICOR settings: BLI and White channels only, 85µm resolution, focus at 0. The bioluminescent intensity (BLI) values were determined using the Image Studio software, version 5.2.5 from LICOR. Regions of interest were drawn around organs (e.g spleen or liver) using software tools and the corresponding bioluminescence intensity (BLI) signal was calculated by the software after subtracting background values. Data were reported as spleen BLI values, liver BLI values, and spleen to liver ratio (i.e the ratio obtained by dividing the BLI value in spleen by the BLI value in the liver of the same animal).

[0259] As shown in **FIGs. 30A-30D**, Lipid Particle Formulation No. 1 stock retains its original critical quality attributes (size, PDI and mRNA encapsulation). Additionally, as shown in **FIGs. 31A-31C**, Lipid Particle Formulation No. 1 maintains functional delivery upon long term storage at -80°C for 6 months. The spleen specificity is also maintained.

**Example 29. Lipid Particle Formulation No. 1 stored at 4°C for 1 month retains original critical quality attributes and functional mRNA delivery *in vivo***

[0260] Lipid Particle Formulation No. 1 was formulated and analyzed for particle size, PDI and mRNA encapsulation. The functional delivery of mRNA was evaluated by injecting the formulations into 6-8 week old C57BL/6J mice (20 µg mRNA). This was the first time point for evaluation (T=0, 4°C).

[0261] Prior to injections with Lipid Particle Formulation No. 1, the mouse fur was clipped along the left lateral aspect of the abdomen up to the spine in order to highlight both splenic and liver expression fields. Mice were injected intravenously via tail vein with 100 µL of Lipid Particle Formulation No. 1 containing equivalent to 20 µg firefly luciferase mRNA. After 6 h, mice were administered 200 µL D-luciferin (3 mg/mouse) *via* intraperitoneal injection (i.p), anaesthetized using isoflurane and imaged within 10 min using a LICOR Pearl imager. Images were obtained in right lateral recumbency under 3% isoflurane for anaesthetic maintenance. LICOR settings: BLI and White channels only, 85um resolution, focus at 0. The bioluminescent intensity (BLI) values were determined using the Image Studio software, version 5.2.5 from LICOR. Regions of interest were drawn around organs (e.g spleen or liver) using software tools and the corresponding bioluminescence intensity (BLI) signal was calculated by the software after subtracting background values. Data were reported as spleen BLI values, liver BLI values and spleen to liver ratio (i.e the ratio obtained by dividing the BLI value in spleen by the BLI value in the liver of the same animal). The formulation was stored at 4°C for a month and reanalyzed for CQAs as well as functional mRNA delivery as mentioned above. At the 1-month (T=1m, 4°C) time point a freshly made batch of Lipid Particle Formulation No. 1 (fresh) served as the control.

[0262] As shown in **FIGs. 32A-32F** and **FIGs. 33A-33C**, Lipid Particle Formulation No. 1 stock retains its original critical quality attributes (size, PDI and mRNA encapsulation) as well as functional mRNA delivery when stored at 4°C for up to a month. The spleen specificity is also maintained

**Example 30. EAE Method**

[0263] C57BL/6J were injected subcutaneously with 200 µL total MOG/CFA containing roughly 200 µg MOG<sub>35-55</sub> under isoflurane anesthesia. For this, two injections of 100 µL were placed on the hind flanks just adjacent to the spinal column. Animals were placed back into their

cages for 3 hours and then injected *via* the i.p. route with 100  $\mu$ L PBS containing 150 ng PTX/mouse (day 0). One day later, they were again injected *via* the i.p. route with 100  $\mu$ L PBS containing 150 ng PTX/mouse (day 1). The animals were injected with Lipid Particle Formulation No. 1 containing MOG, MOG/IL-10, or a control mRNA (enhanced Green Fluorescent Protein (“eGFP”)). Naïve mice did not receive any treatments. One group of mice was administered MOG/IL-10 subcutaneously (on the back). They were administered two doses of Lipid Particle Formulation No. 1 on day 7 and day 10.

**[0264]** Starting from day 10 the mice were monitored for signs of disease and scored daily until day 16 (at the peak of EAE). On day 16 the mice were sacrificed using CO<sub>2</sub> euthanasia and intracardial perfusion with 10-15 mL of PBS. The spleens were harvested, followed by 2 x inguinal lymph nodes (LN) and 2 x brachial/axillary LNs, and kept on ice in PBS. The dissection of the full spinal columns was performed with decapitation just below the cerebellum, and spinal columns were flushed from the distal end with a 19G needle containing PBS. All spinal columns were also kept in PBS on ice until all animals were taken down. The spleens were weighed and subjected to gentleMACS dissociation in 5 mL MACS buffer. LNs were also kept on ice in a 48 well plate.

**[0265]** Next, spinal cords were weighed and digested for 20 min at +37°C in 2.5 mL Multi Tissue Dissociation Kit I per spinal cord and kept on ice. All tissues were crushed with the blunt end of a syringe plunger and filtered through a 70  $\mu$ m filter mesh with MACS buffer, then washed and spun for 5 min at 500 xg. LNs were resuspended to 2 mL MACS buffer. Spleens were subject to a 2 min RBC lysis with ACK lysing buffer, followed by topping up with MACS buffer to 10 mL, washed, and then resuspended to 5 mL MACS buffer. Spinal cords were resuspended in 4 mL of 30% Percoll/PBS mixture with a P1000 pipette tip in a 15 mL conical tube. Then, borosilicate glass Pasteur pipettes were placed into each 15 mL conical tube. To underlay for Percoll gradient, 70% Percoll/PBS mixture was pipetted into the borosilicate glass Pasteur pipette (starting with 2 mL) slowly, taking care not to disrupt the cell suspension during the underlying process. Then, a final 1 mL was pipetted into the borosilicate glass Pasteur pipette for 3 mL total. Each glass pipette was carefully taken out of the Percoll gradient suspension ensuring that the underlaid layer reached 3 mL in the 15 mL conical tube. The suspensions were spun at room temperature for 20 min at 690 xg to separate the mononuclear cell layer in the center of the conical tube from neuronal debris. The top of the Percoll suspension was aspirated out until about ~1 mL of volume above the mononuclear cell layer. Then, mononuclear cells containing the

leukocyte fraction from the CNS were pipetted into a separate 15 mL conical tube and topped up with PBS to roughly 13-14 mL total. All tubes were inverted to mix the spinal cord samples, and then cells were pelleted by spinning at 520 x g for 6 min. Spinal cord mononuclear cells were resuspended in MACS buffer at 150 µL per tube and all sample was placed into a separate 96 well RB plate for flow staining.

**[0266]** The splenocytes and LN cells were all pipetted into a Nexcelom 96 well plate (20 µL per well) and cells were counted by 1:1 resuspension with AOPI viability dye and counted via the Cellaca.

**[0267]** Volumes correlating to 6-8 million cells were pipetted into 1.5 mL microcentrifuge tubes and cells were pelleted for SP and LN cells. Then, cell pellets were resuspended in MACS buffer at 600-800 µL per sample for a concentration of 1 million cells/0.1 mL. Then, 2 million cells corresponding to 0.2 mL from each tube were distributed into the 96 well RB plate for staining for flow cytometric analyses. FMOs were set up accordingly by pooling cells from the MOG/IL10HA groups and then redistributing at 2 million cells per well.

**[0268]** All cells were washed and then first subject to viability staining by addition of 100 µL PBS containing 1:500 Zombie Aqua and 1:100 FcX Plus and incubated for 15 min at room temperature (R.T.). Cells were then washed and resuspended in 50 µL MACS buffer containing 1:10 dilution of MOG 35-55 I-Ab Tetramer PE (MBL International) and incubated at R.T. in the dark for 45 minutes. At the 45 minute mark, the remaining 50 µL of MACS buffer mixture containing cell surface marker antibodies was added per well and the samples were incubated for 20 min at R.T. in the dark. After washing with MACS buffer, cells were fixed with eBioscience Foxp3 Transcription Factor Buffer Set Fix/Perm reagent for 30 min at R.T. in the dark. Then, cells were washed and resuspended in MACS buffer overnight and kept in the refrigerator overnight. Cells were washed and resuspended in 80 µL 1x Perm Buffer and left at room temperature for 30 min to permeabilize after overnight incubation. For Foxp3 staining, 20 µL containing Foxp3 antibody (1:200 dilution in 100 µL) was added for 100 µL total in 1X Perm Buffer and cells were stained for 30 min at R.T. Cells were then run on the BD Celesta after fresh compensation with beads.

**[0269]** The results are shown in **FIGs. 35A-35C**.

**INCORPORATION BY REFERENCE**

**[0270]** All publications and patents cited throughout the text of this specification (including all patents, patent applications, scientific publications, manufacturer's specifications, instructions, etc.) are hereby incorporated by reference in their entirety for all purposes. To the extent the material incorporated by reference contradicts or is inconsistent with this specification, the specification will supersede any such material.

**EQUIVALENTS**

**[0271]** The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting the invention described herein. The scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are intended to be embraced therein.



**WHAT IS CLAIMED IS:**

1. A lipid particle comprising cholesteryl hemisuccinate (“CHEMS”) and a payload and optionally one or more of:
  - a. one or more ionizable lipids;
  - b. one or more neutral lipids;
  - c. one or more sterols; and
  - d. one or more stealth lipids.
2. A method of targeting a payload to a spleen of a subject *in vivo* comprising combining a lipid particle or a composition comprising the payload encapsulated within a lipid particle or a composition comprising CHEMS in a molar amount of at least 11%.
3. The method of claim 2, wherein the lipid particle or composition further comprises one or more ionizable lipids, one or more neutral lipids, one or more sterols, one or more stealth lipids, or any combination thereof.
4. The lipid particle of claim 1 or the method of claim 2 or 3, wherein the lipid particle delivers the payload to the spleen.
5. The lipid particle of claim 1 or 4 or the method of claim 2 or 3, wherein the ratio of the payload delivered to the spleen versus liver is greater than 1.1.
6. The lipid particle of any one of claims 1, 4, and 5 or the method of any one of claims 2-5, wherein the ratio of the payload delivered to the spleen versus liver is about 1.1 to about 12.
7. The lipid particle of any one of claims 1 and 4-6 or the method of any one of claims 2-6, wherein the ratio of payload delivered to the spleen versus liver is about 4 to about 11.
8. The lipid particle of any one of claims 1 and 4-7 or the method of any one of claims 2-7, wherein the ratio of payload delivered to the spleen versus liver is determined using an imaging system.
9. The lipid particle of a claim 8 or the method of claim 8, wherein the imaging system is a LICOR Pearl Trilogy imaging system using Image Studio software from LICOR version 5.2.5.
10. The lipid particle of any one of claims 1 and 4-9 or the method of any one of claims 2-9, wherein the CHEMS in the lipid particle is in a molar amount of at least 12%, at least 13%, at least 14%, at least 15%, at least 16%, at least 17%, at least 18%, at least 19%, or at least 20%.
11. The lipid particle of any one of claims 1 and 4-10 or the method of any one of claims 2-10, wherein the CHEMS in the lipid particle is in a molar amount of about 11% to about 45%, about 15% to about 45%, about 20% to about 45%, about 5% to about 40% about 7.5% to about

40%, about 10% to about 40%, about 15% to about 40%, about 20% to about 40%, about 5% to about 35%, about 7.5% to about 35%, about 10% to about 35%, about 15% to about 35%, about 20% to about 35%, about 5% to about 30%, about 7.5% to about 30%, about 10% to about 30%, about 15% to about 30%, about 20% to about 30%, about 5% to about 25%, about 7.5% to about 25%, about 10% to about 25%, about 15% to about 25%, or about 20% to about 25%.

12. The lipid particle of any one of claims 1 and 4-11 or the method of any one of claims 2-11 wherein the CHEMS in the lipid particle is in a molar amount of about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 25%, about 30%, about 35%, about 40%, or about 45%.

13. The lipid particle of any one of claims 1 and 4-11 or the method of any one of claims 2-11, wherein the CHEMS in the lipid particle is in a molar amount of about 11% to about 20%, about 20% to about 30%, or about 11% to about 30%.

14. The lipid particle of any one of claims 1 and 4-11 or the method of any one of claims 2-11, wherein the CHEMS in the lipid particle is in a molar amount of about 11% to about 20%.

15. The lipid particle of any one of claims 1 and 4-11 or the method of any one of claims 2-11, wherein the CHEMS in the lipid particle is in a molar amount of about 11%.

16. The lipid particle of any one of claims 1 and 4-11 or the method of any one of claims 2-11, wherein the CHEMS in the lipid particle is in a molar amount of about 15%.

17. The lipid particle of any one of claims 1 and 4-11 or the method of any one of claims 2-11, wherein the CHEMS in the lipid particle is in a molar amount of about 20%.

18. The lipid particle of any one of claims 1 and 4-11 or the method of any one of claims 2-11, wherein the CHEMS in the lipid particle is in a molar amount of about 25%.

19. The lipid particle of any one of claims 1 and 4-18 or the method of any one of claims 2-18, wherein the lipid particle comprises one or more ionizable lipids.

20. The lipid particle of any one of claims 1 and 4-19 or the method of any one of claims 2-19, wherein the one or more ionizable lipids in the lipid particle are in a molar amount of at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 11%, at least 12%, at least 13%, at least 14%, at least 15%, at least 16%, at least 17%, at least 18%, at least 19%, or at least 20%.

21. The lipid particle of any one of claims 1 and 4-20 or the method of any one of claims 2-20, wherein the one or more ionizable lipids in the lipid particle are in a molar amount of about 20% to about 70%.

22. The lipid particle of any one of claims 1 and 4-21 or the method of any one of claims 2-21, wherein the one or more ionizable lipids in the lipid particle are in a molar amount of about 25% to about 65%, about 30% to about 65%, about 35% to about 65%, about 45% to about 65%, about 50% to about 65%, about 25% to about 60%, about 30% to about 60%, about 35% to about 60%, about 45% to about 60%, about 50% to about 60%, about 25% to about 55%, about 30% to about 55%, about 35% to about 55%, about 45% to about 55%, about 50% to about 55%, about 25% to about 50%, about 30% to about 50%, about 35% to about 50%, or about 45% to about 50%.
23. The lipid particle of any one of claims 1 and 4-21 or the method of any one of claims 2-21, wherein the one or more ionizable lipids in the lipid particle are in a molar amount of about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, or about 65%.
24. The lipid particle of any one of claims 1 and 4-21 or the method of any one of claims 2-21, wherein the one or more ionizable lipids in the lipid particle are in a molar amount of about 30% to about 60%.
25. The lipid particle of any one of claims 1 and 4-21 or the method of any one of claims 2-21, wherein the one or more ionizable lipids in the lipid particle are in a molar amount of about 40% to about 60%.
26. The lipid particle of any one of claims 1 and 4-21 or the method of any one of claims 2-21, wherein the one or more ionizable lipids in the lipid particle are in a molar amount of about 40% to about 50%.
27. The lipid particle of any one of claims 1 and 4-21 or the method of any one of claims 2-21, wherein the one or more ionizable lipids in the lipid particle are in a molar amount of about 50% to about 60%.
28. The lipid particle of any one of claims 1 and 4-21 or the method of any one of claims 2-21, wherein the one or more ionizable lipids in the lipid particle is present in a molar amount of about 30%.
29. The lipid particle of any one of claims 1 and 4-21 or the method of any one of claims 2-21, wherein the one or more ionizable lipids in the lipid particle are in a molar amount of about 40%.
30. The lipid particle of any one of claims 1 and 4-21 or the method of any one of claims 2-21, wherein the one or more ionizable lipids in the lipid particle are in a molar amount of about 50%.

31. The lipid particle of any one of claims 1 and 4-21 or the method of any one of claims 2-21, wherein the one or more ionizable lipids in the lipid particle are in a molar amount of about 55%.
32. The lipid particle of any one of claims 1 and 4-21 or the method of any one of claims 2-21, wherein the one or more ionizable lipids in the lipid particle are in a molar amount of about 60%.
33. The lipid particle of any one of claims 1 and 4-21 or the method of any one of claims 2-21, wherein the one or more ionizable lipids in the lipid particle are in a molar amount of about 65%.
34. The lipid particle of any one of claims 1 and 4-21 or the method of any one of claims 2-21, wherein the one or more ionizable lipids in the lipid particle are in a molar amount of about 70%.
35. The lipid particle of any one of claims 1 and 4-34 or the method of any one of claims 2-34, wherein the one or more ionizable lipids in the lipid particles are one or more selected from the group consisting of DLin-MC3-DMA ("MC3"), DLin-KC2-DMA ("KC2"), ssPalmO-Phe ("SS-OP"), C12-200, SM-102,  $\alpha$ -D-tocopherolsuccinoyl ("SS-EC"), ALC-0315 ("ALC"), Tri-N-tridecyl 3-(ethyl(methyl)amino)propanoate ("304-O13"), tetrakis(2-(octyldisulfaneyl)ethyl) 3,3',3",3'''-(((methylazanediyl)bis(propane-3,1-diyl))bis(azanetriyl))tetrapropionate ("306-O12B"), 9-[4-(dimethylamino)-1-oxobutoxy]-heptadecanedioic acid, 1,17-di-(2Z)-2-nonen-1-yl ester ("L319"), 9,12-octadecadienoic acid, (9Z,12Z)-1,1',1'',1'''-[(3,6-dioxo-2,5-piperazinediyl)bis(4,1-butanediyl)nitrilodi-4,1-butanediyl] ester ("OF-C4-Deg-Lin"), 2-(dioctylamino)ethyl nonyl hydrogen phosphate ("9A1P9"), 5-(((3-(dibutylamino)propyl)amino)methyl)-6-hydroxyundecane-1,11-diyl (9Z,9'Z,12Z,12'Z)-bis(octadeca-9,12-dienoate) ("IR-117-17"), 9Z,12Z-octadecadienoic acid, 3-[4,4-bis(octyloxy)-1-oxobutoxy]-2-[[[3-(diethylamino)propoxy]carbonyl]oxy]methyl]propyl ester ("LP-01") and 4A3-SC8.
36. The lipid particle of any one of claims 1 and 4-35 or the method of any one of claims 2-35, wherein the one or more ionizable lipids in the lipid particle are one or more selected from the group consisting of DLin-MC3-DMA ("MC3"), DLin-KC2-DMA ("KC2"), ssPalmO-Phe ("SS-OP"), C12-200, SM-102,  $\alpha$ -D-tocopherolsuccinoyl ("SS-EC"), and ALC-0315 ("ALC").
37. The lipid particle of claim 36 or the method of claim 36, wherein the one or more ionizable lipids in the lipid particle are one or more selected from the group consisting of ssPalmO-Phe ("SS-OP"), C12-200, SM-102, and ALC-0315 ("ALC").

38. The lipid particle of any one of claims 1 and 4-37 or the method of any one of claims 2-36, wherein only one ionizable lipid is in the lipid particle.
39. The lipid particle of claim 37 or the method of claim 38, wherein the only ionizable lipid in the lipid particle is SS-OP.
40. The lipid particle of claim 39 or the method of claim 39, wherein when administered to a subject in need thereof, the subject exhibits one or more of: (i) an increase in Treg cells, (ii) reduction in effector T cells, (iii) reduction in B-cells, (iv) reduction in cytokine production, (v) reduction in activated B cells; (vi) reduction in activated effector T cells; or (vii) any combination thereof.
41. The lipid particle of claim 39 or 40 or the method of claim 39 or 40, which when administered to a subject in need thereof, reduces B-cell levels in the subject.
42. The lipid particle of claim 39 or 40 or the method of claim 39 or 40, which when administered to a subject in need thereof, reduces B-cell levels in the subject as compared with a reference lipid particle containing an ionizable lipid other than SS-OP.
43. The lipid particle of claim 39 or 40 or the method of claim 39 or 40, which when administered to a subject in need thereof, reduces B-cell levels in the subject as compared with a reference lipid particle containing MC-3 as the ionizable lipid.
44. The lipid particle of any one of claims 39-43 or the method of any one of claims 39-43, which when administered to a subject in need thereof, reduces T-cell levels in the subject.
45. The lipid particle of any one of claims 39-43 or the method of any one of claims 39-43, which when administered to a subject in need thereof, reduces T-cell levels in the subject as compared with a reference lipid particle containing an ionizable lipid other than SS-OP.
46. The lipid particle of any one of claims 39-43 or the method of any one of claims 39-43, which when administered to a subject in need thereof, reduces T-cell levels in the subject as compared with a reference lipid particle containing MC-3 as the ionizable lipid.
47. The lipid particle of any one of claims 39-46 or the method of any one of claims 39-46, which when administered to a subject in need thereof, reduces levels of IFN- $\alpha$  in the subject.
48. The lipid particle of any one of claims 39-46 or the method of any one of claims 39-46, which when administered to a subject in need thereof, reduces levels of IFN- $\alpha$  in the subject as compared with a reference lipid particle containing an ionizable lipid other than SS-OP.
49. The lipid particle of any one of claims 39-46 or the method of any one of claims 39-46, which when administered to a subject in need thereof, reduces levels of IFN- $\alpha$  in the subject as compared with a reference lipid particle containing MC-3 as the ionizable lipid.

50. The lipid particle of any one of claims 1 and 4-49 or the method of any one of claims 2-49, wherein the one or more ionizable lipids in the lipid particle comprises  $\alpha$ -D-tocopherolsuccinoyl ("SS-EC").
51. The lipid particle of claim 50 or the method of claim 50, which when administered to a subject in need thereof, increases B-cell levels in the subject.
52. The lipid particle of claim 50 or the method of claim 50, which when administered to a subject in need thereof, increases B-cell levels in the subject as compared with a reference lipid particle containing an ionizable lipid other than SS-EC.
53. The lipid particle of claim 50 or the method of claim 50, which when administered to a subject in need thereof, increases B-cell levels in the subject as compared with a reference lipid particle containing MC-3 as the ionizable lipid.
54. The lipid particle of any one of claims 50-53 or the method of any one of claims 50-53, which when administered to a subject in need thereof, increases T-cell levels in the subject.
55. The lipid particle of any one of claims 50-53 or the method of any one of claims 50-53, which when administered to a subject in need thereof, increases T-cell levels in the subject as compared with a reference lipid particle containing an ionizable lipid other than SS-EC.
56. The lipid particle of any one of claims 50-53 or the method of any one of claims 50-53, which when administered to a subject in need thereof, increases T-cell levels in the subject as compared with a reference lipid particle containing MC-3 as the ionizable lipid.
57. The lipid particle of any one of claims 50-53 or the method of any one of claims 50-53, which when administered to a subject in need thereof, increases levels of IFN- $\alpha$  in the subject.
58. The lipid particle of any one of claims 50-53 or the method of any one of claims 50-53, which when administered to a subject in need thereof, increases levels of IFN- $\alpha$  in the subject as compared with a reference lipid particle containing an ionizable lipid other than SS-EC.
59. The lipid particle of any one of claims 50-53 or the method of any one of claims 49-52, which when administered to a subject in need thereof, increases levels of IFN- $\alpha$  in the subject as compared with a reference lipid particle containing MC-3 as the ionizable lipid.
60. The lipid particle of any one of claims 1 and 4-59 or the method of any one of claims 2-59, wherein the lipid particle comprises one or more neutral lipids
61. The lipid particle of claim 60 or the method of claim 60, wherein the one or more neutral lipids in the lipid particle are in a molar amount of at least 0.5%, at least 0.6%, at least 0.7%, at least 0.8%, at least 0.9%, at least 1.1%, at least 1.2%, at least 1.3%, at least 1.4%, at least 1.5%,

at least 1.6%, at least 1.7%, at least 1.8%, at least 1.9%, at least 2%, at least 2.1%, at least 2.2%, at least 2.3%, at least 2.4%, or at least 2.5%.

62. The lipid particle of claim 60 or the method of claim 60, wherein the one or more neutral lipids in the lipid particle are in a molar amount of about 2.5% to about 25%, about 2.5% to about 20%, about 2.5% to about 17.5%, about 2.5% to about 15%, about 2.5% to about 12.5%, about 2.5% to about 10%, about 5% to about 25%, about 5% to about 20%, about 5% to about 17.5%, about 5% to about 15%, about 5% to about 12.5%, about 5% to about 10%, about 7.5% to about 25%, about 7.5% to about 20%, about 7.5% to about 17.5%, about 7.5% to about 15%, about 7.5% to about 12.5%, about 7.5% to about 10%, about 10% to about 25%, about 10% to about 20%, about 10% to about 17.5%, about 10% to about 15%, about 10% to about 12.5%, about 12.5% to about 25%, about 12.5% to about 20%, about 12.5% to about 17.5%, or about 12.5% to about 15%.

63. The lipid particle of claim 60 or the method of claim 60, wherein the one or more neutral lipids in the lipid particle are in a molar amount of about 5%, about 7.5%, about 10%, about 12.5%, about 15%, about 17.5%, about 20%, about 22.5%, or about 25%.

64. The lipid particle of claim 60 or the method of claim 60, wherein the one or more neutral lipids in the lipid particle are in a molar amount of about 5% to about 20%.

65. The lipid particle of claim 60 or the method of claim 60, wherein the one or more neutral lipids in the lipid particle are in a molar amount of about 7.5% to about 17.5%.

66. The lipid particle of claim 60 or the method of claim 60, wherein the one or more neutral lipids in the lipid particle are in a molar amount of about 2.5%.

67. The lipid particle of claim 60 or the method of claim 60, wherein the one or more neutral lipids in the lipid particle are in a molar amount of about 5%.

68. The lipid particle of claim 60 or the method of claim 60, wherein the one or more neutral lipids in the lipid particle are in a molar amount of about 7.5%.

69. The lipid particle of claim 60 or the method of claim 60, wherein the one or more neutral lipids in the lipid particle are in a molar amount of about 10%.

70. The lipid particle of claim 60 or the method of claim 60, wherein the one or more neutral lipids in the lipid particle are in a molar amount of about 12.5%.

71. The lipid particle of claim 60 or the method of claim 60, wherein the one or more neutral lipids in the lipid particle are in a molar amount of about 15%.

72. The lipid particle of claim 60 or the method of claim 60, wherein the one or more neutral lipids in the lipid particle are in a molar amount of about 17.5%.

73. The lipid particle of claim 60 or the method of claim 60, wherein the one or more neutral lipids in the lipid particle are in a molar amount of about 20%.

74. The lipid particle of claim 60 or the method of claim 60, wherein the one or more neutral lipids in the lipid particle are in a molar amount of about 25%.

75. The lipid particle of any one of claims 60-74 or the method of any one of claims 60-74, wherein the one or more neutral lipids in the lipid particles are one or more phosphatidylcholine ("PC"), phosphatidylethanolamine ("PE"), and combination thereof.

76. The lipid particle of any one of claims 60-74 or the method of any one of claims 60-74, wherein the one or more neutral lipids in the lipid particle are one or more selected from the group consisting of 1,2-diastearoyl-sn-glycero-3-phosphocholine ("DSPC"), 1,2-dioleoyl-sn-glycero-3-phosphocholine ("DOPC"), 1,2- dioctadecanoyl-sn-glycerol ("DSDG"), 1,2-dioleoyl-sn-glycerol, 1,2-di-(9Z-octadecenoyl)-sn-glycerol ("DODG"), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine ("DOPE"), diacylphosphatidylcholine, diacylphosphatidylethanolamine, ceramide, sphingomyelin, cephalin, cerebrosides, diacylglycerols dipalmitoylphosphatidylcholine ("DPPC"), palmitoyloleoyl-phosphatidylcholine ("POPC"), palmitoyloleoyl-phosphatidylethanolamine ("POPE"), palmitoyloleyol-phosphatidylglycerol (POPG), dipalmitoyl-phosphatidylethanolamine ("DPPE"), dimyristoyl-phosphatidylethanolamine ("DMPE"), distearoyl-phosphatidylethanolamine ("DSPE"), monomethyl-phosphatidylethanolamine, dimethyl-phosphatidylethanolamine, dielaidoyl-phosphatidylethanolamine ("DEPE"), stearylloleoyl-phosphatidylethanolamine ("SOPE"), and egg phosphatidylcholine ("EPC").

77. The lipid particle of claim 76 or the method of claim 76, wherein the one or more neutral lipids in the lipid particle are DOPE, DODG, or a combination thereof.

78. The lipid particle of claim 76 or 77 or the method of claim 76 or 77, wherein only one neutral lipid is in the lipid particle.

79. The lipid particle of claim 78 or the method of claim 78, wherein the only neutral lipid in the lipid particle is DOPE.

80. The lipid particle of claim 78 or the method of claim 78, wherein the only neutral lipid in the lipid particle is DODG.

81. The lipid particle of any one of claims 1 and 4-80 or the method of any one of claims 2-80, wherein the lipid particle comprises one or more sterols.

82. The lipid particle of claim 81 or the method of claim 81, wherein the one or more sterols in the lipid particle are in a molar amount of at least 5%, at least 6%, at least 7%, at least 8%, at



least 9%, at least 10%, at least 11%, at least 12%, at least 13%, at least 14%, at least 15%, at least 16%, at least 17%, at least 18%, at least 19%, or at least 20%.

83. The lipid particle of claim 81 or the method of claim 81, wherein the one or more sterols in the lipid particle are in a molar amount of about 20% to about 50%, about 20% to about 45%, about 25% to about 45%, about 30% to about 45%, about 35% to about 45%, about 40% to about 45%, about 20% to about 40%, about 25% to about 40%, about 30% to about 40%, about 35% to about 40%, about 20% to about 30%, about 25% to about 30%, or about 20% to about 25%.

84. The lipid particle of claim 81 or the method of claim 81, wherein the one or more sterols in the lipid particle are in a molar amount of about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, or about 50%.

85. The lipid particle of claim 81 or the method of claim 81, wherein the one or more sterols in the lipid particle are in a molar amount of about 20% to about 40%.

86. The lipid particle of claim 81 or the method of claim 81, wherein the one or more sterols in the lipid particle are in a molar amount of about 20% to about 30%.

87. The lipid particle of claim 81 or the method of claim 81, wherein the one or more sterols in the lipid particle are in a molar amount of about 30% to about 40%.

88. The lipid particle of claim 81 or the method of claim 81, wherein the one or more sterols in the lipid particle are in a molar amount of about 20%.

89. The lipid particle of claim 81 or the method of claim 81, wherein the one or more sterols in the lipid particle are in a molar amount of about 28.5%.

90. The lipid particle of claim 81 or the method of claim 81, wherein the one or more sterols in the lipid particle are in a molar amount of about 30%.

91. The lipid particle of claim 81 or the method of claim 81, wherein the one or more sterols in the lipid particle are in a molar amount of about 38.5%.

92. The lipid particle of claim 81 or the method of claim 81, wherein the one or more sterols in the lipid particle are in a molar amount of about 40%.

93. The lipid particle of any one of claims 81-92 or the method of any one of claims 81-92, wherein the one or more sterols in the lipid particle are cholesterol,  $\beta$ -sitosterol, stigmasterol, campesterol, fucosterol, brassicasterol, ergosterol, 9,11-dehydroergosterol, daucosterol,  $\beta$ -sitosterol acetate, or other C-24 alkyl derivatives, and combinations thereof

94. The lipid particle of any one of claims 81-92 or the method of any one of claims 81-92, wherein the one or more sterols in the lipid particle are cholesterol,  $\beta$ -sitosterol, and combinations thereof.

95. The lipid particle of any one of claims 81-93 or the method of any one of claims 81-94, wherein only one sterol is in the lipid particle.
96. The lipid particle of claim 95 or the method of claim 95, wherein the only sterol in the lipid particle is cholesterol.
97. The lipid particle of any one of claims 1 and 4-96 or the method of any one of claims 2-96, wherein the lipid particle comprises one or more stealth lipids.
98. The lipid particle of claim 97 or the method of claim 97, wherein the one or more stealth lipids in the lipid particle are in a molar amount of at least 0.1%, at least 0.15%, at least 0.2%, or at least 0.25%.
99. The lipid particle of claim 97 or the method of claim 97, wherein the one or more stealth lipids in the lipid particle are in a molar amount of about 0.25% to about 3%, about 0.5% to about 3%, about 1% to about 3%, about 1.5% to about 3%, about 2% to about 3%, about 2.5% to about 3%, about 0.25% to about 2.5%, about 0.5% to about 2.5%, about 1% to about 2.5%, about 1.5% to about 2.5%, about 2% to about 2.5%, about 0.25% to about 2%, about 0.5% to about 2%, about 1% to about 2%, about 1.5% to about 2%, about 0.25% to about 1.5%, about 0.5% to about 1.5%, or about 1% to about 1.5%.
100. The lipid particle of claim 97 or the method of claim 97, wherein the one or more stealth lipids in the lipid particle are in a molar amount of about 1% to about 3%.
101. The lipid particle of claim 97 or the method of claim 97, wherein the one or more stealth lipids in the lipid particle are in a molar amount of about 0.5%, about 1%, about 1.5%, about 2%, about 2.5%, or about 3%.
102. The lipid particle of claim 97 or the method of claim 97, wherein the one or more stealth lipids in the lipid particle are in a molar amount of about 1%.
103. The lipid particle of claim 97 or the method of claim 97, wherein the one or more stealth lipids in the lipid particle are in a molar amount of about 1.5%.
104. The lipid particle of claim 97 or the method of claim 97, wherein the one or more stealth lipids in the lipid particle are in a molar amount of about 2%.
105. The lipid particle of claim 97 or the method of claim 97, wherein the one or more stealth lipids in the lipid particle are in a molar amount of about 2.5%.
106. The lipid particle of claim 97 or the method of claim 97, wherein the one or more stealth lipids in the lipid particle are in a molar amount of about 3%.

107. The lipid particle of any one of claims 97-106 or the method of any one of claims 94-103, wherein the one or more stealth lipids are one or more PEG terminated lipids, one or more polysarcosine derivatives, or combinations thereof.

108. The lipid particle of claim 107 or the method of claim 107, wherein the one or more stealth lipids are one or more PEG terminated lipids.

109. The lipid particle of claim 108 or the method of claim 108, wherein the one or more PEG terminated lipids in the lipid particle are one or more selected from the group consisting of 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 ("DMG-PEG2000"), distearoyl-rac-glycerol-PEG2K ("DSG-PEG2k"), [(2R)-2,3-di(octadecanoyloxy)propyl] 2-(2-methoxyethoxycarbonylamino)ethyl phosphate ("C18-mPEG2000"), [3-[3-(2-methoxyethoxy)propylcarbamoyloxy]-2-tetradecanoyloxypropyl] tetradecanoate ("PEG2000-c-DMG"), 3-[hydroxy-[2-[2-(2-methoxyethoxy)ethylamino]ethoxy]phosphoryl]oxy-2-tetradecanoyloxypropyl] tetradecanoate ("DMPE-PEG2000"), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] ("18:1 PEG2000-PE"), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)-2000] ("DSPE-PEG2000-COOH), and Bis(1,2-distearoyl-sn-glycero-3-phosphoethanolamine)-N-[(polyethylene glycol)-2000] ("Bis-DSPE-PEG2000").

110. The lipid particle of claim 109 or the method of claim 109, wherein the one or more PEG terminated lipids in the lipid particle is DMG-PEG2000, DMPE-PEG2000, or a combination thereof.

111. The lipid particle of any one of claims 108-110 or the method of any one of claims 105-107, wherein only one PEG terminated lipid is in the lipid particle.

112. The lipid particle of claim 108 or the method of claim 111, wherein the only PEG terminated lipid in the lipid particle is DMG-PEG2000.

113. The lipid particle of claim 108 or the method of claim 111, wherein the only PEG terminated lipid in the lipid particle is DMPE-PEG2000.

114. The lipid particle of claim 108 or the method of claim 111, wherein the only PEG terminated lipid in the lipid particle is PEG2000-PE.

115. The lipid particle of claim 104 or the method of claim 107, wherein the one or more stealth lipids are one or more polysarcosine derivative.

116. The lipid particle of claim 112 or the method of claim 115, wherein the one or more polysarcosine derivatives in the lipid particle are one or more selected from the group consisting of N-tetradecyl-polysarcosine-25 ("N-tetradecyl-pSar25"), N-hexadecyl-polysarcosine-25 ("N-

hexadecyl-pSar25”), N-octadecyl-polysarcosine-25, N-dodecyl-polysarcosine-25 (“N-octadecyl-pSar25”), 1,2-dimyristoyl-sn-glycero-3-succinyl-N-polysarcosine-25 (“DMG-pSar25”), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-polysarcosine-25 (ammonium salt) (“18:1 PE (DOPE), N,N-ditetradecylamine-N-succinyl[methyl(polysarcosine)45] (“N-TETAMINE-pSar45”), N,N-ditetradecylamine-N-succinyl[methyl(polysarcosine)35] (“N-TETAMINE-pSar35”), N,N-ditetradecyl-polysarcosine-25 (“N-TETAMINE-pSar25”), N-TETAMINE-pSar45-maleimide, or N-TETAMINE-PEOZ-40.

117. The lipid particle of claim 115 or 116 or the method of claim 115 or 116, wherein only one polysarcosine derivative is in the lipid particle.

118. The lipid particle of claim 1 or the method of claim 2 or 3, wherein the lipid particle comprises about 25% to about 45% of SS-OP, about 5% to about 25% of DODG, about 15% to about 35% of cholesterol, about 11% to about 30% CHEMS, and about 0.5% to about 2.5% of DMG-PEG2000 (in molar amounts).

119. The lipid particle of claim 118 or the method of claim 118, wherein the lipid particle comprises about 35% of SS-OP, about 15% of DODG, about 28.5% of cholesterol, about 20% CHEMS, and about 1.5% of DMG-PEG2000 (in molar amounts).

120. The lipid particle of claim 1 or the method of claim 2 or 3, wherein the lipid particle comprises about 25% to about 45% of SS-OP, about 5% to about 25% of DOPE, about 15% to about 35% of cholesterol, about 11% to about 30% CHEMS, and about 0.5% to about 2.5% of DMG-PEG2000 (in molar amounts).

121. The lipid particle of claim 121 or the method of claim 121, wherein the lipid particle comprises about 35% of SS-OP, about 15% of DOPE, about 28.5% of cholesterol, about 20% CHEMS, and about 1.5% of DMG-PEG2000 (in molar amounts).

122. The lipid particle of claim 1 or the method of claim 2 or 3, wherein the lipid particle comprises about 25% to about 45% of SS-OP, about 5% to about 25% of DODG, about 15% to about 35% of cholesterol, about 11% to about 30% CHEMS, and about 0.5% to about 2.5% of DMPE-PEG2000 (in molar amounts).

123. The lipid particle of claim 122 or the method of claim 122, wherein the lipid particle comprises about 35% of SS-OP, about 15% of DODG, about 28.5% of cholesterol, about 20% CHEMS, and about 1.5% of DMPE-PEG2000 (in molar amounts)

124. The lipid particle of claim 1 or the method of claim 2 or 3, wherein the lipid particle comprises about 25% to about 45% of SS-OP, about 5% to about 25% of DOPE, about 15% to

about 35% of cholesterol, about 11% to about 30% CHEMS, and about 0.5% to about 2.5% of DMPE-PEG2000 (in molar amounts).

125. The lipid particle of claim 124 or the method of claim 124, wherein the lipid particle comprises about 35% of SS-OP, about 15% of DOPE, about 28.5% of cholesterol, about 20% CHEMS, and about 1.5% of DMPE-PEG2000 (in molar amounts)

126. The lipid particle of claim 1 or the method of claim 2 or 3, wherein the lipid particle comprises about 25% to about 45% of SS-OP, about 5% to about 25% of DODG, about 15% to about 35% of cholesterol, about 11% to about 30% CHEMS, and about 0.5% to about 2.5% of 18:1 PEG2000-PE (in molar amounts).

127. The lipid particle of claim 126 or the method of claim 126, wherein the lipid particle comprises about 35% of SS-OP, about 15% of DODG, about 28.5% of cholesterol, about 20% CHEMS, and about 1.5% of 18:1 PEG2000-PE (in molar amounts)

128. The lipid particle of claim 1 or the method of claim 2 or 3, wherein the lipid particle comprises about 25% to about 45% of SS-OP, about 5% to about 25% of DOPE, about 15% to about 35% of cholesterol, about 11% to about 30% CHEMS, and about 0.5% to about 2.5% of 18:1 PEG2000-PE (in molar amounts).

129. The lipid particle of claim 128 or the method of claim 128, wherein the lipid particle comprises about 35% of SS-OP, about 15% of DOPE, about 28.5% of cholesterol, about 20% CHEMS, and about 1.5% of 18:1 PEG2000-PE (in molar amounts).

130. The lipid particle of claim 1 or the method of claim 2 or 3, wherein the lipid particle comprises about 30% to about 50% of SS-OP, about 15% to about 55% of cholesterol, about 11% to about 30% CHEMS, and about 0.5% to about 2.5% of DMG-PEG2000 (in molar amounts).

131. The lipid particle of claim 130 or the method of claim 130, wherein the lipid particle comprises about 40% of SS-OP, about 38.5% of cholesterol, about 20% CHEMS, and about 1.5% of DMG-PEG2000 (in molar amounts).

132. The lipid particle of claim 1 or the method of claim 2 or 3, wherein the lipid particle comprises about 20% to about 45% of SS-OP, about 15% to about 55% of cholesterol, about 15% to about 35% CHEMS, and about 0.5% to about 2.5% of DMG-PEG2000 (in molar amounts).

133. The lipid particle of claim 132 or the method of claim 132, wherein the lipid particle comprises about 35% of SS-OP, about 38.5% of cholesterol, about 25% CHEMS, and about 1.5% of DMG-PEG2000 (in molar amounts).

134. The lipid particle of claim 1 or the method of claim 2 or 3, wherein the lipid particle comprises about 20% to about 40% of SS-OP, about 15% to about 55% of cholesterol, about 20% to about 40% CHEMS, and about 0.5% to about 2.5% of DMG-PEG2000 (in molar amounts).

135. The lipid particle of claim 134 or the method of claim 134, wherein the lipid particle comprises about 30% of SS-OP, about 38.5% of cholesterol, about 30% CHEMS, and about 1.5% of DMG-PEG2000 (in molar amounts).

136. The lipid particle of claim 1 or the method of claim 2 or 3, wherein the lipid particle comprises about 15% to about 35% of SS-OP, about 15% to about 55% of cholesterol, about 25% to about 45% CHEMS, and about 0.5% to about 2.5% of DMG-PEG2000 (in molar amounts).

137. The lipid particle of claim 136 or the method of claim 136, wherein the lipid particle comprises about 25% of SS-OP, about 38.5% of cholesterol, about 35% CHEMS, and about 1.5% of DMG-PEG2000 (in molar amounts).

138. The lipid particle of claim 1 or the method of claim 2 or 3, wherein the lipid particle comprises about 30% to about 50% of SS-OP, about 15% to about 55% of cholesterol, about 15% to about 35% CHEMS, and about 0.5% to about 2.5% of DMG-PEG2000 (in molar amounts).

139. The lipid particle of claim 138 or the method of claim 138, wherein the lipid particle comprises about 40% of SS-OP, about 33.5% of cholesterol, about 25% CHEMS, and about 1.5% of DMG-PEG2000 (in molar amounts).

140. The lipid particle of claim 1 or the method of claim 2 or 3, wherein the lipid particle comprises about 30% to about 50% of SS-OP, about 15% to about 55% of cholesterol, about 20% to about 40% CHEMS, and about 0.5% to about 2.5% of DMG-PEG2000 (in molar amounts).

141. The lipid particle of claim 140 or the method of claim 140, wherein the lipid particle comprises about 40% of SS-OP, about 28.5% of cholesterol, about 30% CHEMS, and about 1.5% of DMG-PEG2000 (in molar amounts).

142. A lipid particle comprising:

- a. one or more ionizable lipids in a molar amount of about 20% to about 70%;
- b. one or more neutral lipids in a molar amount of about 2.5% to about 25%;
- c. one or more sterols in a molar amount of about 20% to about 50%;
- d. one or more charged lipids in a molar amount of about 10% or less than 10%; and

e. one or more stealth lipids in a molar amount of about 0.25% to about 3%.

143. The lipid particle of claim 142, wherein the one or more charged lipids in the lipid particle is selected from the group consisting of cholesteryl hemisuccinate ("CHEMS"), 1,2-distearoyl-3-trimethylammonium-propane ("DSTAP"), 18:1 1,2-dioleoyl-3-trimethylammonium-propane ("DOTAP"), 1,2-dioleoyl-sn-glycero-3-phosphate ("18:1 PA"), 18:1 1-stearoyl-2-oleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) ("18:1 PG"), phosphatidylglycerols, cardiolipins, diacylphosphatidylserines, diacylphosphatidic acids, N-dodecanoyl phosphatidylethanolamines, N-succinyl phosphatidylethanolamines, N-glutarylphosphatidylethanolamines, lysylphosphatidylglycerols, palmitoyloleyolphosphatidylglycerol ("POPG"), 1,2-dimyristoyl-3-trimethylammonium-propane ("DMTAP"), 1,2-dipalmitoyl-3-trimethylammonium-propane ("DPTAP"), palmitoyloleyoyl-3-trimethylammonium-propane ("POTAP"), 1,2-dioleoyl-3-dimethylammonium-propane ("DODAP"), palmitoyloleyoyl-3-dimethylammonium-propane ("PODAP"), 1,2-dimyristoyl-3-dimethylammonium-propane ("DMDAP"), 1,2-dipalmitoyl-3-dimethylammonium-propane ("DPDAP"), 1,2-distearoyl-3-dimethylammonium-propane ("DSDAP"), 1,2-dioleoyl-3-dimethylhydroxyethyl-ammonium-propane ("DODMHEAP") (also known as DORI), palmitoyloleyoyl-3-dimethylhydroxyethyl-ammonium-propane ("PODMHEAP") (also known as PORI), 1,2-dimyristoyl-3-dimethylhydroxyethyl-ammonium-propane ("DMDMHEAP") (also known as DMRI), 1,2-dipalmitoyl-3-dimethylhydroxyethyl-ammonium-pPropane ("DPDMHEAP") (also known as DPRI), 1,2-distearoyl-3-dimethylhydroxyethyl-ammonium-propane ("DSDMHEAP") (also known as DSRI), 1,2-diioleoyl-3-methylhydroxyethylammonium-propane ("DOMDHEAP"), palmitoyloleyoyl-3-methylhydroxyethylammonium-propane ("POMDHEAP"), 1,2-dimyristoyl-3-methyldihydroxyethylammonium-propane ("DMMDHEAP"), 1,2-dipalmitoyl-3-methyldihydroxyethylammonium-propane ("DPMDHEAP"), 1,2-distearoyl-3-methyldihydroxyethylammonium-propane ("DSMDHEAP"), 1,2-dioleoyl-3-methyldihydroxyethylammonium-propane ("DOMHEAP"), palmitoyloleyoyl-3-methylhydroxyethylammonium-propane ("POMHEAP"), 1,2-dimyristoyl-3-methylhydroxyethylammonium-propane ("DMMHEAP"), 1,2-dipalmitoyl-3-methylhydroxyethylammonium-propane ("DPMHEAP"), 1,2-distearoyl-3-methylhydroxyethylammonium-propane ("DSMHEAP"), 1,2-dioleoyl-3-dihydroxyethylammonium-propane ("DODHEAP"), palmitoyloleyoyl-3-dihydroxyethylammonium-propane ("PODHEAP"), 1,2-dimyristoyl-3-dihydroxyethylammonium-propane ("DMDHEAP"), 1,2-dipalmitoyl-3-

dihydroxyethylammonium-propane ("DPDHEAP"), 1,2-distearoyl-3-dihydroxyethylammonium-propane ("DSDHEAP"), dimethyldioctadecylammonium bromide ("DDAB"), dioleyldimethylammonium chloride ("DODAC"), 1,2-dioleoyl-sn-glycero-3-ethylphosphocholine ("DOEPC"), 1,2-dimyristoyl-sn-glycero-3-ethylphosphocholine ("DMEPC"), 1,2-dipalmitoyl-sn-glycero-3-ethylphosphocholine ("DPEPC"), 1,2-distearoyl-sn-glycero-3-ethylphosphocholine ("DSEPC"), palmitoyloleoyl-sn-glycero-3-ethylphosphocholine ("POEPC"), 1,2-dioleoyl-3-dimethyl-hydroxyethyl ammonium propane ("DORIE"), 1,2-dimyristyl-3-dimethyl-hydroxyethyl ammonium propane ("DMRIE"), 1,2-dioleoyl-3-methyl-(methoxycarbonyl-ethyl)ammonium-propane ("DOMCAP"), 1,2-dioleoyl-3-methyl-(methoxycarbonylmethyl)ammonium-propane ("DOMGME"), 1,2-dioleoyl-3-N-pyrrolidine-propane ("DOP5P"), 1,2-dioleoyl-3-N-pyrridinium-propane, bromide salt ("DOP6P"), 3b-[N-(N9,N9-dimethylaminoethane)carbamoyl] cholesterol ("DC-Chol"), 3b-[N-(N9,N9-trimethylaminoethane) carbamoyl] cholesterol ("TC-Chol"), 3b(N-(N,N'-Dimethylaminoethan)-carbamoyl)cholesterol ("DAC-Chol"), cholesteryl-oxycarbonyl-methyl-trimethylammonium chloride ("Chol-Betaine"), N-methyl{4-N-amino[(3'- $\beta$ -cholesteryl) carbamoyl]}piperazine ("N-methyl-PipChol"), cetyltrimethylammonium bromide ("CTAB"), N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethyl ammonium chloride ("DOTMA"), 4-(2-aminoethyl)-morpholino-cholesterolhemisuccinate: ("MoChol"), histaminy-Cholesterolhemisuccinate ("HisChol"), cholesterol-(3-imidazol-1-yl propyl)carbamate ("Chim"), (N-2-propylamino[(3'- $\beta$ -cholesteryl) carbamoyl]}morpholine ("MoC3Chol"), [(3-morpholine-4-yl-propylcarbamoyl)-methyl]-carbamic acid cholesteryl ester ("Chol-C3N-Mo3"), ("Chol-C3N-Mo2"), [(2-morpholine-4-yl-ethylcarbamoyl)methyl]-carbamic acid cholesteryl ester ("Chol-C4N-Mo2"), [1-methyl-2-(2-morpholine-4-yl-ethylcarbamoyl)-propyl]-carbamic acid cholesteryl ester ("Chol-DMC3N-Mo2"), 2-(2-morpholine-4-yl-ethylcarbamoyl)-cyclohexane carboxylic acid cholesteryl ester ("CholC4Hex-Mo2"), 4-(2-aminoethyl)-morpholino-cholesterol-2,3-dimethylhemisuccinate ("DmC4Mo2"), 4-(2-aminoethyl)-morpholino-cholesterol-2,2-dimethylhemimalonate ("DmC3Mo2"), 4-(2-aminoethyl)-morpholino-cholesterol-hemimalonate ("C3Mo2"), 4-(2-aminopropyl)-morpholino-cholesterol-hemimalonate ("C3Mo3"), 4-(2-aminoethyl)-morpholino-cholesterol-hemiglutarate ("C5Mo2"), 4-(2-aminoethyl)-morpholino-cholesterol-hemiadipate ("C6Mo2"), 4-(2-aminoethyl)-morpholino-cholesterol-hemiadipate ("C8Mo2"), 4-(2-aminobutyl)-morpholino-cholesterol-hemisuccinate ("C4Mo4"), 4{N-2-ethylamino[(3'- $\beta$ -cholesteryl) carbamoyl]}piperazine ("PipC2Chol"), {N-2-ethylamino[(3'- $\beta$ -cholesteryl) carbamoyl]}morpholine ("MoC2Chol"), {N-2-ethylamino[(3'- $\beta$ -cholesteryl)



carbamoyl]]pyrrolidine ("PyrroC2Chol"), {N-2-propylamino[(3'-β-cholesteryl) carbamoyl]]imidazole ("ImC3Chol"), {N-2-ethylamino[(3'-β-cholesteryl) carbamoyl]]pyridine ("PyC2Chol"), 1,2-dioleoyl-3-N-morpholine-propane ("MoDO"), 1,2-dipalmitoyl-3-N-morpholine-propane ("MoDP"), 4,(2,3-bis-acyloxy-propyl)-1-methyl-1H-imidazole ("DOIM") (also known as DPIM), diacylglycerolhemisuccinate, e.g. dioctadecylamido-glycylspermine ("DOGS"), dimyristoylglycerolhemisuccinate ("DMGS") (also known as DMG-Succ), 1-palmitoyl-2-oleoylglycerolhemisuccinate ("POGS") (also known as POG-Succ), dipalmitoylglycerolhemisuccinate ("DPGS") (also known as POG-Succ), distearoylglycerolhemisuccinate ("DSGS") (also known as DSG-Succ), diacylglycerolhemimalonate, e.g. dioleoylglycerolhemimalonate ("DOGM"), dimyristoylglycerolhemimalonate ("DMGM"), diacylglycerolhemiglutarate, e.g. dioleoylglycerolhemiglutarate ("DOGG"), dimyristoylglycerolhemiglutarate ("DMGG"), diacylglycerolhemiadipate, e.g. dioleoylglycerolhemiadipate ("DOGA"), dimyristoylglycerolhemiadipate ("DMGA"), diacylglycerolhemicyclohexane-1,4-dicarboxylic acid, e.g. dioleoylglycerolhemicyclohexane-1,4-dicarboxylic acid ("DO-cHA"), dimyristoylglycerolhemicyclohexane-1,4-dicarboxylic acid ("DM-cHA"), (2,3-Diacyl-propyl)amino}-oxoalkanoic acid, e.g. 4-{(2,3-dioleoyl-propyl)amino}-4-oxobutanoic acid ("DOAS"), 3-{(2,3-dioleoyl-propyl)amino}-3-oxopropanoic acid ("DOAM"), 5-{(2,3-dioleoyl-propyl)amino}-5-oxopentanoic acid ("DOAG"), 6-{(2,3-dioleoyl-propyl)amino}-6-oxohexanoic acid ("DOAA"), 4-{(2,3-dimyristoyl-propyl)amino}-4-oxobutanoic acid ("DMAS"), 3-{(2,3-dimyristoyl-propyl)amino}-3-oxopropanoic acid ("DMAM"), 5-{(2,3-dimyristoyl-propyl)amino}-5-oxopentanoic acid ("DMAG"), 6-{(2,3-dimyristoyl-propyl)amino}-6-oxohexanoic acid ("DMAA"), diacyl-alkanoic acid, e.g. 2,3-dioleoyl-propanoic acid ("DOP"), 3,4-dioleoyl-butanoic acid ("DOB"), 5,6-dioleoyl-hexanoic acid ("DOS"), 4,5-dioleoyl-pentanoic acid ("DOM"), 6,7-dioleoyl-heptanoic acid ("DOG"), 7,8-dioleoyl-octanoic acid ("DOA"), 2,3-dimyristoyl-propanoic acid ("DMP"), 3,4-dioleoyl-butanoic acid ("DOB"), 5,6-dimyristoyl-hexanoic acid ("DMS"), 4,5-dimyristoyl-pentanoic acid ("DMM"), 6,7-dimyristoyl-heptanoic acid ("DMG"), 7,8-dimyristoyl-octanoic acid ("DMA"), cholesteryloxycarbonylaminocarboxylic acid, e.g. cholesterolhemidodecane dicarboxylic acid ("Chol-C12"), 12-cholesteryloxycarbonylaminododecanoic acid ("CholC13N"), fatty acids, e.g. oleic acid, myristic acid, palmitic acid, stearic acid, nervonic acid, behenic acid, dioleoylphosphatidic acid ("DOPA"), 1,2-dimyristoyl-sn-glycero-3-phosphate ("DMPA"), 1,2-dipalmitoyl-sn-glycero-3-phosphate ("DPPA"), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphate ("POPA"), 1,2-Distearoyl-

sn-glycero-3-phosphate ("DSPA"), cholesterol sulphate ("Chol-SO<sub>4</sub>"), dioleoylphosphatidylglycerol ("DOPG"), 1,2-dimyristoyl-sn-glycero-3-phospho-(1'-rac-glycerol), ("DMPG"), dipalmitoylphosphatidylglycerol ("DPPG"), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol ("POPG"), 1,2-distearoyl-sn-glycero-3-phospho-rac-glycerol ("DSPG"), dioleoylphosphatidylserine ("DOPS"), 1,2-dimyristoyl-sn-glycero-3-phospho-L-serine ("DMPS"), dipalmitoylphosphatidylserine ("DPPS"), 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine ("POPS"), 1,2-distearoyl-sn-glycero-3-phospho-L-serine ("DSPS"), or cetyl-phosphate.

144. The lipid particle of claim 142, wherein the one or more charged lipids in the lipid particle is selected from the group consisting of cholesteryl hemisuccinate ("CHEMS"), 1,2-distearoyl-3-trimethylammonium-propane ("DSTAP"), 18:1 1,2-dioleoyl-3-trimethylammonium-propane ("DOTAP"), 1,2-dioleoyl-sn-glycero-3-phosphate ("18:1 PA"), 18:1 1-stearoyl-2-oleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) ("18:1 PG"), phosphatidylglycerols, cardiolipins, diacylphosphatidylserines, diacylphosphatidic acids, N-dodecanoyl phosphatidylethanolamines, N-succinyl phosphatidylethanolamines, N-glutarylphosphatidylethanolamines, lysylphosphatidylglycerols, and palmitoyl-oleoylphosphatidylglycerol ("POPG").

145. The lipid particle of any one of claims 142-144, wherein only one charged lipid is in the lipid particle.

146. The lipid particle of claim 145, wherein the only charged lipid in the lipid particle is CHEMS.

147. The lipid particle of any one of claims 142-146, wherein the one or more charged lipids in the lipid particle are in a molar of about 10%, less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, less than 4%, less than 3%, less than 2%, less than 1%, or less than 0.1%.

148. The lipid particle of any one of claims 142-146, wherein the one or more charged lipids in the lipid particle are in a molar amount of about 1% to about 10%, about 1% to about 9%, about 1% to about 8%, about 1% to about 7%, about 1% to about 6%, about 1% to about 5%, about 1% to about 4%, about 1% to about 3%, about 1% to about 2%, about 2% to about 10%, about 2% to about 9%, about 2% to about 8%, about 2% to about 7%, about 2% to about 6%, about 2% to about 5%, about 2% to about 4%, about 2% to about 3%, about 3% to about 10%, about 3% to about 9%, about 3% to about 8%, about 3% to about 7%, about 3% to about 6%, about 3% to about 5%, about 3% to about 4%, about 4% to about 10%, about 4% to about 9%, about 4% to about 8%, about 4% to about 7%, about 4% to about 6%, about 4% to about 5%, about 5% to

about 10%, about 5% to about 9%, about 5% to about 8%, about 5% to about 7%, or about 5% to about 6%.

149. The lipid particle of claim 142-146, wherein the one or more charged lipids in the lipid particle are in a molar amount of about 10%.

150. The lipid particle of claim 142, which does not comprise a charged lipid.

151. The lipid particle of any one of claims 142-150, further comprising a payload.

152. A method of targeting a payload to a tissue other than a spleen of a subject in vivo comprising combining a lipid particle or a composition comprising the payload encapsulated within a lipid particle or a composition comprising one or more ionizable lipids, one or more neutral lipids, one or more sterols, one or more stealth lipids, and/or one or more charged lipids, wherein the lipid particle or composition does not comprise CHEMS or comprises a molar amount of about 10% or less than 10% of CHEMS.

153. The method of claim 152, wherein the CHEMS is in a molar of about 10%, less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, less than 4%, less than 3%, less than 2%, less than 1%, or less than 0.1%.

154. The method of claim 152, wherein the CHEMS is in a molar amount of about 1% to about 10%, about 1% to about 9%, about 1% to about 8%, about 1% to about 7%, about 1% to about 6%, about 1% to about 5%, about 1% to about 4%, about 1% to about 3%, about 1% to about 2%, about 2% to about 10%, about 2% to about 9%, about 2% to about 8%, about 2% to about 7%, about 2% to about 6%, about 2% to about 5%, about 2% to about 4%, about 2% to about 3%, about 3% to about 10%, about 3% to about 9%, about 3% to about 8%, about 3% to about 7%, about 3% to about 6%, about 3% to about 5%, about 3% to about 4%, about 4% to about 10%, about 4% to about 9%, about 4% to about 8%, about 4% to about 7%, about 4% to about 6%, about 4% to about 5%, about 5% to about 10%, about 5% to about 9%, about 5% to about 8%, about 5% to about 7%, or about 5% to about 6%.

155. The method of claim 152, wherein the CHEMS is in a molar amount of about 10%.

156. The method of claim 152, which does not comprise CHEMS.

157. The method of any one of claims 152-156, wherein the one or more ionizable lipids are in a molar amount of about 20% to about 70%.

158. The method of any one of claims 152-157, wherein the one or more neutral lipids are in a molar amount of about 2.5% to about 25%.

159. The method of any one of claims 152-158, wherein the one or more sterols is in a molar amount of about 20% to about 50%.

160. The method of any one of claims 152-159, wherein the one or more stealth lipids are in a molar amount of about 0.25% to about 3%.

161. The lipid particle of any one of claims 1142-151 or the method of any one of claims 148-156, wherein the lipid particle delivers the payload to the liver.

162. The lipid particle of claim 161 or the method of claim 161, wherein the ratio of the payload delivered to the liver versus is spleen is greater than 1.1.

163. The lipid particle of claim 161 or the method of claim 161, wherein the ratio of the payload delivered to the liver versus spleen is about 1.1 to about 12.

164. The lipid particle of any one of claims 142-151 and 161-163 or the method of any one of claims 152-163, wherein the ratio of payload delivered to the spleen versus liver is determined using an imaging system.

165. The lipid particle of a claim 164 or the method of claim 164, wherein the imaging system is a LICOR Pearl Trilogy imaging system using Image Studio software from LICOR version 5.2.5.

166. The lipid particle of any one of claims 142-151 and 161-165 or the method of any one of claims 152-166, wherein the lipid particles comprise one or more ionizable lipids.

167. The lipid particle of claim 166 or the method of claim 166, wherein the one or more ionizable lipids in the lipid particle are in a molar amount of about 25% to about 65%, about 30% to about 65%, about 35% to about 65%, about 45% to about 65%, about 50% to about 65%, about 25% to about 60%, about 30% to about 60%, about 35% to about 60%, about 45% to about 60%, about 50% to about 60%, about 25% to about 55%, about 30% to about 55%, about 35% to about 55%, about 45% to about 55%, about 50% to about 55%, about 25% to about 50%, about 30% to about 50%, about 35% to about 50%, or about 45% to about 50%.

168. The lipid particle of claim 166 or the method of claim 166, wherein the one or more ionizable lipids in the lipid particle are in a molar amount of about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, or about 65%.

169. The lipid particle of claim 166 or the method of claim 166, wherein the one or more ionizable lipids in the lipid particle are in a molar amount of about 30% to about 60%.

170. The lipid particle of claim 166 or the method of claim 166, wherein the one or more ionizable lipids in the lipid particle are in a molar amount of about 40% to about 60%.

171. The lipid particle of a claim 166 or the method of claim 166, wherein the one or more ionizable lipids in the lipid particle are in a molar amount of about 40% to about 50%.

172. The lipid particle of claim 166 or the method of claim 166, wherein the one or more ionizable lipids in the lipid particle are in a molar amount of about 50% to about 60%.
173. The lipid particle of claim 166 or the method of claim 166, wherein the one or more ionizable lipids in the lipid particle are in a molar amount of about 30%.
174. The lipid particle of claim 166 or the method of claim 166, wherein the one or more ionizable lipids in the lipid particle are in a molar amount of about 40%.
175. The lipid particle of claim 166 or the method of claim 166, wherein the one or more ionizable lipids in the lipid particle are in a molar amount of about 50%.
176. The lipid particle of claim 166 or the method of claim 166, wherein the one or more ionizable lipids in the lipid particle are in a molar amount of about 55%.
177. The lipid particle of claim 166 or the method of claim 166, wherein the one or more ionizable lipids in the lipid particle are in a molar amount of about 60%.
178. The lipid particle of claim 166 or the method of claim 166, wherein the one or more ionizable lipids in the lipid particle are in a molar amount of about 65%.
179. The lipid particle of claim 166 or the method of claim 166, wherein the one or more ionizable lipids in the lipid particle are in a molar amount of about 70%.
180. The lipid particle of any one of claims 166-179 or the method of any one claims 166-179, wherein the one or more ionizable lipids in the lipid particles are one or more selected from the group consisting of DLin-MC3-DMA ("MC3"), DLin-KC2-DMA ("KC2"), ssPalmO-Phe ("SS-OP"), C12-200, SM-102,  $\alpha$ -D-tocophersuccinoyl ("SS-EC"), ALC-0315 ("ALC"), Tri-N-tridecyl 3-(ethyl(methyl)amino)propanoate ("304-O13"), tetrakis(2-(octyldisulfaneyl)ethyl) 3,3',3",3'''-(((methylazanediyl)bis(propane-3,1-diyl))bis(azanetriyl))tetrapropionate ("306-O12B"), 9-[4-(dimethylamino)-1-oxobutoxy]-heptadecanedioic acid, 1,17-di-(2Z)-2-nonen-1-yl ester ("L319"), 9,12-octadecadienoic acid, (9Z,12Z)-1,1',1'',1'''-[(3,6-dioxo-2,5-piperazinediyl)bis(4,1-butanediyl)nitrilodi-4,1-butanediyl] ester ("OF-C4-Deg-Lin"), 2-(dioctylamino)ethyl nonyl hydrogen phosphate ("9A1P9"), 5-(((3-(dibutylamino)propyl)amino)methyl)-6-hydroxyundecane-1,11-diyl (9Z,9'Z,12Z,12'Z)-bis(octadeca-9,12-dienoate) ("IR-117-17"), 9Z,12Z-octadecadienoic acid, 3-[4,4-bis(octyloxy)-1-oxobutoxy]-2-[[[3-(diethylamino)propoxy]carbonyl]oxy]methyl]propyl ester ("LP-01") and 4A3-SC8
181. The lipid particle of any one of claims 166-179 or the method of any one of claims 166-179, wherein the one or more ionizable lipids in the lipid particle are one or more selected from

the group consisting of DLin-MC3-DMA ("MC3"), DLin-KC2-DMA ("KC2"), ssPalmO-Phe ("SS-OP"), C12-200, SM-102,  $\alpha$ -D-tocopherolsuccinoyl ("SS-EC"), and ALC-0315 ("ALC").

182. The lipid particle of claim 181 or the method of claim 181, wherein the one or more ionizable lipids in the lipid particle are one or more selected from the group consisting of ssPalmO-Phe ("SS-OP"), C12-200, SM-102, and ALC-0315 ("ALC").

183. The lipid particle of any one of claims 166-182 or the method of any one of claims 166-182, wherein only one ionizable lipid is in the lipid particle.

184. The lipid particle of claim 183 or the method of claim 183, wherein the only ionizable lipid in the lipid particle is SS-OP.

185. The lipid particle of claim 183 or 184 or the method of claim 183 or 184, wherein when administered to a subject in need thereof, the subject exhibits one or more of: (i) an increase in Treg cells, (ii) reduction in effector T cells, (iii) reduction in B-cells, (iv) reduction in cytokine production, (v) reduction in activated B cells; (vi) reduction in activated effector T cells; or (vii) any combination thereof.

186. The lipid particle of claim 183 or 184 or the method of claim 183 or 184, which when administered to a subject in need thereof, reduces B-cell levels in the subject.

187. The lipid particle of claim 183 or 184 or the method of claim 183 or 184, which when administered to a subject in need thereof, reduces B-cell levels in the subject as compared with a reference lipid particle containing an ionizable lipid other than SS-OP.

188. The lipid particle of claim 183 or 184 or the method of claim 183 or 184, which when administered to a subject in need thereof, reduces B-cell levels in the subject as compared with a reference lipid particle containing MC-3 as the ionizable lipid.

189. The lipid particle of any one of claims 183-188 or the method of any one of claims 183-188, which when administered to a subject in need thereof, reduces T-cell levels in the subject.

190. The lipid particle of any one of claims 183-188 or the method of any one of claims 183-188, which when administered to a subject in need thereof, reduces T-cell levels in the subject as compared with a reference lipid particle containing an ionizable lipid other than SS-OP.

191. The lipid particle of any one of claims 183-188 or the method of any one of claims 183-188, which when administered to a subject in need thereof, reduces T-cell levels in the subject as compared with a reference lipid particle containing MC-3 as the ionizable lipid.

192. The lipid particle of any one of claims 183-191 or the method of any one of claims 183-191, which when administered to a subject in need thereof, reduces levels of IFN- $\alpha$  in the subject.

193. The lipid particle of any one of claims 183-191 or the method of any one of claims 183-191, which when administered to a subject in need thereof, reduces levels of IFN- $\alpha$  in the subject as compared with a reference lipid particle containing an ionizable lipid other than SS-OP
194. The lipid particle of any one of claims 183-191 or the method of any one of claims 183-191, which when administered to a subject in need thereof, reduces levels of IFN- $\alpha$  in the subject as compared with a reference lipid particle containing MC-3 as the ionizable lipid.
195. The lipid particle of any one of claims 166-179 or the method of any one of claims 166-179, wherein the one or more ionizable lipids in the lipid particle comprises  $\alpha$ -D-tocopherolsuccinoyl ("SS-EC").
196. The lipid particle of claim 195 or the method of claim 195, which when administered to a subject in need thereof, increases B-cell levels in the subject.
197. The lipid particle of claim 195 or the method of claim 195, which when administered to a subject in need thereof, increases B-cell levels in the subject as compared with a reference lipid particle containing an ionizable lipid other than SS-EC.
198. The lipid particle of claim 195 or the method of claim 195, which when administered to a subject in need thereof, increases B-cell levels in the subject as compared with a reference lipid particle containing MC-3 as the ionizable lipid.
199. The lipid particle of any one of claims 195-198 or the method of any one of claims 190-193, which when administered to a subject in need thereof, increases T-cell levels in the subject.
200. The lipid particle of any one of claims 195-198 or the method of any one of claims 190-193, which when administered to a subject in need thereof, increases T-cell levels in the subject as compared with a reference lipid particle containing an ionizable lipid other than SS-EC.
201. The lipid particle of any one of claims 195-198 or the method of any one of claims 190-193, which when administered to a subject in need thereof, increases T-cell levels in the subject as compared with a reference lipid particle containing MC-3 as the ionizable lipid.
202. The lipid particle of any one of claims 195-201 or the method of any one of claims 190-196, which when administered to a subject in need thereof, increases levels of IFN- $\alpha$  in the subject.
203. The lipid particle of any one of claims 195-201 or the method of any one of claims 190-196, which when administered to a subject in need thereof, increases levels of IFN- $\alpha$  in the subject as compared with a reference lipid particle containing an ionizable lipid other than SS-EC.

204. The lipid particle of any one of claims 195-201 or the method of any one of claims 190-196, which when administered to a subject in need thereof, increases levels of IFN- $\alpha$  in the subject as compared with a reference lipid particle containing MC-3 as the ionizable lipid.
205. The lipid particle of any one of claims 142-151 and 161-203 or the method of any one of claims 148-199, wherein the lipid particle comprises one or more neutral lipids.
206. The lipid particle of claim 205 or the method of claim 205, wherein the one or more neutral lipids in the lipid particle are in a molar amount of about 2.5% to about 20%, about 2.5% to about 17.5%, about 2.5% to about 15%, about 2.5% to about 12.5%, about 2.5% to about 10%, about 5% to about 25%, about 5% to about 20%, about 5% to about 17.5%, about 5% to about 15%, about 5% to about 12.5%, about 5% to about 10%, about 7.5% to about 25%, about 7.5% to about 20%, about 7.5% to about 17.5%, about 7.5% to about 15%, about 7.5% to about 12.5%, about 7.5% to about 10%, about 10% to about 25%, about 10% to about 20%, about 10% to about 17.5%, about 10% to about 15%, about 10% to about 12.5%, about 12.5% to about 25%, about 12.5% to about 20%, about 12.5% to about 17.5%, or about 12.5% to about 15%.
207. The lipid particle of claim 205 or the method of claim 205, wherein the one or more neutral lipids in the lipid particle are in a molar amount of about 5%, about 7.5%, about 10%, about 12.5%, about 15%, about 17.5%, about 20%, about 22.5%, or about 25%.
208. The lipid particle of claim 205 or the method of claim 205, wherein the one or more neutral lipids in the lipid particle are in a molar amount of about 5% to about 20%.
209. The lipid particle of claim 205 or the method of claim 205, wherein the one or more neutral lipids in the lipid particle are in a molar amount of about 7.5% to about 17.5%.
210. The lipid particle of claim 205 or the method of claim 205, wherein the one or more neutral lipids in the lipid particle are in a molar amount of about 2.5%.
211. The lipid particle of claim 205 or the method of claim 205, wherein the one or more neutral lipids in the lipid particle are in a molar amount of about 5%.
212. The lipid particle of claim 205 or the method of claim 205, wherein the one or more neutral lipids in the lipid particle are in a molar amount of about 7.5%.
213. The lipid particle of claim 205 or the method of claim 205, wherein the one or more neutral lipids in the lipid particle are in a molar amount of about 10%.
214. The lipid particle of claim 205 or the method of claim 205, wherein the one or more neutral lipids in the lipid particle are in a molar amount of about 12.5%.
215. The lipid particle of claim 205 or the method of claim 205, wherein the one or more neutral lipids in the lipid particle are in a molar amount of about 15%.



216. The lipid particle of claim 205 or the method of claim 205, wherein the one or more neutral lipids in the lipid particle are in a molar amount of about 17.5%.
217. The lipid particle of claim 205 or the method of claim 205, wherein the one or more neutral lipids in the lipid particle are in a molar amount of about 20%.
218. The lipid particle of claim 205 or the method of claim 205, wherein the one or more neutral lipids in the lipid particle are in a molar amount of about 25%.
219. The lipid particle of any one of claims 205-218 or the method of any one of claims 205-218, wherein the one or more neutral lipids in the lipid particles are one or more phosphatidylcholine ("PC"), phosphatidylethanolamine ("PE"), and combination thereof
220. The lipid particle of any one of claims 205-218 or the method of any one of claims 205-218, wherein the one or more neutral lipids in the lipid particle are one or more selected from the group consisting of wherein the one or more neutral lipids in the lipid particle are one or more selected from the group consisting of 1,2-diastearoyl-sn-glycero-3-phosphocholine ("DSPC"), 1,2-dioleoyl-sn-glycero-3-phosphocholine ("DOPC"), 1,2- dioctadecanoyl-sn-glycerol ("DSDG"), 1,2-dioleoyl-sn-glycerol, 1,2-di-(9Z-octadecenoyl)-sn-glycerol ("DODG"), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine ("DOPE"), diacylphosphatidylcholine, diacylphosphatidylethanolamine, ceramide, sphingomyelin, cephalin, cerebroside, diacylglycerols dipalmitoylphosphatidylcholine ("DPPC"), palmitoyl-oleoyl-phosphatidylcholine ("POPC"), palmitoyl-oleoyl-phosphatidylethanolamine ("POPE"), palmitoyl-oleoyl-phosphatidylglycerol (POPG), dipalmitoyl-phosphatidylethanolamine ("DPPE"), dimyristoyl-phosphatidylethanolamine ("DMPE"), distearoyl-phosphatidylethanolamine ("DSPE"), monomethyl-phosphatidylethanolamine, dimethyl-phosphatidylethanolamine, dielaidoyl-phosphatidylethanolamine ("DEPE"), stearyl-oleoyl-phosphatidylethanolamine ("SOPE"), and egg phosphatidylcholine ("EPC").
221. The lipid particle of any one of claims 205-220 or the method of any one of claims 205-220, wherein the one or more neutral lipids in the lipid particle are DOPE, DODG, or a combination thereof.
222. The lipid particle of any one of claims 205-2221 or the method of any one of claims 205-2221, wherein only one neutral lipid is in the lipid particle.
223. The lipid particle of claim 222 or the method of claim 222, wherein the only neutral lipid in the lipid particle is DOPE.
224. The lipid particle of claim 222 or the method of claim 222, wherein the only neutral lipid in the lipid particle is DODG.

225. The lipid particle of any one of claims 142-151 and 161-224 or the method of any one of claims 152-2224, wherein the lipid particle comprises one or more sterols.

226. The lipid particle of claim 225 or the method of claim 225, wherein the one or more sterols in the lipid particle are in a molar amount of about 20% to about 45%, about 25% to about 45%, about 30% to about 45%, about 35% to about 45%, about 40% to about 45%, about 20% to about 40%, about 25% to about 40%, about 30% to about 40%, about 35% to about 40%, about 20% to about 30%, about 25% to about 30%, or about 20% to about 25%.

227. The lipid particle of claim 225 or the method of claim 225, wherein the one or more sterols in the lipid particle are in a molar amount of about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, or about 50%.

228. The lipid particle of claim 225 or the method of claim 225, wherein the one or more sterols in the lipid particle are in a molar amount of about 20% to about 40%.

229. The lipid particle of claim 225 or the method of claim 225, wherein the one or more sterols in the lipid particle are in a molar amount of about 20% to about 30%.

230. The lipid particle of claim 225 or the method of claim 225, wherein the one or more sterols in the lipid particle are in a molar amount of about 30% to about 40%.

231. The lipid particle of claim 225 or the method of claim 225, wherein the one or more sterols in the lipid particle are in a molar amount of about 20%.

232. The lipid particle of claim 225 or the method of claim 225, wherein the one or more sterols in the lipid particle are in a molar amount of about 28.5%.

233. The lipid particle of claim 225 or the method of claim 225, wherein the one or more sterols in the lipid particle are in a molar amount of about 30%.

234. The lipid particle of claim 225 or the method of claim 225, wherein the one or more sterols in the lipid particle are in a molar amount of about 38.5%.

235. The lipid particle of claim 225 or the method of claim 225, wherein the one or more sterols in the lipid particle are in a molar amount of about 40%.

236. The lipid particle of any one of claims 225-235 or the method of any one of claims 225-235 wherein the one or more sterols are cholesterol,  $\beta$ -sitosterol, stigmasterol, campesterol, fucosterol, brassicasterol, ergosterol, 9,11-dehydroergosterol, daucosterol,  $\beta$ -sitosterol acetate, and other C-24 alkyl derivatives, or combinations thereof.

237. The lipid particle of any one of claims 225-236 or the method of any one of claims 225-236, wherein the one or more sterols are cholesterol,  $\beta$ -sitosterol, or combinations thereof.

238. The lipid particle of any one of claims 225-236 or the method of any one of claims 219-230, wherein only sterol is in the lipid particle.

239. The lipid particle of claim 238 or the method of claim 238, wherein the only sterol in the lipid particle is cholesterol.

240. The lipid particle of any one of claims 225-239 or the method of any one of claims 193-206, wherein the one or stealth lipids in the lipid particle are in a molar amount of about 0.5% to about 3%, about 1% to about 3%, about 1.5% to about 3%, about 2% to about 3%, about 2.5% to about 3%, about 0.25% to about 2.5%, about 0.5% to about 2.5%, about 1% to about 2.5%, about 1.5% to about 2.5%, about 2% to about 2.5%, about 0.25% to about 2%, about 0.5% to about 2%, about 1% to about 2%, about 1.5% to about 2%, about 0.25% to about 1.5%, about 0.5% to about 1.5%, or about 1% to about 1.5%.

241. The lipid particle of any one of claims 225-239 or the method of any one of claims 193-206, wherein the one or more stealth lipids in the lipid particle are in a molar amount of about 1% to about 3%.

242. The lipid particle of any one of claims 225-239 or the method of any one of claims 193-206, wherein the one or more stealth lipids in the lipid particle are in a molar amount of about 0.5%, about 1%, about 1.5%, about 2%, about 2.5%, or about 3%.

243. The lipid particle of any one of claims 225-239 or the method of any one of claims 193-206, wherein the one or more stealth lipids in the lipid particle are in a molar amount of about 1%.

244. The lipid particle of any one of claims 225-239 or the method of any one of claims 193-206, wherein the one or more stealth lipids in the lipid particle are in a molar amount of about 1.5%.

245. The lipid particle of any one of claims 225-239 or the method of any one of claims 193-206, wherein the one or more stealth lipids in the lipid particle are in a molar amount of about 2%.

246. The lipid particle of any one of claims 225-239 or the method of any one of claims 193-206, wherein the one or more stealth lipids in the lipid particle are in a molar amount of about 2.5%

247. The lipid particle of any one of claims 225-239 or the method of any one of claims 193-206, wherein the one or more stealth lipids in the lipid particle are in a molar amount of about 3%.

248. The lipid particle of any one of claims 225-247 or the method of any one of claims 219-240, wherein the one or more stealth lipids are one or more PEG terminated lipids, one or more polysarcosine derivatives, or combinations thereof.
249. The lipid particle of claim 248 or the method of claim 248, wherein the one or more stealth lipids are one or more PEG terminated lipids.
250. The lipid particle of claim 248 or 249 or the method of claim 248 or 249, wherein the one or more PEG terminated lipids in the lipid particle are one or more selected from the group consisting of 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 ("DMG-PEG2000"), distearoyl-rac-glycerol-PEG2K ("DSG-PEG2k"), [(2R)-2,3-di(octadecanoyloxy)propyl] 2-(2-methoxyethoxycarbonylamino)ethyl phosphate ("C18-mPEG2000"), [3-[3-(2-methoxyethoxy)propylcarbamoyloxy]-2-tetradecanoyloxypropyl] tetradecanoate ("PEG2000-c-DMG"), 3-[hydroxy-[2-[2-(2-methoxyethoxy)ethylamino]ethoxy]phosphoryl]oxy-2-tetradecanoyloxypropyl] tetradecanoate ("DMPE-PEG2000"), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] ("18:1 PEG2000-PE"), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)-2000] ("DSPE-PEG2000-COOH"), and Bis(1,2-distearoyl-sn-glycero-3-phosphoethanolamine)-N-[(polyethylene glycol)-2000] ("Bis-DSPE-PEG2000").
251. The lipid particle of any one of claims 248-250 or the method of any one of claims 241-243, wherein the one or more PEG terminated lipids in the lipid particle is DMG-PEG2000, DMPE-PEG2000, or a combination thereof.
252. The lipid particle of any one of claims 248-251 or the method of any one of claims 241-244, wherein only one PEG terminated lipid is in the lipid particle.
253. The lipid particle of claim 245 or the method of claim 252, wherein the only PEG terminated lipid in the lipid particle is DMG-PEG2000.
254. The lipid particle of claim 245 or the method of claim 252, wherein the only PEG terminated lipid in the lipid particle is DMPE-PEG2000.
255. The lipid particle of claim 245 or the method of claim 252, wherein the only PEG terminated lipid in the lipid particle is PEG2000-PE.
256. The lipid particle of claim 241 or the method of claim 248, wherein the one or more stealth lipids are one or more polysarcosine derivative.
257. The lipid particle of claim 249 or the method of claim 256, wherein the one or more polysarcosine derivatives in the lipid particle are one or more selected from the group consisting

of N-tetradecyl-polysarcosine-25 (“N-tetradecyl-pSar25”), N-hexadecyl-polysarcosine-25 (“N-hexadecyl-pSar25”), N-octadecyl-polysarcosine-25, N-dodecyl-polysarcosine-25 (“N-octadecyl-pSar25”), 1,2-dimyristoyl-sn-glycero-3-succinyl-N-polysarcosine-25 (“DMG-pSar25”), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-polysarcosine-25 (ammonium salt) (“18:1 PE (DOPE), N,N-ditetradecylamine-N-succinyl[methyl(polysarcosine)45] (“N-TETAMINE-pSar45”), N,N-ditetradecylamine-N-succinyl[methyl(polysarcosine)35] (“N-TETAMINE-pSar35”), N,N-ditetradecyl-polysarcosine-25 (“N-TETAMINE-pSar25”), N-TETAMINE-pSar45-maleimide, or N-TETAMINE-PEOZ-40.

258. The lipid particle of claim 256 or 257 or the method of claim 256 or 257, wherein only one polysarcosine derivative is in the lipid particle.

259. The lipid particle of claim 142 or the method of claim 152, wherein the lipid particle comprises about 40% to about 60% of SS-OP, about 1% to about 20% of DODG, about 25% to about 45% of cholesterol, about 1% to about 10% of CHEMS, and about 0.5% to about 2.5% of DMG-PEG2000 (in molar amounts).

260. The lipid particle of claim 259 or the method of claim 259, wherein the lipid particle comprises about 50% of SS-OP, about 10% of DODG, about 38.5% of cholesterol, about 10% of CHEMS, and about 1.5% of DMG-PEG2000 (in molar amounts).

261. The lipid particle of claim 142 or the method of claim 152 comprising about 40% to about 60% of SS-OP, about 1% to about 20% of DODG, about 25% to about 45% of cholesterol, and about 0.5% to about 2.5% of DMG-PEG2000 (in molar amounts).

262. The lipid particle of claim 261 or the method of claim 261, wherein the lipid particle comprises about 50% of SS-OP, about 10% of DODG, about 38.5% of cholesterol, and about 1.5% of DMG-PEG2000 (in molar amounts).

263. A method of targeting a payload to the pancreas of a subject in need thereof comprising intraperitoneally administering the lipid particle of any one of claims 1, 4-151, and 162-262 to the subject.

264. A method of targeting a payload to a lymph node of a subject in need thereof comprising subcutaneously administering the lipid particle of any one of claims 1, 4-151, and 162-262 to the subject.

265. The lipid particle of any one of claims 1, 4-151, and 162-262 or the method of any one of claims 2-138 and 148-255, wherein the payload comprises a biologically active molecule.

266. The lipid particle of claim 265 or the method of claim 265, wherein the biologically active molecule is a small molecule, a nucleic acid, an aptamer, or any combination thereof.

267. The lipid particle of claim 265 or the method of claim 265, wherein the biologically active molecule comprises a nucleic acid.
268. The lipid particle of claim 267 or the method of claim 267, wherein the nucleic acid comprises a small interfering ribonucleic acid (siRNA), a short hairpin RNA (shRNA), a micro-ribonucleic acid (miRNA), a primary micro-ribonucleic acid (pri-miRNA), a long non-coding RNA (lncRNA), a messenger ribonucleic acid (mRNA), a clustered regularly interspaced short palindromic repeats (CRISPR) related nucleic acid, a CRISPR-RNA (crRNA), a single guide ribonucleic acid (sgRNA), a trans-activating CRISPR ribonucleic acid (tracrRNA), a plasmid deoxyribonucleic acid (pDNA), a transfer ribonucleic acid (tRNA), an antisense oligonucleotide (ASO), an antisense ribonucleic acid (RNA), a guide ribonucleic acid, deoxyribonucleic acid (DNA), a double stranded deoxyribonucleic acid (dsDNA), a single stranded deoxyribonucleic acid (ssDNA), a single stranded ribonucleic acid (ssRNA), a double stranded ribonucleic acid (dsRNA), a CRISPR-associated (Cas) protein, or combinations thereof.
269. The lipid particle of claim 268 or the method of claim 268, wherein the nucleic acid in the lipid particle comprises mRNA.
270. The lipid particle of claim 268 or the method of claim 268, wherein the nucleic acid in the lipid particle encodes a peptide having therapeutic activity.
271. The lipid particle of claim 270 or the method of claim 270, wherein the peptide comprises an epitope amino acid sequence.
272. The lipid particle of claim 270 or the method of claim 270, wherein the peptide induces immune tolerance to cells.
273. A nucleic acid-lipid particle comprising the lipid particle of any one of claims 1, 4-151, and 162-262 and a nucleic acid.
274. A composition comprising the lipid particle of any one of claims 1, 4-151, and 162-262.
275. A pharmaceutical composition comprising the lipid particle of any one of claims 1, 4-151, and 162-262 and one or more pharmaceutically acceptable carriers.
276. The pharmaceutical composition of claim 275, wherein one of the pharmaceutically acceptable carrier is sucrose.
277. The pharmaceutical composition of claim 275 or 276, wherein one of the pharmaceutically acceptable carrier is saline.
278. The pharmaceutical composition of claim 277, wherein the saline is buffered with tris.
279. The pharmaceutical composition of any one of claims 275-278, wherein the pharmaceutical composition is stored at -80 °C.

280. The pharmaceutical composition of any one of claims 275-278, wherein the pharmaceutical composition is stored at 4 °C.
281. A method of producing a nucleic acid-lipid particle, comprising mixing a nucleic acid with the lipid particle of any one of claims 1, 4-151, and 162-262.
282. The method of claim 281, wherein the nucleic acid comprises a small interfering ribonucleic acid (siRNA), a short hairpin RNA (shRNA), a micro-ribonucleic acid (miRNA), a primary micro-ribonucleic acid (pri-miRNA), a long non-coding RNA (lncRNA), a messenger ribonucleic acid (mRNA), a clustered regularly interspaced short palindromic repeats (CRISPR) related nucleic acid, a CRISPR-RNA (crRNA), a single guide ribonucleic acid (sgRNA), a trans-activating CRISPR ribonucleic acid (tracrRNA), a plasmid deoxyribonucleic acid (pDNA), a transfer ribonucleic acid (tRNA), an antisense oligonucleotide (ASO), an antisense ribonucleic acid (RNA), a guide ribonucleic acid, deoxyribonucleic acid (DNA), a double stranded deoxyribonucleic acid (dsDNA), a single stranded deoxyribonucleic acid (ssDNA), a single stranded ribonucleic acid (ssRNA), a double stranded ribonucleic acid (dsRNA), a CRISPR-associated (Cas) protein, or combinations thereof.
283. The method of claim 282, wherein the nucleic acid comprises mRNA.
284. The method of claim 282, wherein the nucleic acid encodes a peptide having therapeutic activity.
285. The method of claim 284, wherein the peptide comprises an epitope amino acid sequence.
286. The method of claim 284, wherein the peptide induces immune tolerance to cells
287. A method for introducing a nucleic acid into a cell comprising contacting the cell with a lipid particle of any one of claims 1, 4-151, and 162-262.
288. A method for treating a disease or disorder in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition of any one of claims 275-280.
289. A method of preventing or delaying the onset or recurrence of a disease or disorder in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition of any one of claims 275-280.
290. A method for restoring immune homeostasis in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition of any one of claims 275-280.

291. A method for limiting or reducing immunogenicity responses in a subject in need thereof comprising administering to the subject in need thereof a therapeutically effective amount of a pharmaceutical composition of any one of claims 275-280.

292. The method of any one of claims 288-291, wherein the subject, after the administration to a subject in need thereof, the subject exhibits one or more of: (i) an increase in Treg cells, (ii) reduction in effector T cells, (iii) reduction in B-cells, (iv) reduction in cytokine production, (v) reduction in activated B cells; (vi) reduction in activated effector T cells; or (vii) any combination thereof.

293. A method for limiting or reducing inflammatory responses in a subject in need thereof comprising administering to the subject in need thereof a therapeutically effective amount of a pharmaceutical composition of any one of claims 275-280.

294. The method of any one of claims 288-293, wherein the pharmaceutical composition is administered intranasally, intracranially, intrathecally, intradermally, intratracheally, transdermally, intravenously, intraperitoneally, by injection, by infusion, intramuscularly, or subcutaneously.

295. The method of any one of claims 288-293, wherein the pharmaceutical composition is administered intravenously, intraperitoneally, by injection, by infusion, intramuscularly, or subcutaneously to the subject.

296. The method of any one of claims 288-295, wherein the subject is a mammal.

297. The method of claim 296, wherein the mammal is a human.

298. The method of any one of claims 288, wherein the disease is an autoimmune disease.

299. The method of claim 298, wherein the autoimmune disease is selected from the group consisting of autoimmune hepatitis, inflammatory bowel disease (including Crohn's disease and ulcerative colitis), nonalcoholic fatty pancreas disease, scleroderma, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, celiac disease, Type 1 diabetes, Guillain-Barré syndrome, Hashimoto's thyroiditis, polymyalgia rheumatic, alopecia areata, fibrosis, psoriasis, pemphigus vulgaris, vitiligo, ankylosing spondylitis, juvenile idiopathic arthritis, psoriatic arthritis, mixed connective tissue disease, neuromyelitis optica, latent autoimmune diabetes in adults ("LADA"), autoimmune thyroid disease, Grave's disease, Addison's disease, autoimmune atrophic gastritis, pernicious anemia, atopic dermatitis, bullous pemphigoid, myasthenia gravis, poly/dermatomyositis), rheumatic fever, primary sclerosing cholangitis, autoimmune uveitis and Behcet's disease), diseases that affect the blood or bone marrow (e.g., autoimmune haemolytic anemia, idiopathic thrombocytopenic purpura, idiopathic leucopenia,



Goodpasture's syndrome, autoimmune nephritis, glomerulonephritis, Wegener's granulomatosis, chronic inflammatory demyelinating polyradiculoneuropathy, Sjogren's syndrome, primary biliary cholangitis, Parkinson's disorder, and antiphospholipid syndrome.

300. The method of claim 299, wherein the autoimmune disease is autoimmune hepatitis, Type 1 diabetes or multiple sclerosis.

301. The method of claim 288, wherein the disease is an infectious disease.

302. The method of claim 288, wherein the disease is a cancer.