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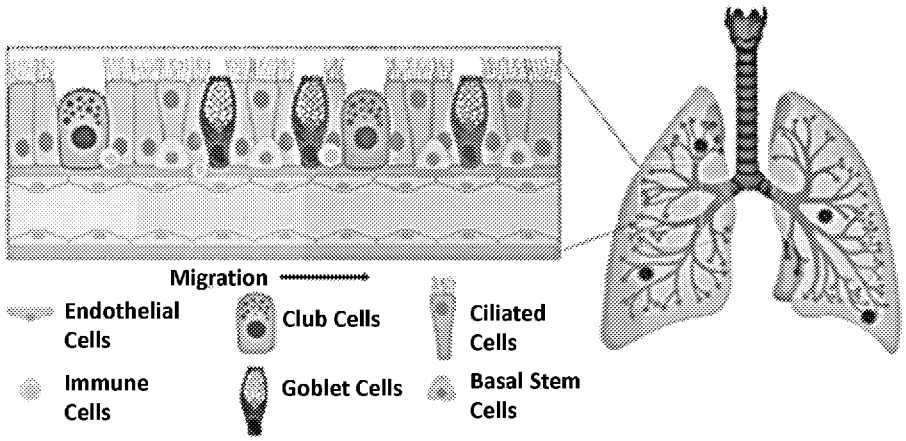
(54) Title: GENE EDITING METHODS AND COMPOSITIONS FOR TREATING CYSTIC FIBROSIS

FIG. 1A



(57) Abstract: Provided are methods to treat cystic fibrosis in a subject. Also provided are methods of modifying the nucleic acid sequence in the CFTR gene in a lung cell type, methods of increasing the expression of the CFTR gene in a lung cell type, and methods of modulating the activity of a CFTR gene in a lung cell type. The present disclosure further relates to the method of treating cystic fibrosis comprising the administration of a lipid nanoparticle (LNP) and a gene editing system, wherein administration of the LNP mediates genome editing in lung cells.

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GENE EDITING METHODS AND COMPOSITIONS FOR TREATING CYSTIC FIBROSIS

Cross-Reference to Related Applications

[0001] This application claims priority from U.S. provisional application No. 63/614,394 filed December 22, 2023, entitled "GENE EDITING METHODS AND COMPOSITIONS FOR TREATING CYSTIC FIBROSIS" and U.S. provisional application No. 63/659,813, filed June 13, 2024, entitled "GENE EDITING METHODS AND COMPOSITIONS FOR TREATING CYSTIC FIBROSIS", the contents of which are incorporated by reference in its entirety.

Incorporation by Reference of Sequence Listing

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled 282152000140SeqList.xml, created December 19, 2024, which is 9,430 bytes in size. The information in the electronic format of the Sequence Listing is incorporated by reference in its entirety.

Field

[0003] The present disclosure relates in some aspects to methods and uses of lipid nanoparticles comprising nucleic acids encoding a base editor and a guide RNA, for treating subjects with cystic fibrosis, and related methods, uses, and articles of manufacture.

Background

[0004] Cystic Fibrosis (CF) is a genetic disorder that impacts tens of thousands of people worldwide. CF is caused by mutations in the *cystic fibrosis transmembrane conductance regulator* (*CFTR*) gene, which encodes an ion channel. These mutations result in dysregulated chloride and bicarbonate transport across the membrane of epithelial cells, causing cells to secrete thick, viscous mucus. This leads to inflammation, infections, tissue damage, respiratory issues, and organ failure, and result in patients having shortened life expectancy. Many CF-causing mutations in *CFTR* have been identified, but the most common is the gene variant F508del, in which the deletion of three base pairs cause the loss of phenylalanine at position 508. Efforts for CF treatment include options that focus on preventing infections and clearing

mucus in the lungs. In terms of curing CF, gene therapy approaches have been considered as a promising avenue. To this end, lipid nanoparticles (LNPs) are being utilized as a delivery platform for delivering adenine base editors (ABEs) in order to correct mutated *CFTR* genes and cure patients of this life-threatening genetic disease.

[0005] Various strategies for delivering nucleic acid molecules into cells are available, including transfection- and transduction-based techniques. However, there remains a need for improved delivery strategies of nucleic acid molecules into cells and treatments for cystic fibrosis. Provided herein are embodiments that meet such needs.

Summary

[0006] The present application provides methods of treating a subject with cystic fibrosis by administering to the subject a composition comprising a lipid nanoparticle (LNP) that comprises a gene editing system.

[0007] Provided herein is a method of treating a subject with cystic fibrosis, the method comprising administering to the subject a composition comprising a lipid nanoparticle (LNP) that comprises a gene editing system, wherein the gene editing system comprises: (i) a first nucleic acid encoding a base editor; and (ii) a second nucleic acid encoding a guide RNA (gRNA), and wherein the composition treats the cystic fibrosis in the subject. In some embodiments, the *CFTR* gene of the subject comprises a R553X stop codon mutation. In some embodiments, the administration of the composition results in an increase in the expression of the full-length cystic fibrosis transmembrane conductance regulator (CFTR) protein in the subject, as compared to a subject with cystic fibrosis and whose *CFTR* gene comprises a R553X stop codon mutation, and that is not administered the composition.

[0008] In some embodiments, the administration of the composition results in an increase in the function of the cystic fibrosis transmembrane conductance regulator (CFTR) protein in the subject, as compared to a subject with cystic fibrosis and whose *CFTR* gene comprises a R553X stop codon mutation, and that is not administered the composition. In other embodiments, the nucleic acid encoding the base editor is RNA. In some embodiments, the base editor is an adenine base editor (ABE). In other embodiments, the ABE comprises a tRNA adenosine deaminase (TadA) protein, or a variant thereof, fused to a catalytically impaired Cas protein capable of binding to a specific nucleotide sequence. In some embodiments, the ABE is ABE8e.

[0009] In some of any embodiments, the base editor is a cytosine base editor (CBE). In some embodiments, the CBE comprises a cytidine deaminase protein, or a variant thereof, fused to a catalytically impaired Cas protein capable of binding to a specific nucleotide sequence. In some embodiments, the ABE or the CBE further comprises a Cas9 enzyme that does not have any nuclease activity. In some embodiments, the LNP comprises an ionizable cationic lipid, a zwitterionic phospholipid, a cholesterol, and a PEG lipid. In some of any embodiments, the LNP comprises 5A2-SC8, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), cholesterol, DMG-PEG, and one or more selective organ targeting (SORT) molecules.

[0010] Provided herein is a method of delivering a gene editing system to a lung cell type in a subject, the method comprising administering to the subject a composition comprising a lipid nanoparticle (LNP) that comprises a gene editing system, wherein the gene editing system comprises (i) a first nucleic acid encoding an endonuclease or a base editor; and (ii) a second nucleic acid encoding a guide RNA (gRNA), and wherein the gene editing system is delivered to a lung cell type in a subject.

[0011] In some embodiments, the lung cell type is an endothelial cell or an epithelial cell. In some of any embodiments, the lung cell type is an immune cell. In some embodiments, the lung cell type is a stem cell. In some embodiments, the endonuclease is a Cas nuclease of the CRISPR-Cas system. In other embodiments, the Cas nuclease is a Cas9 nuclease, a Cas12 nuclease, or a Cas13 nuclease. In some embodiments, the nucleic acid encoding the endonuclease is DNA. In some embodiments, the nucleic acid encoding the endonuclease is RNA. In some embodiments, the nucleic acid encoding the base editor is DNA. In some of any embodiments, the nucleic acid encoding the base editor is RNA.

[0012] In some embodiments, the base editor is an adenine base editor (ABE). In some embodiments, the ABE comprises a tRNA adenosine deaminase (TadA) protein, or a variant thereof, fused to a catalytically impaired Cas protein capable of binding to a specific nucleotide sequence. In some embodiments, the ABE is ABE8e. In some embodiments, the base editor is a cytosine base editor (CBE). In some embodiments, the CBE comprises a cytidine deaminase protein, or a variant thereof, fused to a catalytically impaired Cas protein capable of binding to a specific nucleotide sequence. In some embodiments, the ABE or the CBE further comprises a Cas9 enzyme that does not have any nuclease activity.

[0013] In some embodiments, the ratio of the first nucleic acid to the second nucleic acid is 1:1 on a molecule:molecule basis. In some embodiments, the ratio of the first nucleic acid to the

second nucleic acid is 1:1 on a weight basis. In some embodiments, the ratio of the first nucleic acid to the second nucleic acid is 2:1 on a molecule:molecule basis. In some embodiments, the ratio of the first nucleic acid to the second nucleic acid is 2:1 on a weight basis.

[0014] In some embodiments, the LNP comprises an ionizable cationic lipid, a zwitterionic phospholipid, a cholesterol, and a PEG lipid. In some embodiments, the LNP comprises 5A2-SC8, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), cholesterol, DMG-PEG, and one or more selective organ targeting (SORT) molecules. In some embodiments, the one or more SORT molecules comprises permanently positively charged moiety. In some embodiments, the one or more SORT molecules is selected from the group consisting of 18:1 DOTMA (DOTMA); DORI, DC-6-14; 12:0 EPC (Chloride Salt); 14:0 EPC (Chloride Salt); 16:0 EPC (Chloride Salt); 18:0 EPC (Chloride Salt); 18:1 EPC (Chloride Salt); 16:0-18:1 EPC (Chloride Salt); 14:1 EPC (Triflate Salt); 18:0 DDAB (Dimethyldioctadecylammonium (Bromide Salt)); 14:0 TAP; 16:0 TAP; 18:0 TAP; 18:1 TAP (DOTAP); 18:1 TAP (DOTAP, MS Salt); 18:1 DODAP, or 18:1 PA (1,2-dioleoyl-sn-glycero-3-phosphate (sodium salt)) (18PA).

[0015] In some embodiments, the one or more SORT molecule comprises DOTAP (1,2-dioleoyl-3-trimethylammonium propane). In some embodiments, the one or more SORT molecule comprises 18PA. In some embodiments, the one or more SORT molecule comprises DODAP. In some embodiments, the DODAP comprises about 20% molar ratio of the total lipids. In some embodiments, the DOTAP comprises about 50% molar ratio of the total lipids. In some embodiments, the 18PA comprises about 10% molar ratio of the total lipids. In some of any embodiments, the one or more SORT molecule comprises DOTMA. In some embodiments, the LNP comprises a ratio of DOPE:DOTMA between 3:1 and 1:3. In embodiments, the ratio of DOPE:DOTMA is about 3:1. In some embodiments, the ratio of DOPE:DOTMA is about 1:1.

[0016] In some embodiments, the SORT molecule comprises from about 5% to about 60% molar percentage of the LNP. In some embodiments, the SORT molecule comprises about 40% molar percentage of the LNP. In some embodiments, the SORT molecule comprises about 50% molar percentage of the LNP. In some embodiments, the LNP binds vitronectin.

[0017] In some embodiments, the guide RNA comprises a circular RNA. In some of any embodiments, the guide RNA comprises a linear RNA. In some embodiments, the guide RNA is a single guide RNA (sgRNA). In some embodiments, the guide RNA comprises a target sequence that is complementary with a target sequence of a cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. In some embodiments, the nucleotide sequence of the guide

RNA is AAGTAAAACCTCTACAAATG (SEQ ID NO: 1) or TTGCTCATTGACCTCCACTC (SEQ ID NO: 2).

[0018] In some embodiments, the composition comprises a pharmaceutically acceptable carrier. In some embodiments, the composition is administered intravenously. In some of any embodiments, the subject is a human. In some embodiments, the subject has cystic fibrosis.

[0019] Provided herein is a method of modifying the nucleic acid sequence of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene in a lung cell type, wherein the *CFTR* gene comprises a R553X stop codon mutation, the method comprising: (a) contacting the lung cell type with a composition comprising a lipid nanoparticle (LNP), wherein the LNP comprises (i) a first nucleic acid encoding a base editor; and (ii) a second nuclec acid encoding a guide RNA; (b) determining the nucleic acid sequence of the *CFTR* gene in the lung cell type, wherein the nucleic acid sequence of the *CFTR* gene in the lung cell type is modified to remove the R553X stop codon mutation. In some embodiments, the modification comprises the replacing of the thymine at 1789 base in exon 11 of the *CFTR* gene with cytosine.

[0020] Provided herein is a method of increasing the expression of full-length cystic fibrosis transmembrane conductance regulator (CFTR) protein in a lung cell type, wherein a *CFTR* gene in the lung cell type comprises a R553X mutation, the method comprising: (a) contacting the lung cell type with a composition comprising a lipid nanoparticle (LNP), wherein the LNP comprises (i) a first nucleic acid encoding a base editor; and (ii) a second nucleic acid encoding a guide RNA; (b) determining the expression of full-length CFTR protein in the lung cell type, wherein the expression of full-length CFTR protein is increased in the lung cell type, as compared to a lung cell type comprising a *CFTR* gene comprising a R553X mutation, and that is not contacted with the composition.

[0021] Provided herein is a method of modulating the activity of the cystic fibrosis transmembrane conductance regulator (CFTR) protein in a lung cell type, wherein the *CFTR* gene in the lung cell type comprises a R553X mutation, the method comprising: (a) contacting the lung cell type with a composition comprising a lipid nanoparticle (LNP), wherein the LNP comprises (i) a first nucleic acid encoding a base editor; and (ii) a second nucleic acid encoding a guide RNA; (b) determining the activity of the CFTR protein in the lung cell type, wherein the activity of the CFTR protein is modulated in the lung cell type, as compared to a lung cell type comprising a *CFTR* gene comprising a R553X mutation, and that is not contacted with the composition.

[0022] In some embodiments, expression of the CFTR protein is determined in a lung cell type in a subject, wherein the subject has been administered the composition, and wherein the expression is determined by one or more bioassays comprising sweat chloride concentration assay, β-adrenergic sweat assay, and nasal potential difference assay. In some embodiments, expression of the CFTR protein is determined by analysis of chloride levels in the sweat of the subject. In some embodiments, the chloride levels in the sweat of the subject after being administered the composition are decreased as compared to the chloride levels in the sweat of the subject before being administered the composition. In some embodiments, the expression is measured using western blotting, immunoprecipitation, and anti-CFTR antibodies. In some embodiments, the activity of the CFTR protein is increased in the lung cell type, as compared to a lung cell type comprising a *CFTR* gene comprising a R553X mutation, and that is not contacted with the composition.

[0023] In some embodiments, the lung cell type is an endothelial cell or an epithelial cell. In some embodiments, the lung cell type is an immune cell. In some embodiments, the lung cell type is a stem cell.

[0024] In some embodiments, the nucleic acid encoding the base editor is DNA. In some embodiments, the nucleic acid encoding the base editor is RNA. In some embodiments, the base editor is an adenine base editor (ABE). In some embodiments, the ABE comprises a tRNA adenosine deaminase (TadA) protein, or a variant thereof, fused to a catalytically impaired Cas protein capable of binding to a specific nucleotide sequence. In some embodiments, the base editor is a cytosine base editor (CBE). In some embodiments, the CBE comprises a cytidine deaminase protein, or a variant thereof, fused to a catalytically impaired Cas protein capable of binding to a specific nucleotide sequence. In some embodiments, the ABE or the CBE further comprises a Cas9 enzyme that does not have any nuclease activity.

[0025] In some embodiments, the ratio of the first nucleic acid to the second nucleic acid is 1:1 on a molecule:molecule basis. In some embodiments, the ratio of the first nucleic acid to the second nucleic acid is 1:1 on a weight basis. In some embodiments, the ratio of the first nucleic acid to the second nucleic acid is 2:1 molecule:molecule basis. In some embodiments, the ratio of the first nucleic acid to the second nucleic acid is 2:1 on a weight basis.

[0026] In some embodiments, the LNP comprises an ionizable cationic lipid, a zwitterionic phospholipid, a cholesterol, and a PEG lipid. In some embodiments, the LNP comprises 5A2-SC8, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), cholesterol, DMG-PEG, and one

or more selective organ targeting (SORT) molecules. In some embodiments, the one or more SORT molecules comprises permanently positively charged moiety. In some embodiments, the one or more SORT molecule is selected from the group consisting of 18:1 DOTMA (DOTMA); DORI, DC-6-14; 12:0 EPC (Chloride Salt); 14:0 EPC (Chloride Salt); 16:0 EPC (Chloride Salt); 18:0 EPC (Chloride Salt); 18:1 EPC (Chloride Salt); 16:0-18:1 EPC (Chloride Salt); 14:1 EPC (Triflate Salt); 18:0 DDAB (Dimethyldioctadecylammonium (Bromide Salt)); 14:0 TAP; 16:0 TAP; 18:0 TAP; 18:1 TAP (DOTAP); 18:1 TAP (DOTAP, MS Salt); 18:1 DODAP, or 18:1 PA (1,2-dioleoyl-sn-glycero-3-phosphate (sodium salt)) (18PA).

[0027] In some embodiments, the one or more SORT molecules comprises DOTAP (1,2-dioleoyl-3-trimethylammonium propane). In some embodiments, the one or more SORT molecules comprises 18PA. In some embodiments, the one or more SORT molecules comprises DODAP. In some embodiments, the DODAP comprises about 20% molar ratio of the total lipids. In some embodiments, the DOTAP comprises about 50% molar ratio of the total lipids. In some embodiments, the 18PA comprises about 10% molar ratio of the total lipids.

[0028] In some embodiments, the SORT molecule comprises DOTMA. In some of any embodiments, the LNP comprises a ratio of DOPE:DOTMA of between 3:1 and 1:3. In some embodiments, the ratio of DOPE:DOTMA is about 3:1. In some embodiments, the ratio of DOPE:DOTMA is about 1:1. In some embodiments, the SORT molecule comprises from about 5% to about 60% molar percentage of the LNP. In some embodiments, the SORT molecule comprises about 40% molar percentage of the LNP. In some embodiments, the SORT molecule comprises from 50% molar percentage of the LNP. In some embodiments, the LNP binds vitronectin.

[0029] In some embodiments, the guide RNA comprises a circular RNA. In some of any embodiments, the guide RNA comprises a linear RNA. In some embodiments, the guide RNA is a single guide RNA (sgRNA). In some embodiments, the guide RNA comprises a target sequence that is complementary with a target sequence of a cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. In some embodiments, the nucleotide sequence of the guide RNA is AAGTAAAACCTCTACAAATG (SEQ ID NO: 1) or TTGCTCATTGACCTCCACTC (SEQ ID NO: 2).

[0030] In some embodiments, the function of the CFTR protein is determined by one or more bioassays comprising sweat chloride concentration assay, β-adrenergic sweat assay, and nasal potential difference assay. In some embodiments, the function of the CFTR protein is

determined by analysis of chloride levels in the sweat of the subject. In some embodiments, the chloride levels in the sweat of the subject after being administered the composition are decreased as compared to the chloride levels in the subject before being administered the composition.

[0031] In some embodiments, the composition comprises a pharmaceutically acceptable carrier. In some embodiments, the subject is a human. In some embodiments, the administration of the composition to the subject is by intravenous administration.

[0032] Provided herein is a method of restoring the function of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene in a subject with cystic fibrosis, the method comprising: (a) administering to the subject a composition comprising a lipid nanoparticle (LNP), wherein the LNP comprises (i) a first nucleic acid encoding a base editor; and (ii) a second nucleic acid encoding a guide RNA; (b) determining the function of the *CFTR* gene in the subject, wherein the function of the *CFTR* gene is restored in the subject.

[0033] In some embodiments, about 5% to about 95% of the function of the *CFTR* gene is restored. In some embodiments, the restoring of the function of the *CFTR* gene is determined by the increase of CFTR protein expression. In some embodiments, expression of the CFTR protein is determined by one or more bioassays comprising sweat chloride concentration assay, β-adrenergic sweat assay, and nasal potential difference assay. In some embodiments, expression of the CFTR protein is determined by analysis of chloride levels in the sweat of the subject. In some embodiments, chloride levels in the sweat of the subject after being administered the composition are decreased as compared to levels in a subject before being administered the composition. In some embodiments, the expression is measured using western blotting, immunoprecipitation, and anti-CFTR antibodies.

[0034] In some embodiments, the nucleic acid encoding the base editor is DNA. In some embodiments, the nucleic acid encoding the base editor is RNA. In some embodiments, the base editor is an adenine base editor (ABE). In some embodiments, the base editor is ABE8e. In some embodiments, the ABE comprises a tRNA adenosine deaminase (TadA) protein, or a variant thereof, fused to a catalytically impaired Cas protein capable of binding to a specific nucleotide sequence. In some embodiments, the base editor is a cytosine base editor (CBE). In some embodiments, the CBE comprises a cytidine deaminase protein, or a variant thereof, fused to a catalytically impaired Cas protein capable of binding to a specific nucleotide sequence. In some

of any embodiments, the ABE or the CBE further comprises a Cas9 enzyme that does not have any nuclease activity.

[0035] In some embodiments, the ratio of the first nucleic acid to the second nucleic acid is 1:1 on a molecule:molecule basis. In some embodiments, the ratio of the first nucleic acid to the second nucleic acid is 1:1 on a weight basis. In some embodiments, the ratio of the first nucleic acid to the second nucleic acid is 2:1 molecule:molecule basis. In some embodiments, the ratio of the first nucleic acid to the second nucleic acid is 2:1 on a weight basis.

[0036] In some embodiments, the LNP comprises an ionizable cationic lipid, a zwitterionic phospholipid, a cholesterol, and a PEG lipid. In some embodiments, the LNP comprises 5A2-SC8, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), cholesterol, DMG-PEG, and one or more selective organ targeting (SORT) molecules. In some embodiments, the one or more SORT molecules comprises permanently positively charged moiety. In some of any embodiments, the one or more SORT molecule is selected from the group consisting of 18:1 DOTMA (DOTMA); DORI, DC-6-14; 12:0 EPC (Chloride Salt); 14:0 EPC (Chloride Salt); 16:0 EPC (Chloride Salt); 18:0 EPC (Chloride Salt); 18:1 EPC (Chloride Salt); 16:0-18:1 EPC (Chloride Salt); 14:1 EPC (Triflate Salt); 18:0 DDAB (Dimethyldioctadecylammonium (Bromide Salt)); 14:0 TAP; 16:0 TAP; 18:0 TAP; 18:1 TAP (DOTAP); 18:1 TAP (DOTAP, MS Salt); 18:1 DODAP, or 18:1 PA (1,2-dioleoyl-sn-glycero-3-phosphate (sodium salt)) (18PA).

[0037] In some embodiments, the one or more SORT molecules comprises DOTAP (1,2-dioleoyl-3-trimethylammonium propane). In some embodiments, the one or more SORT molecules comprises 18PA. In some embodiments, the one or more SORT molecules comprises DODAP. In some embodiments, the DODAP comprises about 20% molar ratio of the total lipids. In some embodiments, the DOTAP comprises about 50% molar ratio of the total lipids. In some embodiments, the 18PA comprises about 10% molar ratio of the total lipids. In some embodiments, the one or more SORT molecules comprises DOTMA. In some embodiments, the LNP comprises a ratio of DOPE:DOTMA of between 3:1 and 1:3. In some embodiments, the ratio of DOPE:DOTMA is about 3:1. In some embodiments, the ratio of DOPE:DOTMA is about 1:1. In some embodiments, the one or more SORT molecules comprises from about 5% to about 60% molar percentage of the LNP. In some embodiments, the one or more SORT molecules comprises about 40% molar percentage of the LNP. In some embodiments, the SORT molecule comprises from 50% molar percentage of the LNP. In some embodiments according to any one of the methods described above, the LNP binds vitronectin.

[0038] In some embodiments, the guide RNA comprises a circular RNA. In some embodiments, the guide RNA comprises a linear RNA. In some embodiments, the guide RNA is a single guide RNA (sgRNA). In some embodiments, the guide RNA comprises a target sequence that is complementary with a target sequence of a cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. In some embodiments, the nucleotide sequence of the guide RNA is AAGTAAAACCTCTACAAATG (SEQ ID NO: 1) or TTGCTCATTGACCTCCACTC (SEQ ID NO: 2).

[0039] In some embodiments, the composition comprises a pharmaceutically acceptable carrier. In some embodiments, the subject is a human. In some embodiments, the administration of the composition to the subject is by intravenous administration. In some embodiments, the LNP is localized to the lungs of the subject. In some embodiments, the LNP is capable of delivering the first and second nucleic acids to the lungs of the subject.

[0040] Provided herein is a lung cell type comprising a modified cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, wherein the modification comprises the replacement of the thymine at 1789 base in exon 11 of the *CFTR* gene with cytosine.

[0041] Provided herein is a method of treating cystic fibrosis in a subject, the method comprising administering to the subject a composition comprising a lipid nanoparticle (LNP), wherein the LNP comprises (i) a first nucleic acid encoding a base editor; and (ii) a second nucleic acid encoding a guide RNA, wherein the first nucleic acid and the second nucleic acid are delivered to a lung cell in the subject.

Brief Description of the Drawings

[0042] **FIG. 1A-1M** shows that direct *in vivo* gene editing was achieved in mouse lungs that persisted for one year. Diagram showing Lung SORT LNPs delivery of mRNA-encoded gene editors to various lung cell types, including stem cells (**FIG. 1A**). Diagram showing the experimental procedure used to evaluate the efficiency of lung cell editing in Ai14 tdTom reporter mice, in which Cre recombinase can excise the loxP flanked stop cassette enabling tdTom fluorescence protein expression. Mice were injected with LNP-Cre at 2 mg/kg total RNA (20:1, total lipid to RNA weight ratio) with two sequential doses, 48 hours apart. Mice treated with PBS were used as negative control (**FIG. 1B**). *Ex vivo* fluorescence imaging analyses of mouse lungs 2, 7, 21, 42, 60, 120 ,180, and 360 days after the last injection (**FIG. 1C**). Quantification analysis of *ex vivo* lung images was shown as average radiance (**FIG. 1D**) and as

total Flux (**FIG. 1E**). Flow cytometry gating strategy for lung endothelial, epithelial, and immune cells. Single cells prepared from Ai14 mouse lungs were gated. Viable (Ghost Red negative) total lung cells, endothelial cells (CD31 positive), epithelial cells (EpCam positive), or immune cells (CD45 positive) expressing tdTomato fluorescence (tdTom positive) were analyzed by flow cytometry (n=3) (**FIG. 1F**). Single cells prepared from Ai14 mouse lungs were gated. Viable (Ghost Red negative) lung basal stem cells (EpCam positive, Ngfr positive) and lung stem cells (EpCam positive, Krt5 or CK5 positive) expressing tdTomato fluorescence (tdTom positive) were analyzed by flow cytometry (n=3) (**FIG. 1G**). Time-course flow cytometry analyses showing the percentage of tdTom-positive ($tdTOM^+$) cell was reported among (**FIG. 1H**) lung cells, (**FIG. 1I**) endothelial cells, (**FIG. 1J**) immune cells, (**FIG. 1K**) epithelial cells, (**FIG. 1L**) $Ngfr^+$ stem cells, and (**FIG. 1M**) $Krt5^+$ stem cells.

[0043] **FIG. 2A-2G** depicts durable i*n vivo* gene editing in mouse lung with LNP-Cas9. Schematic representation of experimental procedure used to evaluate the efficiency of lung cell editing in Ai14 tdTomato reporter mice, in which Cas9/sgTOM1 can delete the stop cassette enabling tdTomato fluoresce protein expression (**FIG. 2A**). Lung SORT LNPs were used to co-deliver Cas9 mRNA and sgTOM1 (LNP-Cas9). Mice were intravenously injected with LNP-Cas9 at 2 mg/kg total RNA (Cas9 mRNA: sgTOM1=2:1, wt/wt; total lipid to total RNA=20:1, wt/wt) with three sequential doses, one week apart. Mice treated with PBS was used as negative control. Time-course flow cytometry analyses 7, 21, 60, and 240 days after the last injection showing the percentage of tdTomato-positivie (tdTom+) cell was reported among (**FIG. 2B**) total lung cells, (**FIG. 2C**) lung endothelial cells, (**FIG. 2D**) lung immune cells, (**FIG. 2E**) lung epithelial cells, (**FIG. 2F**) Ngfr+ lung basal stem cells, and (**FIG. 2G**) Krt5+lung basal stem cells. Similar to the result of Cre editing, tdTom+ cells retained persistent expression across the lungs including 38.7% of endothelial cells, 32.5% of epithelial cells, 6.1% of immune cells, 16.7% of $Ngfr^+$ lung basal stem cells, and 7.2% of $Krt5^+$ lung basal stem cells for up to 240 days.

[0044] **FIG. 3A-3D** depicts the minimal toxicity observed after LNP-Cas9 treatments. *In vivo* toxicity of Lung SORT LNPs were evaluated by measuring liver function parameters, ALT (**FIG. 3A**) and AST (**FIG. 3B**) and kidney function parameters, BUN (**FIG. 3C**) and CREA (**FIG. 3D**) in mouse serum 7, 21, 60, and 240 days after three sequential doses of LNP-Cas9, one week apart (2 mg/kg total RNA, i.v., total lipid/total RNA=20:1). PBS-treated mice were used as a negative control.

[0045] **FIG. 4A-4G** depicts lung SORT LNP-mediated editing in tracheal and bronchus regions. LNP-Cre were intravenously administered to Ai14 mice in two successive doses, each being 2 mg/kg total RNA, 48 hours apart. The tracheas and bronchus regions were extracted 48 hours following the final injection, and tdTom expression (tdTom+) across various cell types was quantified using flow cytometry. The composition of cells markedly differed between the trachea and bronchus regions of the lung (**FIG. 4A**). The trachea harbored more immune cells (~55.8%) and fewer epithelial (~13.7%) and endothelial cells (~8.4%) compared to the bronchus (35.4% immune cells, 28.7% epithelial cells, and 23.5% endothelial cells). Percentages of total edited cells, endothelial cells, immune cells, epithelial cells, $Ngfr^+$ basal stem cells, and $Krt5^+$ basal stem cells in the trachea compared to the bronchus region (**FIG. 4B-FIG. 4G**).

[0046] **FIG. 5A-5B** depicts gene editing in mouse lung endothelial progenitor cells with LNP-Cre. Ai14 mice were dosed with two sequential LNP-Cre treatments (two days apart) at 2 mg/kg total RNA (20:1, total lipid to RNA weight ratio). The lungs were collected at 2 days, 7 days, 21 days, 42 days, 60 days, 120 days, 180 days, 270 days, and 360 days after the last injection. Flow cytometry gating strategy for lung endothelial progenitor cells (**FIG. 5A**). Single cells prepared from Ai14 mouse lungs were gated. Single cells prepared from Ai14 mouse lungs were gated. Viable (Ghost Red negative) lung endothelial progenitor cells (CD45 negative, CD31 positive, CD157 positive) expressing tdTomato fluorescence (tdTomato positive) were analyzed by flow cytometry. Gene editing in mouse lung hematopoietic lung endothelial progenitor cells were obtained from three mice per each time point (**FIG. 5B**). Saline treated mice were served as negative control.

[0047] **FIG. 6A-6C** depicts gene editing in mouse lung hematopoietic progenitor cells with LNP-Cre. Ai14 mice were dosed with two sequential LNP-Cre treatments (two days apart) at 2 mg/kg total RNA (20:1, total lipid to RNA weight ratio). The lungs were collected at 2 days, 7 days, 21 days, 42 days, 60 days, 120 days, 180 days, 270 days, and 360 days after the last injection. Flow cytometry gating strategy for lung hematopoietic progenitor cells (**FIG. 6A**). Single cells prepared from Ai14 mouse lungs were gated. Viable (Ghost Red negative) lung multipotent progenitor cells (lineage negative, CD45 positive, Sca1 negative, c-kit positive) or lung hematopoietic stem cells (lineage negative, CD45 positive, Sca1 positive, c-kit positive) expressing tdTomato fluorescence (tdTomato positive) were analyzed by flow cytometry. Gene editing in mouse lung hematopoietic lung multipotent progenitor cells (**FIG. 6B**) and lung

hematopoietic stem cells (**FIG. 6C**) were obtained from three mice per each time point. Saline treated mice were served as negative control.

[0048] **FIG. 7A-7E** depicts lung SORT LNP-mediated efficient delivery into mature lung epithelial cells where vitronectin receptor expressing lung cell types exhibit enhanced editing efficiency. Representative immunofluorescence images of lung sections from LNP-Cre treated Ai14 reporter mice 2, 7, 60, 120, 270, and 360 days after the last injection. Lung sections were stained with 5 different biomarker of various lung epithelial cell types (HOPX for AT1 cells, ABCA3 for AT2 cells, MUC5AC for globet cells, Tubulin for ciliated cells and SCGB1A1 for club cells) to quantify LNP-Cre-mediated editing in mature lung epithelial cells from Ai14 tdTom reporter mice lung. PBS serves as a negative control. Scale bar = 30 μm. Biomarkers (white); tdTom (red); nuclei (blue) (**FIG. 7A**). Quantification analysis of LNP-Cre-mediated editing in mature lung epithelium base on IHC images. Results were obtained from five to six random airway per whole slide IHC images and are presented as mean ± SEM (**FIG. 7B**). Representative native whole slide immunofluorescence images of lung sections from a PBS treated mouse (**FIG. 7C**) and a LNP-Cre treated mouse (**FIG. 7D**), DAPI shown as blue and tdTom shown as Red. Representative native tissuecyte image of a single lung left lobe whole section from a LNP-Cre treated mouse (**FIG. 7E**).

[0049] **FIG. 8A-8B** depicts quantitative TissueCyte analysis of mTmG mice lung following LNP-Cre treatment. Schematic representation of LNP-Cre mediated eGFP fluorescence protein expression replacing the red fluorescence in lung cells after systemic administrations (**FIG. 8A**). A mouse was injected intravenously with a single LNP-Cre treatment at 2 mg/kg total RNA (20:1, total lipid to RNA weight ratio). The mouse lung was collected two days after the injection. PBS-treated mTmG mouse was used as negative control. Quantitative analysis of GFP positive (GFP^+) area % in LNP-Cre treated mTmG mouse lung left lobe by TissueCyte 3D imaging and analysis (**FIG. 8B**).

[0050] **FIG. 9A-9B** depicts the protein corona composition adsorbed onto Lung SORT LNP surface as determined by unbiased mass spectrometry proteomics. The most abundant proteins were ranked and plotted as a heat map (**FIG. 9A**) and classified into physiological classes of the identified proteins (**FIG. 9B**).

[0051] **FIG. 10A-10E** depicts vitronectin receptor-expressing lung cell types exhibit enhanced editing efficiency. Quantification of tdTom positivity in vitronectin receptor ($CD51^+CD61^+$) expressing cells and the vitronectin receptor population in lung endothelial cell

(**FIG. 10A**), immune cells (**FIG. 10B**), epithelial cells (**FIG. 10C**), $Ngfr^+$ cells (**FIG. 10D**) and $Krt5^+$ stem cells (**FIG. 10E**). Data are mean ± SEM ($n = 4$ independent replicates). 2-way ANOVA.

[0052] **FIG. 11A-11E** depicts flow cytometry gating strategy for vitronectin receptor. Single cells prepared from Ai14 mouse lungs were gated. Viable (Ghost Red negative) total lung cells, vitronectin receptor expressing (CD51 positive CD61 positive) endothelial cells (CD31 positive; **FIG. 11A**), immune cells (CD45 positive; **FIG. 11B**), epithelial cells (EpCam positive; **FIG. 11C**), lung basal stem cells (EpCam positive Ngfr positive (**FIG. 11D**) or EpCam positive Krt5 positive (**FIG. 11E**) with tdTomato fluorescence (tdTom positive) were analyzed by flow cytometry (n=3).

[0053] **FIG. 12A-12B** depicts the efficient adenine base editing in 16HBEge R553X cells. LNP-ABE (ABE mRNA:sgR553X=2:1 by weight, 1.5 μg total RNA per well) mediated high level base editing efficiency (>95%) in 16HBEge R553X cells at the target T7 position (**FIG. 12A**). The A•T to G•C conversion on T7 position was analyzed using EditR analysis with Sanger sequencing data. The stoichiometry of ABE mRNA and sgR553X was investigated by measuring the editing level after transfecting 16HBEge cell using a series of LNP-ABE (0.8 μg total RNA per well) with altered ABE mRNA to sgRNA weight ratios (**FIG. 12B**). Data are shown as mean±SEM (n=2 biologically independent samples).

[0054] **FIG. 13A-13F** depicts efficient adenine base editing was achieved in lung basal cells across CF models. Workflow for differentiation of HBE from a healthy donor and a CF patient with $CFTR^{R553X/F508del}$ into airway epithelium and base editing strategy to correct CF R553X mutation (**FIG. 13A**). Untreated CF HBE cells were used as negative control and HBE from healthy donor with wild-type CFTR gene was used for comparison. LNP-ABE-mediated 60% of allelic base editing in both undifferentiated P2 and fully differentiated P3 culture (**FIG. 13B**). The frequency of desired product (the box highlighted in blue) and bystander editing was evaluated using NGS sequencing (**FIG. 13C**). Efficacy of LNP-ABE with or without Trikafta in CFTR protein restoration in $CFTR^{R553X/F508del}$ HBE culture measured by JESS western blotting (**FIG. 13D**). Band B (**FIG. 13E**) and Band C (**FIG. 13F**) intensities were normalized by vinculin (20 μg/mL) as an internal standard. Data are mean ± SEM of $n = 3$ independent replicates. Unpaired t-test.

[0055] **FIG. 14A-14B** depicts raw data of gel images of CFTR (**FIG. 14A**) and Vinculin as internal standard (**FIG. 14B**) from JESS western blotting.

[0056] **FIG. 15A-15B** depicts the ability of LNP-ABE to restore HBE culture function. Quantitative data from average current calculated from area under the curve (AUC) representing CFTR activity further confirmed CFTR restoration. Data are mean ± SEM ($n = 4$ independent replicates); One-way ANOVA (**FIG. 15A**). CFTR function in HBE culture from non-cystic fibrosis individuals who are wild type for the *CFTR* gene; Data are mean ± SEM ($n = 4$ independent replicates) (**FIG. 15B**).

[0057] **FIG. 16A-16G** depicts in vivo stem cell editing in CF mouse lungs. Workflow of R553X correction in intestinal organoids using LNP-ABEs and the mechanism of Forskolin-induced swelling (FIS) assay (**FIG. 16A**). Intestinal stem cells were isolated from R553X homozygous mice to generate intestinal organoids as an *ex vivo* model to evaluate CFTR function restoration following LNP-ABE treatment. Forskolin-induced CFTR activation can facilitate ion/water transportation leading to organoid swelling. No swelling was observed from untreated group (**FIG. 16B**), while LNP-ABE treated group showed over 80% of intestinal organoid swelling (**FIG. 16C, FIG. 16D**); Scale bar: 500 μm. Approximately 50% of base editing was confirmed using DNA sequencing; Data are mean ± SD ($n = 8$ independent replicates); Unpaired t-test (**FIG. 16E**). Workflow of assessing LNP-ABE-mediated base editing in mouse lung basal cells after a single administration (**FIG. 16F**). CF heterozygous R553X mice were injected intravenously with LNP-ABE (1.5 mg kg^{-1} total RNA, ABE mRNA:sgR553X=2:1, weight ratio). Mice were sacrificed 10 days after the injection. Whole lung tissue, isolated lung basal stem cells, and trachea populations were used for DNA extraction, PCR amplification, and NGS sequencing. Base editing efficiency of all adenines within the target protospacer in three lung populations. Data are mean ± SEM ($n = 3$ independent replicates) (**FIG. 16G**).

[0058] **FIG. 17A-17M** shows that direct *in vivo* gene editing was achieved in mouse lungs that persisted for one year. Schematic representation of LNP-mediated gene editor delivery into lung cells after systemic administration (**FIG. 17A**). Diagram showing the experimental procedure used to evaluate the efficiency of lung cell editing in Ai14 tdTom reporter mice, in which Cre recombinase can excise the loxP flanked stop cassette enabling tdTom fluorescence protein expression. Mice were injected with LNP-Cre at 2 mg/kg total RNA (20:1, total lipid to RNA weight ratio) with two sequential doses, 48 hours apart. Mice treated with PBS were used as negative control (**FIG. 17B**). *Ex vivo* fluorescence imaging analyses of mouse lungs 2, 7, 21, 42, 60, 120 ,180, 360, and 660 days after the last injection (**FIG. 17C**). Quantification analysis

of *ex vivo* lung images was shown as average radiance (**FIG. 17D**) and as total Flux (**FIG. 17E**). Flow cytometry gating strategy for lung endothelial, epithelial, and immune cells. Single cells prepared from Ai14 mouse lungs were gated. Viable (Ghost Red negative) total lung cells, endothelial cells (CD31 positive), epithelial cells (EpCam positive), or immune cells (CD45 positive) expressing tdTomato fluorescence (tdTom positive) were analyzed by flow cytometry (n=3) (**FIG. 17F**). Flow cytometry gating strategy for lung endothelial, epithelial, and immune cells. Single cells prepared from Ai14 mouse lungs were gated. Viable (Ghost Red negative) lung basal stem cells (EpCam positive, Ngfr positive) and lung stem cells (EpCam positive, Krt5 or CK5 positive) expressing tdTomato fluorescence (tdTom positive) were analyzed by flow cytometry (n=3) (**FIG. 17G**). Time-course flow cytometry analyses showing the percentage of tdTom-positive ($tdTOM^+$) cell was reported among (**FIG. 17H**) lung cells, (**FIG. 17I**) endothelial cells, (**FIG. 17J**) immune cells, (**FIG. 17K**) epithelial cells, (**FIG. 17L**) $Ngfr^+$ stem cells, and (**FIG. 17M**) $Krt5^+$ stem cells. Data are means ± SEMs (n = 3 biologically independent replicates) for treated groups.

[0059] **FIG. 18A-18H** depicts durable i*n vivo* gene editing in mouse lung with LNP-Cas9. Schematic representation of experimental procedure used to evaluate the efficiency of lung cell editing in Ai14 tdTomato reporter mice, in which Cas9/sgTOM1 can delete the stop cassette enabling tdTomato fluoresce protein expression (**FIG. 18A**). The location of sgTOM1 on the stop cassette (**FIG. 18B**). Lung SORT LNPs were used to co-deliver Cas9 mRNA and sgTOM1 (LNP-Cas9). Mice were intravenously injected with LNP-Cas9 at 2 mg/kg total RNA (Cas9 mRNA: sgTOM1=2:1, wt/wt; total lipid to total RNA=20:1, wt/wt) with three sequential doses, one week apart. Mice treated with PBS was used as negative control. Time-course flow cytometry analyses 7, 21, 60, and 240 days after the last injection showing the percentage of tdTomato-positivie (tdTom+) cell was reported among (**FIG. 18C**) total lung cells, (**FIG. 18D**) lung endothelial cells, (**FIG. 18E**) lung immune cells, (**FIG. 18F**) lung epithelial cells, (**FIG. 18G**) Ngfr+ lung stem cells, and (**FIG. 18H**) Krt5+lung stem cells. Similar to the result of Cre editing, tdTom+ cells retained persistent expression across the lungs including 38.7% of endothelial cells, 32.5% of epithelial cells, 6.1% of immune cells, 16.7% of $Ngfr^+$ lung basal stem cells, and 7.2% of $Krt5^+$ lung basal stem cells for up to 240 days. Data are presented as individual data points or mean ± SEM, (n = 3 biologically independent replicates).

[0060] **FIG. 19A-19E** depicts the minimal toxicity observed after LNP-Cas9 treatments. *In vivo* toxicity of Lung SORT LNPs were evaluated by measuring liver function parameters, ALT

(**FIG. 19A**) and AST (**FIG. 19B**) and kidney function parameters, BUN (**FIG. 19C**) and CREA (**FIG. 19D**) in mouse serum 7, 21, 60, and 240 days after three sequential doses of LNP-Cas9, one week apart (2 mg/kg total RNA, i.v., total lipid/total RNA=20:1) (n = 3 biologically independent replicates). PBS-treated mice were used as a negative control. Data are presented as individual data points or mean ± SEM. Histopathology evaluation performed by H&E staining of heart and spleen tissues 7, 60, and 120 days after LNP-Cas9 treatment (**FIG. 19E**). PBS-treated mice were used as a negative control. Scale bar: 100 μm.

[0061] **FIG. 20A-20G** depicts lung SORT LNP-mediated editing in tracheal and bronchus regions. LNP-Cre were intravenously administered to Ai14 mice in two successive doses, each being 2 mg/kg total RNA, 48 hours apart. The tracheas and bronchus regions were extracted 48 hours following the final injection, and tdTom expression (tdTom+) across various cell types was quantified using flow cytometry. The composition of cells markedly differed between the trachea and bronchus regions of the lung (**FIG. 20A**). The trachea harbored more immune cells (55.8%) and fewer epithelial (13.7%) and endothelial cells (8.4%) compared to the bronchus (35.4% immune cells, 28.7% epithelial cells, and 23.5% endothelial cells). Percentages of total edited cells (**FIG. 20B**), endothelial cells (**FIG. 20C**), immune cells (**FIG. 20D**), epithelial cells (**FIG. 20E**), $Ngfr^+$ stem cells (**FIG. 20F**), and $Krt5^+$ stem cells (**FIG. 20G**) in the trachea compared to the bronchus region. Data are presented as mean ± SEM in **FIG. 20B-FIG. 20G** (n = 5 biologically independent replicates).

[0062] **FIG. 21A-21B** depicts gene editing in endothelial beds of various organs with LNP-Cre. LNP-Cre was intravenously administered to Ai14 mice in two successive doses, 2 mg/kg total RNA, 48 hours apart. tdTom expression ($tdTom^+$) across $CD31^+$ endothelial beds of various organs (**FIG. 21A**) and total cells of various organs (**FIG. 21B**) were quantified by flow cytometry. Tested organs include heart, lung, liver, spleen, kidney, pancreas, stomach, duodenum, jejunum, ileum, cecum, colon, and rectum. PBS-treated mice were used as negative control. Data are presented as mean ± SEM (n = 3 independent replicates).

[0063] **FIG. 22A-22B** depicts lung SORT LNP-mediated editing in lung immune cells. LNP-Cre were intravenously administered to Ai14 mice in two successive doses, each being 2 mg/kg total RNA, 48 hours apart. Flow cytometry gating strategy for various lung immune cells (**FIG. 22A**). Single cells prepared from Ai14 mouse lungs were gated. Viable (Ghost Red negative) lung immune cells (CD45 positive) including B cells (B220 positive), CD4 T cells (CD3 positive CD4 positive), CD8 T cells (CD3 positive CD8 positive), dendritic cells (CD11c

positive), neutrophils (Ly-6G positive) and macrophages (F4/80 positive) were analyzed by flow cytometry to evaluate tdTomato expression level (tdTomato positive). PBS treated mice were served as negative control. The results showed that 22.6% of neutrophils, 41.7% of macrophage, 32.4% of dendritic cells, 14.9% B cells, 14.9% $CD4^+$ T cells and 14.5% of $CD8^+$ T cells were edited (**FIG. 22B**). Data are presented as mean ± SEM (n=3 biologically independent replicates).

[0064] **FIG. 23A-23D** depicts lung SORT LNP-mediated editing in *P. aeruginosa* infected mouse lungs. Ai14 mice were randomly allocated to *P. aeruginosa* infection group or non-infection group. Mice in infection group were inoculated intranasally with 50 µl of *P. aeruginosa* at 3.5 x 105 CFU to generate acute infection model (**FIG. 23A**). LNP-Cre were intravenously administered to Ai14 mice, 2 mg/kg total RNA, 9 h post infection. Neutrophil invasion was observed in infected lungs 9 h after the intranasal infection compared to non-infected lungs (**FIG. 23B**). VtnR abundance (**FIG. 23C**) and tdTom expression ($tdTom^+$) across various cell types in infected and non-infected mouse lung (**FIG. 23D**) were measured using flow cytometry. No significant difference was observed in VtnR abundance and editing efficiency among various tested lung cell types except immune cells between infected and non-infected lungs. PBS-treated infected mice were used as negative control. Data are presented as mean ± SEM (n = 3 independent replicates), Unpaired t-test. P values < 0.05 were considered statistically significant.

[0065] **FIG. 24A-24B** depicts gene editing in mouse lung endothelial progenitor cells with LNP-Cre. Ai14 mice were dosed with two sequential LNP-Cre treatments (two days apart) at 2 mg/kg total RNA (20:1, total lipid to RNA weight ratio). The lungs were collected at 2, 7, 21, 42, 60, 120, 180, 270, 360, and 660 days after the last injection. Flow cytometry gating strategy for lung endothelial progenitor cells (**FIG. 24A**). Single cells prepared from Ai14 mouse lungs were gated. Single cells prepared from Ai14 mouse lungs were gated. Viable (Ghost Red negative) lung endothelial progenitor cells (CD45 negative, CD31 positive, CD157 positive) expressing tdTomato fluorescence (tdTomato positive) were analyzed by flow cytometry. Gene editing in mouse lung hematopoietic lung endothelial progenitor cells were obtained from three mice per each time point (**FIG. 24B**). PBS treated mice were served as negative control. Data are presented as individual data points or mean ± SEM (n=3 biologically independent replicates).

[0066] **FIG. 25A-25C** depicts gene editing in mouse lung hematopoietic progenitor cells with LNP-Cre. Ai14 mice were dosed with two sequential LNP-Cre treatments (two days apart) at 2 mg/kg total RNA (20:1, total lipid to RNA weight ratio). The lungs inclusive of tracheas were collected at 2, 7, 21, 42, 60, 120, 180, 270, 360, and 660 days after the last injection. Flow cytometry gating strategy for lung hematopoietic progenitor cells (**FIG. 25A**). Single cells prepared from Ai14 mouse lungs were gated. Viable (Ghost Red negative) lung multipotent progenitor cells (lineage negative, CD45 positive, Sca1 negative, c-kit positive) or lung hematopoietic stem cells (lineage negative, CD45 positive, Sca1 positive, c-kit positive) expressing tdTomato fluorescence (tdTomato positive) were analyzed by flow cytometry. Gene editing in mouse lung hematopoietic lung multipotent progenitor cells (**FIG. 25B**) and lung hematopoietic stem cells (**FIG. 25C**) were obtained from three mice per each time point. PBS treated mice were served as negative control. Data are presented as individual data points or mean ± SEM (n=3 biologically independent replicates).

[0067] **FIG. 26** depicts a representative whole slide images showing five to six random segments selection for quantification analysis.

[0068] **FIG. 27A-27K** depicts lung SORT LNP-mediated efficient delivery into diverse lung cell types with enhanced delivery to VtnR-expressing cells. Representative immunofluorescence images of lung sections from LNP-Cre treated Ai14 reporter mice (2, 7, 60, 120, 270, and 360 days after treatment) to assess LNP-mediated editing in mature lung epithelial cells (HOPX for AT1 cells, ABCA3 for AT2 cells, MUC5AC for goblet cells, tubulin for ciliated cells, and SCGB1A1 for club cells). PBS-treated mice served as negative controls. Scale bar = 30 μm. Markers (white); tdTom (red); nuclei (blue) (**FIG. 27A**). Quantification of LNP-Cre-mediated editing in mature lung epithelium based on immunofluorescence images. Results were obtained from five to six random airway segments per whole slide. Data are presented as means ± SEMs (**FIG. 27B**). Representative whole slide immunofluorescence images from PBS treated (**FIG. 27C**) and LNP-Cre treated (**FIG. 27D**) mice. DAPI is blue and tdTom is red. Scale bars = 1mm. Representative tissuecyte image of a LNP-Cre-treated mouse's lung whole left lobe (**FIG. 27E**). Scale bar = 1mm. VtnR (CD51+CD61+) abundance (**FIG. 27F**) and quantification of tdTom positivity in VtnR+ (CD51+CD61+) cells and VtnR- (CD51-CD61-) fraction in lung endothelial cells (**FIG. 27G**), immune cells (**FIG. 27H**), epithelial cells (**FIG. 27I**), NGFR+ cells (**FIG. 27J**), and KRT5+ stem cells (**FIG. 27K**). Data are means ± SEMs (n = 3 biologically

independent replicates), Unpaired t-test. P values < 0.05 were considered statistically significant. ns = not significant.

[0069] **FIG. 28A-28B** depicts gene editing and persistence of gene editing in mouse lung ionocytes following systemic LNP-Cre administration**.** Ai14 mice were dosed with two sequential LNP-Cre treatments (two days apart) at 2 mg/kg total RNA (20:1, total lipid to RNA weight ratio). The lungs were collected 2 days and 660 days after the last injection. Flow cytometry gating strategy for lung ionocytes. FOXI1 positive lung ionocytes were gated and analyzed for the expression of tdtomato fluorescence (**FIG. 28A**). Editing levels, correlated with tdtomato expression in mouse lung ionocytes, were measured from three mice at each time point (**FIG. 28B**). PBS treated mice served as the negative control. Data are presented as mean ± SEM (n=3 biologically independent replicates).

[0070] **FIG. 29A-29B** depicts quantitative TissueCyte analysis of mTmG mice lung following LNP-Cre treatment. Schematic representation of LNP-Cre mediated eGFP fluorescence protein expression replacing the red fluorescence in lung cells after systemic administrations. A mouse was injected intravenously with a single LNP-Cre treatment at 2 mg/kg total RNA (20:1, total lipid to RNA weight ratio). The mouse lung was collected two days after the injection. PBS-treated mTmG mouse was used as negative control (**FIG. 29A**). Quantitative analysis of GFP positive (GFP^+) area % in LNP-Cre treated mTmG mouse lung left lobe by TissueCyte 3D imaging and analysis (**FIG. 29B**). Data are presented as individual data points (n=1).

[0071] **FIG. 30A-30B** depicts the protein corona composition adsorbed onto Lung SORT LNP surface determined by unbiased mass spectrometry proteomics. The most abundant proteins were ranked and plotted as a heat map (**FIG. 30A**) and classified into physiological classes of the identified proteins (**FIG. 30B**).

[0072] **FIG. 31A-31D** depicts how vitronectin regulates SORT and non-SORT LNP efficacy in vitronectin receptor (VtnR) expressing cells in vitro. 5-component 5A2-SC8 SORT LNPs, 5-component DLin-MC3-DMA (MC3) SORT LNPs, and 4-component non-SORT LNPs (standard MC3 LNPs) were preincubated with 0.25 g Vtn/g lipid prior to treating Vtn-negative liver cancer cells (Huh-7), Vtn-expressing kidney cancer cells (A-498), and Vtn-expressing human lung epithelial (16HBE14o-) cells to measure functional mRNA delivery (bioluminescence) (**FIG. 31A**). The activity of functional luciferase translated from mRNA delivered by uncoated LNPs (labeled "-") or Vtn-coated LNPs (labeled "+") in relevant cell lines (25 ng mRNA, 24 h,

n = 4) (**FIG. 31B**). Cells were saturated with 0.2 μg Vtn/mL prior to treatment with Vtn-coated SORT and non-SORT LNPs (which do not bind Vtn) to assess functional mRNA delivery (bioluminescence) (**FIG. 31C**). The activity of functional luciferase translated from mRNA delivered by Vtn-coated LNPs in cells with or without Vtn saturation (**FIG. 31D**). (25 ng mRNA, 24 h, n = 4). Statistical significance was determined using an unpaired t test. P values < 0.05 were considered statistically significant. Data are shown as mean ± SEM.

[0073] **FIG. 32** depicts the flow cytometry gating strategy for vitronectin receptor. Single cells prepared from Ai14 mouse organs were gated. Viable (Ghost Red negative) total cells, vitronectin receptor expressing (CD51 positive CD61 positive) endothelial cells (CD31 positive), immune cells (CD45 positive), epithelial cells (EpCAM positive), lung stem cells (EpCAM positive NGFR positive or EpCAM positive KRT5 positive) with tdTomato fluorescence (tdTom positive) were analyzed by flow cytometry (n=3).

[0074] **FIG. 33A-33B** depicts LNP delivery in VtnR-expressing lung endothelial cells, lung multipotent progenitor cells, and lung hematopoietic stem cells. VtnR ($CD51^+CD61^+$) abundance (**FIG. 33A**) and quantification of tdTom positivity in $VtnR^+$ ($CD51^+CD61^+$) cells and $VtnR^-$ ($CD51^-CD61^-$) fraction in lung endothelial cells, lung multipotent progenitor cells, and lung hematopoietic stem cells (**FIG. 33B**). Ai14 mice were dosed with two sequential LNP-Cre treatments (two days apart) at 2 mg/kg total RNA (20:1, total lipid to RNA weight ratio). The lungs were collected 2 days after the last injection. Single cells prepared from Ai14 mouse lungs were gated. Viable (Ghost Red negative) total lung cells, vitronectin receptor expressing (CD51 positive CD61 positive) endothelial progenitor cells (CD31 positive CD157 positive), lung resident multipotent immune progenitor cells (lineage negative, CD45 positive, Sca1 negative, c-kit positive), or lung resident hematopoietic stem cells (lineage negative, CD45 positive, Sca1 positive, c-kit positive) with tdTomato fluorescence (tdTom positive) were analyzed by flow cytometry. Lung endothelial cells (22.4%), lung multipotent progenitor cells (22.5%), and lung hematopoietic stem cells (38.6%) all exhibit high levels of VtnR expression. Lung SORT LNPs transfect a very high fraction of endothelial and lung hematopoietic stem cells, which may mask any potential contribution of VtnR versus other receptor interactions. Data are mean ± SEM (n = 3 independent replicates).

[0075] **FIG. 34A-34C** depicts profiling of VtnR expression in mouse tissues and LNP delivery in VtnR-expressing lung endothelial and non-endothelial cells. VtnR ($CD51^+CD61^+$) abundance (**FIG. 34A**) and quantification of tdTom positivity in $VtnR^+$ ($CD51^+CD61^+$) cells and

$VtnR^-$ ($CD51^-CD61^-$) fraction in endothelial cells (**FIG. 34B**) or non-endothelial cells (**FIG. 34C**) across various organs. Ai14 mice were dosed with two sequential LNP-Cre treatments (two days apart) at 2 mg/kg total RNA (20:1, total lipid to RNA weight ratio). Organs were collected 2 days after the last injection. Single cells prepared from Ai14 mouse organs were gated. Viable (Ghost Red negative) total cells, vitronectin receptor expressing (CD51 positive CD61 positive) endothelial cells (CD31 positive) or non-endothelial cells (CD31 negative) with tdTomato fluorescence (tdTom positive) were analyzed by flow cytometry. Comparing to heart (3.0%), liver (2.5%), spleen (3.7%), kidney (1.1%), pancreas (1.4%), duodenum (2.2%), ovaries/testes (4.3%), lung exhibit highest level of VtnR expression (24.4%). Endothelial cells of all tested organs displayed no preference for tdTom in $VtnR^+$ or VtnR- fraction. However, in non-endothelial cell population, tdTom expression was enriched within the $VtnR^+$ fraction compared to the $VtnR^-$ fraction in heart (24.8% vs 2.9%), lung (23.3% vs 2.9%), liver (88.8% vs 52.3%), spleen (10.4% vs 4.6%) and kidney (24.6% vs 2.7%). Data are mean ± SEM (n = 3 independent replicates), unpaired t-test. P values < 0.05 were considered statistically significant.

[0076] **FIG. 35A-35B** depicts efficient adenine base editing in 16HBEge R553X cells. LNP-ABE (ABE mRNA:sgR553X=2:1 by weight, 1.5 μg total RNA per well) mediated high level base editing efficiency (>95%) in 16HBEge R553X cells at the target T_7 position (**FIG. 35A**). The A•T to G•C conversion on T_7 position was analyzed using EditR analysis with Sanger sequencing data. The stoichiometry of ABE mRNA and sgR553X was investigated by measuring the editing level after transfecting 16HBEge cell using a series of LNP-ABE (0.8 μg total RNA per well) with altered ABE mRNA to sgRNA weight ratios (**FIG. 35B**). Data are shown as mean ± SEM (n=2 independent samples).

[0077] **FIG. 36A-36O** depicts efficient adenine base editing was achieved in lung basal cells in patient-derived HBE cells and CF mouse model. Workflow for differentiation of HBE from a healthy donor and a CF patient with $CFTR^{R553X/F508del}$ into airway epithelium and base editing strategy to correct CF R553X mutation (**FIG. 36A**). Untreated CF HBE cells were used as negative control and HBEs from a healthy donor with wild-type CFTR gene was used for comparison. LNP-ABE-mediated 60% of allelic base editing in both undifferentiated P2 (n=1) and fully differentiated P3 culture (n=4) (**FIG. 36B**). The frequency of desired product (the box highlighted in blue) and bystander editing was evaluated using NGS sequencing (**FIG. 36C**). Efficacy of LNP-ABE with or without Trikafta in CFTR protein restoration in $CFTR^{R553X/F508del}$ HBE culture measured by capillary western blotting (**FIG. 36D**). Band B (**FIG. 36E**) and Band

C (**FIG. 36F**) intensities from **FIG. 36D** were normalized to vinculin (20 μg/mL) as an internal standard. Data are means ± SEMs of n = 3 independent replicates. One-way analysis of variance (ANOVA); P values of <0.05 were considered statistically significant. Quantitative data for average current calculated from AUC representing CFTR activity further confirmed CFTR restoration. Data are means ± SEMs of n=4. One-way ANOVA; P values of <0.05 were considered statistically significant (**FIG. 36G**). CFTR function in HBE culture from non-CF patients who are wild-type for the CFTR gene (n=4 independent replicates) (**FIG. 36H**). Workflow of R553X correction in intestinal organoids using LNP-ABEs and the mechanism of Forskolin-induced swelling (FIS) assay (**FIG. 36I**). Intestinal stem cells were isolated from R553X homozygous mice to generate intestinal organoids as an *ex vivo* model to evaluate CFTR function restoration after LNP-ABE treatment. Forskolin-induced CFTR activation can facilitate ion/water transportation leading to organoid swelling. No swelling was observed from untreated group (**FIG. 36J**), while LNP-ABE treated group showed over 80% of intestinal organoid swelling (**FIG. 36K, FIG. 36L**); Scale bars: 1000 μm. Approximately 50% of base editing was confirmed using DNA sequencing (**FIG. 36M**); Data are means ± SEMs of n = 8 independent replicates in **FIG. 36L** and **FIG. 36M**. Unpaired t-test; P values of <0.05 were considered statistically significant. Workflow of assessing LNP-ABE-mediated base editing in mouse lung basal cells after a single administration (**FIG. 36N**). CF heterozygous R553X mice were injected intravenously with LNP-ABE (1.5 mg kg^{-1} total RNA, ABE mRNA:sgR553X=2:1, weight ratio). Mice were euthanized 10 days after the injection. Whole lung tissue, trachea, and isolated lung NGFR+ basal stem cells populations were used for DNA extraction, PCR amplification, and NGS sequencing. Base editing efficiency of all adenines within the target protospacer in three lung populations (**FIG. 36O**). Data are mean ± SEM (n = 4 independent replicates).

[0078] **FIG. 37A-37B** depicts raw data of gel images of CFTR (**FIG. 37A**) and Vinculin as internal standard (**FIG. 37B**) from Jess™ capillary western blotting.

[0079] **FIG. 38A-38B** depicts apical and basolateral delivery of LNP-tdTom in P3 differentiated HBE R553X/F508del cultures. Cells were then treated with 12 μg LNP-tdTom (12 μg tdTomato mRNA per well) either to the apical side in liquid bolus or to the basolateral side. Untreated HBE cells were used as control. Single cells prepared from inserts were gated (**FIG. 38A**). Viable (Scarlet 723 negative) basal cells (KRT5 positive), club cells (SCGB1A1 positive), goblet cells (MUC5AC positive), and ciliated cells (Acetyl-Tubulin positive) with tdTomato fluorescence (tdTom positive) were analyzed by flow cytometry. Quantitative analysis of

tdTom$^+$ cells in HBE basal and differentiated cell populations (n=3) (**FIG. 38B**). Data are shown as mean ± SEM. Unpaired t-test. P values < 0.05 were considered statistically significant.

Detailed Description

[0080] Cystic fibrosis, also known as mucoviscidosis, is an autosomal recessive genetic disorder that affects most critically the lungs, and also the pancreas, liver, and intestine (Gibson et al., Am J Respir Crit Care Med. (2003) 168(8):918-951; Ratjen et al., Lancet Lond Engl. (2003) 361(9358):681-689; O'Sullivan et al., Lancet Lond Engl. (2009) 373(9678):1891-1904). Cystic fibrosis is caused by mutations in the gene encoding for the Cystic Fibrosis Transmembrane conductance Regulator (CFTR) protein. This protein functions as a channel that transports chloride ions across the membrane of cells and is required to regulate the components of mucus, sweat, saliva, tears, and digestive enzymes. Disease-causing mutations in the CFTR protein cause dysfunction of its channel activity resulting in abnormal transport of chloride and sodium ions across the epithelium, leading to the thick, viscous secretions in the lung, pancreas and other organs characteristic of cycstic fibrosis disease (O'Sulliven et al., Lancet Lond Engl. (2009) 373(9678):1891-1904; Rowe et al., N Engl J Med. (2005) 352(19):1992-2001). Most cycstic fibrosis patients develop severe, chronic lung disease related to airway obstruction partly due to increased levels of sulfated mucins, inflammation, and recurrent infections that are eventually lethal; the median predicted survival age in the US is 40.7 years. Cystic fibrosis is the most frequent lethal genetic disease in the white population.

[0081] Cystic fibrosis (CF) is an autosomal recessive disorder with an estimated 89,000 individuals affected worldwide. Patients with CF have mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, mainly affecting the lungs, liver, pancreas, and intestine. CFTR is a membrane protein that functions as a chloride channel in the apical membrane of epithelial cells. Individuals with CF have dysregulated chloride and bicarbonate transport across the apical surface of secretory epithelia. Almost 2,000 variants have been cataloged in the Cystic Fibrosis Mutation database, including variants that cause substitution of a single amino acid, nonsense mutations, frameshifts, mis-splicing variants, or affect the promoter region. The most common cause of CF is the gene variant F508del, which is defined by a deletion of three base pairs that cause the loss of the amino acid phenylalanine at position 508. The loss of phenylalanine causes incorrect folding of the CFTR protein and subsequent

degradation. Overall, the variants affect the amount of CFTR protein available in the apical cell membrane, reducing the activity and efficiency of the ion channel.

[0082] The lungs of individuals with cystic fibrosis are colonized and infected by bacteria from an early age. This leads to chronic airway infection and inflammation, progressing to bronchiectasis, gas trapping, hypoxemia, and hypercarbia. Pulmonary insufficiency is responsible for 68.1% of CF-related deaths in the US. In the initial stage, common bacteria such as *Staphylococcus aureus* and *Hemophilus influenzae* colonize and infect the lungs. Eventually, *Pseudomonas aeruginosa* (and sometimes *Burkholderia cepacia*) dominates. By 18 years of age, 80% of patients with classic CF harbor *P. aeruginosa*, and 3.5% harbor *B. cepacia*. Once within the lungs, these bacteria adapt to the environment and develop resistance to commonly used antibiotics.

[0083] The decrease or loss of function of CFTR protein leads to multiorgan dysfunction and a shortened life expectancy. The abnormally viscous secretions in the lung airways cause obstructions that lead to inflammation, tissue damage, frequent respiratory infections, and organ failure. Other organ systems that contain epithelia are also affected, including sweat glands and the male reproductive tract. Currently, obstructive lung disease is the primary cause of morbidity.

[0084] Several treatments for cystic fibrosis have been developed since the first causal CFTR mutation was identified in 1989. The gene-targeting therapies aim to augment the function of mutant CFTR or restore CFTR function by correcting the mutations. Other therapies are focused on improving mucociliary clearance and preventing infections. Therapies comprised of a group of small molecules that aim to modulate and restore mutant CFTR function are known as CFTR modulators. The compounds elexacaftor-tezacaftor-ivacaftor enhance the activity of mutant CFTR, and patients with the F508del variant taking this combination have improved lung function from 0.2% in the placebo group to 13.6%. However, these modulators are ineffective for CFTR mutations that lead to premature stop codons or splicing defects. For example, patients with mutations that cause premature stop codons (R553X, G542X, and W1282X) or with a splice-site mutation (3849 + 10 kb C>T) cannot benefit from CFTR modulators. Using gene therapy and gene editing approaches to treat patients with nonsense and splicing CFTR mutations can be a viable alternative. *See e.g.,* Ong, T. et al., , *JAMA* 2023; Cutting, G., *Nature reviews. Genetics* 2015; Esposito C. et al., *Life (Basel)* 2023; Hisert, K. et

al., *The Lancet* 2023; McHugh, D. et al., *PloS one* 2018; and Krishnamurthy, S. et al., *Nucleic acids research* 2021, which are incorporated by reference herein in its entirety.

[0085] Gene editing is anticipated to revolutionize the field of medicine by creating durable therapies across a wide spectrum of diseases (J. Y. Wang, J. A. Doudna, CRISPR technology: A decade of genome editing is only the beginning. *Science* **379**, eadd8643 (2023); F. A. Ran *et al.*, Genome engineering using the CRISPR-Cas9 system. *Nat. Protoc.* **8**, 2281-2308 (2013); C. A. Hodges, R. A. Conlon, Delivering on the promise of gene editing for cystic fibrosis. *Genes Dis* **6**, 97-108 (2019)). Direct delivery of gene editors to target cells using synthetic nanoparticle- or virus-based systems could facilitate efficient and precise gene editing directly within the patient's body, bypassing the complexities, risks, and costs associated with ex vivo procedures (K. A. Hajj, K. A. Whitehead, Tools for translation: Non-viral materials for therapeutic mRNA delivery. *Nat. Rev. Mater.* **2**, 17056 (2017); T. Wei *et al.*, Delivery of tissue-targeted scalpels: Opportunities and challenges for in vivo CRISPR/Cas-based genome editing. *ACS Nano* **14**, 9243-9262 (2020); M. J. Mitchell *et al.*, Engineering precision nanoparticles for drug delivery. *Nat. Rev. Drug Discov.* **20**, 101-124 (2021); A. Raguram, S. Banskota, D. R. Liu, Therapeutic in vivo delivery of gene editing agents. *Cell* **185**, 2806-2827 (2022)). Although progress has been made targeting differentiated cells such as hepatocytes (J. D. Gillmore *et al.*, CRISPR-Cas9 in vivo gene editing for transthyretin amyloidosis. *New. Engl. J. Med.* **385**, 493-502 (2021)) and T cells (J. G. Rurik *et al.*, CAR T cells produced in vivo to treat cardiac injury. *Science* **375**, 91-96 (2022)), nanoparticle delivery of gene editors to stem cells has remained elusive. Achieving durable therapeutic responses likely requires editing in stem cells to overcome the loss of corrected DNA in differentiated cells that regularly turnover (C. A. Hodges, R. A. Conlon, Delivering on the promise of gene editing for cystic fibrosis. *Genes Dis* **6**, 97-108 (2019); L. Xu *et al.*, CRISPR/Cas9-mediated CCR5 ablation in human hematopoietic stem/progenitor cells confers HIV-1 resistance in vivo. *Mol. Ther.* **25**, 1782-1789 (2017)).

[0086] Genetic lung diseases constitute a large unmet medical need (N. E. King *et al.*, Correction of airway stem cells: Genome editing approaches for the treatment of cystic fibrosis. *Hum. Gene Ther.* **31**, 956-972 (2020); S. Suzuki *et al.*, Highly efficient gene editing of cystic fibrosis patient-derived airway basal cells results in functional CFTR correction. *Mol. Ther.* **28**, 1684-1695 (2020)) hindered by delivery challenges to protect genome editors from degradation, achieve cell targeted delivery, and minimize off-target effects while overcoming significant physiological barriers including mucus, macrophages, and endothelial tissues (A. McCarron, P.

Cmielewski, V. Drysdale, D. Parsons, M. Donnelley, Effective viral-mediated lung gene therapy: Is airway surface preparation necessary? *Gene Ther.* **30**, 469-477 (2023)).

[0087] Lipid nanoparticles (LNPs) are the most clinically advanced non-viral delivery platform. More than a billion doses of mRNA LNP COVID-19 vaccines have been administered intramuscularly worldwide, demonstrating high safety, efficacy, and repeat dose ability (F. P. Polack *et al.*, Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *New. Engl. J. Med.* **383**, 2603-2615 (2020); L. R. Baden *et al.*, Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *New. Engl. J. Med.* **384**, 403-416 (2020)). Treatment of genetic lung diseases including Cystic Fibrosis (CF) would benefit a direct intravenous (IV) delivery approach to bypass formidable local intratracheal (B. Li *et al.*, Combinatorial design of nanoparticles for pulmonary mRNA delivery and genome editing. *Nat. Biotechnol.*, (2023)) and inhalation (A. K. Patel *et al.*, Inhaled nanoformulated mRNA polyplexes for protein production in lung epithelium. *Adv. Mater.* **31**, 1805116 (2019)) barriers including thick sticky mucus (F. Ratjen *et al.*, Cystic fibrosis. *Nat. Rev. Dis. Primers* **1**, 15010 (2015)) and poor access to stem cells that line the basement of the epithelium. Yet, LNPs predominantly accumulate in the liver when administered intravenously, which hampers extrahepatic therapeutic utility.

[0088] Whether Lung Selective Organ Targeting (SORT) LNPs (Q. Cheng *et al.*, Selective ORgan Targeting (SORT) nanoparticles for tissue specific mRNA delivery and CRISPR/Cas gene editing. *Nat. Nanotechnol.* **15**, 313-320 (2020)) could access tissue-resident stem cells was investigated. It was shown that optimized lung-targeting LNPs can deliver CRISPR-Cas9 (M. Jinek *et al.*, A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* **337**, 816-821 (2012)) and adenine base editors (ABE) (N. M. Gaudelli *et al.*, Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. *Nature* **551**, 464-471 (2017); E. M. Porto, A. C. Komor, I. M. Slaymaker, G. W. Yeo, Base editing: advances and therapeutic opportunities. *Nat. Rev. Drug Discov.* **19**, 839-859 (2020)) to facilitate reconstitution of lung epithelium with corrected genes, thereby achieving enduring and significant therapeutic effects. Provided herein are methods to treat a disease or disorder (e.g. cystic fibrosis).

[0089] In some embodiments, the methods comprise administering to a subject a lipid nanoparticle (LNP) that comprises a gene editing system. In some embodiments, the gene editing system comprises an adenine base editor (ABE), wherein the ABE comprises a tRNA adenosine deaminase (TadA) protein, or a variant thereof, fused to a catalytically impaired Cas

protein capable of binding to a specific nucleotide sequence. In some embodiments, the gene editing system is used to treat a subject with cystic fibrosis.

[0090] The adenine base editor deaminates an adenosine leading to a point mutation from adenine (A) to guanine (G). For example, the adenosine can be converted to an inosine residue. Within the constraints of a DNA polymerase active site, inosine pairs most stably with C and therefore is read or replicated by the cell's replication machinery as a guanine (G). Such base editors are useful for targeted editing of nucleic acid sequences. Such base editors can be used for targeted editing of DNA *in vitro*, e.g., for the generation of mutant cells or animals.

[0091] Such base editors can be used for the introduction of targeted mutations in the cell of a living mammal. Such base editors may also be used for the introduction of targeted mutations for the correction of genetic defects in cells *ex vivo*, e.g., in cells obtained from a subject that are subsequently reintroduced into the same or another subject, or for multiplexed editing of a genome. These base editors may be used for the introduction of targeted mutations *in vivo*, e.g., the correction of genetic defects or the introduction of deactivating mutations in disease-associated genes in a subject, or for multiplexed editing of a genome. In some embodiments, the ABEs described herein are utilized for the targeted editing of G to A mutations (*e.g.,* targeted genome editing). Reference is made to WO2021/158921 published August 12, 2021, which is incorporated herein in its entirety.

[0092] All publications, including patent documents, scientific articles and databases, referred to in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication were individually incorporated by reference. If a definition set forth herein is contrary to or otherwise inconsistent with a definition set forth in the patents, applications, published applications and other publications that are herein incorporated by reference, the definition set forth herein prevails over the definition that is incorporated herein by reference.

[0093] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

I. METHODS OF USE

[0094] In some embodiments, the methods provided herein comprise the treatment of a lung disease or lung disorder in a subject. In some embodiments, the methods provided herein comprise the treatment of cystic fibrosis. In some embodiments, the methods provided herein for the treatment of a lung disease or lung disorder in a subject comprise administering the

described LNPs comprising a gene editing system or administering compositions comprising the described LNPs comprising a gene editing system to the subject. In some embodiments, the methods provided herein for the treatment of cyctic fibrosis in a subject comprise administering the described LNPs comprising a gene editing system or administering compositions comprising the described LNPs comprising a gene editing system to the subject. In some embodiments, the method comprises the treatment of cystic fibrosis in a subject, wherein the method comprises administering to a subject a LNP composition comprising a gene editing system to the subject. LNPs as described in this section are described further in Section II below. Gene editing systems as described in this section are described further in Section III below.

[0095] In some embodiments, the methods provided herein comprise the delivery of a composition to a subject. Compositions as described in this section are described further in Section IV below. In some embodiments, the methods provided herein comprise the delivery of a composition comprising a gene editing system to a lung cell type in a subject. In some embodiments, the composition comprises a LNP and a gene editing system. In some embodiments, the composition comprises a LNP, wherein the LNP comprises a gene editing system. In some embodiments, the composition comprises a LNP, wherein the LNP comprises a gene editing system, wherein the gene editing system comprises a first nucleic acid and a second nucleic acid. In some embodiments, the first nucleic acid encodes an endonuclease. In some embodiments, the first nucleic acid encodes a base editor. In some embodiments, the second nucleic acid encodes a guide RNA (gRNA). In some embodiments, the gene editing system is delivered to a lung cell type in a subject. In some embodiments, a composition comprising a LNP comprising a gene editing system, wherein the gene editing system comprises a first nucleic acid encoding a base editor and a second nucleic acid encoding a gRNA, is delivered to a lung cell type in a subject. In some embodiments, the composition is delivered to a lung cell type in a subject with cystic fibrosis. In some embodiments, the cell is a lung cell. In some embodiments, the lung cell comprises lung cell types that include, but are not limited to, an endothelial cell or an epithelial cell. In some embodiments, the lung cell type is an immune cell. In some embodiments, the lung cell type is a stem cell. In some embodiments, the epithelial cell is a ciliated cell, non-ciliated cell, a goblet cell, a brush cell (alveolar macrophage), an airway basal cell, a small granule cell, a bronchial epithelial cell, a small airway epithelial cell, and/or a tracheal epithelial cell. In some embodiments, the lung cell is a secretory cell and or ionocyte.

[0096] In some embodiments, the methods provided herein comprise the modification of the nucleic acid sequence of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene in a lung cell type in a subject. In some embodiments, the *CFTR* gene in a lung type in a subject comprises a point mutation. In some embodiments, the *CFTR* gene in a lung type in a subject comprises a deletion. In some embodiments, the *CFTR* gene in a lung type in a subject comprises a F508del deletion. In some embodiments, the *CFTR* gene in a lung type in a subject comprises a R553X stop codon mutation. In some embodiments, the methods provided herein comprise the modification of the nucleic acid sequence of the *CFTR* gene wherein the CFTR gene comprises a R553X stop codon mutation. In some embodiments, the modification of the nucleic acid comprises the contacting of the lung cell type with a composition comprising a LNP. In some embodiments, the composition comprises a LNP and a gene editing system. In some embodiments, the composition comprises a LNP, wherein the LNP comprises a first nucleic acid and a second nucleic acid. In some embodiments, the composition comprises an LNP comprising a first nucleic acid, wherein the first nucleic acid encodes a base editor, and a second nucleic acid, wherein the second nucleic acid encodes a gRNA. In some embodiments, the modification of the nucleic acid comprises the determination of the nucleic acid sequence of the *CFTR* gene in the lung cell type. In some embodiments, the modification of the nucleic acid of the *CFTR* gene comprises the removal of the R553X stop codon mutation. In some embodiments, the modification of the nucleic acid sequence of the *CFTR* gene in a lung cell type in a subject, wherein the *CFTR* gene comprises a R553X stop codon mutation, comprises the contacting of the lung cell type with a composition comprising a LNP, wherein the LNP comprises a first nucleic acid encoding a base editor and a second nucleic acid encoding a gRNA, wherein the nucleic acid sequence of the *CFTR* gene in the lung cell type is modified to remove the R553X stop codon mutation. In some embodiments, the modification of the nucleic acid of the *CFTR* gene in a lung cell type occurs in a subject with cystic fibrosis. In some embodiments, the cell is a lung cell. In some embodiments, the lung cell comprises lung cell types that include, but are not limited to, an endothelial cell or an epithelial cell. In some embodiments, the lung cell type is an immune cell. In some embodiments, the lung cell type is a stem cell. In some embodiments, the epithelial cell is a ciliated cell, non-ciliated cell, a goblet cell, a brush cell (alveolar macrophage), an airway basal cell, a small granule cell, a bronchial epithelial cell, a small airway epithelial cell, and/or a tracheal epithelial cell. In some embodiments, the lung cell is a secretory cell and or ionocyte.

[0097] In some embodiments, the methods provided herein comprise the increase of the expression of a full-length cystic fibrosis transmembrane conductance regulator (CFTR) protein in a lung cell type in a subject. In some embodiments, the lung cell type in a subject comprises a *CFTR* gene comprising a R553X stop codon mutation. In some embodiments, the *CFTR* gene in a lung type in a subject comprises a point mutation. In some embodiments, the *CFTR* gene in a lung type in a subject comprises a deletion. In some embodiments, the *CFTR* gene in a lung type in a subject comprises a F508del deletion. In some embodiments, the methods provided herein comprise the increase of the expression of a full length CFTR protein in a lung cell type in a subject, wherein the *CFTR* gene in the lung cell type comprises a R553X mutation, wherein the lung cell type is contacted with a composition comprising a LNP. In some embodiments, the composition comprises a LNP and a gene editing system. In some embodiments, the composition comprises a LNP, wherein the LNP comprises a first nucleic acid and a second nucleic acid. In some embodiments, the composition comprises an LNP comprising a first nucleic acid, wherein the first nucleic acid encodes a base editor, and a second nucleic acid, wherein the second nucleic acid encodes a gRNA. In some embodiments, the increase of the expression of a full-length CFTR protein comprises the determination of the expression of full-length CFTR protein in the lung cell type. In some embodiments, the increase of the expression of a full-length CFTR protein in a lung cell type in a subject, wherein the *CFTR* gene comprises a R553X stop codon mutation, comprises the contacting of the lung cell type with a composition comprising a LNP, wherein the LNP comprises a first nucleic acid encoding a base editor and a second nucleic acid encoding a gRNA, wherein the expression of full-length CFTR protein is increased in the lung cell type as compared to a lung cell type that is not contacted with the composition. In some embodiments, the increase of the expression of a full-length CFTR protein in a lung cell type occurs in a subject with cystic fibrosis. In some embodiments, the cell is a lung cell. In some embodiments, the lung cell comprises lung cell types that include, but are not limited to, an endothelial cell or an epithelial cell. In some embodiments, the lung cell type is an immune cell. In some embodiments, the lung cell type is a stem cell. In some embodiments, the epithelial cell is a ciliated cell, non-ciliated cell, a goblet cell, a brush cell (alveolar macrophage), an airway basal cell, a small granule cell, a bronchial epithelial cell, a small airway epithelial cell, and/or a tracheal epithelial cell. In some embodiments, the lung cell is a secretory cell and or ionocyte.

[0098] In some embodiments, the methods provided herein comprise the modulation of the activity of a cystic fibrosis transmembrane conductance regulator (CFTR) protein in a lung cell type in a subject. In some embodiments, the lung cell type in a subject comprises a *CFTR* gene comprising a R553X stop codon mutation. In some embodiments, the methods provided herein comprise the modulation of the activity of a CFTR protein in a lung cell type in a subject, wherein the *CFTR* gene in the lung cell type comprises a R553X mutation, wherein the lung cell type is contacted with a composition comprising a LNP. In some embodiments, the composition comprises an LNP and a gene editing system. In some embodiments, the composition comprises a LNP, wherein the LNP comprises a first nucleic acid and a second nucleic acid. In some embodiments, the composition comprises a LNP comprising a first nucleic acid, wherein the first nucleic acid encodes a base editor, and a second nucleic acid, wherein the second nucleic acid encodes a gRNA. In some embodiments, the modulation of the activity of the CFTR protein comprises the determination of the activity of the CFTR protein in the lung cell type. In some embodiments, the modulation of the activity of the CFTR protein in a lung cell type in a subject, wherein the *CFTR* gene comprises a R553X stop codon mutation, comprises the contacting of the lung cell type with a composition comprising a LNP, wherein the LNP comprises a first nucleic acid encoding a base editor and a second nucleic acid encoding a gRNA, wherein the activity of the CFTR protein is modulated in the lung cell type as compared to a lung cell type that is not contacted with the composition. In some embodiments, the modulation of the activity of the CFTR protein in a lung cell type occurs in a subject with cystic fibrosis. In some embodiments, the cell is a lung cell. In some embodiments, the lung cell comprises lung cell types that include, but are not limited to, an endothelial cell or an epithelial cell. In some embodiments, the lung cell type is an immune cell. In some embodiments, the lung cell type is a stem cell. In some embodiments, the epithelial cell is a ciliated cell, non-ciliated cell, a goblet cell, a brush cell (alveolar macrophage), an airway basal cell, a small granule cell, a bronchial epithelial cell, a small airway epithelial cell, and/or a tracheal epithelial cell. In some embodiments, the lung cell is a secretory cell and or ionocyte.

[0099] In some embodiments, the methods provided herein comprise the restoration of the function of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene in a subject. In some embodiments, the methods provided herein comprise the restoration of the function of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene in a subject with cystic fibrosis. In some embodiments, the *CFTR* gene in a lung type in a subject comprises a point

mutation. In some embodiments, the *CFTR* gene in a lung type in a subject comprises a deletion. In some embodiments, the *CFTR* gene in a lung type in a subject comprises a F508del deletion. In some embodiments, the *CFTR* gene in a subject comprises a R553X stop codon mutation. In some embodiments, the *CFTR* gene in a lung type in a subject comprises a point mutation. In some embodiments, the *CFTR* gene in a lung type in a subject comprises a deletion. In some embodiments, the *CFTR* gene in a lung type in a subject comprises a F508del deletion. In some embodiments, the restoration of the function of the *CFTR* gene comprises the administration of a composition to a subject with cystic fibrosis. In some embodiments, the composition comprises a LNP and a gene editing system. In some embodiments, the composition comprises a LNP, wherein the LNP comprises a first nucleic acid and a second nucleic acid. In some embodiments, the composition comprises a LNP comprising a first nucleic acid, wherein the first nucleic acid encodes a base editor, and a second nucleic acid, wherein the second nucleic acid encodes a gRNA. In some embodiments, the restoration of the function of the *CFTR* gene comprises the determination of the function of the *CFTR* gene in the subject. In some embodiments, the restoration of the function of the *CFTR* gene comprises the administration of a composition to a subject, wherein the composition comprises a LNP, wherein the LNP comprises a first nucleic acid encoding a base editor and a second nucleic acid encoding a gRNA, wherein the function of the *CFTR* gene is restored in the subject. In some embodiments, the restorationof the function of the *CFTR* gene occurs in a subject with cystic fibrosis.

[0100] In some embodiments, a patient in need of treatment is a male or female of 2 years or older, of 3 years or older, of 6 years or older, of 7 years or older, of 12 years or older, of 13 years or older, of 18 years or older, of 19 years or older, of 25 years or older, of 25 years or older, of 30 years or older, of 35 years or older, of 40 years or older, of 45 years or older, or of 50 years or older. In some embodiments, a patient in need of treatment is less than 50 years old, less than 45 years old, less than 40 years old, less than 35 years old, less than 30 years old, less than 25 years old, less than 20 years old, less than 19 years old, less than 18 years old, less than 13 years old, less than 12 years old, less than 7 years old, less than 6 years old, less than 3 years old, or less than 2 years old. In some embodiments, a patient in need of treatment is a male or female from 2 to 18 years old, from 2 to 12 years old, from 2 to 6 years old, from 6 to 12 years old, from 6 to 18 years old, from 12 to 16 years old, from 2 to 50 years old, from 6 to 50 years old, from 12 to 50 years old, or from 18 to 50 years old. In some embodiments, a patient in need of treatment is a female who is pregnant or who may become pregnant.

[0101] Patients with cystic fibrosis have more chloride in their sweat than people who do not have cycstic fibrosis. For a child who has cyctic fibrosis, the sweat chloride test results will confirm the diagnosis by showing a high chloride level. A baby has to sweat enough to do the test. Full-term babies usually produce enough sweat by 2 weeks of age. In some embodiments, a patient in need of treatment has a sweat chloride value of ≥60 mmol/L, ≥65 mmol/L, ≥70 mmol/L, ≥75 mmol/L, ≥80 mmol/L, ≥85 mmol/L, ≥90 mmol/L, ≥95 mmol/L, ≥100 mmol/L, ≥110 mmol/L, ≥120 mmol/L, ≥130 mmol/L, ≥140 mmol/L or ≥150 mmol/L by quantitative pilocarpine iontophoresis (documented in the subject's medical record). In some embodiments, a patient in need of treatment has chronic sinopulmonary disease and/or gastrointestinal/nutritional abnormalities consistent with cystic fibrosis.

[0102] In some embodiments, forced expiratory volume in 1 second (FEV1) is an established marker of cystic fibrosis disease progression that is used to capture clinical course and evaluate therapeutic efficacy. Thus, in various embodiments a patient in need of treatment has FEV1≥50% and ≤90% (e.g., ≤85%, ≤80%, ≤75%, ≤70%, ≤65%, ≤60%, or ≤55%) of the predicted normal (i.e., the average FEV of non-cycstic fibrosis patients) based on the patient's age, gender, and height. In some embodiments, a patient in need of treatment has resting oxygen saturation ≥92% on room air (pulse oximetry). In some embodiments, a patient in need of treatment has a body mass index ≥17.5 kg/m2 and weight ≥40 kg.

[0103] In some embodiments, the method results in the production of CFTR protein in the subject. In some embodiments, the method results in an increase of CFTR protein in the subject of at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, or at least about 25-fold compared to baseline.

[0104] In some embodiments, the increase in CFTR protein is detectable within about 6 hours, 8 hours, 12 hours, 24 hours, 36 hours, or 48 hours of administration of the pharmaceutical composition. In some embodiments, the increase in the CFTR protein is detectable by qPCR on RNA purified from tissue samples.

[0105] In some embodiments, a patient in need of treatment has received or is concurrently receiving other lung disease medications. For example, a patient in need of treatment may be receiving lumacaftor/ivacaftor combination drug (ORKAMBI®) or may have been on this treatment for at least 28 days prior to commencement of the treatment according to the present disclosure. Other cystic fibrosis medications may include, but are not limited to, routine inhaled

therapies directed at airway clearance and management of respiratory infections, such as bronchodilators, rhDNase (PULMOZYME (Dornase alfa)), hypertonic saline, antibiotics, and steroids; and other routine CF-related therapies such as systemic antibiotics, pancreatic enzymes, multivitamins, and diabetes and liver medications.

[0106] In some embodiments, the method of treatment comprises (1) providing: a) a nebulizer, and b) a container including the LNP and base editor formulation in a pharmaceutically acceptable carrier, and (2) administering the formulation using the nebulizer. In some embodiments, the volume of the LNP and base editor formulation in the container has a volume of 10 mL, 9 mL, 8 mL, 7 mL, 6 mL, 5 mL, 4 mL, 3 mL, 2 mL, or 1 mL. In some embodiments, formulations and compositions generally include a pharmaceutically acceptable carrier. The carrier is preferably a liquid carrier. In some embodiments, the carrier preferably includes water and may include other components. In some embodiments, the composition including the LNP and base editor formulation is stored in an ampule, a vial, or a single-use vial prior to administrating. In some embodiments, the composition is stored in a single-use vial prior to administering.

[0107] In some embodiments, the administration of the pharmaceutical composition results in the expression of a protein in a lung of the subject. In some embodiments, administration of the LNP and base editor composition results in detection of a protein in a lung of the subject between 6 and 12 hours after delivery to the subject. In some embodiments, detection of the protein in the lung is at 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours or 12 hours. In some embodiments, the protein is detected using known techniques in the art, including, but not limited to, Western blot analysis.

[0108] In some embodiments, a polypeptide delivered according to the described method results in increased protein level or activity an upper airway, a central airway, or peripheral airway of a lung of the subject by, e.g., at least approximately 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold, 500-fold, 1000-fold, or 1500-fold as compared to a control (e.g., endogenous level of protein or activity without or before the treatment according to the disclosure, or a historical reference level).

[0109] In some embodiments, a CFTR gRNA delivered according to the described method results in increased CFTR protein level or activity an upper airway, a central airway, or peripheral airway of a lung of the subject by, e.g., at least approximately 10%, 20%, 30%, 40%,

50%, 60%, 70%, 80%, 90%, 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold, 500-fold, 1000-fold, or 1500-fold as compared to a control (e.g., endogenous level of protein or activity without or before the treatment according to the disclosure, or a historical reference level).

[0110] In some embodiments, mRNA expression may be detected or quantified by qPCR on RNA purified from tissue samples. The protein or expression may be determined by measuring immune responses to the protein. Qualitative assessment of the protein may also be performed by Western blot analysis. The protein activity may be measured by an appropriate activity assay. Various other methods are known in the art and may be used to determine the protein expression or activity.

[0111] The CFTR mRNA expression may be detected or quantified by qPCR on RNA purified from tissue samples. The CFTR protein or expression may be determined by measuring immune responses to CFTR protein. In some embodiments, IgG antibody to CFTR protein is measured by an enzyme-linked immunosorbent assay in collected serum samples. In some embodiments, CFTR-specific T cell responses are assessed using collected peripheral blood mononuclear cells. In some embodiments, T cell responses to CFTR are measured by a human interferon-γ enzyme-linked immunospot assay as described by Calcedo et al. (Calcedo et al., Hum Gene Ther Clin Dev. (2013) 24:108-15). Qualitative assessment of CFTR protein may also be performed by Western blot analysis. The CFTR protein activity may be measured by CFTR chloride channel activity in appropriate tissue cells. A stable potential with the mean value of a 10 second scoring interval after perfusion of solution is recorded. CFTR activity is estimated by the change in potential difference following perfusion with chloride-free isoproterenol. Various other methods are known in the art and may be used to determine the CFTR mRNA and CFTR protein expression or activity.

[0112] In some embodiments, the administration of the LNP comprising a gene editing system in accord with the provided methods effectively treats a subject with a mutation in the CFTR gene. In some embodiments, the mutation is a R553X CFTR nonsense mutation. In some embodiments, the expression level of the CFTR gene in a subject administered the LNP comprising a gene editing system is increased at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least

90%, at least 95%, or at least 100% as compared to the expression level in the subject prior to administration of the LNP.

[0113] In some embodiments, the expression level of the CFTR gene in a subject administered the LNP comprising a gene editing system is increased between 0 to 10%, between 5% to 15%, between 20% to 30%, between 25% to 35%, between 30% to 40%, between 35% to 45%, between 40% to 50%, between 45% to 55%, between 50% to 60%, between 55% to 65%, between 60% to 70%, between 70% to 80%, between 75% to 85%, between 80% to 90%, between 85% to 95%, between 90% to 100% as compared to the expression level in the subject prior to administration of the LNP.

[0114] In some embodiments, administration of the LNP comprising a gene editing system enhances the expression or activity of CFTR protein by way of increasing the amount of a functional CFTR gene, transcript or protein in the cell (e.g., by at least about 1.1-fold) relative to a corresponding control. In some embodiments, the method yields a therapeutically effective amount of a functional of CFTR gene, transcript or protein in the cell. In some embodiments, the administration of the LNP comprising a gene editing system yields at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% by mole or by weight, increase in detectable CFTR gene, transcript or protein in the cell as compared to the cell prior to administration of the LNP composition.

[0115] In some embodiments, the method for enhancing the expression or activity of CFTR protein comprises the enhancement (e.g., chloride) ion transport in cell(s) (e.g., by at least about 1.1 -fold) relative to a cell prior to administration of the LNP composition. In some embodiments, the method reduces defective export from or import to cell(s) of chloride, such as chloride anion or in the form of a chloride salt or other chloride-containing compound. In some embodiments, the method enhances or stimulates ion (e.g., chloride) transport in cell(s). In some embodiments, the enhanced or stimulated ion (e.g., chloride) transport results in secretion or absorption of (e.g., chloride) ions. In some embodiments, enhanced (e.g., chloride) ion transport is determined by evaluating CFTR-mediated currents across cell(s) by employing standard Ussing chamber (see Ussing and Zehrahn, Acta. Physiol. Scand. 23:110-127, 1951) or nasal potential difference measurements (see Knowles et al., Hum. Gene Therapy 6:445-455,

[0116] 1995). In some embodiments, the enhanced chloride transport is be determined by the Ieq (equivalent current) assay using the TECC-24 system as described in Vu et al., J. Med. Chem. 2017, 60, 458-473, which is hereby incorporated by reference in its entirety.

[0117] In some embodiments, the enhanced (e.g., chloride) ion transport is determined by CFTR-dependent whole-cell current measurement(s), as described in International Patent Application No. PCT/US2017/032967, published as W02017201091, which is hereby incorporated by reference in its entirety. In some embodiments, the method further comprises deriving (e.g., by cell culturing) a cell composition (e.g., a lung cell composition) from the cell.

[0118] In some embodiments, response assessment may be performed at baseline (e.g. prior to any of the methods provided herein), 1 month, 3 months, 6 months, 9 months, 12 months, 18 months, and/or 24 months following administration of the LNP. In some aspects, response assessment is be performed at baseline, 1 month, 3 months, 6 months, 9 months, 12 months, 18 months, and 24 months following administration of the LNP.

[0119] In some embodiments, the method described herein comprises delivery of one or more polynucleotides to a cell. In some embodiments, the method comprises contacting a cell with a LNP comprising a gene editing system. In some embodiments, the method comprises expressing a protein or an RNA in a cell.

[0120] In some embodiments, the method comprises a method of increasing chloride flux in a cell. In some embodiments, the method comprises contacting the cell with a LNP and base editor composition, wherein optionally the cell comprises homozygous inactivating mutations in the CFTR gene. In some embodiments, the method maintains transpeithelial electrical resistance (TEER) or reduces TEER by at most 10%, at most 20%, or at most 30%. In some embodiments, the cell is a lung cell. In some embodiments, the lung cell comprises lung cell types that include, but are not limited to, an endothelial cell or an epithelial cell. In some embodiments, the lung cell type is an immune cell. In some embodiments, the lung cell type is a stem cell. In some embodiments, the epithelial cell is a ciliated cell, non-ciliated cell, a goblet cell, a brush cell (alveolar macrophage), an airway basal cell, a small granule cell, a bronchial epithelial cell, a small airway epithelial cell, and/or a tracheal epithelial cell. In some embodiments, the lung cell is a secretory cell and or ionocyte.

[0121] In some embodiments, the method specifically transduces secretory cells and/or ionocytes compared to other lung cells. In some embodiments, the lung cell is a ciliated cell. In some embodiments, the method specifically transduces ciliated cells compared to other lung cells. In some embodiments, the method comprises nebulizing the LNP and base editor composition to generate an aerosolized composition, then contacting the aerosolized

composition with the cell. In some embodiments, the composition is an aerosolized composition, and the method comprises contacting the aerosolized composition with the cell.

[0122] In some embodiments, the method comprises the delivery of the one or more polynucleotides to the lung of a subject, wherein the method comprises administering to the subject a composition comprising a LNP comprising a gene editing system. In some embodiments, the method comprises the treating or preventing lung disease in a subject, wherein the method comprises administering to the subject a composition comprising a LNP comprising a gene editing system.

[0123] In some embodiments, the methods provided herein comprise a method for lung cell editing. In some embodiments, the methods comprise a method for genetic correction of cystic fibrosis transmembrane conductance regulator (CFTR) in a lung (e.g., basal) cell, comprising: contacting the lung (e.g., basal) cell with a composition that comprises a nucleic acid editing system assembled with a lipid composition, thereby delivering the nucleic acid editing system to the lung (e.g., basal) cell.

[0124] In some embodiments, the methods provided herein comprise a method for genetic correction of cystic fibrosis transmembrane conductance regulator (CFTR) in a cell composition, comprising: contacting the cell composition comprising a plurality of lung (e.g., basal) cells with a composition that comprises a nucleic acid editing system assembled with a lipid composition, thereby delivering the nucleic acid editing system, e.g., to at least about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 65%, or 70% of the plurality of lung (e.g., basal) cells.

[0125] In some embodiments, the methods provided herein comprise a method for genetic correction of cystic fibrosis transmembrane conductance regulator (CFTR) in a cell composition, comprising: contacting the cell composition with a composition that comprises a nucleic acid editing system assembled with a lipid composition, which cell composition comprise a lung (e.g., basal) cell and a lung non-basal cell, thereby delivering the nucleic acid editing system to the lung (e.g., basal) cell in a greater amount than that delivered to the lung non-basal cell. The non-basal cell may be an ionocyte (e.g., exhibiting or determined to exhibit to FOXI1), a ciliated cell, or a secretory cell (such as goblet cell and club cell).

[0126] In some embodiments, the methods provided herein comprise methods for genetic correction of CFTR. In some embodiments, the lung (e.g., basal) cell or the plurality of lung (e.g., basal) cells is/are determined to exhibit a mutation in CFTR gene. In some embodiments,

the lung (e.g., basal) cell or the plurality of lung (e.g., basal) cells exhibit(s) a mutation in CFTR gene.

[0127] In some embodiments, the methods provided herein comprise methods for genetic correction of CFTR wherein the contacting is ex vivo. In some embodiments, the contacting is in vitro. In some embodiments, the contacting is in vivo. In some embodiments, a cell or pluralty of cells is isolated from the subject. The compositions as described elsewhere here may be contacted with the cell outside of the subject. Upon administration of the composition or therapeutic, the cell may be re-injected or otherwise re-introduced into the subject. In some embodiments, the cell is a cell line. In some embodiments, the cell is a lung cell. In some embodiments, the lung cell is a lung airway cell. Examples of lung airway cells that can be targeted by the delivery of the present application includes but is not limited to basal cell, secretory cell such as goblet cell and club cell, ciliated cell and any combination thereof.

[0128] In some embodiments, the method comprising nebulizing the composition prior to the administering step. In some embodiments, the composition is administered, as an aerosolized composition, by inhalation. In some embodiments, the method delivers to the lung an effective amount of the composition. In some embodiments, the composition comprises a LNP comprising a gene editing system.

[0129] In some embodiments, the method delivers to the lung an amount effective of the composition to treat the lung disease. In some embodiments, the method is more effective than contacting the cell with and/or adminsitering to the subject elexacaftor, tezacaftor, lumacaftor, ivacaftor, or a combination thereof. In some embodiments, the method is at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, or at least 60% more effective. In some embodiments, the method is 10%-70%, 20%-70%, 30%-70%, 40%-70%, 50%-70%, or 60%-70% more effective.

II. LIPID NANOPARTICLES

[0130] Provided herein are methods of increasing the expression of a gene in a cell, and methods of modifying the nucleic acid sequences of a gene in a cell, including administration and uses, such as therapeutic uses, comprising administration of a lipid nanoparticle (LNP) comprising a gene editing system. In some embodiments, the LNP comprises a nucleic acid encoding a base editor that is administered to a subject.

[0131] Also provided herein are methods of treating a subject with cystic fibrosis. In some emebodiments, the method comprises the administration of a LNP comprising a gene editing

system to the subject. In some embodiments, the LNP comprises a nucleic acid encoding a recombinase. In some embodiments, the method comprises administration of an LNP comprising a first nucleic acid and a second nucleic acid.

A. Components of LNPs

[0132] In some embodiments, the methods comprise the administration of a LNP comprising a gene editing system to a subject. In some embodiments, the LNP comprises an ionizable lipid, a phospholipid, a polyethylene glycol (PEG) lipid, a sterol, and one or more DNA molecules or an RNA molecules. In some embodiments, the methods comprise the delivery of the nucleic acids enveloped by the LNP to a target organ and/or target cell.

1. Ionizable Lipids

[0133] In some embodiments, the LNP comprises an ionizable lipid. In some embodiments, the ionizable lipid is an ionizable cationic lipid. In some embodiments, the cationic ionizable lipids contain one or more groups which is protonated at physiological pH but may deprotonated and has no charge at a pH above 8, 9, 10, 11, or 12. The ionizable cationic group comprises one or more protonatable amines which are able to form a cationic group at physiological pH. The cationic ionizable lipid compound may also further comprise one or more lipid components such as two or more fatty acids with C6-C24 alkyl or alkenyl carbon groups. In some embodiments, these lipid groups may be attached through an ester linkage or may be further added through a Michael addition to a sulfur atom. In some embodiments, these compounds may be a dendrimer, a dendron, a polymer, or a combination thereof.

[0134] In some embodiments, the LNP comprises one or more ionizable (e.g., ionizable amino) lipids (e.g., lipids that may have a positive or partial positive charge at physiological pH). Ionizable lipids may be selected from the group comprising, but not limited to, 3-(didodecylamino)-N1,N1,4-tridodecy1-1-piperazineethanamine (KL10), N1-[2-(didodecylamino)ethyl] N1,N4,N4-tridodecy1-1,4- piperazinediethanamine (KL22), 14,25-ditridecy1-15,18,21,24-tetraaza-octatriacontane (KL25), 1,2-dilinoleyloxy-N,N-dimethylaminopropane (DLin-DMA), 2,2-dilinoley1-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA), heptatriaconta-6,9,28,31-tetraen-19-y1-4-(dimethylamino)butanoate (DLin-MC3-DMA), 2,2-dilinoleyl-4-(2 dimethylaminoethyl)-[1,3]-dioxolane (DLin-KC2-DMA), 1,2-dioleyloxy-N,Ndimethylaminopropane (DODMA), 2-({8 [(3(3)-cholest-5-en-3-yloxy]octylIoxy)N,N dimethy1-3-[(9Z,12Z)-octadeca-9,12-dien-l-yloxy]propan-1-amine (Octyl-

CLinDMA), (2R)-2-({8-[(3(3)-cholest-5-en-3-yloxy]octylIoxy)-N,N-dimethy1-3-[(9Z,12Z)-octadeca-9,12-dien-l-yloxy]propan-1-amine (Octyl-CLinDMA (2R)), and (2S) 2- ({8-[(3(3)-chol e st-5-en-3 -yloxy] octyl } oxy)-N,N-dimethyl-3 -[(9Z,12Z)-octadeca-9,12-di en-1 -yloxy]propan-l-amine (Octyl-CLinDMA (2S)), 4-hydroxybutyl) azanediyl)bis (hexane-6,1-diyl)bis(2-hexyldecanoate (ALC-0315), or heptadecan-9-yl 8-((2-hydroxyethyl) (6-oxo-6-(undecyloxy) hexyl) amino) octanoate (SM-102). In some embodiments, the ionizable lipid comprises a cyclic amine group.

[0135] In some embodiments, ionizable lipids can also be the compounds disclosed in International Publication No. WO 2017/075531 Al, hereby incorporated by reference in its entirety. In some embodiments, ionizable lipids can also be the compounds disclosed in International Publication No. WO 2015/199952 Al, hereby incorporated by reference in its entirety. In some embodiments, the ionizable lipid may be selected from, but not limited to, an ionizable lipid described in International Publication Nos. WO2012040184, WO2011153120, WO2011149733, WO2011090965, WO2011043913, WO2011022460, WO2012061259, WO2012054365, WO2012044638, WO2010080724, WO201021865, WO2008103276, WO2013086373 and WO2013086354, US Patent Nos. 7,893,302, 7,404,969, 8,283,333, and 8,466,122 and US Patent Publication No. US20100036115, US20120202871, US20130064894, US20130129785, US20130150625, US20130178541 and S20130225836; the contents of each of which are herein incorporated by reference in their entirety.

[0136] In some embodiments, a cationic lipid may be selected from (20Z,23Z)-N,N-dimethylnonacosa-20,23-dien-10-amine, (17Z,20Z)-N,Ndimemylhexacosa-17,20-dien-9-amine, (1Z,19Z)-N5N-dimethylpentacosa-1 6, 19-dien-8-amine, (13Z,16Z)-N,N-dimethyldocosa-13,16-dien-5-amine, (12Z,15Z)-N,N dimethylhenicosa-12,15- dien-4-amine, (14Z,17Z)-N,N-dimethyltricosa-14,1 7-di en-6-amine, (15Z,18Z)-N,N-dimethyltetracosa-15,18-dien-7-amine, (18Z,21Z)-N,Ndimethylheptacosa-18,21-dien-10-amine, (15Z,18Z)-N,N-dimethyltetracosa-15,18-dien-5-amine, (14Z,17Z)-N,N-dimethyltricosa-14,17-dien-4-amine, (19Z,22Z)-N,Ndimeihyloctacosa-19,22-dien-9-amine, (18Z,21 Z)-N,N-dimethylheptacosa- 18 ,21 -dien-8—amine, (17Z,20Z)-N,N-dimethylhexacosa- 17,20-dien-7-amine, (16Z,19Z)-N,Ndimethylpentacosa-16,19-dien-6-amine, (22Z,25Z)-N,N-dimethylhentriaconta-22,25- dien-10-amine, (21 Z ,24Z)-N,N-dimethyltriaconta-21,24-dien-9-amine, (18Z)-N,Ndimetylheptacos-18-en-10-amine, (17Z)-N,N-dimethylhexacos-17-en-9-amine, (19Z,22Z)-N,N-dimethyloctacosa-19,22-dien-7-amine, N,N-dimethylheptacosan-10-amine, (20Z,23Z)-N-ethyl-

N-methylnonacosa-20,23-dien-10-amine, 1-[(11Z,14Z)-1-nonylicosa-11,14-dien-l-yl] pyrrolidine, (20Z)-N,N-dimethylheptacos-20-en-1 0-amine, (15Z)-N,N-dimethyl eptacos-15-en-1 0-amine, (14Z)-N,N-dimethylnonacos-14-en-10- amine, (17Z)-N,N-dimethylnonacos-17-en-10-amine, (24Z)-N,N-dimethyltritriacont-24-en-10-amine, (20Z)-N,N-dimethylnonacos-20-en-1 0-amine, (22Z)-N,Ndimethylhentriacont- 22-en-10-amine, (16Z)-N,N-dimethylpentacos-16-en-8-amine, (12Z,15Z)-N,N-dimethy1-2-nonylhenicosa-12,15-dien- 1—amine, (13Z,16Z)-N,Ndimethy1-3-nonyldocosa-13,16-dien-l—amine, N,N-dimethyl-1- [(1S,2R)-2-oetyleyclopropyl] eptadecan-8-amine, 1-[(1S,2R)-2-hexylcyclopropy1]-N,Ndimethylnonadecan-10-amine, N,N-dimethy1-1-[(1S ,2R)-2-oetylcyclopropyl]nonadecan-10-amine, N,N-dimethy1-21-[(1S,2R)-2-octylcyclopropyl]henicosan-10-amine,N,N-dimethy1-1- [(1S,2S)-2- { [(1R,2R)-2- p entyl cy cIopropyl] methyl } cy cl opropyl]nonadecan-10-amine,N,N-di methyl -1 -[(1S,2R)-2-octylcyclopropyl]hexadecan-8-amine, N,N-dimethyl-[(1R,2S)-2 undecyIcyclopropyl]tetradecan-5-amine, N,N-dimethy1-3- {7- [(1S,2R)-2-octylcyclopropyl]heptylI dodecan-l—amine, 1-[(1R,2S)-2-hepty lcyclopropy1]-N,Ndimethyloctadecan- 9—amine, 1-[(1S,2R)-2-decylcyclopropy1]-N,N-dimethylpentadecan-6-amine, N,N-dimethy1-1-[(1S,2R)-2-octylcyclopropyl]pentadecan-8-amine, R-N,Ndimethy1-1-[(9Z,12Z)-octadeca-9,12- dien-l-yloxy]-3-(octyloxy)propan-2-amine, S-N,Ndimethy1-1-[(9Z,12Z)-octadeca- 9,12-dien-l-yloxy]-3-(octyloxy)propan-2-amine, 1- {2-[(9Z,12Z)-octadeca-9,12-dien-l-yloxy]-1-[(octyloxy)methyl]ethyllpyrrolidine, (2S)-N,Ndimethy1-1- [(9Z,12Z)-octadeca-9,12-dien-l-yloxy]-3-[(5Z)-oct-5-en-l-yloxy]propan-2-amine, 1- {2-[(9Z,12Z)-octadeca-9,12-dien-l-yloxy]-1- [(octyloxy)methyl]ethylIazetidine, (2S)-1- (hexyloxy)-N,N-dimethy1-3-[(9Z,12Z)-octadeca-9,12-dien-l-yloxy]propan-2- amine, (2S)-1-(heptyloxy)-N,N-dimethy1-3-[(9Z,12Z)-octadeca-9,12-dien-lyloxy] propan-2-amine, N,N-dimethy1-1-(nonyloxy)-3-[(9Z,12Z)-octadeca-9,12-dien-1- yl oxy] propan-2-amine, N,N-di methyl-1 -[(9Z)-octadec-9-en-1 -yloxy] -3 - (octyl oxy)prop an-2-amine; (2 S)-N,N-dimethy1-1-[(6Z, 9Z,12Z)-octadeca-6, 9,12-tri en-1- yloxy]-3 - (octyloxy)propan-2-amine, (2 S)-1-[(11Z,14Z)-icosa-11,14-dien-l-yloxy]-N,Ndimethy1- 3-(pentyloxy)propan-2-amine, (2 S)-1-(hexyl oxy)-3 - [(11Z,14Z)-i co sa-11,14- di en-1 -yloxy]-N,N-dimethylpropan-2-amine, 1- [(11Z,14Z)-icosa-11,14-dien-1 -yloxy]- N,N-dimethy 1-3 -(octyloxy)propan-2-amine, 1 -[(13Z,16Z)-docosa-13,16-dien-l-yloxy]-N,N-dimethy1-3 -(octyloxy)propan-2-amine, (2 S)-1- [(13Z,16Z)-docosa-13,16-dien-1 -yl oxy] -3 -(hexyl oxy)-N,N-dimethylprop an-2-amine, (2 S)-1- [(13Z)-doco s-13 -en-l-yl oxy]-3 -(hexyloxy)-N,N-dimethylpropan-2-amine, 1 -[(13Z)-docos-13 -en-1 -yloxy]-N,Ndimethy1- 3

-(octyl oxy)propan-2-amine, 1-[(9Z)-hexadec-9-en-l-yloxy]-N,N-dimethy1-3- (octyloxy)propan-2-amine, (2R)-N,N-dimethyl-H(1-metoyloctyl)oxy]-3-[(9Z,12Z)- octadeca-9,12-dien-l-yloxy]propan-2-amine, (2R)-1-[(3,7- dim ethyl octyl)oxy] -N,Ndimethy1- 3 - [(9Z,12Z)-octadeca-9,12-di en-l-yloxy] propan-2-amine, N,N-dimethy1-1 - (octyloxy)-3-({ 8-[(1 S,2 S)-2-[(1R,2R)-2- pentylcyclopropyl]methyllcyclopropyl]octylloxy)propan-2-amine, N,N-dimethy1-1 - [8- (2-oc lyl cycl opropyl)octyl] oxy}-3 -(octyl oxy)prop an-2-amine and (11E,20Z,23Z)-N,Ndimethylnonacosa- 11,20,2-trien-10-amine or a pharmaceutically acceptable salt or stereoisomer thereof.

[0137] In some embodiments, the ionizable cationic lipids refer to lipid and lipid-like molecules with nitrogen atoms that can acquire charge (pKa). These molecules with amino groups typically have between 2 and 6 hydrophobic chains, often alkyl or alkenyl such as C_6-C_{24} alkyl or alkenyl groups, but may have at least 1 or more that 6 tails. In some embodiments, these cationic ionizable lipids are dendrimers, which are a polymer exhibiting regular dendritic branching, formed by the sequential or generational addition of branched layers to or from a core and are characterized by a core, at least one interior branched layer, and a surface branched layer. (See *Petar R. Dvornic and Donald A. Tomalia in Chem. in Britain, 641-645, August 1994.*) In other embodiments, the term "dendrimer" as used herein is intended to include, but is not limited to, a molecular architecture with an interior core, interior layers (or "generations") of repeating units regularly attached to this initiator core, and an exterior surface of terminal groups attached to the outermost generation. A "dendron" is a species of dendrimer having branches emanating from a focal point which is or can be joined to a core, either directly or through a linking moiety to form a larger dendrimer. In some embodiments, the dendrimer structures have radiating repeating groups from a central core which doubles with each repeating unit for each branch. In some embodiments, the dendrimers described herein may be described as a small molecule, medium-sized molecules, lipids, or lipid-like material. These terms may be used to describe compounds described herein which have a dendron like appearance (*e.g.* molecules which radiate from a single focal point).

[0138] While dendrimers are polymers, dendrimers may be preferable to traditional polymers because they have a controllable structure, a single molecular weight, numerous and controllable surface functionalities, and traditionally adopt a globular conformation after reaching a specific generation. Dendrimers can be prepared by sequentially reactions of each repeating unit to produce monodisperse, tree-like and/or generational structure polymeric

structures. Individual dendrimers consist of a central core molecule, with a dendritic wedge attached to one or more functional sites on that central core. The dendrimeric surface layer can have a variety of functional groups disposed thereon including anionic, cationic, hydrophilic, or lipophilic groups, according to the assembly monomers used during the preparation.

[0139] Modifying the functional groups and/or the chemical properties of the core, repeating units, and the surface or terminating groups, their physical properties can be modulated. Some properties which can be varied include, but are not limited to, solubility, toxicity, immunogenicity and bioattachment capability. Dendrimers are often described by their generation or number of repeating units in the branches. A dendrimer consisting of only the core molecule is referred to as Generation 0, while each consecutive repeating unit along all branches is Generation 1, Generation 2, and so on until the terminating or surface group. In some embodiments, half generations are possible resulting from only the first condensation reaction with the amine and not the second condensation reaction with the thiol.

[0140] Preparation of dendrimers requires a level of synthetic control achieved through series of stepwise reactions comprising building the dendrimer by each consecutive group. Dendrimer synthesis can be of the convergent or divergent type. During divergent dendrimer synthesis, the molecule is assembled from the core to the periphery in a stepwise process involving attaching one generation to the previous and then changing functional groups for the next stage of reaction. Functional group transformation is necessary to prevent uncontrolled polymerization. Such polymerization would lead to a highly branched molecule that is not monodisperse and is otherwise known as a hyperbranched polymer. Due to steric effects, continuing to react dendrimer repeat units leads to a sphere shaped or globular molecule, until steric overcrowding prevents complete reaction at a specific generation and destroys the molecule's monodispersity. Thus, in some embodiments, the dendrimers of G1-G10 generation are specifically contemplated. In some embodiments, the dendrimers comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 repeating units, or any range derivable therein. In some embodiments, the dendrimers used herein are G0, G1, G2, or G3. However, the number of possible generations (such as 11, 12, 13, 14, 15, 20, or 25) may be increased by reducing the spacing units in the branching polymer.

[0141] Additionally, dendrimers have two major chemical environments: the environment created by the specific surface groups on the termination generation and the interior of the dendritic structure which due to the higher order structure can be shielded from the bulk media

and the surface groups. Because of these different chemical environments, dendrimers have found numerous different potential uses including in therapeutic applications.

[0142] In some embodiments, the dendrimers are assembled using the differential reactivity of the acrylate and methacrylate groups with amines and thiols. The dendrimers may include secondary or tertiary amines and thioethers formed by the reaction of an acrylate group with a primary or secondary amine and a methacrylate with a mercapto group. Additionally, the repeating units of the dendrimers may contain groups which are degradable under physiological conditions. In some embodiments, these repeating units may contain one or more germinal diethers, esters, amides, or disulfides groups. In some embodiments, the core molecule is a monoamine which allows dendritic polymerization in only one direction. In other embodiments, the core molecule is a polyamine with multiple different dendritic branches which each may comprise one or more repeating units. The dendrimer may be formed by removing one or more hydrogen atoms from this core. In some embodiments, these hydrogen atoms are on a heteroatom such as a nitrogen atom. In some embodiments, the terminating group is a lipophilic groups such as a long chain alkyl or alkenyl group. In other embodiments, the terminating group is a long chain haloalkyl or haloalkenyl group. In other embodiments, the terminating group is an aliphatic or aromatic group containing an ionizable group such as an amine ($-NH_2$) or a carboxylic acid ($-CO_2H$). In still other embodiments, the terminating group is an aliphatic or aromatic group containing one or more hydrogen bond donors such as a hydroxide group, an amide group, or an ester.

[0143] In some embodiments, the cationic ionizable lipids contain one or more asymmetrically-substituted carbon or nitrogen atoms, and may be isolated in optically active or racemic form. Thus, all chiral, diastereomeric, racemic form, epimeric form, and all geometric isomeric forms of a chemical formula are intended, unless the specific stereochemistry or isomeric form is specifically indicated. Cationic ionizable lipids may occur as racemates and racemic mixtures, single enantiomers, diastereomeric mixtures and individual diastereomers. In some embodiments, a single diastereomer is obtained. The chiral centers of the cationic ionizable lipids of the present application can have the *S* or the *R* configuration. Furthermore, it is contemplated that one or more of the cationic ionizable lipids may be present as constitutional isomers. In some embodiments, the compounds have the same formula but different connectivity to the nitrogen atoms of the core. Without wishing to be bound by any theory, it is believed that such cationic ionizable lipids exist because the starting monomers react first with the primary

amines and then statistically with any secondary amines present. Thus, the constitutional isomers may present the fully reacted primary amines and then a mixture of reacted secondary amines.

[0144] Chemical formulas used to represent cationic ionizable lipids of the present application will typically only show one of possibly several different tautomers. For example, many types of ketone groups are known to exist in equilibrium with corresponding enol groups. Similarly, many types of imine groups exist in equilibrium with enamine groups. Regardless of which tautomer is depicted for a given formula, and regardless of which one is most prevalent, all tautomers of a given chemical formula are intended.

[0145] In some embodiments, the cationic ionizable lipids have the advantage that they may be more efficacious than, be less toxic than, be longer acting than, be more potent than, produce fewer side effects than, be more easily absorbed than, and/or have a better pharmacokinetic profile (*e.g.*, higher oral bioavailability and/or lower clearance) than, and/or have other useful pharmacological, physical, or chemical properties over, compounds known in the prior art, whether for use in the indications stated herein or otherwise.

[0146] In some embodiments, atoms making up the cationic ionizable lipids include all isotopic forms of such atoms. Isotopes, as used herein, include those atoms having the same atomic number but different mass numbers. By way of general example and without limitation, isotopes of hydrogen include tritium and deuterium, and isotopes of carbon include ^{13}C and ^{14}C.

[0147] It should be recognized that the particular anion or cation forming a part of any salt form of a cationic ionizable lipids provided herein is not critical, so long as the salt, as a whole, is pharmacologically acceptable. Additional examples of pharmaceutically acceptable salts and their methods of preparation and use are presented in *Handbook of Pharmaceutical Salts: Properties, and Use* (2002), which is incorporated herein by reference.

[0148] In some embodiments, the ionizable clipid is a dendrimer or dendron. In some embodiments, the ionizable lipid comprises an ammonium group which is positively charged at physiological pH and contains at least two hydrophobic groups. In some embodiments, the ammonium group is positively charged at a pH from about 6 to about 8. In some embodiments, the ionizable lipid is a dendrimer or dendron. In some embodiments, the ionizable lipid comprises at least two C_6-C_{24} alkyl or alkenyl groups.

[0149] Modifying the functional groups and/or the chemical properties of the core, repeating units, and the surface or terminating groups, their physical properties can be modulated. Some

properties which can be varied include, but are not limited to, solubility, toxicity, immunogenicity and bioattachment capability. Dendrimers are often described by their generation or number of repeating units in the branches. A dendrimer consisting of only the core molecule is referred to as Generation 0, while each consecutive repeating unit along all branches is Generation 1, Generation 2, and so on until the terminating or surface group. In some embodiments, half generations are possible resulting from only the first condensation reaction with the amine and not the second condensation reaction with the thiol.

[0150] Preparation of dendrimers requires a level of synthetic control achieved through series of stepwise reactions comprising building the dendrimer by each consecutive group. Dendrimer synthesis can be of the convergent or divergent type. During divergent dendrimer synthesis, the molecule is assembled from the core to the periphery in a stepwise process involving attaching one generation to the previous and then changing functional groups for the next stage of reaction. Functional group transformation is necessary to prevent uncontrolled polymerization. Such polymerization would lead to a highly branched molecule that is not monodisperse and is otherwise known as a hyperbranched polymer. Due to steric effects, continuing to react dendrimer repeat units leads to a sphere shaped or globular molecule, until steric overcrowding prevents complete reaction at a specific generation and destroys the molecule's monodispersity. Thus, in some embodiments, the dendrimers of G1-G10 generation are specifically contemplated. In some embodiments, the dendrimers comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 repeating units, or any range derivable therein. In some embodiments, the dendrimers used herein are G0, G1, G2, or G3. However, the number of possible generations (such as 11, 12, 13, 14, 15, 20, or 25) may be increased by reducing the spacing units in the branching polymer.

[0151] Additionally, dendrimers have two major chemical environments: the environment created by the specific surface groups on the termination generation and the interior of the dendritic structure which due to the higher order structure can be shielded from the bulk media and the surface groups. Because of these different chemical environments, dendrimers have found numerous different potential uses including in therapeutic applications.

[0152] In some embodiments, the dendrimers are assembled using the differential reactivity of the acrylate and methacrylate groups with amines and thiols. In some embodiments, the dendrimers include secondary or tertiary amines and thioethers formed by the reaction of an acrylate group with a primary or secondary amine and a methacrylate with a mercapto group.

Additionally, in some embodiments, the repeating units of the dendrimers contain groups which are degradable under physiological conditions. In some embodiments, these repeating units may contain one or more germinal diethers, esters, amides, or disulfides groups. In some embodiments, the core molecule is a monoamine which allows dendritic polymerization in only one direction. In other embodiments, the core molecule is a polyamine with multiple different dendritic branches which each may comprise one or more repeating units. The dendrimer may be formed by removing one or more hydrogen atoms from this core. In some embodiments, these hydrogen atoms are on a heteroatom such as a nitrogen atom. In some embodiments, the terminating group is a lipophilic groups such as a long chain alkyl or alkenyl group. In other embodiments, the terminating group is a long chain haloalkyl or haloalkenyl group. In other embodiments, the terminating group is an aliphatic or aromatic group containing an ionizable group such as an amine ($-NH_2$) or a carboxylic acid ($-CO_2H$). In still other embodiments, the terminating group is an aliphatic or aromatic group containing one or more hydrogen bond donors such as a hydroxide group, an amide group, or an ester.

[0153] In some embodiments, the cationic ionizable lipids contain one or more asymmetrically-substituted carbon or nitrogen atoms, and can be isolated in optically active or racemic form. Thus, all chiral, diastereomeric, racemic form, epimeric form, and all geometric isomeric forms of a chemical formula are intended, unless the specific stereochemistry or isomeric form is specifically indicated. In some embodiments, cationic ionizable lipids occur as racemates and racemic mixtures, single enantiomers, diastereomeric mixtures and individual diastereomers. In some embodiments, a single diastereomer is obtained. The chiral centers of the cationic ionizable lipids of the present application can have the *S* or the *R* configuration. Furthermore, it is contemplated that one or more of the cationic ionizable lipids may be present as constitutional isomers. In some embodiments, the compounds have the same formula but different connectivity to the nitrogen atoms of the core. Without wishing to be bound by theory, in some embodiments, such cationic ionizable lipids exist because the starting monomers react first with the primary amines and then statistically with any secondary amines present. Thus, the constitutional isomers may present the fully reacted primary amines and then a mixture of reacted secondary amines.

[0154] Chemical formulas used to represent cationic ionizable lipids of the present application will typically only show one of possibly several different tautomers. For example, many types of ketone groups are known to exist in equilibrium with corresponding enol groups.

Similarly, many types of imine groups exist in equilibrium with enamine groups. Regardless of which tautomer is depicted for a given formula, and regardless of which one is most prevalent, all tautomers of a given chemical formula are intended.

[0155] The cationic ionizable lipids of the present application may also have the advantage that they may be more efficacious than, be less toxic than, be longer acting than, be more potent than, produce fewer side effects than, be more easily absorbed than, and/or have a better pharmacokinetic profile (*e.g.*, higher oral bioavailability and/or lower clearance) than, and/or have other useful pharmacological, physical, or chemical properties over, compounds known in the prior art, whether for use in the indications stated herein or otherwise.

[0156] In addition, atoms making up the cationic ionizable lipids of the present application are intended to include all isotopic forms of such atoms. Isotopes, as used herein, include those atoms having the same atomic number but different mass numbers. By way of general example and without limitation, isotopes of hydrogen include tritium and deuterium, and isotopes of carbon include ^{13}C and ^{14}C.

[0157] It should be recognized that the particular anion or cation forming a part of any salt form of a cationic ionizable lipids provided herein is not critical, so long as the salt, as a whole, is pharmacologically acceptable. Additional examples of pharmaceutically acceptable salts and their methods of preparation and use are presented in *Handbook of Pharmaceutical Salts: Properties, and Use* (2002), which is incorporated herein by reference.

[0158] In some embodiments, the ionizable cationic lipid is a dendrimer or dendron. In some embodiments, the ionizable lipid comprises an ammonium group which is positively charged at physiological pH and contains at least two hydrophobic groups. In some embodiments, the ammonium group is positively charged at a pH from about 6 to about 8. In some embodiments, the ionizable lipid is a dendrimer or dendron. In some embodiments, the ionizable lipid comprises at least two C_6-C_{24} alkyl or alkenyl groups.

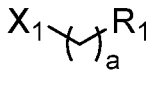
Dendrimers of Formula (I)

[0159] In some embodiments, the ionizable lipid comprises at least two C_8-C_{24} alkyl groups. In some embodiments, the ionizable lipid is a dendrimer further defined by the formula:

$$\text{Core-(Repeating Unit)}_n\text{-Terminating Group} \quad \text{(D-I)}$$

wherein one or more hydrogen atoms of the core are replaced with a repeating unit and wherein:

the core has the formula:



(D-II)

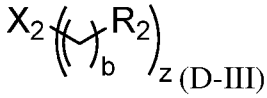
wherein:

X_1 is amino or C_1-C_{12} alkylamino, C_1-C_{12} dialkylamino, C_3-C_{12} heterocycloalkyl, C_5-C_{12} heteroaryl, or a substituted version thereof;

R_1 is amino, hydroxy, mercapto, C_1-C_{12} alkylamino, or C_1-C_{12} dialkylamino, or a substituted version of either of these groups; and

a is 1, 2, 3, 4, 5, or 6; or

the core has the formula:



(D-III)

wherein:

X_2 is $N(R_5)_y$;

R_5 is hydrogen, C_1-C_{18} alkyl, or substituted C_1-C_{18} alkyl; and

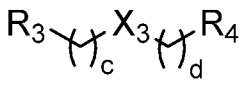
y is 0, 1, or 2, provided that the sum of y and z is 3;

R_2 is amino, hydroxy, mercapto, C_1-C_{12} alkylamino, or C_1-C_{12} dialkylamino, or a substituted version of either of these groups;

b is 1, 2, 3, 4, 5, or 6; and

z is 1, 2, or 3; provided that the sum of z and y is 3; or

the core has the formula:

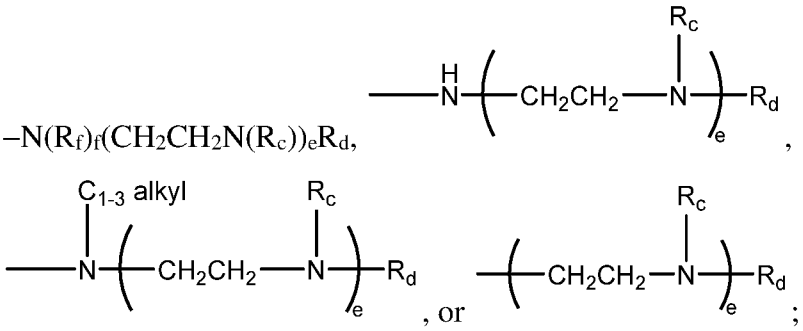


(D-IV)

wherein:

X_3 is $-NR_6-$, wherein R_6 is hydrogen, C_1-C_8 alkyl, or C_1-C_8 substituted alkyl, $-O-$, or C_1-C_8 alkylaminodiyl, C_1-C_8 alkoxydiyl, C_6-C_8 arenediyl, C_5-C_8 heteroarenediyl, C_3-C_8 heterocycloalkanediyl, or a substituted version of any of these groups;

R_3 and R_4 are each independently amino, hydroxy, mercapto, C_1-C_{12} alkylamino, or C_1-C_{12} dialkylamino, or a substituted version of either of these groups; or a group of the formula:



wherein:

e and f are each independently 1, 2, or 3; provided that the sum of e and f is 3;

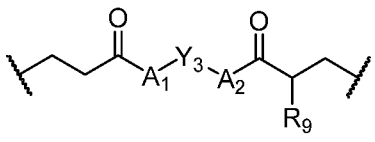
R_c, R_d, and R_f are each independently hydrogen, C_1-C_6 alkyl, or substituted C_1-C_6 alkyl;

c and d are each independently 1, 2, 3, 4, 5, or 6; or

the core is C_1-C_{18} alkylamine, C_1-C_{36} dialkylamine, C_3-C_{12} heterocycloalkane, or a substituted version of any of these groups;

wherein the repeating unit comprises a degradable diacyl or a degradable diacyl and a linker;

the degradable diacyl group has the formula:



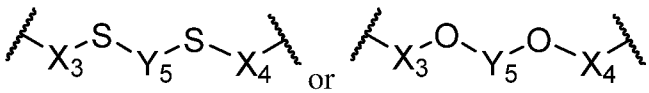
(D-VII)

wherein:

A_1 and A_2 are each independently –O– , -S-, or –NR_a–, wherein:

R_a is hydrogen, C_1-C_6 alkyl, or substituted C_1-C_6 alkyl;

Y_3 is C_1-C_{12} alkanediyl, C_1-C_{12} alkenediyl, C_6-C_{12} arenediyl, or a substituted version of any of these groups; or a group of the formula:



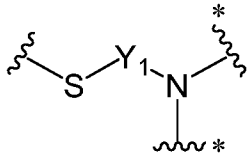
wherein:

X_3 and X_4 are C_1-C_{12} alkanediyl, C_2-C_{12} alkenediyl, C_6-C_{12} arenediyl, or a substituted version of any of these groups;

Y_5 is a covalent bond, C_1-C_{12} alkanediyl, C_1-C_{12} alkenediyl, C_6-C_{12} arenediyl, or a substituted version of any of these groups; and

R_9 is C_1-C_8 alkyl or substituted C_1-C_8 alkyl;

the linker group has the formula:

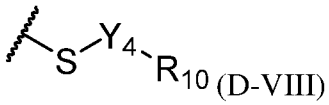

(D-VI)

wherein:

Y_1 is C_1-C_{12} alkanediyl, C_1-C_{12} alkenediyl, C_6-C_{12} arenediyl, or a substituted version of any of these groups; and

wherein each  independently denotes a point of attachment to another repeating unit or a terminating group; and

the terminating group has the formula:


(D-VIII)

wherein:

Y_4 is alkanediyl or an C_1-C_{18} alkanediyl wherein one or more of the hydrogen atoms on the C_1-C_{18} alkanediyl has been replaced with –OH, –F, –Cl, –Br, –I, –SH, $–OCH_3$, $–OCH_2CH_3$, $–SCH_3$, or $–OC(O)CH_3$;

R_{10} is hydrogen, carboxy, hydroxy,

C_6-C_{12} aryl, C_1-C_{12} alkylamino, C_1-C_{12} dialkylamino, C_3-C_{12} *N*-heterocycloalkyl, $–C(O)N(R_{11})–$ C_1-C_6 alkanediyl– C_3-C_{12} heterocycloalkyl, –C(O)– C_1-C_{12} alkylamino, –C(O)– C_1-C_{12} dialkylamino, or –C(O)– C_3-C_{12} *N*-heterocycloalkyl, wherein:

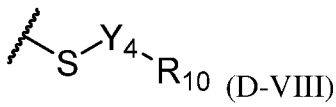
R_{11} is hydrogen, C_1-C_6 alkyl, or substituted C_1-C_6 alkyl;

wherein the final degradable diacyl in the chain is attached to a terminating group;

n is 0, 1, 2, 3, 4, 5, or 6;

or a pharmaceutically acceptable salt thereof.

[0160] In some embodiments, the terminating group is further defined by the formula:


(D-VIII)

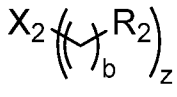
wherein:

Y4 is C1-C18 alkanediyl; and

R_{10} is hydrogen. In some embodiments, A_1 and A_2 are each independently –O– or $–NR_a–$.

[0161] In some embodiments of the dendrimer of formula (D-I), the terminating group is a structure selected from the structures in **Table 2.**

[0162] In some embodiments of the dendrimer of formula (D-I), the core is further defined by the formula:


(D-III)

[0163] wherein:

X_2 is $N(R_5)_y$;

R_5 is hydrogen or C_1-C_8 alkyl, or substituted C_1-C_{18} alkyl; and

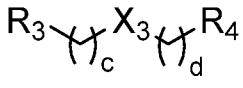
y is 0, 1, or 2, provided that the sum of y and z is 3;

R_2 is amino, hydroxy, or mercapto, or C_1-C_{12} alkylamino, C_1-C_{12} dialkylamino, or a substituted version of either of these groups;

b is 1, 2, 3, 4, 5, or 6; and

z is 1, 2, 3; provided that the sum of z and y is 3.

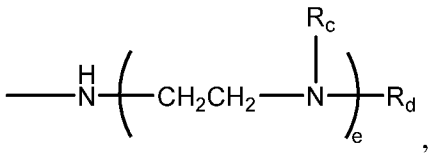
[0164] In some embodiments of the dendrimer of formula (D-I), the core is further defined by the formula:

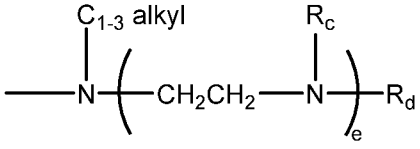
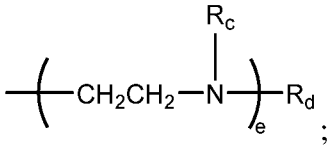

(D-IV)

wherein:

X_3 is $-NR_6-$, wherein R_6 is hydrogen, C_1-C_8 alkyl, or substituted C_1-C_8 alkyl, –O–, or C_1-C_8 alkylaminodiyl, C_1-C_8 alkoxydiyl, C_1-C_8 arenediyl, C_1-C_8 heteroarenediyl, C_1-C_8 heterocycloalkanediyl, or a substituted version of any of these groups;

R_3 and R_4 are each independently amino, hydroxy, or mercapto, or C_1-C_{12} alkylamino, dialkylamino, or a substituted version of either of these groups; or a group of the formula:

$-N(R_f)_f(CH_2CH_2N(R_c))_eR_d$,  ,

 , or  ;

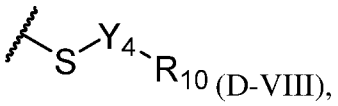
wherein:

e and f are each independently 1, 2, or 3; provided that the sum of e and f is 3;

R_c, R_d, and R_f are each independently hydrogen, C_1-C_6 alkyl, or substituted C_1-C_6 alkyl;

c and d are each independently 1, 2, 3, 4, 5, or 6.

[0165] In some embodiments of the dendrimer of formula (I), the terminating group is represented by the formula:



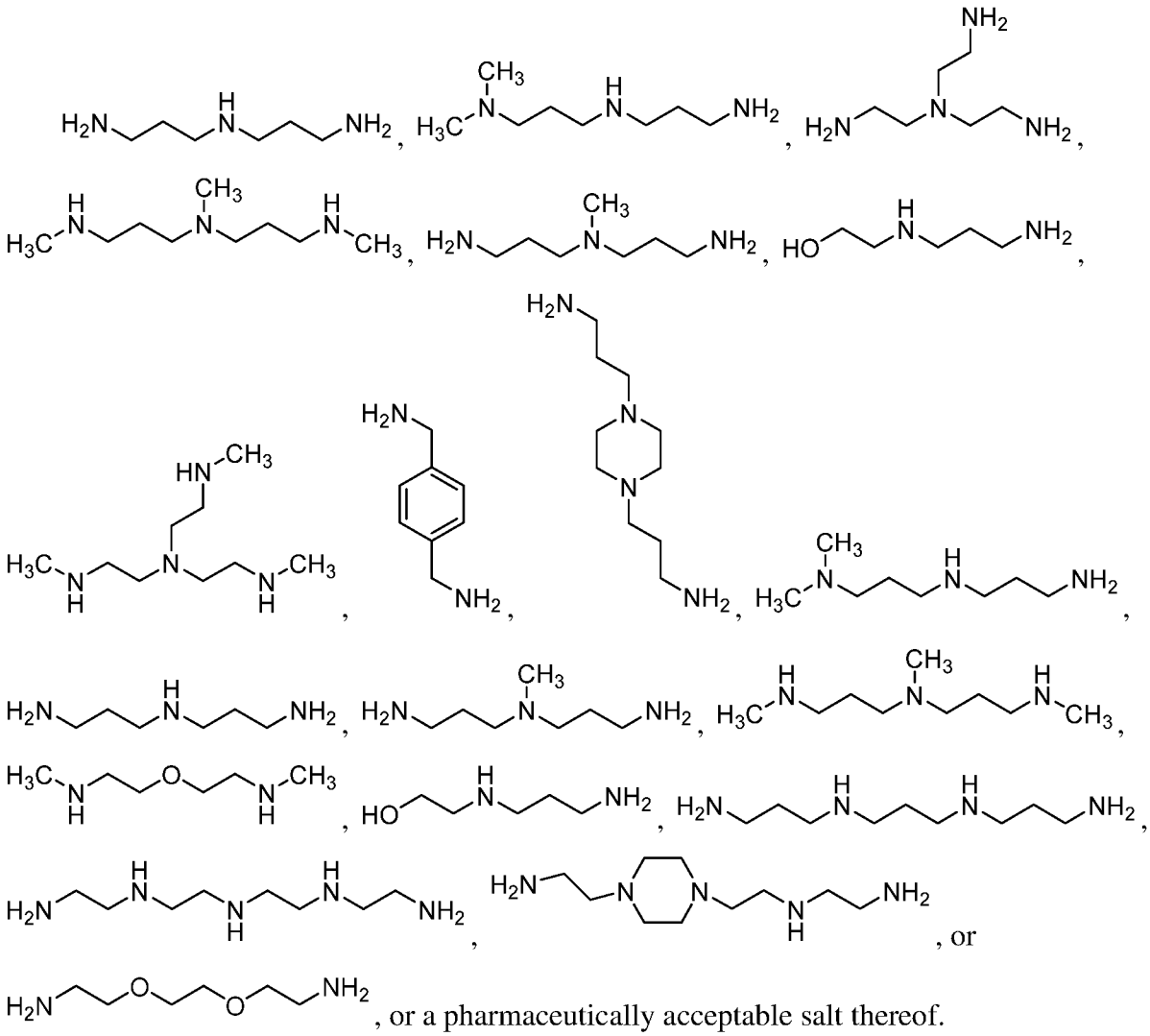
(D-VIII),

wherein:

Y_4 is alkanediyl$_{(C\leq 18)}$; and

R_{10} is hydrogen.

[0166] In some embodiments of the dendrimer of formula (D-I), a core of the structure of formula (D-IV) is:

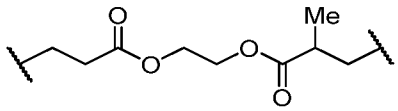


, or a pharmaceutically acceptable salt thereof.

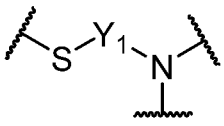
[0167] In some embodiments of the dendrimer of formula (D-I), the core comprises a structural formula set forth in **Table 2** and pharmaceutically acceptable salts thereof, wherein *

indicates a point of attachment of the core to a repeating unit (*i.e.*, where a hydrogen of the core is relaced with a repeating unit).

[0168] In some embodiments of the dendrimer of formula (D-I), the degradable diacyl is further defined as:



In some embodiments of the dendrimer of formula (D-I), the linker is further defined as



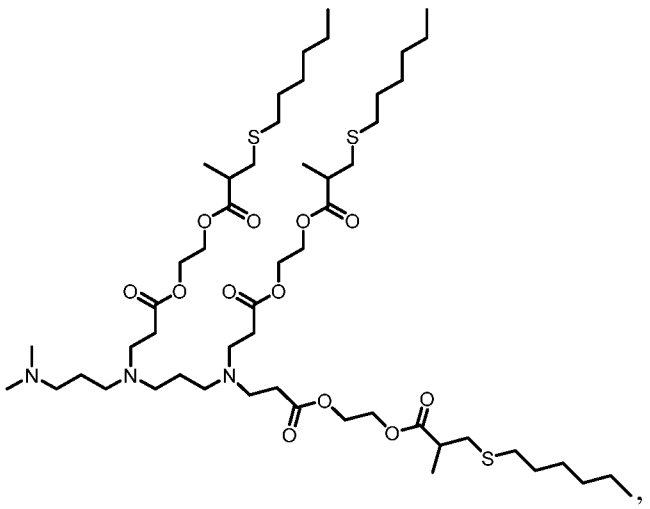
(D-VI),

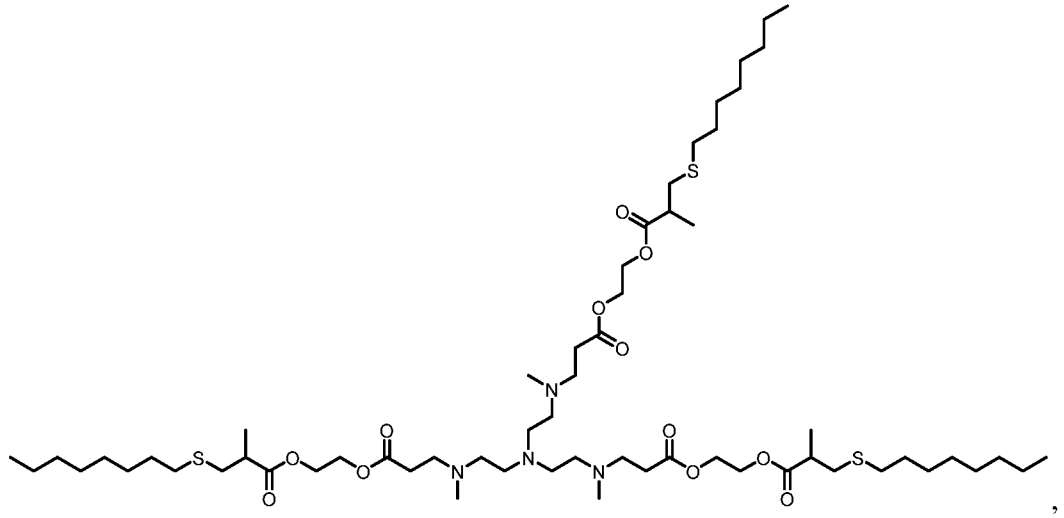
wherein Y_1 is C_1-C_8 alkanediyl or substituted C_1-C_{12} alkanediyl.

[0169] In some embodiments, in the core of formula (D-IV), R_6 is H. In some embodiments, in the core of formula (D-IV), R_6 is C_1-C_8 alkyl. In some embodiments, in the core of formula (D-IV), R_6 is substituted alkyl (*e.g.*, alkyl substituted with -NH_2, alkyl substituted with -$NHCH_3$, or alkyl substituted with -$NHCH_2CH_3$).

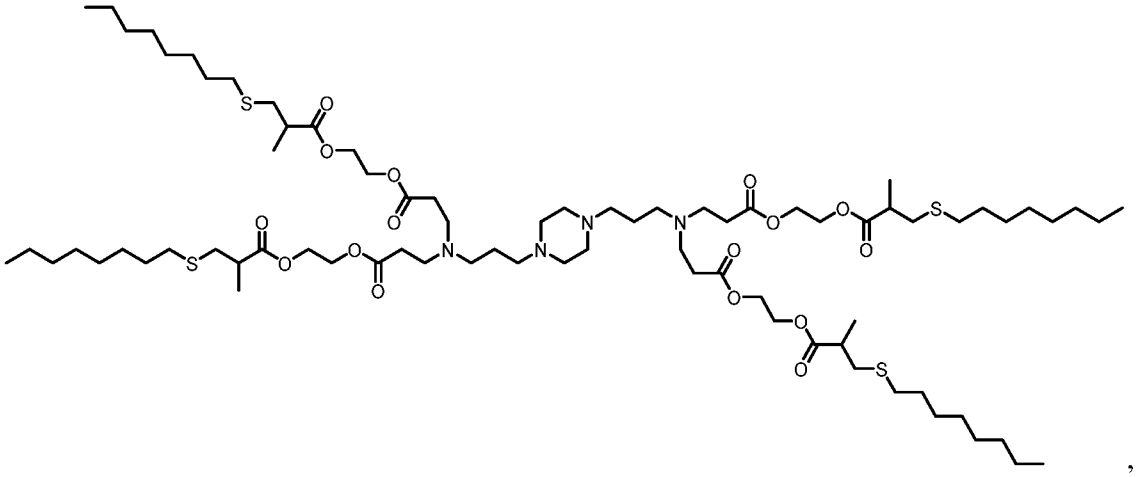
[0170] In some embodiments one or two hydrogen atoms of the core are replaced with a repeating unit. In some embodiments three or four hydrogen atoms of the core is replaced with a repeating unit. In some embodiments five hydrogen atoms of the core is replaced with a repeating unit. In some embodiments six hydrogen atoms of the core is replaced with a repeating unit.

[0171] In some embodiments of the dendrimer of formula (D-I), the dendrimer is selected from the group consisting of:





,



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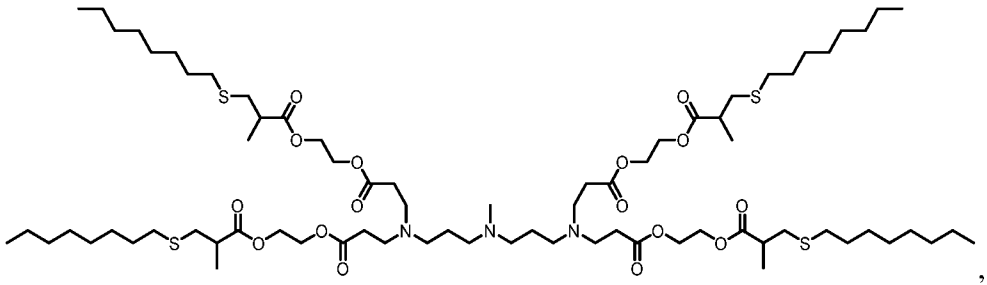

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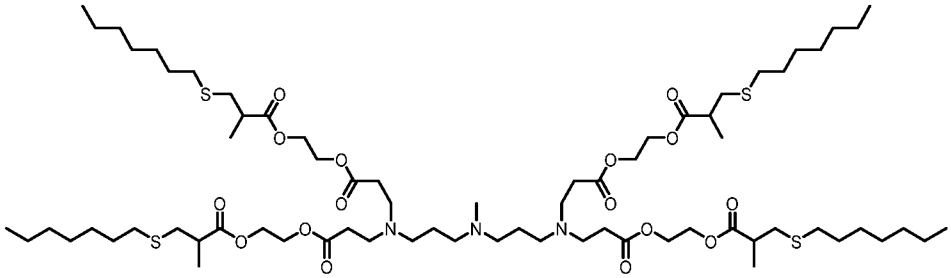
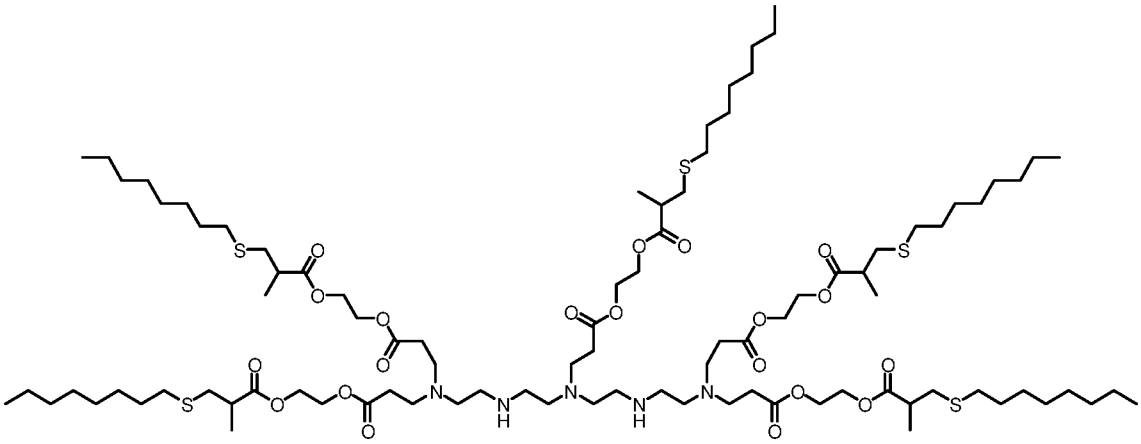
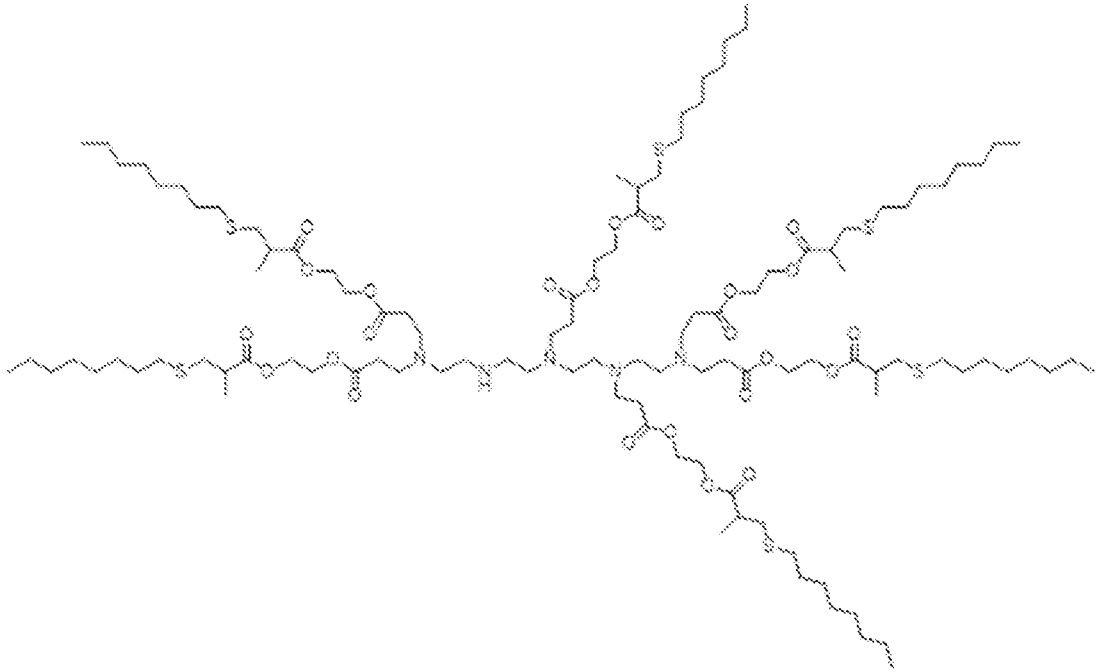




, and

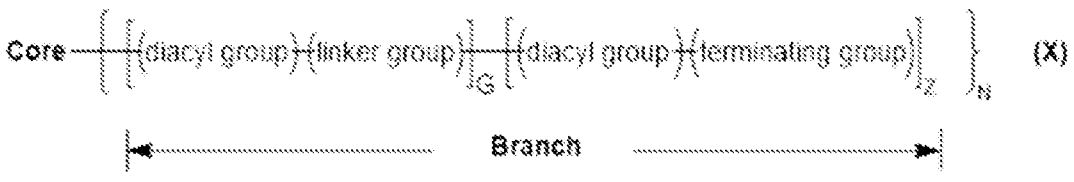


;

and pharmaceutically acceptable salts thereof.

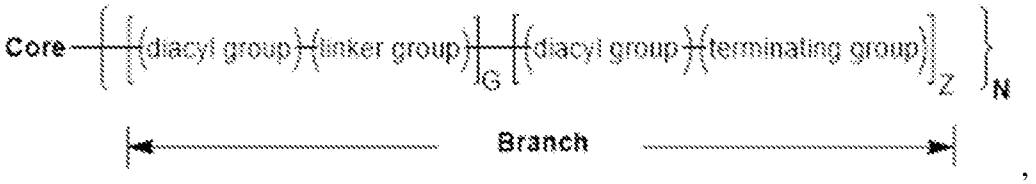
Dendrimers of Formula (X)

[0172] In some embodiments of the lipid composition, the ionizable lipid is a dendrimer of the formula  . In some embodiments, the ionizable lipid is a dendrimer of the formula



.

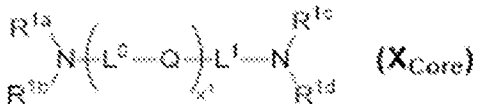
[0173] In some embodiments of the lipid composition, the ionizable lipid is a dendrimer of a generation (g) having a structural formula:



,

or a pharmaceutically acceptable salt thereof, wherein:

(a) the core comprises a structural formula (X_{Core}):



,

wherein:

Q is independently at each occurrence a covalent bond, -O-, -S-, -NR^2-, or -$CR^{3a}R^{3b}$-;

R^2 is independently at each occurrence R^{1g} or -L^2-$NR^{1e}R^{1f}$;

R^{3a} and R^{3b} are each independently at each occurrence hydrogen or an optionally substituted (*e.g.*, C_1-C_6, such as C_1-C_3) alkyl;

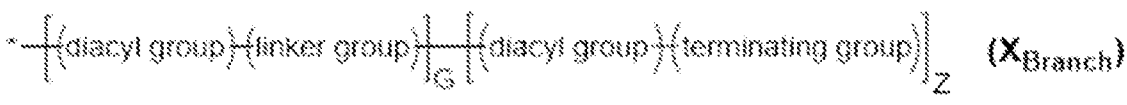
R^{1a}, R^{1b}, R^{1c}, R^{1d}, R^{1e}, R^{1f}, and R^{1g} (if present) are each independently at each occurrence a point of connection to a branch, hydrogen, or an optionally substituted (*e.g.*, C_1-C_{12}) alkyl;

L^0, L^1, and L^2 are each independently at each occurrence selected from a covalent bond, alkylene, heteroalkylene, [alkylene]-[heterocycloalkyl]-[alkylene], [alkylene]-(arylene)-[alkylene], heterocycloalkyl, and arylene; or,

alternatively, part of L^1 form a (*e.g.*, C_4-C_6) heterocycloalkyl (*e.g.*, containing one or two nitrogen atoms and, optionally, an additional heteroatom selected from oxygen and sulfur) with one of R^{1c} and R^{1d}; and

x^1 is 0, 1, 2, 3, 4, 5, or 6; and

(b) each branch of the plurality (N) of branches independently comprises a structural formula (X_{Branch}):



,

wherein:

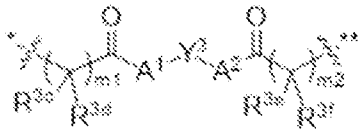
* indicates a point of attachment of the branch to the core;

g is 1, 2, 3, or 4;

$Z = 2^{(g-1)}$;

G=0, when g=1; or G = $\sum_{i=0}^{i=g-2} 2^i$, when g≠1;

(c) each diacyl group independently comprises a structural formula



, wherein:

* indicates a point of attachment of the diacyl group at the proximal end thereof;

** indicates a point of attachment of the diacyl group at the distal end thereof;

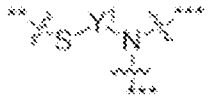
Y^3 is independently at each occurrence an optionally substituted (*e.g.*, C_1-C_{12}); alkylene, an optionally substituted (*e.g.*, C_1-C_{12}) alkenylene, or an optionally substituted (*e.g.*, C_1-C_{12}) arenylene;

A^1 and A^2 are each independently at each occurrence -O-, -S-, or -NR^4-, wherein:

R^4 is hydrogen or optionally substituted (*e.g.*, C_1-C_6) alkyl;

m^1 and m^2 are each independently at each occurrence 1, 2, or 3; and

R^{3c}, R^{3d}, R^{3e}, and R^{3f} are each independently at each occurrence hydrogen or an optionally substituted (*e.g.*, C_1-C_8) alkyl; and

(d) each linker group independently comprises a structural formula  , wherein:

** indicates a point of attachment of the linker to a proximal diacyl group;

*** indicates a point of attachment of the linker to a distal diacyl group; and

Y_1 is independently at each occurrence an optionally substituted (*e.g.*, C_1-C_{12}) alkylene, an optionally substituted (*e.g.*, C_1-C_{12}) alkenylene, or an optionally substituted (*e.g.*, C_1-C_{12}) arenylene; and

(e) each terminating group is independently selected from optionally substituted (*e.g.*, C_1-C_{18}, such as C_4-C_{18}) alkylthiol, and optionally substituted (*e.g.*, C_1-C_{18}, such as C_4-C_{18}) alkenylthiol.

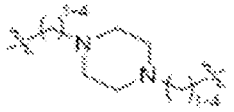
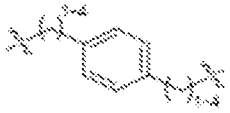
[0174] In some embodiments of X_{Core}, Q is independently at each occurrence a covalent bond, -O-, -S-, -NR^2-, or -$CR^{3a}R^{3b}$. In some embodiments of X_{Core} Q is independently at each occurrence a covalent bond. In some embodiments of X_{Core} Q is independently at each occurrence an -O-. In some embodiments of X_{Core} Q is independently at each occurrence a -S-. In some embodiments of X_{Core} Q is independently at each occurrence a -NR^2 and R^2 is

independently at each occurrence R^{1g} or $-L^2-NR^{1e}R^{1f}$. In some embodiments of X_{Core} Q is independently at each occurrence a $-CR^{3a}R^{3b}R^{3a}$, and R^{3a} and R^{3b} are each independently at each occurrence hydrogen or an optionally substituted alkyl (*e.g.*, C_1-C_6, such as C_1-C_3).

[0175] In some embodiments of X_{Core}, R^{1a}, R^{1b}, R^{1c}, R^{1d}, R^{1e}, R^{1f}, and R^{1g} (if present) are each independently at each occurrence a point of connection to a branch, hydrogen, or an optionally substituted alkyl. In some embodiments of X_{Core}, R^{1a}, R^{1b}, R^{1c}, R^{1d}, R^{1e}, R^{1f}, and R^{1g} (if present) are each independently at each occurrence a point of connection to a branch, hydrogen. In some embodiments of X_{Core}, R^{1a}, R^{1b}, R^{1c}, R^{1d}, R^{1e}, R^{1f}, and R^{1g} (if present) are each independently at each occurrence a point of connection to a branch an optionally substituted alkyl (*e.g.*, C_1-C_{12}).

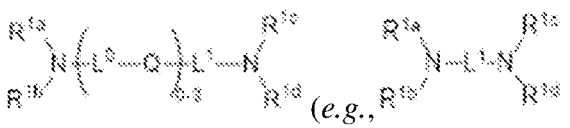
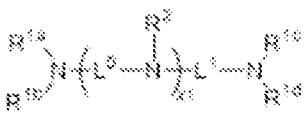
[0176] In some embodiments of X_{Core}, L^0, L^1, and L^2 are each independently at each occurrence selected from a covalent bond, alkylene, heteroalkylene, [alkylene]-[heterocycloalkyl]-[alkylene], [alkylene]-(arylene)-[alkylene], heterocycloalkyl, and arylene; or, alternatively, part of L^1 form a heterocycloalkyl (*e.g.*, C_4-C_6 and containing one or two nitrogen atoms and, optionally, an additional heteroatom selected from oxygen and sulfur) with one of R^{1c} and R^{1d}. In some embodiments of X_{Core}, L^0, L^1, and L^2 are each independently at each occurrence can be a covalent bond. In some embodiments of X_{Core}, L^0, L^1, and L^2 are each independently at each occurrence can be a hydrogen. In some embodiments of X_{Core}, L^0, L^1, and L^2 are each independently at each occurrence can be an alkylene (*e.g.*, C_1-C_{12}, such as C_1-C_6 or C_1-C_3). In some embodiments of X_{Core}, L^0, L^1, and L^2 are each independently at each occurrence can be a heteroalkylene (*e.g.*, C_1-C_{12}, such as C_1-C_8 or C_1-C_6). In some embodiments of X_{Core}, L^0, L^1, and L^2 are each independently at each occurrence can be a heteroalkylene (*e.g.*, C_2-C_8 alkyleneoxide, such as oligo(ethyleneoxide)). In some embodiments of X_{Core}, L^0, L^1, and L^2 are each independently at each occurrence can be a [alkylene]-[heterocycloalkyl]-[alkylene] [(*e.g.*, C_1-C_6) alkylene]-[(*e.g.*, C_4-C_6) heterocycloalkyl]-[(*e.g.*, C_1-C_6) alkylene]. In some embodiments of X_{Core}, L^0, L^1, and L^2 are each independently at each occurrence can be a [alkylene]-(arylene)-[alkylene] [(*e.g.*, C_1-C_6) alkylene]-(arylene)-[(*e.g.*, C_1-C_6) alkylene]. In some embodiments of X_{Core}, L^0, L^1, and L^2 are each independently at each occurrence can be a [alkylene]-(arylene)-[alkylene] (*e.g.*, [(*e.g.*, C_1-C_6) alkylene]-phenylene-[(*e.g.*, C_1-C_6) alkylene]). In some embodiments of X_{Core}, L^0, L^1, and L^2 are each independently at each occurrence can be a heterocycloalkyl (*e.g.*, C_4-C_6heterocycloalkyl). In some embodiments of X_{Core}, L^0, L^1, and L^2 are each independently at each occurrence can be an arylene (*e.g.*, phenylene). In some

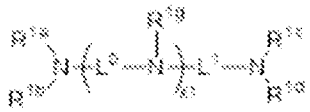
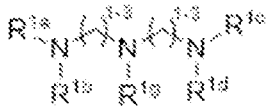
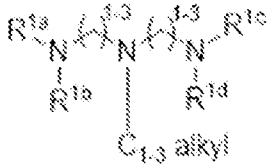
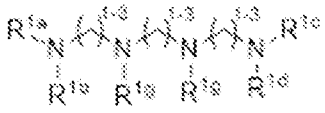
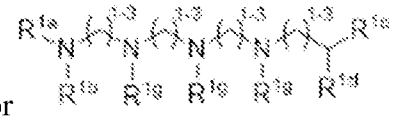
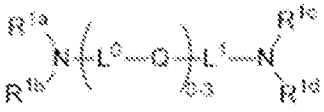
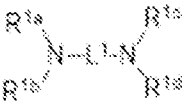
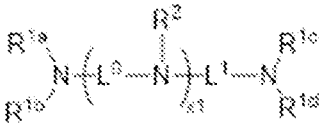
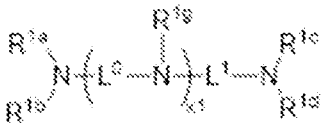
embodiments of X_{Core}, part of L^1 form a heterocycloalkyl with one of R^{1c} and R^{1d}. In some embodiments of X_{Core}, part of L^1 form a heterocycloalkyl (*e.g.*, C_4-C_6 heterocycloalkyl) with one of R^{1c} and R^{1d} and the heterocycloalkyl can contain one or two nitrogen atoms and, optionally, an additional heteroatom selected from oxygen and sulfur.

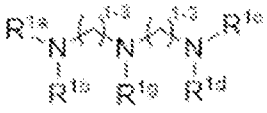
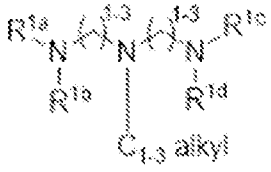
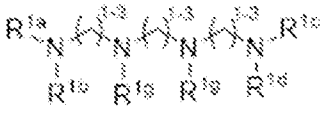
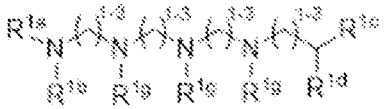
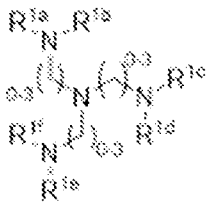
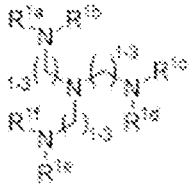
[0177] In some embodiments of X_{Core}, L^0, L^1, and L^2 are each independently at each occurrence selected from a covalent bond, C_1-C_6 alkylene (*e.g.*, C_1-C_3 alkylene), C_2-C_{12} (*e.g.*, C_2-C_8) alkyleneoxide (*e.g.*, oligo(ethyleneoxide), such as -$(CH_2CH_2O)_{1-4}$-(CH_2CH_2)-), [(C_1-C_4) alkylene]-[(C_4-C_6) heterocycloalkyl]-[(C_1-C_4) alkylene] (*e.g.*, ), and [(C_1-C_4) alkylene]-phenylene-[(C_1-C_4) alkylene] (*e.g.*, ). In some embodiments of X_{Core}, L^0, L^1, and L^2 are each independently at each occurrence selected from C_1-C_6 alkylene (*e.g.*, C_1-C_3 alkylene), -(C_1-C_3 alkylene-O)$_{1-4}$-(C_1-C_3 alkylene), -(C_1-C_3 alkylene)-phenylene-(C_1-C_3 alkylene)-, and -(C_1-C_3 alkylene)-piperazinyl-(C_1-C_3 alkylene)-. In some embodiments of X_{Core}, L^0, L^1, and L^2 are each independently at each occurrence C_1-C_6 alkylene (*e.g.*, C_1-C_3 alkylene). In some embodiments, L^0, L^1, and L^2 are each independently at each occurrence C_2-C_{12} (*e.g.*, C_2-C_8) alkyleneoxide (*e.g.*, -(C_1-C_3 alkylene-O)$_{1-4}$-(C_1-C_3 alkylene)). In some embodiments of X_{Core}, L^0, L^1, and L^2 are each independently at each occurrence selected from [(C_1-C_4) alkylene]-[(C_4-C_6) heterocycloalkyl]-[(C_1-C_4) alkylene] (*e.g.*, -(C_1-C_3 alkylene)-phenylene-(C_1-C_3 alkylene)-) and [(C_1-C_4) alkylene]-[(C_4-C_6) heterocycloalkyl]-[(C_1-C_4) alkylene] (*e.g.*, -(C_1-C_3 alkylene)-piperazinyl-(C_1-C_3 alkylene)-).

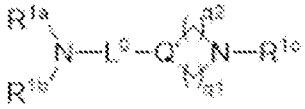
[0178] In some embodiments of X_{Core}, x^1 is 0, 1, 2, 3, 4, 5, or 6. In some embodiments of X_{Core}, x^1 is 0. In some embodiments of X_{Core}, x^1 is 1. In some embodiments of X_{Core}, x^1 is 2. In some embodiments of X_{Core}, x^1 is 0, 3. In some embodiments of X_{Core} x^1 is 4. In some embodiments of X_{Core} x^1 is 5. In some embodiments of X_{Core}, x^1 is 6.

[0179] In some embodiments of X_{Core}, the core comprises a structural formula:

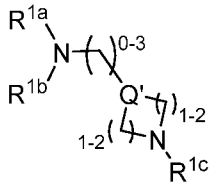
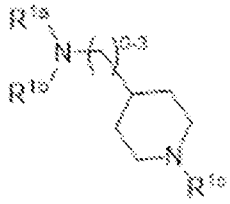
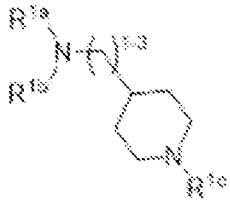
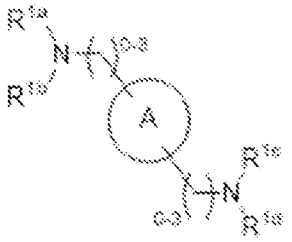
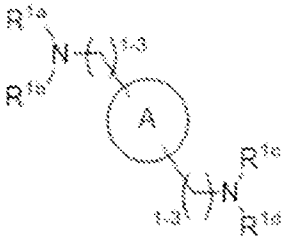
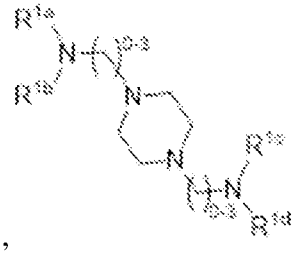
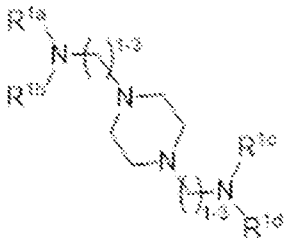
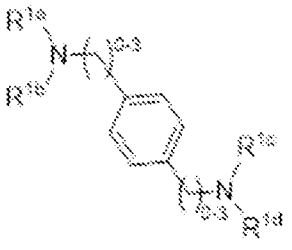
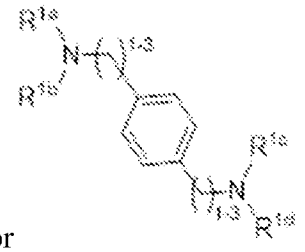
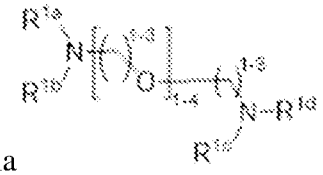

(*e.g.*,). In some embodiments of X_{Core}, the core comprises a structural formula:  . In some embodiments of X_{Core}, the core comprises

a structural formula:  (*e.g.*,  ,  ,  , or ). In some embodiments of X_{Core}, the core comprises a structural formula:  (*e.g.*, ). In some embodiments of X_{Core}, the core comprises a structural formula:  . In some embodiments of X_{Core}, the core comprises a structural formula: 

(*e.g.*,  ,  ,  , or ). In some embodiments of X_{Core}, the core comprises a structural formula:  , such as  or ). In some embodiments of X_{Core}, the core comprises a structural formula:

 , wherein Q' is $-NR^2-$ or $-CR^{3a}R^{3b}-$; q^1 and q^2 are each independently 1


or 2. In some embodiments of X_{Core}, the core comprises a structural formula: or


(*e.g.*,  ,  ,  , or ). In some embodiments of X_{Core}, the core comprises a structural formula 
or 
(*e.g.*,  ,  ,  , or ), wherein ring A is an optionally substituted aryl or an optionally substituted (*e.g.*, C_3-C_{12}, such as C_3-C_5) heteroaryl. In some embodiments of X_{Core}, the core comprises has a structural formula  .

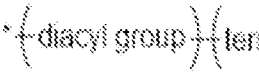
[0180] In some embodiments of X_{Core}, the core comprises a structural formula set forth in **Table 2** and pharmaceutically acceptable salts thereof, wherein * indicates a point of attachment of the core to a branch of the plurality of branches.

[0181] In some embodiments, the plurality (N) of branches comprises at least 3 branches, at least 4 branches, at least 5 branches. In some embodiments, the plurality (N) of branches

comprises at least 3 branches. In some embodiments, the plurality (N) of branches comprises at least 4 branches. In some embodiments, the plurality (N) of branches comprises at least 5 branches.

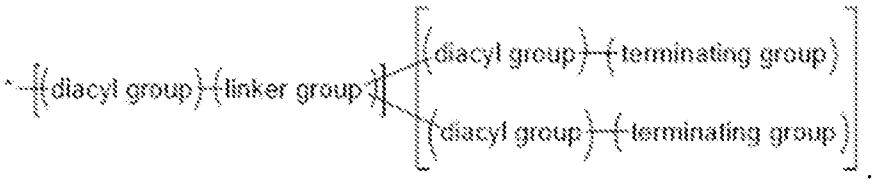
[0182] In some embodiments of X_{Branch}, g is 1, 2, 3, or 4. In some embodiments of X_{Branch}, g is 1. In some embodiments of X_{Branch}, g is 2. In some embodiments of X_{Branch}, g is 3. In some embodiments of X_{Branch}, g is 4.

[0183] In some embodiments of X_{Branch}, $Z = 2^{(g-1)}$ and when g=1, G=0. In some embodiments of X_{Branch}, $Z = 2^{(g-1)}$ and $G = \sum_{i=0}^{i=g-2} 2^i$, when g≠1.

[0184] In some embodiments of X_{Branch}, g=1, G=0, Z=1, and each branch of the plurality of branches comprises a structural formula each branch of the plurality of branches comprises a structural formula 

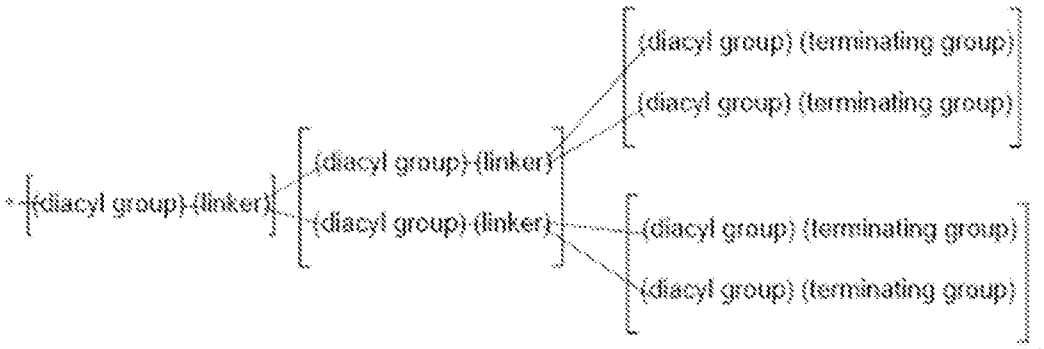
.

[0185] In some embodiments of X_{Branch}, g=2, G=1, Z=2, and each branch of the plurality of branches comprises a structural formula



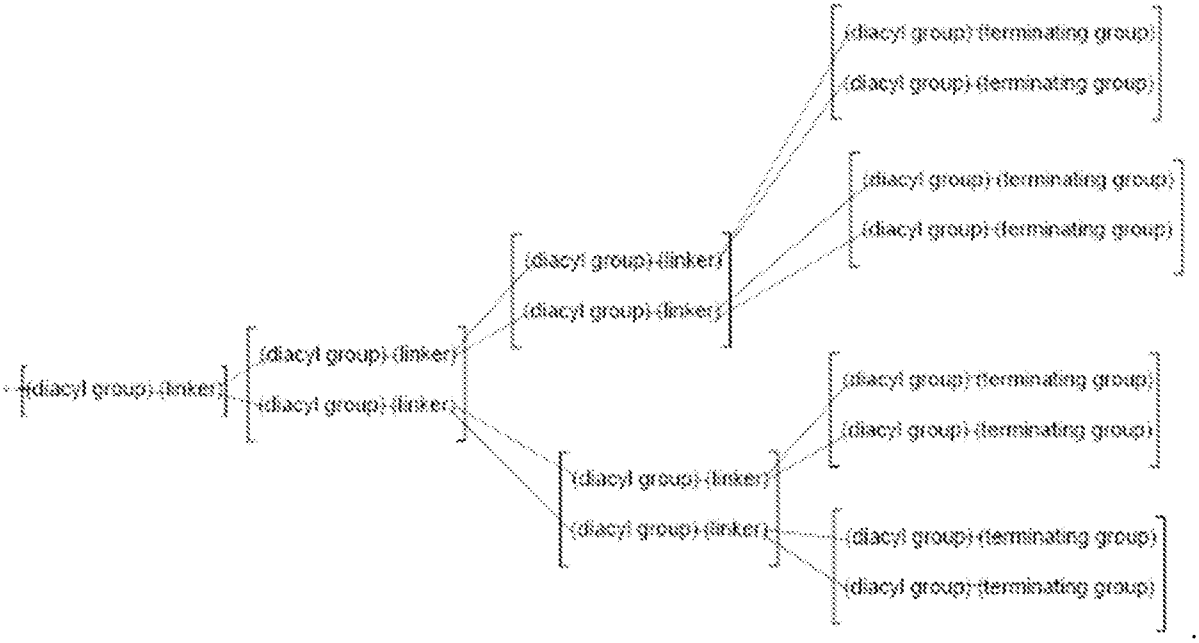
.

[0186] In some embodiments of X_{Branch}, g=3, G=3, Z=4, and each branch of the plurality of branches comprises a structural formula



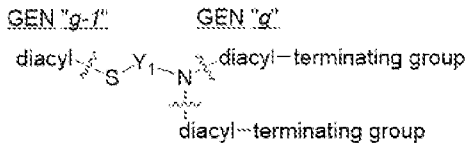
.

[0187] In some embodiments of X_{Branch}, g=4, G=7, Z=8, and each branch of the plurality of branches comprises a structural formula



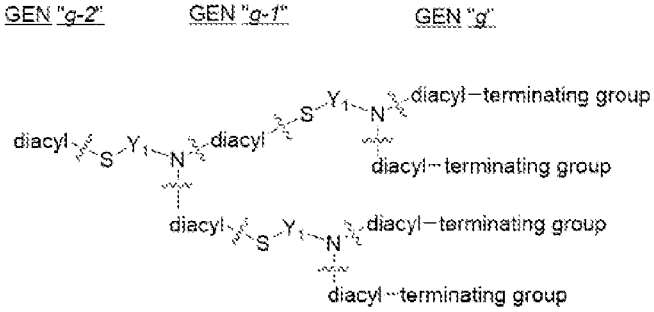
.

[0188] In some embodiments, the dendrimers described herein with a generation (g) = 1 has



the structure: .

[0189] In some embodiments, the dendrimers described herein with a generation (g) = 1 has



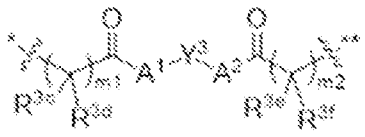
the structure: .

[0190] An example formulation of the dendrimers described herein for generations 1-4 is shown in **Table 1**. The number of diacyl groups, linker groups, and terminating groups can be calculated based on g.

Table 1. Formulation of Dendrimer Groups Based on Generation (g)

	$g = 1$	$g = 2$	$g = 3$	$g = 4$	
# of diacyl grp	1	1+2=3	$1+2+2^2=7$	$1+2+2^2+2^3=15$	$1+2+\ldots+2^{g-1}$
# of linker grp	0	1	1+2	$1+2+2^2$	$1+2+\ldots+2^{g-2}$

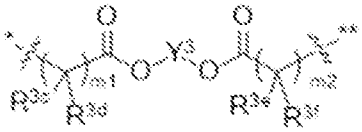
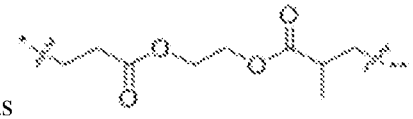
# of terminating grp	1	2	2^2	2^3	$2^{(g-1)}$

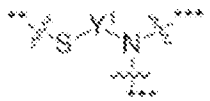
[0191] In some embodiments, the diacyl group independently comprises a structural formula  , * indicates a point of attachment of the diacyl group at the proximal end thereof, and ** indicates a point of attachment of the diacyl group at the distal end thereof.

[0192] In some embodiments of the diacyl group of X_{Branch}, Y^3 is independently at each occurrence an optionally substituted; alkylene, an optionally substituted alkenylene, or an optionally substituted arenylene. In some embodiments of the diacyl group of X_{Branch}, Y^3 is independently at each occurrence an optionally substituted alkylene (*e.g.*, C_1-C_{12}). In some embodiments of the diacyl group of X_{Branch}, Y^3 is independently at each occurrence an optionally substituted alkenylene (*e.g.*, C_1-C_{12}). In some embodiments of the diacyl group of X_{Branch}, Y^3 is independently at each occurrence an optionally substituted arenylene (*e.g.*, C_1-C_{12}).

[0193] In some embodiments of the diacyl group of X_{Branch}, A^1 and A^2 are each independently at each occurrence -O-, -S-, or -NR^4-. In some embodiments of the diacyl group of X_{Branch}, A^1 and A^2 are each independently at each occurrence -O-. In some embodiments of the diacyl group of X_{Branch}, A^1 and A^2 are each independently at each occurrence -S-. In some embodiments of the diacyl group of X_{Branch}, A^1 and A^2 are each independently at each occurrence -NR^4- and R^4 is hydrogen or optionally substituted alkyl (*e.g.*, C_1-C_6). In some embodiments of the diacyl group of X_{Branch}, m^1 and m^2 are each independently at each occurrence 1, 2, or 3. In some embodiments of the diacyl group of X_{Branch}, m^1 and m^2 are each independently at each occurrence 1. In some embodiments of the diacyl group of X_{Branch}, m^1 and m^2 are each independently at each occurrence 2. In some embodiments of the diacyl group of X_{Branch}, m^1 and m^2 are each independently at each occurrence 3. In some embodiments of the diacyl group of X_{Branch}, R^{3c}, R^{3d}, R^{3e}, and R^{3f} are each independently at each occurrence hydrogen or an optionally substituted alkyl. In some embodiments of the diacyl group of X_{Branch}, R^{3c}, R^{3d}, R^{3e}, and R^{3f} are each independently at each occurrence hydrogen. In some embodiments of the diacyl group of X_{Branch}, R^{3c}, R^{3d}, R^{3e}, and R^{3f} are each independently at each occurrence an optionally substituted (*e.g.*, C_1-C_8) alkyl.

[0194] In some embodiments of the diacyl group, A^1 is -O- or -NH-. In some embodiments of the diacyl group, A^1 is -O-. In some embodiments of the diacyl group, A^2 is -O- or -NH-. In some embodiments of the diacyl group, A^2 is -O-. In some embodiments of the diacyl group, Y^3 is C_1-C_{12} (*e.g.*, C_1-C_6, such as C_1-C_3) alkylene.

[0195] In some embodiments of the diacyl group, the diacyl group independently at each occurrence comprises a structural formula  (*e.g.*,  , such as ), and optionally R^{3c}, R^{3d}, R^{3e}, and R^{3f} are each independently at each occurrence hydrogen or C_1-C_3 alkyl.

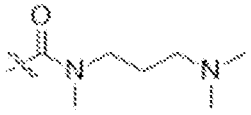
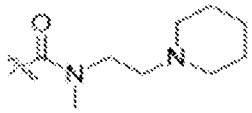
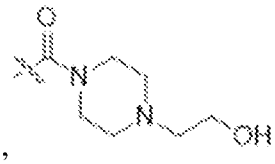
[0196] In some embodiments, linker group independently comprises a structural formula  , ** indicates a point of attachment of the linker to a proximal diacyl group, and *** indicates a point of attachment of the linker to a distal diacyl group.

[0197] In some embodiments of the linker group of X_{Branch} if present, Y_1 is independently at each occurrence an optionally substituted alkylene, an optionally substituted alkenylene, or an optionally substituted arenylene. In some embodiments of the linker group of X_{Branch} if present, Y_1 is independently at each occurrence an optionally substituted alkylene (*e.g.*, C_1-C_{12}). In some embodiments of the linker group of X_{Branch} if present, Y_1 is independently at each occurrence an optionally substituted alkenylene (*e.g.*, C_1-C_{12}). In some embodiments of the linker group of X_{Branch} if present, Y_1 is independently at each occurrence an optionally substituted arenylene (*e.g.*, C_1-C_{12}).

[0198] In some embodiments of the terminating group of X_{Branch}, each terminating group is independently selected from optionally substituted alkylthiol and optionally substituted alkenylthiol. In some embodiments of the terminating group of X_{Branch}, each terminating group is an optionally substituted alkylthiol (*e.g.*, C_1-C_{18}, such as C_4-C_{18}). In some embodiments of the terminating group of X_{Branch}, each terminating group is optionally substituted alkenylthiol (*e.g.*, C_1-C_{18}, such as C_4-C_{18}).

[0199] In some embodiments of the terminating group of X_{Branch}, each terminating group is independently C_1-C_{18} alkenylthiol or C_1-C_{18} alkylthiol, and the alkyl or alkenyl moiety is

optionally substituted with one or more substituents each independently selected from halogen, C_6-C_{12} aryl, C_1-C_{12} alkylamino, C_4-C_6 *N*-heterocycloalkyl , -OH, -C(O)OH, –C(O)N(C_1-C_3 alkyl)–(C_1-C_6 alkylene)–(C_1-C_{12} alkylamino), –C(O)N(C_1-C_3 alkyl)–(C_1-C_6 alkylene)–(C_4-C_6 *N*-heterocycloalkyl), –C(O)–(C_1-C_{12} alkylamino), and –C(O)–(C_4-C_6 *N*-heterocycloalkyl), and the C_4-C_6 *N*-heterocycloalkyl moiety of any of the preceding substituents is optionally substituted with C_1-C_3 alkyl or C_1-C_3 hydroxyalkyl.

[0200] In some embodiments of the terminating group of X_{Branch}, each terminating group is independently C_1-C_{18} (*e.g.*, C_4-C_{18}) alkenylthiol or C_1-C_{18} (*e.g.*, C_4-C_{18}) alkylthiol, wherein the alkyl or alkenyl moiety is optionally substituted with one or more substituents each independently selected from halogen, C_6-C_{12} aryl (*e.g.*, phenyl), C_1-C_{12} (*e.g.*, C_1-C_8) alkylamino (*e.g.*, C_1-C_6 mono-alkylamino (such as -$NHCH_2CH_2CH_2CH_3$) or C_1-C_8 di-alkylamino (such as )), C_4-C_6 *N*-heterocycloalkyl (*e.g.*, *N*-pyrrolidinyl (), *N*-piperidinyl (), *N*-azepanyl ()), -OH, -C(O)OH, –C(O)N(C_1-C_3 alkyl)–(C_1-C_6 alkylene)–(C_1-C_{12} alkylamino (*e.g.*, mono- or di-alkylamino)) (*e.g.*, ), –C(O)N(C_1-C_3 alkyl)–(C_1-C_6 alkylene)–(C_4-C_6 *N*-heterocycloalkyl) (*e.g.*, ), –C(O)–(C_1-C_{12} alkylamino (*e.g.*, mono- or di-alkylamino)), and –C(O)–(C_4-C_6 *N*-heterocycloalkyl) (*e.g.*, ), wherein the C_4-C_6 *N*-heterocycloalkyl moiety of any of the preceding substituents is optionally substituted with C_1-C_3 alkyl or C_1-C_3 hydroxyalkyl. In some embodiments of the terminating group of X_{Branch}, each terminating group is independently C_1-C_{18} (*e.g.*, C_4-C_{18}) alkylthiol, wherein the alkyl moiety is optionally substituted with one substituent -OH. In some embodiments of the terminating group of X_{Branch}, each terminating group is independently C_1-C_{18} (*e.g.*, C_4-C_{18}) alkylthiol, wherein the alkyl moiety is optionally substituted with one substituent selected from C_1-C_{12} (*e.g.*, C_1-C_8) alkylamino (*e.g.*, C_1-C_6 mono-alkylamino (such as -$NHCH_2CH_2CH_2CH_3$) or C_1-C_8 di-

alkylamino (such as )) and C_4-C_6 *N*-heterocycloalkyl (*e.g.*, *N*-pyrrolidinyl (), *N*-piperidinyl (), *N*-azepanyl ()). In some embodiments of the terminating group of X_{Branch}, each terminating group is independently C_1-C_{18} (*e.g.*, C_4-C_{18}) alkenylthiol or C_1-C_{18} (*e.g.*, C_4-C_{18}) alkylthiol. In some embodiments of the terminating group of X_{Branch}, each terminating group is independently C_1-C_{18} (*e.g.*, C_4-C_{18}) alkylthiol.

Table 2. Example core structures

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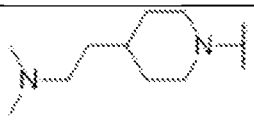
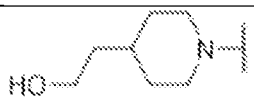
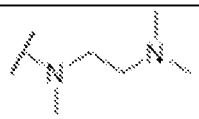
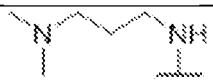
1A2	
1A3	
1A4	
1A5	
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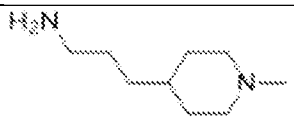
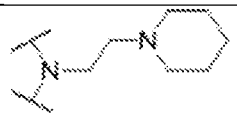
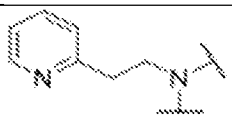
2A2	
2A3	
2A4	
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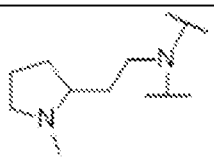
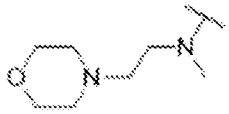
2A6	
2A7	
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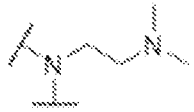
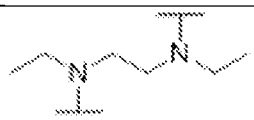
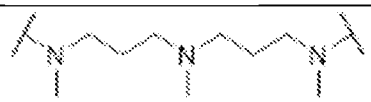
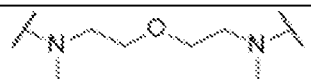
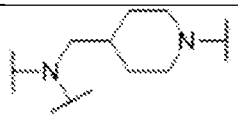
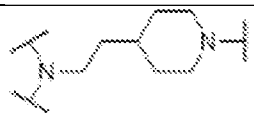
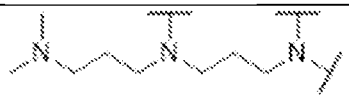
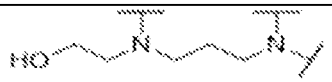
2A9	
2A10	
2A11	
2A12	
3A1	
3A2	
3A3	
3A4	
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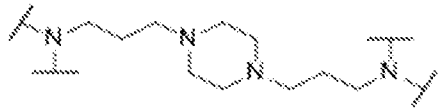
4A1	

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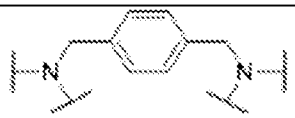
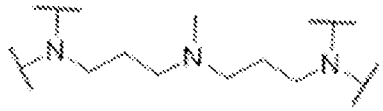
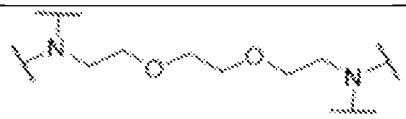
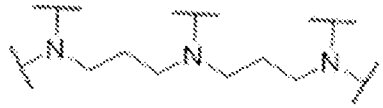
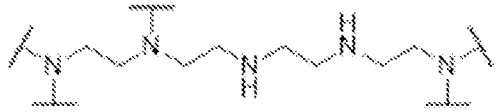
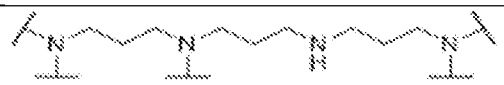
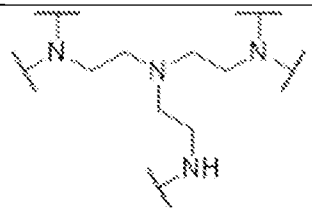
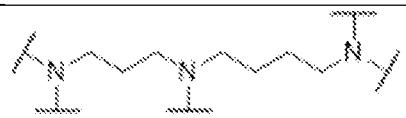
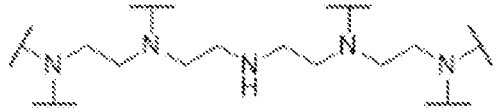
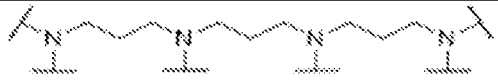
4A2	
4A3	
4A4	
5A1	
5A2	
5A3	
5A4	
5A5	
6A1	
6A2	
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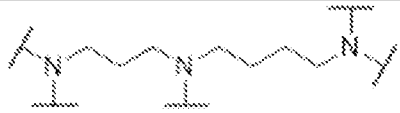
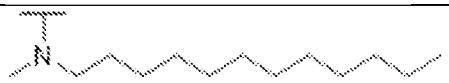
6A4	
1H1	
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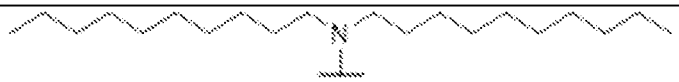
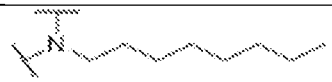
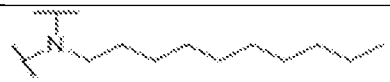
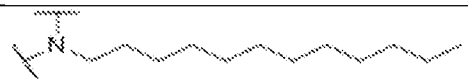
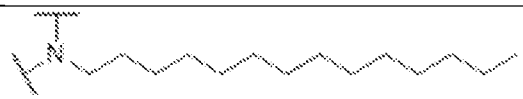
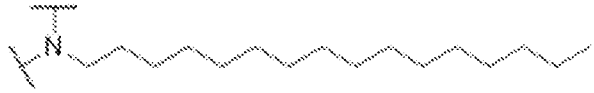
1H3	
2H1	
2H2	
2H3	
2H4	
2H5	
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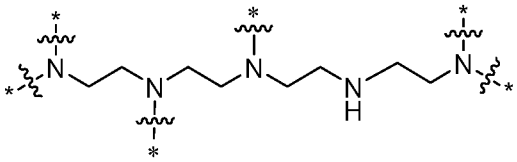
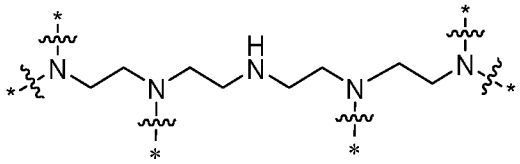
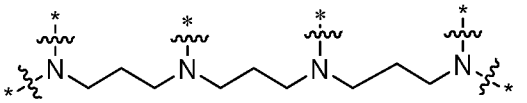
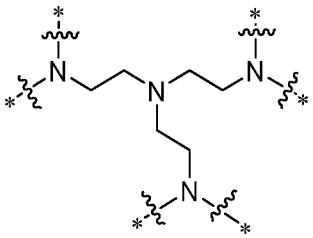
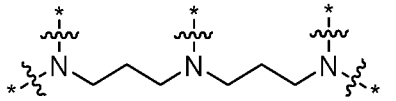
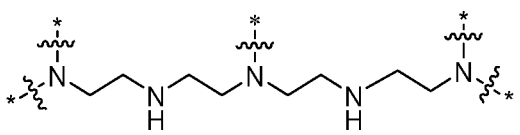
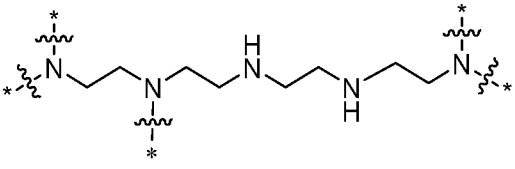
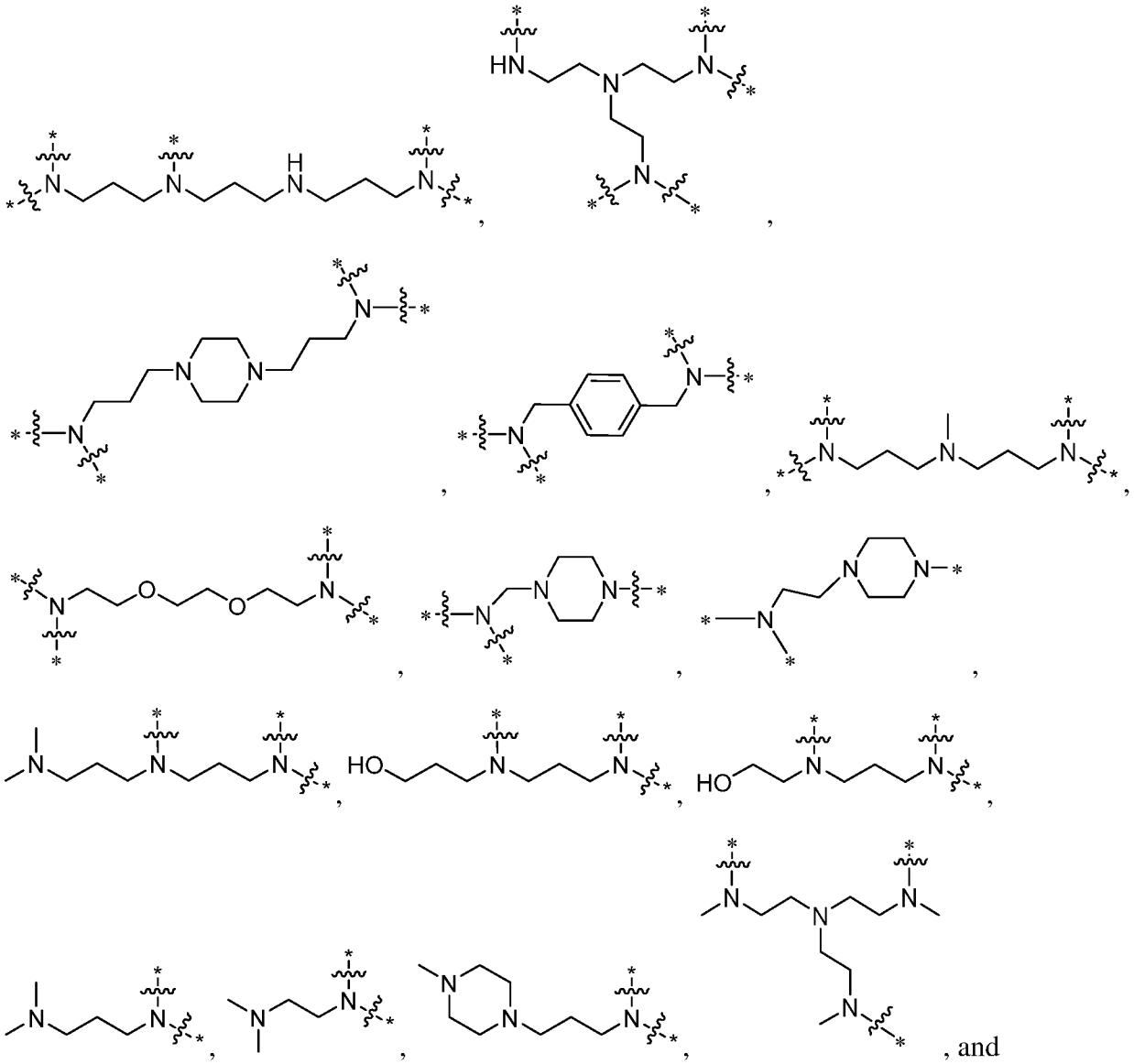
[0201] In some embodiments of X_{Core}, the core comprises a structural formula selected from the group consisting of:  ,

 ,  ,

 ,  ,

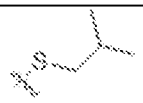
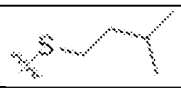
 ,  ,

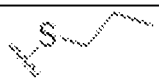
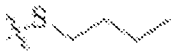
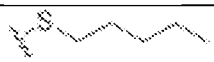
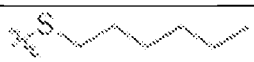
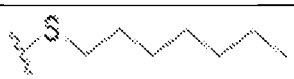
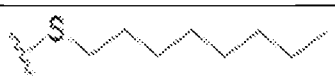
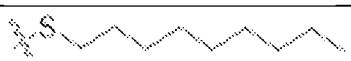
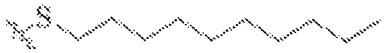
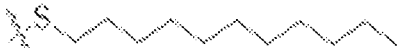


pharmaceutically acceptable salts thereof, wherein * indicates a point of attachment of the core to a branch of the plurality of branches.

[0202] In some embodiments of the terminating group of X_{Branch}, each terminating group is independently a structure selected from the structurea in **Table 3**. In some embodiments, the dendrimers described herein can comprise a terminating group or pharmaceutically acceptable salt, or thereof selected in **Table 3**.

Table 3. Example terminating group / peripheries structures

ID #	Structure
SC1	
SC2	

ID #	Structure
SC3	
SC4	
SC5	
SC6	
SC7	
SC8	
SC9	
SC10	
SC11	
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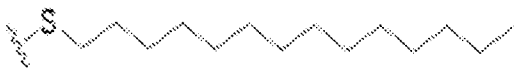
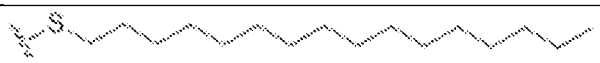
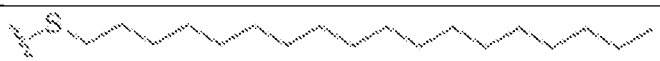
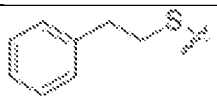
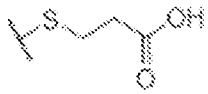
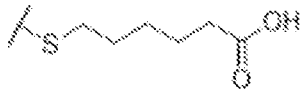
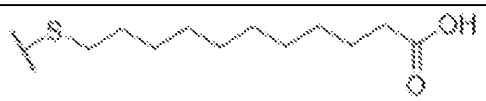
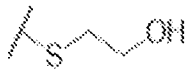
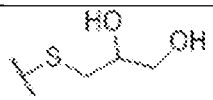
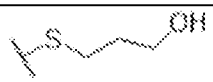
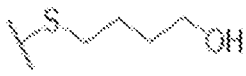
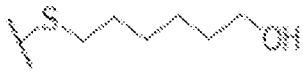
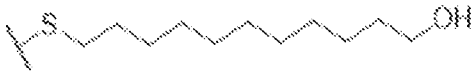
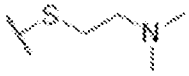
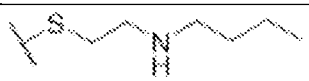
SC14	
SC16	
SC18	
SC19	
SO1	
SO2	
SO3	
SO4	
SO5	
SO6	
SO7	
SO8	

ID #	Structure
SO9	
SN1	
SN2	
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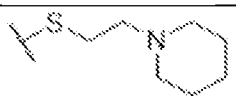
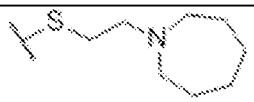
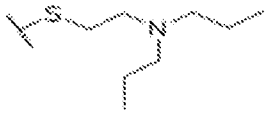
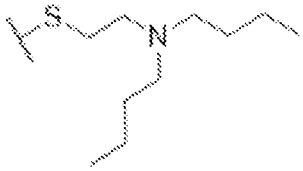
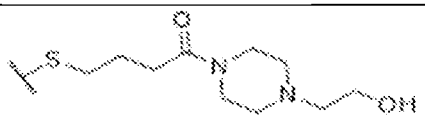
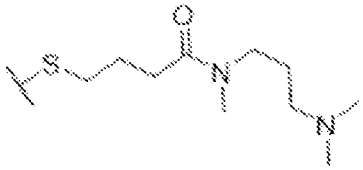
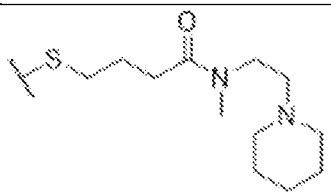
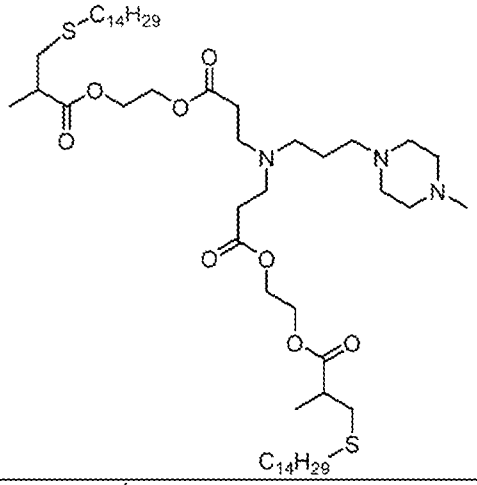
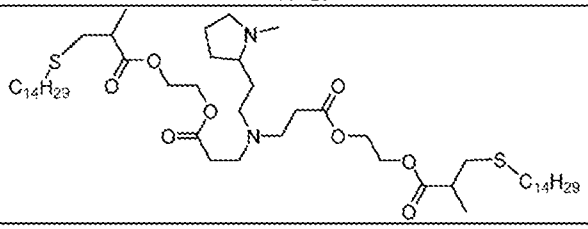
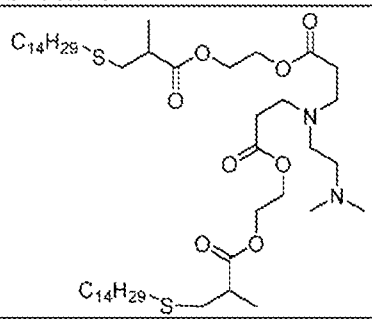
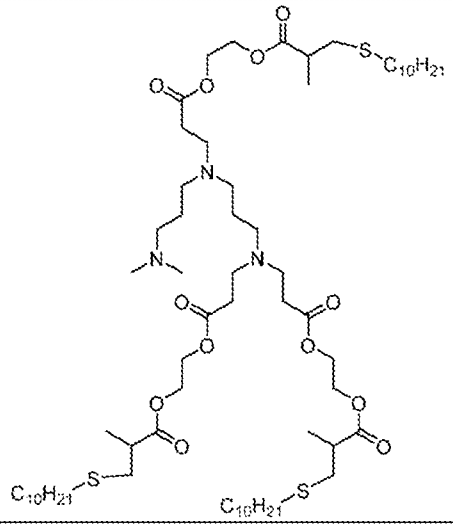
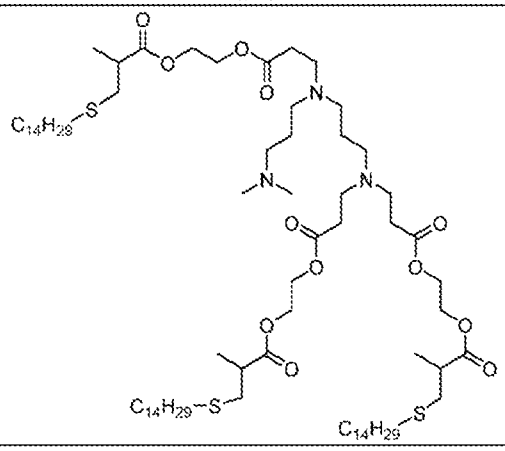
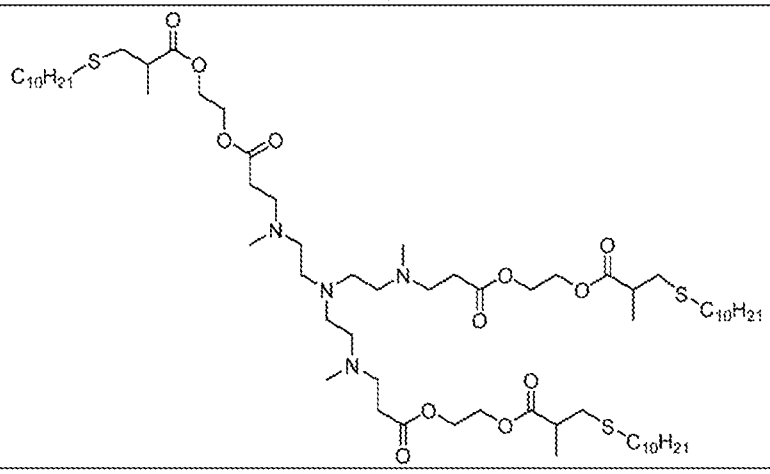
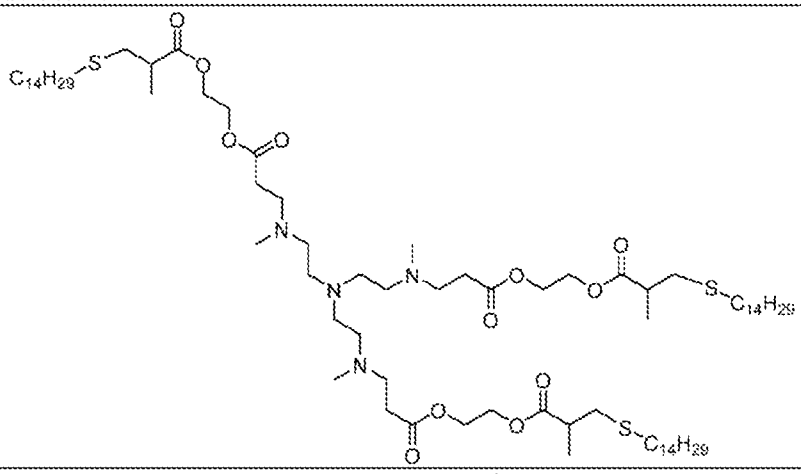
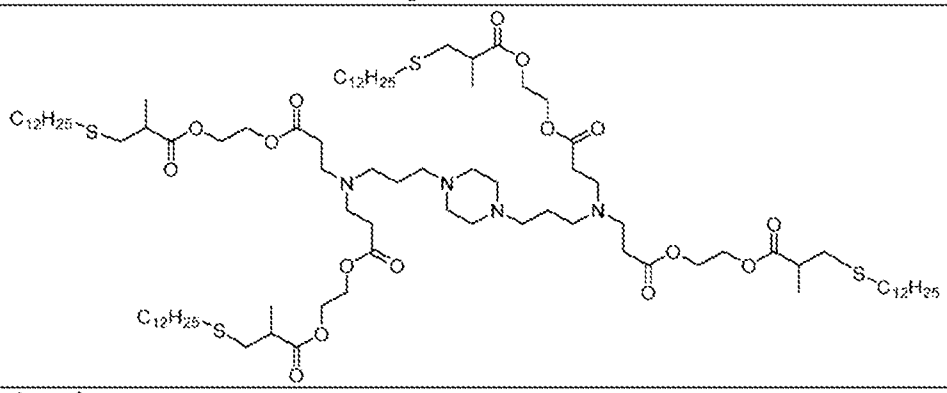
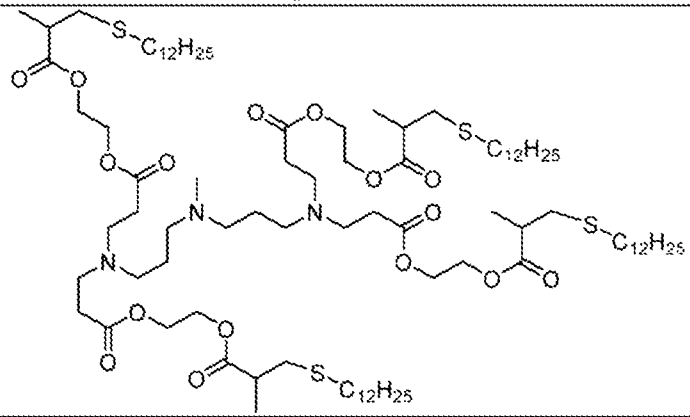
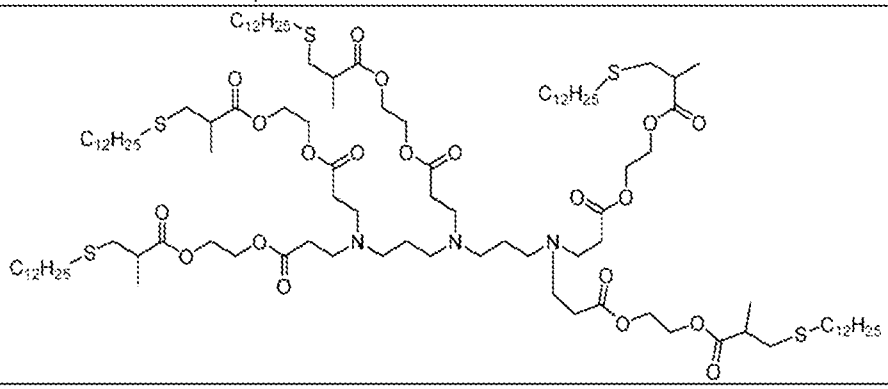
SN4	
SN5	
SN6	
SN7	
SN8	
SN9	
SN10	
SN11	

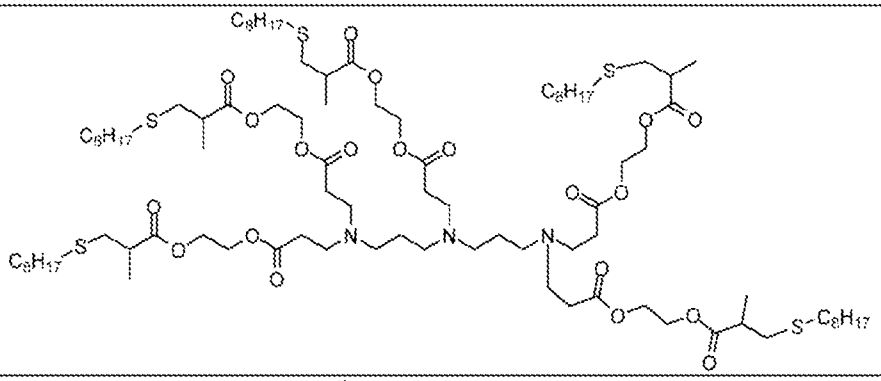
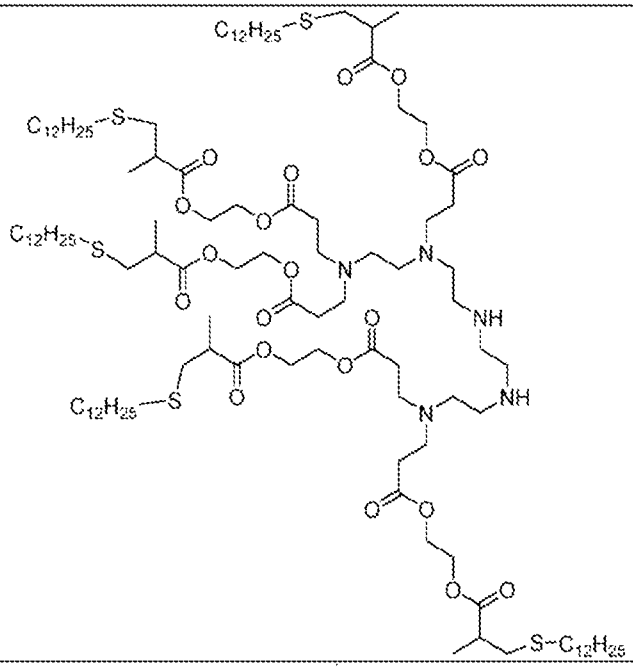
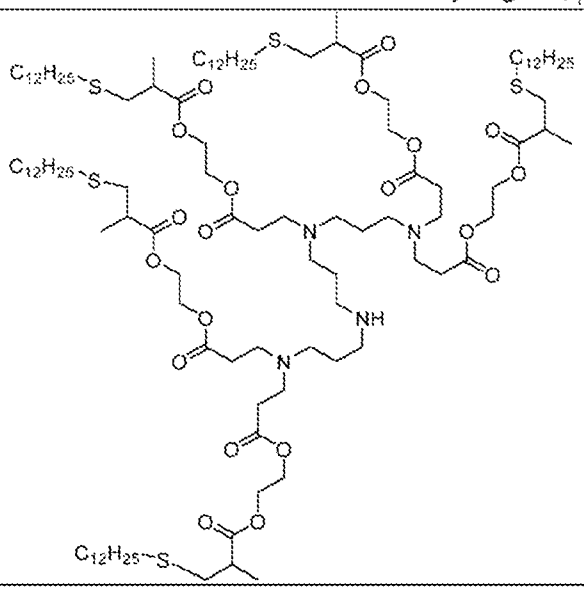
[0203] In some embodiments, the dendrimer of Formula (X) is selected from those set forth in **Table 4** and pharmaceutically acceptable salts thereof.

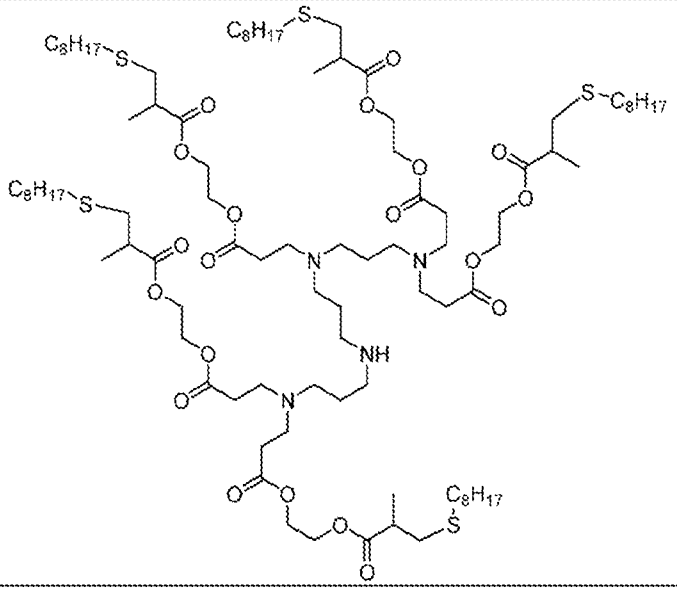
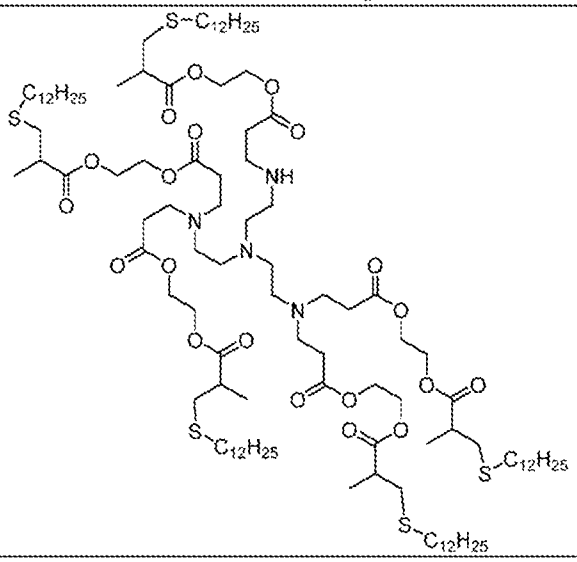
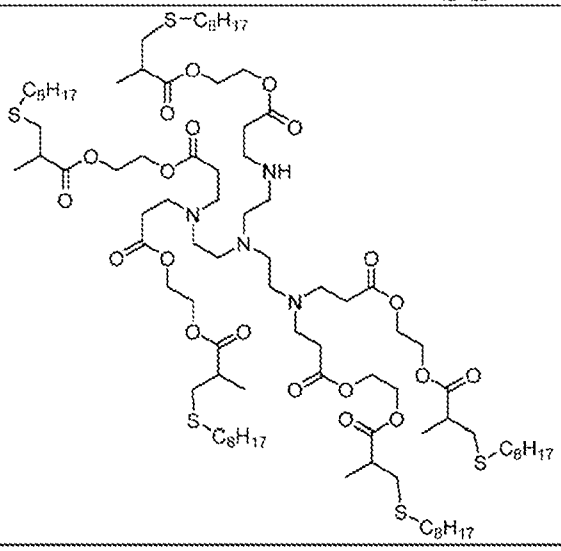
Table 4. Example ionizable cationic lipo-dendrimers

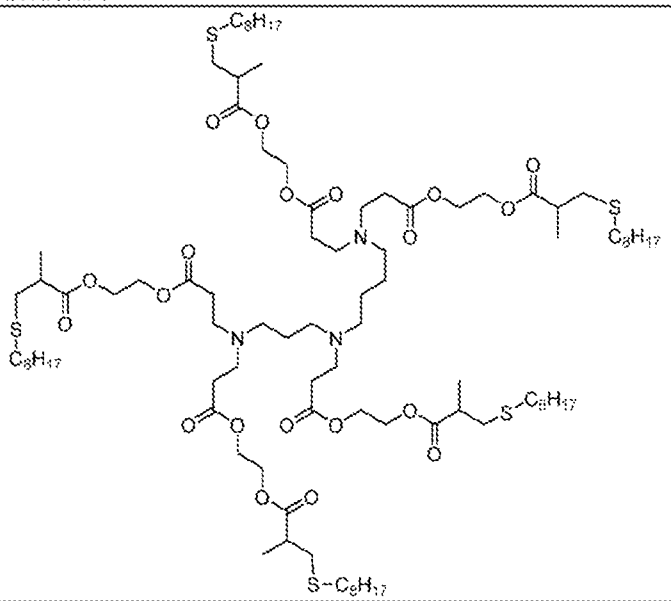
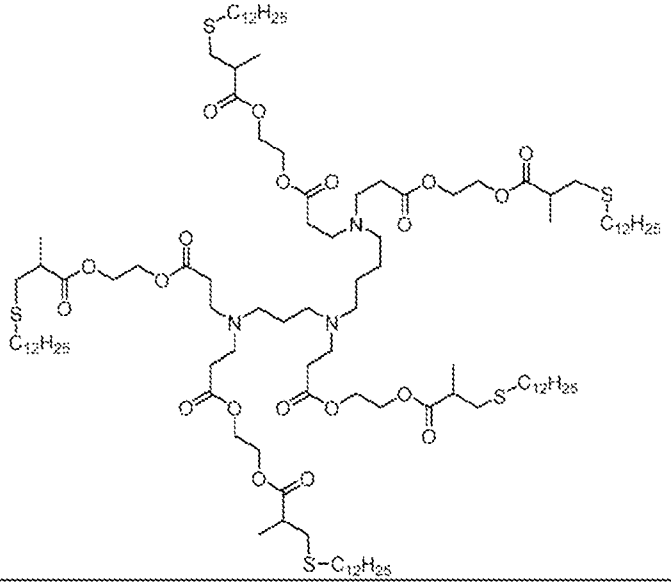
ID #	Structure
2A2-SC14	
2A6-SC14	

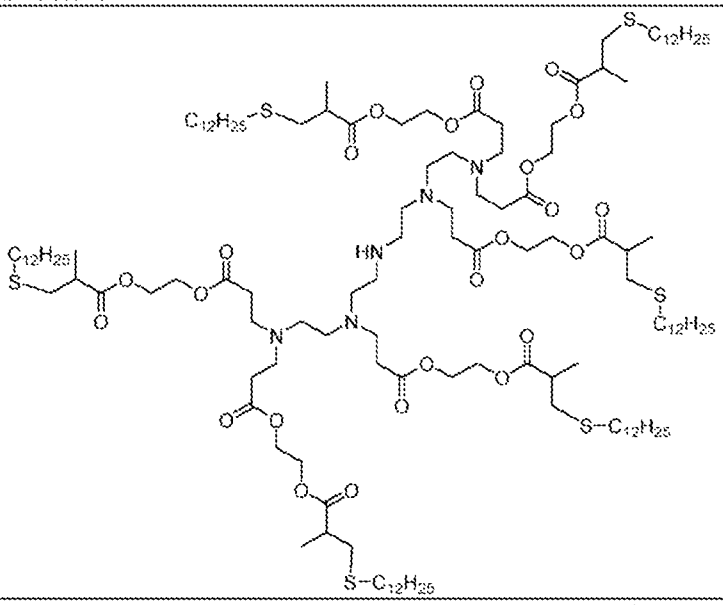
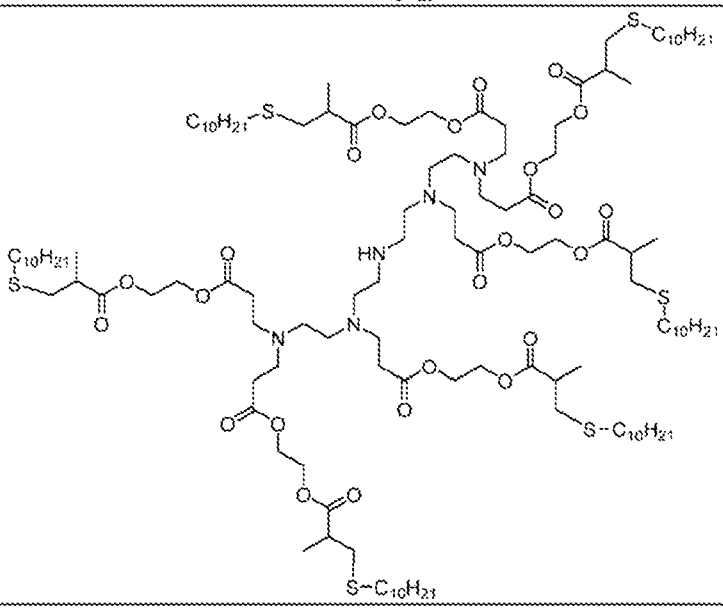
ID #	Structure
2A9-SC14	
3A3-SC10	
3A3-SC14	
3A5-SC10	

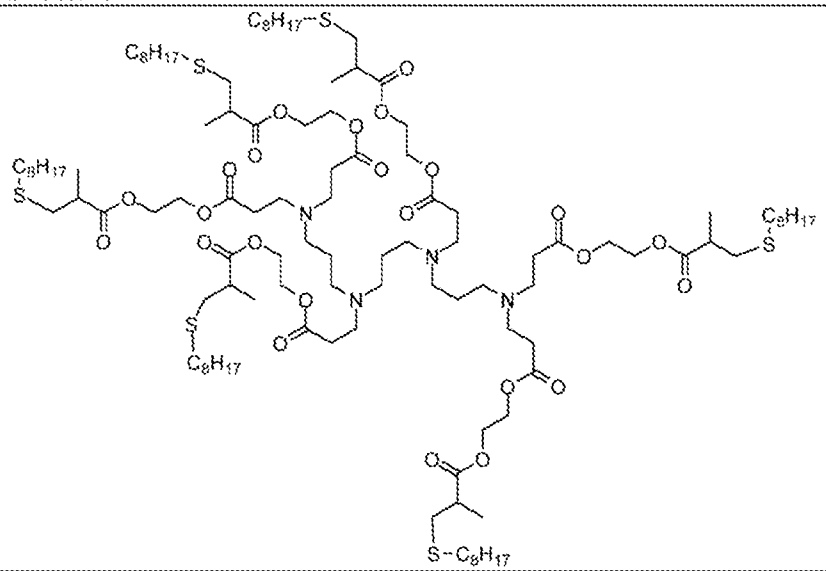
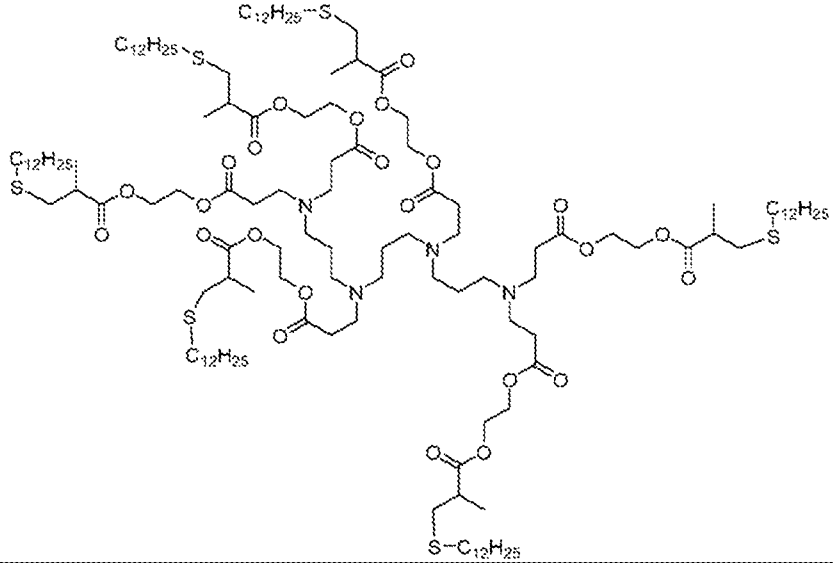
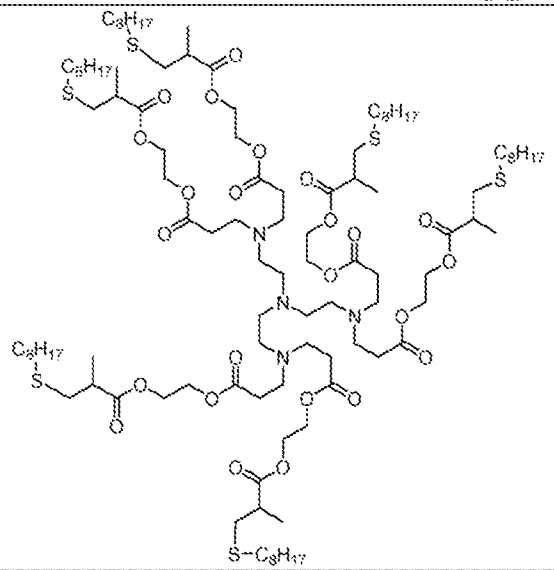
ID #	Structure
3A5-SC14	
4A1-SC12	
4A3-SC12	
5A1-SC12	

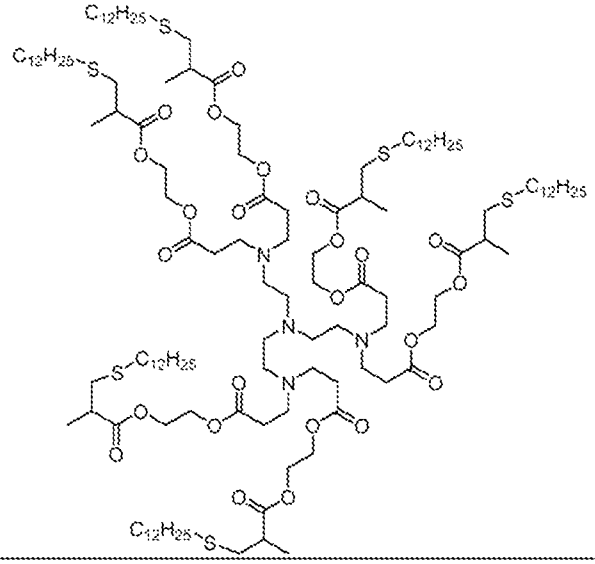
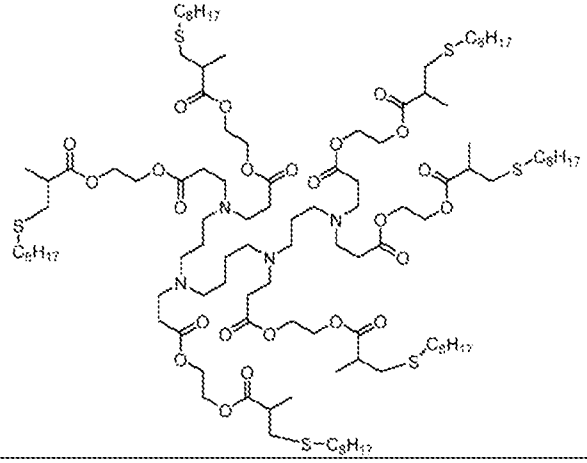
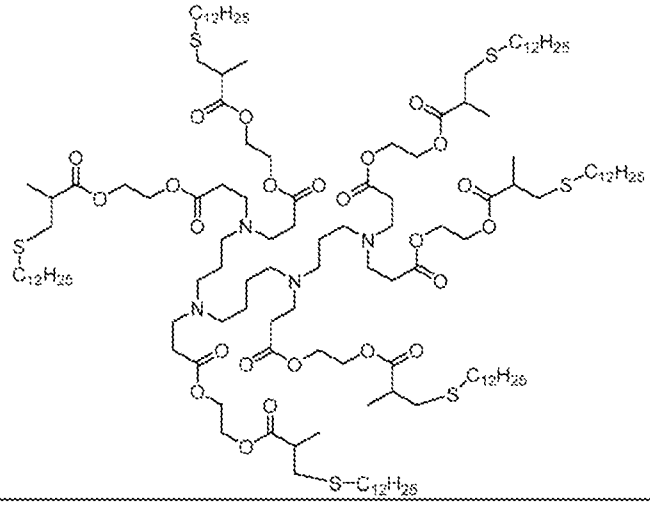
ID #	Structure
5A1-SC8	
5A2-2-SC12 (5-arm)	
5A3-1-SC12 (5 arm)	

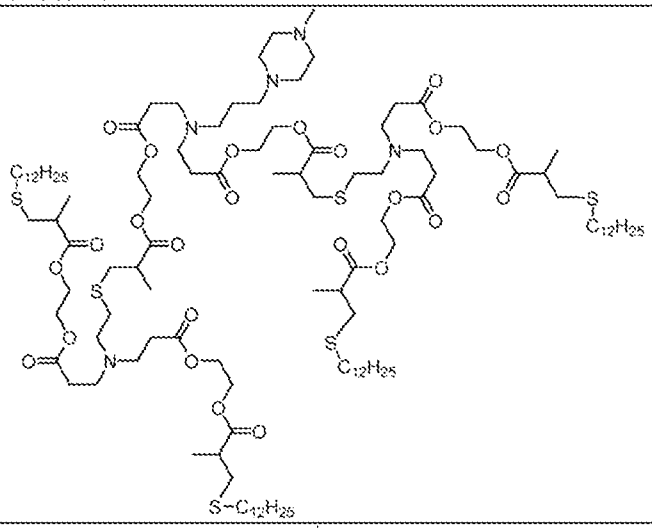
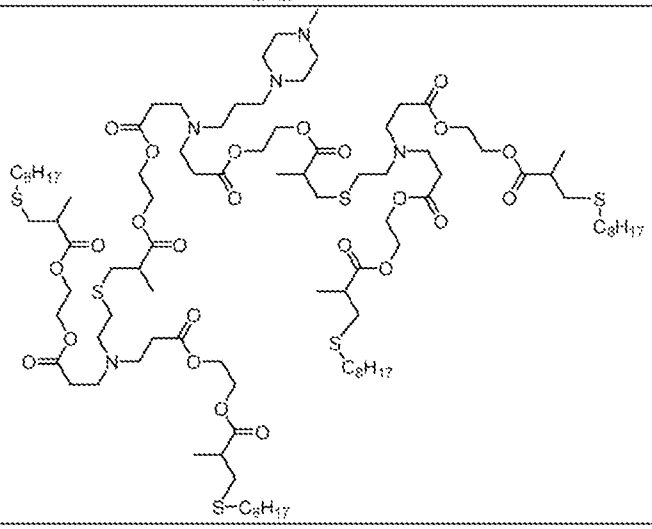
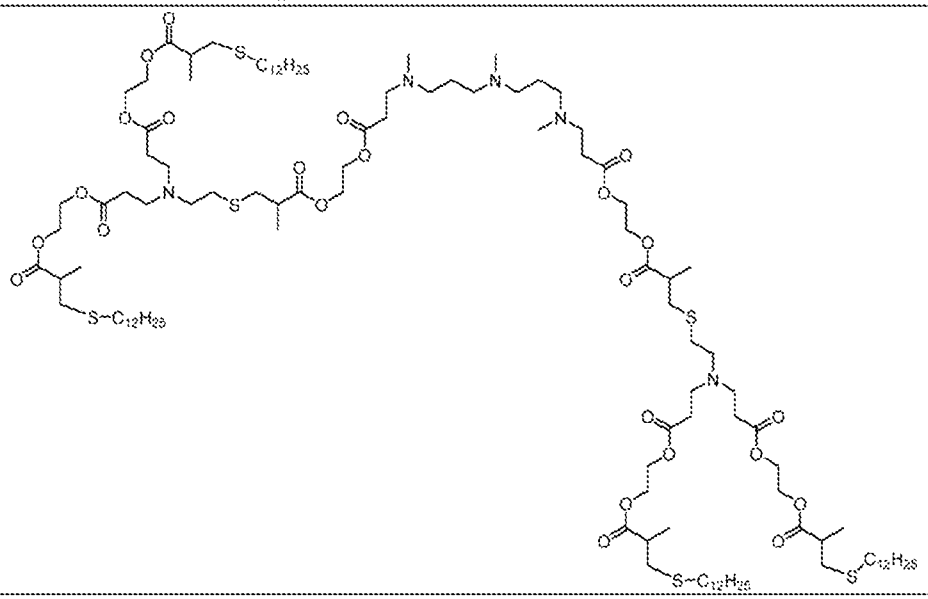
ID #	Structure
5A3-1-SC8 (5-arm)	
5A4-1-SC12 (5-arm)	
5A4-1-SC8 (5-arm)	

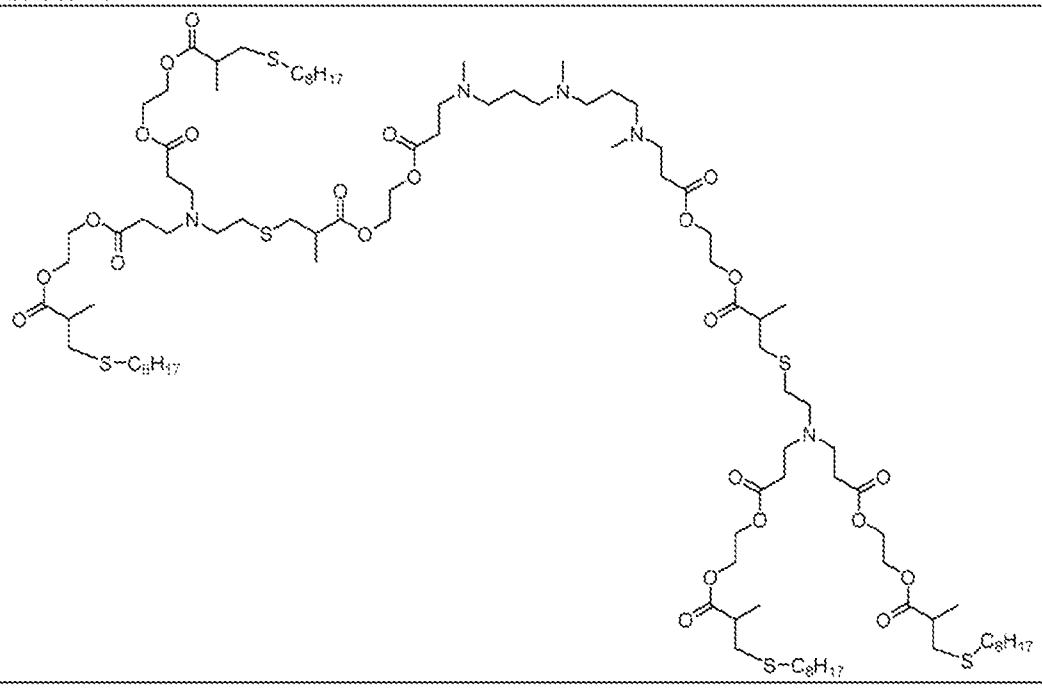
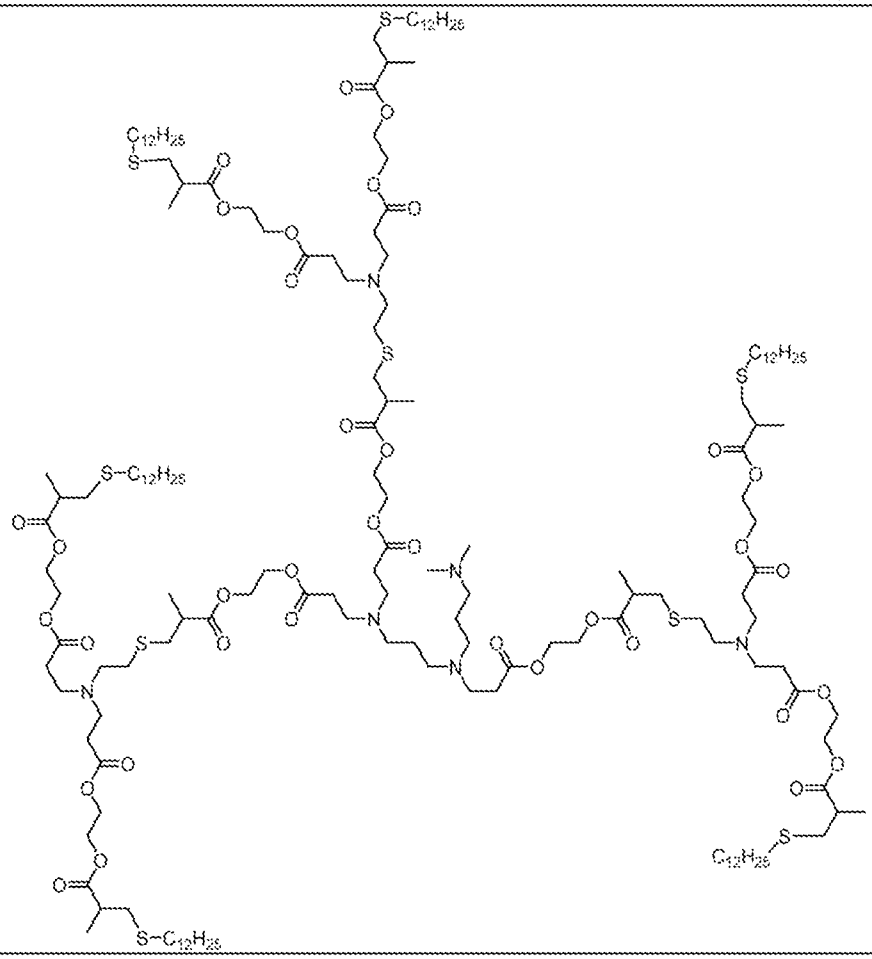
ID #	Structure
5A5-SC8	
5A5-SC12	

ID #	Structure
5A2-4-SC12 (6-arm)	
5A2-4-SC10 (6-arm)	

ID #	Structure
5A3-2-SC8 (6-arm)	
5A3-2-SC12 (6-arm)	
5A4-2-SC8 (6-arm)	

ID #	Structure
5A4-2-SC12 (6-amn)	
6A4-SC8	
6A4-SC12	

ID #	Structure
2A2-g2-SC12	
2A2-g2-SC8	
2A11-g2-SC12	

ID #	Structure
2A11-g2-SC8	
3A3-g2-SC12	

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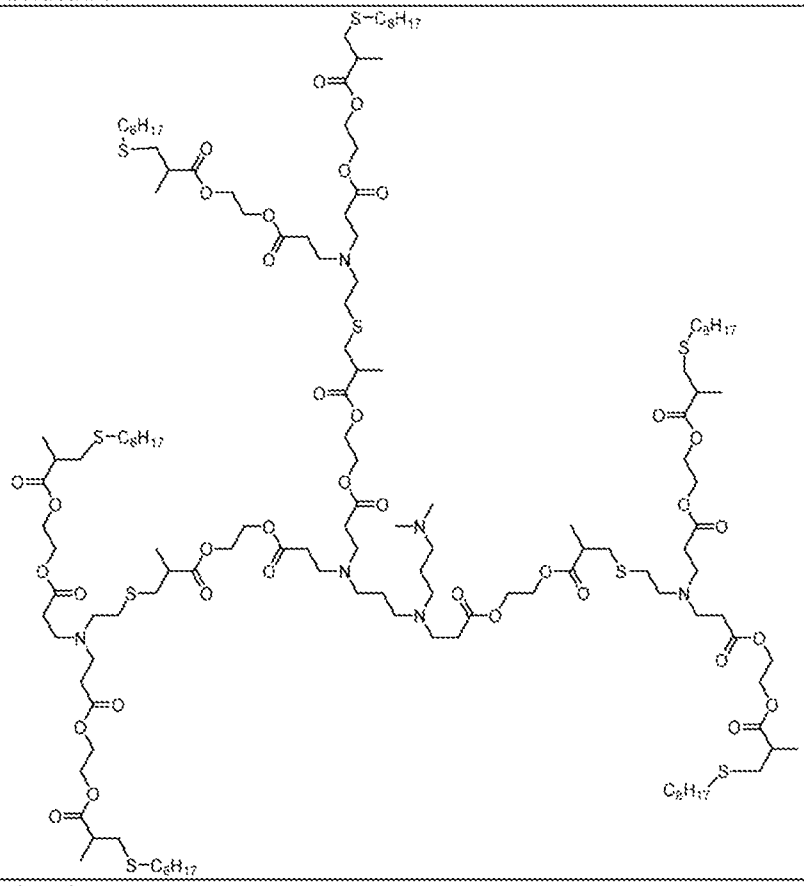
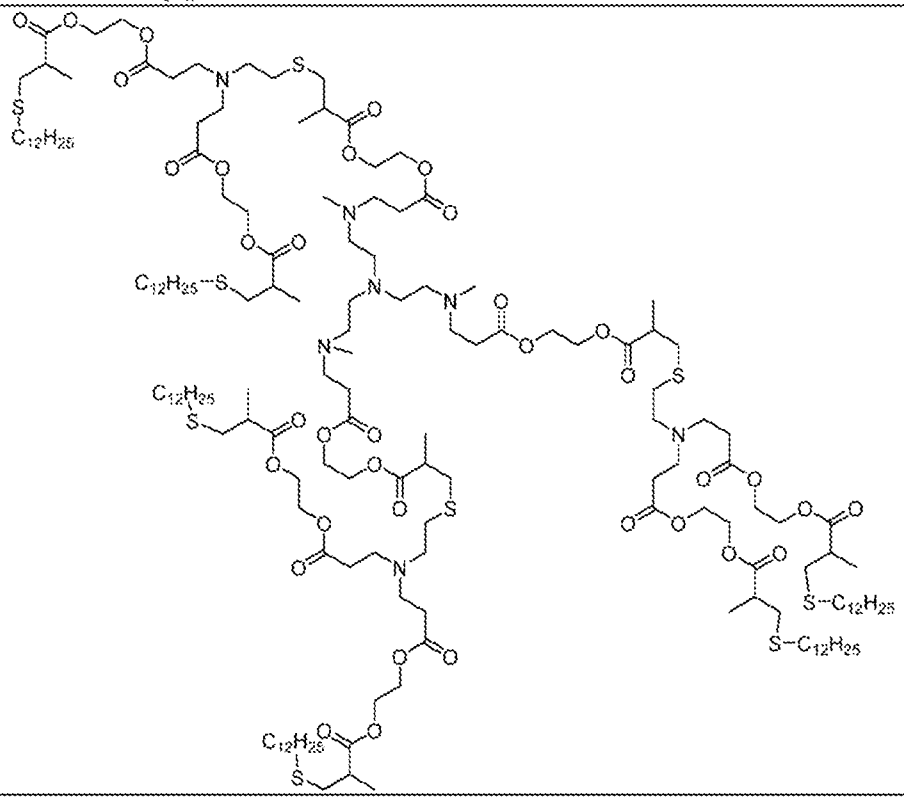
3A3-g2-SC8	
3A5-g2-SC12	

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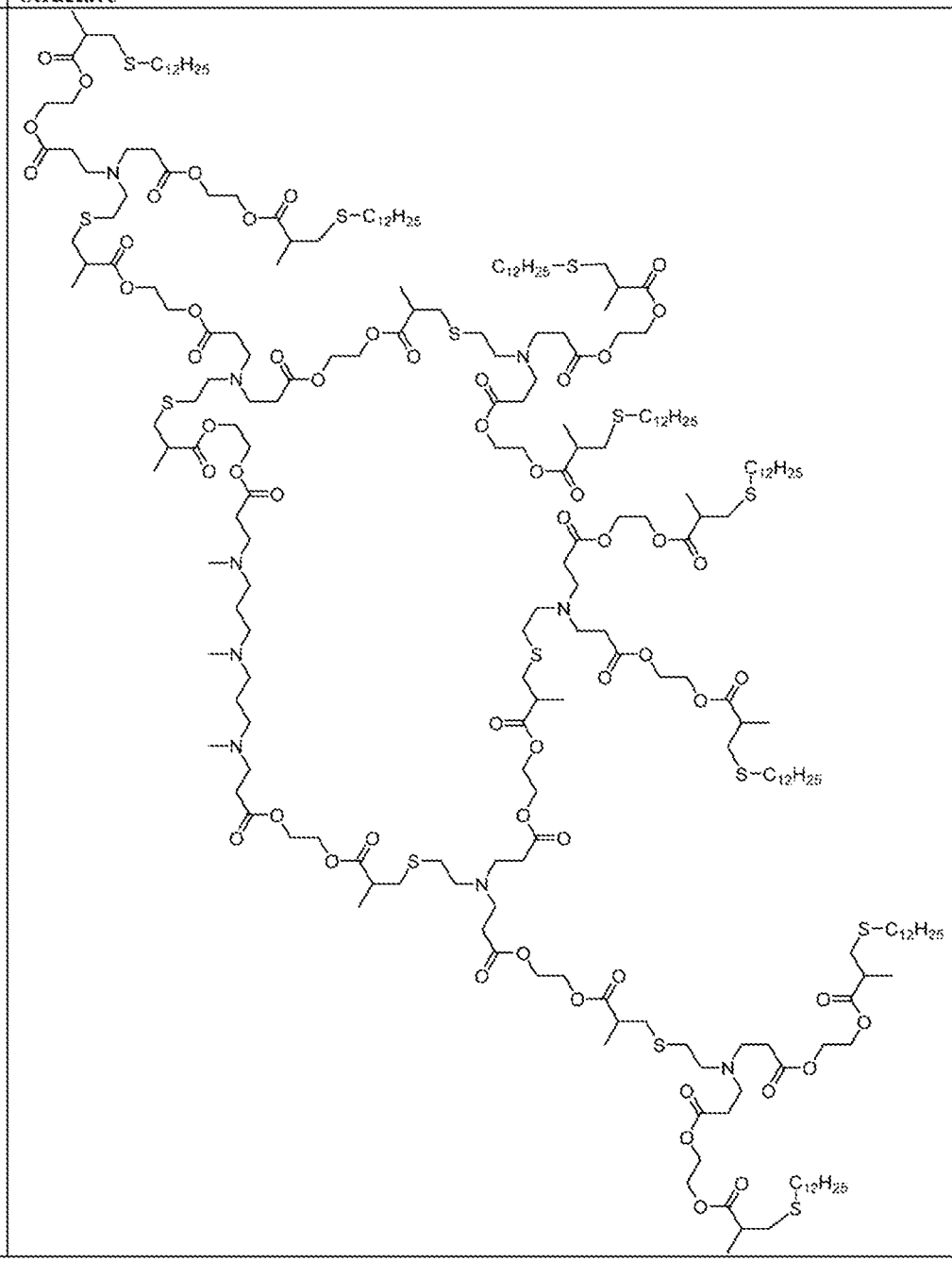
2A11-g3-SC12	

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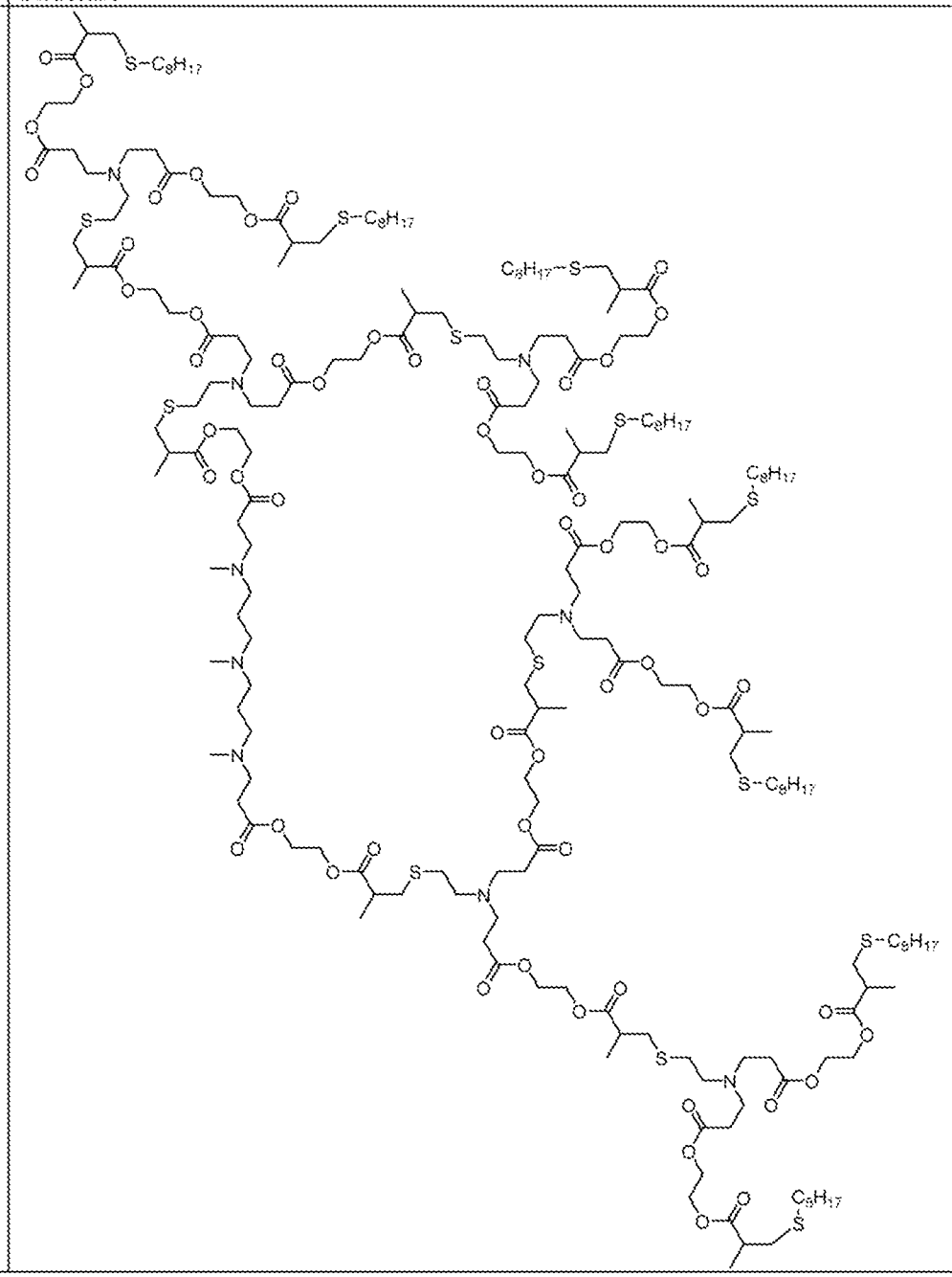
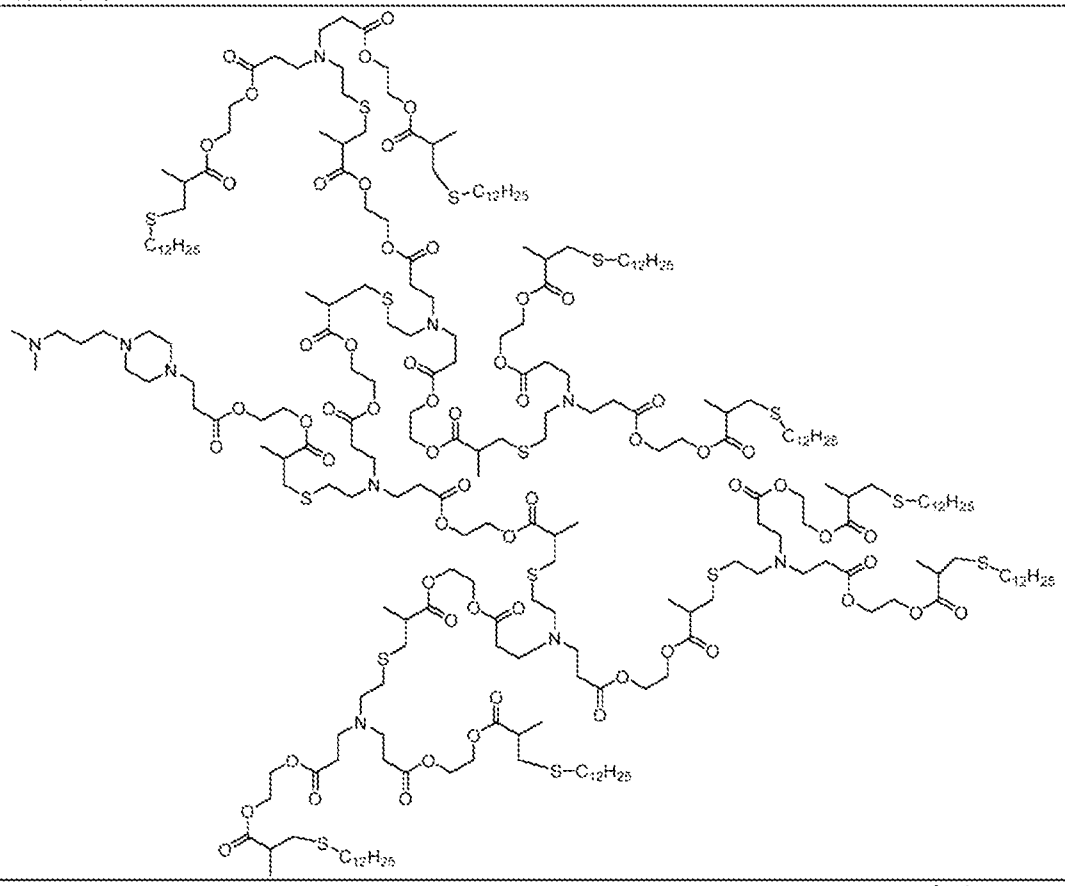
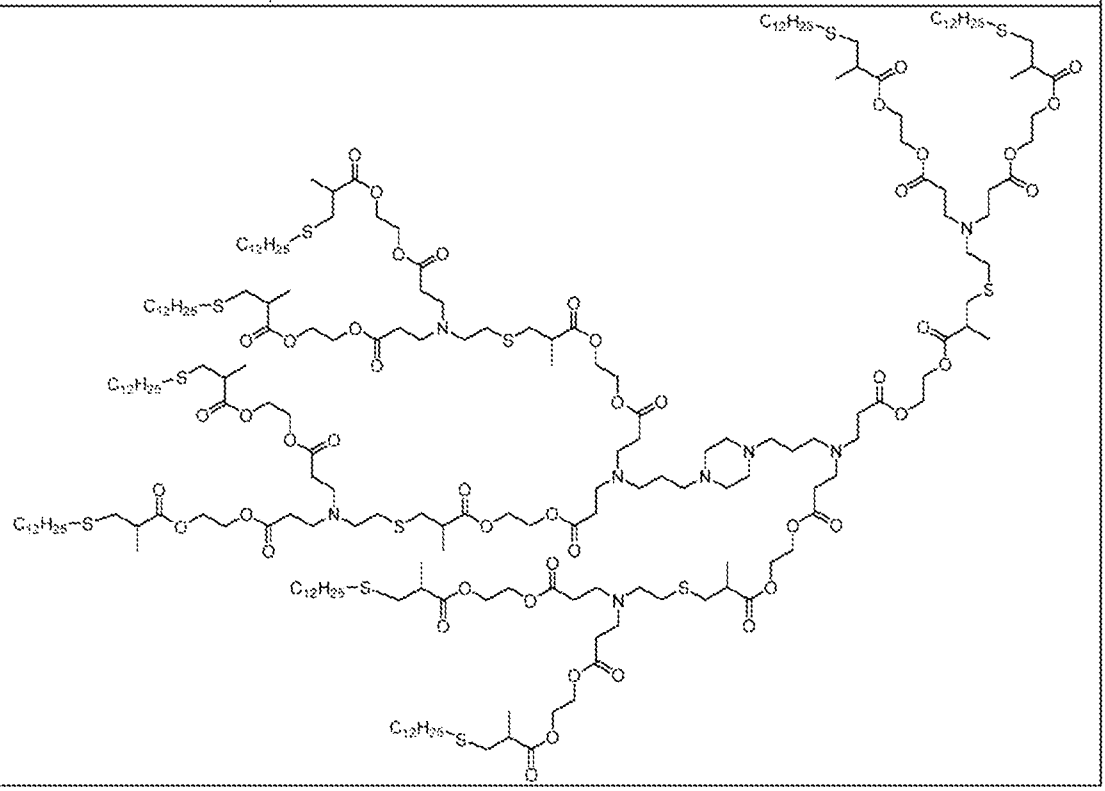
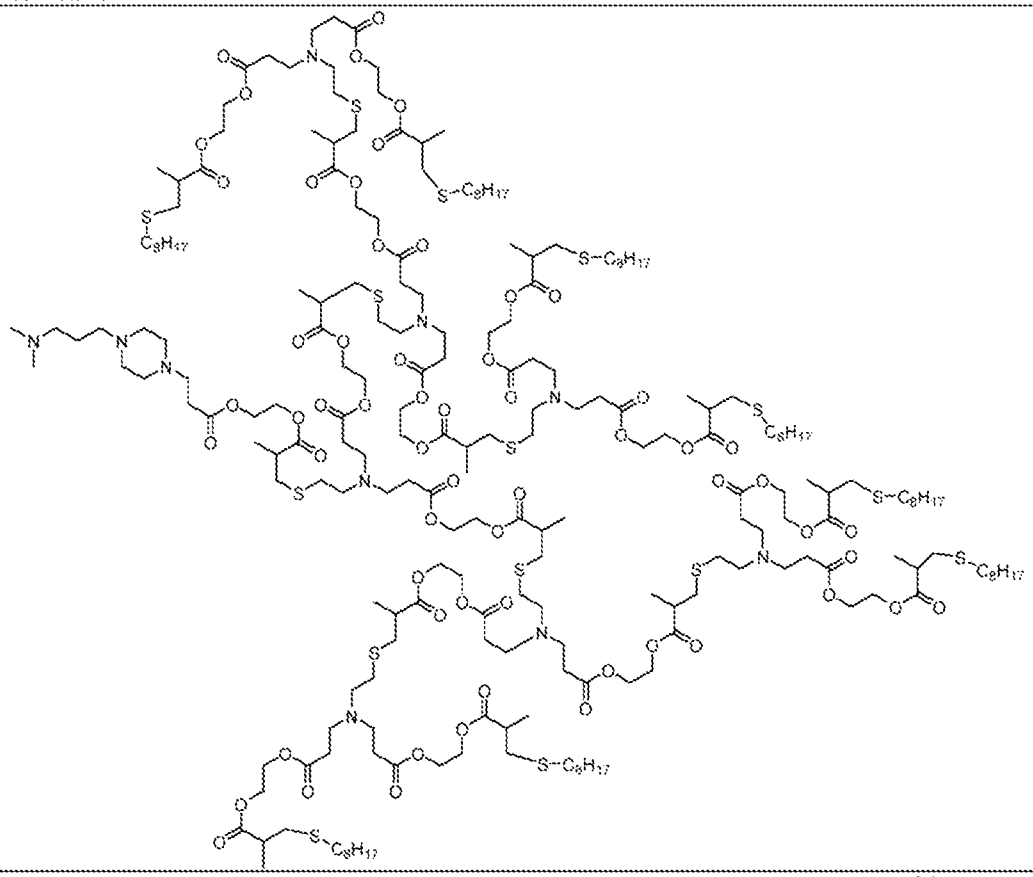
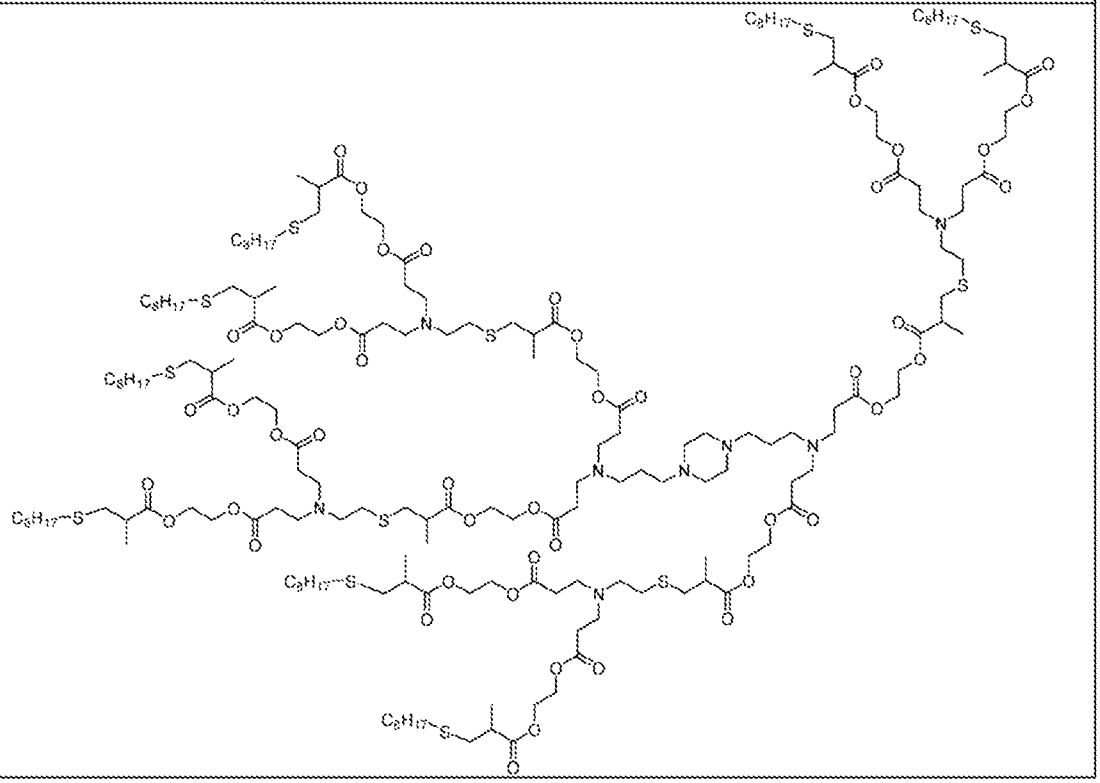
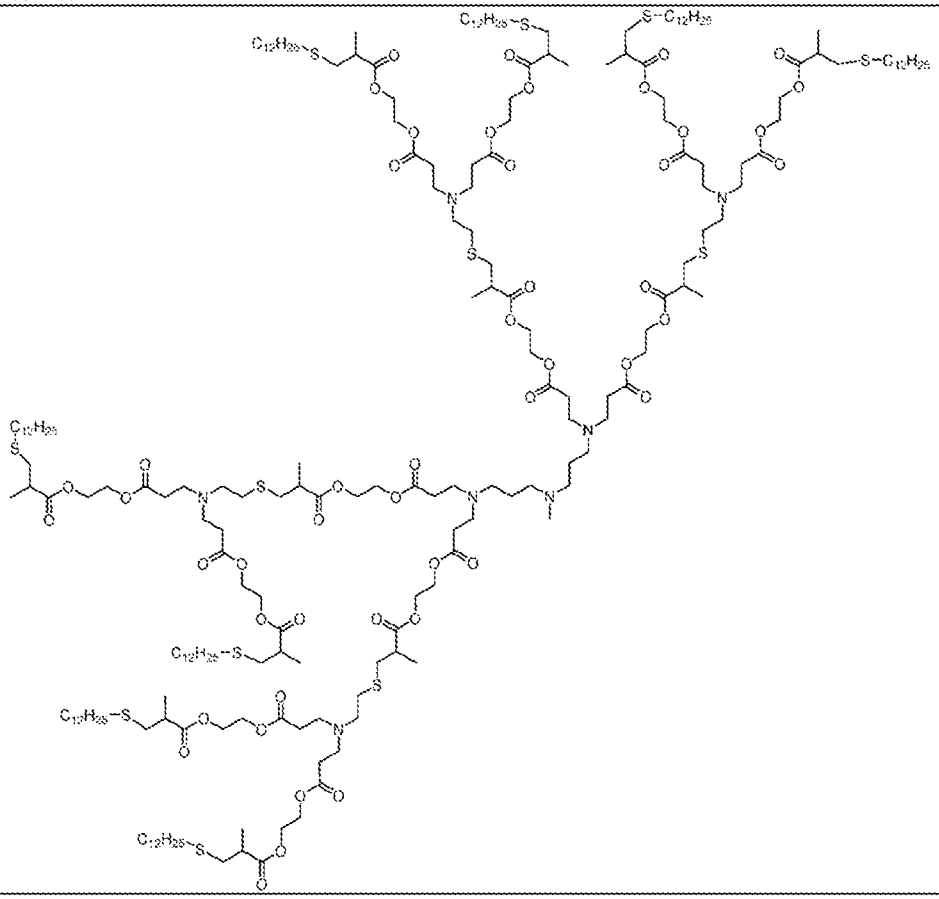
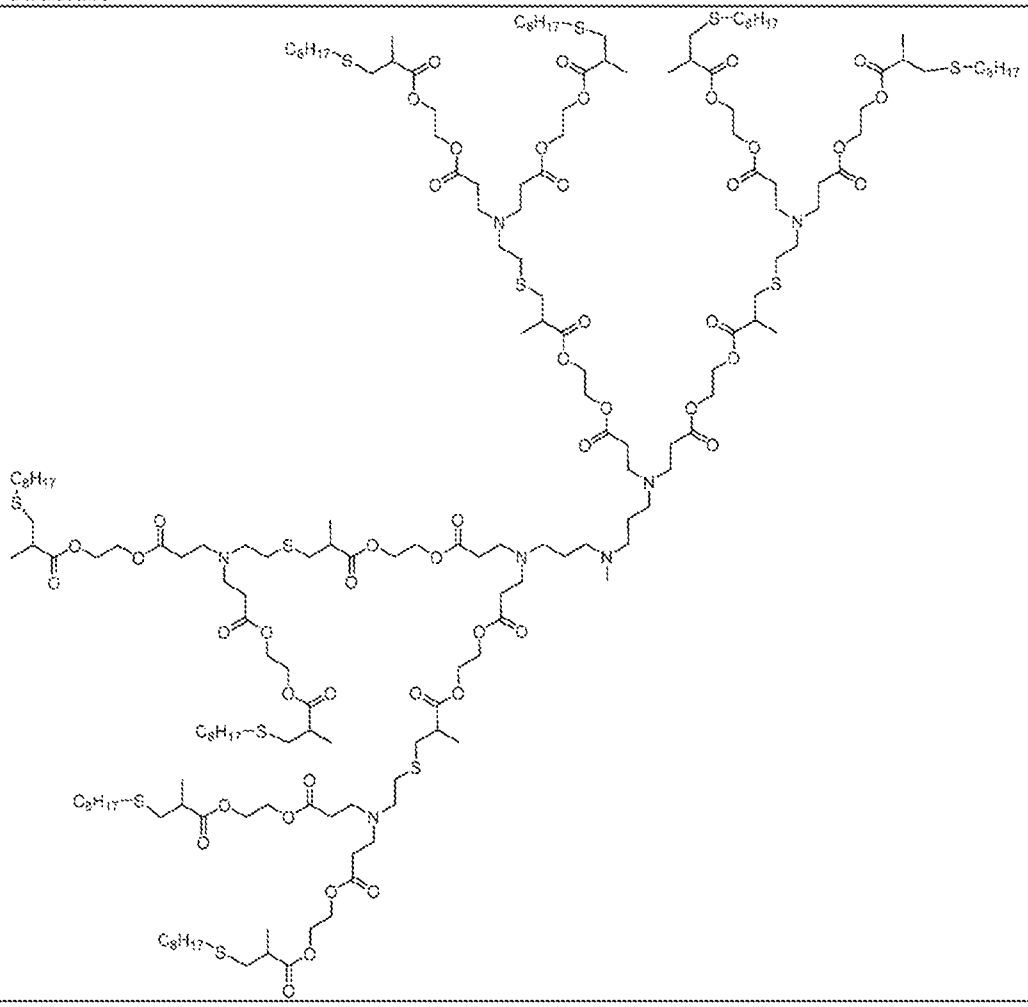
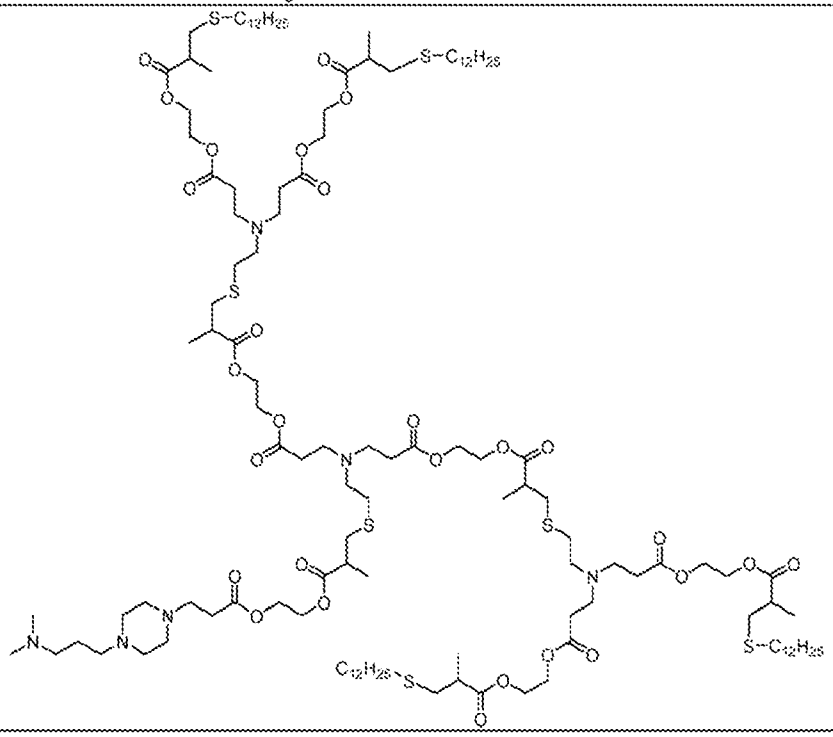
2A11-g3-SC8	

ID #	Structure
1A2-g4-SC12	
4A1-g2-SC12	

ID #	Structure
1A2-g4-SC8	
4A1-g2-SC8	

ID #	Structure
4A3-g2-SC12	

ID #	Structure
4A3-g2-SC8	
1A2-g3-SC12	

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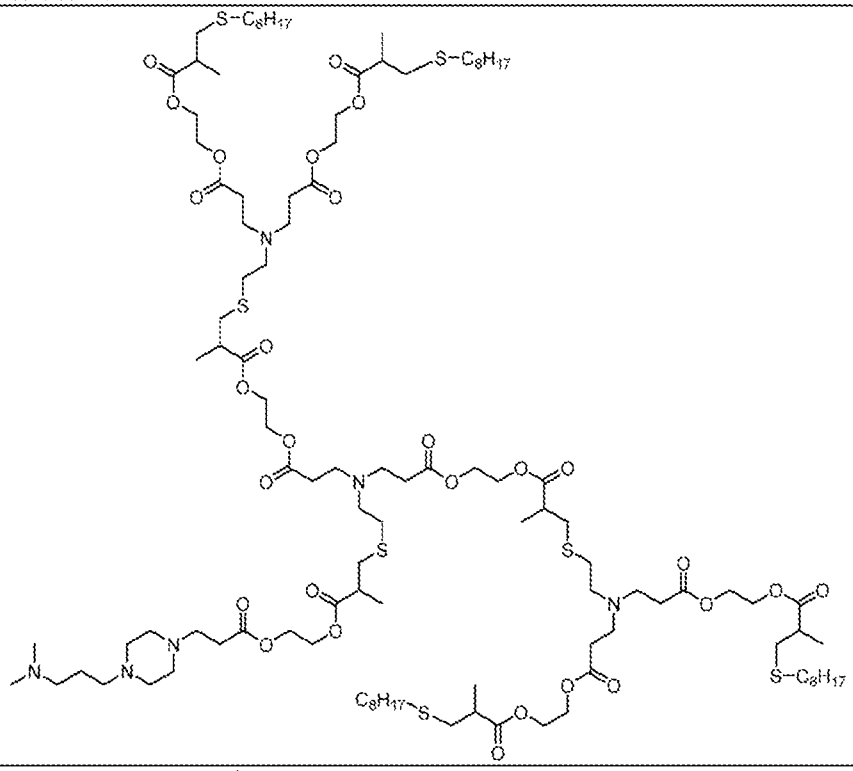
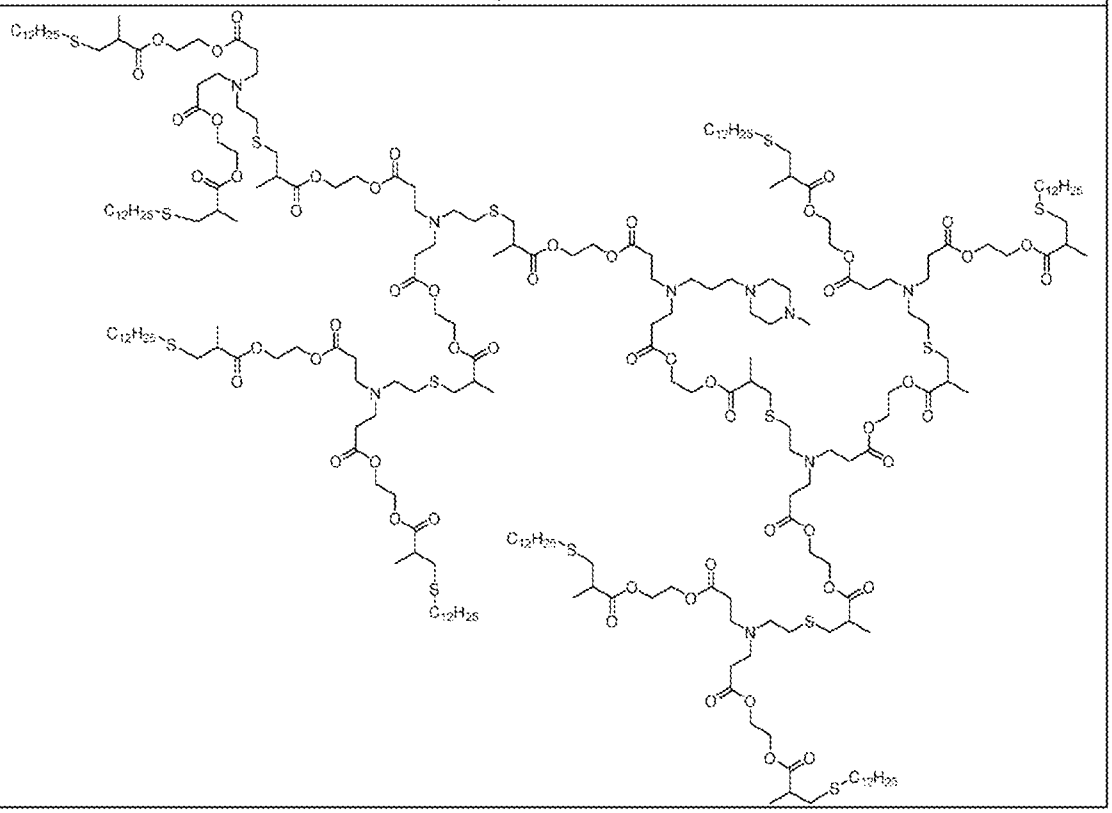
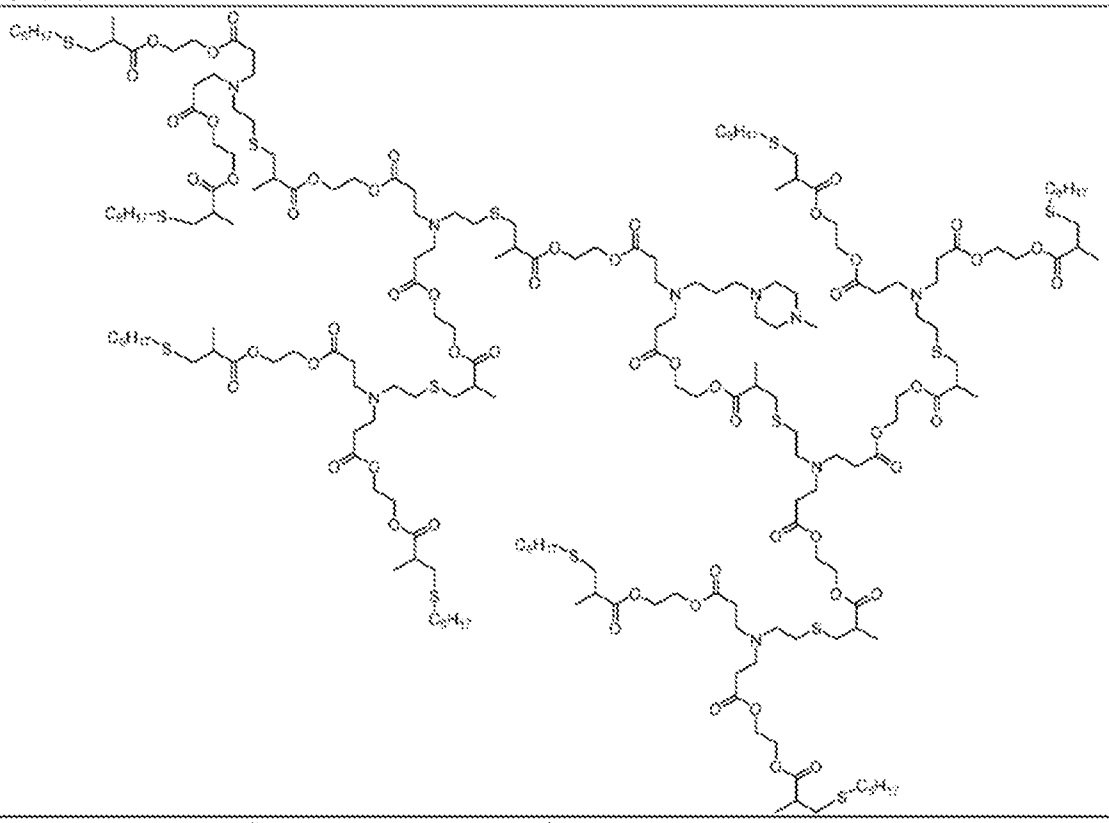
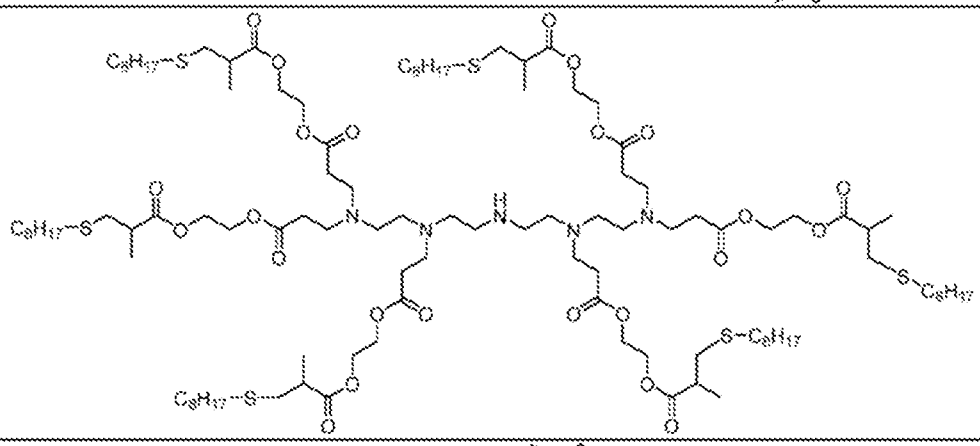
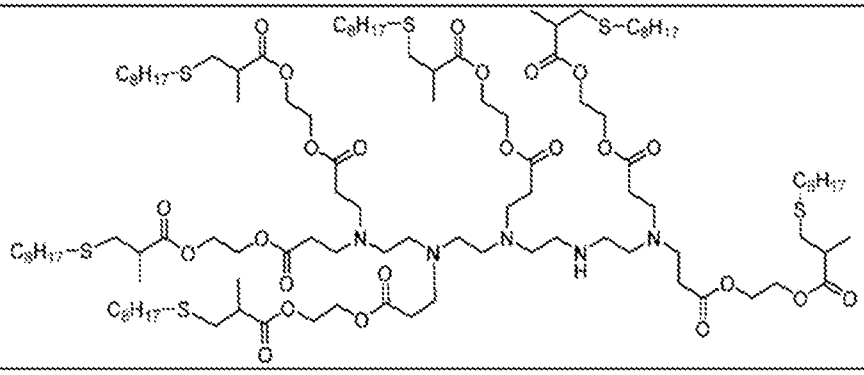
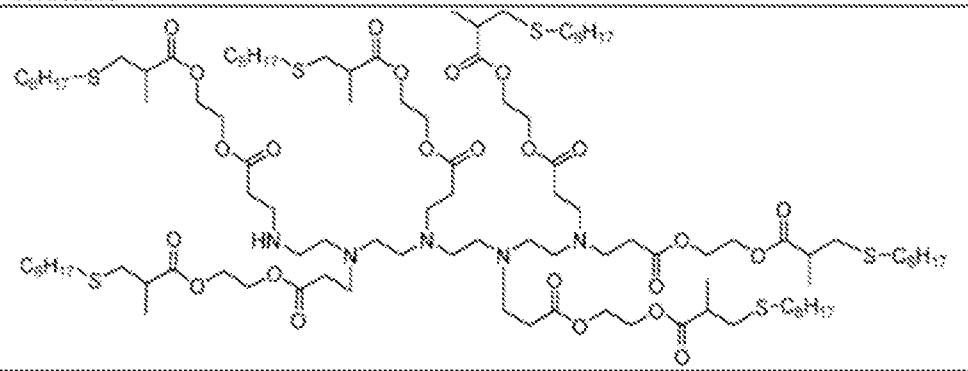
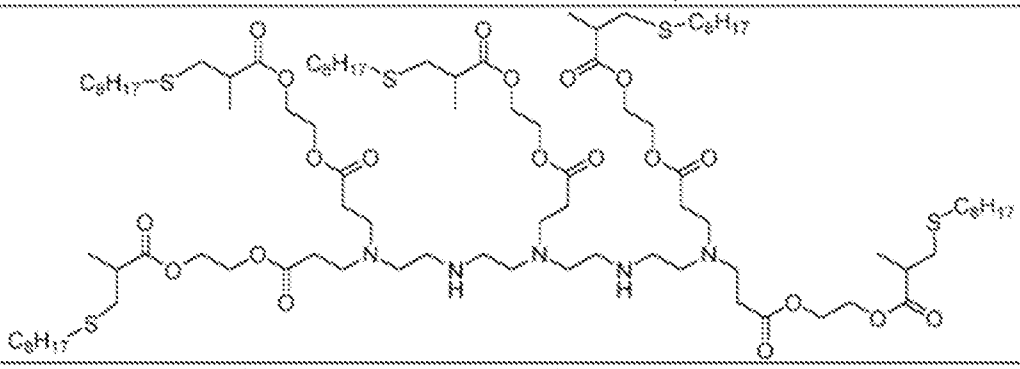
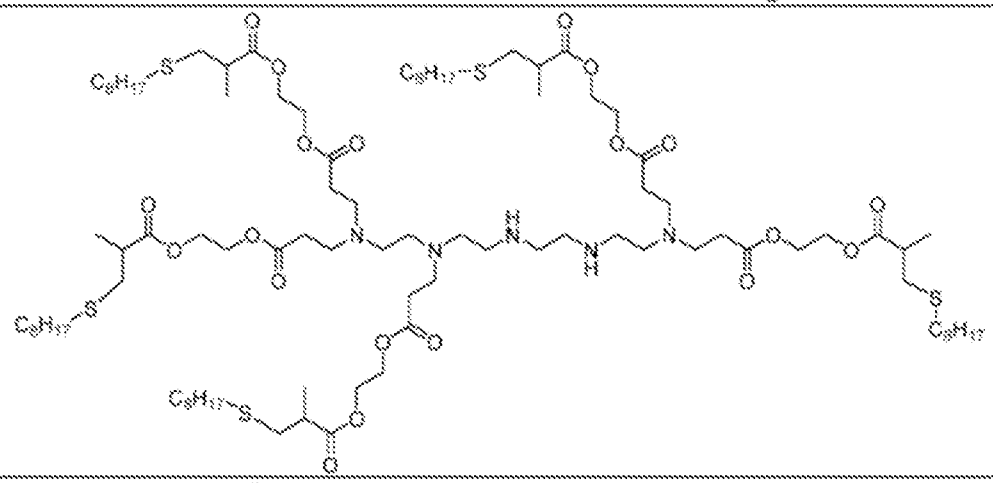
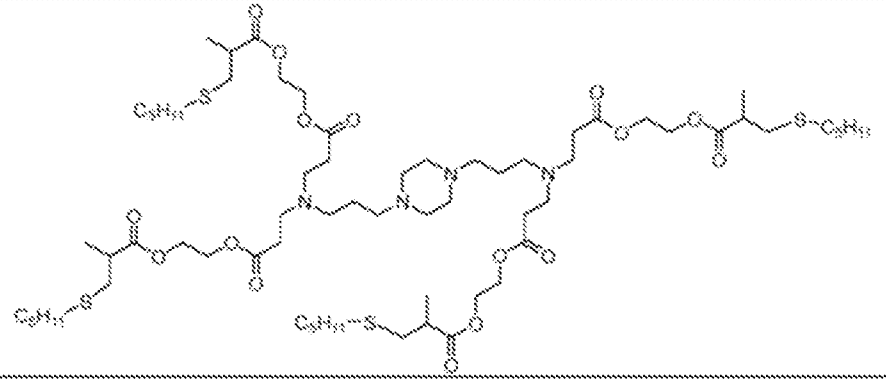
1A2-g3-SC8	
2A2-g3-SC12	

ID #	Structure
2A2-g3-SC8	
5A2-4-SC8 (6-arm)	
5A-5-SC8 (6 arm)	

ID #	Structure
5A2-6-SC8 (6 arm)	
5A2-1-SC8 (5-arm)	
5A2-2-SC8	
4A1-SC5	

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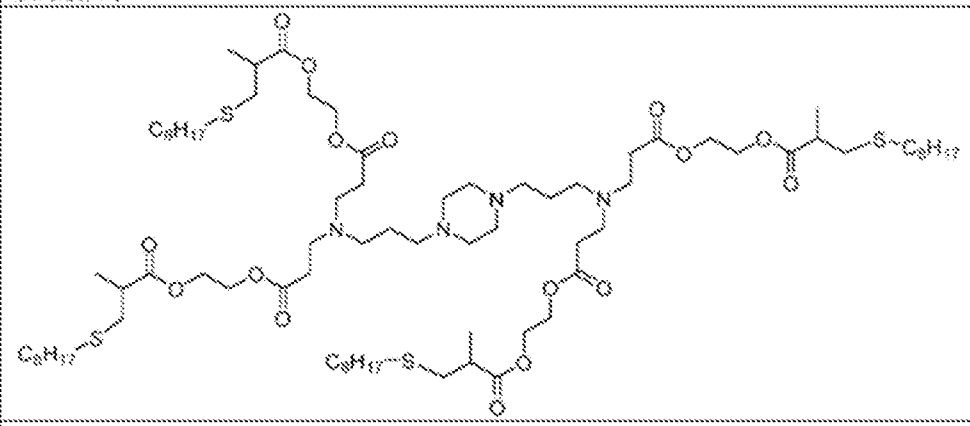
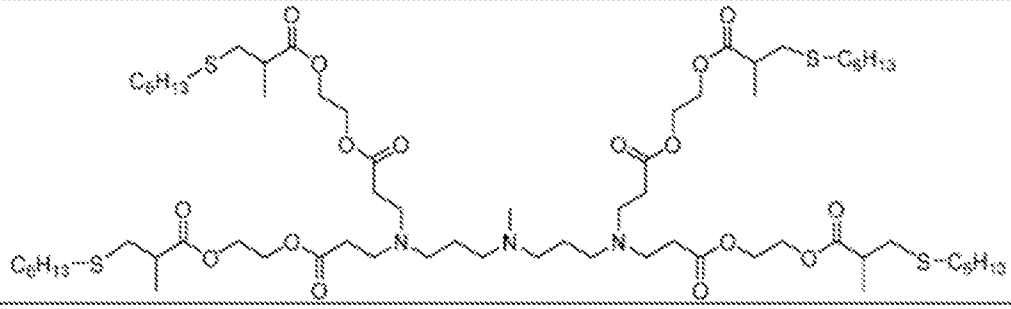
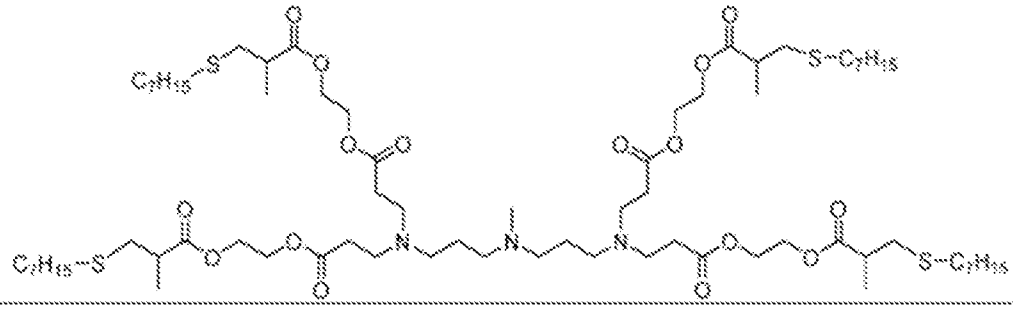
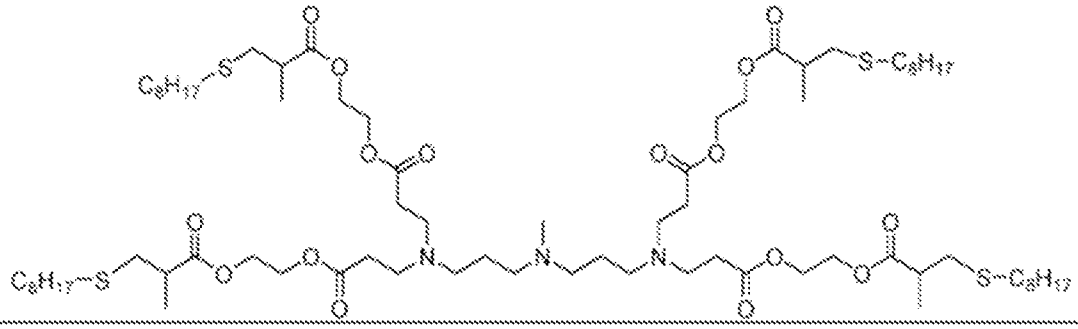
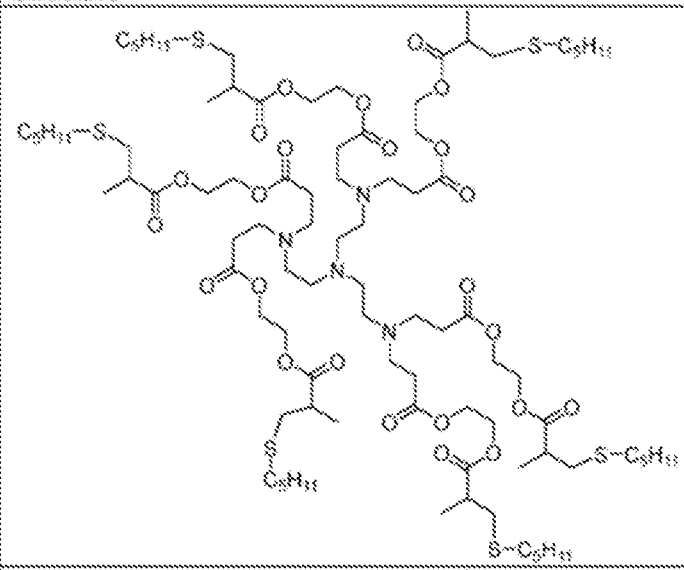
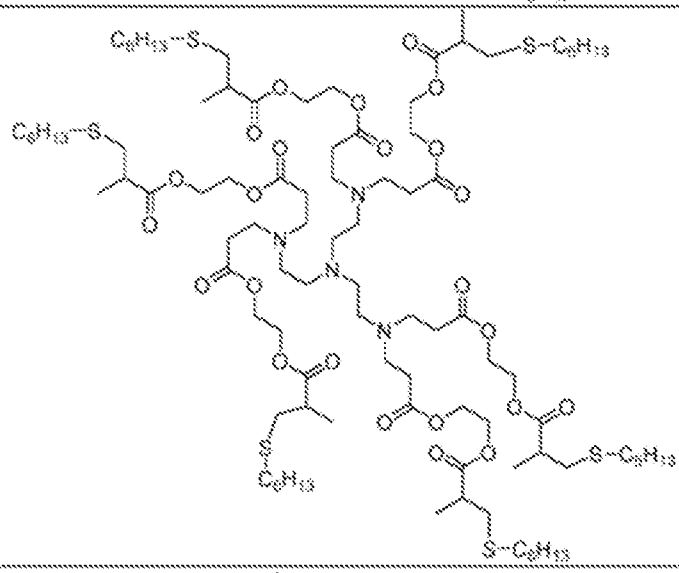
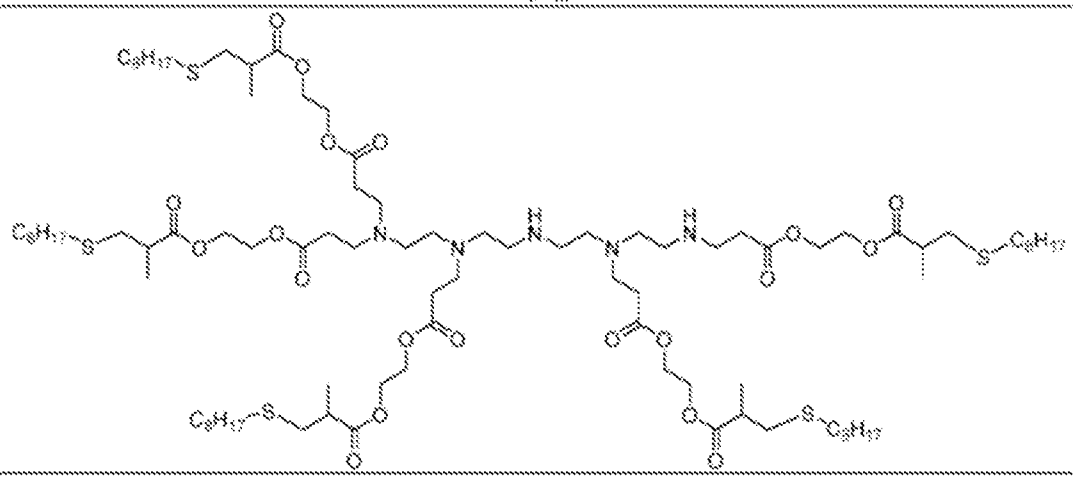
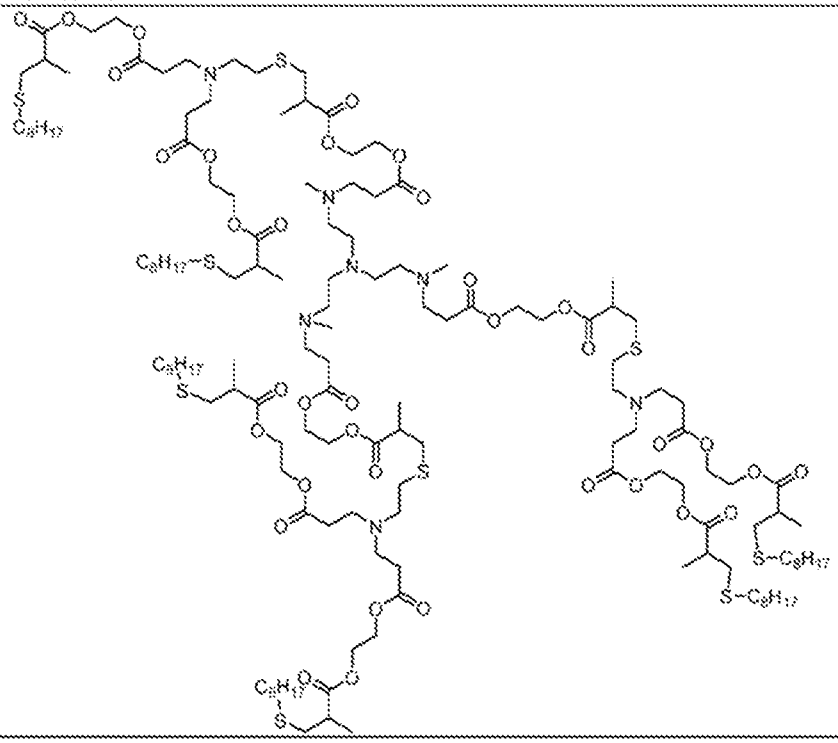
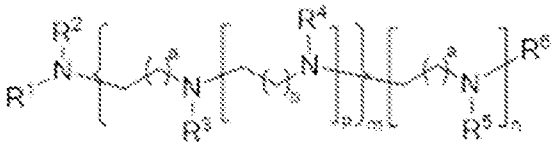
4A1-SC8	
4A3-SC6	
4A3-SC7	
4A3-SC8	

ID #	Structure
5A4-2-SC5 (6 arm)	
5A4-2-SC6 (6 arm)	
5A2-4-SC8 (5-arm)	

ID #	Structure
3A5-g2-SC8	

Other Ionizable lipids

[0204] In some embodiments of the lipid composition, the cationic lipid comprises a structural formula (D-I'):

 (D-I'),

wherein:

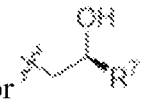
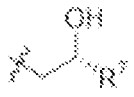
a is 1 and b is 2, 3, or 4; or, alternatively, b is 1 and a is 2, 3, or 4;

m is 1 and n is 1; or, alternatively, m is 2 and n is 0; or, alternatively, m is 2 and n is 1; and

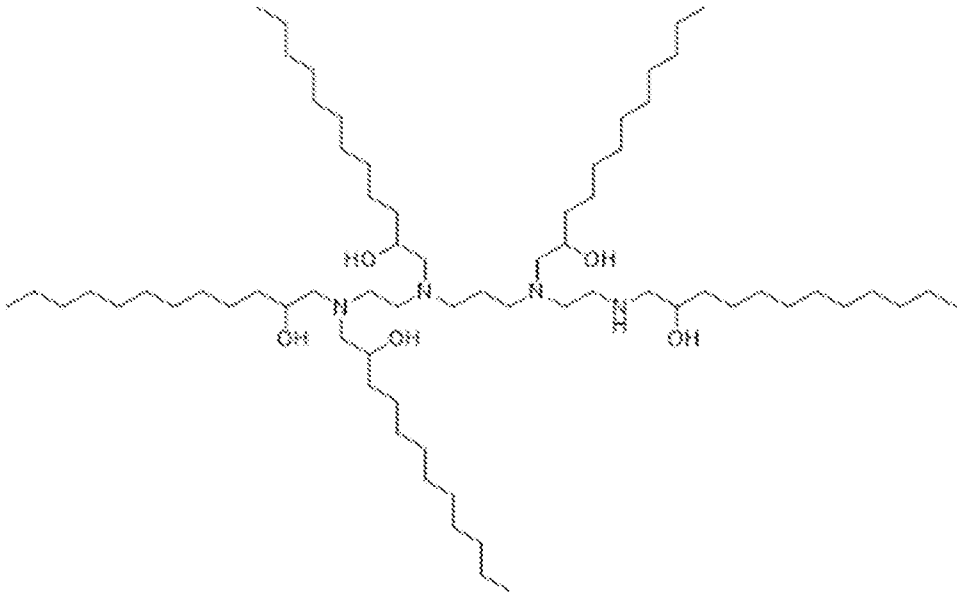
R^1, R^2, R^3, R^4, R^5, and R^6 are each independently selected from the group consisting of H, -$CH_2CH(OH)R^7$, -$CH(R^7)CH_2OH$, -$CH_2CH_2C(=O)OR^7$, -$CH_2CH_2C(=O)NHR^7$, and -CH_2R^7, wherein R^7 is independently selected from C_3-C_{18} alkyl, C_3-C_{18} alkenyl having one C=C double bond, a protecting group for an amino group, -$C(=NH)NH_2$, a poly(ethylene glycol) chain, and a receptor ligand;

provided that at least two moieties among R^1 to R^6 are independently selected from -$CH_2CH(OH)R^7$, -$CH(R^7)CH_2OH$, -$CH_2CH_2C(=O)OR^7$, -$CH_2CH_2C(=O)NHR^7$, or -CH_2R^7, wherein R^7 is independently selected from C_3-C_{18} alkyl or C_3-C_{18} alkenyl having one C=C

double bond; and wherein one or more of the nitrogen atoms indicated in formula (D-I') may be protonated to provide a cationic lipid.

[0205] In some embodiments of the cationic lipid of formula (D-I'), a is 1. In some embodiments of the cationic lipid of formula (D-I'), b is 2. In some embodiments of the cationic lipid of formula (D-I'), m is 1. In some embodiments of the cationic lipid of formula (D-I'), n is 1. In some embodiments of the cationic lipid of formula (D-I'), R^1, R^2, R^3, R^4, R^5, and R^6 are each independently H or $-CH_2CH(OH)R^7$. In some embodiments of the cationic lipid of formula (D-I'), R^1, R^2, R^3, R^4, R^5, and R^6 are each independently H or  . In some embodiments of the cationic lipid of formula (D-I'), R^1, R^2, R^3, R^4, R^5, and R^6 are each independently H or  . In some embodiments of the cationic lipid of formula (D-I'), R^7 is C_3-C_{18} alkyl (*e.g.*, C_6-C_{12} alkyl).

[0206] In some embodiments, the cationic lipid of formula (D-I') is 13,16,20-tris(2-hydroxydodecyl)-13,16,20,23-tetraazapentatricontane-11,25-diol:



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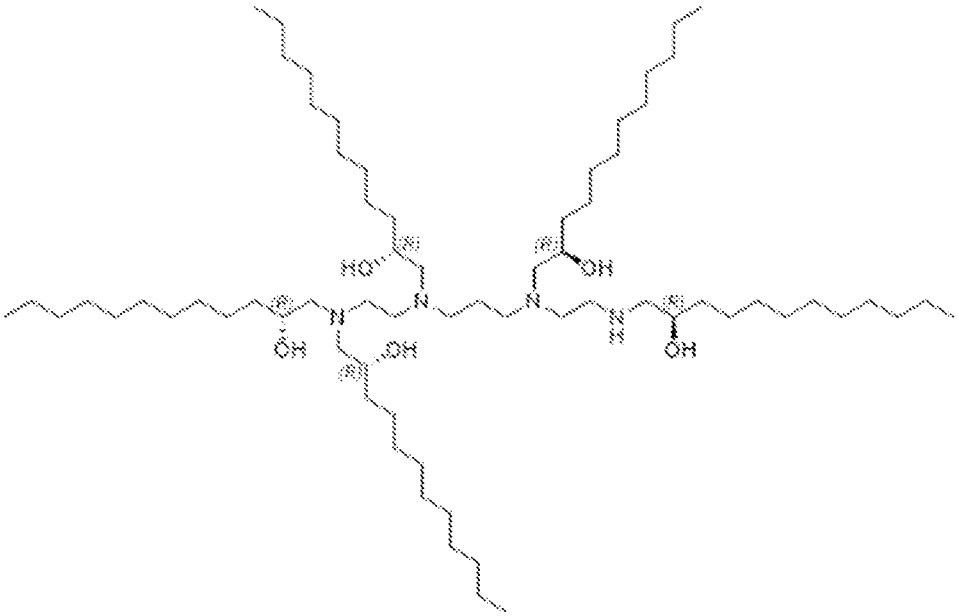
[0207] In some embodiments, the cationic lipid of formula (D-I') is (11*R*,25*R*)-13,16,20-tris((*R*)-2-hydroxydodecyl)-13,16,20,23-tetraazapentatricontane-11,25-diol:



[0208] Additional cationic lipids that can be used in the compositions and methods of the present application include those cationic lipids as described in J. McClellan, M. C. King, Cell 2010, 141, 210-217, and International Patent Publication WO 2010/144740, WO 2013/149140, WO 2016/118725, WO 2016/118724, WO 2013/063468, WO 2016/205691, WO 2015/184256, WO 2016/004202, WO 2015/199952, WO 2017/004143, WO 2017/075531, WO 2017/117528, WO 2017/049245, WO 2017/173054 and WO 2015/095340, which are incorporated herein by reference for all purposes. Examples of those ionizable cationic lipids include but are not limited to those as shown in **Table 5**.

Table 5. Example Ionizable Cationic Lipids

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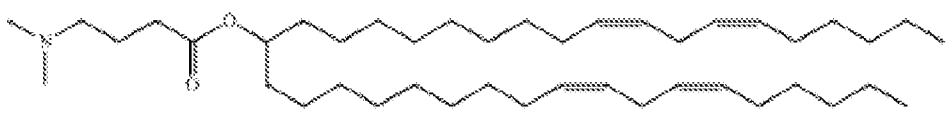
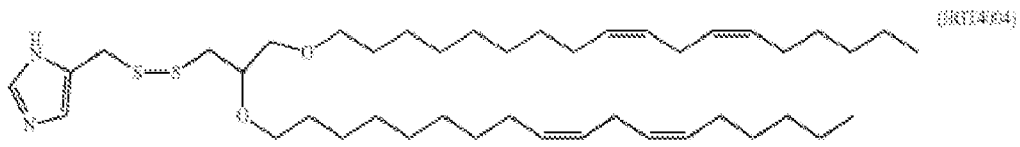
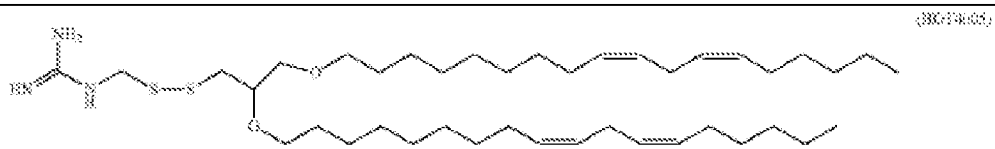
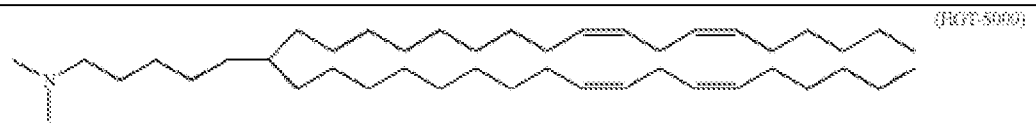
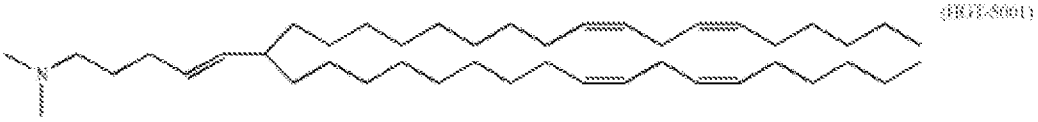
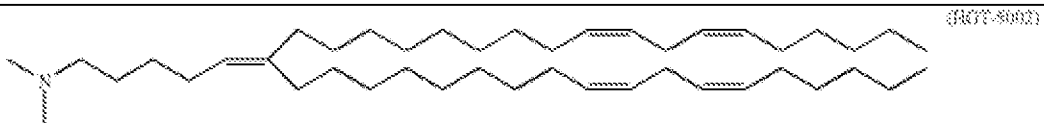
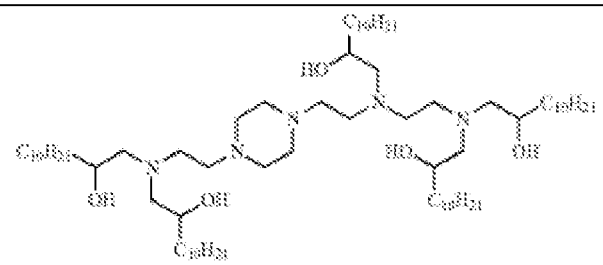
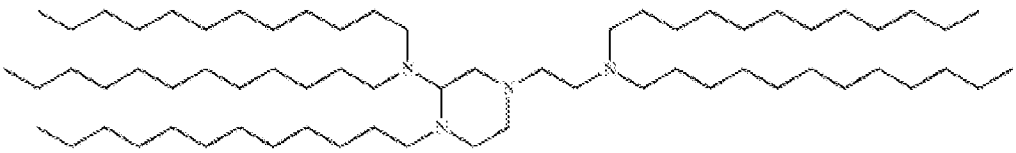
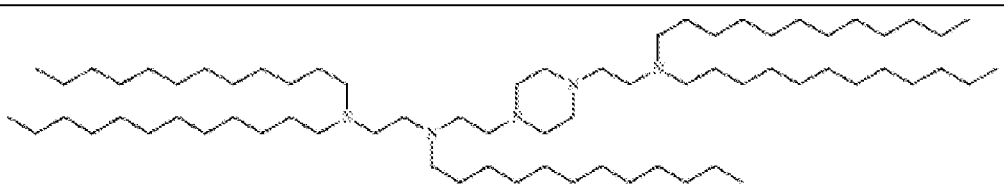
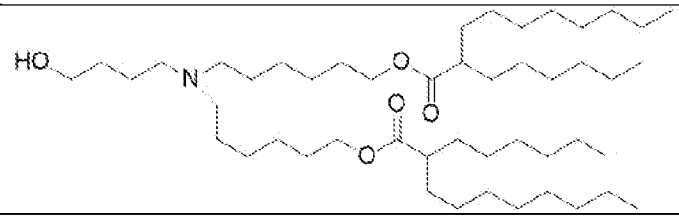
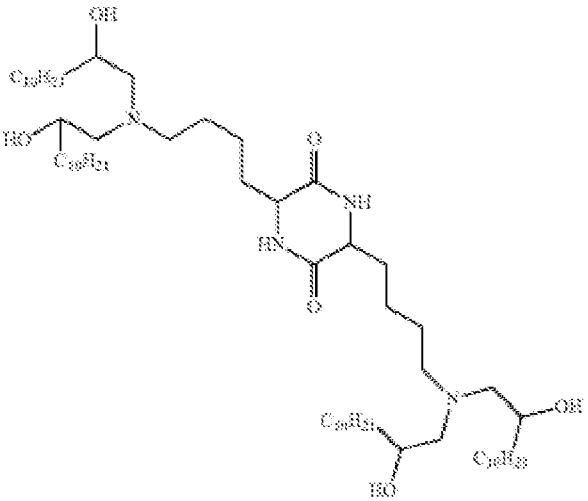
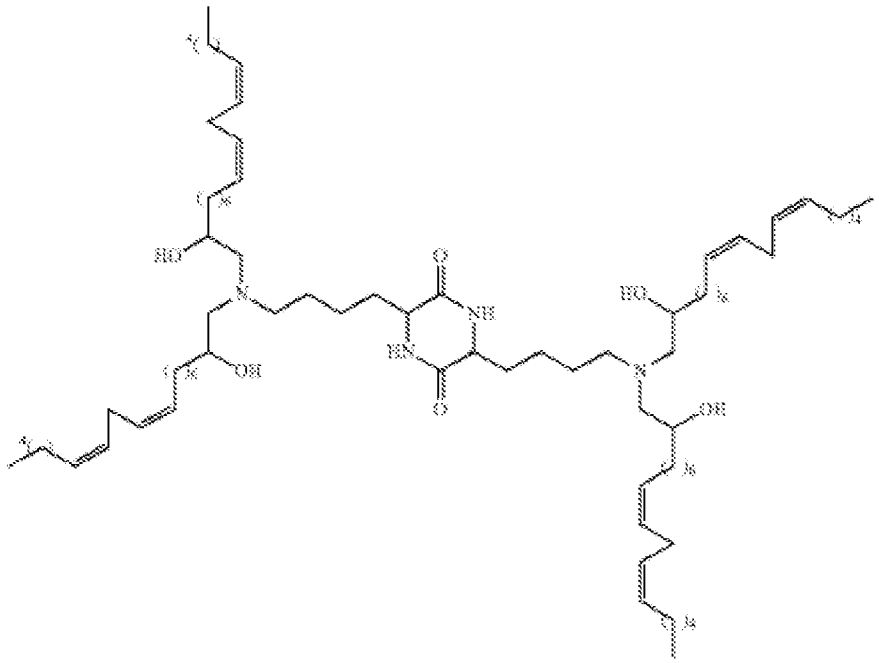
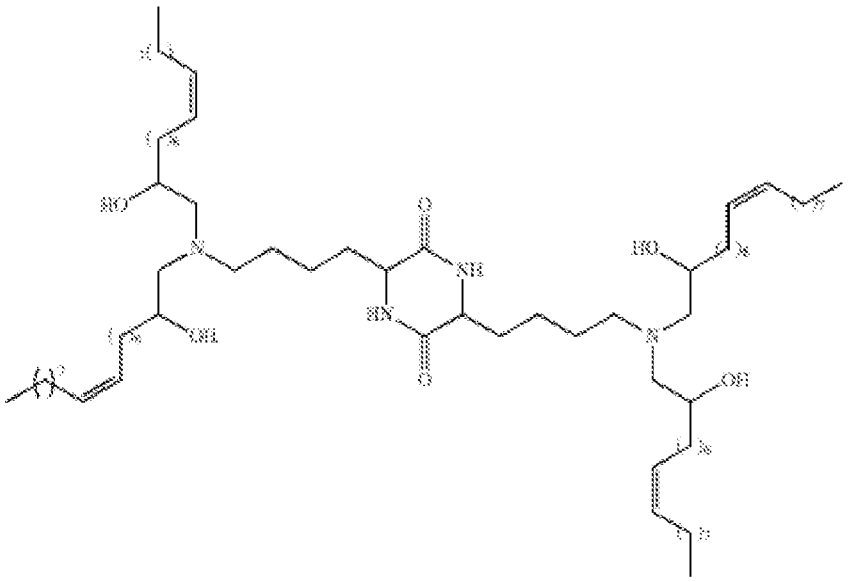
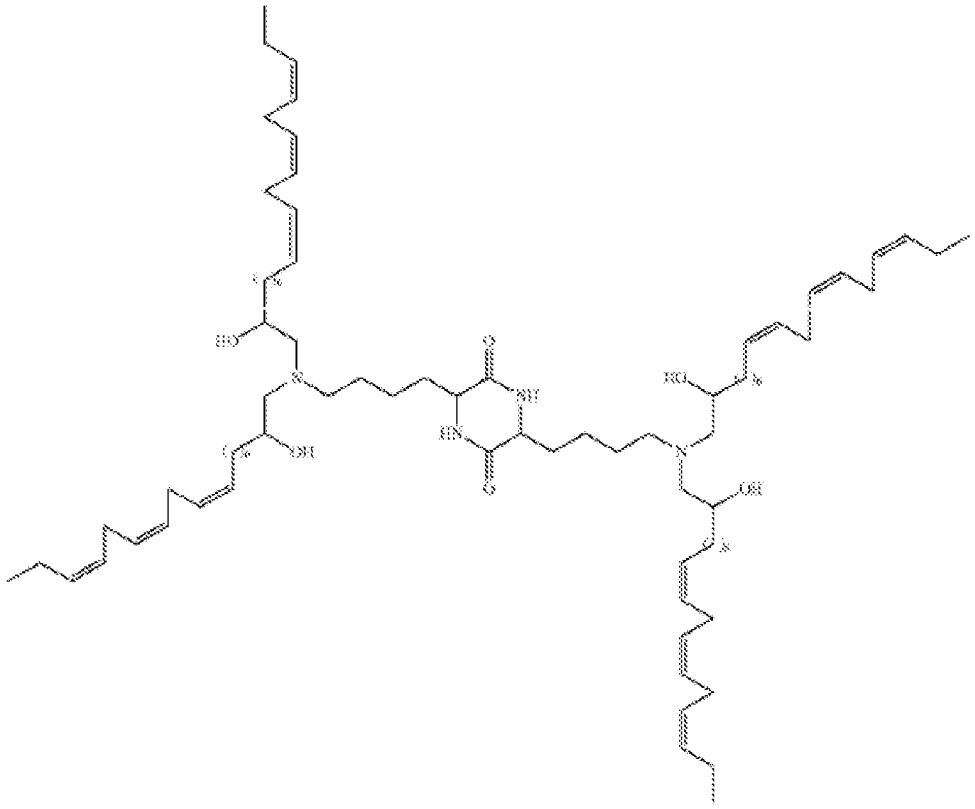
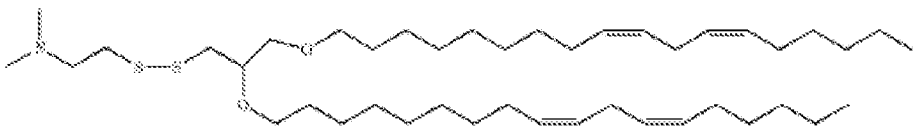
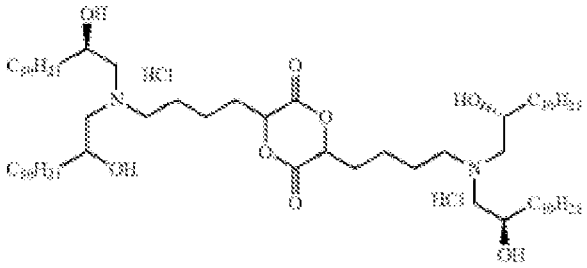
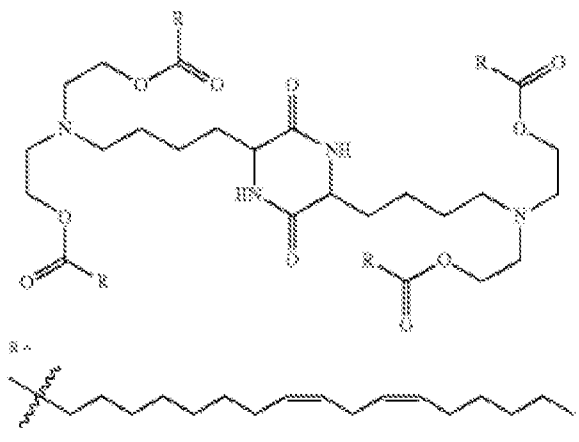
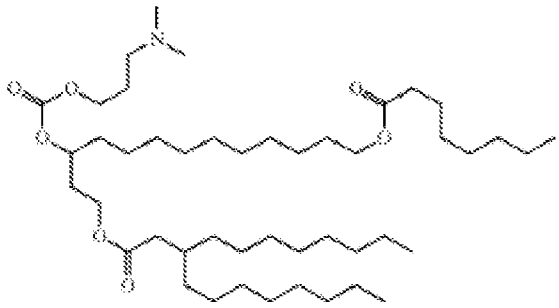
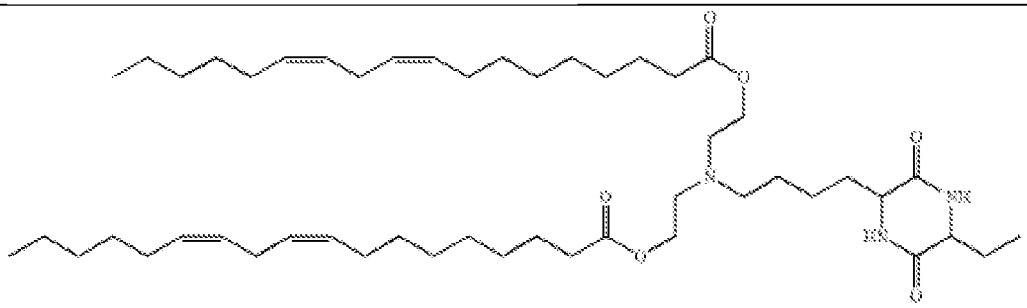
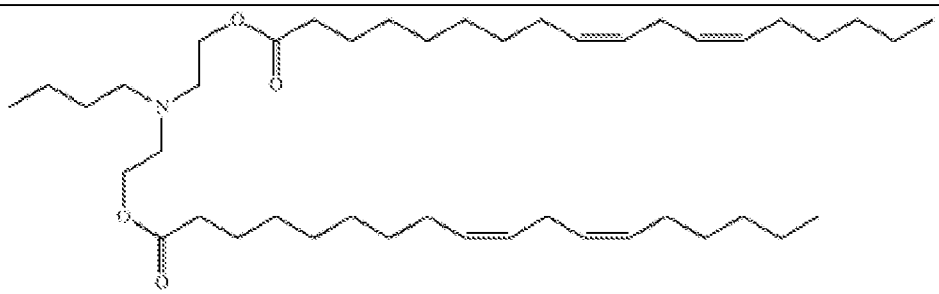
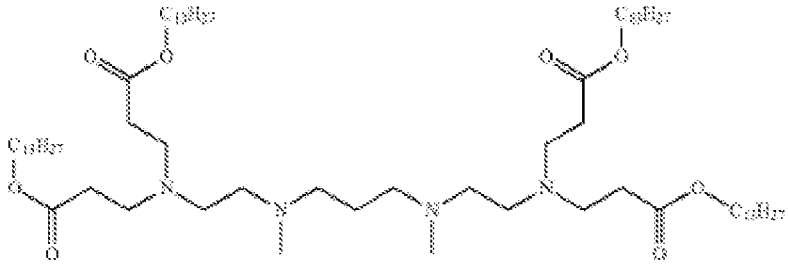
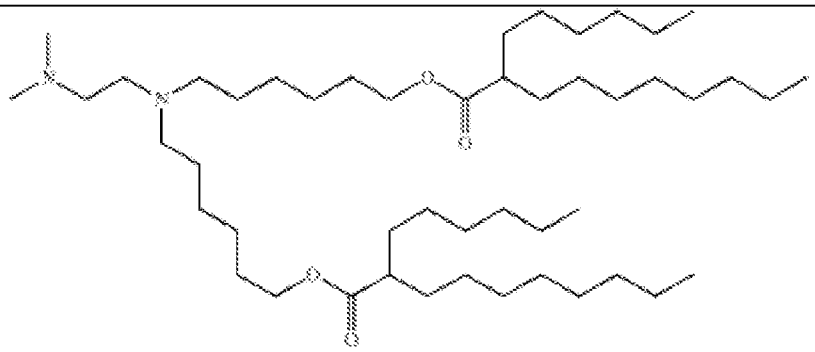
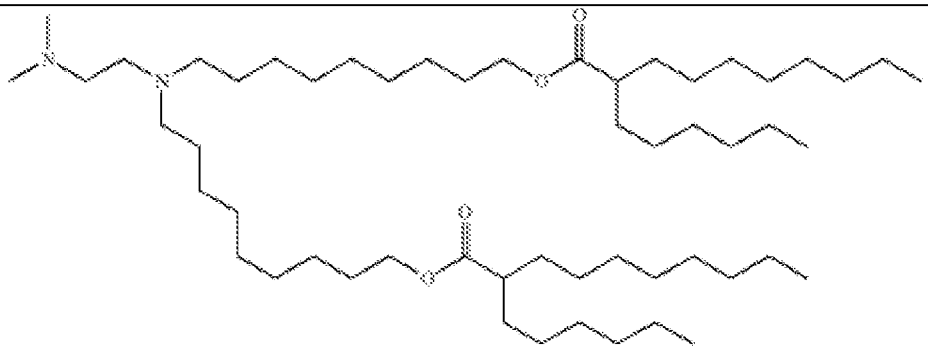
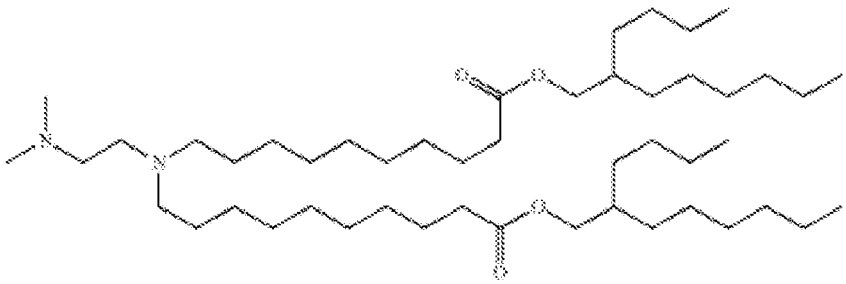
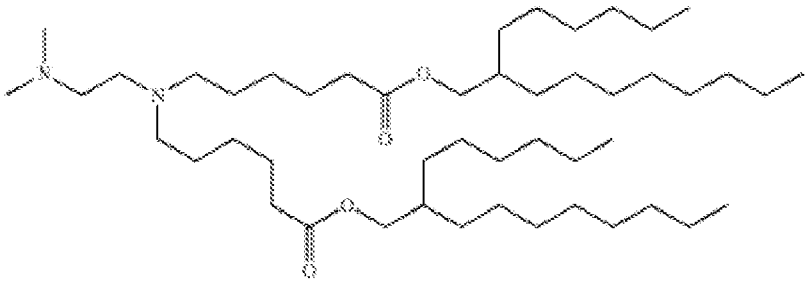
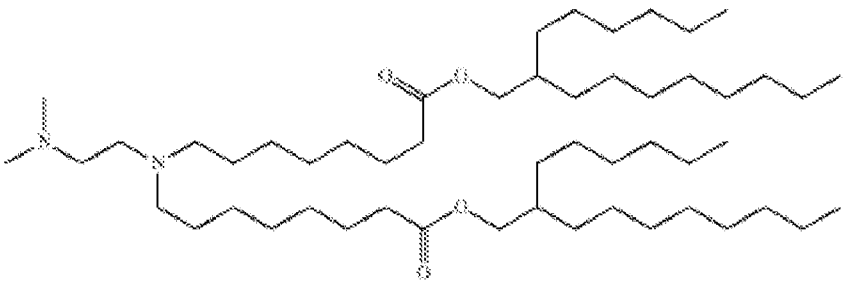
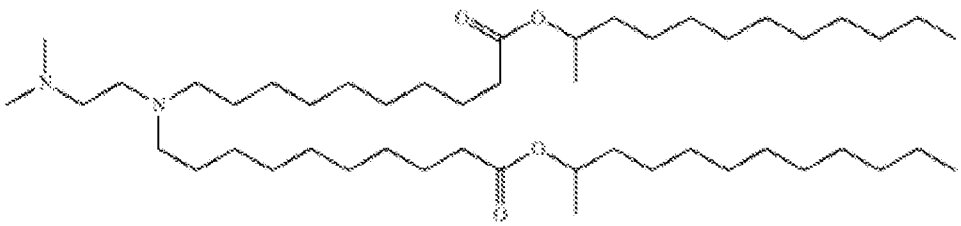
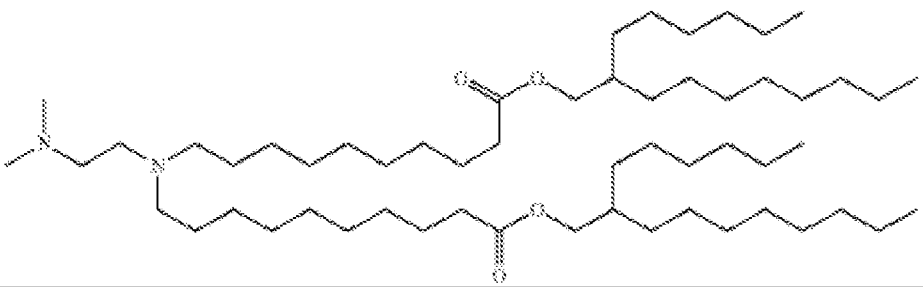
1	
2	
3	

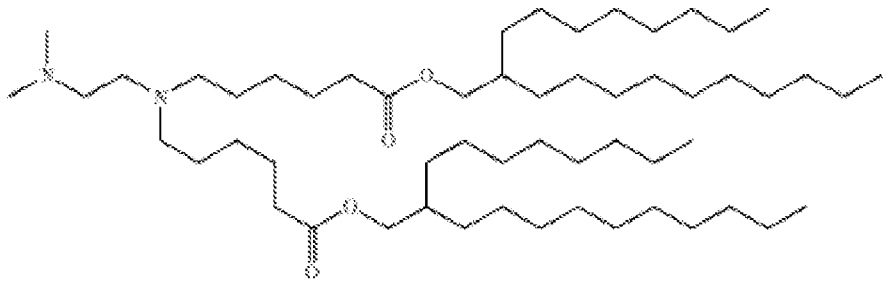
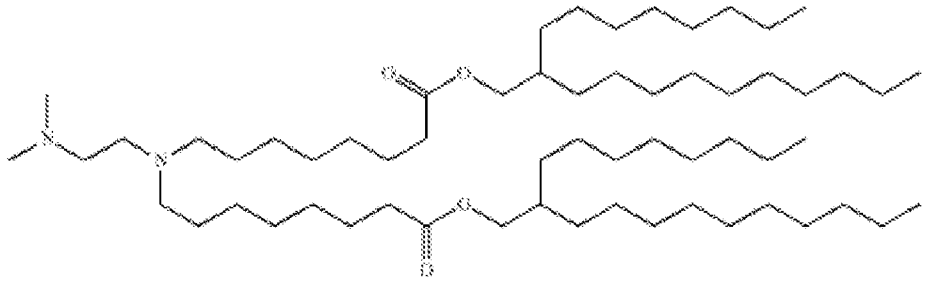
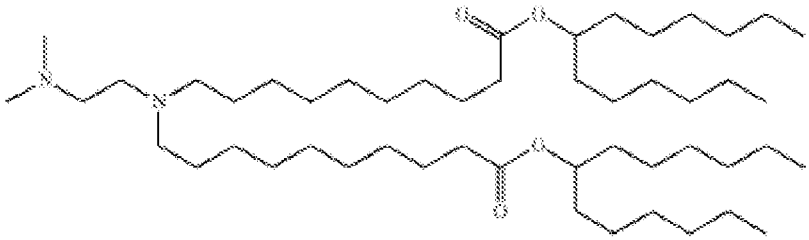
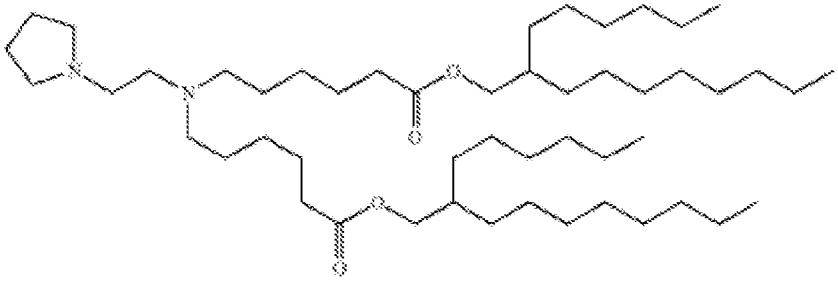
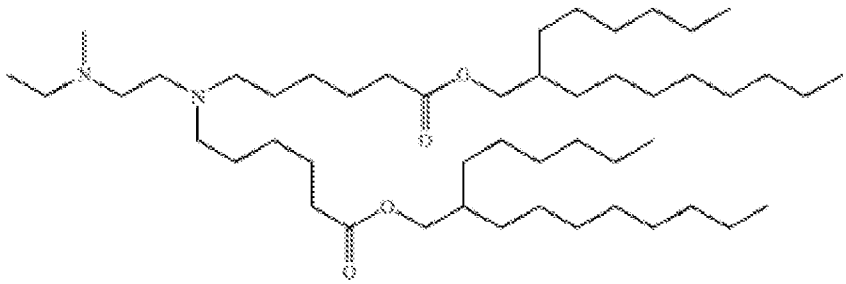
4	
5	
6	
7	
8	
9	
10	
11	

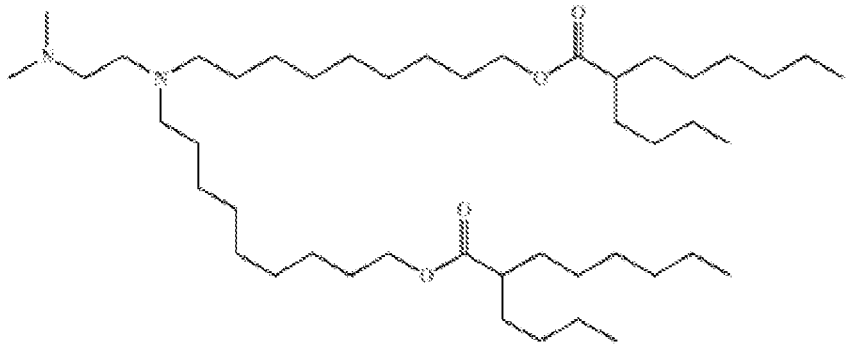
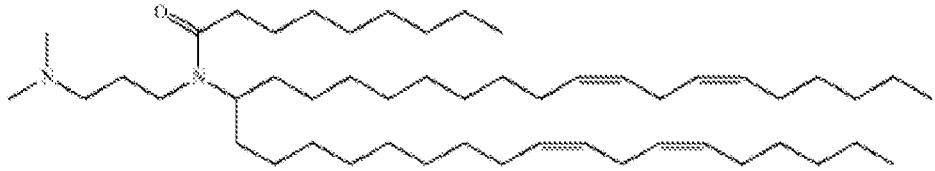
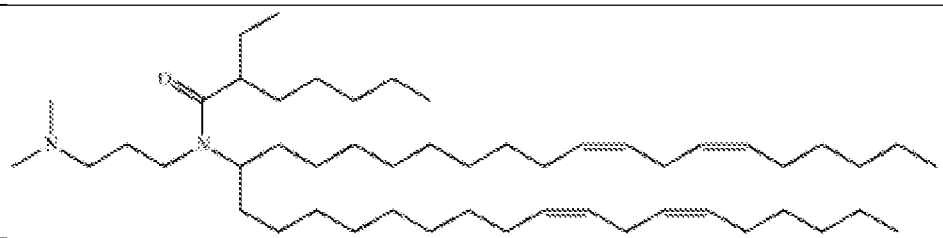
12	
13	

14	
15	
16	
17	

18	
19	
20	
21	
22	

23	
24	
25	
26	
27	
28	

29	
30	
31	
32	
33	

34	
35	
36	
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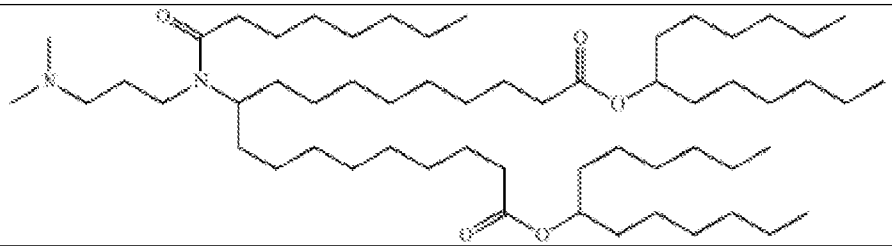
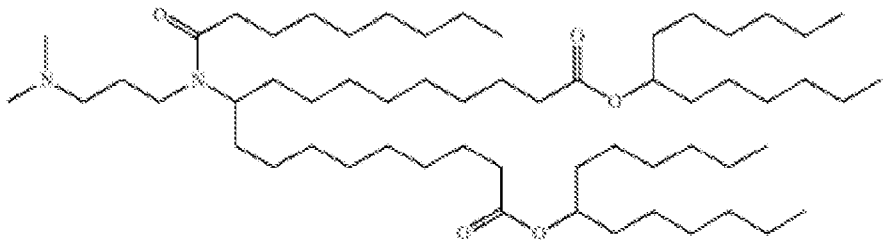
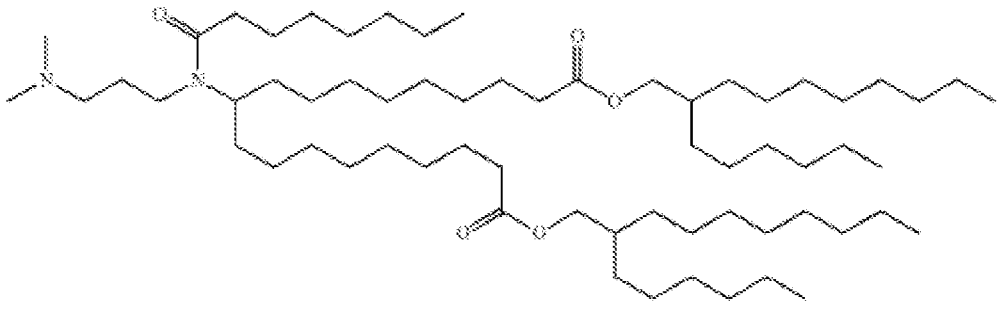
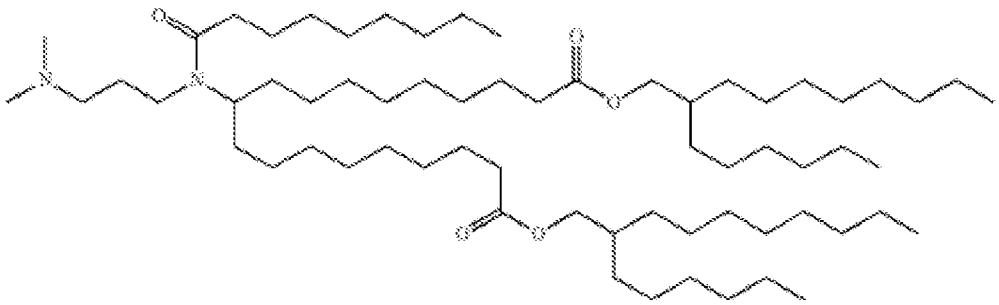
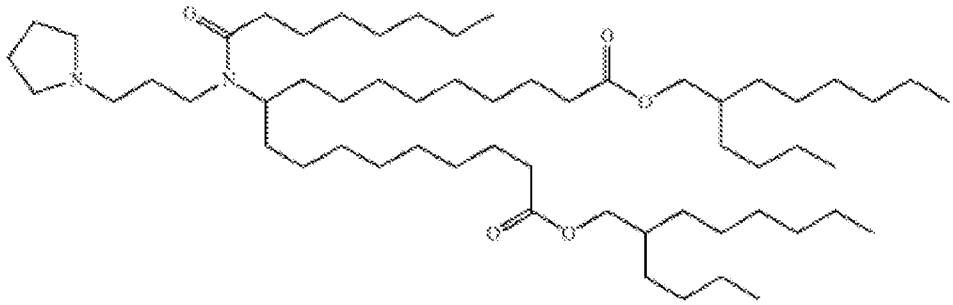
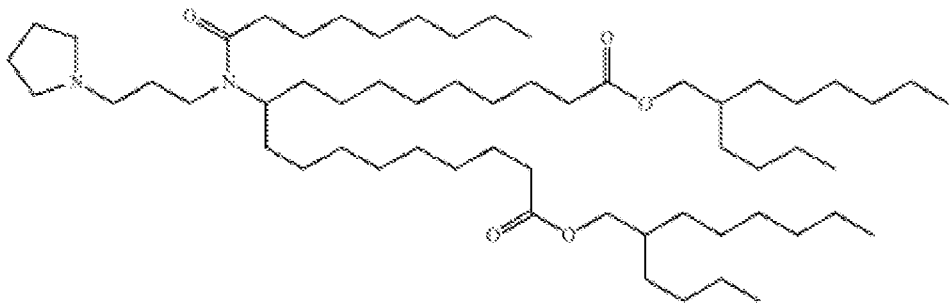
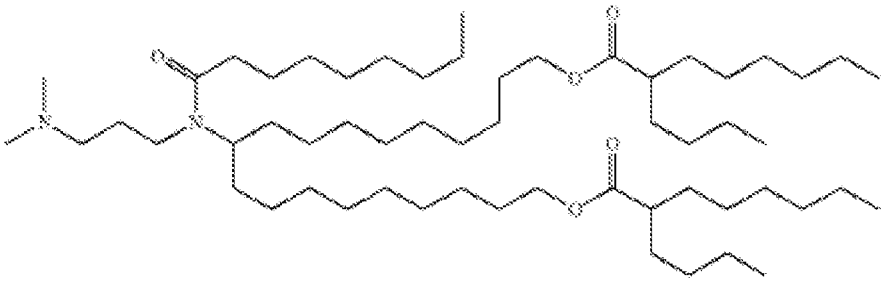
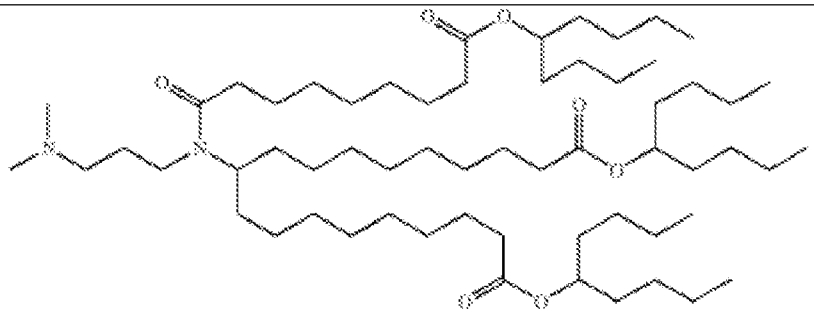
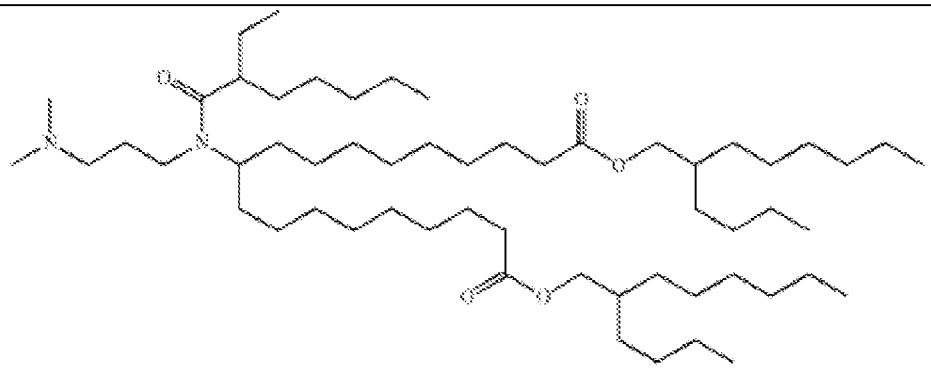
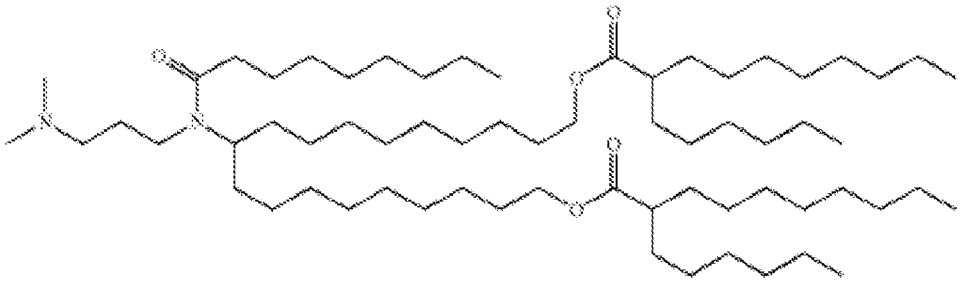
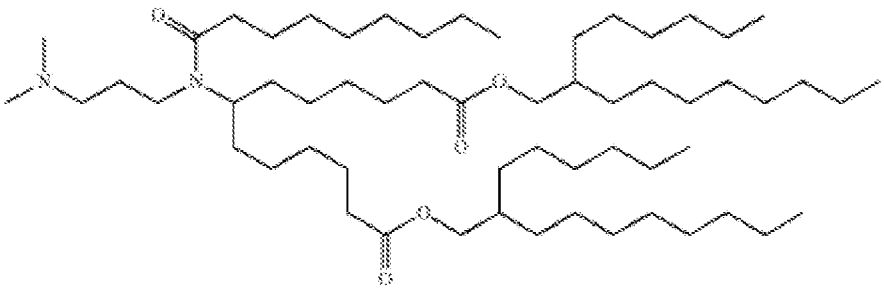
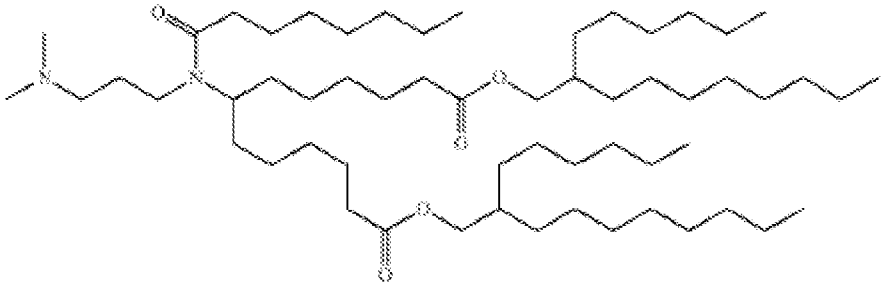
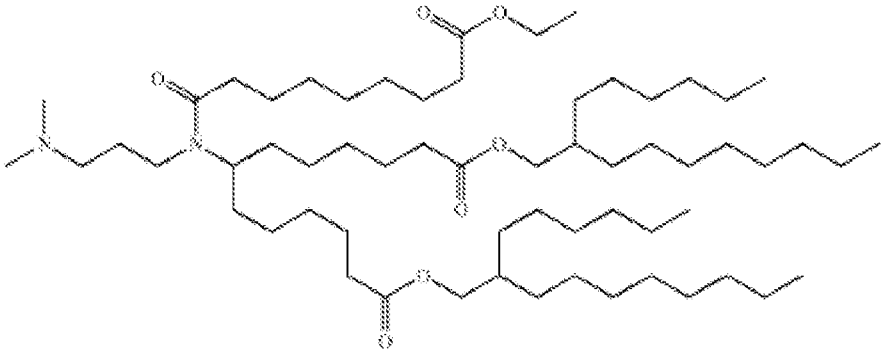
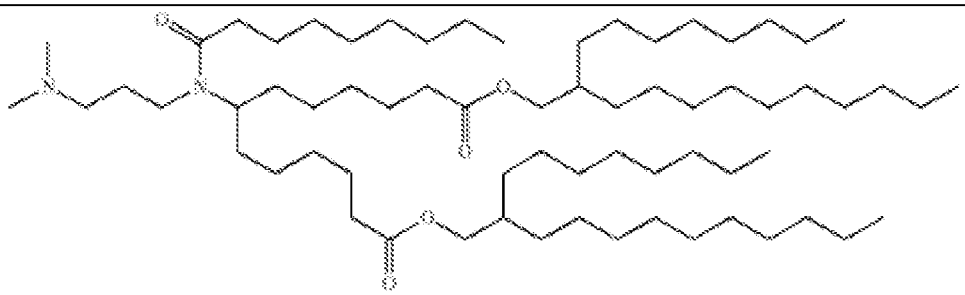
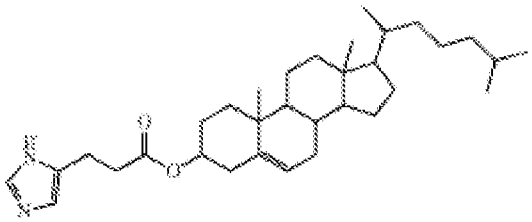
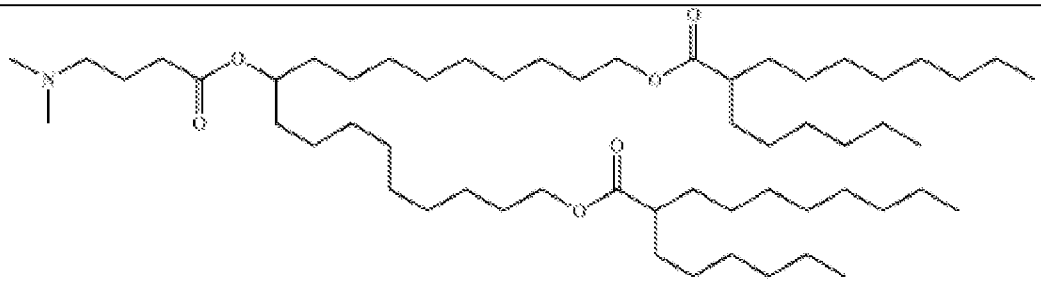
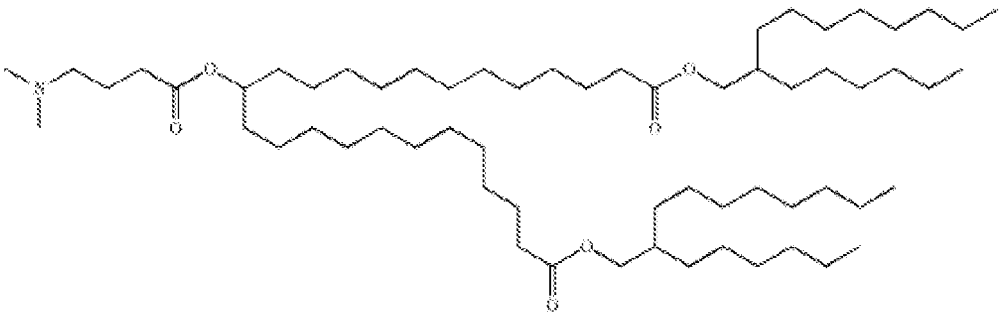
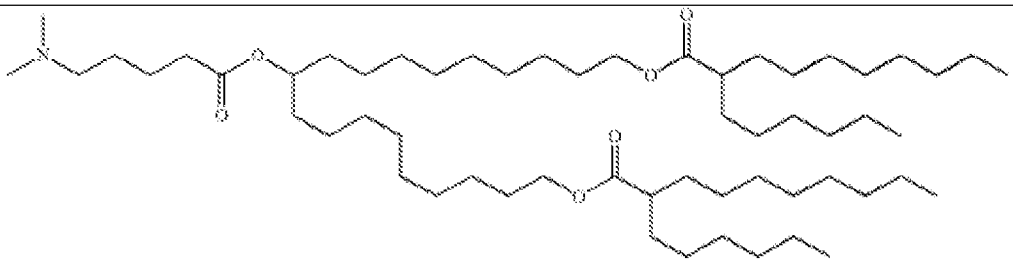
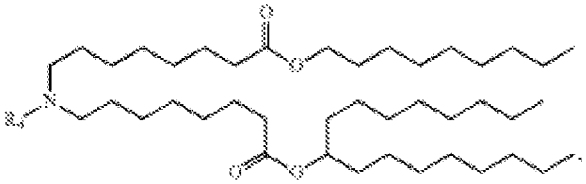
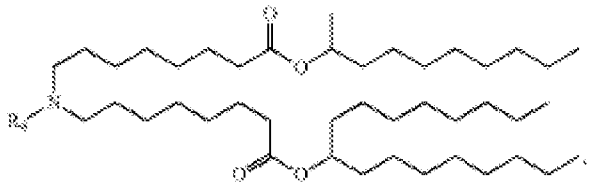
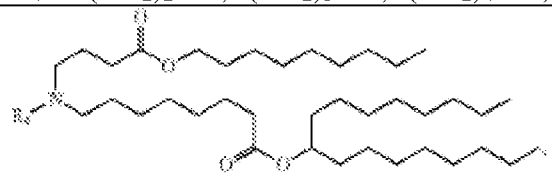
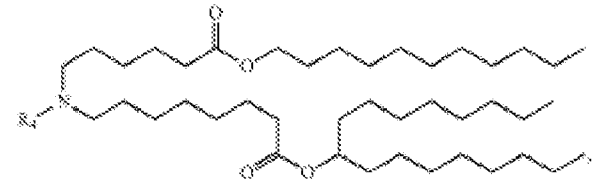
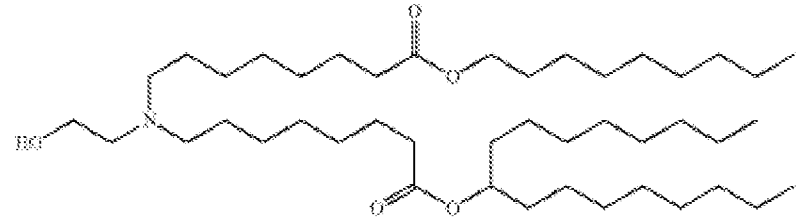
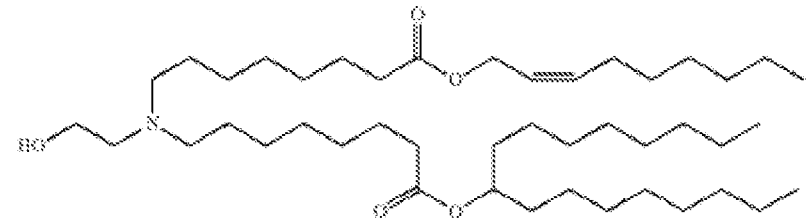
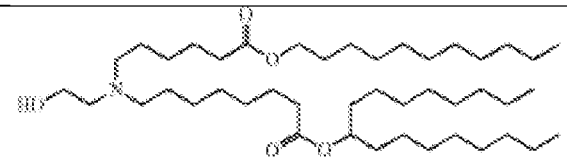
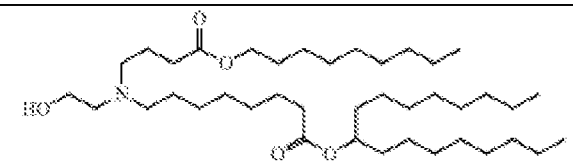
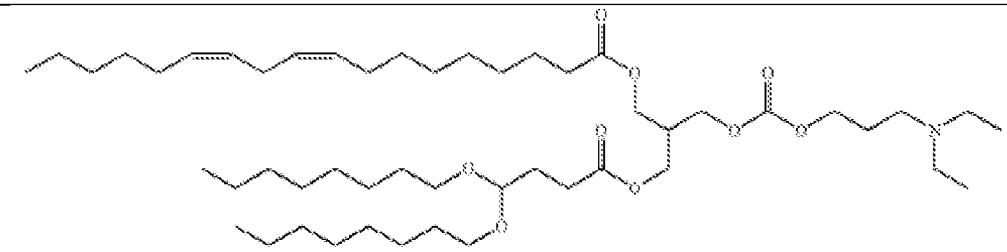
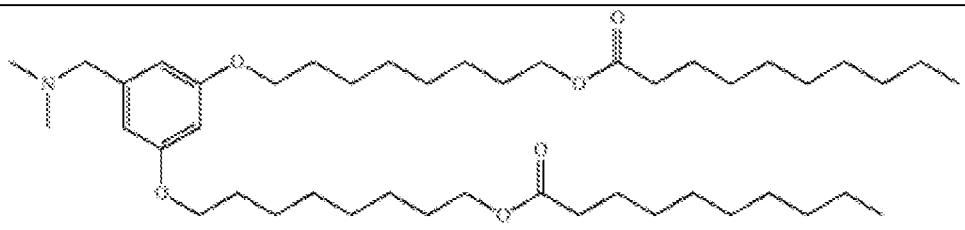
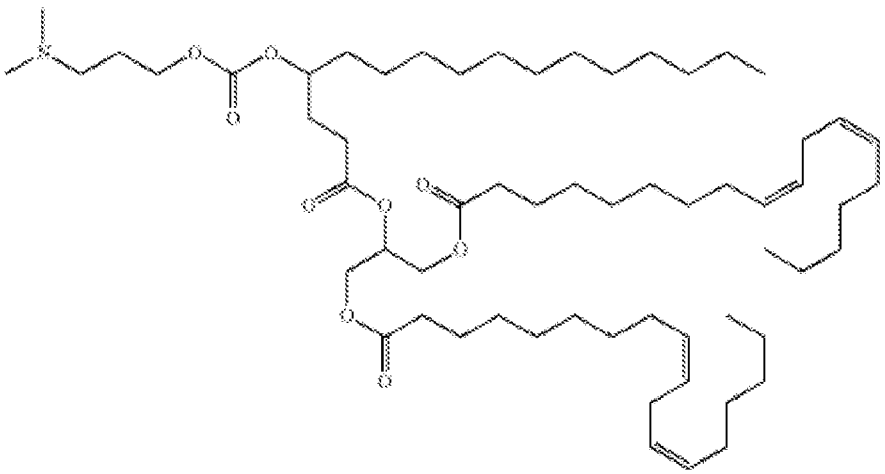
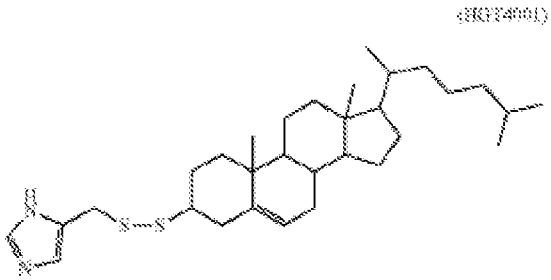
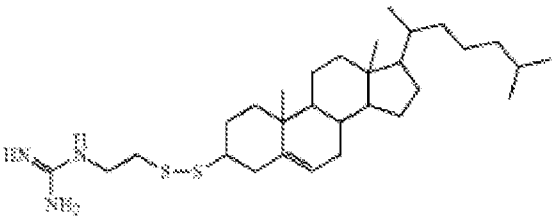
38	
39	
40	

41	
42	
43	
44	
45	

46	
47	
48	
49	
50	

51	
52	
53	
54	
55	
56	 R_4 = -$(CH_2)_2$OH, -$(CH_2)_3$OH, -$(CH_2)_4$OH;

57	 R_4 = -$(CH_2)_2$OH, -$(CH_2)_3$OH, -$(CH_2)_4$OH;
58	 R_4 = -$(CH_2)_2$OH, -$(CH_2)_3$OH, -$(CH_2)_4$OH;
59	
60	
61	
62	
63	
64	

65	
66	
67	 (IGT4001)
68	 (IGT4002)

[0209] In some embodiments of the lipid composition of the present application, the ionizable lipid is present in an amount of from about from about 20 mol% to about 23 mol%. In some embodiments, the ionizable lipid is present in an amount of about 20 mol%, about 20.5 mol%, about 21 mol%, about 21.5 mol%, about 22 mol%, about 22.5 mol%, or about 23 mol%. In other embodiments, the ionizable lipid is present in an amount of from about 7.5 mol% to about 20 mol%. In some embodiments, the ionizable lipid is present in an amount of about 7.5 mol%, about 8 mol%, about 9 mol%, about 10 mol%, about 11 mol%, about 12 mol%, about 13

mol%, about 14 mol%, about 15 mol%, about 16 mol%, about 17 mol%, about 18 mol%, about 19 mol%, or about 20 mol%.

[0210] In some embodiments of the lipid composition of the present application, the lipid composition comprises the ionizable lipid in an amount of from about 5 mol% to about 30 mol%. In some embodiments of the lipid composition of the present application, the lipid composition comprises the ionizable lipid in an amount of from about 10 mol% to about 25 mol%. In some embodiments of the lipid composition of the present application, the lipid composition comprises the ionizable lipid in an amount of from about 15 mol% to about 20 mol%. In some embodiments of the lipid composition of the present application, the lipid composition comprises the ionizable lipid in an amount of from about 10 mol% to about 20 mol%. In some embodiments of the lipid composition of the present application, the lipid composition comprises the ionizable lipid in an amount of from about 20mol% to about 30mol%. In some embodiments of the lipid composition of the present application, the lipid composition comprises the ionizable lipid in an amount of of at least (about) 5 mol%, at least (about) 10 mol%, at least (about) 15 mol%, at least (about) 20 mol%, at least (about) 25 mol%, or at least (about) 30 mol%. In some embodiments of the lipid composition of the present application, the lipid composition comprises the ionizable lipid in an amount of of at most (about) 5 mol%, at most (about) 10 mol%, at most (about) 15 mol%, at most (about) 20 mol%, at most (about) 25 mol%, or at most (about) 30mol%.

2. Phospholipids

[0211] In some embodiments, the LNP comprises a phospholipid. Phospholipids are a subset of non-cationic lipids that comprise a phosphate group. In some embodiments, the lipid component of LNP may include one or more phospholipids, such as one or more (poly) unsaturated lipids. Phospholipids may assemble into one or more lipid bilayers. In general, phospholipids may include a phospholipid moiety and one or more fatty acid moieties. In some embodiments, the phospholipid moiety may be selected from the group including, but not limited to, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl glycerol, phosphatidyl serine, phosphatidic acid, 2-lysophosphatidyl choline, and a sphingomyelin. A fatty acid moiety may be selected from the group including, but not limited to, lauric acid, myristic acid, myristoleic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, erucic acid, phytanoic acid, arachidic acid, arachidonic acid, eicosapentaenoic acid, behenic acid, docosapentaenoic acid, and docosahexaenoic acid.

[0212] In some embodiments, non-natural species including natural species with modifications and substitutions including branching, oxidation, cyclization, and alkynes are also contemplated. For example, a phospholipid may be functionalized with or cross-linked to one or more alkynes (*e.g.*, an alkenyl group in which one or more double bonds is replaced with a triple bond). Under appropriate reaction conditions, an alkyne group may undergo a copper-catalyzed cycloaddition upon exposure to an azide. Such reactions may be useful in functionalizing a lipid bilayer of a nanoparticle composition to facilitate membrane permeation or cellular recognition or in conjugating a nanoparticle composition to a useful component such as targeting or imaging moiety (*e.g.*, a dye).

[0213] In some embodiments, the LNP described herein comprises about 5 mol% to about 30 mol% of phospholipid. In some embodiments, the LNP comprises about 10 mol% to about 30 mol%, or about 12 mol% to about 30 mol%, or about 14 mol% to about 30 mol%, or about 16 mol% to about 30 mol%, or about 18 mol% to about 30 mol%, or about 20 mol% to about 30 mol%, or about 22 mol% to about 30 mol%, or about 24 mol% to about 30 mol%, or about 26 mol% to about 30 mol%, or about 28 mol% to about 30 mol%. In some embodiments, the LNP comprises about 10 mol%, or about 11 mol%, or about 12 mol%, or about 13 mol%, or about 14 mol%, or about 15 mol%, or about 16 mol%, or about 17 mol%, or about 18 mol%, or about 19 mol%, or about 20 mol%, or about 21 mol%, or about 22 mol%, or about 23 mol%, or about 24 mol%, or about 25 mol%, or about 26 mol%, or about 27 mo%, or about 28 mol%, or about 29 mol%, or about 30 mol%.

[0214] In some embodiments, the LNP comprises about 5% to about 30% weight of phoshpolipid. In some embodiments, the LNP comprises about 5% weight, or 10%, or 12%, or 15%, or 18%, or 20%, or 25%, or 30% weight of phospholipid.

[0215] In some embodiments of the LNP comprises a molar percentage of the phospholipid to the total lipid composition from about 20 to about 23. In some embodiments, the molar percentage is from about 20, 20.5, 21, 21.5, 22, 22.5, to about 23 or any range derivable therein. In other embodiments, the molar percentage is from about 7.5 to about 60. In some embodiments, the molar percentage is from about 7.5, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, to about 20 or any range derivable therein.

[0216] In some embodiments, the LNP comprises the phospholipid at a molar percentage from about 8% to about 23%. In some embodiments, the LNP comprises the phospholipid at a molar percentage from about 10% to about 20%. In some embodiments, the LNP comprises the

phospholipid at a molar percentage from about 15% to about 20%. In some embodiments, the LNP comprises the phospholipid at a molar percentage from about 8% to about 15%. In some embodiments, the LNP comprises the phospholipid at a molar percentage from about 10% to about 15%. In some embodiments, the LNP comprises the phospholipid at a molar percentage from about 12% to about 18%. In some embodiments, the LNP comprises the phospholipid at a molar percentage of at least about 8%, at least about 10%, at least about 12%, at least about 15%, at least about 18%, at least about 20%, or at least about 23%. In some embodiments, the LNP comprises the phospholipid at a molar percentage of at most (about) 8%, at most about 10%, at most about 12%, at most about 15%, at most about 18%, at most about 20%, or at most about 23%.

[0217] In some embodiments, phospholipids contained in the LNP are selected from the group comprising, but not limited to, 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycero-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-diundecanoyl-snglycero-phosphocholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-sn-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemsPC), 1-hexadecyl-snglycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine, 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE), 1,2-diphytanoyl-sn-glycero-3-phosphocholine (4ME 16:0 PC), 1,2-diphytanoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (sodium salt) (4ME 16:0 PG), 1,2-diphytanoyl-sn-glycero-3-phospho-L-serine (sodium salt) (4ME 16:0 PS), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinolenoylsn-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine, and 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), and sphingomyelin.

[0218] In some embodiments, the phospholipid contains one or two long chain (*e.g.*, C6-C24) alkyl or alkenyl groups, a glycerol or a sphingosine, one or two phosphate groups, and, optionally, a small organic molecule. The small organic molecule may be an amino acid, a sugar, or an amino substituted alkoxy group, such as choline or ethanolamine. In some

embodiments, the phospholipid is a phosphatidylcholine. In some embodiments, the phospholipid is distearoylphosphatidylcholine or dioleoylphosphatidylethanolamine. In some embodiments, other zwitterionic lipids are used, where zwitterionic lipid defines lipid and lipid-like molecules with both a positive charge and a negative charge. In some embodiments of the lipid compositions, the phospholipid is not an ethylphosphocholine.

3. Polymer conjugated lipids

[0219] In some embodiments, the LNP comprises lipids conjugated to polymers, such as lipids conjugated to polyethylene glycol ("PEG-lipid"). Methods for making and using PEG-lipids are described, for example, in Int'l Pat. Pub. No. WO 2012/099755 and U.S. Pat. Pub No. 2014/0200257.

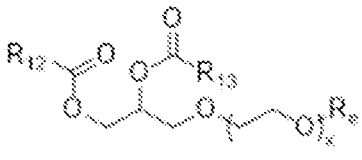
[0220] In some embodiments, the LNP comprises a PEG-lipid selected from the non-limiting group including PEG-modified phosphatidylethanolamines, PEG-modified phosphatidic acids, PEG-modified ceramides, PEG-modified dialkylamines, PEG-modified diacylglycerols, PEG-modified dialkylglycerols, and mixtures thereof. For example, a PEG-lipid may be PEG-c-DOMG, PEG-DMG, PEG-DLPE, PEG-DMPE, PEG-DPPC, or a PEG-DSPE lipid.

[0221] In some embodiments, PEG-lipids contained in the LNP are described in Int'l Pat. Pub. No. WO 2012/099755, the contents of which is herein incorporated by reference in its entirety. In some embodiments, any of these exemplary PEG-lipids described herein may be modified to comprise a hydroxyl group on the PEG chain. In some embodiments, the PEG-lipid is a PEG-OH lipid. As generally defined herein, a "PEG-OH lipid" is a PEG-lipid having one or more hydroxyl (-OH) groups on the lipid. In some embodiments, the PEG-OH lipid includes one or more hydroxyl groups on the PEG chain. In certain embodiments, a PEG-OH or hydroxy-PEG-lipid comprises an -OH group at the terminus of the PEG chain.

[0222] In some embodiments, the lipid composition further comprises a polymer conjugated lipid. In some embodiments, the polymer conjugated lipid is a PEG-lipid. In some embodiments, the PEG-lipid is a diglyceride which also comprises a PEG chain attached to the glycerol group. In other embodiments, the PEG-lipid is a compound which contains one or more C_6-C_{24} long chain alkyl or alkenyl group or a C_6-C_{24} fatty acid group attached to a linker group with a PEG chain. Some non-limiting examples of a PEG-lipid includes a PEG modified phosphatidylethanolamine and phosphatidic acid, a PEG ceramide conjugated, PEG modified dialkylamines and PEG modified 1,2-diacyloxypropan-3-amines, PEG modified diacylglycerols and dialkylglycerols. In some embodiments, PEG modified

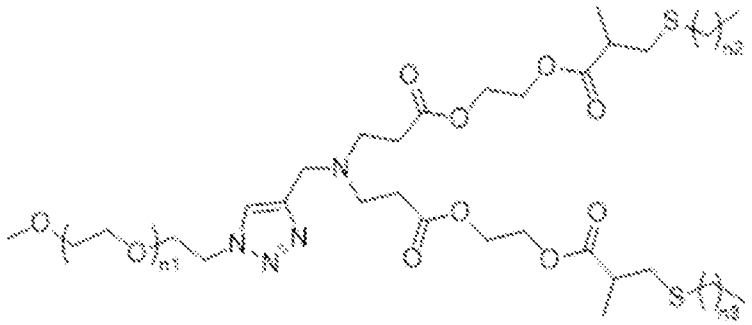
diastearoylphosphatidylethanolamine or PEG modified dimyristoyl-*sn*-glycerol. In some embodiments, the PEG modification is measured by the molecular weight of PEG component of the lipid. In some embodiments, the PEG modification has a molecular weight from about 100 to about 15,000. In some embodiments, the molecular weight is from about 200 to about 500, from about 400 to about 5,000, from about 500 to about 3,000, or from about 1,200 to about 3,000. The molecular weight of the PEG modification is from about 100, 200, 400, 500, 600, 800, 1,000, 1,250, 1,500, 1,750, 2,000, 2,250, 2,500, 2,750, 3,000, 3,500, 4,000, 4,500, 5,000, 6,000, 7,000, 8,000, 9,000, 10,000, 12,500, to about 15,000. Some non-limiting examples of lipids that may be used in the present application are taught by U.S. Patent 5,820,873, WO 2010/141069, or U.S. Patent 8,450,298, which is incorporated herein by reference.

[0223] In some embodiments, the PEG-lipid has a structural formula:



, wherein: R_{12} and R_{13} are each independently $alkyl_{(C\leq 24)}$, $alkenyl_{(C\leq 24)}$, or a substituted version of either of these groups; R_e is hydrogen, $alkyl_{(C\leq 8)}$, or substituted $alkyl_{(C\leq 8)}$; and x is 1-250. In some embodiments, R_e is $alkyl_{(C\leq 8)}$ such as methyl. R_{12} and R_{13} are each independently $alkyl_{(C\leq 4\text{-}20)}$. In some embodiments, x is 5-250. In one embodiment, x is 5-125 or x is 100-250. In some embodiments, the PEG-lipid is 1,2-dimyristoyl-*sn*-glycerol, methoxypolyethylene glycol.

[0224] In some embodiments, the PEG-lipid has a structural formula:



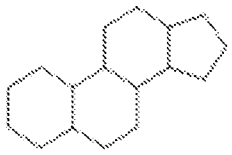
, wherein: n_1 is an integer between 1 and 100 and n_2 and n_3 are each independently selected from an integer between 1 and 29. In some embodiments, n_1 is 5, 10, 15, 20, 25, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100, or any range derivable therein. In some embodiments, n_1 is from about 30 to about 50. In some embodiments, n_2 is from 5 to 23. In some embodiments, n_2 is 11 to about 17. In some embodiments, n_3 is from 5 to 23. In some embodiments, n_3 is 11 to about 17.

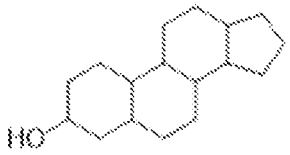
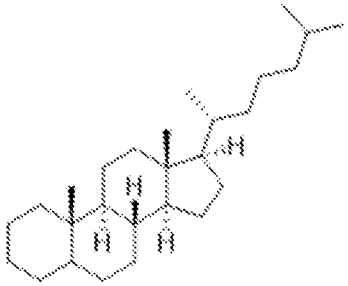
[0225] In some embodiments, the compositions may further comprise a molar percentage of the PEG-lipid to the total lipid composition from about 4.0 to about 4.6. In some embodiments, the molar percentage is from about 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, to about 4.6 or any range derivable therein. In other embodiments, the molar percentage is from about 1.5 to about 4.0. In some embodiments, the molar percentage is from about 1.5, 1.75, 2, 2.25, 2.5, 2.75, 3, 3.25, 3.5, 3.75, to about 4.0 or any range derivable therein.

[0226] In some embodiments, the lipid composition comprises the polymer-conjugated lipid at a molar percentage from about 0.5% to about 10%. In some embodiments, the lipid composition comprises the polymer-conjugated lipid at a molar percentage from about 1% to about 8%. In some embodiments, the lipid composition comprises the polymer-conjugated lipid at a molar percentage from about 2% to about 7%. In some embodiments, the lipid composition comprises the polymer-conjugated lipid at a molar percentage from about 3% to about 5%. In some embodiments, the lipid composition comprises the polymer-conjugated lipid at a molar percentage from about 5% to about 10%. In some embodiments, the lipid composition comprises the polymer-conjugated lipid at a molar percentage of at least (about) 0.5%, at least (about) 1%, at least (about) 1.5%, at least (about) 2%, at least (about) 2.5%, at least (about) 3%, at least (about) 3.5%, at least (about) 4%, at least (about) 4.5%, at least (about) 5%, at least (about) 5.5%, at least (about) 6%, at least (about) 6.5%, at least (about) 7%, at least (about) 7.5%, at least (about) 8%, at least (about) 8.5%, at least (about) 9%, at least (about) 9.5%, or at least (about) 10%. In some embodiments, the lipid composition comprises the polymer-conjugated lipid at a molar percentage of at most (about) 0.5%, at most (about) 1%, at most (about) 1.5%, at most (about) 2%, at most (about) 2.5%, at most (about) 3%, at most (about) 3.5%, at most (about) 4%, at most (about) 4.5%, at most (about) 5%, at most (about) 5.5%, at most (about) 6%, at most (about) 6.5%, at most (about) 7%, at most (about) 7.5%, at most (about) 8%, at most (about) 8.5%, at most (about) 9%, at most (about) 9.5%, or at most (about) 10%.

4. Sterol

[0227] In some embodiments, the LNP comprises a steroid or steroid derivative. As used herein, in some embodiments, the term “steroid” is a class of compounds with a four ring 17 carbon cyclic structure which can further comprises one or more substitutions including alkyl groups, alkoxy groups, hydroxy groups, oxo groups, acyl groups, or a double bond between two

or more carbon atoms. In one aspect, the ring structure of a steroid comprises three fused cyclohexyl rings and a fused cyclopentyl ring as shown in the formula:  .

[0228] In some embodiments, a steroid derivative comprises the ring structure above with one or more non-alkyl substitutions. In some embodiments, the steroid or steroid derivative is a sterol wherein the formula is further defined as:  . In some embodiments, the steroid or steroid derivative is a cholestane or cholestane derivative. In a cholestane, the ring structure is further defined by the formula:  . As described above, a cholestane derivative includes one or more non-alkyl substitution of the above ring system. In some embodiments, the cholestane or cholestane derivative is a cholestene or cholestene derivative or a sterol or a sterol derivative. In other embodiments, the cholestane or cholestane derivative is both a cholestere and a sterol or a derivative thereof.

[0229] In some embodiments, the compositions may further comprise a molar percentage of the steroid to the total lipid composition from about 40 to about 46. In some embodiments, the molar percentage is from about 40, 41, 42, 43, 44, 45, to about 46 or any range derivable therein. In other embodiments, the molar percentage of the steroid relative to the total lipid composition is from about 15 to about 40. In some embodiments, the molar percentage is 15, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, or 40, or any range derivable therein.

[0230] In some embodiments, the lipid composition comprises the steroid or steroid derivative at a molar percentage from about 15% to about 46%. In some embodiments, the lipid composition comprises the steroid or steroid derivative at a molar percentage from about 20% to about 40%. In some embodiments, the lipid composition comprises the steroid or steroid derivative at a molar percentage from about 25% to about 35%. In some embodiments, the lipid composition comprises the steroid or steroid derivative at a molar percentage from about 30% to

about 40%. In some embodiments, the lipid composition comprises the steroid or steroid derivative at a molar percentage from about 20% to about 30%. In some embodiments, the lipid composition comprises the steroid or steroid derivative at a molar percentage of at least (about) 15%, of at least (about) 20%, of at least (about) 25%, of at least (about) 30%, of at least (about) 35%, of at least (about) 40%, of at least (about) 45%, or of at least (about) 46%. In some embodiments, the lipid composition comprises the steroid or steroid derivative at a molar percentage of at most (about) 15%, of at most (about) 20%, of at most (about) 25%, of at most (about) 30%, of at most (about) 35%, of at most (about) 40%, of at most (about) 45%, or of at most (about) 46%.

[0231] In some embodiments, the cationic agent is a cationic lipid which is a sterol amine. A sterol amine has, for its hydrophobic portion, a sterol, and for its hydrophilic portion, an amine group. The sterol group is selected from, but not limited to, cholesterol, sitosterol, campesterol, stigmasterol or derivatives thereof. The amine group can comprise one to five primary, secondary, tertiary amines, or mixtures thereof. At least one of the amines has a pKa of 8 or greater and is charged at physiological pH. The primary, secondary, or tertiary amines can be part of a larger amine containing functional group selected from, but not limited to -C(=N-)-N-, -C=C-N-, -C=N-, or -N-C(=N-)-N-. The amine can be contained in a three to eight membered heteroalkyl or heteroaryl ring.

B. Selective Organ Targeting (SORT) Molecules

[0232] In some embodiments, the method comprises the administration of a LNP comprising a gene editing system, wherein the LNP comprises one or more selective organ targeting (SORT) molecules.

[0233] Selective organ targeting enables the controllable delivery of the nucleic acids encased by the LNP to specific target tissues. Traditionally, LNPs have been limited to intramuscular and intravenous administration where the LNPs target and accumulate in the liver due to physiological factors. In some embodiments, LNPs comprising SORT molecules (i.e. SORT LNPs) include a supplemental SORT molecule, wherein the chemical structure of the SORT molecule determines the tissue-specific activity of the LNP. In some embodiments, the method comprises administration of the LNP comprising a SORT molecule, wherein LNP is delivered to organs and cells other than the liver.

[0234] In some embodiments of the LNP comprising a gene editing system comprising a SORT molecule is preferentially delivered to a target organ. In some embodiments, the target

organ is a lung, a lung tissue or a lung cell. In some embodiments, the lung cell type is an endothelial cell or an epithelial cell. In some embodiments, the lung cell type is an immune cell. In some embodiments, the lung cell is a stem cell. In some embodiments, the epithelial cell is a ciliated cell, non-ciliated cell, a goblet cell, a brush cell (alveolar macrophage), an airway basal cell, a small granule cell, a bronchial epithelial cell, a small airway epithelial cell, and/or a tracheal epithelial cell. In some embodiments, the lung cell is a secretory cell and or ionocyte.

[0235] As used herein, the term "preferentially delivered" is used to refer to a composition, upon being delivered, which is delivered to the target organ (e.g., lung), tissue, or cell in at least 25% (e.g., at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75%) of the amount administered.

[0236] In some embodiments, the LNP comprises one or more SORT molecules. In some embodiments, the SORT molecule comprises a cationic lipid or an anionic lipid. In some embodiments, the SORT molecule comprises a permanently cationic lipid or a permanently anionic lipid. In some embodiments, the SORT molecule comprises a cationic lipid or a permanently cationic lipid. In some embodiments, the SORT molecule comprises permanently positively charged moiety. The permanently positively charged moiety may be positively charged at a physiological pH such that the SORT molecule comprises a positive charge upon delivery of a polynucleotide to a cell. In some embodiments, the positively charged moiety is quaternary amine or quaternary ammonium ion. In some embodiments, the SORT molecule comprises, or is otherwise complexed to or interacting with, a counterion. In some embodiments, the SORT molecule comprises two or more alkyl or alkenyl chains of C6-C24.

[0237] In some embodiments, the one or more SORT molecule is a permanently cationic lipid (i.e., comprising one or more hydrophobic components and a permanently cationic group). In some embodiments, the permanently cationic lipid may contain a group which has a positive charge regardless of the pH. One permanently cationic group that may be used in the permanently cationic lipid is a quaternary ammonium group.

[0238] In some embodiments, the one or more SORT molecule is ionizable cationic lipid (i.e., comprising one or more hydrophobic components and an ionizable cationic group). The ionizable positively charged moiety may be positively charged at a physiological pH. One ionizable cationic group that may be used in the ionizable cationic lipid is a tertiary ammine group.

[0239] In some embodiments, the one or more SORT molecule comprises 18:1 DOTMA; DORI, DC-6-14; 12:0 EPC (Chloride Salt); 14:0 EPC (Chloride Salt); 16:0 EPC (Chloride Salt); 18:0 EPC (Chloride Salt); 18:1 EPC (Chloride Salt); 16:0-18:1 EPC (Chloride Salt); 14:1 EPC (Triflate Salt); 18:0 DDAB (Dimethyldioctadecylammonium (Bromide Salt)); 14:0 TAP; 16:0 TAP; 18:0 TAP; 18:1 TAP (DOTAP); 18:1 TAP (DOTAP, MS Salt); 18:1 DODAP, or 18:1 PA (1,2-dioleoyl-sn-glycero-3-phosphate (sodium salt)). In some embodiments, the SORT molecule comprises DOTAP (1,2-dioleoyl-3-trimethylammonium propane). In some embodiments, the SORT molecule comprises 18:1 PA. In some embodiments, the SORT molecule comprises DODAP.

[0240] In some embodiments, the SORT molecule comprises between 5% to 70% molar percentage of the LNP. In some embodiments, the SORT molecule comprises up to about 5%, up to about 10%, up to about 15%, up to about 20%, up to about 25%, up to about 30%, up to about 35%, up to about 40%, up to about 45%, up to about 50%, up to about 55%, up to about 60%, up to about 65%, or up to about 70% molar percentage of the LNP. In some embodiments, the SORT molecule comprises about 20% molar percentage of the LNP. In some embodiments, the SORT molecule comprises about 25% molar percentage of the LNP. In some embodiments, the SORT molecule comprises about 30% molar percentage of the LNP. In some embodiments, the SORT molecule comprises about 35% molar percentage of the LNP. In some embodiments, the SORT molecule comprises about 40% molar percentage of the LNP. In some embodiments, the SORT molecule comprises about 45% molar percentage of the LNP.

[0241] In some embodiments, the SORT molecule comprises from about 5% to about 70% molar percentage of the LNP, from about 10% to about 70% molar percentage of the LNP, from about 15% to about 70% molar percentage of the LNP, from about 20% to about 70% molar percentage of the LNP, from about 25% to about 70% molar percentage of the LNP, from about 30% to about 70% molar percentage of the LNP, from about 35% to about 70% molar percentage of the LNP, from about 40% to about 70% molar percentage of the LNP, from about 45% to about 70% molar percentage of the LNP, from about 50% to about 70% molar percentage of the LNP, from about 55% to about 70% molar percentage of the LNP, from about 60% to about 70% molar percentage of the LNP, from about 65% to about 70% molar percentage of the LNP, from about 5% to about 65% molar percentage of the LNP, from about 10% to about 65% molar percentage of the LNP, from about 15% to about 65% molar percentage of the LNP, from about 20% to about 65% molar percentage of the LNP, from about

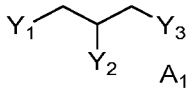
25% to about 65% molar percentage of the LNP, from about 30% to about 65% molar percentage of the LNP, from about 35% to about 65% molar percentage of the LNP, from about 40% to about 65% molar percentage of the LNP, from about 45% to about 65% molar percentage of the LNP, from about 50% to about 65% molar percentage of the LNP, from about 55% to about 65% molar percentage of the LNP, from about 60% to about 65% molar percentage of the LNP, from about 5% to about 60% molar percentage of the LNP, from about 10% to about 60% molar percentage of the LNP, from about 15% to about 60% molar percentage of the LNP, from about 20% to about 60% molar percentage of the LNP, from about 25% to about 60% molar percentage of the LNP, from about 30% to about 60% molar percentage of the LNP, from about 35% to about 60% molar percentage of the LNP, from about 40% to about 60% molar percentage of the LNP, from about 45% to about 60% molar percentage of the LNP, from about 50% to about 60% molar percentage of the LNP, from about 55% to about 60% molar percentage of the LNP, from about 5% to about 55% molar percentage of the LNP, from about 10% to about 55% molar percentage of the LNP, from about 15% to about 55% molar percentage of the LNP, from about 20% to about 55% molar percentage of the LNP, from about 25% to about 55% molar percentage of the LNP, from about 30% to about 55% molar percentage of the LNP, from about 35% to about 55% molar percentage of the LNP, from about 40% to about 55% molar percentage of the LNP, from about 50% to about 55% molar percentage of the LNP, from about 5% to about 50% molar percentage of the LNP, from about 10% to about 50% molar percentage of the LNP, from about 15% to about 50% molar percentage of the LNP, from about 20% to about 50% molar percentage of the LNP, from about 25% to about 50% molar percentage of the LNP, from about 30% to about 50% molar percentage of the LNP, from about 35% to about 50% molar percentage of the LNP, from about 40% to about 50% molar percentage of the LNP, from about 45% to about 50% molar percentage of the LNP, from about 5% to about 45% molar percentage of the LNP, from about 10% to about 45% molar percentage of the LNP, from about 15% to about 45% molar percentage of the LNP, from about 20% to about 45% molar percentage of the LNP, from about 25% to about 45% molar percentage of the LNP, from about 30% to about 45% molar percentage of the LNP, from about 35% to about 45% molar percentage of the LNP, from about 40% to about 45% molar percentage of the LNP, from about 5% to about 40% molar percentage of the LNP, from about 10% to about 40% molar percentage of the LNP, from about 15% to about 40% molar percentage of the LNP, from about 20% to about 40% molar percentage of the

LNP, from about 25% to about 40% molar percentage of the LNP, from about 30% to about 40% molar percentage of the LNP, from about 30% to about 40% molar percentage of the LNP, from about 5% to about 35% molar percentage of the LNP, from about 10% to about 35% molar percentage of the LNP, from about 15% to about 35% molar percentage of the LNP, from about 20% to about 35% molar percentage of the LNP, from about 25% to about 35% molar percentage of the LNP, from about 30% to about 35% molar percentage of the LNP, from about 5% to about 30% molar percentage of the LNP, from about 10% to about 30% molar percentage of the LNP, from about 15% to about 30% molar percentage of the LNP, from about 20% to about 30% molar percentage of the LNP, from about 25% to about 30% molar percentage of the LNP, from about 5% to about 25% molar percentage of the LNP, from about 10% to about 25% molar percentage of the LNP, from about 15% to about 25% molar percentage of the LNP, from about 20% to about 25% molar percentage of the LNP, from about 5% to about 20% molar percentage of the LNP, from about 10% to about 20% molar percentage of the LNP, from about 15% to about 20% molar percentage of the LNP, from about 5% to about 15% molar percentage of the LNP, from about 10% to about 15% molar percentage of the LNP, or from about 5% to about 15% molar percentage of the LNP. In some embodiments, the SORT molecule comprises from about 20% to about 40% molar percentage of the LNP. In some embodiments, the SORT molecule comprises from about 35% to about 40% molar percentage of the LNP. In some embodiments, the SORT molecule comprises from about 40% to about 45% molar percentage of the LNP. In some embodiments, the SORT molecule comprises from about 35% to about 45% molar percentage of the LNP.

[0242] In some embodiments, the SORT molecule facilitates the preferential delivery of the one or more polynucleotides comprising one or more nucleic acids encapsulated in the LNP to a target organ and/or a target cell. In some embodiments, an LNP comprising a SORT molecule delivers a greater percentage of the enveloped nucleic acid than a reference LNP without a SORT molecule. In some embodiments, the LNP comprising a SORT molecule delivers at least 5% more, at least 10% more, at least 15% more, at least 20% more, at least 25% more, at least 30% more, at least 35% more, at least 40% more, at least 45% more, at least 50% more, at least 55% more, at least 60% more, at least 65% more, at least 70% more, at least 75% more, at least 80% more, at least 85% more, at least 90% more, at least 95% more, or at least 99% more encapsulated nucleic acids to the target organ and/or the target cell than a reference LNP not comprising a SORT molecule. In some embodiments, the target organ is the lung. In some

embodiments, target cells may comprise, but are not limited to, basal cells, secretory cells such as goblet cells and club cells, ciliated cells, and any combination thereof.

[0243] In some embodiments, the LNP comprising a SORT molecule achieves a greater therapeutic effect compare to a reference LNP that does not comprise a SORT molecule. In some embodiments of the method, the LNP comprising a SORT molecule achieves about 1.1-fold to about 20-fold therapeutic effect compared to that achieved with a reference LNP. In some embodiments of the method, the LNP comprising a SORT molecule achieves about 1.1 - fold to about 10-fold therapeutic effect compared to that achieved with a reference LNP. In some embodiments of the method, the LNP comprising a SORT molecule achieves about 1.1-fold to about 5-fold therapeutic effect compared to that achieved with a reference LNP. In some embodiments of the method, the LNP comprising a SORT molecule achieves about 5-fold to about 10-fold therapeutic effect compared to that achieved with a reference LNP. In some embodiments of the method, the LNP comprising a SORT molecule achieves about 10-fold to about 20-fold therapeutic effect compared to that achieved with a reference LNP. In some embodiments of the method, the LNP comprising a SORT molecule achieves at least about 1.1 - fold, at least about 1.5-fold, at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 11-fold, at least about 12-fold, at least about 13-fold, at least about 14-fold, at least about 15-fold, at least about 16-fold, at least about 17-fold, at least about 18-fold, at least about 19-fold, or at least about 20-fold therapeutic effect compared to that achieved with a reference LNP.

[0244] In some embodiments of the lipid compositions, the additional lipid is a permanently cationic lipid (*i.e.*, comprising one or more hydrophobic components and a permanently cationic group). The permanently cationic lipid may contain a group which has a positive charge regardless of the pH. One permanently cationic group that may be used in the permanently cationic lipid is a quaternary ammonium group. The permanently cationic lipid may comprise a structural formula:  (S-I), wherein:

Y_1, Y_2, or Y_3 are each independently $X_1C(O)R_1$ or $X_2N^+R_3R_4R_5$;

provided at least one of Y_1, Y_2, and Y_3 is $X_2N^+R_3R_4R_5$;

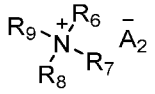
R_1 is C_1-C_{24} alkyl, C_1-C_{24} substituted alkyl, C_1-C_{24} alkenyl, C_1-C_{24} substituted alkenyl;

X_1 is O or NR_a, wherein R_a is hydrogen, C_1-C_4 alkyl, or C_1-C_4 substituted alkyl;

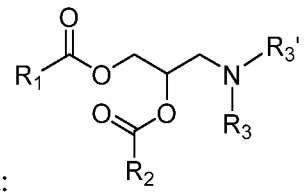
X_2 is C_1-C_6 alkanediyl or C_1-C_6 substituted alkanediyl;

R_3, R_4, and R_5 are each independently C_1-C_{24} alkyl, C_1-C_{24} substituted alkyl, C_1-C_{24} alkenyl, C_1-C_{24} substituted alkenyl; and

A_1 is an anion with a charge equal to the number of $X_2N^+R_3R_4R_5$ groups in the compound.

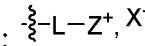
[0245] In some embodiments, the permanently cationic additonal lipid (e.g., SORT lipid) has a structural formula:  (S-II), wherein:

R_6-R_9 are each independently C_1-C_{24} alkyl, C_1-C_{24} substituted alkyl, C_1-C_{24} alkenyl, C_1-C_{24} substituted alkenyl; provided at least one of R_6-R_9 is a group of C_8-C_{24}; and A_2 is a monovalent anion.

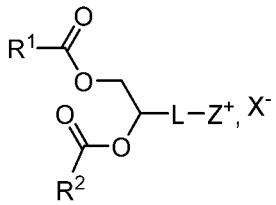
[0246] In some embodiments of the lipid compositions, the SORT (additional) lipid is an ionizable cationic lipid (*i.e.*, comprising one or more hydrophobic components and an ionizable cationic group, *e.g.* a tertiary amino group). The ionizable positively charged moiety may be positively charged at a physiological pH. One ionizable cationic group that may be used in the ionizable lipid is a tertiary ammine group. In some embodiments of the lipid compositions disclosed herein, the additonal lipid (e.g., additonal lipid (e.g., SORT lipid)) has a structural formula:  (S-I'a), wherein:

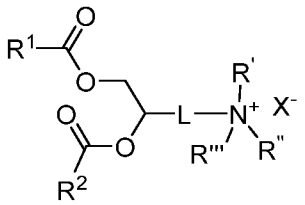
R_1 and R_2 are each independently C_8-C_{24} alkyl, C_8-C_{24} alkenyl, or a substituted version of either group; and R_3 and R_3' are each independently C_1-C_6 alkyl or substituted C_1-C_6 alkyl.

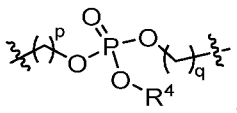
[0247] In some embodiments of formula (S-I'a) R_1 and R_2 are each independently C_8-C_{24} alkenyl (*e.g.* hexadecane, heptadecene, or octadecene). In some embodiments of formula (S-I'a), R_3 and R_3' are each independently C_1-C_6 alkyl (*e.g.*, methyl or ethyl). In some embodiments of formula (S-I'a) R_1 and R_2 are each independently C_8-C_{24} alkenyl, (*e.g.* hexadecane, heptadecene, or octadecene) and R_3 and R_3' are each independently C_1-C_6 alkyl (*e.g.*, methyl or ethyl).

[0248] In some embodiments of the lipid compositions, the additional ionizable lipid or permanently cationic lipid comprises a head group of a particular structure. In some embodiments, the additonal lipid (e.g., additonal lipid (e.g., SORT lipid)) comprises a headgroup having a structural formula:  , wherein L is a linker; Z^+ is positively charged moiety and X^- is a counterion. In some embodiment, the linker is a biodegradable linker. The

biodegradable linker may be degradable under physiological pH and temperature. The biodegradable linker may be degraded by proteins or enzymes from a subject. In some embodiments, the positively charged moiety is a quaternary ammonium ion or quaternary amine.

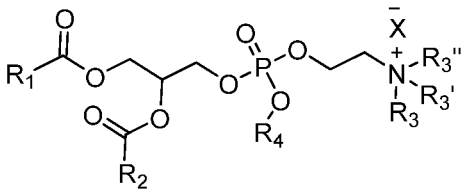
[0249] In some embodiments of the lipid compositions, the SORT (additional ionizable lipid or permanently cationic) lipid has a structural formula:  , wherein R^1 and R^2 are each independently an optionally substituted C_6-C_{24} alkyl, or an optionally substituted C_6-C_{24} alkenyl.

[0250] In some embodiments of the lipid compositions, the additonal lipid (e.g., additonal lipid (e.g., SORT lipid)) has a structural formula:  .

[0251] In some embodiments of the lipid compositions, the additonal lipid (e.g., additonal lipid (e.g., SORT lipid)) comprises a Linker (L). In some embodiments, L is  , wherein:

p and q are each independently 1, 2, or 3; and

R^4 is an optionally substituted C_1-C_6 alkyl.

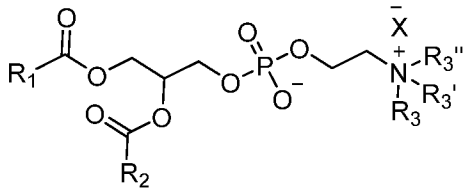
[0252] In some embodiments of the lipid compositions, the additonal lipid (e.g., additonal lipid (e.g., SORT lipid)) has a structural formula:  (IA), wherein:

R_1 and R_2 are each independently C_8-C_{24} alkyl, C_8-C_{24} alkenyl, or a substituted version of either group;

R_3, R_3', and R_3'' are each independently C_1-C_6 alkyl or substituted C_1-C_6 alkyl;

R_4 is C_1-C_6 alkyl or substituted C_1-C_6 alkyl; and

X^- is a monovalent anion.

[0253] In some embodiments of the lipid compositions, the additonal lipid (e.g., additonal lipid (e.g., SORT lipid)) is a phosphatidylcholine (*e.g.*, 14:0 EPC). In some embodiments, the phosphatidylcholine compound is further defined as:  (IA), wherein:

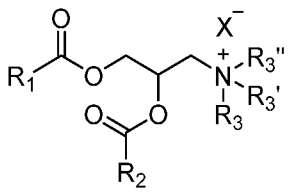
R_1 and R_2 are each independently C_8-C_{24} alkyl, C_8-C_{24} alkenyl, or a substituted version of either group;

R_3, R_3', and R_3'' are each independently C_1-C_6 alkyl or substituted C_1-C_6 alkyl; and

X^- is a monovalent anion.

[0254] In some embodiments of the lipid compositions, the additonal lipid (e.g., additonal lipid (e.g., SORT lipid)) is a phosphocholine lipid. In some embodiments, the additonal lipid (e.g., additonal lipid (e.g., SORT lipid)) is an ethylphosphocholine. The ethylphosphocholine may be, by way of example, without being limited to, 1,2-dimyristoleoyl-sn-glycero-3-ethylphosphocholine, 1,2-dioleoyl-sn-glycero-3-ethylphosphocholine, 1,2-distearoyl-sn-glycero-3-ethylphosphocholine, 1,2-dipalmitoyl-sn-glycero-3-ethylphosphocholine, 1,2-dimyristoyl-sn-glycero-3-ethylphosphocholine, 1,2-dilauroyl-sn-glycero-3-ethylphosphocholine, 1-palmitoyl-2-oleoyl-sn-glycero-3-ethylphosphocholine.

[0255] In some embodiments of the lipid compositions, the lipid has a structural formula:

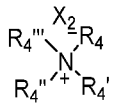
 (S-I'), wherein:

R_1 and R_2 are each independently C_8-C_{24} alkyl, C_8-C_{24} alkenyl, or a substituted version of either group;

R_3, R_3', and R_3'' are each independently C_1-C_6 alkyl or substituted C_1-C_6 alkyl;

X^- is a monovalent anion.

[0256] By way of example, and without being limited thereto, a additonal lipid (e.g., additonal lipid (e.g., SORT lipid)) of the structural formula of the immediately preceding paragraph is 1,2-dioleoyl-3-trimethylammonium-propane (18:1 DOTAP) (*e.g.*, chloride salt).

[0257] In some embodiments of the lipid compositions, the additonal lipid (e.g., additonal lipid (e.g., SORT lipid)) has a structural formula:  (S-II'), wherein:

R_4 and R_4' are each independently $alkyl_{(C6\text{-}C24)}$, $alkenyl_{(C6\text{-}C24)}$, or a substituted version of either group;

R_4'' is $alkyl_{(C\leq 24)}$, $alkenyl_{(C\leq 24)}$, or a substituted version of either group;

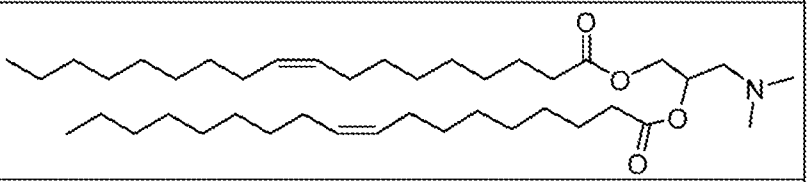
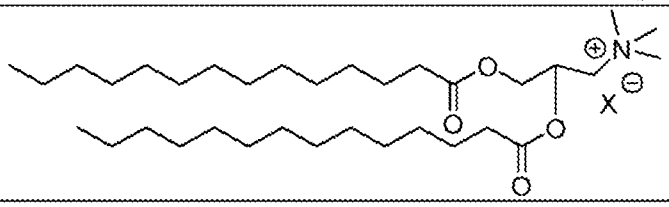
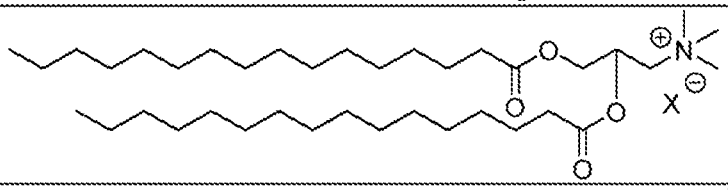
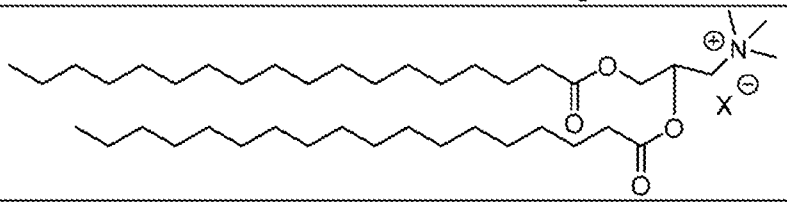
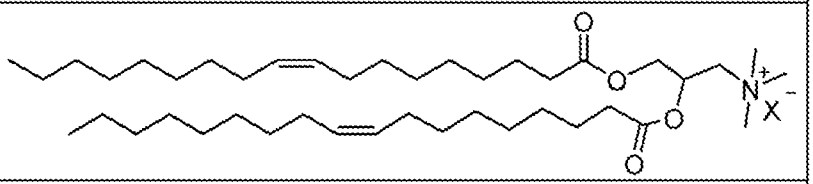
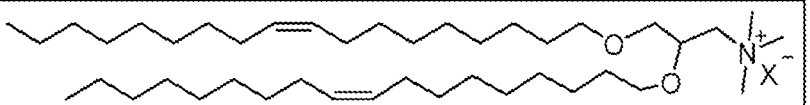
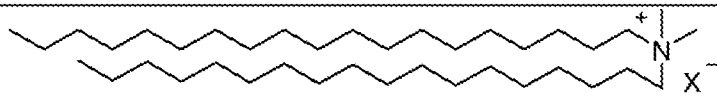
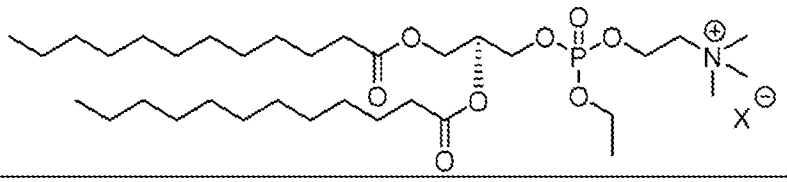
R_4''' is $alkyl_{(C1\text{-}C8)}$, $alkenyl_{(C2\text{-}C8)}$, or a substituted version of either group; and

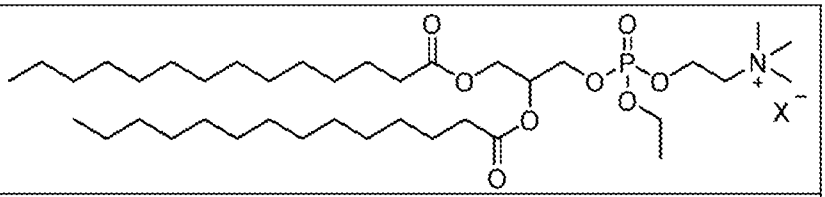
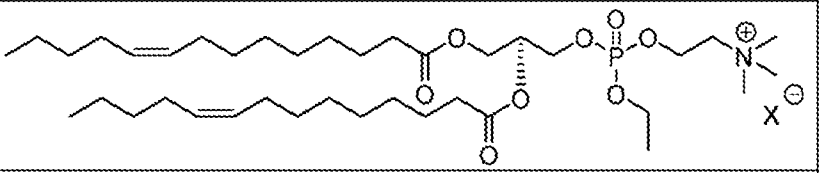
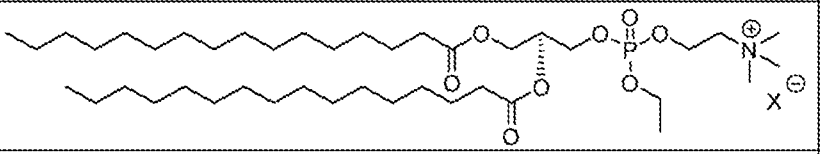
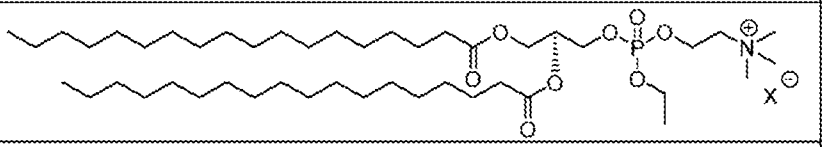
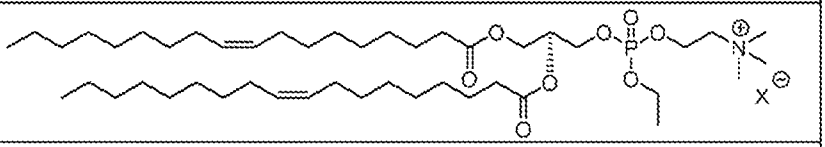
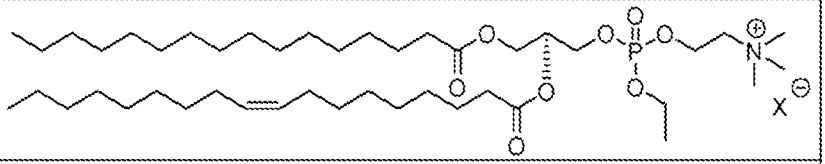
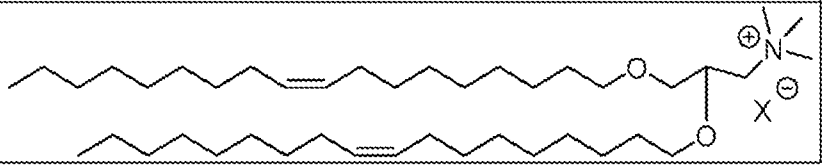
X_2 is a monovalent anion.

[0258] By way of example, and without being limited thereto, a additonal lipid (e.g., additonal lipid (e.g., SORT lipid)) of the structural formula of the immediately preceding paragraph is dimethyldioctadecylammonium (DDAB).

[0259] In some embodiments of the lipid compositions, the additional lipid is selected from the lipids set forth in **Table 6**.

Table 6. Example additonal lipid (*e.g.*, SORT lipids)

Lipid Name	Structure
1,2-Dioleoyl-3-dimethylammonium-propane (18:1 DODAP)	
1,2-dimyristoyl-3-trimethylammonium-propane (14:0 TAP) (e.g., chloride salt)	
1,2-dipalmitoyl-3-trimethylammonium-propane (16:0 TAP) (e.g., chloride salt)	
1,2-stearoyl-3-trimethylammonium-propane (18:0 TAP) (e.g., chloride salt)	
1,2-Dioleoyl-3-trimethylammonium-propane (18:1 DOTAP) (e.g., chloride salt)	
1,2-Di-O-octadecenyl-3-trimethylammonium propane (DOTMA) (e.g., chloride salt)	
Dimethyldioctadecylammonium (DDAB) (e.g., bromide salt)	
1,2-dilauroyl-sn-glycero-3-ethylphosphocholine (12:0 EPC) (e.g., chloride salt)	

1,2-Dioleoyl-sn-glycero-3-ethylphosphocholine (14:0 EPC) (e.g., chloride salt)	
1,2-dimyristoleoyl-sn-glycero-3-ethylphosphocholine (14:1 EPC) (e.g., triflate salt)	
1,2-dipalmitoyl-sn-glycero-3-ethylphosphocholine (16:0 EPC) (e.g., chloride salt)	
1,2-distearoyl-sn-glycero-3-ethylphosphocholine (18:0 EPC) (e.g., chloride salt)	
1,2-dioleoyl-sn-glycero-3-ethylphosphocholine (18:1 EPC) (e.g., chloride salt)	
1-palmitoyl-2-oleoyl-sn-glycero-3-ethylphosphocholine (16:0-18:1 EPC) (e.g., chloride salt)	
1,2-di-O-octadecenyl-3-trimethylammonium propane (18:1 DOTMA) (e.g., chloride salt)	

X^- is a counterion (e.g., Cl^-, Br^-, etc.)

[0260] In some embodiments of the lipid composition of the present application, the lipid composition comprises the additonal lipid (e.g., SORT lipid) at a molar percentage from about 20% to about 65%. In some embodiments of the lipid composition of the present application, the lipid composition comprises the additonal lipid (e.g., SORT lipid) at a molar percentage from about 25% to about 60%. In some embodiments of the lipid composition of the present application, the lipid composition comprises the additonal lipid (e.g., SORT lipid) at a molar percentage from about 30% to about 55%. In some embodiments of the lipid composition of the present application, the lipid composition comprises the additonal lipid (e.g., SORT lipid) at a molar percentage from about 20% to about 50%. In some embodiments of the lipid composition of the present application, the lipid composition comprises the additonal lipid (e.g., SORT lipid) at a molar percentage from about 30% to about 60%. In some embodiments of the lipid

composition of the present application, the lipid composition comprises the additonal lipid (e.g., SORT lipid) at a molar percentage from about 25% to about 60%. In some embodiments of the lipid composition of the present application, the lipid composition comprises the additonal lipid (e.g., SORT lipid) at a molar percentage of at least (about) 25%, at least (about) 30%, at least (about) 35%, at least (about) 40%, at least (about) 45%, at least (about) 50%, at least (about) 55%, at least (about) 60%, or at least (about) 65%. In some embodiments of the lipid composition of the present application, the lipid composition comprises the additonal lipid (e.g., SORT lipid) at a molar percentage of at most (about) 25%, at most (about) 30%, at most (about) 35%, at most (about) 40%, at least (about) 45%, at most (about) 50%, at most (about) 55%, at most (about) 60%, or at most (about) 65%. In some embodiments of the lipid composition of the present application, the lipid composition comprises the additonal lipid (e.g., SORT lipid) at a molar percentage of about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, or 65%, or of a range between (inclusive) any two of the foregoing values. Illustrative LNP compositions are presented below in **Table 7**.

[0261] Table 7: Illustrative LNP compositions.

Dendrimer SORT Helper Chol PEG	**Mol%**	**Wt%**	**Lipid:RNA (wt/ wt)**
4A3-SC7 DODAP DOPE Chol DMG-PEG	19.05 20 19.05 38.09 3.81	34.33 16.54 18.1 18.81 12.21	40
5A2-SC8 DOPE Chol DMG-PEG	23.81 23.81 47.62 4.76	51.83 17.75 18.45 11.97	40
5A2-SC8 DOTAP DOPE Chol DMG-PEG	11.9 50 11.9 23.82 2.38	30.5 41.15 10.45 10.86 7.05	40
5A2-SC8 DODAP DOPE	19.05 20 19.05	44.59 15.27 15.87	40

Chol DMG-PEG	38.09 3.81	10.3 13.96	
4A3-SC7 14:0 EPC DOPE Chol DMG-PEG	19.05 20 19.05 38.09 3.81	33.53 18.5 17.68 18.37 11.92	30
4A3-SC7 14:0 TAP DOPE Chol DMG-PEG	19.05 20 19.05 38.09 3.81	34.85 15.28 18.38 19.1 12.39	30
5A2-SC8 18:1 PA DOPE Chol DMG-PEG	22.62 5 22.62 45.24 4.52	49.93 3.67 17.1 17.77 11.52	40
5A2-SC8 14:0 TAP DOPE Chol DMG-PEG	14.29 40 14.29 28.57 2.86	37.19 28.25 12.74 13.23 8.6	40

C. Polynucleotides

[0262] In some embodiments, the method disclosed herein comprises the administration of a LNP, wherein the LNP comprises one or more polynucleotides. In some embodiments, delivery of the one or more polynucleotides to a target cell can be used for therapeutic potential. For instance, it can allow delivery of nucleic acids (e.g. mRNA) encoding a protein that stimulates the immune system near the target cell or leads the target cells to express the protein in such a way that immune cells kill the target cell. In some aspects, such delivery is beneficial in the context of treating or preventing tumors or cancer, for prophylactic uses or in the context of vaccination. In some embodiments, the LNP comprises a one or more polynucleotides. In some embodiments, the polynucleotide comprises a gRNA. In some embodiments, the polynucleotide comprises the mRNA of a base editor. In some embodiments, the polynucleotide comprises a gRNA and the mRNA of a base editor.

[0263] In some embodiments, the LNP is used to deliver a polynucleotide to a target cell. In some embodiments, the polynucleotide is a DNA. In some embodiments the polynucleotide comprises a ribonucleic acid (RNA). In some embodiments the RNA is a circRNA, an mRNA, a

siRNA, an RNAi, and/or a microRNA. In some embodiments, the RNA enhances the anti-tumor effect or the therapeutic effect of the nanoparticle. In some embodiments, the polynucleotide comprises a deoxyribonucleic acid (DNA). In some embodiments, the RNA and/or enhances the therapeutic effect of the LNP.

[0264] siRNA or small interfering RNA or silencing RNA are RNA molecules that are double stranded, non-coding RNA molecules. These RNA molecules are typically 20-24 base pairs in length and produced through the Dicer enzyme catalyzing production from long double-stranded RNA molecules. siRNA can bind to mRNA with a complementary sequence and lead to mRNA degradation. Delivery of siRNA to a cell can be used to reduce specific mRNA transcripts, leading to decreased protein production from the mRNA. This is a form of RNA interference or RNAi. Another form of RNAi includes microRNA, which are similar to siRNAs but may contain post-transcriptional modifications. MicroRNAs also work to deplete target mRNAs and thereby decrease proteins produced by the mRNAs. Delivery of microRNAs to target cells offer therapeutic potentials by decreasing production of negative proteins.

[0265] mRNA is RNA that can be translated into a protein and its delivery into target cells can lead to localized production of protein within the target cell. mRNA is rapidly degraded and so needs to be efficiently delivered to the target cell. In some embodiments, the nanoparticle is used to deliver mRNA to a target cell.

[0266] In some embodiments, the RNA is a linear RNA molecule. In some embodiments, the linear RNA is at least 600 nucleotides in length, at least 1000 nucleotides in length, or at least 1200 nucleotides in length. In some embodiments, the linear RNA is less than 2000 nucleotides in length. In some embodiments, the linear RNA is at least 600 nucleotides in length but less than 2000 nucleotides in length, at least 1000 nucleotides in length but less than 2000 nucleotides in length, at least 1200 nucleotides in length but less than 2000 nucleotides in length, at least 1400 nucleotides in length but less than 2000 nucleotides in length, at least 600 nucleotides in length but less than 1400 nucleotides in length, or at least 600 nucleotides in length but less than 2000 nucleotides in length. In some embodiments, the RNA comprises a cleaved linear RNA comprising a hydroxyl group at the 5′ terminus, and a 2′,3′-cyclic phosphate at the 3′ terminus.

[0267] In some embodiments, the RNA is a messenger RNA (mRNA) molecule. In some embodiments, the nucleic acid is a capped mRNA. In some embodiments, the mRNA molecule is greater than 2000 nucleotides, greater than 2500 nucleotides, greater than 3000 nucleotides,

greater than 3500 nucleotides, greater than 4000 nucleotides, greater than 4500 nucleotides, or greater than 5000 nucleotides in length. In some embodiments, the mRNA molecule is about 2000 nucleotides in length. In some embodiments, the mRNA molecule is about 2500 nucleotides in length. In some embodiments, the mRNA molecule is about 3000 nucleotides in length. In some embodiments, the mRNA molecule is about 3500 nucleotides in length. In some embodiments, the mRNA molecule is about 4000 nucleotides in length. In some embodiments, the mRNA molecule is about 4500 nucleotides in length. In some embodiments, the mRNA molecule is about 5000 nucleotides in length.

[0268] CircRNA is a covalently closed continuous loop of single-stranded RNA. CircRNA can be divided into four categories including exonic circRNA (ecircRNA), circular intronic RNA (ciRNA), exon-intron circRNA (ElciRNA), and intergenic circRNA.

[0269] The configuration of circRNA results in several advantages compared to linear mRNA, including resistance to exonuclease-mediated degradation, increased stability, extended half-life, increased protein expression, and reduced immunogenicity (Chen, RNA Biol, 12(4):381-388 (2015); Wesselhoeft et al., Nat Commun, 9(1):2629 (2018)). circRNA generally has a longer half-life compared to their linear mRNA counterparts (Wesselhoeft et al., Nat Commun, 9(1):2629 (2018)). Accordingly, circRNA improves protein expression (e.g., of the encoded gene product) over its lifetime compared to linear mRNAs. CircRNA can be synthesized in vitro by chemical, enzymatic, and ribozymatic approaches. One approach, using permuted intron-exon (PIE) splicing to result in RNA circularization, has been developed to express gene products from circRNA. PIE splicing systems based on Group I introns that are naturally found in the rRNA, tRNA, and mRNA genes of bacteria and non-metazoan eukaryotes can produce circRNA by self-splicing.

[0270] In some embodiments, the circular RNA is at least 600 nucleotides in length, at least 1000 nucleotides in length, or at least 1200 nucleotides in length. In some embodiments, the circular RNA is less than 2000 nucleotides in length. In some embodiments, the circular RNA is at least 600 nucleotides in length but less than 2000 nucleotides in length, at least 1000 nucleotides in length but less than 2000 nucleotides in length, at least 1200 nucleotides in length but less than 2000 nucleotides in length, at least 1400 nucleotides in length but less than 2000 nucleotides in length, at least 600 nucleotides in length but less than 1400 nucleotides in length, or at least 600 nucleotides in length but less than 2000 nucleotides in length.

[0271] In some embodiments, the circular RNA molecule is greater than 2000 nucleotides, greater than 2500 nucleotides, greater than 3000 nucleotides, greater than 3500 nucleotides, greater than 4000 nucleotides, greater than 4500 nucleotides, or greater than 5000 nucleotides in length. In some embodiments, the circular RNA molecule is about 2000 nucleotides in length. In some embodiments, the circular RNA molecule is about 2500 nucleotides in length. In some embodiments, the circular RNA molecule is about 3000 nucleotides in length. In some embodiments, the circular RNA molecule is about 3500 nucleotides in length. In some embodiments, the circular RNA molecule is about 4000 nucleotides in length. In some embodiments, the circular RNA molecule is about 4500 nucleotides in length. In some embodiments, the circular RNA molecule is about 5000 nucleotides in length.

[0272] In some embodiments, the polynucleotide comprises a DNA molecule. In some embodiments, the DNA is a naked DNA molecule. In some embodiments, the DNA is a double-stranded DNA molecule. In some embodiments, the DNA is a single-stranded DNA molecule. In some embodiments, the DNA is a modified DNA molecule. In some embodiments, the DNA is modified to enhance its stability. In some embodiments, the DNA is a closed-ended DNA molecule. In some embodiments, the DNA is a naked closed-ended DNA molecule.

[0273] In some embodiments, the DNA is at least 600 nucleotides, at least 1000 nucleotides, or at least 1200 nucleotides in length. In some embodiments, the DNA is less than 2000 nucleotides in length. In some embodiments, the DNA is at least 600 nucleotides but less than 2000 nucleotides in length, at least 1000 nucleotides but less than 2000 nucleotides in length, at least 1200 nucleotides but less than 2000 nucleotides in length, at least 1400 nucleotides but less than 2000 nucleotides in length, at least 600 nucleotides but less than 1400 nucleotides in length, or at least 600 nucleotides but less than 2000 nucleotides in length.

[0274] In some embodiments, the polynucleotide is from 2000 nucleotides to 5000 nucleotides in length. In some embodiments, the polynucleotide is from 2500 to 5000 nucleotides in length. In some embodiments, the polynucleotide is from 3000 to 5000 nucleotides in length. In some embodiments, the polynucleotide is from 3500 to 5000 nucleotides in length. In some embodiments, the polynucleotide is from 4000 to 5000 nucleotides in length. In some embodiments, the polynucleotide is from 4500 to 5000 nucleotides in length.

[0275] In some embodiments, the polynucleotide has a concentration of 0.5-3.0 mg/mL, of 1.0-3.0 mg/mL, of 2.0-3.0 mg/mL of 1.0 mg/mL. In some embodiments, the polynucleotide has

a concentration of 0.5 mg/mL, 0.6 mg/mL, 0.7 mg/mL, 0.8 mg/mL, 0.9 mg/mL, 1.0 mg/mL, 1.1 mg/mL, 1.2 mg/mL, 1.3 mg/mL, 1.4 mg/mL, or 1.5 mg/mL. In some embodiments, the polynucleotide has a concentration of 1.0 mg/mL.

[0276] In some embodiments, the polynucleotide is from 2000 to 5000 nucleotides in length and at a concentration of 1.0 mg/mL. In some embodiments, the polynucleotide is from 2500 to 5000 nucleotides in length and at a concentration of 1.0 mg/mL. In some embodiments, the polynucleotide is from 3000 to 5000 nucleotides in length and at a concentration of 1.0 mg/mL. In some embodiments, the polynucleotide is from 3500 to 5000 nucleotides in length and at a concentration of 1.0 mg/mL. In some embodiments, the polynucleotide is from 4000 to 5000 nucleotides in length and at a concentration of 1.0 mg/mL. In some embodiments, the polynucleotide is from 4500 to 5000 nucleotides in length and at a concentration of 1.0 mg/mL.

[0277] In some embodiments, the polynucleotide is from 2000 to 5000 nucleotides in length and at a concentration of 0.9 mg/mL. In some embodiments, the polynucleotide is from 2500 to 5000 nucleotides in length and at a concentration of 0.9 mg/mL. In some embodiments, the polynucleotide is from 3000 to 5000 nucleotides in length and at a concentration of 0.9 mg/mL. In some embodiments, the polynucleotide is from 3500 to 5000 nucleotides in length and at a concentration of 0.9 mg/mL. In some embodiments, the polynucleotide is from 4000 to 5000 nucleotides in length and at a concentration of 0.9 mg/mL. In some embodiments, the polynucleotide is from 4500 to 5000 nucleotides in length and at a concentration of 0.9 mg/mL.

[0278] In some embodiments, the polynucleotide is from 2000 to 5000 nucleotides in length and at a concentration of 0.8 mg/mL. In some embodiments, the polynucleotide is from 2500 to 5000 nucleotides in length and at a concentration of 0.8 mg/mL. In some embodiments, the polynucleotide is from 3000 to 5000 nucleotides in length and at a concentration of 0.8 mg/mL. In some embodiments, the polynucleotide is from 3500 to 5000 nucleotides in length and at a concentration of 0.8 mg/mL. In some embodiments, the polynucleotide is from 4000 to 5000 nucleotides in length and at a concentration of 0.8 mg/mL. In some embodiments, the polynucleotide is from 4500 to 5000 nucleotides in length and at a concentration of 0.8 mg/mL.

[0279] In some embodiments, the polynucleotide is from 2000 to 5000 nucleotides in length and at a concentration of 0.7 mg/mL. In some embodiments, the polynucleotide is from 2500 to 5000 nucleotides in length and at a concentration of 0.7 mg/mL. In some embodiments, the polynucleotide is from 3000 to 5000 nucleotides in length and at a concentration of 0.7 mg/mL. In some embodiments, the polynucleotide is from 3500 to 5000 nucleotides in length and at a

concentration of 0.7 mg/mL. In some embodiments, the polynucleotide is from 4000 to 5000 nucleotides in length and at a concentration of 0.7 mg/mL. In some embodiments, the polynucleotide is from 4500 to 5000 nucleotides in length and at a concentration of 0.7 mg/mL.

[0280] In some embodiments, the polynucleotide is from 2000 to 5000 nucleotides in length and at a concentration of 0.6 mg/mL. In some embodiments, the polynucleotide is from 2500 to 5000 nucleotides in length and at a concentration of 0.6 mg/mL. In some embodiments, the polynucleotide is from 3000 to 5000 nucleotides in length and at a concentration of 0.6 mg/mL. In some embodiments, the polynucleotide is from 3500 to 5000 nucleotides in length and at a concentration of 0.6 mg/mL. In some embodiments, the polynucleotide is from 4000 to 5000 nucleotides in length and at a concentration of 0.6 mg/mL. In some embodiments, the polynucleotide is from 4500 to 5000 nucleotides in length and at a concentration of 0.6 mg/mL.

[0281] In some embodiments, the polynucleotide has an average molecular weight of up to 20,000,000 Da. In some embodiments, the polynucleotide can have an average molecular weight of up to 2,000,000 Da. In some embodiments, the polynucleotide may have an average molecular weight of up to 150,000 Da. In some embodiments, the polynucleotide has an average molecular weight of up to 15,000 Da, 5,000 Da or 1,000 Da.

D. Formulations

[0282] In some embodiments, the method described herein comprises the LNP composition, comprising an LNP comprising a gene editing system, wherein the LNP comprises 1,2-dioleoyl-3-dimethylammonium propane (DODAP) at a molar percentage less then 25% or less then 20%; cholesterol at a molar percentage greater than 40%; and/or messenger RNA (mRNA) at a lipid:mRNA ratio less than 40:1.

[0283] In some embodiments, the method described herein comprises a LNP composition, comprising an LNP comprising a gene editing system, wherein the LNP specifically transduces lung cells; and/or the LNP delivers mRNA to lung cells or in an amount effective to increase expression and/or function of a polypeptide or polynucleotide encoded by the mRNA. In some embodiments, the lung cells comprise lung cell types that include, but are not limited to, endothelial cells or epithelial cells. In some embodiments, the lung cell type is an immune cell. In some embodiments, the lung cell type is a stem cell. In some embodiments, the epithelial cell is a ciliated cell, non-ciliated cell, a goblet cell, a brush cell (alveolar macrophage), an airway basal cell, a small granule cell, a bronchial epithelial cell, a small airway epithelial cell, and/or a

tracheal epithelial cell. In some embodiments, the lung cell type is a secretory cell and or ionocyte.

[0284] In some embodiments, the LNP specifically transduces secretory cells and/or ionocytes; and/or wherein the LNP delivers mRNA to lung cells in an amount effective to increase expression and/or function of a polypeptide or polynucleotide encoded by the mRNA.

[0285] In some embodiments, the LNP comprises an ionizable cationic lipid; a neutral phospholipid; a polyethylene-glycol (PEG)-lipid; and/or a/the cholesterol. In some embodiments, the LNP comprises a second ionizable cationic lipid. In some embodiments, the LNP comprises an anionic lipid. In some embodiments, the LNP comprises a permanently cationic lipid.

[0286] In some embodiments, the LNP comprises DODAP at a molar percentage less than 25% or less then 20%. In some embodiments, the LNP comprises a DODAP at a molar percentage of less than 5%, less than 10%, less than 15%, less than 16%, than 17%, than 18%, than 19%, than 20%, than 21%, than 22%, than 22%, than 23%, than 24% or of less than 25%.

[0287] In some embodiments, the LNP comprises DODAP at a molar percentage between 5% and 25%, between 7.5% and 25%, between 10% and 25%, between 15% and 25%, between 20% and 25%, between 5% and 20%, between 7.5% and 20%, between 10% and 20%, between 15% and 20%, between 5% and 15%, between 7.5% and 15%, between 10% and 15%, between 5% and 10%, or between 7.5% and 10%.

[0288] In some embodiments, the LNP comprises DODAP at a molar percentage between 17.5% and 20%, between 17.5% and 22.5%, between 17.5% and 25%, between 5% and 17.5%, between 7.5% and 17.5%, between 10% and 17.5%, between 12.5% and 17.5% or between 15% and 17.5%. In some embodiments, the LNP comprises DODAP at a molar percentage of 16%.

[0289] In some embodiments, the LNP comprises cholesterol at a molar percentage greater than 40%, greater than 45%, greater than 50%, greater than 55%, greater than 60%, greater than 65%, greater than 70%, greater than 75%, greater than 80%, greater than 85%, greater than 90%, greater than 95% or greater than 99%.

[0290] In some embodiments, the LNP comprises cholesterol at a molar percentage between 40% and 60%, between 45% and 60%, between 50% and 60%, between 55% and 60%, between 40% and 55%, between 40% and 50%, between 40% and 45%, between 45% and 55%, between 45% and 50% or between 50% and 55%. In some embodiments, the LNP comprises cholesterol at a molar percentage of 50%. Exemplary LNP formulations are presented below in **Table 8**.

Table 8: Exemplary fomulations

	Ionizable Lipid	SORT Lipid	Helper Lipid	Sterol	PEGylated lipid	Lipid:RNA ratio
1	4A3-SC7	DODAP	DOPE	Cholesterol	DMG-PEG	20:1-40:1
	13%-15%	15%-25%	10%-25%	40%-60%	2%-6%	
2	4A3-SC7	DODAP	DOPE	Cholesterol	DMG-PEG	25:1-40:1
	13.5%-15%	15%-25%	10%-25%	40%-55%	2%-4%	
3	4A3-SC7	DODAP	DOPE	Cholesterol	DMG-PEG	30:1-40:1
	14%-15%	10%-20%	15%-25%	40%-50%	2.5%-3%	
4	4A3-SC8	DODAP	DOPE	Cholesterol	DMG-PEG	30:1-40:1
	14%-15	15%-20%	15%-25%	40%-45%	2%-3%	
5	4A3-SC7	DODAP	DOPE	Cholesterol	DMG-PEG	30:1-40:1
	14.5%-15	7.5%-17.5%	15%-25%	40%-45%	2.5%-3%	
6	4A3-SC7	DODAP	DOPE	Cholesterol	DMG-PEG	35:1-40:1
	14.5%-15	10%-17.5%	20%-25%	40%-50%	2%-3%	
7	4A3-SC7	DODAP	DOPE	Cholesterol	DMG-PEG	35:1-40:1
	14%-15	12.5%-17.5%	20%-25%	40%-50%	2.5%-3%	
8	4A3-SC7	DODAP	DOPE	Cholesterol	DMG-PEG	35:1-40:1
	14.5%-15%	15%-17.5%	20%-25%	40%-45%	2%-3%	
9	4A3-SC7	DODAP	DOPE	Cholesterol	DMG-PEG	20:1-35:1
	13%-14.5%	20%-25%	10%-20%	45%-60%	2.5%-3.5%	
10	4A3-SC7	DODAP	DOPE	Cholesterol	DMG-PEG	25:1-35:1
	13.5%-14.5%	20%-25%	10%-20%	50%-60%	3%-3.5%	
11	4A3-SC7	DODAP	DOPE	Cholesterol	DMG-PEG	25:1-30:0
	13%-14%	17.5%-22.5%	10%-20%	45%-50%	2.5%-3.5%	
12	4A3-SC7	DODAP	DOPE	Cholesterol	DMG-PEG	20:0-30:0
	13.5%-14%	17.5%-22.5%	10%-12.5%	45%-50%	3%-3.5%	
13	4A3-SC7	DODAP	DOPE	Cholesterol	DMG-PEG	20:0-30:0
	13.5%-14%	17.5%-25%	10%-12.5%	50%-60%	2.5%-3.5%	
14	4A3-SC7	DODAP	DOPE	Cholesterol	DMG-PEG	20:1-35:1
	13%-14%	17.5%-25%	10%-12.5%	45%-60%	3%-4%	
15	5A2-SC8	DODAP	DOPE	Cholesterol	DMG-PEG	25:1-40:1
	13%-15%	15%-25%	10%-25%	40%-60%	2%-6%	
16	5A2-SC8	DODAP	DOPE	Cholesterol	DMG-PEG	25:1-40:1
	13.5%-15%	15%-25%	10%-25%	40%-55%	2%-4%	
17	4A3-SC7	DODAP	DOPE	Sitosterol	DMG-PEG	25:1-40:1
	13.5%-15%	15%-25%	10%-25%	40%-60%	2%-4%	
18	4A3-SC7	DOTMA	DOPE	Cholesterol	DMG-PEG	20:1-40:1
	13%-15%	15%-25%	10%-25%	40%-60%	2%-6%	
19	5A2-SC8	DOTMA	DOPE	Cholesterol	DMG-PEG	25:1-40:1
	13.5%-15%	15%-25%	10%-25%	40%-55%	2%-4%	
20	4A3-SC7	DODAP	DSPC	Cholesterol	DMG-PEG	25:1-40:1
	13%-15%	15%-25%	10%-25%	40%-60%	2%-6%	
21	5A2-SC8	DODAP	DSPC	Cholesterol	DMG-PEG	20:1-40:1
	13.5%-15%	15%-25%c	10%-25%	40%-55%	2%-4%	

22	4A3-SC7	DODAP	DSPC	Cholesterol	DMG-PEG2000	20:1-40:1
	13.5%-15%	15%-25%	10%-25%	40%-55%	2%-4%	
23	4A3-SC7	14:0 TAP	DOPE	Cholesterol	DMG-PEG2000	20:1-40:1
	13%-15%	15%-25%	10%-25%	40%-60%	2%-4%	
24	5A2-SC8	14:0 EPC	DOPE	Sitosterol	DMG-PEG2000	20:1-40:1
	13%-15%	15%-25%	10%-25%	40%-60%	2%-6%	
25	5A2-SC8	DOTMA	DOPE	Sitosterol	DMG-PEG2000	20:1-40:1
	13%-15%	15%-25%	10%-25%	40%-55%	2%-6%	

[0291] In some embodiments, the LNP comprises messenger RNA (mRNA). In some embodiments, the LNP comprises mRNA at a lipid:mRNA ratio less than 40:1. In some embodiments, the lipid:mRNA ratio is between 20:1 and 40:1, between 25:1 and 40:1, between 30:1 and 40:1, between 35:1 and 40:1, between 20:1 and 35:1, between 25:1 and 35:1, between 30:1 and 35:1, between 20:1 and 30:1, between 25:1 and 30:1, between 20:1 and 25:1, between 25:1 and 30:1, between 25:1 and 35:1, between 20:1 and 36:1, or between 25:1 and 36:1.

[0292] In some embodiments, the lipid:mRNA ratio is 36:1. In some embodiments, the lipid:mRNA ratio is 25:1. In some embodiments, the ionizable cationic lipid is 5A2-SC8 or 4A3-SC7; the neutral phospholipid is 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) or 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC); and/or the polyethylene-glycol (PEG)-lipid is DMG-PEG,optionally DMG-PEG2000.In some embodiments, the ionizable cationic lipid is 4A3-SC7; the neutral phospholipid is DOPE; and the polyethylene-glycol (PEG)-lipid is DMG-PEG.

[0293] In some embodiments, the LNP comprises a second cationic lipid and the second cationic lipid is DODAP.

[0294] In some embodiments, the LNP comprises a second cationic lipid and the second cationic lipid is 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA). In some embodiments, the ionizable cationic lipid is 4A3-SC7 and the LNP comprises 4A3-SC7 at a molar percentage between 13% and 15%, between 13.5% and 15%, between 14% and 15%, between 14.5 and 15%, between 13% and 14.5%, between 13.5% and 14.5%, between 14% and 14.5%, between 13% and 14%, between 13.5% and 14% or between 13% and 13.5%.

[0295] In some embodiments, the LNP comprises PEG-lipid at a molar percentage between 2% and 8%, between 4% and 8%, between 6% and 8%, between 2% and 6%, between 4% and 6%, between 2% and 4%, between 2% and 3%, between 3% and 4%, between 2.5% and 3.5%,

between 2.5% and 3% or between 3% and 3.5%. In some embodiments, the PEG-lipid at a molar percentage of (about) 3%.

[0296] In some embodiments, the LNP comprises a neutral phospholipid and the neutral phospholipid is DOPE.

[0297] In some embodiments, the LNP comprises DOPE at a molar percentage between 10% and 25%, between 10% and 20%, between 10% and 15%, between 10% and 12.5%, between 15% and 25, between 15% and 20% or between 20% and 25%. In some embodiments, the LNP comprises DOPE at a molar percentage of 11% or 22%.

[0298] In some embodiments, the LNP comprises a polynucleotide.In some embodiments, the polynucleotide is a messenger RNA (mRNA). In some embodiments, the mRNA comprises between 100 bases and 8 kilobases (kb). In some embodiments, the mRNA comprises between 1 lkb and 8 kb, between 2 kb and 8 kb, between 3 kb and 8 kb, between 4 kb and 8 kb, between 5 kb and 8 kb, between 6 kb and 8 kb, between 7 kb and 8 kb, between 1 kb and 7 kb, between 2 kb and 7 kb, between 3 kb and 7 kb, between 4 kb and 7 kb, between 5 kb and 7 kb, or between 6 kb and 7 kb, between 1 kb and 6 kb, between 2 kb and 6 kb, between 3 kb and 6 kb, between 4 kb and 6 kb, or between 5 kb and 6 kb.

[0299] In some embodiments, the mRNA comprises (about) 2 kb. In some embodiments, the mRNA comprises (about) 4.6 kb. In some embodiments, the mRNA encodes a cystic fibrosis transmembrane conductance regulator (CFTR) protein. In some embodiments, the nucleic acid sequence of the mRNA is set forth in SEQ ID NO:3. In some embodiments, the mRNA encodes a Cre recombinase. In some embodiments, the mRNA encodes a CRISPR-Cas protein. In some embodiments, the CRISPR-Cas protein is Cas9, or a variant thereof. In other embodiments, the CRISPR-Cas protein is Cas12, or a variant thereof. In some embodiments, the mRNA encodes a base editor. In some embodiments, the mRNA encodes an adenine base editor (ABE). In some embodiments, the mRNA encodes a cytosine base editor (CBE).

[0300] In some embodiments, the composition is a pharmaceutical composition. In some embodiments, the composition is an aerosolized composition. In some embodiments, the LNP composition has an encapsulation efficiency of between 50% and 99%, between 60% and 99%, between 70% and 99%, between 80% and 99%, between 90% and 99%, between 95% and 99%, between 50% and 95%, between 60% and 95%, between 70% and 95%, between 80% and 95%, between 85% and 95%, or between 90% and 95%.

[0301] In some embodiments, the LNP composition is substantively free of any anionic lipid, of any permanently cationic lipid, or of any anionic lipid and any permanently cationic lipid. In some embodiments, the LNP composition is substantively free of any ionizable cationic lipids.

III. GENE EDITING AND BASE EDITORS

[0302] In some embodiments, the methods disclosed herein comprise the administration of a LNP, wherein the LNP encapsulates a gene editing system. In some embodiments, the LNP comprises a nucleic acid encoding a base editor. In some embodiments, the LNP comprises a nucleic acid encoding a nuclease. In some embodiments, the LNP comprises a nucleic acid encoding a CRISPR-associated (Cas) polypeptide or a variant thereof. In some embodiments, the LNP comprises a guide RNA (gRNA).

A. Overview CRISPR/Cas Systems

1. *CRISPR/Cas*

[0303] The discovery and engineering of clustered regularly interspaced short palindromic repeats (CRISPR)-Cas systems for genome editing has substantially expanded and improved the editing capabilities in eukaryotic cells. Initially identified in bacteria and archaeal organisms, CRISPR-Cas systems are classified into two main groups based on the number of effector proteins involved in the cleavage of nucleic acids: class 1, which cleaves nucleic acids with multiprotein complexes and class 2, which uses single-protein effectors for cleavage (Koonin EV, Makarova KS, Zhang F. Diversity, classification and evolution of CRISPR-Cas systems. Curr Opin Microbiol. 2017 Jun; 37:67-78, which is incorporated by reference herein in its entirety). Class 2 is further subdivided by the type of Cas protein, including the DNA-targeting type-II Cas9 and type-V Cas12, and the RNA-targeting type-VI Cas13; these systems are more widely used due to the technical advantages of using a single-protein effector domain. Other important characteristics to consider when selecting an editing strategy include cell type, cellular environment, expression method of the CRISPR-Cas system, and method of delivery, as they can affect editing efficiency and frequency of undesired genomic editing events.

[0304] CRISPR-Cas class 2 systems are principally comprised of an endonuclease protein encoded by a set of CRISPR-associated (cas) genes and a short RNA sequence called guide RNA (gRNA) that guides the protein. In naturally occurring systems, CRISPR RNAs (crRNAs)

pair with trans-activating crRNAs (tracrRNAs) to facilitate the formation of the ribonucleoprotein complex that precedes editing. Engineered approaches can utilize a single gRNA (sgRNA). sgRNA is a single RNA molecule that contains both the crRNA sequence fused to the tracrRNA sequence. sgRNA can be synthetically generated or made *in vitro* or *in vivo* from a DNA template. The DNA-targeting systems also require a protospacer-adjacent motif (PAM), a short-required sequence, to occur near the target DNA site. Type-II CRISPR-Cas9 derived from *Streptococcus pyogenes* is one of the most commonly used types, and its main components are RNA-guided Cas9 endonuclease and a sgRNA. For Cas9 effectors, the PAM sequence is located 3' of the protospacer on the DNA strand not complementary to the guide RNA (Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science. 2012 Aug 17;337(6096):816-21, which is incorporated by reference herein in its entirety). The sgRNA and Cas9 nuclease form a Cas9 ribonucleoprotein that can search, bind, and cleave the specific target sequence. Once the target site is located, the endonuclease generates a double-stranded break that is followed by two self-repair mechanisms: the error-prone non-homologous end joining (NHEJ) pathway or the homology-directed repair (HDR) pathway. Editing of the nucleic acids occurs after the nuclease treatment. NHEJ can introduce insertions or deletions (indels), generating frameshift mutations or premature stop codons that inactivate the target gene. The HDR pathway can introduce precise genomic modifications but requires a homologous DNA repair template and is typically less efficient than NHEJ.

[0305] In addition to nucleases that cleave target sequences, there are three other classes of CRISPR-Cas tools: base editors, transposases, and prime editors. These four classes can mediate different types of genomic edits, including conversion, deletion, or insertion of nucleic acids. These tools can use a nuclease dead Cas or deactivated Cas (dCas) system. The nuclease domains are mutated to abolish cleavage activity and obtain a dCas, such as for Cas9, where two point mutations are introduced to attain dCas9 (Xu, Y., & Li, Z. (2020). CRISPR-Cas systems: Overview, innovations and applications in human disease research and gene therapy. Computational and Structural Biotechnology Journal., 18, 2401–15, which is incorporated by reference herein in its entirety). Importantly, the DNA binding activity of dCas9 is not affected. Fusing dCas systems with other effector domains can further extend CRISPR-Cas applications as catalytically inactive Cas nucleases are useful programmable proteins that localize the fused proteins to the target regions. For example, the CRISPR-dCas9 system fused to transcriptional

activators (CRISPRa) or repressors (CRISPRi) can be used to activate or inhibit the transcription of target genes, respectively (Xu, Y., & Li, Z. (2020). CRISPR-Cas systems: Overview, innovations and applications in human disease research and gene therapy. Computational and Structural Biotechnology Journal., 18, 2401–15, which is incorporated by reference herein in its entirety). Furthermore, the development of the prime editing system, a versatile fusion between Cas9, a reverse transcriptase, and a prime editing gRNA (pegRNA), can mediate insertions, deletions, and all 12 types of base substitutions without double-strand breaks or donor templates (Anzalone, A.V., Randolph, P.B., Davis, J.R. *et al.* Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature* **576**, 149–157 (2019), which is incorporated by reference herein in its entirety).

2. *Base editors*

[0306] In some embodiments, the methods provided herein comprises the administration of an LNP, wherein the LNP comprises a nucleic acid encoding a base editor. Base editors comprise fusions between impaired Cas enzymes which are unable to create double stranded breaks (DSBs), and a base-modification enzyme that modifies single-stranded nucleic acids only. Base editing can precisely convert one nucleic acid or base pair into another in genomic DNA or cellular RNA without double-strand breaks, DNA repair templates, or relying on repair mechanisms. Two classes of DNA base editors can convert a base pair to another: cytosine base editors (CBEs) can convert a C-G base pair into a T-A base pair, and adenine base editors (ABEs) can convert an A-T base pair to a G-C base pair. These editors can be used to perform all possible transition mutations. However, while base editors avoid the generation of indels, due to target sequence requirements, the base editing window is more restricted. Of note, cytosine to guanine base editors (CBGEs) have recently also been under development (Kurt, I.C., Zhou, R., Iyer, S. *et al.* CRISPR C-to-G base editors for inducing targeted DNA transversions in human cells. *Nat Biotechnol* **39**, 41–46 (2021), which is incorporated by reference herein in its entirety).

3. *Cytosine base editors (CBEs)*

[0307] In some embodiments, the base editor is a cytosine base editor. The first generation of CBEs were made up of a cytidine deaminase enzyme, such as an Apolipoprotein B MRNA Editing Enzyme Catalytic Subunit 1 (APOBEC1), fused to the amino terminus of a catalytically impaired Cas, which can only edit single-stranded DNA. The Cas protein can either be a catalytically inactive dCas, or a partially inactive Cas nickase (nCas), which includes mutations

that only allow the enzyme to nick the non-edited strand (Porto, E.M., Komor, A.C., *et al.* Base editing: advances and therapeutic opportunities. *Nat Rev Drug Discov* **19**, 839–859 (2020); Huang, T.P., Newby, G.A. & Liu, D.R. Precision genome editing using cytosine and adenine base editors in mammalian cells. *Nat Protoc* **16**, 1089–1128 (2021), which are incorporated by reference herein in its entirety). The use of nCas9 promotes repair of the non-edited strand using the deaminated strand as template, which increases editing efficiency (Huang, T.P., Newby, G.A. & Liu, D.R. Precision genome editing using cytosine and adenine base editors in mammalian cells. *Nat Protoc* 16, 1089–1128 (2021), which is incorporated by reference herein in its entirety). Improved versions also include uracil glycosylase inhibitor (UGI) in the CBE fusion complex to improve editing efficiency. UGI inhibits uracil DNA glycosylase (UNG), an enzyme which eliminates uracil bases through the base-excision repair (BER) pathway.

[0308] The CBE base editing process begins with sgRNA directing the Cas protein to the target locus. Cas binding to the target denatures the DNA duplex to generate a ssDNA R-loop formation that exposes a region of DNA with target cytosines that the cytidine deaminase enzyme can deaminate. CBEs convert a C-G base pair to a T-A base pair by deaminating the target cytosine to generate uracil, which will be read as a thymine by polymerases (Porto, E.M., Komor, A.C., *et al.* Base editing: advances and therapeutic opportunities. *Nat Rev Drug Discov* 19, 839–859 (2020), which is incorporated by reference herein in its entirety). Further fusion proteins have been developed as cytosine base-editors to improve base-editing efficiency, modify the editing window, and reduce indel formation during base-editing (Kim, Y., Komor, A., Levy, J. *et al.* Increasing the genome-targeting scope and precision of base editing with engineered Cas9-cytidine deaminase fusions. *Nat Biotechnol* **35**, 371–376 (2017), which is incorporated by reference herein in its entirety).

4. ***Adenine base editors (ABEs)***

[0309] As methylated cytosines undergo high rates of spontaneous cytosine deamination, and almost half of the pathogenic point mutations identified can be corrected by a base pair conversion from an A-T base pair to a G-C base pair, ABEs are highly relevant in the context of correcting disease-causing mutations. ABEs contain a catalytically impaired Cas protein, either a dCas, with no endonuclease activity, or nCas, which yield single-stranded breaks, fused to a DNA modifying enzyme, *Escherichia coli* tRNA adenosine deaminase (ecTadA). As ssDNA adenosine deaminase enzymes are not naturally occurring, TadA required extensive engineering and development through the directed mutagenesis to produce the first generation of ABE.

Similar to CBEs, sgRNA guides the Cas domain to the intended target locus, which exposes a stretch of ssDNA in an R-loop for editing. TadA deaminates an adenine's exocyclic amine to yield inosine, which is read as guanine by polymerases, converting A-T base pairs to G-C base pairs (Porto, E.M., Komor, A.C., *et al.* Base editing: advances and therapeutic opportunities. *Nat Rev Drug Discov* **19**, 839–859 (2020), which is incorporated by reference herein in its entirety).

[0310] In contrast to CBEs, which are compatible with a variety of Cas homologs, ABEs are more restricted. Optimization and protein engineering of ABEs have been performed to improve editing efficiency and expand the targeting range. For example, a previous version of ABE, ABE7.10, was compatible with limited Cas9 enzymes and exhibited lower DNA editing efficiency than CBEs. Through directed evolution, the adenosine deaminase enzyme of ABE7.10, TadA-7.10, was evolved to include 8 additional mutations, yielding TadA-8e, which allowed for greater compatibility with more Cas9 and Cas12a homologs, higher deamination rates, and overall improved DNA editing efficiency. The base editor variant ABE8e contains an ecTadA-8e fused to a *Streptococcus pyogenes* Cas9 nickase (SpCas9n). ABE8e has a broader base-editing window than ABE7.10, resulting in an editing window that is on par with that of CBEs (Richter MF, Zhao KT, Eton E, Lapinaite A, Newby GA, Thuronyi BW, Wilson C, Koblan LW, Zeng J, Bauer DE, Doudna JA, Liu DR. Phage-assisted evolution of an adenine base editor with improved Cas domain compatibility and activity. Nat Biotechnol. 2020 Jul;38(7):883-891, which is incorporated by reference herein in its entirety).

5. *Applications of CRISPR/Cas*

[0311] CRISPR-Cas systems can be used in a number of disease-relevant contexts. The generation of animal and cell models of human disease has been facilitated by CRISPR-Cas systems as it allows for the generation of knockout, knock-in, and mutagenesis models. The high sensitivity and single-base specificity also allow CRISPR-Cas systems to be used in the molecular diagnosis of disease by screening for susceptibility genes and pathogenic genes. Additionally, CRISPR-mediated genome-editing therapies are increasingly relevant in treating specific conditions caused by mutated or defective genes, including monogenic diseases caused by mutations of a single allele or a pair of alleles on homologous chromosomes.

[0312] Several important issues and challenges need to be considered during the application of CRISPR-Cas. The editing efficiency and editing byproducts are important considerations when performing genome-editing alterations. There are also challenges associated with off-target effects and delivery methods. Modifications that enhance specificity and fidelity and

reduce off-target effects have been made to Cas proteins. Improving the specificity when designing sgRNAs has also decreased the frequency of off-target effects. Lastly, effective delivery of CRISPR-Cas components remains a challenge. The ideal delivery methods should have reduced toxicity, high efficiency, low mutagenicity of the chromosomal DNA, and be cost-effective. *See e.g.,* Jinek, M. et al., A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity, *Science* 2012, Koonin, E. et al., Diversity, classification and evolution of CRISPR-Cas systems, *Current opinion in microbiology* 2017, Rees, H. and Liu, D., Base editing: precision chemistry on the genome and transcriptome of living cells, *Nature Reviews Genetics* 2018, Adli, M., The CRISPR tool kit for genome editing and beyond, *Nature communications* 2018, and Xu, Y. and Li, Z., CRISPR-Cas systems: Overview, innovations and applications in human disease research and gene therapy, *Computational and structural biotechnology journal* 2020, which are incorporated by reference herein in its entirety.

B. Gene Editing Systems

[0313] The present disclosure provides a method comprising administering a lipid nanoparticle (LNP) that comprises a gene editing system. As used herein, the term "gene editing system" refers to a DNA or RNA editing system that comprises an enzyme element that can bind to DNA or RNA. The enzyme element can comprise an enzyme with nuclease activity, including but not limited to endonuclease activity, or a nucleic acid encoding such an enzyme. The enzyme element can comprise an enzyme with recombinase activity, or a nucleic acid encoding such an enzyme. The gene editing system can further comprise a guide RNA (gRNA) element that comprises a RNA molecule comprising a nucleotide sequence substantially complementary to a nucleotide sequence at one or more target genomic regions. The enzyme element can comprise an enzyme that is guided or brought to a target genomic region(s) by a guide RNA element, or a nucleic acid encoding such an enzyme. In some embodiments, the enzyme element can be naturally occurring. In some embodiments, the enzyme element can comprise a fusion protein.

[0314] In some embodiments, the gene editing system comprises a CRISPR-Cas enzyme, or a variant thereof, and a guide RNA (gRNA). In some embodiments, the gene editing system comprises a fusion enzyme. In some embodiments, the fusion enzyme is a fusion of a Cas enzyme, or a variant thereof, with another enzyme, such as a recombinase, a polymerase, a deaminase, a reverse transcriptase, or another enzyme that binds to nucleic acids. In one embodiment, the fusion enzyme is a fusion between a catalytically impaired Cas enzyme

capable of binding to a specific nucleotide sequence and a base-modifying enzyme. In one embodiment, the base-modifying enzyme is an adenosine deaminase. In another embodiment, the base-modifying enzyme is a cytidine deaminase. A base editor comprises a base-modifying enzyme, such as an adenosine deaminase or a cytidine deaminase, fused to a catalytically impaired Cas protein capable of binding to a specific nucleotide sequence.

[0315] In some embodiments, the gene editing system comprises a base editor and a guide RNA (gRNA). In some embodiments, the base editor is an adenine base editor (ABE). In other embodiments, the base editor is a cytosine base editor (CBE). An ABE comprises a tRNA adenosine deaminase (TadA) protein, or a variant thereof, fused to a catalytically impaired Cas protein capable of binding to a specific nucleotide sequence. A CBE comprises a cytidine deaminase protein, or a variant thereof, fused to a catalytically impaired Cas protein capable of binding to a specific nucleotide sequence. In some embodiments, the catalytically impaired Cas protein is a dead or deactivated Cas9 (dCas). In some embodiments, the catalytically impaired Cas is a Cas9 nickase (nCas). In some embodiments, a dCas has no endonuclease activity. In other embodiments, a nCas creates single-stranded breaks in a nucleic acid.

[0316] In some embodiments, the methods described herein include a gene editing system, a composition comprising a gene editing system, or a gene editing system assembled with the lipid composition. In some embodiment, the lipid composition comprises one or more polypeptides. Some polypeptides may include endonucleases such as any one of the nuclease enzymes described herein. For example, the nuclease enzyme may include from CRISPR-associated (Cas) proteins or Cas nucleases including type I CRISPR-associated (Cas) polypeptides, type II CRISPR-associated (Cas) polypeptides, type III CRISPR-associated (Cas) polypeptides, type IV CRISPR-associated (Cas) polypeptides, type V CRISPR-associated (Cas) polypeptides, and type VI CRISPR-associated (Cas) polypeptides; zinc finger nucleases (ZFN); transcription activator-like effector nucleases (TALEN); meganucleases; RNA-binding proteins (RBP); CRISPR-associated RNA binding proteins; recombinases; flippases; transposases; Argonaute (Ago) proteins (*e.g.*, prokaryotic Argonaute (pAgo), archaeal Argonaute (aAgo), eukaryotic Argonaute (eAgo), and Natronobacterium gregoryi Argonaute (NgAgo)); Adenosine deaminases acting on RNA (ADAR); CIRT, PUP, homing endonuclease, or any functional fragment thereof, any derivative thereof; any variant thereof; and any fragment thereof.

[0317] In some embodiments, the gene editing system comprises a zinc finger nuclease (ZFN). A zinc-finger nuclease (ZFN) comprises a zinc-finger DNA binding domain fused to a

DNA cleavage domain. For example, fusion proteins comprise the cleavage domain (or cleavage half-domain) from at least one Type IIS restriction enzyme and one or more zinc finger binding domains, which may or may not be engineered. In some embodiments, the cleavage domain is from the Type IIS restriction endonuclease FokI, which generally catalyzes double-stranded cleavage of DNA, at 9 nucleotides from its recognition site on one strand and 13 nucleotides from its recognition site on the other. See, e.g., U.S. Pat. Nos. 5,356,802; 5,436,150 and 5,487,994; Li et al. (1992) Proc. Natl. Acad. Sci. USA 89:4275-4279; Li et al. (1993) Proc. Natl. Acad. Sci. USA 90:2764-2768; Kim et al. (1994a) Proc. Natl. Acad. Sci. USA 91:883-887; Kim et al. (1994b) J. Biol. Chem. 269: 978-982. Some gene-specific engineered zinc fingers are available commercially. For example, a platform called CompoZr, for zinc-finger construction is available that provides specifically targeted zinc fingers for thousands of targets. See, e.g., Gaj et al., Trends in Biotechnology, 2013, 31(7), 397-405. In some cases, commercially available zinc fingers are used or are custom designed.

[0318] In some embodiments, the gene editing system comprises a Transcription Activator like Effector (TALE). TALE proteins are from the bacterial species *Xanthomonas* and comprise a plurality of repeated sequences, each repeat comprising di-residues in position 12 and 13 (RVD) that are specific to each nucleotide base of the nucleic acid targeted sequence. Binding domains with similar modular base-per-base nucleic acid binding properties (MBBBD) can also be derived from different bacterial species. In some embodiments, a "TALE DNA binding domain" or "TALE" is a polypeptide comprising one or more TALE repeat domains/units. The repeat domains, each comprising a repeat variable diresidue (RVD), are involved in binding of the TALE to its cognate target DNA sequence. A single "repeat unit" (also referred to as a "repeat") is typically 33-35 amino acids in length and exhibits at least some sequence homology with other TALE repeat sequences within a naturally occurring TALE protein. TALE proteins may be designed to bind to a target site using canonical or non-canonical RVDs within the repeat units. See, e.g., U.S. Pat. Nos. 8,586,526 and 9,458,205.

[0319] In some embodiments, the gene editing system comprises a TAL-effector nuclease (TALEN). In some embodiments, a "TALE-nuclease" (TALEN) is a fusion protein comprising a nucleic acid binding domain typically derived from a Transcription Activator Like Effector (TALE) and a nuclease catalytic domain that cleaves a nucleic acid target sequence. The catalytic domain comprises a nuclease domain or a domain having endonuclease activity, like for instance I-TevI, ColE7, NucA and Fok-I. In a particular embodiment, the TALE domain can

be fused to a meganuclease like for instance I-CreI and I-OnuI or functional variant thereof. In some embodiments, the TALEN is a monomeric TALEN. A monomeric TALEN is a TALEN that does not require dimerization for specific recognition and cleavage, such as the fusions of engineered TAL repeats with the catalytic domain of I-TevI described in WO2012138927. TALENs have been described and used for gene targeting and gene modifications (see, e.g., Boch et al. (2009) Science 326(5959): 1509-12.; Moscou and Bogdanove (2009) Science 326(5959): 1501; Christian et al. (2010) Genetics 186(2): 757-61; Li et al. (2011) Nucleic Acids Res 39(1): 359-72).

1. *CRISPR-Cas proteins*

[0320] As used herein, the term "Cas protein" or "CRISPR-Cas protein" refers to a full-length Cas protein obtained from nature, a recombinant Cas protein having a sequence that differs from a naturally occurring Cas protein, or any fragment of a Cas protein that nevertheless retains all or a significant amount of the requisite basic functions needed for the disclosed methods, *i.e.,* (i) possession of nucleic-acid binding of the Cas protein to a target DNA, (ii) ability to create double-strand breaks in the target DNA sequence; and/or (iii) ability to nick the target DNA sequence on one strand. The Cas proteins contemplated herein comprise CRISPR Cas9 proteins, as well as Cas9 equivalents, variants (*e.g.,* Cas9 nickase (nCas9) or nuclease inactive Cas9 (dCas9) homologs, orthologs, or paralogs, whether naturally occurring or non-naturally occurring (*e.g.,* engineered or recombinant), and may include a Cas9 equivalent from any type of CRISPR system (*e.g.,* type II, V, VI), including Cpf1 (a type-V CRISPR-Cas systems), C2c1 (a type V CRISPR-Cas system), C2c2 (a type VI CRISPR-Cas system) and C2c3 (a type V CRISPR-Cas system). Further Cas-equivalents are described in Makarova *et al.,* "C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector," *Science* 2016; 353(6299), the contents of which are incorporated herein by reference.

[0321] The term "Cas9" or "Cas9 domain" comprises any naturally occurring Cas9 from any organism, any naturally-occurring Cas9 equivalent or functional fragment thereof, any Cas9 homolog, ortholog, or paralog from any organism, and any mutant or variant of a Cas9, naturally-occurring or engineered. The term Cas9 is not meant to be particularly limiting and may be referred to as a "Cas9 or equivalent." Additional Cas9 sequences and structures are well known to those of skill in the art (see, *e.g.,* "Complete genome sequence of an M1 strain of Streptococcus pyogenes." Ferretti et al., J.J., McShan W.M., Ajdic D.J., Savic DJ., Savic G., Lyon K., Primeaux C., Sezate S., Suvorov A.N., Kenton S., Lai H.S., Lin S.P., Qian Y., Jia

H.G., Najar F.Z., Ren Q., Zhu H., Song L., White J., Yuan X., Clifton S.W., Roe B.A., McLaughlin R.E., *Proc. Natl. Acad. Sci.* U.S.A. 98:4658-4663(2001); "CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III." De1tcheva E., Chylinski K., Sharma C.M., Gonzales K., Chao Y., Pirzada Z.A., Eckert M.R., Vogel J., Charpentier E., Nature 47 l:602-607(2011); and "A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity." Jinek M., Chylinski K., Fonfara I., Hauer M., Doudna J.A., Charpentier E. *Science* 337:816-821(2012), the entire contents of each of which are incorporated herein by reference).

[0322] Examples of Cas9 and Cas9 equivalents are provided as follows; however, these specific examples are not meant to be limiting. The base editors of the present disclosure may use any suitable CRISPR-Cas domain, including any suitable Cas9 or Cas9 equivalent.

[0323] In some aspects, the disclosure provides base editors comprising one or more adenosine deaminase variants disclosed herein and a CRISPR-Cas protein. In some embodiments, the CRISPR-Cas protein comprises a Cas homolog. The CRISPR-Cas protein may be selected from any CRISPR associated protein, including but not limited to a Cas9, a Cas9n, a dCas9, a CasX, a CasY, a C2c1, a C2c2, a C2c3, a GeoCas9, a CjCas9, a Cas12a, a Cas12b, a Cas12g, a Cas12h, a Cas12i, a Cas13b, a Cas13c, a Cas13d, a Cas14, a Csn2, an xCas9, an SpCas9-NG, an SpCas9-NG-CP1041, an SpCas9-NG-VRQR, an LbCas12a, an AsCas12a, a Cas9-KKH, a circularly permuted Cas9, an Argonaute (Ago) domain, a SmacCas9, a Spy-macCas9, an SpCas9-VRQR, an SpCas9-NRRH, an SpaCas9-NRTH, an SpCas9-NRCH. Other non-limiting examples of Cas proteins include Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csn1 and Csx12), Cas10, Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csxl, Csx15, Csf1, Csf2, Csf3, Csf4, homologs thereof, or modified versions thereof. In certain embodiments, the CRISPR-Cas protein comprises or is a Cas9 protein or a Cas12a protein derived from *S. pyogenes* or *S. aureus*. In some embodiments, the CRISPR-Cas protein comprises a nuclease dead Cas9 (dCas9) protein, a Cas9 nickase (nCas9) protein, or a nuclease active Cas9 protein. In some embodiments, the Cas protein may be complexed with a guide polynucleotide.

[0324] Exemplary CRISPR-Cas proteins include but are not limited to *S. pyogenes* Cas9 nickase (SpCas9n) and *S. aureus* Cas9 nickase (SaCas9n). Additional exemplary CRISPR-Cas proteins include *S. aureus* Cas9-KKH (SaCas9-KKH), LbCas12a, enAsCas12a (an engineered

AsCas12a recently reported by Joung *et al.),* SpCas9-NG, SpCas9-VRQR, SpCas9-NG-CPl041, SpCas9-NG-VRQR, SpCas9-NRCH, CP1028-SpCas9, and CP1041-SpCas9. In some embodiments, the CRISPR-Cas protein comprises a Cas9 nickase (nCas9) protein. In some embodiments, the CRISPR-Cas protein comprises an SpCas9n protein. In certain embodiments, the CRISPR-Cas protein of any of the disclosed base editors is a SaCas9n. In certain embodiments, the CRISPR-Cas protein of any of the disclosed base editors is an SpCas9-NRCH. In certain embodiments, the CRISPR-Cas protein of any of the disclosed base editors is an LbCas12a, *e.g.,* a catalytically inactive or "dead" LbCas12a. In certain embodiments, the CRISPR-Cas protein of any of the disclosed base editors is an AsCas12a, *e.g.,* an enAsCas12a. In certain embodiments, the CRISPR-Cas protein of any of the disclosed base editors is a circular permuted variant of SpCas9, *e.g.,* a CP1028 SpCas9 or a CP1041 SpCas9. In certain embodiments, the CRISPR-Cas protein of any of the disclosed base editors is an evolved SpCas9, *e.g.,* an SpCas9-NG. In certain embodiments, the CRISPR-Cas protein of any of the disclosed base editors is an SpCas9-NG-CP1041. In certain embodiments, the CRISPR-Cas protein of any of the disclosed based editors is SpCas9-NG-VRQR.

[0325] The nuclease in the compositions described herein may be Cas9 (*e.g.*, from *S. pyogenes* or *S. pneumonia*). The CRISPR-Cas protein can direct cleavage of one or both strands at the location of a target sequence, such as within the target sequence and/or within the complement of the target sequence of any one of the genes described herein. For example, the CRISPR enzyme may be directed and cleaved a genomic locus of CFTR.

[0326] The CRISPR-Cas protein may be mutated with respect to a corresponding wild-type enzyme such that the mutated CRISPR-Cas protein lacks the ability to cleave one or both strands of a target polynucleotide containing a target sequence. For example, an aspartate-to-alanine substitution (D10A) in the RuvC catalytic domain of Cas9 from *S. pyogenes* converts Cas9 from a nuclease that cleaves both strands to a nickase (cleaves a single strand). In some embodiments, a Cas9 nickase may be used in combination with guide sequence(s), *e.g.*, two guide sequences, which target respectively sense and antisense strands of the DNA target. This combination allows both strands to be nicked and used to induce non-homologous end-joining (NHEJ) or homology directed repair (HDR). *See e.g.*, WO2022216619, which is incorporated herein by reference in its entirety.

[0327] Adenine base editors can deaminate an adenosine that leads to a point mutation from adenine (A) to guanine (G). The adenine base editors can comprise the canonical SpCas9, or any

ortholog Cas9 protein, or any variant Cas9 protein including any naturally occurring variant, mutant, or otherwise engineered version of Cas9 that is known or which can be made or evolved through a directed evolutionary or otherwise mutagenesis process. In various embodiments, the CRISPR-Cas protein has nickase activity, i.e., can only cleave one strand of the target DNA sequence. In other embodiments, the CRISPR-Cas protein has an inactive nuclease, e.g., are "dead" or deactivated proteins. Other variant Cas9 proteins that may be used are those having a smaller molecular weight than the canonical SpCas9 (e.g., for easier delivery) or having modified or rearranged primary amino acid sequence (e.g., the circular permutant forms). The adenine base editors described herein can also comprise Cas9 equivalents, including Cas 12a/Cpf1 and Cas12b proteins. The CRISPR-Cas proteins used herein (e.g., SpCas9, SaCas9, SaCas9 variant or SpCas9 variant) can also contain various modifications that alter/enhance their PAM specificities. The disclosure contemplates any Cas9, Cas9 variant, or Cas9 equivalent which has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.9% sequence identity to a reference Cas9 sequence, such as a reference SpCas9 canonical sequence , a reference SaCas9 canonical sequence or a reference Cas9 equivalent (e.g., Cas12a/Cpf1).

[0328] In certain embodiments, the adenine base editors contemplated herein can include a Cas9 protein that is of smaller molecular weight than the canonical SpCas9 sequence. In some embodiments, the smaller-sized Cas9 variants may facilitate delivery to cells, e.g., by an expression vector, nanoparticle, or other means of delivery. The canonical SpCas9 protein is 1368 amino acids in length and has a predicted molecular weight of 158 kilodaltons. Smaller-sized Cas9 variants can be at least 1300 amino acids, or at least less than 1290 amino acids, or than less than 1280 amino acids, or less than 1270 amino acid, or less than 1260 amino acid, or less than 1250 amino acids, or less than 1240 amino acids, or less than 1230 amino acids, or less than 1220 amino acids, or less than 1210 amino acids, or less than 1200 amino acids, or less than 1190 amino acid, or less than 1180 amino acids, or less than 1170 amino acids, or less than 1160 amino acids, or less than 1150 amino acids, or less than 1140 amino acids, or less than 1130 amino acids, or less than 1120 amino acids, or less than 1110 amino acids, or less than 1100 amino acids, or less than 1050 amino acids, or less than 1000 amino acids, or less than 950 amino acids, or less than 900 amino acids, or less than 850 amino acids, or less than 800 amino acids, or less than 750 amino acids, or less than 700 amino acids, or less than 650 amino acids,

or less than 600 amino acids, or less than 550 amino acids, or less than 500 amino acids, but at least larger than about 400 amino acids and retaining the required functions of the Cas9 protein.

[0329] In one embodiment, the base editors may comprise the "canonical SpCas9" nuclease from *S. pyogenes,* which has been widely used as a tool for genome engineering. This Cas9 protein is a large, multi-domain protein containing two distinct nuclease domains. Point mutations can be introduced into Cas9 to abolish one or both nuclease activities, resulting in a nickase Cas9 (nCas9) or dead Cas9 (dCas9), respectively, that still retains its ability to bind DNA in a sgRNA programmed manner. In principle, when fused to another protein or domain, Cas9 or variant thereof (*e.g.*, nCas9) can target that protein to virtually any DNA sequence simply by co-expression with an appropriate sgRNA. As used herein, the canonical SpCas9 protein refers to the wild type protein from *S. pyogenes* having the following amino acid sequence. The base editors described herein may include canonical SpCas9, or any variant thereof having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity with a wild type Cas9 sequence provided above. The adenine base editors described herein may include any of the above SpCas9 sequences, or any variant thereof having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity thereto.

[0330] In other embodiments, the Cas9 protein can be a wild type Cas9 ortholog from another bacterial species. In some embodiments, the Cas9 protein is an ortholog comprising a sequence of at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity to any of the below orthologs. In some embodiments, the adenine base editor may include any of the above Cas9 ortholog sequences, or any variants thereof having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity thereto.

[0331] The CRISPR-Cas domain may include any suitable homologs and/or orthologs or naturally occurring enzymes, such as Cas9. Cas9 homologs and/or orthologs have been described in various species, including, but not limited to, *S. pyogenes* and *S. thermophilus.* Preferably, the Cas moiety is configured (*e.g.*, mutagenized, recombinantly engineered, or otherwise obtained from nature) as a nickase. Such Cas9 nucleases and sequences include Cas9 sequences from the organisms and loci disclosed in Chylinski, Rhun, and Charpentier, "The tracrRNA and Cas9 families of type II CRlSPR-Cas immunity systems" (2013) RNA Biology 10:5, 726-737; the entire contents of which are incorporated herein by reference. In some embodiments, a Cas9 nuclease has an inactive (*e.g.*, an inactivated) DNA cleavage domain, that is, the Cas9 is a nickase.

[0332] In some embodiments, the disclosed base editors may comprise a catalytically inactive, deactivated, or "dead," CRISPR-Cas domain. Exemplary catalytically inactive domains in the disclosed adenine base editors are dead *S. pyogenes* Cas9 (dSpCas9), dead *S. aureus* Cas9 (dSaCas9) and dead *Lachnospiraceae bacterium* Cas12a (dLbCas12a).

[0333] In certain embodiments, the base editors described herein may include a dead Cas9, *e.g.*, dead SpCas9, which has no nuclease activity due to one or more mutations that inactivate both nuclease domains of SpCas9, namely the RuvC domain (which cleaves the nonprotospacer DNA strand) and HNH domain (which cleaves the protospacer DNA strand). The nuclease inactivation may be due to one or mutations that result in one or more substitutions and/or deletions in the amino acid sequence of the encoded protein, or any variants thereof having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity thereto. The D10A and N580A mutations in the wild-type *S. aureus* Cas9 amino acid sequence may be used to form a dSaCas9. Accordingly, in some embodiments, the CRISPR-Cas domain of the base editors provided herein comprises a dSaCas9 that has D10A and N580A mutations relative to the wild-type SaCas9 sequence.

[0334] As used herein, the term "dCas9" refers to a nuclease-inactive Cas9 or nuclease-dead Cas9. The term dCas9 is not meant to be particularly limiting and may be referred to as a "dCas9 or equivalent." Any suitable mutation which inactivates both Cas9 endonucleases may be used to form the dCas9.

[0335] In other embodiments, dCas9 corresponds to, or comprises in part or in whole, a Cas9 amino acid sequence having one or more mutations that inactivate the Cas9 nuclease activity. In other embodiments, Cas9 variants having mutations may result in the full or partial inactivation of the endogenous Cas9 nuclease activity (*e.g.*, dCas9 or nCas9, respectively). In some embodiments, variants or homologues of Cas9 (*e.g.*, variants of Cas9 from *Streptococcus pyogenes*) are provided which are at least about 70% identical, at least about 80% identical, at least about 90% identical, at least about 95% identical, at least about 98% identical, at least about 99% identical, at least about 99.5% identical, or at least about 99.9% identical to the sequence of wild type Cas9 from *Streptococcus pyogenes*.

[0336] In some embodiments, the CRISPR-Cas protein of any of the disclosed base editors comprises a dead *S. pyogenes* Cas9 (dSpCas9). In some embodiments, the CRISPR-Cas protein of any of the disclosed base editors comprises a dead *Lachnospiraceae bacterium* Cas12a (dLbCas12a).

[0337] In some embodiments, the disclosed base editors may comprise a CRISPR-Cas protein that comprises a nickase. In some embodiments, the base editors described herein comprise a Cas9 nickase. The term "Cas9 nickase" or "nCas9" refers to a variant of Cas9 which is capable of introducing a single-strand break in a double strand DNA molecule target. In some embodiments, the Cas9 nickase comprises only a single functioning nuclease domain. The wild type Cas9 (*e.g.*, the canonical SpCas9) comprises two separate nuclease domains, namely, the RuvC domain (which cleaves the non-protospacer DNA strand) and HNH domain (which cleaves the protospacer DNA strand). In one embodiment, the Cas9 nickase comprises a mutation in the RuvC domain which inactivates the RuvC nuclease activity.

[0338] The catalytically impaired Cas9 protein can be, but is not limited to NRRH, NRTH, NRCH, xCas9, SpCas9-NG, SpCas9, SpG, SpRY, SauriCas9, SaCas9, Nme2Cas9, VRER-SpCas9, and VQRSpCas9. In some embodiments, the catalytically impaired Cas9 protein is SpCas9-NG.

[0339] In some embodiments, the CRISPR-Cas protein of any of the disclosed base editors comprises an *S. pyogenes* Cas9 nickase (SpCas9n). In some embodiments, the CRISPR-Cas protein of any of the disclosed base editors comprises an *S. aureus* Cas9 nickase (SaCas9n).

[0340] The CRISPR-Cas proteins used in the base editors described herein may also include other Cas9 variants that area at least about 80% identical, at least about 90% identical, at least about 95% identical, at least about 96% identical, at least about 97% identical, at least about 98% identical, at least about 99% identical, at least about 99.5% identical, or at least about 99.9% identical to any reference Cas9 protein, including any wild type Cas9, or mutant Cas9 (*e.g.*, a dead Cas9 or Cas9 nickase), or circular permutant Cas9, or other variant of Cas9 disclosed herein or known in the art.

[0341] In some embodiments, the Cas9 variant comprises a fragment of a reference Cas9 (*e.g.,* a gRNA binding domain or a DNA-cleavage domain), such that the fragment is at least about 70% identical, at least about 80% identical, at least about 90% identical, at least about 95% identical, at least about 96% identical, at least about 97% identical, at least about 98% identical, at least about 99% identical, at least about 99.5% identical, or at least about 99.9% identical to the corresponding fragment of wild type Cas9. In some embodiments, the fragment is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%

identical, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% of the amino acid length of a corresponding wild type Cas9.

[0342] In some embodiments, the disclosure also may utilize Cas9 fragments which retain their functionality and which are fragments of any herein disclosed Cas9 protein. In some embodiments, the Cas9 fragment is at least 100 amino acids in length. In some embodiments, the fragment is at least 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, or at least 1300 amino acids in length.

[0343] In various embodiments, the base editors disclosed herein may comprise one of the Cas9 variants described as follows, or a Cas9 variant thereof having at least about 70% identical, at least about 80% identical, at least about 90% identical, at least about 95% identical, at least about 96% identical, at least about 97% identical, at least about 98% identical, at least about 99% identical, at least about 99.5% identical, or at least about 99.9% identical to any reference Cas9 variants.

[0344] In some embodiments, the base editors described herein can include any Cas9 equivalent. As used herein, the term "Cas9 equivalent" is a broad term that encompasses any CRISPR-Cas protein that serves the same function as Cas9 in the present adenine base editors despite that its amino acid primary sequence and/or its three-dimensional structure may be different and/or unrelated from an evolutionary standpoint. Thus, while Cas9 equivalents include any Cas9 ortholog, homolog, mutant, or variant described or embraced herein that are evolutionarily related, the Cas9 equivalents also embrace proteins that may have evolved through convergent evolution processes to have the same or similar function as Cas9, but which do not necessarily have any similarity with regard to amino acid sequence and/or three dimensional structure. The adenine base editors described here embrace any Cas9 equivalent that would provide the same or similar function as Cas9 despite that the Cas9 equivalent may be based on a protein that arose through convergent evolution.

[0345] However, the Cas9 equivalents contemplated herein may also be obtained from archaea, which constitute a domain and kingdom of single-celled prokaryotic microbes different from bacteria.

2. *Base Editors*

[0346] In some embodiments, the gene editing system comprises a base editor and a guide RNA (gRNA). In some embodiments, the base editor is an adenine base editor (ABE). In other embodiments, the base editors is a cytosine base editor (CBE). An ABE comprises a tRNA

adenosine deaminase (TadA) protein, or a variant thereof, fused to a catalytically impaired Cas protein capable of binding to a specific nucleotide sequence. A CBE comprises a cytidine deaminase protein, or a variant thereof, fused to a catalytically impaired Cas protein capable of binding to a specific nucleotide sequence. In some embodiments, the present disclosure provides base editors having adenosine deaminase domains that are mutated (*e.g.* evolved to have mutations) that enable the deaminase domain to have improved activity when used with Cas homologs (*e.g.,* homologs other than SpCas9). Accordingly, the present disclosure provides variants of adenosine deaminases (*e.g.,* variants of TadA-7.10). One example of an adenosine deaminase variant is TadA-8e, which contains eight additional mutations relative to the TadA-7.10 deaminase domain (where TadA-7.10 contains the mutations W23R, H36L, P48A, R51L, L84F, A106V, D108N, H123Y, S146C, D147Y, R152P, E155V, I156F, and K157N in the ecTadA sequence). TadA-8e is broadly compatible with diverse Cas9 or Cas12 homologs, and exhibits improved editing efficiencies when paired with previously incompatible Cas9 or Cas12 homologs. For instance, disclosed adenosine deaminase variants such as TadA-8e exhibit higher editing efficiencies when paired in a base editor with certain Cas9 variants, such as circularly permuted variants CP1041 and CP1028, than exhibited by the TadA-7.10 deaminase. In some embodiments, the adenosine deaminase is TadA-7.10. In some embodiments, the adenosine deaminase is TadA-8e.

[0347] In one embodiment, the base editor is ABE 0.1, ABE 0.2, ABE 1.1, ABE 1.2, ABE 2.1, ABE 2.2, ABE 2.3, ABE 2.4, ABE 2.5, ABE 2.6, ABE 2.7, ABE 2.8, ABE 2.9, ABE 2.10, ABE 2.11, ABE 2.12, ABE 3.1, ABE 3.2, ABE 3.3, ABE 3.4, ABE 3.5, ABE 3.6, ABE 3.7, ABE 3.8, ABE 4.1, ABE 4.2, ABE 4.3, ABE 5.1, ABE 5.2, ABE 5.3, ABE 5.4, ABE 5.5, ABE 5.6, ABE 5.7, ABE 5.8, ABE 5.9, ABE 5.10, ABE 5.11, ABE 5.12, ABE 5.13, ABE 5.14, ABE 6.1, ABE 6.2, ABE 6.3, ABE 6.4, ABE 6.5, ABE 6.6, ABE 7.1, ABE 7.2, ABE 7.3, ABE 7.4, ABE 7.5, ABE 7.6, ABE 7.7, ABE 7.8, ABE 7.9, ABE 7.10, or ABEmax, as described in US 2020/0308571, which is hereby incorporated by reference in its entirety. In another embodiment, the base editor is an ABE8 variant. In some embodiments, the base editor is ABE8e.

[0348] Exemplary ABEs include, but are not limited to, ABE7.10 (or ABEmax), ABE8e, SaKKH-ABE8e, NG-ABE8e, ABE-xCas9, SaKKH-ABE7.10, NG-ABE7.10, ABE7.10-VRQR, ABE8e-NRTH, ABE8e-NRRH, ABE8e-NRCH, NG-CP1041-ABE8e, ABE8eCP1041, ABE8e-CP 1028, and ABE8e-VRQR. In certain embodiments, the ABE used in the disclosed methods is an ABE8e or an ABE7.10. ABE8e may be referred to herein as "ABE8" or "ABE8.0." The

ABE8e base editor and variants thereof may comprise an adenosine deaminase domain containing a TadA-8e adenosine deaminase monomer (monomer form) or a TadA-8e adenosine deaminase homodimer or heterodimer (dimer form). ABE8e is further described in Richter MF, Zhao KT, Eton E, Lapinaite A, Newby GA, Thuronyi BW, Wilson C, Koblan LW, Zeng J, Bauer DE, Doudna JA, Liu DR. Phage-assisted evolution of an adenine base editor with improved Cas domain compatibility and activity. *Nat Biotechnol.* 2020 Jul;38(7):883-891, which is incorporated by reference herein in its entirety. Other ABEs can be used to deaminate a target adenosine in accordance with the disclosure.

[0349] In some embodiments, any of the disclosed base editors are capable of deaminating adenosine in a nucleic acid sequence (*e.g.,* DNA or RNA). In various embodiments, the adenosine deaminases of the base editors hydrolytically deaminate a targeted adenosine in a nucleic acid of interest to an inosine, which is read as a guanosine (G) by DNA polymerase enzymes. In some embodiments, the base editor is an ABE. In another embodiment, the base editor is ABE8e. In other embodiments, the base editor is a CBE. In some embodiments, the gene editing system comprises a ABE and a guide RNA (gRNA). In some embodiments, the gene editing system comprises a CBE and a guide RNA (gRNA).

[0350] In some embodiments, any of the adenosine deaminases provided herein are capable of deaminating adenine, *e.g.,* deaminating adenine in a deoxyadenosine residue of DNA. The adenosine deaminase may be derived from any suitable organism (*e.g., E. coli).* In some embodiments, the adenosine deaminase is a naturally-occurring adenosine deaminase that includes one or more mutations corresponding to any of the mutations provided herein (*e.g.,* mutations in ecTadA). In some embodiments, the adenosine deaminase is derived from a prokaryote. In some embodiments, the adenosine deaminase is from a bacterium. In some embodiments, the disclosed adenosine deaminases are variants of a TadA derived from a species other than *Escherichia coli,* such as *Staphylococcus aureus, Salmonella typhi, Shewanella putrefaciens, Haemophilus influenzae, Caulobacter crescentus,* or *Bacillus subtilis.* In some embodiments, the adenosine deaminase is from *E. coli.*

[0351] In certain embodiments, the base editor includes mutations that confer reduced off-target effects, such as reduced RNA editing activity and off-target DNA editing activity, on the adenine base editor. In various embodiments, the disclosure provides an ABE that has one or more amino acid variations introduced into the amino acid sequence of the adenosine deaminase domain relative to the amino acid sequence of the reference adenosine deaminase domain. The

ABE may include variants in one or more components or domains of the base editor (*e.g.*, variations introduced into the adenosine deaminase domain, or variations introduced into both the adenine deaminase domain and the CRISPR-Cas domain).

[0352] For example, the disclosed adenosine deaminase variants may be at least about 70% identical, at least about 80% identical, at least about 90% identical, at least about 95% identical, at least about 96% identical, at least about 97% identical, at least about 98% identical, at least about 99% identical, at least about 99.5% identical, or at least about 99.9% identical to a reference adenosine deaminase domain.

[0353] In some embodiments, the adenosine deaminase domain of any of the disclosed base editors comprises a single adenosine deaminase, or a monomer. In some embodiments, the adenosine deaminase domain comprises 2, 3, 4 or 5 adenosine deaminases. In some embodiments, the adenosine deaminase domain comprises two adenosine deaminases, or a dimer. In some embodiments, the deaminase domain comprises a dimer of an engineered (or evolved) deaminase and a wild-type deaminase, such as a wild-type *E. coli*-derived deaminase. Base editors are further described in International Publication No. WO 2018/027078, published August 2, 2018; International Publication No. WO 2019/079347 on April 25, 2019; international Application No PCT/US2019/033848, filed May 23, 2019, which published as International Publication No. WO 2019/226953 on November 28, 2019; U.S. Patent Publication No. 2018/0073012, published March 15, 2018, which issued as U.S. Patent No. 10,113,163, on October 30, 2018; U.S. Patent Publication No. 2017/0121693, published May 4, 2017, Which issued as U.S. Patent No. 10,167,457 on January 1, 2019; International Publication No. WO 2017/070633, published April 27, 2017; U.S. Patent Publication No. 2015/0166980, published June 18, 2015; U.S. Patent No. 9,840,699, issued December 12, 2017; and U.S. Patent No. 10,077,453, issued September 18, 2018, and International Patent Application No. PCT/US2020/28568, filed April 16, 2020: all of which are incorporated herein by reference in their entireties.

[0354] Exemplary ABEs of this disclosure comprise the monomer and dimer versions of the following editors: ABE8e, SaABE8e, SaKKH-ABE8e, NG-ABE8e, ABE-xCas9, ABE8e-NRTH, ABE8e-NRRH, ABE8e-NRCH, ABE8e-NG-CP1041, ABE8e-VRQR-CP1041, ABE8e-CP1041, ABE8e-CP1028, ABE8e-VRQR. ABE8e-LbCas12a (LbABE8e), ABE8eAsCas12a (enAsABE8e), ABE8e-SpyMac, ABE8e (TadA-8e V 106W), ABE8e (K20A, R21A), and ABE8e (TadA-8e V82G). The monomer version refers to an editor having an adenosine

deaminase domain that comprises a TadA-8e and does not comprise a second adenosine deaminase enzyme. The dimer version refers to an editor having an adenosine deaminase domain that comprises a first and second adenosine deaminase, *i.e.,* a wild-type TadA enzyme and a TadA-8e enzyme. In some embodiments, the ABE is a ABE8e.

[0355] Any two or more of the adenosine deaminases described herein may be connected to one another (*e.g.,* by a linker, such as a peptide linker) within an adenosine deaminase domain of the base editors provided herein. In some embodiments, the base editor comprises two adenosine deaminases (*e.g.,* a first adenosine deaminase and a second adenosine deaminase). For instance, in certain embodiments, the base editors provided herein may contain exactly two adenosine deaminases. In some embodiments, the first and second adenosine deaminases are any of the adenosine deaminases provided herein. In some embodiments, the adenosine deaminases are the same. In some embodiments, the adenosine deaminases are different. In some embodiments, the first adenosine deaminase and second adenosine deaminase are derived from the same bacterial species. In some embodiments, the first adenosine deaminase and second adenosine deaminase are derived from different bacterial species.

[0356] In some embodiments, the base editor comprises a heterodimer of a first adenosine deaminase and a second adenosine deaminase. In some embodiments, the first adenosine deaminase is N-terminal to the second adenosine deaminase in the base editor. In some embodiments, the first adenosine deaminase is C-terminal to the second adenosine deaminase in the base editor. In some embodiments, the first adenosine deaminase and the second deaminase are fused directly to each other or via a linker. In some embodiments, the first adenosine deaminase is fused N-terminal to the CRISPR-Cas protein via a linker, and the second deaminase is fused C-terminal to the CRISPR-Cas protein via a linker. In other embodiments, the second adenosine deaminase is fused N-terminal to the CRISPR-Cas protein via a linker, and the first deaminase is fused C-terminal to the CRISPR-Cas protein via a linker.

[0357] In some embodiments, the base editors described herein may comprise one or more heterologous protein domains (*e.g.*, about or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more domains in addition to the base editor components). A base editor may comprise any additional protein sequence, and optionally a linker sequence between any two domains. Other exemplary features that may be present are localization sequences, such as cytoplasmic localization sequences, export sequences, such as nuclear export sequences, or other localization sequences, as well as sequence tags.

[0358] In certain embodiments, linkers may be used to link any of the peptides or peptide domains or domains of the base editor (*e.g.,* a CRISPR-Cas protein covalently linked to an adenosine deaminase domain). In some embodiments, the linker is 5-100 amino acids in length, for example, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35-40, 40-45, 45-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100- 110, 110-120, 120-130, 130-140, 140-150, or 150-200 amino acids in length. Longer or shorter linkers are also contemplated. In some embodiments, the linker is 32 amino acids in length.

[0359] The linker may be as simple as a covalent bond, or it may be a polymeric linker many atoms in length. In certain embodiments, the linker is a polypeptide or based on amino acids. In other embodiments, the linker is not peptide-like. In certain embodiments, the linker is a covalent bond (*e.g.,* a carbon-carbon bond, disulfide bond, carbon-heteroatom bond, *etc.*). In certain embodiments, the linker is a carbon-nitrogen bond of an amide linkage. In certain embodiments, the linker is a cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic or heteroaliphatic linker. In certain embodiments, the linker is polymeric (*e.g.,* polyethylene, polyethylene glycol, polyamide, polyester, *etc.*). In certain embodiments, the linker comprises a monomer, dimer, or polymer of aminoalkanoic acid. In certain embodiments, the linker comprises an aminoalkanoic acid (*e.g.,* glycine, ethanoic acid, alanine, beta-alanine, 3-aminopropanoic acid, 4-aminobutanoic acid, 5-pentanoic acid, *etc.*). In certain embodiments, the linker comprises a monomer, dimer, or polymer of aminohexanoic acid (Ahx). In certain embodiments, the linker is based on a carbocyclic moiety (*e.g.,* cyclopentane, cydohexane). In other embodiments, the linker comprises a polyethylene glycol moiety (PEG). In other embodiments, the linker comprises amino acids. In certain embodiments, the linker comprises a peptide. In certain embodiments, the linker comprises an aryl or heteroaryl moiety. In certain embodiments, the linker is based on a phenyl ring. The linker may include functionalized moieties to facilitate attachment of a nucleophile (*e.g.*, thiol, amino) from the peptide to the linker. Any electrophile may be used as part of the linker. Exemplary electrophiles include, but are not limited to, activated esters, activated amides, Michael acceptors, alkyl halides, aryl halides, acyl halides, and isothiocyanates.

[0360] In some aspects, the present disclosure provides compositions comprising the ABE as described herein and one or more guide RNAs, *e.g.,* a single-guide RNA ("sgRNA"). In addition, the present disclosure provides for nucleic acid molecules encoding and/or expressing the adenine base editors as described herein, as well as expression vectors or constructs for

expressing the adenine base editors described herein and a gRNA, host cells comprising said nucleic acid molecules and expression vectors, and optionally one or more gRNAs, and compositions for delivering and/or administering nucleic acid-based embodiments described herein.

[0361] In some embodiments, the LNP comprises a gene editing system, wherein the gene editing system comprises a gRNA and the mRNA of the base editor. In some embodiments, the gRNA and the mRNA of the base editor are present at a molar ratio that is 1: 1. In some embodiments, the gRNA and the mRNA of the base editor are present at a molar ratio that is not 1:1. In some embodiments, the gRNA and the mRNA of the base editor are present at a molar or weight ratio less than 1:1. In some embodiments, the gRNA and the mRNA of the base editor are present at a molar or weight ratio of at most about 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:11, 1:12, 1:13, 1:14, 1:15, 1:16, 1:17, 1:18, 1:19, 1:20, 1:21, 1:22, 1:23, 1:24, 1:25, 1:26, 1:27, 1:28, 1:29, or 1:30. In some embodiments, the gRNA and the mRNA of the base editor are present at a molar or weight ratio of at least about 1:30, 1:29, 1:28, 1:27, 1:26, 1:25, 1:24, 1:23, 1:22, 1:21, 1:20, 1:19, 1:18, 1:17, 1:16, 1:15, 1:14, 1:13, 1:12, 1:11, 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, or 1:1. In some embodiments, the gRNA and the mRNA of the base editor are present at a molar or weight ratio of about 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:11, 1:12, 1:13, 1:14, 1:15, 1:16, 1:17, 1:18, 1:19, 1:20, 1:21, 1:22, 1:23, 1:24, 1:25, 1:26, 1:27, 1:28, 1:29, or 1:30, or a range between any two of the foregoing values.

[0362] In some embodiments, the mRNA of the base editor and the gRNA are present at a molar or weight ratio that is not 1:1. In some embodiments, the mRNA of the base editor and the gRNA are present at a molar or weight ratio less than 1:1. In some embodiments, the mRNA of the base editor and the gRNA are present at a molar or weight ratio of at most about 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:11, 1:12, 1:13, 1:14, 1:15, 1:16, 1:17, 1:18, 1:19, 1:20, 1:21, 1:22, 1:23, 1:24, 1:25, 1:26, 1:27, 1:28, 1:29, or 1:30. In some embodiments, the mRNA of the base editor and the gRNA are present at a molar or weight ratio of at least about 1:30, 1:29, 1:28, 1:27, 1:26, 1:25, 1:24, 1:23, 1:22, 1:21, 1:20, 1:19, 1:18, 1:17, 1:16, 1:15, 1:14, 1:13, 1:12, 1:11, 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, or 1:1. In some embodiments, the mRNA of the base editor and the gRNA are present at a molar or weight ratio of about 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:11, 1:12, 1:13, 1:14, 1:15, 1:16, 1:17, 1:18, 1:19, 1:20, 1:21, 1:22, 1:23, 1:24, 1:25, 1:26, 1:27, 1:28, 1:29, or 1:30, or a range between any two of the foregoing values.

3. *Guide RNA*

[0363] In some embodiments, the one or more polynucleotides comprise a guide RNA (gRNA). In some embodiments, gRNAs are designed to recognize target sequences in a gene or genome of interest. Such gRNAs may be designed to have guide sequences (or "spacers") having complementarity to a protospacer within the target sequence.

[0364] In some embodiments, the gRNA is complexed with a recombinant nuclease capable of inducing a DNA break. In some embodiments, the gRNA is a single guide RNA (sgRNA). In some embodiments, the gRNA comprises a crispr RNA (crRNA) and a tracrRNA. In some embodiments, the gRNA is modified. In some embodiments, the gRNA is modified with an end modification (e.g. (3 x 2′-O-methyl-3′-phosphorothioate (MS) on 5' end and 3 x 2′-O-methyl-3′-phosphonoacetat (MP) on 3'end (K. A. Hajj, K. A. Whitehead, *Nat. Rev. Mater.* 2, 17056 (2017)). In some embodiments, the gRNA can be heavily modified (T. Wei et al., *ACS Nano* 14, 9243-9262 (2020).

[0365] In some embodiments, gRNAs can be used with one or more of the disclosed ABEs, e.g., in the disclosed methods of editing a nucleic acid molecule. Such gRNAs may be designed to have guide sequences having complementarity to a protospacer within a target sequence to be edited, and to have backbone sequences that interact specifically with the CRISPR-Cas protein of any of the disclosed base editors, such as Cas9 nickase proteins of the disclosed base editors. The guide sequence becomes associated or bound to the base editor and directs its localization to a specific target sequence having complementarity to the guide sequence or a portion thereof. The particular design of a guide sequence will depend upon the nucleotide sequence of a genomic target sequence (i.e., the desired site to be edited) and the type of CRISPR-Cas protein (e.g., type of Cas9 protein) present in the base editor, among other factors, such as PArv1 sequence locations, percent G/C content in the target sequence, the degree of microhomology regions, and/or secondary structures.

[0366] In some embodiments, the polynucleotide encodes a guide polynucleotide (such as guide RNA (gRNA) or guide DNA (gDNA)) that is at least partially complementary to the genomic region of a gene, where upon binding of the guide polynucleotide to the gene the guide polynucleotide recruits the guide polynucleotide guided CRISPR-Cas protein to cleave and genetically modified the region. In some embodiments, a CFTR gene can be modified by the guide polynucleotide-guided CRISPR-Cas protein.

[0367] In general, a guide sequence is any polynucleotide sequence having sufficient complementarity with a target polynucleotide sequence to hybridize with the target sequence and direct sequence-specific binding of the CRISPR-Cas protein (e.g., a Cas9 or Cas9 variant) to the target sequence. In some embodiments, the degree of complementarity between a guide sequence and its corresponding target sequence, when optimally aligned using a suitable alignment algorithm, is about or more than about 50%, 60%, 75%, 80%, 85%, 90%, 95%, 97.5%, 99%, or more. Optimal alignment may be determined with the use of any suitable algorithm for aligning sequences, non-limiting example of which include the Smith-Waterman algorithm, the Needleman-Wunsch algorithm, algorithms based on the Burrows-Wheeler Transform (e.g., the Burrows Wheeler Aligner), ClustalW, Clustal X, BLAT, Novoalign (Novocraft Technologies), ELAND (Illumina, San Diego, Calif.), SOAP, and Maq (available at maq.sourceforge.net).

[0368] In some embodiments, a guide sequence is above or more than about 5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 75, or more nucleotides in length. In some embodiments, each gRNA comprises a guide sequence of at least 10 contiguous nucleotides (e.g., 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides) that is complementary to a target sequence (or off target site).

[0369] In some embodiments, a guide sequence is less than about 75, 50, 45, 40, 35, 30, 25, 20, 15, 12, or fewer nucleotides in length. The ability of a guide sequence to direct sequence-specific binding of a base editor to a target sequence may be assessed by any suitable assay. For example, the components of a base editor, including the guide sequence to be tested, may be provided to a host cell having the corresponding target sequence, such as by transfection with vectors encoding the components of a base editor disclosed herein, followed by an assessment of preferential cleavage within the target sequence. Similarly, cleavage of a target polynucleotide sequence may be evaluated in situ by providing the target sequence, components of a base editor, including the guide sequence to be tested and a control guide sequence different from the test guide sequence, and comparing binding or rate of cleavage at the target sequence between the test and control guide sequence reactions. Other assays are possible, and will occur to those skilled in the art.

[0370] In some embodiments, the gRNA comprises a sequence that is complementary to a target sequence. In some embodiments, the gRNA comprises a sequence that is complementary

to a target sequence of a cystic fibrosis transmembrane conductance regulator (CFTR) gene or transcript. In some embodiments, the gRNA comprises a sgRNA. In some embodiments, the sequence of the gRNA is shown in **Table 9**. In some embodiments, the gRNA comprises the nucleic acid sequence of SEQ ID NO: 1 or a nucleic acid sequence having at least at or about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 1. In some embodiments, the gRNA comprises the nucleic acid sequence of SEQ ID NO: 2 or a nucleic acid sequence having at least at or about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 2.

Table 9: Sequences of sgRNA

sgRNA	Sequence (5' to 3')	PAM (5' to 3')
sgTOM1	AAGTAAAACCTCTACAAATG (SEQ ID NO: 1)	TGG
sgR553X	TTGCTCATTGACCTCCACTC (SEQ ID NO: 2)	AG

[0371] In some embodiments, a guide RNA (gRNA) comprising a sequence that is complementary to a target sequence (e.g. CFTR gene) and is used with RNA-guided nucleases, e.g., Cas, to induce a DNA break at the target site or target position. Methods for designing gRNAs and exemplary targeting domains can include those described in, e.g., WO2015/161276, WO2017/193107, WO2017/093969, US2016/272999 and US2015/056705, the contents of which are incorporated by reference. Methods for introducing a genetic disruption at one or more target sites and gRNAs that target the target sites include those described in, e.g., WO2015/161276, WO2015/070083, WO2019/070541, WO2019/195491, WO2019/195492, WO2019/089884, and WO2020/223535, the contents of which are incorporated by reference.

[0372] Several exemplary gRNA structures, with domains indicated thereon, are described in WO2015/161276. While not wishing to be bound by theory, with regard to the three dimensional form, or intra- or inter-strand interactions of an active form of a gRNA, regions of high complementarity are sometimes shown as duplexes in WO2015/161276.

[0373] In some embodiments, the gRNA is a unimolecular or chimeric gRNA comprising, from 5' to 3': a targeting domain which targets a target site (e.g., a locus in the CFTR gene); a first complementarity domain; a linking domain; a second complementarity domain (which is complementary to the first complementarity domain); a proximal domain; and optionally, a tail domain.

[0374] In some embodiments, the gRNA is a modular gRNA comprising first and second strands. In these cases, the first strand preferably includes, from 5' to 3': a targeting domain (which targets a target site e.g., at CFTR gene); and a first complementarity domain. The second strand generally includes, from 5' to 3': optionally, a 5' extension domain; a second complementarity domain; a proximal domain; and optionally, a tail domain.

IV. COMPOSITIONS

[0375] Provided herein are methods comprising compositions comprising lipid nanoparticles (LNPs), such as LNPs comprising a gene editing system. In some embodiments, the composition comprises LNPs in any formulation such as those described in Section IID. In some embodiments, the method comprises administration of the LNPs comprising a gene editing system and/or compositions containing said LNPs to a subject for the treatment of disease or condition. In some aspects, the LNPs or compositions comprising said LNPs are administered to a subject, such as a subject with a disease or condition, or to prevent or reduce the severity of a disease or condition. In some embodiments, the method comprises administration of the LNPs comprising a gene editing system and/or compositions containing said LNPs to a subject for the treatment of cystic fibrosis. In some embodiments, the composition is a pharmaceutical composition. In some embodiments, the composition comprises a pharmaceutically acceptable excipient.

[0376] In some embodiments, the LNPs, or compositions comprising the same, can be administered by any suitable means, for example, by injection, e.g., intravenous or subcutaneous injections, intraocular injection, periocular injection, subretinal injection, intravitreal injection, trans-septal injection, subscleral injection, intrachoroidal injection, intracameral injection, subconjectval injection, subconjuntival injection, sub-Tenon's injection, retrobulbar injection, peribulbar injection, or posterior juxtascleral delivery. In some embodiments, they are administered by parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. In some embodiments, the nanoparticle is administered (e.g., in a lipoplex particle or liposome) intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, intracranial, intrathoracic, or subcutaneous administration. Dosing and administration may depend in part on whether the administration is brief or chronic. Various dosing schedules include but are not limited to single or multiple administrations over various

time-points, bolus administration, and pulse infusion. In some embodiments, the LNPs, or compositions comprising the same are administered intravenously. In some embodiments, the LNPs or compositions comprising the same are administered by way of aerosolized delivery.

[0377] In some embodiments, the LNPs or compositions are administered in the presence of an adjuvant. In some embodiments, the LNPs or compositions can be administered by repeat administration of the peptide a plurality of times. In some embodiments, repeated administration is with a low dose of peptide. In some embodiments, the repeat administration of the LNPs or compositions occurs over an extended time period, such as for up to three days, four days, five days, six days, one week, two weeks, three weeks or a month. In some embodiments, the LNPs or compositions can be administered in a high dose amount. In some embodiments, one or more administration modes, schedules or frequency of administration are employed. Other methods that can be used to deliver nucleic acids to the subject include viral, e.g., retroviral or lentiviral, transduction, transposons, and electroporation.

[0378] In some embodiments, the LNPs are formulated with a pharmaceutically acceptable carrier. In some aspects, the choice of carrier is determined in part by the particular cell or agent and/or by the method of administration. Accordingly, there are a variety of suitable formulations. For example, the pharmaceutical composition can contain preservatives. Suitable preservatives may include, for example, methylparaben, propylparaben, sodium benzoate, and benzalkonium chloride. In some aspects, a mixture of two or more preservatives is used. The preservative or mixtures thereof are typically present in an amount of about 0.0001% to about 2% by weight of the total composition. Carriers are described, e.g., by Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980). Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars

such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG).

[0379] Buffering agents in some aspects are included in the compositions. Suitable buffering agents include, for example, citric acid, sodium citrate, phosphoric acid, potassium phosphate, and various other acids and salts. In some aspects, a mixture of two or more buffering agents is used. The buffering agent or mixtures thereof are typically present in an amount of about 0.001% to about 4% by weight of the total composition. Methods for preparing administrable pharmaceutical compositions are known. Exemplary methods are described in more detail in, for example, Remington: The Science and Practice of Pharmacy, Lippincott Williams & Wilkins; 21st ed. (May 1, 2005).

[0380] The formulations can include aqueous solutions. The formulation or composition may also contain more than one active ingredient useful for the particular indication, disease, or condition being treated with the cells or agents, where the respective activities do not adversely affect one another. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended. Thus, in some embodiments, the pharmaceutical composition further includes other pharmaceutically active agents or drugs.

[0381] In some embodiments, the pharmaceutical composition comprises the LNP comprising a gene editing system in amounts effective to treat the disease or condition, such as a therapeutically effective or prophylactically effective amount. Therapeutic efficacy in some embodiments is monitored by periodic assessment of treated subjects. For repeated administrations over several days or longer, depending on the condition, the treatment is repeated until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful and can be determined. The desired dosage can be delivered by a single bolus administration of the composition, by multiple bolus administrations of the composition, or by continuous infusion administration of the composition.

[0382] In some embodiments, the LNP comprising a gene editing system may be administered using standard administration techniques, formulations, and/or devices. Provided are formulations and devices, such as syringes and vials, for storage and administration of the compositions. When administering a therapeutic composition (e.g., a pharmaceutical composition containing a LNP), it will generally be formulated in a unit dosage injectable form (solution, suspension, emulsion).

[0383] In some embodiments, pharmaceutical compositions are provided as sterile liquid preparations, e.g., isotonic aqueous solutions, suspensions, emulsions, dispersions, or viscous compositions, which may in some aspects be buffered to a selected pH. Liquid preparations are normally easier to prepare than gels, other viscous compositions, and solid compositions. Additionally, liquid compositions are somewhat more convenient to administer, especially by injection. Viscous compositions, on the other hand, can be formulated within the appropriate viscosity range to provide longer contact periods with specific tissues. Liquid or viscous compositions can comprise carriers, which can be a solvent or dispersing medium containing, for example, water, saline, phosphate buffered saline, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol) and suitable mixtures thereof.

[0384] Sterile injectable solutions can be prepared by incorporating the LNPs in a solvent, such as in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose, dextrose, or the like. The formulations to be used for in vivo administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.

[0385] Also provided herein are pharmaceutical compositions (e.g, in liquid form prior to aerosolization) comprising the LNPs described herein. Such compositions can be used for the treatment of a lung disease in a patient or subject. The pharmaceutical compositions of the disclosure may include a pharmaceutically acceptable carrier, and a thorough discussion of such carriers is available in Chapter 30 of Remington: The Science and Practice of Pharmacy (23rd ed., 2021).

[0386] In some embodiments, aerosolized composition comprises LNPs for selective delivery to one or more of goblet cells, secretory cells, club cells, basal cells or ionocytes. In some embodiments, the aerosolized pharmaceutical composition comprises LNPs for selective delivery to one or more of ciliated cells, club cells, or basal cells.

[0387] In some embodiments, the aerosolized pharmaceutical compositions include one or more of a poloxamer (e.g., Poloxamer 188) polyethylene glycol ("PEG"), sucrose, and a buffer, wherein the buffer comprises a citrate buffer, an acetate buffer, or a Tris buffer. In some embodiments, the PEG has a concentration from 1% to 4% (w/v). In some embodiments, the PEG has a concentration from 1% to 5%, or 2 to 4%. In some embodiments, the aerosolized pharmaceutical compositions includes Poloxamer 188 at a concentration of between about 0.001% w/v and 0.5% w/v.

[0388] In some embodiments, the composition includes sucrose. In some embodiments, the sucrose is at a concentration from 1% to 15% w/v, 5% to 15% w/v, 1% to 10% w/v, or 5% to 10% w/v. In some embodiments, the composition includes a citrate buffer. For example, the citrate buffer is at a pH from 4 to 8. In various examples, the buffer is an acetate buffer and has a pH from 4 to 8. In still other embodiments, the composition includes a Tris buffer, and the Tris buffer has a pH from 4 to 8. In some embodiments, the composition has a pH of 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, or 8.0. In some embodiments, composition has pKa 4 to 7.

[0389] In some embodiments, the pharmaceutical compositions comprising the LNPs described have at least one pharmaceutically acceptable excipient or carrier. In some embodiments, the pharmaceutically acceptable excipient or carrier is for nebuilzation of the composition.

[0390] In some embodiments, the pharmaceutical compositions can also include excipients and/or additives. Examples of these are surfactants, stabilizers, complexing agents, antioxidants, or preservatives which prolong the duration of use of the finished pharmaceutical formulation, flavorings, vitamins, or other additives known in the art. Complexing agents include, but are not limited to, ethylenediaminetetraacetic acid (EDTA) or a salt thereof, such as the disodium salt, citric acid, nitrilotriacetic acid and the salts thereof. In some embodiments, preservatives include, but are not limited to, those that protect the solution from contamination with pathogenic particles, including benzalkonium chloride or benzoic acid, or benzoates such as sodium benzoate. Antioxidants include, but are not limited to, vitamins, provitamins, ascorbic acid, vitamin E, salts or esters thereof.

[0391] In some embodiments, one or more tonicity agents may be added to provide the desired ionic strength. Tonicity agents for use herein include those which display no or only negligible pharmacological activity after administration. Both inorganic and organic tonicity adjusting agents may be used. In some embodiments, the disclosure provides a method of making an LNP composition comprising mixing lipid components and polynucleotides in conditations effective to assemble LNPs comprising the polynucleotides.

[0392] In some embodiments, the method comprises nebulizing the composition to generate an aerosolized LNP composition.

V. ARTICLES OF MANUFACTURE AND KITS

[0393] Also provided are kits and articles of manufacture, such as those containing reagents for performing the methods provided herein, e.g., reagents for producing LNPs and

compositions thereof and/or reagents for introducing one or more nucleic acid molecules into a lung type cell using LNPs and/or compositions thereof. In some aspects, the kits or articles of manufacture can contain reagents and/or nucleic acids for use in engineering or manufacturing processes to generate the LNP.

[0394] In some embodiments, the kits can contain reagents and/or consumables required for producing LNPs and compositions thereof. In some embodiments, the kits can contain reagents and/or consumables required for delivery of nucleic acid into the lung type cells using such LNPs and/or compositions thereof. The various components of the kit may be present in separate containers or certain compatible components may be precombined into a single container. In some embodiments, the kits further contain instructions for using the components of the kit to practice the provided methods. In some embodiments, the kit includes a lipid nanoparticle composition comprising one or more of a phospholipid, an ionizable lipid, a PEG-lipid, cholesterol, and a base editor. In some embodiments, the kit comprises a LNP composition and a base editor and a nebulizer mask and/or a mesh suitable for use in a nebulizer.

[0395] Also provided are articles of manufacture, which may include a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container in some embodiments holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition. In some embodiments, the container has a sterile access port. Exemplary containers include an intravenous solution bags, vials, including those with stoppers pierceable by a needle for injection, or bottles or vials for orally administered agents. The label or package insert may indicate that the composition is used for treating a disease or condition.

[0396] The article of manufacture may further include a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further include another or the same container comprising a pharmaceutically-acceptable buffer. It may further include other materials such as other buffers, diluents, filters, needles, and/or syringes.

VI. DEFINITIONS

[0397] Unless defined otherwise, all terms of art, notations and other technical and scientific terms or terminology used herein are intended to have the same meaning as is commonly understood by one of ordinary skill in the art to which the claimed subject matter pertains. In

some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art.

[0398] As used herein, "percent (%) amino acid sequence identity" and "percent identity" and "sequence identity" when used with respect to an amino acid sequence (reference polypeptide sequence) is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino 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 or Megalign (DNASTAR) 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.

[0399] As used herein, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. For example, "a" or "an" means "at least one" or "one or more." It is understood that aspects, embodiments, and variations described herein include "comprising," "consisting," and/or "consisting essentially of" aspects, embodiments and variations.

[0400] Throughout this disclosure, various aspects of the claimed subject matter are presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the claimed subject matter. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, where a range of values is provided, it is understood that each intervening value, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the claimed subject matter. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the claimed subject matter, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of

the limits, ranges excluding either or both of those included limits are also included in the claimed subject matter. This applies regardless of the breadth of the range.

[0401] The term "about" as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X".

[0402] As used herein, a "composition" refers to any mixture of two or more products, substances, or compounds, including cells. It may be a solution, a suspension, liquid, powder, a paste, aqueous, non-aqueous or any combination thereof.

[0403] As used herein, "abasic editor" refers any one of the abasic editors described in PCT/JP2015/080958 and US20170321210, which are incorporated herein by reference.

[0404] As used herein, the term "lipid composition" generally refers to a composition comprising lipid compound(s), including but not limited to, a lipoplex, a liposome, a lipid particle. Examples of lipid compositions include suspensions, emulsions, and vesicular compositions.

[0405] As used herein, the term "lipid nanoparticle" refers to a vesicle formed by one or more lipid components typically used as carriers for nucleic acid, protein, or oligonucleotide delivery in the context of pharmaceutical development. Generally, lipid nanoparticle compositions for delivery are composed of synthetic ionizable or cationic lipids, phospholipids, cholesterol, and a polyethylene glycol (PEG) lipid. However, these compositions may also include other lipids.

[0406] As used herein, the term "neutral phospholipid" refers to phospholipids that have little or no net charge at physiological pH. In many embodiments, neutral phospholipids are zwitterions, although other types of net neutral phospholipids are known and may be used.

[0407] As used herein, the term "PEG-lipid" refers to a lipid modified with a polyethylene glycol unit. In some embodiments, the PEG-lipid comprises DMG. In some embodiments, the PEG-lipid comprises DSPE.

[0408] As used herein, the term "PEG-OH lipid" refers to a PEG-lipid having one or more hydroxyl (-OH) groups on the lipid.

[0409] As used herein, "adenosine deaminase" refers to a polypeptide or fragment thereof capable of catalyzing the hydrolytic deamination of adenine or adenosine. In some embodiments, the deaminase or deaminase domain is an adenosine deaminase catalyzing the

hydrolytic deamination of adenosine to inosine or deoxy adenosine to deoxyinosine. In some embodiments, the adenosine deaminase catalyzes the hydrolytic deamination of adenine or adenosine in deoxyribonucleic acid (DNA). The adenosine deaminases (e.g., engineered adenosine deaminases, evolved adenosine deaminases) provided herein may be from any organism, such as a bacterium.

[0410] As used herein, "hydrogen" means −H; "hydroxy" means −OH; "oxo" means =O; "carbonyl" means −C(=O)−; "carboxy" means −C(=O)OH (also written as −COOH or −CO2H); "halo" means independently −F, −Cl, −Br or −I; "amino" means $-NH_2$; "hydroxyamino" means −NHOH; "nitro" means $-NO_2$; imino means =NH; "cyano" means −CN; "isocyanate" means −N=C=O; "azido" means −N3; in a monovalent context "phosphate" means $-OP(O)(OH)_2$ or a deprotonated form thereof; in a divalent context "phosphate" means –OP(O)(OH)O– or a deprotonated form thereof; "mercapto" means −SH; and "thio" means =S; "sulfonyl" means $-S(O)_2-$; "hydroxysulfonyl" means $-S(O)_2OH$; "sulfonamide" means $-S(O)_2NH_2$; and "sulfinyl" means –S(O)–.

[0411] As used herein, "ionizable lipid" refers to a lipid comprising one or more charged moieties. In some embodiments, an ionizable lipid may be positively charged or negatively charged. For instance, an ionizable lipid may be positively charged at lower pHs, in which case it could be referred to as "cationic lipid." In certain embodiments, an ionizable lipid molecule may comprise an amine group, and can be referred to as an ionizable amino lipids.

[0412] As used herein, a "charged moiety" refers to a chemical moiety that carries a formal electronic charge, *e.g.*, monovalent (+1, or -1), divalent (+2, or -2), trivalent (+3, or -3), etc. The charged moiety may be anionic (*i.e.*, negatively charged) or cationic (*i.e.*, positively charged). Examples of positively-charged moieties include amine groups (*e.g.*, primary, secondary, and/or tertiary amines), ammonium groups, pyridinium group, guanidine groups, and imidazolium groups. In a particular embodiment, the charged moieties comprise amine groups. Examples of negatively- charged groups or precursors thereof, include carboxylate groups, sulfonate groups, sulfate groups, phosphonate groups, phosphate groups, hydroxyl groups, and the like. The charge of the charged moiety may vary, in some cases, with the environmental conditions, for example, changes in pH may alter the charge of the moiety, and/or cause the moiety to become charged or uncharged. In general, the charge density of the molecule may be selected as desired.

[0413] As used herein "partial negative charge" and "partial positive charge" are given its ordinary meaning in the art. A "partial negative charge" may result when a functional group

comprises a bond that becomes polarized such that electron density is pulled toward one atom of the bond, creating a partial negative charge on the atom. Those of ordinary skill in the art will, in general, recognize bonds that can become polarized in this way.

[0414] As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to complete or partial amelioration or reduction of a disease or condition or disorder, or a symptom, adverse event, effect, or outcome, or phenotype associated therewith. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. The terms do not imply complete curing of a disease or complete elimination of any symptom or effect(s) on all symptoms or outcomes.

[0415] As used herein, "adverse event" refers to any new untoward medical occurrence or worsening of a pre-existing medical condition occurring in a clinical investigation participant after signing of informed consent, whether or not considered related to the study intervention. An adverse event can therefore be any unfavorable and unintended sign (such as an abnormal laboratory test result), symptom, or disease temporally associated with the study intervention.

[0416] As used herein, "delaying development of a disease" means to defer, hinder, slow, retard, stabilize, suppress and/or postpone development of the disease (such as cancer). This delay can be of varying lengths of time, depending on the history of the disease and/or subject being treated. As sufficient or significant delay can, in effect, encompass prevention, in that the subject does not develop the disease. For example, a late stage cancer, such as development of metastasis, may be delayed.

[0417] "Preventing," as used herein, includes providing prophylaxis with respect to the occurrence or recurrence of a disease in a subject that may be predisposed to the disease but has not yet been diagnosed with the disease. In some embodiments, the provided molecules and compositions are used to delay development of a disease or to slow the progression of a disease.

[0418] As used herein, to "suppress" a function or activity is to reduce the function or activity when compared to otherwise same conditions except for a condition or parameter of interest, or alternatively, as compared to another condition. For example, an antibody or composition or cell which suppresses tumor growth reduces the rate of growth of the tumor

compared to the rate of growth of the tumor in the absence of the antibody or composition or cell.

[0419] The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

[0420] A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[0421] An "effective amount" of an agent, e.g., a pharmaceutical formulation, binding molecule, antibody, cells, or composition, in the context of administration, refers to an amount effective, at dosages/amounts and for periods of time necessary, to achieve a desired result, such as a therapeutic or prophylactic result.

[0422] A "therapeutically effective amount" of an agent, e.g., a pharmaceutical formulation, binding molecule, antibody, cells, or composition refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result, such as for treatment of a disease, condition, or disorder, and/or pharmacokinetic or pharmacodynamic effect of the treatment. The therapeutically effective amount may vary according to factors such as the disease state, age, sex, and weight of the subject, and the populations of cells administered. In some embodiments, the provided methods involve administering the molecules, antibodies, cells, and/or compositions at effective amounts, e.g., therapeutically effective amounts.

[0423] A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, but not necessarily, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

[0424] As used herein, a "subject" or an "individual" is a mammal. In some embodiments, a "mammal" includes humans, non-human primates, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, rabbits, cattle, pigs, hamsters, gerbils, mice, ferrets, rats, cats, monkeys, *etc.* In some embodiments, the subject is human.

[0425] As used herein, the terms "lung disease," "pulmonary disease," "pulmonary disorder," broadly refer to diseases or disorders of the lung. Lung diseases may be characterized by symptoms including but not limited to difficulty breathing, coughing, airway discomfort and

inflammation, increased mucus, and/or pulmonary fibrosis. Examples of lung diseases include Primary Ciliary Dyskinesia (PCD) (also referred to as Kartageners Syndrome, or Immotile Cilia Syndrome), cystic fibrosis, asthma, lung cancer, Chronic Obstructive Pulmonary Disease (COPD), bronchitis, emphysema, bronchiectasis, pulmonary edema, pulmonary fibrosis, sarcoidosis, pulmonary hypertension, pneumonia, tuberculosis, Interstitial Pulmonary Fibrosis (IPF), Interstitial Lung Disease (ILD), Acute Interstitial Pneumonia (AlP), Respiratory Bronchiolitis-associated Interstitial Lung Disease (RBILD), Desquamative Interstitial Pneumonia (DIP), Non-Specific Interstitial Pneumonia (NSIP), Idiopathic Interstitial Pneumonia (IIP), Bronchiolitis obliterans, with Organizing Pneumonia (BOOP), restrictive lung disease, or pleurisy.

VII. EXEMPLARY EMBODIMENTS

[0426] Among the provided embodiments are:

[0427] Embodiment 1: A method of treating a subject with cystic fibrosis, the method comprising administering to the subject a composition comprising a lipid nanoparticle (LNP) that comprises a gene editing system, wherein the gene editing system comprises:

(i) a first nucleic acid encoding a base editor; and

(ii) a second nucleic acid encoding a guide RNA (gRNA),

and wherein the composition treats the cystic fibrosis in the subject.

[0428] Embodiment 2: The method of embodiment 1, wherein the *CFTR* gene of the subject comprises a R553X stop codon mutation.

[0429] Embodiment 3: The method of embodiment 2, wherein the administration of the composition results in an increase in the expression of the full-length cystic fibrosis transmembrane conductance regulator (CFTR) protein in the subject, as compared to a subject with cystic fibrosis and whose *CFTR* gene comprises a R553X stop codon mutation, and that is not administered the composition.

[0430] Embodiment 4: The method of embodiment 2, wherein the administration of the composition results in an increase in the function of the cystic fibrosis transmembrane conductance regulator (CFTR) protein in the subject, as compared to a subject with cystic fibrosis and whose *CFTR* gene comprises a R553X stop codon mutation, and that is not administered the composition.

[0431] Embodiment 5: The method of any of embodiments 1-4, wherein the nucleic acid encoding the base editor is RNA.

[0432] Embodiment 6: The method of any of embodiments 1-5, wherein the base editor is an adenine base editor (ABE).

[0433] Embodiment 7: The method of embodiment 6, wherein the ABE comprises a tRNA adenosine deaminase (TadA) protein, or a variant thereof, fused to a catalytically impaired Cas protein capable of binding to a specific nucleotide sequence.

[0434] Embodiment 8: The method of embodiment 6, wherein the ABE is ABE8e.

[0435] Embodiment 9: The method of any of embodiments 1-5, wherein the base editor is a cytosine base editor (CBE).

[0436] Embodiment 10: The method of embodiment 9, wherein the CBE comprises a cytidine deaminase protein, or a variant thereof, fused to a catalytically impaired Cas protein capable of binding to a specific nucleotide sequence.

[0437] Embodiment 11: The method of any of embodiments 6-10, wherein the ABE or the CBE further comprises a Cas9 enzyme that does not have any nuclease activity.

[0438] Embodiment 12: The method of any of embodiments 1-11, wherein the LNP comprises an ionizable cationic lipid, a zwitterionic phospholipid, a cholesterol, and a PEG lipid.

[0439] Embodiment 13: The method of any of embodiments 1-12, wherein the LNP comprises 5A2-SC8, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), cholesterol, DMG-PEG, and one or more selective organ targeting (SORT) molecules.

[0440] Embodiment 14: A method of delivering a gene editing system to a lung cell type in a subject, the method comprising administering to the subject a composition comprising a lipid nanoparticle (LNP) that comprises a gene editing system, wherein the gene editing system comprises (i) a first nucleic acid encoding an endonuclease or a base editor; and (ii) a second nucleic acid encoding a guide RNA (gRNA), and wherein the gene editing system is delivered to a lung cell type in a subject.

[0441] Embodiment 15: The method of embodiment 14, wherein the lung cell type is an endothelial cell or an epithelial cell.

[0442] Embodiment 16: The method of embodiment 14 or 15, wherein the lung cell type is an immune cell.

[0443] Embodiment 17: The method of any of embodiments 14-16, wherein the lung cell type is a stem cell.

[0444] Embodiment 18: The method of any of embodiments 14-17, wherein the endonuclease is a Cas nuclease of the CRISPR-Cas system.

[0445] Embodiment 19: The method of embodiment 18, wherein the Cas nuclease is a Cas9 nuclease, a Cas12 nuclease, or a Cas13 nuclease.

[0446] Embodiment 20: The method of any of embodiments 14-19, wherein the nucleic acid encoding the endonuclease is DNA.

[0447] Embodiment 21: The method of any of embodiments 14-19, wherein the nucleic acid encoding the endonuclease is RNA.

[0448] Embodiment 22: The method of any of embodiments 14-17, wherein the nucleic acid encoding the base editor is DNA.

[0449] Embodiment 23: The method of embodiment 14-17, wherein the nucleic acid encoding the base editor is RNA.

[0450] Embodiment 24: The method of any of embodiments 14-17, 22, and 23, wherein the base editor is an adenine base editor (ABE).

[0451] Embodiment 25: The method of embodiment 24, wherein the ABE comprises a tRNA adenosine deaminase (TadA) protein, or a variant thereof, fused to a catalytically impaired Cas protein capable of binding to a specific nucleotide sequence.

[0452] Embodiment 26: The method of embodiment 24, wherein the ABE is ABE8e.

[0453] Embodiment 27: The method of any of embodiments 14-17, 22, and 23, wherein the base editor is a cytosine base editor (CBE).

[0454] Embodiment 28: The method of embodiment 27, wherein the CBE comprises a cytidine deaminase protein, or a variant thereof, fused to a catalytically impaired Cas protein capable of binding to a specific nucleotide sequence.

[0455] Embodiment 29: The method of any of embodiments 14-17 and 22-28, wherein the ABE or the CBE further comprises a Cas9 enzyme that does not have any nuclease activity.

[0456] Embodiment 30: The method of any of embodiments 1-29, wherein the ratio of the first nucleic acid to the second nucleic acid is 1:1 on a molecule:molecule basis.

[0457] Embodiment 31: The method of any of embodiments 1-29, wherein the ratio of the first nucleic acid to the second nucleic acid is 1:1 on a weight basis.

[0458] Embodiment 32: The method of any of embodiments 1-29, wherein the ratio of the first nucleic acid to the second nucleic acid is 2:1 on a molecule:molecule basis.

[0459] Embodiment 33: The method of any of embodiments 1-29, wherein the ratio of the first nucleic acid to the second nucleic acid is 2:1 on a weight basis.

[0460] Embodiment 34: The method of any one of embodiments 14-33, wherein the LNP comprises an ionizable cationic lipid, a zwitterionic phospholipid, a cholesterol, and a PEG lipid.

[0461] Embodiment 35: The method of any one of embodiments 14-34, wherein the LNP comprises 5A2-SC8, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), cholesterol, DMG-PEG, and one or more selective organ targeting (SORT) molecules.

[0462] Embodiment 36: The method of embodiment 13 or 35, wherein the one or more SORT molecules comprises permanently positively charged moiety.

[0463] Embodiment 37: The method of embodiment 36, wherein the one or more SORT molecules is selected from the group consisting of 18:1 DOTMA (DOTMA); DORI, DC-6-14; 12:0 EPC (Chloride Salt); 14:0 EPC (Chloride Salt); 16:0 EPC (Chloride Salt); 18:0 EPC (Chloride Salt); 18:1 EPC (Chloride Salt); 16:0-18:1 EPC (Chloride Salt); 14:1 EPC (Triflate Salt); 18:0 DDAB (Dimethyldioctadecylammonium (Bromide Salt)); 14:0 TAP; 16:0 TAP; 18:0 TAP; 18:1 TAP (DOTAP); 18:1 TAP (DOTAP, MS Salt); 18:1 DODAP, or 18:1 PA (1,2-dioleoyl-sn-glycero-3-phosphate (sodium salt)) (18PA).

[0464] Embodiment 38: The method of embodiment 13 or 35, wherein the one or more SORT molecule comprises DOTAP (1,2-dioleoyl-3-trimethylammonium propane).

[0465] Embodiment 39: The method of embodiment 36 or 37, wherein the one or more SORT molecule comprises 18PA.

[0466] Embodiment 40: The method of embodiment 36 or 37, wherein the one or more SORT molecule comprises DODAP.

[0467] Embodiment 41: The method of embodiment 40, wherein the DODAP comprises about 20% molar ratio of the total lipids.

[0468] Embodiment 42: The method of embodiment 38, wherein the DOTAP comprises about 50% molar ratio of the total lipids.

[0469] Embodiment 43: The method of embodiment 39, wherein the 18PA comprises about 10% molar ratio of the total lipids.

[0470] Embodiment 44: The method of embodiment 36 or 37, wherein the one or more SORT molecule comprises DOTMA.

[0471] Embodiment 45: The method of embodiments 35, 36 or 37, wherein the LNP comprises a ratio of DOPE:DOTMA between 3:1 and 1:3.

[0472] Embodiment 46: The method of embodiment 45, wherein the ratio of DOPE:DOTMA is about 3:1.

[0473] Embodiment 47: The method of embodiment 45, wherein the ratio of DOPE:DOTMA is about 1:1.

[0474] Embodiment 48: The method of any of embodiments 13, 35-47, wherein the SORT molecule comprises from about 5% to about 60% molar percentage of the LNP.

[0475] Embodiment 49: The method of any of embodiments 13, 35-47, wherein the SORT molecule comprises about 40% molar percentage of the LNP.

[0476] Embodiment 50: The method of any of embodiments 13, 35-47, wherein the SORT molecule comprises about 50% molar percentage of the LNP.

[0477] Embodiment 51: The method of any of embodiments 1-50, wherein the LNP binds vitronectin.

[0478] Embodiment 52: The method of any one of embodiments 1-51, wherein the guide RNA comprises a circular RNA.

[0479] Embodiment 53: The method of any one of embodiments 1-51, wherein the guide RNA comprises a linear RNA.

[0480] Embodiment 54: The method of any of embodiments 1-51, wherein the guide RNA is a single guide RNA (sgRNA).

[0481] Embodiment 55: The method of any of embodiments 1-54, wherein the guide RNA comprises a target sequence that is complementary with a target sequence of a cystic fibrosis transmembrane conductance regulator (*CFTR*) gene.

[0482] Embodiment 56: The method of any of embodiments 1-55, wherein the nucleotide sequence of the guide RNA is AAGTAAAACCTCTACAAATG (SEQ ID NO: 1) or TTGCTCATTGACCTCCACTC (SEQ ID NO: 2).

[0483] Embodiment 57: The method of any one of embodiments 1 to 56, wherein the composition comprises a pharmaceutically acceptable carrier.

[0484] Embodiment 58: The method of any one of embodiments 1-57, wherein the composition is administered intravenously.

[0485] Embodiment 59: The method of any one of embodiments 1-58, wherein the subject is a human.

[0486] Embodiment 60: The method of any one of embodiments 14-59, wherein the subject has cystic fibrosis.

[0487] Embodiment 61: A method of modifying the nucleic acid sequence of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene in a lung cell type, wherein the *CFTR* gene comprises a R553X stop codon mutation, the method comprising:

1. contacting the lung cell type with a composition comprising a lipid nanoparticle (LNP), wherein the LNP comprises (i) a first nucleic acid encoding a base editor; and (ii) a second nucleic acid encoding a guide RNA;
2. determining the nucleic acid sequence of the *CFTR* gene in the lung cell type, wherein the nucleic acid sequence of the *CFTR* gene in the lung cell type is modified to remove the R553X stop codon mutation.

[0488] Embodiment 62: The method of embodiment 61, wherein the modification comprises the replacing of the thymine at 1789 base in exon 11 of the *CFTR* gene with cytosine.

[0489] Embodiment 63: A method of increasing the expression of full-length cystic fibrosis transmembrane conductance regulator (CFTR) protein in a lung cell type, wherein a *CFTR* gene in the lung cell type comprises a R553X mutation, the method comprising:

1. contacting the lung cell type with a composition comprising a lipid nanoparticle (LNP), wherein the LNP comprises (i) a first nucleic acid encoding a base editor; and (ii) a second nucleic acid encoding a guide RNA;
2. determining the expression of full-length CFTR protein in the lung cell type, wherein the expression of full-length CFTR protein is increased in the lung cell type, as compared to a lung cell type comprising a *CFTR* gene comprising a R553X mutation, and that is not contacted with the composition.

[0490] Embodiment 64: A method of modulating the activity of the cystic fibrosis transmembrane conductance regulator (CFTR) protein in a lung cell type, wherein the *CFTR* gene in the lung cell type comprises a R553X mutation, the method comprising:

1. contacting the lung cell type with a composition comprising a lipid nanoparticle (LNP), wherein the LNP comprises (i) a first nucleic acid encoding a base editor; and (ii) a second nucleic acid encoding a guide RNA;
2. determining the activity of the CFTR protein in the lung cell type, wherein the activity of the CFTR protein is modulated in the lung cell type, as compared to a lung cell type comprising a *CFTR* gene comprising a R553X mutation, and that is not contacted with the composition.

[0491] Embodiment 65: The method of embodiment 63, wherein expression of the CFTR protein is determined in a lung cell type in a subject, wherein the subject has been administered the composition, and wherein the expression is determined by one or more bioassays comprising sweat chloride concentration assay, β-adrenergic sweat assay, and nasal potential difference assay.

[0492] Embodiment 66: The method of embodiment 65, wherein expression of the CFTR protein is determined by analysis of chloride levels in the sweat of the subject.

[0493] Embodiment 67: The method of embodiment 65, wherein the chloride levels in the sweat of the subject after being administered the composition are decreased as compared to the chloride levels in the sweat of the subject before being administered the composition.

[0494] Embodiment 68: The method of embodiment 63, wherein the expression is measured using western blotting, immunoprecipitation, and anti-CFTR antibodies.

[0495] Embodiment 69: The method of embodiment 64, wherein the activity of the CFTR protein is increased in the lung cell type, as compared to a lung cell type comprising a *CFTR* gene comprising a R553X mutation, and that is not contacted with the composition.

[0496] Embodiment 70: The method of any one of embodiments 61-69 wherein the lung cell type is an endothelial cell or an epithelial cell.

[0497] Embodiment 71: The method of any one of embodiments 61-69, wherein the lung cell type is an immune cell.

[0498] Embodiment 72: The method of any one of embodiments 61-69, wherein the lung cell type is a stem cell.

[0499] Embodiment 73: The method of any one of embodiments 61-72, wherein the nucleic acid encoding the base editor is DNA.

[0500] Embodiment 74: The method of any one of embodiments 61-72, wherein the nucleic acid encoding the base editor is RNA.

[0501] Embodiment 75: The method of any one of embodiments 61-72, wherein the base editor is an adenine base editor (ABE).

[0502] Embodiment 76: The method of embodiment 75, wherein the ABE comprises a tRNA adenosine deaminase (TadA) protein, or a variant thereof, fused to a catalytically impaired Cas protein capable of binding to a specific nucleotide sequence.

[0503] Embodiment 77: The method of any one of embodiments 61-72, wherein the base editor is a cytosine base editor (CBE).

[0504] Embodiment 78: The method of embodiment 77, wherein the CBE comprises a cytidine deaminase protein, or a variant thereof, fused to a catalytically impaired Cas protein capable of binding to a specific nucleotide sequence.

[0505] Embodiment 79: The method of any of embodiments 75-78, wherein the ABE or the CBE further comprises a Cas9 enzyme that does not have any nuclease activity.

[0506] Embodiment 80: The method of any one of embodiments 61-79, wherein the ratio of the first nucleic acid to the second nucleic acid is 1:1 on a molecule:molecule basis.

[0507] Embodiment 81: The method of any one of embodiments 61-79, wherein the ratio of the first nucleic acid to the second nucleic acid is 1:1 on a weight basis.

[0508] Embodiment 82: The method of any one of embodiments 61-79, wherein the ratio of the first nucleic acid to the second nucleic acid is 2:1 molecule:molecule basis.

[0509] Embodiment 83: The method of any one of embodiments 61-79, wherein the ratio of the first nucleic acid to the second nucleic acid is 2:1 on a weight basis.

[0510] Embodiment 84: The method of any one of embodiments 61-83, wherein the LNP comprises an ionizable cationic lipid, a zwitterionic phospholipid, a cholesterol, and a PEG lipid.

[0511] Embodiment 85: The method of any one of embodiments 61-84, wherein the LNP comprises 5A2-SC8, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), cholesterol, DMG-PEG, and one or more selective organ targeting (SORT) molecules.

[0512] Embodiment 86: The method of embodiment 85, wherein the one or more SORT molecules comprises permanently positively charged moiety.

[0513] Embodiment 87: The method of embodiment 85 or 86, wherein the one or more SORT molecule is selected from the group consisting of 18:1 DOTMA (DOTMA); DORI, DC-6-14; 12:0 EPC (Chloride Salt); 14:0 EPC (Chloride Salt); 16:0 EPC (Chloride Salt); 18:0 EPC (Chloride Salt); 18:1 EPC (Chloride Salt); 16:0-18:1 EPC (Chloride Salt); 14:1 EPC (Triflate Salt); 18:0 DDAB (Dimethyldioctadecylammonium (Bromide Salt)); 14:0 TAP; 16:0 TAP; 18:0 TAP; 18:1 TAP (DOTAP); 18:1 TAP (DOTAP, MS Salt); 18:1 DODAP, or 18:1 PA (1,2-dioleoyl-sn-glycero-3-phosphate (sodium salt)) (18PA).

[0514] Embodiment 88: The method of any of embodiments 85-87, wherein the one or more SORT molecules comprises DOTAP (1,2-dioleoyl-3-trimethylammonium propane).

[0515] Embodiment 89: The method of any of embodiments 85-87, wherein the one or more SORT molecules comprises 18PA.

[0516] Embodiment 90: The method of any of embodiments 85-87, wherein the one or more SORT molecules comprises DODAP.

[0517] Embodiment 91: The method of embodiment 90, wherein the DODAP comprises about 20% molar ratio of the total lipids.

[0518] Embodiment 92: The method of embodiment 88, wherein the DOTAP comprises about 50% molar ratio of the total lipids.

[0519] Embodiment 93: The method of embodiment 89, wherein the 18PA comprises about 10% molar ratio of the total lipids.

[0520] Embodiment 94: The method of any of embodiments 85-87, wherein the SORT molecule comprises DOTMA.

[0521] Embodiment 95: The method of embodiment 85, 86 or 87, wherein the LNP comprises a ratio of DOPE:DOTMA of between 3:1 and 1:3.

[0522] Embodiment 96: The method of embodiment 95, wherein the ratio of DOPE:DOTMA is about 3:1.

[0523] Embodiment 97: The method of embodiment 95, wherein the ratio of DOPE:DOTMA is about 1:1.

[0524] Embodiment 98: The method of any of embodiments 85-97, wherein the SORT molecule comprises from about 5% to about 60% molar percentage of the LNP.

[0525] Embodiment 99: The method of any of embodiments 85-98, wherein the SORT molecule comprises about 40% molar percentage of the LNP.

[0526] Embodiment 100: The method of any of embodiments 85-98, wherein the SORT molecule comprises from 50% molar percentage of the LNP.

[0527] Embodiment 101: The method of any of embodiments 61-100, wherein the LNP binds vitronectin.

[0528] Embodiment 102: The method of any one of embodiments 61-101, wherein the guide RNA comprises a circular RNA.

[0529] Embodiment 103: The method of any one of embodiments 61-101, wherein the guide RNA comprises a linear RNA.

[0530] Embodiment 104: The method of any one of embodiments 61-101, wherein the guide RNA is a single guide RNA (sgRNA).

[0531] Embodiment 105: The method of any of embodiments 61-104, wherein the guide RNA comprises a target sequence that is complementary with a target sequence of a cystic fibrosis transmembrane conductance regulator (*CFTR*) gene.

[0532] Embodiment 106: The method of any of embodiments 61-105, wherein the nucleotide sequence of the guide RNA is AAGTAAAACCTCTACAAATG (SEQ ID NO: 1) or TTGCTCATTGACCTCCACTC (SEQ ID NO: 2).

[0533] Embodiment 107: The method of embodiment 4, wherein the function of the CFTR protein is determined by one or more bioassays comprising sweat chloride concentration assay, β-adrenergic sweat assay, and nasal potential difference assay.

[0534] Embodiment 108: The method of embodiment 107, wherein the function of the CFTR protein is determined by analysis of chloride levels in the sweat of the subject.

[0535] Embodiment 109: The method of embodiment 108, wherein the chloride levels in the sweat of the subject after being administered the composition are decreased as compared to the chloride levels in the subject before being administered the composition.

[0536] Embodiment 110: The method of any one of embodiments 61-109, wherein the composition comprises a pharmaceutically acceptable carrier.

[0537] Embodiment 111: The method of any one of embodiments 66-110, wherein the subject is a human.

[0538] Embodiment 112: The method of any one of embodiments 66-111, wherein the administration of the composition to the subject is by intravenous administration.

[0539] Embodiment 113: A method of restoring the function of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene in a subject with cystic fibrosis, the method comprising:

1. administering to the subject a composition comprising a lipid nanoparticle (LNP), wherein the LNP comprises (i) a first nucleic acid encoding a base editor; and (ii) a second nucleic acid encoding a guide RNA;
2. determining the function of the *CFTR* gene in the subject,

wherein the function of the *CFTR* gene is restored in the subject.

[0540] Embodiment 114: The method of embodiment 113, wherein about 5% to about 95% of the function of the *CFTR* gene is restored.

[0541] Embodiment 115: The method of embodiment 113 or 114, wherein the restoring of the function of the *CFTR* gene is determined by the increase of CFTR protein expression.

[0542] Embodiment 116: The method of embodiment 115, wherein expression of the CFTR protein is determined by one or more bioassays comprising sweat chloride concentration assay, β-adrenergic sweat assay, and nasal potential difference assay.

[0543] Embodiment 117: The method of embodiment 115 or 116 wherein expression of the CFTR protein is determined by analysis of chloride levels in the sweat of the subject.

[0544] Embodiment 118: The method of embodiment 117, wherein chloride levels in the sweat of the subject after being administered the composition are decreased as compared to levels in a subject before being administered the composition.

[0545] Embodiment 119: The method of embodiment 115, wherein the expression is measured using western blotting, immunoprecipitation, and anti-CFTR antibodies.

[0546] Embodiment 120: The method of any one of embodiments 113-119, wherein the nucleic acid encoding the base editor is DNA.

[0547] Embodiment 121: The method of any one of embodiments 113-119, wherein the nucleic acid encoding the base editor is RNA.

[0548] Embodiment 122: The method of any one of embodiments 113-121, wherein the base editor is an adenine base editor (ABE).

[0549] Embodiment 123: The method of embodiment 122, wherein the base editor is ABE8e.

[0550] Embodiment 124: The method of embodiment 122, wherein the ABE comprises a tRNA adenosine deaminase (TadA) protein, or a variant thereof, fused to a catalytically impaired Cas protein capable of binding to a specific nucleotide sequence.

[0551] Embodiment 125: The method of any one of embodiments 113-121, wherein the base editor is a cytosine base editor (CBE).

[0552] Embodiment 126: The method of embodiment 122, wherein the CBE comprises a cytidine deaminase protein, or a variant thereof, fused to a catalytically impaired Cas protein capable of binding to a specific nucleotide sequence.

[0553] Embodiment 127: The method of embodiments 122-126, wherein the ABE or the CBE further comprises a Cas9 enzyme that does not have any nuclease activity.

[0554] Embodiment 128: The method of any one of embodiments 113-126, wherein the ratio of the first nucleic acid to the second nucleic acid is 1:1 on a molecule:molecule basis.

[0555] Embodiment 129: The method of any one of embodiments 113-126, wherein the ratio of the first nucleic acid to the second nucleic acid is 1:1 on a weight basis.

[0556] Embodiment 130: The method of any one of embodiments 113-129, wherein the ratio of the first nucleic acid to the second nucleic acid is 2:1 molecule:molecule basis.

[0557] Embodiment 131: The method of any one of embodiments 113-129, wherein the ratio of the first nucleic acid to the second nucleic acid is 2:1 on a weight basis.

[0558] Embodiment 132: The method of any one of embodiments 113-131, wherein the LNP comprises an ionizable cationic lipid, a zwitterionic phospholipid, a cholesterol, and a PEG lipid.

[0559] Embodiment 133: The method of any one of embodiments 113-132, wherein the LNP comprises 5A2-SC8, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), cholesterol, DMG-PEG, and one or more selective organ targeting (SORT) molecules.

[0560] Embodiment 134: The method of embodiment 133, wherein the one or more SORT molecules comprises permanently positively charged moiety.

[0561] Embodiment 135: The method of embodiment 133 or 134, wherein the one or more SORT molecule is selected from the group consisting of 18:1 DOTMA (DOTMA); DORI, DC-6-14; 12:0 EPC (Chloride Salt); 14:0 EPC (Chloride Salt); 16:0 EPC (Chloride Salt); 18:0 EPC (Chloride Salt); 18:1 EPC (Chloride Salt); 16:0-18:1 EPC (Chloride Salt); 14:1 EPC (Triflate Salt); 18:0 DDAB (Dimethyldioctadecylammonium (Bromide Salt)); 14:0 TAP; 16:0 TAP; 18:0 TAP; 18:1 TAP (DOTAP); 18:1 TAP (DOTAP, MS Salt); 18:1 DODAP, or 18:1 PA (1,2-dioleoyl-sn-glycero-3-phosphate (sodium salt)) (18PA).

[0562] Embodiment 136: The method of any of embodiments 133-135, wherein the one or more SORT molecules comprises DOTAP (1,2-dioleoyl-3-trimethylammonium propane).

[0563] Embodiment 137: The method of any of embodiments 133-135, wherein the one or more SORT molecules comprises 18PA.

[0564] Embodiment 138: The method of any of embodiments 133-135, wherein the one or more SORT molecules comprises DODAP.

[0565] Embodiment 139: The method of embodiment 138, wherein the DODAP comprises about 20% molar ratio of the total lipids.

[0566] Embodiment 140: The method of embodiment 136, wherein the DOTAP comprises about 50% molar ratio of the total lipids.

[0567] Embodiment 141: The method of embodiment 137, wherein the 18PA comprises about 10% molar ratio of the total lipids.

[0568] Embodiment 142: The method of any of embodiments 133-135, wherein the one or more SORT molecules comprises DOTMA.

[0569] Embodiment 143: The method of embodiment 133, 134, or 135, wherein the LNP comprises a ratio of DOPE:DOTMA of between 3:1 and 1:3.

[0570] Embodiment 144: The method of embodiment 143, wherein the ratio of DOPE:DOTMA is about 3:1.

[0571] Embodiment 145: The method of embodiment 143, wherein the ratio of DOPE:DOTMA is about 1:1.

[0572] Embodiment 146: The method of any of embodiments 133-145, wherein the one or more SORT molecules comprises from about 5% to about 60% molar percentage of the LNP.

[0573] Embodiment 147: The method of any of embodiments 133-145, wherein the one or more SORT molecules comprises about 40% molar percentage of the LNP.

[0574] Embodiment 148: The method of any of embodiments 114-145, wherein the SORT molecule comprises from 50% molar percentage of the LNP.

[0575] Embodiment 149: The method of any of embodiments 114-148, wherein the LNP binds vitronectin.

[0576] Embodiment 150: The method of any one of embodiments 114-149, wherein the guide RNA comprises a circular RNA.

[0577] Embodiment 151: The method of any one of embodiments 114-149, wherein the guide RNA comprises a linear RNA.

[0578] Embodiment 152: The method of any one of embodiments 114-149, wherein the guide RNA is a single guide RNA (sgRNA).

[0579] Embodiment 153: The method of any of embodiments 114-152, wherein the guide RNA comprises a target sequence that is complementary with a target sequence of a cystic fibrosis transmembrane conductance regulator (*CFTR*) gene.

[0580] Embodiment 154: The method of any of embodiments 111-150, wherein the nucleotide sequence of the guide RNA is AAGTAAAACCTCTACAAATG (SEQ ID NO: 1) or TTGCTCATTGACCTCCACTC (SEQ ID NO: 2).

[0581] Embodiment 155: The method of any one of embodiments 114-154, wherein the composition comprises a pharmaceutically acceptable carrier.

[0582] Embodiment 156: The method of any one of embodiments 114-155, wherein the subject is a human.

[0583] Embodiment 157: The method of any one of embodiments 114-156, wherein the administration of the composition to the subject is by intravenous administration.

[0584] Embodiment 158: The method of any of embodiments 1-157, wherein the LNP is localized to the lungs of the subject.

[0585] Embodiment 159: The method of any of embodiments 1-157, wherein the LNP is capable of delivering the first and second nucleic acids to the lungs of the subject.

[0586] Embodiment 160: A lung cell type comprising a modified cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, wherein the modification comprises the replacement of the thymine at 1789 base in exon 11 of the *CFTR* gene with cytosine.

[0587] Embodiment 161: A method of treating cystic fibrosis in a subject, the method comprising administering to the subject a composition comprising a lipid nanoparticle (LNP), wherein the LNP comprises (i) a first nucleic acid encoding a base editor; and (ii) a second nucleic acid encoding a guide RNA, wherein the first nucleic acid and the second nucleic acid are delivered to a lung cell in the subject.

VIII. EXAMPLES

[0588] The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

Example 1: Lung SORT LNPs mediate stem cell delivery *in vivo* to achieve durable editing for over one year

[0589] The potential of Lung SORT LNPs to deliver mRNA-encoded gene editors to various lung cell types, including stem cells, was investigated. Ai14 LoxP-stop-LoxP tdTomato (tdTom) reporter mice (Q. Cheng *et al.*, Selective Organ Targeting (SORT) nanoparticles for tissue specific mRNA delivery and CRISPR/Cas gene editing. *Nat. Nanotechnol.* **15**, 313-320 (2020); L. Madisen *et al.*, A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nat. Neurosci.* **13**, 133-140 (2010); J. B. Miller *et al.*, Non-viral CRISPR/Cas gene editing in vitro and in vivo enabled by synthetic nanoparticle co-delivery of Cas9 mRNA and sgRNA. *Angew. Chem. Int. Ed.* **56**, 1059-1063 (2017); T. Wei, Q. Cheng, Y.-L. Min, E. N. Olson, D. J. Siegwart, Systemic nanoparticle delivery of CRISPR-Cas9 ribonucleoproteins for effective tissue specific genome editing. *Nat. Commun.* **11**, 3232 (2020);

X. Wang *et al.*, Preparation of selective organ targeting (SORT) lipid nanoparticles (LNPs) using multiple technical methods for tissue-specific mRNA delivery. *Nat. Protoc.* **18**, 265-291 (2022)), in which removal of the stop cassette orchestrated by Cre recombinase or CRISPR-Cas9 translated from mRNA activates tdTom expression, were used allowing identification of gene edited cells. Mice were administrated with Cre mRNA Lung SORT LNPs (LNP-Cre) IV (2 mg/kg, 2 doses 48 hours apart) to evaluate the long-term efficacy of lung editing. Lung tissues were collected at various intervals post the second injection, including baseline 48 hours, 7 days, 21 days, 42 days, 60 days, 120 days, 180 days, 270 days, and 360 days (**FIG. 1A**) for *ex-vivo* imaging and flow cytometry. tdTom expression was uniformly spread throughout the mouse lung at every time point (**FIG. 1B**). tdTom quantification by average radiance (**FIG. 1C**) and total flux (**FIG. 1D**) indicated persistence of the tdTom for the entire 360-day experiment.

[0590] Basal cells are regarded as the tissue-specific stem cells of the mouse and human airway epithelium due to their ability to self-renew and differentiate into multiple lineages, including ciliated cells, secretory cells, goblet cells and ionocytes (D. T. Montoro *et al.*, A revised airway epithelial hierarchy includes CFTR-expressing ionocytes. *Nature* **560**, 319-324 (2018); J. R. Rock *et al.*, Basal cells as stem cells of the mouse trachea and human airway epithelium. *Proc. Natl. Acad. Sci. USA* **106**, 12771-12775 (2009); J. R. Rock, S. H. Randell, B. L. Hogan, Airway basal stem cells: a perspective on their roles in epithelial homeostasis and remodeling. *Dis. Models Mech.* **3**, 545-556 (2010); Y. Zhou *et al.*, Airway basal cells show regionally distinct potential to undergo metaplastic differentiation. *Elife* **11**, (2022)). These cells in the mouse and human airway epithelium can be identified by the expression of nerve growth factor receptor (Ngfr) (J. R. Rock *et al.*, Basal cells as stem cells of the mouse trachea and human airway epithelium. *Proc. Natl. Acad. Sci. USA* **106**, 12771-12775 (2009)) or cytoskeletal protein keratin 5 (Krt5) (W. Zuo *et al.*, p63+Krt5+ distal airway stem cells are essential for lung regeneration. *Nature* **517**, 616-620 (2015)). Ngfr-positive cells, when isolated from mouse and human airway epithelium, are enriched in cells possessing stem cell properties (J. R. Rock *et al.*, Basal cells as stem cells of the mouse trachea and human airway epithelium. *Proc. Natl. Acad. Sci. USA* **106**, 12771-12775 (2009)). Subsequent lineage tracing studies in mice revealed that Krt5-positive basal cells exhibit stem cell characteristics and are vital for mouse lung regeneration (A. E. Vaughan *et al.*, Lineage-negative progenitors mobilize to regenerate lung epithelium after major injury. *Nature* **517**, 621-625 (2015)).

[0591] Based on flow cytometry analysis, mice had durable editing in a diverse array of lung cell types encompassing endothelial cells ($CD31^+$), epithelial cells ($EpCam^+$), immune cells ($CD45^+$), and stem cells ($Ngfr^+$ and $Krt5^+$) (**FIG. 1E** and **FIG. 1F**). Over 32% of all lung cells manifested tdTom-positive ($tdTom^+$) expression 48 hours after the second LNP dose (baseline 48-hour mark), which subsequently escalated to 51% on day 7 and then retained this level throughout the 360-day experiment (**FIG. 1G**). Editing level was consistently high among major lung cell types at one-year end point (>95% in $CD31^+$ cells, >27% in $CD45^+$ cells, >68% in $EpCam^+$ cells) (**FIG. 1H-FIG. 1J**). Among $EpCam^+Ngfr^+$ stem cells, 28% showed tdTom expression on day 2, climbing to 54% on day 7, and reaching 70% on day 360 (**FIG. 1K**). Within $EpCam^+$Krt5+ stem cells, 27% displayed tdTom expression at 48 hours, peaking to 84% on day 21 and then remaining between 50-80% for up to 360 days (**FIG. 1L**). This dynamic tdTom expression within stem cells may be indicative of mouse stem cell turnover rate over different time intervals (J. R. Rock *et al.*, Basal cells as stem cells of the mouse trachea and human airway epithelium. *Proc. Natl. Acad. Sci. USA* **106**, 12771-12775 (2009); J. R. Rock, S. H. Randell, B. L. Hogan, Airway basal stem cells: a perspective on their roles in epithelial homeostasis and remodeling. *Dis. Models Mech.* **3**, 545-556 (2010); W. Zuo *et al.*, p63+Krt5+ distal airway stem cells are essential for lung regeneration. *Nature* **517**, 616-620 (2015); A. E. Vaughan *et al.*, Lineage-negative progenitors mobilize to regenerate lung epithelium after major injury. *Nature* **517**, 621-625 (2015); Pooja *et al.*, Distal airway stem cells yield alveoli in vitro and during lung regeneration following H1N1 influenza infection. *Cell* **147**, 525-538 (2011)).

[0592] Gene editing was further confirmed through application of CRISPR-Cas9 as a second editing approach. Lung SORT LNPs containing Cas9 mRNA and sgTOM1 (B. T. Staahl *et al.*, Efficient genome editing in the mouse brain by local delivery of engineered Cas9 ribonucleoprotein complexes. *Nat. Biotechnol.* **35**, 431-434 (2017)) (2:1, wt:wt) (LNP-Cas9) were administered IV to Ai14 reporter mice (2 mg/kg per dose, 3 doses 7 days apart). Sequences of the sgRNA are below in **Table E1**.

Table E1. Sequences of sgRNA used in this study.

sgRNA	**Sequence (5' to 3')**	**PAM (5' to 3')**
sgTOM1	AAGTAAAACCTCTACAAATG	TGG
sgR553X	TTGCTCATTGACCTCCACTC	AG

[0593] LNP-Cas9 mediated *in vivo* editing yielded persistent tdTom expression across the lungs at eight-month end point including >16% in whole lung cells, >40% in endothelial cells, >7% in immune cells, >25% in epithelial cells, >10% in stem cells (**FIG. 2A-FIG. 2G**). Confirming editing in stem cells at these levels was encouraging since CRISPR-Cas9 underestimates editing because double or triple stop deletion is required to activate tdTom. Next, *in vivo* toxicity was evaluated and it was found that LNP-Cas9 did not alter kidney and liver function up to 240 days at the tested dose (2 mg/kg total RNA three times) (**FIG. 3A-FIG. 3D**), suggesting minimal toxicity and repeat dose feasibility. Additionally, we examined whether Lung SORT LNPs facilitate delivery to the tracheal region of the lung. High levels of gene editing were quantified, with overall lower editing rates in the trachea compared to the bronchus (**FIG. 4A-FIG. 4G**). Lung stem cells were again edited by Lung SORT LNPs in the trachea.

Example 2: In vivo editing of lung resident endothelial and immune progenitor cells

[0594] The lifespans of both lung endothelial cells and lung resident immune cells range from a few days to several weeks (D. H. Bowden, Cell turnover in the lung. *Am. Rev. Respir. Dis.* **128**, S46-S48 (1983); I. Y. R. Adamson, in *Toxicology of Inhaled Materials: General Principles of Inhalation Toxicology,* H. Witschi, J. D. Brain, Eds. (Springer Berlin Heidelberg, Berlin, Heidelberg, 1985), chap. 11, pp. 289-317). Given this high turnover rate, it raises the question of how the editing efficiencies in these cell types are sustained for an entire year. It was determined whether the lung SORT LNPs would transfect and edit lung resident endothelial and immune progenitor cells, ultimately leading to a pool of edited mature cells. Recently, it has been demonstrated that CD157 (BST1) serves as a marker for tissue-resident endothelial progenitors in a range of organs, including the lungs (T. Wakabayashi *et al.*, CD157 marks tissue-resident endothelial stem cells with homeostatic and regenerative properties. *Cell Stem Cell* **22**, 384-397.e386 (2018)). Flow cytometry was employed to track the expression of tdTom in lung tissue-resident endothelial progenitor cells ($CD31^+CD157^+$) after an IV injection of LNP-Cre (**FIG. 5A**). Over 87% of $CD31^+CD157^+$ cells expressed tdTom at 48 hours, which rose to 95% by day 7, and was sustained for a year (**FIG. 5B**). Studies also indicate the existence of a pool of lung resident hematopoietic stem/progenitor cells. These cells display a similar marker expression profile to that found in bone marrow, $Lin^-Sca1^+c\text{-}kit^+$ (LSK) for hematopoietic stem cells (HSCs) and $Lin^-Sca1^-c\text{-}kit^+$ for multipotent progenitors (MPs) (X. Wang *et al.*, Preparation of selective organ targeting (SORT) lipid nanoparticles (LNPs) using multiple technical methods for tissue-specific mRNA delivery. *Nat. Protoc.* **18**, 265-291 (2022)). Following IV

administration of LNP-Cre, 74% of lung resident HSCs and 42% of lung resident MPs expressed tdTom at 48 hours, which remained persistent over 360 days (**FIG. 6A-FIG. 6C**).

Example 3: Long-term editing of diverse lung epithelial cell types in vivo

[0595] The lung's epithelial lining is made up of a diverse assortment of cell types, each performing unique roles in preserving lung functionality and homeostasis (D. N. Kotton, E. E. Morrisey, Lung regeneration: mechanisms, applications and emerging stem cell populations. *Nat. Med.* **20**, 822-832 (2014); Brigid *et al.*, Repair and regeneration of the respiratory system: Complexity, plasticity, and mechanisms of lung stem cell function. *Cell Stem Cell* **15**, 123-138 (2014)). Key epithelial cell types in the lung include alveolar type 1 (AT1) cells for gas-change function, type 2 (AT2) cells for maintaining homeostasis in the lung alveolar region, goblet cells for mucus production, ciliated cells for mucus removal, and club cells for bronchiolar epithelium protection. To delve deeper into transfected cell types, lung tissues at various time points following two IV injections of LNP-Cre, 48 hours apart, were harvested. Tissue section imaging with a whole-slide scanner allowed for the identification of co-localization of tdTom expression and cell-specific markers. The primary antibody staining conditions are listed below in **Table E2**. All primary antibodies are used at a 1:100 dilution and incubated for 20 minutes.

Table E2: Primary Antibody Staining Conditions used for Immunohistochemistry

Cell type	**Primary Antibody (1:100)**
AT1 cells	Anti-Hop Antibody (E-1): sc-398703 (SantaCruz; sc-398703)
AT2 cells	Purified anti-ABCA3 Antibody (Biolegend; 911001)
Goblet cells	Recombinant Anti-Mucin 5AC antibody [45M1] (AbCam; ab3649)
Ciliated cells	Monoclonal Anti-Tubulin, Acetylated antibody produced in mouse (Millipore Sigma; T6793-100UL)
Club cells	Anti- SCGB1A1 antibody (AbCam; ab40873)

[0596] Nearly all cell types displayed co-localization of tdTom and their respective cell marker, indicating widespread editing (**FIG. 7A**). Approximately 18% of AT1 cells, 20% of AT2 cells, 10% of goblet cells, 6% of ciliated cells, and 2% of club cells showed evidence of

editing and tdTom expression, which persisted for up to 360 days (**FIG. 7B**). Whole section images (**FIG. 7C-FIG. 7E**) of the entire lung after LNP-Cre treatment reveal a uniform distribution of tdTom expression throughout lung lobe. A second reporter mouse, double-fluorescent mTmG (M. D. Muzumdar, B. Tasic, K. Miyamichi, L. Li, L. Luo, A global double-fluorescent Cre reporter mouse. *Genesis* **45**, 593-605 (2007)) was used to further visualize and quantify edited cells (~8% by tissue volume) two days after a single LNP-Cre administration that mediated excision to turn on eGFP expression replacing pre-existing tdTom fluorescent proteins (**FIG. 8A, FIG. 8B**). This further supports lung SORT LNP enabled genome editing in the majority of different lung cell types.

Example 4: Vitronectin receptor expressing lung cell types exhibit enhanced editing efficiency

[0597] Recent work has demonstrated that SORT LNPs avidly bind distinct plasma proteins after IV administration, thereby facilitating endogenous targeting of organs and cell types (S. A. Dilliard, Q. Cheng, D. J. Siegwart, On the mechanism of tissue-specific mRNA delivery by selective organ targeting nanoparticles. *Proc. Natl. Acad. Sci. USA* **118**, e2109256118 (2021)). Whether that the protein corona of the Lung SORT LNPs used was most highly enriched in vitronectin using proteomic analysis was verified (**FIG. 9A, FIG. 9B**). It was previously reported that bound serum vitronectin aided the uptake of LNPs by vitronectin receptor-expressing kidney cancer cells (A-498) in vitro (S. A. Dilliard, Q. Cheng, D. J. Siegwart, On the mechanism of tissue-specific mRNA delivery by selective organ targeting nanoparticles. *Proc. Natl. Acad. Sci. USA* **118**, e2109256118 (2021)). Yet, the mechanism through which the serum vitronectin, bound to Lung SORT LNPs, impacts the targeting of lung cells *in vivo* remains unclear.

[0598] To pursue this goal, the expression of the vitronectin receptor ($CD51^+CD61^+$) by different lung cell types was analyzed. Most cell types exhibit high levels of vitronectin receptor expression (24.2% in $CD31^+$ cells 23.5% in $EpCam^+$ cells, 29.5% in $EpCam^+Ngfr^+$ cells and 33.6% in $EpCam^+Krt5^+$ cells) (**FIG. 10A, FIG. 10C-FIG. 10E**). However, in the case of $CD45^+$ cells, only a small proportion (5.0%) express the vitronectin receptor (**FIG. 10B; FIG. 11A-FIG. 11E**). To evaluate the correlation between vitronectin receptor expression with Lung SORT LNP mediated editing, the level of tdTom expression in the vitronectin receptor-positive lung cells was compared to their vitronectin receptor-negative counterparts 24 hours after a single LNP-Cre treatment. $EpCam^+$ (36.2% in $CD51^+CD61^+$ fraction vs. 14.7% in $CD51^-CD61^-$

fraction), $EpCam^+Ngfr^+$(38.9% vs. 13.7%), $EpCam^+Krt5^+$ (35.3% vs. 4.2%), and $CD45^+$ cells (38.2% vs. 3.1%) demonstrated significant tdTom expression enrichment within the vitronectin receptor-positive cells (**FIG. 10A-FIG. 10E**). These results emphasize the essential role of vitronectin receptor-mediated uptake of serum vitronectin bound lung SORT LNPs *in vivo*. Data show that other factors such as transcytosis (W. Zuo *et al.*, p63+Krt5+ distal airway stem cells are essential for lung regeneration. *Nature* **517**, 616-620 (2015)), passage through tight junctions (A. E. Vaughan *et al.*, Lineage-negative progenitors mobilize to regenerate lung epithelium after major injury. *Nature* **517**, 621-625 (2015)), and exosome transfer (Pooja *et al.*, Distal airway stem cells yield alveoli in vitro and during lung regeneration following H1N1 influenza infection. *Cell* **147**, 525-538 (2011)) could contribute to this intricate process of cellular LNP uptake (S. A. Dilliard, D. J. Siegwart, Passive, active and endogenous organ-targeted lipid and polymer nanoparticles for delivery of genetic drugs. *Nat. Rev. Mater.* **8**, 282-300 (2023)). Endothelial cells, which represent the first cellular barrier faced by Lung SORT LNPs displayed no preference for tdTom expression (~40% tdTom expression for each fraction), suggesting vitronectin dependent and independent processes. Further studies exploring these areas are instrumental in deepening the understanding of these complex mechanisms.

Example 5: Efficient editing of primary human CF patient-derived basal cells

[0599] To assess the therapeutic potential of Lung SORT LNP for genetic lung diseases, CF was used as a representative disease model which is caused by mutations in cystic fibrosis transmembrane conductance regulator (CFTR) gene. Among editing strategies, base editing is an attractive method for efficient single base pair correction with no reliance on double-stranded break repair pathways, resulting in minimal undesired byproducts, even in non-diving cells (N. M. Gaudelli *et al.*, Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. *Nature* **551**, 464-471 (2017); G. A. Newby, D. R. Liu, In vivo somatic cell base editing and prime editing. *Mol. Ther.* **29**, 3107-3124 (2021); W.-H. Yeh, H. Chiang, H. A. Rees, A. S. B. Edge, D. R. Liu, In vivo base editing of post-mitotic sensory cells. *Nat. Commun.* **9**, (2018)). A base editing strategy, A•T to G•C conversion of the currently untreatable CF nonsense mutation $CFTR^{R553X}$ to wild-type CFTR, has been reported in cells (M. H. Geurts *et al.*, CRISPR-Based Adenine Editors Correct Nonsense Mutations in a Cystic Fibrosis Organoid Biobank. *Cell Stem Cell* **26**, 503-510.e507 (2020); S. Krishnamurthy *et al.*, Functional correction of CFTR mutations in human airway epithelial cells using adenine base editors. *Nucleic Acids Res* **49**, 10558-10572 (2021)). This therapeutic strategy using the LNP-ABE

system co-encapsulating ABE mRNA and sgR553X was applied to correct CFTR R553X mutation in primary and immortalized human bronchial epithelial (HBE) cells.

[0600] In engineered 16HBEge CFTR-R553X cells (S. Krishnamurthy *et al.*, Functional correction of CFTR mutations in human airway epithelial cells using adenine base editors. *Nucleic Acids Res* **49**, 10558-10572 (2021)), LNP-ABE treatment led to 95.0% of correction revealed by DNA sequencing at 1.5 ug/well dose (**FIG. 12A**). Then the stoichiometry of ABE mRNA and sgR553X was investigated using 16HBEge cells and found that editing efficiency increased with higher ABE mRNA content peaking at 1:1 ABE mRNA to sgRNA (wt/wt) (**FIG. 12B**).

[0601] Next, the R553X mutation was corrected in primary CF patient-derived human bronchial epithelial (HBE) cells (D. C. Gruenert, W. E. Finkbeiner, J. H. Widdicombe, Culture and transformation of human airway epithelial cells. *Am. J. Physiol.* **268**, L347-360 (1995)) carrying the R553X/F508del heterozygous mutation. The difficult-to-treat HBE model has been used to develop CFTR modulator small molecule therapies due to its capability of differentiation into a pseudostratified epithelium in air-liquid interface (ALI) culture that mimic characteristics of *in vivo* airway biology, allowing strong prediction of therapeutic efficacy in humans (J. P. Clancy *et al.*, CFTR modulator theratyping: Current status, gaps and future directions. *J. Cyst. Fibros.* **18**, 22-34 (2019); F. Van Goor *et al.*, Rescue of CF airway epithelial cell function in vitro by a CFTR potentiator, VX-770. *Proc. Natl. Acad. Sci. USA* **106**, 18825-18830 (2009); F. Van Goor *et al.*, Correction of the F508del-CFTR protein processing defect in vitro by the investigational drug VX-809. *Proc. Natl. Acad. Sci. USA* **108**, 18843-18848 (2011)). The experimental workflow is described in **FIG. 13A**. Following LNP-ABE (1.4 ug total RNA, ABE mRNA:sgRNA=2:1, weight ratio) treatment, approximately 60% of allelic editing was achieved in both undifferentiated P2 culture composed mainly of basal cells and fully differentiated P3 culture analyzed by Editr (M. G. Kluesner *et al.*, EditR: A method to quantify base editing from Sanger sequencing. *CRISPR J.* **1**, 239-250 (2018)) (**FIG. 13B**). Using next generation sequencing (NGS) and CRISPResso2 (K. Clement *et al.*, CRISPResso2 provides accurate and rapid genome editing sequence analysis. *Nat. Biotechnol.* **37**, 224-226 (2019)) analysis, a mean 83.7% frequency of the desired product (C nucleotide at T_7), with a moderate level of bystander editing (14.5%) at adjacent T_{11} position for amino acid G553, but no off-target DNA editing at a further T_{17} position (**FIG. 13C**) was confirmed. No therapeutic outcome was affected by the bystander editing at T_{11} position since both GG**T** and GG**C** codon encoding glycine.

Additionally, no evidence of off-target DNA editing using sgR553X has been previously reported (S. Krishnamurthy *et al.*, Functional correction of CFTR mutations in human airway epithelial cells using adenine base editors. *Nucleic Acids Res* **49**, 10558-10572 (2021)). To evaluate the CFTR protein expression restoration after LNP-ABE treatment, JESS capillary western blotting (**FIG. 13D, FIG. 14A, FIG. 14B**) was performed. LNP-ABE alone doubled the expression of core glycosylated CFTR (Band B, **FIG. 13E**) and increased the expression of fully glycosylated, mature CFTR by 5.5-fold (Band C, **FIG. 13F**). Combined treatment with LNP-ABE and the CFTR small molecule modulator Trikafta (elexacaftor/tezacaftor/ivacaftor, the standard of care for CF patients with a single F508del allele (P. G. Middleton *et al.*, Elexacaftor–Tezacaftor–Ivacaftor for cystic fibrosis with a single Phe508del allele. *New. Engl. J. Med.* **381**, 1809-1819 (2019))), further increased the expression of fully glycosylated CFTR by 7.8-fold (Band C, **FIG. 13F**).

[0602] Lastly, the ability of LNP-ABE to restore HBE culture function was tested by measuring CFTR-dependent Cl^- channel activity 4 weeks after the LNP-ABE treatment using transepithelial current clamp (TECC) assay. Unlike non-CF derived HBE cultures, fully differentiated CF HBE cultures display similar function characteristics associated with CF airway phenotype *in vivo*, including the accumulation of thick sticky mucus on the top of epithelium and abnormal ion and fluid transport (T. Neuberger, B. Burton, H. Clark, F. Van Goor. (Humana Press, 2011), pp. 39-54). By measuring CFTR-dependent current changes, the area under the curve (AUC) between forskolin and ivacaftor (VX-770) stimulation, one can evaluate the level of functional restoration of CFTR. LNP-ABE alone effectively restored 53.4% of the CFTR function (AUC 11.6 μA/cm2*min) (**FIG. 15A**) compared to the activity of wild-type CFTR measured from HBE culture derived from an individual without cystic fibrosis presumed to have 100% CFTR activity (AUC 21.7 μA/cm2*min) (**FIG. 15B**), which greatly exceeds the widely accepted therapeutic threshold for CFTR activity restoration of > 10% to prevent CF disease phenotypes (S. M. Rowe, F. Accurso, J. P. Clancy, Detection of cystic fibrosis transmembrane conductance regulator activity in early-phase clinical trials. *Proc. Am. Thorac. Soc.* **4**, 387-398 (2007); M. D. Amaral, Processing of CFTR: Traversing the cellular maze-How much CFTR needs to go through to avoid cystic fibrosis? *Pediatr. Pulmonol.* **39**, 479-491 (2005)). CFTR function was further boosted and reached to the level of nearly 85% restoration in combination of LNP-ABE and Trikafta (AUC 18.5 μA/cm2*min) (**FIG. 15B**). Considering the nature of heterozygous CF HBE model, higher efficacy of restoration of CFTR

function might be achieved in homozygous model by treating with LNP-ABE alone. Together, these results suggest that efficient correction of undifferentiated lung basal cells can successfully produce corrected mature epithelium and restore CFTR function.

Example 6: In vivo stem cell editing in CF mouse lungs

[0603] Given the successful remodeling and regeneration of healthy human bronchial epithelium in primary HBE cells and Lung SORT LNP's ability to deliver genome editors *in vivo*, LNP-ABE-mediated editing in genetically engineered CF mice was evaluated. Despite having the homozygous R553X nonsense mutation in their genomic DNA, the model does not exhibit pathological features in the lung, consistent with other CF animal models (D. R. McHugh *et al.*, A G542X cystic fibrosis mouse model for examining nonsense mutation directed therapies. *Plos One* **13**, e0199573 (2018)). To address this constraint, intestinal stem cells were isolated from homozygous R553X mice and generated intestinal organoids as an *ex vivo* model (D. K. Crawford *et al.*, Targeting G542X CFTR nonsense alleles with ELX-02 restores CFTR function in human-derived intestinal organoids. *J. Cyst. Fibros.* **20**, 436-442 (2021)) to assess the restoration of CFTR function following LNP-ABE treatment (**FIG. 16A**). Using the forksolin-induced swelling (FIS) assay, forskolin activates CFTR, stimulates intracellular pathways, and phosphorylates CFTR to open the CFTR channel, permitting ion/water uptake, and ultimately organoid swelling. Once the mutated CFTR gene is corrected, organoid swelling should be observed, while untreated organoids will remain at the baseline volume (**FIG. 16B**). Organoid swelling after LNP-ABE treatment was quantified (**FIG. 16C**), with over 82% of organoids swelled (**FIG. 16D**), suggesting a high percentage of organoids being corrected by LNP-ABE. After extracting genomic DNA from those intestinal organoids, 47.8% of A•T to G•C conversion was confirmed by Editr analysis of sequencing results (**FIG. 16E**).

[0604] To assess the feasibility of base editing in lung stem cells for potential long-lasting effect, heterozygous R553X mice with one allele carrying human R553X mutation was used and the other one has normal mouse CFTR gene. By using a specific pair of primer design, only the human R553X region was amplified by PCR for sequencing analysis. The primers are listed below in **Table E3**.

Table E3. Primers used for sequencing analyses

Target	**Primer**	**Sequence (5' to 3')**
R553X (HBE model)	Forward	GGAAGATGTGCCTTTCAAATTCAG

	Reverse	ATGTGATTCTTAACCCACTAGCCA
R553X (Mouse model)	Forward	GGAAGATGTGCCTTTCAAATTCAG
	Reverse	ACAGCAAATGCTTGCTAG

[0605] LNP-ABE was administrated intravenously at 1.5 $mgkg^{-1}$ total RNA (ABE mRNA: sgR553X=2:1, weight ratio). Characteristics of the LNPs produced are shown in **Table E4**.

Table E4: Characterization of Lung SORT LNPs encapsuling ABE mRNA/sgR553X made by vortex mixing or T-mixing.

Mixing	Particle size (nm)	PDI	Zeta potential	% encapsulation
Vortex mixing	130.0 ± 0.8	0.14 ± 0.01	6.29 ± 0.34	82.9 ± 1.1
T-mixing	90.0 ± 0.7	0.14 ± 0.03	6.28 ± 0.56	98.2 ± 0.0

[0606] Ten days after a single treatment, whole mouse lungs were collected. Genomic DNA was extracted from whole lungs, trachea, and lung stem cells isolated from whole lung single cell suspension using magnetic based cell separation (**Figure 16F**) for PCR amplification and NGS sequencing. Surprisingly, a mean 51.7% of correction at T_7 position in lung stem cells, with 6.6% of frequency of bystander editing at T_{11} position and minimal or no conversion on T_{17} position, compared to a mean 11.5% and 28.8% of desired correction on T_7 in the whole lung and in trachea populations respectively was observed (**Figure 16G**). These results suggest that LNP-ABE can efficiently correct the target pathogenic mutation in mouse lung stem cells.

Example 7: Lung SORT LNPs mediate stem cell delivery to achieve durable editing for >1.5 years

[0607] The potential of lung SORT LNPs to deliver mRNA-encoded gene editors to various lung cell types, including stem cells, was investigated. Ai14 LoxP-stop-LoxP tdTomato (tdTom) reporter mice (Q. Cheng *et al.*, Selective Organ Targeting (SORT) nanoparticles for tissue specific mRNA delivery and CRISPR/Cas gene editing. *Nat. Nanotechnol.* **15**, 313-320 (2020); L. Madisen *et al.*, A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nat. Neurosci.* **13**, 133-140 (2010); J. B. Miller *et al.*, Non-viral CRISPR/Cas gene editing in vitro and in vivo enabled by synthetic nanoparticle co-delivery of Cas9 mRNA and sgRNA. *Angew. Chem. Int. Ed.* **56**, 1059-1063 (2017); T. Wei, Q. Cheng, Y.-L. Min, E. N. Olson, D. J. Siegwart, Systemic nanoparticle delivery of CRISPR-Cas9 ribonucleoproteins for effective tissue specific genome editing. *Nat. Commun.* **11**, 3232 (2020);

X. Wang *et al.*, Preparation of selective organ targeting (SORT) lipid nanoparticles (LNPs) using multiple technical methods for tissue-specific mRNA delivery. *Nat. Protoc.* **18**, 265-291 (2022)), in which removal of the stop cassette orchestrated by Cre recombinase or CRISPR-Cas9 translated from mRNA activates tdTom expression, were used allowing identification of gene edited cells (**FIG. 17A**). Mice were administrated with Cre mRNA Lung SORT LNPs (LNP-Cre) IV (2 mg/kg, 2 doses 48 hours apart) to evaluate the rates and long-term persistance of genetic changes in the lung after Cre editing. Lung tissues were collected at 10 intervals after the second injection, from 2 to 660 days (**FIG. 17B**) for *ex-vivo* imaging and flow cytometry. tdTom expression was uniformly spread throughout the mouse lung at every time point (**FIG. 17C**). tdTom quantification by average radiance (**FIG. 17D**) and total flux (**FIG. 17E**) indicated persistence of the tdTom for the entire 660-day experiment.

[0608] Basal cells are regarded as the tissue-specific stem cells of the mouse and human airway epithelium due to their ability to self-renew and differentiate into various mature cell lineages, including ciliated cells, secretory cells, goblet cells and ionocytes (D. T. Montoro *et al.*, A revised airway epithelial hierarchy includes CFTR-expressing ionocytes. *Nature* **560**, 319-324 (2018); J. R. Rock *et al.*, Basal cells as stem cells of the mouse trachea and human airway epithelium. *Proc. Natl. Acad. Sci. USA* **106**, 12771-12775 (2009); J. R. Rock, S. H. Randell, B. L. Hogan, Airway basal stem cells: a perspective on their roles in epithelial homeostasis and remodeling. *Dis. Models Mech.* **3**, 545-556 (2010); Y. Zhou *et al.*, Airway basal cells show regionally distinct potential to undergo metaplastic differentiation. *Elife* **11**, (2022)). These cells in the mouse and human airway epithelium can be identified by the expression of nerve growth factor receptor (Ngfr) (J. R. Rock *et al.*, Basal cells as stem cells of the mouse trachea and human airway epithelium. *Proc. Natl. Acad. Sci. USA* **106**, 12771-12775 (2009)) or cytoskeletal protein keratin 5 (Krt5) (W. Zuo *et al.*, p63+Krt5+ distal airway stem cells are essential for lung regeneration. *Nature* **517**, 616-620 (2015)), both of which were used to ensure a thorough and comprehensive investigation of editing efficiencies. Lineage tracing and clonal growth studies have supported their roles in differentiation and regeneration (J. R. Rock, S. H. Randell, B. L. Hogan, Airway basal stem cells: a perspective on their roles in epithelial homeostasis and remodeling. *Dis. Models Mech.* **3**, 545-556 (2010); J. R. Rock *et al.*, Basal cells as stem cells of the mouse trachea and human airway epithelium. *Proc. Natl. Acad. Sci. USA* **106**, 12771-12775 (2009); W. Zuo *et al.*, p63+Krt5+ distal airway stem cells are essential for lung regeneration.

Nature **517**, 616-620 (2015); Kumar *et al.*, Distal airway stem cells yield alveoli in vitro and during lung regeneration following H1N1 influenza infection. *Cell* **147**, 525-538 (2011)).

[0609] Based on flow cytometry analysis, mice had durable editing in a diverse array of lung cell types encompassing endothelial cells ($CD31^+$), epithelial cells ($EpCam^+$), immune cells ($CD45^+$), and stem cells ($Ngfr^+$ and $Krt5^+$) (**FIG. 17F** and **FIG. 17G**). Over 32% of all lung cells manifested tdTom-positive ($tdTom^+$) expression 48 hours after the second LNP dose (baseline mark), which subsequently escalated to 51% on day 7 and was sustained throughout the 660-day experiment (**Figure 17F**). Editing level was consistently high at the 660-day end point (>93% in endothelial cells, >23% in immune cells, and >48% in epithelial cells) (**FIG. 17I-FIG. 17K**). Within $EPCAM_+KRT5_+$ stem cells, 27% displayed tdTom expression at 48 hours, peaking to 97% on day 120 and then remaining between 45 and 80% for up to 660 days (**Figure 17M**). This dynamic tdTom expression within stem cells may be indicative of mouse stem cell turnover rate over different time intervals (J. R. Rock *et al.*, Basal cells as stem cells of the mouse trachea and human airway epithelium. *Proc. Natl. Acad. Sci. USA* **106**, 12771-12775 (2009); J. R. Rock, S. H. Randell, B. L. Hogan, Airway basal stem cells: a perspective on their roles in epithelial homeostasis and remodeling. *Dis. Models Mech.* **3**, 545-556 (2010); W. Zuo *et al.*, p63+Krt5+ distal airway stem cells are essential for lung regeneration. *Nature* **517**, 616-620 (2015); A. E. Vaughan *et al.*, Lineage-negative progenitors mobilize to regenerate lung epithelium after major injury. *Nature* **517**, 621-625 (2015); Pooja *et al.*, Distal airway stem cells yield alveoli in vitro and during lung regeneration following H1N1 influenza infection. *Cell* **147**, 525-538 (2011)).

[0610] The general applicability of lung SORT LNPs was further examined with CRISPR-Cas9 as a second editing approach. Lung SORT LNPs encapsulating Cas9 mRNA and sgTOM1 (B. T. Staahl *et al.*, Efficient genome editing in the mouse brain by local delivery of engineered Cas9 ribonucleoprotein complexes. *Nat. Biotechnol.* **35**, 431-434 (2017)) (2:1, wt:wt) (**Table E5**) (LNP-Cas9) were administered intravenously to Ai14 reporter mice (2 mg/kg per dose, three doses 7 days apart).

[0611] Sequences of the sgRNA are below in **Table E5**.

Table E5. Sequences of sgRNA used in this study.

sgRNA	Sequence (5' to 3')	PAM (5' to 3')
sgTOM1	AAGTAAAACCTCTACAAATG	TGG
sgR553X	TTGCTCATTGACCTCCACTC	AG

[0612] LNP-Cas9 editing yielded persistent tdTom expression across the lungs through the 8-month end point, including in stem cells (**FIG. 18A-FIG. 18H**). Confirming editing in stem cells at these levels was encouraging since multiple stop deletions are required to activate tdTom in this model, which can underestimate CRISPR-Cas9 editing outcomes.

[0613] Regarding tolerability, LNP-Cas9 did not alter kidney and liver function up to 240 days at the tested dose and did not cause tissue damage based on hematoxylin and eosin (H&E) staining of mouse hearts and spleens (2 mg/kg total RNA three times) (**FIG. 19A-FIG. 19E**). Lung SORT LNPs facilitated delivery to the trachea, which is involved in the pathophysiology of lung diseases, albeit at a lower overall rate (12.3%) compared to the bronchus (46.9%) (**FIG. 20A-FIG. 20G**). Editing was also observed in CF-affected organs, including the liver, pancreas, kidney, and gastrointestinal tract (**FIG. 21A-FIG. 21B**). A more in-depth characterization revealed 13.5-41.7% editing in various immune cell types, including neutrophils (L. M. Yonker *et al.*, Neutrophil dysfunction in cystic fibrosis. *J. Cyst. Fibros.* **20**, 1062-1071 (2021)) and macrophages (J. L. Gillan *et al.*, CAGE sequencing reveals CFTR-dependent dysregulation of type I IFN signaling in activated cystic fibrosis macrophages. *Sci. Adv.* **9**, eadg5128(2023)) (**FIG. 22A-FIG. 22B**). Moreover, delivery efficacy was unchanged in a bacterial infection model with inflamed lungs (S. Abid *et al.*, 17β-Estradiol dysregulates innate immune responses to pseudomonas aeruginosa respiratory infection and is modulated by estrogen receptor antagonism. *Infect. Immun.* **85**, (2017)) (**FIG. 23A-FIG. 23D**), suggesting infections common in people with CF may not reduce gene editing.

Example 8: In vivo editing of lung resident endothelial and immune progenitor cells

[0614] The lifespans of both lung endothelial cells and lung resident immune cells range from a few days to several weeks (D. H. Bowden, Cell turnover in the lung. *Am. Rev. Respir. Dis.* **128**, S46-S48 (1983); I. Y. R. Adamson, in *Toxicology of Inhaled Materials: General Principles of Inhalation Toxicology,* H. Witschi, J. D. Brain, Eds. (Springer Berlin Heidelberg, Berlin, Heidelberg, 1985), chap. 11, pp. 289-317). Given this high turnover rate, it raises the question of how the editing efficiencies in these cell types are sustained for more than 1.5 years. It was determined whether the lung SORT LNPs would transfect and edit lung resident endothelial and immune progenitor cells, ultimately leading to a pool of edited mature cells. Recently, it has been demonstrated that CD157 (BST1) serves as a marker for tissue-resident endothelial progenitors in a range of organs, including the lungs (T. Wakabayashi *et al.*, CD157 marks tissue-resident endothelial stem cells with homeostatic and regenerative properties. *Cell*

Stem Cell **22**, 384-397.e386 (2018)). Flow cytometry was employed to track the expression of tdTom in lung tissue-resident endothelial progenitor cells ($CD31^+CD157^+$) after an IV injection of LNP-Cre (**FIG. 24A**). Over 87% of $CD31^+CD157^+$ cells expressed tdTom at 48 hours, which rose to 95% by day 7, and was sustained for 660 days (**FIG. 24B**). Studies also indicate the existence of a pool of lung resident hematopoietic stem/progenitor cells. These cells display a marker expression profile of $Lin^-Sca1^+c\text{-}kit^+$ (LSK) for hematopoietic stem cells (HSCs) and $Lin^-Sca1^-c\text{-}kit^+$ for multipotent progenitors (MPs) (X. Wang *et al.*, Preparation of selective organ targeting (SORT) lipid nanoparticles (LNPs) using multiple technical methods for tissue-specific mRNA delivery. *Nat. Protoc.* **18**, 265-291 (2022)). Following IV administration of LNP-Cre, 74% of lung resident HSCs and 42% of lung resident MPs expressed tdTom at 48 hours, which remained persistent over 660 days (**FIG. 25A-FIG. 25C**).

Example 9: Long-term editing of diverse lung epithelial cell types in vivo

[0615] The lung's epithelial lining is made up of a diverse assortment of cell types, each performing unique roles in preserving lung functionality and homeostasis (D. N. Kotton, E. E. Morrisey, Lung regeneration: mechanisms, applications and emerging stem cell populations. *Nat. Med.* **20**, 822-832 (2014); Brigid *et al.*, Repair and regeneration of the respiratory system: Complexity, plasticity, and mechanisms of lung stem cell function. *Cell Stem Cell* **15**, 123-138 (2014)). Key epithelial cell types in the lung include alveolar type 1 (AT1) cells for gas-change function, type 2 (AT2) cells for maintaining homeostasis in the lung alveolar region, goblet cells for mucus production, ciliated cells for mucus removal, club cells for bronchiolar epithelium protection, and rare ionocytes that highly express CFTR (Plasschaert *et al.*, A single-cell atlas of the airway epithelium reveals the CFTR-rich pulmonary ionocyte. *Nature.* **560**(7718):377-381 (2018)). To delve deeper into transfected cell types, lung tissues at various time points following two IV injections of LNP-Cre, 48 hours apart, were harvested (**FIG. 26**). Tissue section imaging with a whole-slide scanner allowed for the identification of co-localization of tdTom expression and cell-specific markers. The primary antibody staining conditions are listed below in **Table E6**. All primary antibodies are used at a 1:100 dilution and incubated for 20 minutes.

Table E6: Primary Antibody Staining Conditions used for Immunohistochemistry

Cell type	Antibody Name	Vendor	Antibody Catalog #	Primary Antibody Dilution	Primary Antibody

					Incubation (min)
AT1 cells	Anti-Hop Antibody (E-1): sc-398703	SantaCruz	sc-398703	1:100	20 minutes
AT2 cells	Purified anti-ABCA3 Antibody	Biolegend	911001	1:100	20 minutes
Goblet cells	Recombinant Anti-Mucin 5AC antibody [45M1]	AbCam	ab3649	1:100	20 minutes
Ciliated cells	Monoclonal Anti-Tubulin, Acetylated antibody produced in mouse	Millipore Sigma	T6793-100UL	1:100	20 minutes
Club sells	Anti- SCGB1A1 antibody	AbCam	ab40873	1:100	20 minutes

[0616] Nearly all cell types displayed co-localization of tdTom and their respective cell marker, indicating widespread editing (**FIG. 27A**). Approximately 18% of AT1 cells, 20% of AT2 cells, 10% of goblet cells, 6% of ciliated cells, and 2% of club cells showed evidence of editing, which persisted for up to 360 days (**FIG. 27B**). A total of 21.4% of ionocytes were edited by day 2, increasing to 58% at the 660-day mark (**FIG. 28A**- **FIG. 28B**), which is important because ionocytes control airway surface liquid absorption (Yuan *et al.*, Transgenic ferret models define pulmonary ionocyte diversity and function. *Nature*. **621**(7980):857-867 (2023); Lei *et al.*, CFTR-rich ionocytes mediate chloride absorption across airway epithelia. *J Clin Invest*. **133**(20):e171268 (2023)). Whole section images (**FIG. 27C-FIG. 27E**) and three-dimensional rendering of the entire lung after LNP-Cre treatment reveal a uniform distribution of tdTom expression throughout lung lobe. A second reporter mouse, double-fluorescent mTmG (M. D. Muzumdar, B. Tasic, K. Miyamichi, L. Li, L. Luo, A global double-fluorescent Cre reporter mouse. *Genesis* **45**, 593-605 (2007)) was used to further visualize and quantify edited cells (~8% by tissue volume) two days after a single LNP-Cre administration that mediated

excision to turn on eGFP expression replacing pre-existing tdTom fluorescent proteins (**FIG. 29A, FIG. 29B**). This further supports lung SORT LNP-enabled genome editing.

Example 10: Vitronectin receptor expressing lung cell types exhibit enhanced editing efficiency

[0617] Recent work has demonstrated that SORT LNPs avidly bind distinct plasma proteins after IV administration, thereby facilitating endogenous targeting of organs and cell types (S. A. Dilliard, Q. Cheng, D. J. Siegwart, On the mechanism of tissue-specific mRNA delivery by selective organ targeting nanoparticles. *Proc. Natl. Acad. Sci. USA* **118**, e2109256118 (2021)). Whether that the protein corona of the Lung SORT LNPs used was most highly enriched in vitronectin using proteomic analysis was verified (**FIG. 30A, FIG. 30B**). Preincubating lung SORT LNPs with vitronectin improved transfection of $VtnR^+$ human bronchial epithelial ($16HBE14o^-$) cells (H. C. Valley *et al.*, Isogenic cell models of cystic fibrosis-causing variants in natively expressing pulmonary epithelial cells. *J. Cyst. Fibros.* **18**, 476-483 (2019)), but not VtnR- cells (**FIG. 31A-FIG. 31D**). To further study this mechanism, VtnR expression *in vivo* was analyzed. Most lung cell types exhibit high levels of VtnR (25.5% of endothelial, 23.5% of epithelial, 29.5% of $EpCAM^+NGFR^+$, and 33.6% of $EpCAM^+KRT5^+$ cells) (**FIG. 27F, FIG. 32**). To evaluate the correlation between VtnR expression and lung SORT LNP-mediated editing, tdTom activation in VtnR-positive ($VtnR^+$, $CD51^+CD61^+$) lung cells were compared between their VtnR-negative ($VtnR^-$, $CD51^-CD61^-$) counterparts 24 hours after a single LNP-Cre treatment. Lung SORT LNP delivery was greater in $VtnR^+$ epithelial, immune, $EpCAM^+NGFR^+$ stem, and $EpCAM^+KRT5^+$ stem cells compared with $VtnR^-$ fractions (**FIG. 27G-FIG. 27K**), indicative of preferential uptake. Endothelial cell delivery was high in $VtnR^+$ and $VtnR^-$ cells at the tested dose (**FIG. 33A**, **FIG. 33B**). The lung is overall the most VtnR-enriched organ (24.4%) compared with all other organs with <4% VtnR positivity, which suggests a role for tissue specificity, especially in nonendothelial cell populations (**FIG. 34A-FIG. 34C**). These results emphasize the role of VtnR-mediated uptake of plasma vitronectin-bound lung SORT LNPs.

Example 11: Efficient editing of primary human CF patient-derived basal cells

[0618] To assess the therapeutic potential of Lung SORT LNP for genetic lung diseases, CF was used as a representative disease model which is caused by mutations in cystic fibrosis transmembrane conductance regulator (CFTR) gene. Among editing strategies, base editing is an

attractive method for efficient single base pair correction with no reliance on double-stranded break repair pathways, resulting in minimal undesired byproducts, even in non-diving cells (N. M. Gaudelli *et al.*, Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. *Nature* **551**, 464-471 (2017); G. A. Newby, D. R. Liu, In vivo somatic cell base editing and prime editing. *Mol. Ther.* **29**, 3107-3124 (2021); W.-H. Yeh, H. Chiang, H. A. Rees, A. S. B. Edge, D. R. Liu, In vivo base editing of post-mitotic sensory cells. *Nat. Commun.* **9**, (2018)). A base editing strategy, A•T to G•C conversion of the currently untreatable CF nonsense mutation $CFTR^{R553X}$ to wild-type CFTR, has been reported in cells (M. H. Geurts *et al.*, CRISPR-Based Adenine Editors Correct Nonsense Mutations in a Cystic Fibrosis Organoid Biobank. *Cell Stem Cell* **26**, 503-510.e507 (2020); S. Krishnamurthy *et al.*, Functional correction of CFTR mutations in human airway epithelial cells using adenine base editors. *Nucleic Acids Res* **49**, 10558-10572 (2021)). This therapeutic strategy using the LNP-ABE system co-encapsulating ABE mRNA and sgR553X was applied to correct CFTR R553X mutation in primary and immortalized human bronchial epithelial (HBE) cells (**Table E4**).

[0619] In engineered 16HBEge CFTR-R553X cells (S. Krishnamurthy *et al.*, Functional correction of CFTR mutations in human airway epithelial cells using adenine base editors. *Nucleic Acids Res* **49**, 10558-10572 (2021)), LNP-ABE (ABE mRNA:sgRNA = 2:1, wt/wt) treatment led to 95.0% of correction by DNA sequencing at 1.5 ug/well dose (**FIG. 35A, FIG. 35B**).

[0620] Next, the R553X mutation was corrected in primary CF patient-derived human bronchial epithelial (HBE) cells (D. C. Gruenert, W. E. Finkbeiner, J. H. Widdicombe, Culture and transformation of human airway epithelial cells. *Am. J. Physiol.* **268**, L347-360 (1995)) carrying the R553X/F508del heterozygous mutation. The difficult-to-treat HBE model has been used to develop CFTR modulator small molecule therapies due to its capability of differentiation into a pseudostratified epithelium in air-liquid interface (ALI) culture that mimic characteristics of *in vivo* airway biology, allowing strong prediction of therapeutic efficacy in humans (J. P. Clancy *et al.*, CFTR modulator theratyping: Current status, gaps and future directions. *J. Cyst. Fibros.* **18**, 22-34 (2019); F. Van Goor *et al.*, Rescue of CF airway epithelial cell function in vitro by a CFTR potentiator, VX-770. *Proc. Natl. Acad. Sci. USA* **106**, 18825-18830 (2009); F. Van Goor *et al.*, Correction of the F508del-CFTR protein processing defect in vitro by the investigational drug VX-809. *Proc. Natl. Acad. Sci. USA* **108**, 18843-18848 (2011)). The experimental workflow is described in **FIG. 36A**. Following LNP-ABE (1.4 ug total RNA, ABE

mRNA:sgRNA=2:1, wt/wt) treatment, approximately 60% of allelic editing was achieved in both undifferentiated P2 culture composed mainly of basal cells and fully differentiated P3 culture analyzed by EditR (M. G. Kluesner *et al.*, EditR: A method to quantify base editing from Sanger sequencing. *CRISPR J.* **1**, 239-250 (2018)) (**FIG. 36B**). Using next generation sequencing (NGS) and CRISPResso2 (K. Clement *et al.*, CRISPResso2 provides accurate and rapid genome editing sequence analysis. *Nat. Biotechnol.* **37**, 224-226 (2019)) analysis, a mean 83.7% frequency of the desired product (C nucleotide at T_7), with moderate bystander editing (14.5%) at T_{11} and no editing at T_{17} (**FIG. 36C**) was confirmed. Bystander editing at T_{11} position had a neutral effect since both GG**T** and GG**C** codons encode glycine.

[0621] To evaluate the CFTR protein expression restoration after LNP-ABE treatment, capillary western blotting (**FIG. 36D, FIG. 37A, FIG. 37B**) was performed. LNP-ABE alone doubled the expression of core glycosylated CFTR (Band B, **FIG. 36D, FIG. 36E**) and increased the expression of fully glycosylated, mature CFTR by 5.5-fold (Band C, **FIG. 36D, FIG. 36F**). Combined treatment with LNP-ABE and the CFTR small molecule modulator Trikafta (elexacaftor/tezacaftor/ivacaftor, the standard of care for CF patients with F508del mutations (P. G. Middleton *et al.*, Elexacaftor–Tezacaftor–Ivacaftor for cystic fibrosis with a single Phe508del allele. *New. Engl. J. Med.* **381**, 1809-1819 (2019))), further increased the expression of fully glycosylated CFTR by 7.8-fold (Band C, **FIG. 36D, FIG. 36F**). Lastly, the ability of LNP-ABE to restore HBE culture function was tested by measuring CFTR-dependent Cl^- channel activity 4 weeks after the LNP-ABE treatment using transepithelial current clamp (TECC) assay. LNP-ABE alone effectively restored 53.4% of the CFTR function (AUC 11.6 μA/cm2*min) (**FIG. 36G**) compared to the activity of wild-type CFTR measured from HBE culture derived from an individual without cystic fibrosis presumed to have 100% CFTR activity (AUC 21.7 μA/cm2*min) (**FIG. 36H**), which greatly exceeds the widely accepted therapeutic threshold for CFTR activity restoration of > 10% to prevent CF disease phenotypes (S. M. Rowe, F. Accurso, J. P. Clancy, Detection of cystic fibrosis transmembrane conductance regulator activity in early-phase clinical trials. *Proc. Am. Thorac. Soc.* **4**, 387-398 (2007); M. D. Amaral, Processing of CFTR: Traversing the cellular maze-How much CFTR needs to go through to avoid cystic fibrosis? *Pediatr. Pulmonol.* **39**, 479-491 (2005)). CFTR function was further boosted to 85% restoration by the combination of LNP-ABE and Trikafta (AUC 18.5 μA/cm2*min) (**FIG. 36G**). Considering the nature of heterozygous CF HBE model, higher CFTR function can be achieved with LNP-ABE in homozygous models. Lung SORT LNP

delivery of tdTomato mRNA (LNP-tdTom) in differentiated HBEs from the basolateral side to mimic *in vivo* systemic delivery to the lungs was also assessed, which confirmed >50% transfection of basal cells (**FIG. 38A, FIG. 38B**). Together, these results indicate that efficient correction of undifferentiated lung basal cells can successfully produce corrected mature epithelium and restore CFTR function.

Example 12: In vivo stem cell editing in CF mouse lungs

[0622] LNP-ABE-mediated editing in genetically engineered CF mice harboring the whole human exon 12 containing R553X replacing the endogenous mouse exon was evaluated to study R553X correction in the local human sequence context. Despite having the R553X nonsense mutation, the model does not exhibit pathological features in the lung, consistent with other CF animal models (D. R. McHugh *et al.*, A G542X cystic fibrosis mouse model for examining nonsense mutation directed therapies. *Plos One* **13**, e0199573 (2018)). To address this constraint, intestinal stem cells were isolated from homozygous R553X mice and generated intestinal organoids (D. K. Crawford *et al.*, Targeting G542X CFTR nonsense alleles with ELX-02 restores CFTR function in human-derived intestinal organoids [forskolin-induced swelling (FIS) assay] (*J. Cyst. Fibros.* **20**, 436-442 (2021)) to assess the restoration of CFTR function (**FIG. 36I-FIG. 36K**). Using the forksolin-induced swelling (FIS) assay, forskolin activates CFTR, stimulates intracellular pathways, and phosphorylates CFTR to open the CFTR channel, permitting ion/water uptake, and ultimately organoid swelling. Once the mutated CFTR gene is corrected, organoid swelling should be observed, while untreated organoids will remain at the baseline volume (**FIG. 40B**). More than 82% of organoids swelled after LNP-ABE treatment (**FIG. 36L**). In total, 47.8% of A•T to G•C conversion was confirmed by DNA sequencing (**FIG. 36M**). These findings demonstrate the capacity to remedy pathological features of CF in cells derived from R553X mice.

[0623] To assess the feasibility of base editing in lung stem cells for potential long-lasting effect, heterozygous R553X mice with one allele carrying the locally humanized R553X mutation and one normal mouse CFTR allele was used. By using a specific pair of primer design, only the human R553X region was amplified by PCR for sequencing analysis. The primers are listed below in **Table E7**.

Table E7. Primers used for sequencing analyses.

Target	Primer	Sequence (5' to 3')

R553X (HBE model)	Forward	GGAAGATGTGCCTTTCAAATTCAG
	Reverse	ATGTGATTCTTAACCCACTAGCCA
R553X (Mouse model)	Forward	GGAAGATGTGCCTTTCAAATTCAG
	Reverse	ACAGCAAATGCTTGCTAG

[0624] LNP-ABE was administrated intravenously at 1.5 $mgkg^{-1}$ total RNA (ABE mRNA: sgR553X=2:1, weight ratio). Characteristics of the LNPs produced are shown in **Table E8**.

Table E8: Characterization of Lung SORT LNPs encapsuling ABE mRNA/sgR553X made by vortex mixing or T-mixing.

Mixing	**Particle size (nm)**	**PDI**	**Zeta potential**	**% encapsulation**
Vortex mixing	130.0 ± 0.8	0.14 ± 0.01	6.29 ± 0.34	82.9 ± 1.1
T-mixing	90.0 ± 0.7	0.14 ± 0.03	6.28 ± 0.56	98.2 ± 0.0

[0625] Ten days after a single treatment, whole mouse lungs were collected. Genomic DNA was extracted from whole lungs, trachea, and lung stem cells isolated from whole lung single cell suspension using magnetic based cell separation (**Figure 36N**) for PCR amplification and NGS sequencing. In total, 50.0% correction at the desired T_7 position in lung stem cells, 12.2% in whole lung, and 28.7% in the trachea were quantified (**Figure 36O**). These results indicate that LNP-ABE can efficiently correct the target pathogenic mutation in mouse lung stem cells.

[0626] Genome editing in lung stem cells can provide a lasting therapy for genetic lung diseases. Acknowledging historical and recent advances in viral vectors for gene therapy (A. L. Cooney, P. B. McCray, Jr., P. L. Sinn, Cystic fibrosis gene therapy: Looking back, looking forward. *Genes* **9**, 538 (2018); E. W. F. W. Alton *et al.*, Preparation for a first-in-man lentivirus trial in patients with cystic fibrosis. *Thorax* **72**, 137-147 (2017); D. Vidović *et al.*, rAAV-CFTRΔR Rescues the Cystic Fibrosis Phenotype in Human Intestinal Organoids and Cystic Fibrosis Mice. *Am. J. Respir. Crit. Care Med.* **193**, 288-298 (2016); L. S. Ostedgaard *et al.*, CFTR with a partially deleted R domain corrects the cystic fibrosis chloride transport defect in human airway epithelia in vitro and in mouse nasal mucosa in vivo. *Proc. Natl. Acad. Sci. USA* **99**, 3093-3098 (2002)) and lung delivery (Z. Yan *et al.*, Recombinant adeno-associated virus-mediated editing of the G551D cystic fibrosis transmembrane conductance regulator mutation in ferret airway basal cells. *Hum. Gene Ther.* **33**, 1023-1036 (2022); M. K. Yanda *et al.*, Transduction of ferret surface and basal cells of airways, lung, liver, and pancreas via intratracheal or intravenous delivery of adeno-associated virus 1 or 6. *Hum. Gene Ther.* **34**,

1135-1144 (2023)), packaging limits, immunogenicity, and the risk of gene disruption have hindered success in genome editing. Compared with viral and other nonviral aerosolized delivery approaches, IV-administered SORT LNPs can have greater access to lung basal stem cells given the lack of disease-related mucosal barriers and the immediate proximity of the lung endothelial bed to LNPs (N. Bertrand, J.-C. Leroux, The journey of a drug-carrier in the body: An anatomo-physiological perspective. *J. Control. Release* **161**, 152-163 (2012)). It was demonstrated herein that lung SORT LNPs deliver mRNA, CRISPR-Cas9, and base editor components to various lung cells, including stem cells. Tracking of edited cells for 22 months revealed that the degree of editing was consistently maintained across the lungs during this entire period, indicating further differentiation to mature cells.

[0627] Endogenous targeting is a mechanism to aid lung accumulation and cellular uptake. Other factors, such as transcytosis (G. W. Liu, E. B. Guzman, N. Menon, R. S. Langer, Lipid nanoparticles for nucleic acid delivery to endothelial cells. *Pharm. Res.* **40**, 3-25 (2023)), recycling endosomes (P. Paramasivam *et al.*, Endosomal escape of delivered mRNA from endosomal recycling tubules visualized at the nanoscale. *J. Cell Biol.* **221**, (2022)), passage through tight junctions (Y. Zhang, W.-X. Yang, Tight junction between endothelial cells: the interaction between nanoparticles and blood vessels. *Beilstein J. Nanotechnol.* **7**, 675-684 (2016)), and exosome-macrophage transfer (M. Maugeri *et al.*, Linkage between endosomal escape of LNP-mRNA and loading into EVs for transport to other cells. *Nat. Commun.* **10**, 4333 (2019)), can contribute to the delivery process (S. A. Dilliard, D. J. Siegwart, Passive, active and endogenous organ-targeted lipid and polymer nanoparticles for delivery of genetic drugs. *Nat. Rev. Mater.* **8**, 282-300 (2023)). Although VtnR expression can explain enrichment of SORT LNPs in lung cells, there can be vitronectin-dependent and -independent processes involved in endothelial cell delivery. Given that SORT LNPs access deeper tissue structures through the vasculature, reduced expression of VtnR in nonlung organs (B. Singh, C. Fu, J. Bhattacharya, Vascular expression of the αvβ3-integrin in lung and other organs. *Am. J. Physiol. Lung Cell Mol. Physiol.* **278**, L217-L226 (2000)) can be one factor that contributes to the selectivity of SORT LNPs. Additional protein corona–related factors could contribute to the observed lung tissue enrichment by reduced binding of proteins associated with liver uptake (A. Akinc *et al.*, Targeted delivery of RNAi therapeutics with endogenous and exogenous ligand-based mechanisms. *Mol. Ther.* **18**, 1357-1364 (2010)) and increased binding of proteins involved in avoiding clearance mechanisms (S. A. Dilliard, Q. Cheng, D. J. Siegwart, On the mechanism of

tissue-specific mRNA delivery by selective organ targeting nanoparticles. *Proc. Natl. Acad. Sci. USA* **118**, e2109256118 (2021); S. A. Dilliard *et al.*, The interplay of quaternary ammonium lipid structure and protein corona on lung-specific mRNA delivery by selective organ targeting (SORT) nanoparticles. *J. Control. Release* **361**, 361-372 (2023)).

[0628] Lung SORT LNPs facilitated delivery of base editors to achieve gene correction in nearly 50% of lung stem cells in a CF mouse model carrying the R553X mutation, paving the way for therapeutic intervention in CF and other diseases. SORT LNPs effectively delivered base editors to correct the CFTR and restore function in primary R553X–F508del HBEs. These transwell functional readouts can indicate clinical benefit, having supported the approval of clinically used CFTR modulators (J. P. Clancy *et al.*, CFTR modulator theratyping: Current status, gaps and future directions. *J. Cyst. Fibros.* **18**, 22-34 (2019); F. Van Goor *et al.*, Rescue of CF airway epithelial cell function in vitro by a CFTR potentiator, VX-770. *Proc. Natl. Acad. Sci. USA* **106**, 18825-18830 (2009); F. Van Goor *et al.*, Correction of the F508del-CFTR protein processing defect in vitro by the investigational drug VX-809. *Proc. Natl. Acad. Sci. USA* **108**, 18843-18848 (2011)).

Example 13: Materials, Methods and Supplementary Information

Materials and Methods

Lipid nanoparticle formulation

[0629] 5A2-SC8 was synthesized and purified by following published protocols (K. Zhou *et al.*, Modular degradable dendrimers enable small RNAs to extend survival in an aggressive liver cancer model. *Proc. Natl. Acad. Sci. USA* **113**, 520-525 (2016); X. Wang *et al.*, Preparation of selective organ targeting (SORT) lipid nanoparticles (LNPs) using multiple technical methods for tissue-specific mRNA delivery. *Nat. Protoc.* **18**, 265-291 (2022)). 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE, Cat# 850725) and 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP, Cat# 890890) were purchased from Avanti Polar Lipids. Cholesterol (Cat# C3045) was purchased from Sigma-30 Aldrich. 1,2-Dimyristoyl-rac-glycero-3-methylpolyoxyethylene (DMG-PEG2000, Cat# GM-020) was purchased from NOF America Corporation. D-Lin-MC3-DMA (Cat# 555308) was purchased from MedKoo Biosciences. LNPs were prepared by following published protocol (X. Wang *et al.*, Preparation of selective organ targeting (SORT)

lipid nanoparticles (LNPs) using multiple technical methods for tissue-specific mRNA delivery. *Nat. Protoc.* **18**, 265-291 (2022)). In brief, an aqueous phase with RNA cargos was rapidly mixed with an organic phase congaing the lipids at a 3:1 volume ratio. The organic phase was prepared by dissolving 5A2-SC8, DOPE, cholesterol, DMG-PEG and DOTAP in ethanol at given molar ratio (32.4:18:36:3.6:10 for in vitro studies; 21.6:12:24:2.4:40 for in vivo studies). The aqueous phase contains RNA cargos in citrate buffer (pH 4). The total lipid to RNA weight ratio was 20:1. LNPs were formed by either vortex mixing or T-mixing, then dialyzed (Pur-A-Lyzer Midi Dialysis Kits, WMCO 3.5 kDa, Cat# PURX35100) against 1 x phosphate buffered saline (PBS) for 3 hours before usage. The particle size, polydispersity index, and zeta potential of LNPs were measured using dynamic light scattering (DLS) (Zetasizer Nano ZS machine, Malvern, v.7.13) and the RNA encapsulation efficiency were measured using Quant-iT RiboGreen RNA assay (Invitrogen, Cat# R11491) following published protocols (X. Wang *et al.*, Preparation of selective organ targeting (SORT) lipid nanoparticles (LNPs) using multiple technical methods for tissue-specific mRNA delivery. *Nat. Protoc.* **18**, 265-291 (2022)).

In vitro transcribed mRNA and chemically modified sgRNA

[0630] mRNAs encoding Cre recombinase, Cas9, NG-ABE8e were synthesized by in vitro transcription (IVT). NG-ABE8e was a gift from Professor David Liu (Addgene plasmid # 138491 ; http://n2t.net/addgene:138491 ; RRID:Addgene_138491) (M. F. Richter *et al.*, Phage-assisted evolution of an adenine base editor with improved Cas domain compatibility and activity. *Nat. Biotechnol.* **38**, 883-891 (2020)). The coding fragments of each protein were cloned into a pCS2+MT plasmid backbone featuring a SP6 promoter, customized 5' and 3' untranslated regions (UTR) as well as a poly(A) segment through NEBuilder HiFi DNA assembly (NEB, Cat# E2621S). IVT was conducted using the MEGAscript SP6 transcription kit (Invitrogen, Cat# AM1330) with N1-methylpseudouridine-5'-triphosphate replacing the typical uridine triphosphate. Next, a Cap1 cap structure was installed to the 5' end of the mRNA using the Cap1 Capping System (Hongene, Cat# ON-028 & ON-014). Luciferase mRNA and tdTomato mRNA was provided by ReCode Therapeutics. All mRNAs were purified using LiCl and the integrity of the purified mRNAs was monitored by High Sensitivity RNA ScreenTape System (Agilent, Cat# 5067-5579).

[0631] sgRNAs (sgTOM1 and sgR553X) were purchased from and synthesized by Agilent using solid phase synthesis and phosphoramidite chemistry. All sgRNAs were incorporated with end modification (3 x 2′-O-methyl-3′-phosphorothioate (MS) on 5' end and 3 x 2′-O-methyl-3′-phosphonoacetat (MP) on 3' end (D. E. Ryan *et al.*, Phosphonoacetate modifications enhance the stability and editing yields of guide RNAs for Cas9 editors. *Biochemistry*, (2022))). Heavily modified sgR553X (HM sgR553X) was utilized for *in vivo* base editing experiment by following a previously reported modification pattern (H. Yin *et al.*, Structure-guided chemical modification of guide RNA enables potent non-viral in vivo genome editing. *Nat. Biotechnol.* **35**, 1179-1187 (2017)). The sequences of sgRNA used are shown in **Table E5**.

Animal experiments

[0632] Ai14 mice (Strain #:007914, RRID:IMSR_JAX:007914) and mT/mG mice (Strain #:007676, RRID:IMSR_JAX:007676) were purchased from The Jackson Laboratory and bred to maintain the homozygous strains at the UTSW Animal Facility. All animals were maintained on a 12/12-h light/dark schedule at a mean temperature of 22 °C. Heterozygous and homozygous R553X CF mice created at Case Western Reserve University were used for *in vivo* base editing studies at CWRU. All experiments were performed with sample sizes calculated by power analyses.

Cell lines and primary cells

[0633] The immortalized, CFTR wild-type expressing human bronchial epithelial cells, 16HBE14o- (generated by D. Gruenert) was gene edited at the endogenous CFTR locus using CRISPR-Cas9 to create isogenic 16HBEge CFTR R553X and 16HBEge CFTR F508del (M470) cell lines (H. C. Valley *et al.*, Isogenic cell models of cystic fibrosis-causing variants in natively expressing pulmonary epithelial cells. *J. Cyst. Fibros.* **18**, 476-483 (2019)). The 16HBEge CFTR R553X and 16HBEge CFTR F508del (M470) cells were provided by the Cystic Fibrosis Foundation Therapeutics Lab.

[0634] Primary HBE cells were collected from transplanted lungs from a healthy donor (DD0059J) who has wild-type $CFTR^{wt/wt}$ and an individual with cystic fibrosis (20180717CF) who was heterozygous for $CFTR^{R553X/F508del}$. Primary HBE cells were provided by the Cystic Fibrosis Foundation Therapeutic Lab.

In vivo long-term lung cell editing study

[0635] For gene editing in mice lungs with LNP-Cre, 8-week-old Ai14 mice were randomly allocated to either LNP-Cre-treated group or PBS-treated group. The mice were injected with 200 μL of LNP-Cre formulation at 2 mg/kg (*Cre* mRNA) twice, 2 days apart. Animals were euthanized at different time points over a total of 660 days (2 days, 7 days, 21 days, 42 days, 60 days, 120 days, 180 days, 270 days,360, and 660 days after the last injection). To study in vivo lung cell editing, lungs were either collected for *ex vivo* fluorescence imaging (AMI-HTX, Spectral Instruments Imaging) followed by FACS analysis or fixed with 4% PFA for two days followed by 10%-18% sucrose for frozen sectioning with the assistance of the UTSW Histo Pathology Core and immunofluorescence staining with HOPX, MUC5AC, Tubulin, SCGB1A1 and ABCA3 antibodies (**Table E6**).

[0636] To investigate the potential of Lung SORT LNPs to deliver CRISPR-Cas9/sgRNA system in Ai14 mice, a previously developed sgRNA (sg298 or sgTOM1, **Table E5**) was utilized (B. T. Staahl *et al.*, Efficient genome editing in the mouse brain by local delivery of engineered Cas9 ribonucleoprotein complexes. *Nat. Biotechnol.* **35**, 431-434 (2017)). Unlike Cre recombinase, which is highly efficient in LoxP deletion, tdTom activation induced by the CRISPR-Cas9 system necessitates double or triple deletion of the STOP cassettes which underestimates the efficacy of LNP-Cas9-mediated editing. For gene editing in mice lungs with LNP-Cas9, 8-week-old Ai14 mice were randomly allocated to either LNP-Cas9-treated group or PBS-treated group. The mice were injected with 200 μL of LNP-Cre formulation at 2 mg/kg total RNA (*Cas9* mRNA:sgTOM1=2:1, wt/wt) three times, one week apart. Animals were euthanized at different time points over a total of 240 days (7 days, 21 days, 60 days, 120 days, 240 days after the last injection) (**FIG. 18**). Lung tissues were collected for FACS analysis to study *in vivo* lung cell editing, heart and spleen tissues were collected for histopathology evaluation (sections and H&E staining were prepared by UT Southwestern Tissue Management Shared Resource, the slides were scanned using Vectra Polaris from Akoya Biosciences at 20X magnification and digitalized using Phenochart software), and serum separated from whole blood were collected to measure liver function (AST and ALT) and kidney function (BUN and CREA).

LNP treatment in mice with acute infection and inflammation in lungs

[0637] The acute infection mouse model was generated by following a published protocol (S. Abid *et al.*, 17β-Estradiol dysregulates innate immune responses to pseudomonas aeruginosa respiratory infection and is modulated by estrogen receptor antagonism. *Infect. Immun.* **85**, (2017)). 8-week-old Ai14 mice were randomly allocated to *P. aeruginosa* infection group or non-infection group. *P. aeruginosa* strain PAO1 was obtained from ATCC. Bacteria colonies were streaked onto Trypticase soy agar (TSA) plates from frozen glycerol stocks and incubated overnight at 37°C. Overnight cultures were washed in 150 nM sodium chloride, and the resulting pellets were resuspended in 1 ml of Luria-Bertani (LB) broth. Culture concentrations were determined based on the optical density at 600 nm (OD600) and the previously determined number of CFU for each strain, to give the CFU/OD600. Mice in the infection group were inoculated intranasally with 50 ul of *P. aeruginosa* at 3.5 x 10^5 CFU to generate acute infection model. The previous study found neutrophil number and cytokine expression evaluated 9 h after *P. aeruginosa* infection in mouse lungs (S. Abid *et al.*, 17β-Estradiol dysregulates innate immune responses to pseudomonas aeruginosa respiratory infection and is modulated by estrogen receptor antagonism. *Infect. Immun.* **85**, (2017)). To investigate the delivery efficiency of LNP in infected and inflamed lungs, mice were injected with either LNP-Cre formulation at 2 mg/kg (*Cre* mRNA) or PBS 9 hr post *P. aeruginosa infection*. Animals were euthanized 24 hr after LNP-Cre treatment, followed by FACS analysis of lung cells to study editing efficiency.

Isolation of single cells from mouse tissues

[0638] Mouse lungs, tracheas and other organs were extracted and immediately placed in ice-cold PBS. These tissues were then cut into small pieces and moved into a 50 mL tube containing 10 mL of 1X lung tissue digestion media [RPMI digestion medium (1:1 vol/vol): RPMI supplemented with 2% wt/vol BSA, 300 U/mL collagenase, and 100 U/mL hyaluronidase]. The 50 mL tube was placed in an incubator set at 37 °C for an hour, with continuous shaking at a speed of 180 rpm. After incubation, the homogenized lung cell solution was agitated using a pipette to disperse any clumps of cells, then it was filtered into a new 50 mL Falcon tube through a 100-micron strainer. The strainer was rinsed using 10 mL of a wash buffer composed of chilled PBS and 2% Fetal Bovine Serum (FBS). Afterwards, the sample was centrifuged at a speed of 1200 rpm for 5 minutes. Following this step, the supernatant was removed, and the remaining cell pellet was reconstituted in 10 mL of the chilled wash buffer. Subsequently, the sample was spun at 1200 rpm for 5 minutes. Next, the cell pellet was treated

with 5 mL of 1X RBC lysis buffer (BioLegend, Cat# 420301) at room temperature for 5 minutes to eliminate any red blood cells. After the lysis procedure, 10 mL of wash media was added to the sample, which was subsequently spun at 1200 rpm for another 5 minutes. The final cell pellet, which was now free from red blood cells, was reconstituted in 5 mL of cell staining buffer (BioLegend, Cat# 420201). Finally, the sample was subjected to antibody staining in preparation for flow cytometry.

[0639] To examine whether Lung SORT LNPs facilitate mRNA delivery to the tracheal region of the lung and enable cell editing, LNP-Cre were intravenously administered to Ai14 mice in two successive doses, each being 2 mg/kg 48 hours apart. Tracheas (**FIG. 20A**) were extracted 48 hours following the final injection, and tdTom expression across various cell types was quantified using flow cytometry. The composition of cells markedly differed between the trachea and bronchus regions of the lung. The trachea harbored more immune cells (~55.8%) and fewer epithelial (~13.7%) and endothelial cells (~8.4%) compared to the bronchus (35.4% immune cells, 28.7% epithelial cells, and 23.5% endothelial cells) (**FIG. 20A**). Overall, the percentage of total edited cells was lower in the trachea (~12%) compared to the bronchus (~46%) (**FIG. 20B**). Among the various cell types, barring edited endothelial cells which were 70.7% in the trachea and 88.3% bronchus (high in both trachea and bronchus), the percentage of edited immune cells (5.6% in trachea vs. 37.8% in bronchus), epithelial cells (16.4% in trachea vs. 71.7% in bronchus), basal epithelial cells (12.0% in trachea vs. 59.9% in bronchus), and epithelial stem cells (12.2% in trachea vs. 67.9% in bronchus) was lower in the trachea compared to the bronchus region (**FIG. 20B-FIG. 20G**). This difference could be attributed to the challenges faced by the lung SORT LNPs in navigating and reaching the tracheal part of the lung. Regardless, Lung SORT LNPs also reach the trachea following IV administration and achieve genome editing.

Antibody staining of single cells from mouse tissues and analysis by flow cytometry

[0640] Single-cell suspensions, derived from the mouse lungs and other organs, were initially blocked with mouse Fc-receptor blocker (Thermo Fisher, Cat# 14-9161-73) for a period of 15 minutes. After that, the cells were marked with a variety of antibodies, namely Alexa Fluor 488-conjugated anti-mouse CD31 (BioLegend, Cat# 102414), Pacific Blue-conjugated anti-mouse CD45 (BioLegend, Cat# 103126), Alexa Fluor 647-conjugated anti-mouse EpCAM (BioLegend, Cat# 118212), APC-conjugated anti-mouse CD157 (BioLegend, Cat# 140207),

PerCP Cy5-5-conjugated anti-mouse Sca1 (Invitrogen, Cat# 45-5981-80), Alexa Fluor 700-conjugated anti-mouse CD117 (Thermo Fisher, Cat# 56-1172-80), FITC-conjugated anti-mouse lineage cocktail antibodies (BioLegend, Cat# 133302), unconjugated anti-mouse NGFR (LS Bio, Cat# LS-C179536), PE/Cyanine7-conjugated anti-mouse/rat CD61 (BioLegend, Cat# 104317),Brilliant Violet 711-conjugated anti-mouse CD51 (Fisher Scientific, Cat# BDB740755), FITC-conjugated anti-mouse CD3 (BioLegend, Cat# 100203), FITC-conjugated anti-mouse Ly-6G (BioLegend, Cat# 108405), FITC-conjugated anti-mouse CD11b (BioLegend, Cat# 101205), FITC-conjugated anti-mouse TER-119 (BioLegend, Cat# 116205), APC-conjugated anti-mouse B220 (BioLegend, Cat# 103211), Alexa Fluor 700-conjugated anti-mouse CD8 (Thermo Fisher, Cat# 56-0081-80), PerCP-conjugated anti-mouse CD4 (BioLegend, Cat# 100431), APC-conjugated anti-mouse Ly-6G (BioLegend, Cat# 127613), Alexa Fluor 594-conjugated anti-mouse F4/80 (BioLegend, Cat# 123140), PerCP/Cy5.5-conjugated anti-mouse CD11c (BioLegend, Cat# 117327). This labeling process involved incubating a 100 µL portion of the cell suspension with 1 µL these antibodies for 15 minutes, while keeping on ice. Dead cells within the suspension were identified using Ghost Dye Red (Tonbo Bioscience, Cat# 13-0865-T100). Following this, the cell pellet was washed thrice with cell staining buffer to eliminate excess antibodies. The NGFR-stained cells were further stained with a rat secondary Alexa Fluor 488-conjugated antibody (Thermo Fisher, Cat# A48262) for 15 minutes on ice. Finally, the cell pellet was resuspended in 500 µL of cold cell staining buffer and kept on ice until it was ready for analysis through a flow cytometer. The cells were subsequently analyzed using a Becton Dickenson (BD) LSR Fortessa flow cytometer. Finally, the data collected from the flow cytometer were processed and analyzed using Flowjo software (BD).

[0641] For intracellular flow cytometry, the EpCAM-stained cell pellet was resuspended in 500 µL of fix/perm solution, part of the BD Fix/Perm kit (BD Bioscience, Cat# 554714) and left on ice for 20 minutes. Following this, the cells were centrifuged at 1200 rpm for 5 minutes to obtain cell pellet. The pellet was then washed three times with 1X fix/perm wash buffer (the kit supplied a 10X concentration, which was diluted to 1X with MilliQ water), before being prepared for antibody staining. Subsequently, 100 µL of the fixed and permeabilized EpCAM-stained cells were incubated with 1 µL of an unconjugated anti-mouse KRT5 antibody (Abcam, Cat# ab52635) for a duration of 30 minutes. After this incubation period, the cells were washed three times with 1X fix/perm wash buffer to remove any surplus antibody, and then resuspended in 100 µL of 1X fix/perm buffer. In the subsequent step, 100 µL of the KRT5-labelled cells were

incubated with 1 µL of an Alexa Fluor 488-conjugated anti-rabbit secondary antibody on ice for an additional 30 minutes. Finally, after another three washes with 1X fix/perm wash buffer, the cell pellet was resuspended in 500 µL of cold 1X fix/perm wash buffer and kept on ice until it was ready for analysis through a flow cytometer.

[0642] For intracellular FOXI1 staining, the cell pellet was resuspended in 500 µL of fix/perm solution, part of the BD Fix/Perm kit (BD Bioscience, Cat# 554714) and left on ice for 20 minutes. Following this, the cells were centrifuged at 1200 rpm for 5 minutes to obtain cell pellet. The pellet was then washed three times with 1X fix/perm wash buffer (the kit supplied a 10X concentration, which was diluted to 1X with MilliQ water), before being prepared for antibody staining. Subsequently, 100 µL of the fixed and permeabilized cells were incubated with 1 µL of an Alexa Fluor 647 conjugated anti-mouse FOXI1 antibody (Novus Biologicals, Cat# NBP2-70747AF647) for a duration of 30 minutes. After this incubation period, the cells were washed three times with 1X fix/perm wash buffer to remove any surplus antibody. Finally, the cell pellet was resuspended in 500 µL of cold 1X fix/perm wash buffer and kept on ice until it was ready for analysis by a flow cytometer.

Magnetic cell separation of cells isolated from mouse lungs

[0643] To isolate NGFR positive cells from the snap freezed lungs, the mouse biotin positive selection kit (Stem Cell Technologies, Cat# 17665) was employed based on the manufacturer's instructions. In brief, 5 x10^6 RBC-depleted cells were incubated in 100 µL of cell staining buffer (BioLegend, Cat# 420201) with 1 µL of FcR blocker (Thermo Fisher, Cat# 14-9161-73) and 1 µL of biotin-conjugated anti-mouse NGFR antibody (Thermo Fisher, Cat# P75NTR-BIOTIN) at room temperature for 15 minutes. Next, 10 µL of biotin selection cocktail was added to the cell suspension and incubated for another 15 minutes at room temperature. Finally, 5 µL of streptavidin-bound magnetic nanoparticles was added to the cell suspension and incubated for 10 minutes at room temperature. The cell suspension was then adjusted to a total volume of 2.5 mL by adding 2.4 mL of cell staining buffer in a 5 mL falcon tube and was subjected to a magnet for 5 minutes. The magnet was then placed at a 45-degree angle, enabling us to decant off the supernatant. The NGFR positive cell fraction, which had adhered to the tube, was dislodged from the magnet by washing the tubes with 500 µL of fresh cell staining buffer.

Lastly, the magnetic nanoparticles bound cells were centrifuged at 1200 rpm for 5 minutes to obtain the final cell pellet.

Immunofluorescence

[0644] For Immunofluorescence studies, mouse lung samples were fixed with 4% paraformaldehyde for two days at 4 °C then equilibrated in 10% sucrose for 12 hr followed by 18% sucrose for another 12 hr before proceeding with cryosectioning with assistance from the UTSW Histo Pathology Core. Slides were then stained with HOPX, MUC5AC, Tubulin, SCGB1A1, and ABCA3 antibodies (**Table E6**) of lung cells and later imaged with assistance from the UTSW Tissue Management Core. The frozen tissues mounted on charged glass slides were dried for 30 minutes at 37 °C on a slide warmer (Slide Moat from Boekel Scientific, model 240000) followed by 15 minutes at room temperature. The slides were rehydrated in a wash buffer (Leica, Cat# AR9590) for 10 minutes and then loaded into the Leica Bond RX. The slides were incubated with unconjugated primary antibody for 20 minutes followed by goat anti-mouse secondary antibody with Alexa Fluor 647 (Biolegend, Cat# 405322, 1:200) or donkey anti-rabbit secondary antibody with Alexa Fluor 647 (Biolegend, Cat# 406414, 1:200) for 30 minutes. All slides were mounted with ProLong Gold Antifade Mountant with DNA Stain DAPI (Invitrogen, Cat# P36931). The slides were scanned using Vectra Polaris from Akoya Biosciences at 20X magnification and digitalized using Phenochart software. Five to six random segments from each whole slide immunofluorence images for each mouse lung were used to generate quantification analysis. Editing efficiency was calculated as tdTomato$^+$Alexa Fluor 647$^+$ cells/Fluor 647$^+$ cells and expressed as a percentage.

TissueCyte 3D imaging and analysis

[0645] Inflated lung tissues were prepared by following a published protocol (T. Klouda *et al.*, From 2D to 3D: Promising advances in imaging lung structure. *Front. Med.* **7**, 343 (2020)) and used for vibratome sectioning and tissuecyte 3D imaging. In brief, mouse trachea was cannulated with 22 g blunt-ended needle and inflated with 2.5–4 mL 2% agarose solution. The dissected lungs were incubated in 4% paraformaldehyde solution overnight at 4 °C. The next day, the left lobe of the lungs was washed with 1 X PBS at 4 °C and stored for future use.

[0646] A modified agarose embedding method was employed for the lungs used in this study, which incorporated infiltration of the inflated lung with acrylamide prior to agarose embedding. The lungs were soaked overnight at 4 °C in a solution of Surecast (ThermoFisher, Cat# HC2040), with a total concentration of 4% Surecast and 0.5% VA-044 activator, in excess volume (5 mL per lung lobe), diluted in phosphate buffer (PB; 0.42 g/L monobasic sodium phosphate, 0.92 g/L dibasic sodium phosphate). The lungs were then allowed to equilibrate to room temperature for 1 hour while gently shaking. Each lung was transferred to a cryomold (VWR, Cat# 15160-215), which was filled with the prepared acrylamide solution. The mold was covered securely with foil and incubated in an oven at 40 °C for two hours. After polymerization was complete, the excess acrylamide was carefully removed from around the lung. The acrylamide-infused tissue was then embedded in oxidized agarose for vibratome sectioning and TissueCyte imaging as previously described (D. M. O. Ramirez, A. D. Ajay, M. P. Goldberg, J. P. Meeks. (Springer New York, 2019), pp. 195-224).

[0647] Images of serial tissue sections were collected throughout the volume of the lung using the Tissuecyte 1000 Two-Photon Tomography system with integrated vibratome. Samples were imaged using a two photon laser with 920 nm excitation wavelength and 3-channel PMT detectors (Red >560 nm, Green 500-560 nm, Blue <500 nm) as contiguous overlapping tiles on each serial section. Serial blockface sections were cut with the integrated vibratome (60 hz) spaced 75 μm apart and each serial section was imaged at three Z-focal plane levels spaced 25 μm apart. Contiguous overlapping image tiles for each section and focal plane were stitched using the UT Autostitch software (Tissuevision). Overlapping tile regions were aligned based on xy coordinates and specified overlap parameter (95%) and alpha blended to form the whole section image. Average tile intensity values from the first 60 slices were used to perform brightness adjustment on all tiles prior to stitching to improve section uniformity. Stitched whole section images from the three focal planes collected for each section were downsampled to 1.5 microns per pixel, merged as a maximum intensity projection (MIP), and color adjusted for visual contrast fidelity by eye using a Matlab script. For the Ai14 mouse lung rendering, whole lung volumes of the red channel of the contrast adjusted MIP tissue images were resampled to 800 z slices using bilinear interpolation to improve rendering smoothness, and visualized in Fiji's 3D viewer. For the mTmG mouse lung rendering, once the merging and color adjustment were complete, a subsample of MIP color adjusted section images from the whole lung volume were selected for the training portion of the machine learning based signal segmentation

pipeline. Machine learning training for GFP signal segmentation was done in the Ilastik software, using the default parallel random forest implementation. Selected features used as discriminators in the model were Gaussian Smoothing (sigma = 0.3-5.0), Laplacian of Gaussian (sigma = 0.3-5.0), Gaussian Gradient Magnitude (sigma = 0.3-5.0), Difference of Gaussians (sigma = 0.3-5.0), Structure Tensor Eigenvalues (sigma = 0.3-5.0), Hessian of Gaussian Eigenvalues (sigma = 0.3-5.0). In Ilastik, an annotator labeled pixels from selected section images as either GFP signal or background classes (background, tissue autofluorescence, and bubbles). These annotations were used to train the classifier. Once the annotator labeled enough pixels to be satisfied with the classification accuracy, predictions for GFP signal on all of the lung volume sections were exported as 8 bit probability maps: per pixel intensity mappings separated by class, in which higher intensity values represent a higher classifier certainty that the classification of that pixel is correct.

[0648] The exported probability maps were used to perform quantification on the classified GFP signal. Using a thresholded version of the MIP images, a selection mask of the tissue area in the images was created. This mask was applied to the GFP probability map to select predicted GFP signal that was within the tissue boundaries. Then the summed intensity values and summed intensity values per unit volume (mm^3) were calculated using a Matlab script. The percent area calculation was done by determining a threshold level (threshold = 101) for the probability map by eye to determine the classifier certainty level where visible GFP signal looked correctly segmented, and then applying the same determined threshold to both samples. Then the total volume of positive thresholded GFP signal within the tissue area was quantified and divided by the total tissue volume using an ImageJ script to obtain the final percent area measurement. Whole lung volumes were resampled to 800 – z slices using bilinear interpolation to improve rendering smoothness, and visualized in Fiji's 3D viewer.

Mass spectrometry proteomics analysis of plasma proteins absorbed on Lung SORT LNPs

[0649] The previously described approach was used to isolate plasma proteins that interact with the Lung SORTLNPs used in this study (S. A. Dilliard, Q. Cheng, D. J. Siegwart, On the mechanism of tissue-specific mRNA delivery by selective organ targeting nanoparticles. *Proc. Natl. Acad. Sci. USA* **118**, e2109256118 (2021)). In brief, C57BL6/J mouse plasma (Fisher Scientific, Cat# NC0961764) was added to LNP-Cre solution (1 g/L, total lipid content), at a 1:1 volume ratio and incubated for 15 min at 37 °C. The LNP/plasma mixture was loaded onto a 0.7

M sucrose in MilliQ water cushion of equal volume to the mixture and centrifuged for 1 hour at 15,300 g and 4 °C. The supernatant was removed, and the pellet was washed with 1× PBS. The pellet was centrifuged at 15,300 g and 4 °C for another 5 min to remove washing buffer. Washing was repeated twice more for a total of three washes. After the final wash, the pellet was resuspended in 2 wt. % SDS. Excess lipids were removed using ReadyPrep 2-D Cleanup (Bio-Rad, Cat# 1632130) following the manufacturer recommended protocol. The resulting pellet was resuspended in 2× Laemmli buffer.

[0650] To prepare the sample for mass spectrometry proteomics, 10 μL of the plasma protein mixture isolated from the LNPs was loaded on to a 4-20% TGX gel. The sample was run at 90 V for 10 min, to enable stacking. To fix and visualize the proteins, the gel was stained with Bio-Safe Coomassie. After 1 hour of destaining, the protein band was cut out using a razor blade and diced into a small cube with a volume of nearly 1 mm^3. The cubes were then placed in a 1.5 mL Eppendorf tube and kept at 4 °C until they were analyzed by mass spectrometry at the UTSW Proteomics Core. The protein contents were identified and quantified using a Thermo QExactive HF mass spectrometer. The identified proteins were sorted according to their abundance/MW. A custom Python Script was created to rank the most abundant proteins and plot them as a heat map and classify the physiological classes of the identified proteins.

Code used for protein corona composition analysis

```
import pandas as pd
import numpy as np
import requests as r
import matplotlib.pyplot as plt
import seaborn as sns
from Bio import SeqIO
from Bio.SeqUtils.ProtParam import ProteinAnalysis
from io import StringIO
def load_protein_corona(corona_file):
    corona = pd.read_excel(corona_file, index_col = 'Accession')
    corona = corona.fillna(0)
    return corona

def collate_corona_files(corona_list, to_copy, sample_names):
    corona_data_subset = []
```

```
        for i in range(len(corona_list)):
            corona_data_subset.append(corona_list[i][['Description', to_copy]])
            corona_data_subset[i].columns = ['Description', sample_names[i]]
        all_coronas = pd.concat(corona_data_subset, axis=1, copy=False)
        all_coronas['Description'] = all_coronas['Description'].fillna('')
        grouped_coronas = pd.DataFrame()
        grouped_coronas['Description'] =
all_coronas['Description'].groupby(level=0,axis=1).max()
        for sample_name in sample_names:
            grouped_coronas = grouped_coronas.join(all_coronas[sample_name])
        grouped_coronas = grouped_coronas.fillna(0)
        return grouped_coronas

    def filter_proteins(corona_df, threshold):
        clean_corona_df = corona_df.copy()
        for i in range(len(corona_df)):
            single_protein = corona_df.iloc[i,1:]
            row_name = single_protein.name
            if single_protein.max() < threshold:
                clean_corona_df = clean_corona_df.drop(row_name)
        return clean_corona_df

    def proteins_heatmap(corona_df, show_variant=False):
        sample_start = corona_df.columns.get_loc('Description') + 1
        corona_abundances = corona_df.iloc[:,sample_start:]
        new_names = []
        if show_variant:
            protein_names = corona_df.Description.tolist()
            for protein in protein_names:
                split_name = protein.split(' OS')
                var_num_location = split_name[1].find('SV')
                var_num = split_name[1][var_num_location:]
                new_names.append(split_name[0] + ' ' + var_num)
            corona_abundances.index = new_names
        else:
            protein_names = corona_df.Description.tolist()
            for protein in protein_names:
                split_name = protein.split(' OS')
                new_names.append(split_name[0])
            corona_abundances.index = new_names
        color_choice = sns.cubehelix_palette(as_cmap=True)
        plt.figure(figsize=(50,25))
        sns.heatmap(corona_abundances, cmap=color_choice, yticklabels=1)
        plt.xticks(rotation=0)
        sns.set(font_scale=2)

    def cluster_proteins(corona_df, show_variant=False):
        sample_start = corona_df.columns.get_loc('Description') + 1
```

```
    corona_abundances = corona_df.iloc[:,sample_start:]
        new_names = []
    if show_variant:
        protein_names = corona_df.Description.tolist()
        for protein in protein_names:
            split_name = protein.split(' OS')
            var_num_location = split_name[1].find('SV')
            var_num = split_name[1][var_num_location:]
            new_names.append(split_name[0] + ' ' + var_num)
        corona_abundances.index = new_names
    else:
        protein_names = corona_df.Description.tolist()
        for protein in protein_names:
            split_name = protein.split(' OS')
            new_names.append(split_name[0])
        corona_abundances.index = new_names
    color_choice = sns.cubehelix_palette(as_cmap=True)
    sns.clustermap(corona_abundances, cmap=color_choice, yticklabels=1, figsize = (50,40))

def average_proteins(corona_df, replicates, sample_names):
    replicate_average = []
    column_names = [sample_names[i][:-2] for i in range(0,len(sample_names),replicates)]
    average_corona_df = pd.DataFrame(index = corona_df.index)
    average_corona_df['Description'] = corona_df['Description']
    for i in range(1, len(sample_names),replicates):
        replicate_df = corona_df.iloc[:,i:i+replicates]
        replicate_average = replicate_df.mean(axis=1).tolist()
        sample_index = int((i-1)/replicates)
        average_corona_df[column_names[sample_index]] = replicate_average
    return average_corona_df

def classify_proteins(corona_df):
    protein_classes = pd.read_excel('Protein Classification.xlsx')
    protein_classes = protein_classes.fillna('')
    classified_proteins = pd.DataFrame(index= corona_df.columns[1:],
    columns= protein_classes.columns)
    classified_proteins = classified_proteins.fillna(0)
    for i in range(1,len(corona_df)-1):
        row = corona_df.iloc[i,:]
        prot_acc = row.name
        for column in protein_classes:
            if prot_acc in protein_classes[column].values:
                classified_proteins[column] += row
    classified_proteins = classified_proteins.transpose()
    return(classified_proteins)

def get_prot_sequences(accession_list):
    proteins = []
```

```
    baseUrl="http://www.uniprot.org/uniprot/"
    for accession in accession_list:
        currentUrl=baseUrl+accession+".fasta"
        response = r.post(currentUrl)
        cData=''.join(response.text)
        Seq=StringIO(cData)
        pSeq=list(SeqIO.parse(Seq,'fasta'))
        if len(pSeq) > 0:
            proteins.append(pSeq[0].seq)
    return proteins

def prot_analysis(prot_seqs, prot_names):
    pI_list = [] # isoelectric point
    aromaticity_list = [] # aromaticity
    hydropathy_list = [] # GRAVY score
    helix_list = [] # % protein with helix structure
    turn_list = [] # % protein with turn structure
    sheet_list= [] # % protein with sheet structure
    charge_list = [] # protein charge at pH 7.4
    for seq in prot_seqs:
        seq = str(seq)
        seq = seq.replace("X","G")
        seq = seq.replace("U","G")
        seq = seq.replace("B","G")
        seq = seq.replace("Z","G")
        prot_data = ProteinAnalysis(seq)
        pI_list.append(prot_data.isoelectric_point())
        aromaticity_list.append(prot_data.aromaticity())
        hydropathy_list.append(prot_data.gravy())
        charge_list.append(prot_data.charge_at_pH(7.4))
        secondary_structure = prot_data.secondary_structure_fraction()
        helix_list.append(secondary_structure[0])
        turn_list.append(secondary_structure[1])
        sheet_list.append(secondary_structure[2])

    protein_properties = pd.DataFrame(index=prot_names)
    protein_properties['pI'] = pI_list
    protein_properties['Aromaticity'] = aromaticity_list
    protein_properties['Hydropathy'] = hydropathy_list
    protein_properties['Charge at pH = 7.4'] = charge_list
    protein_properties['Helix'] = helix_list
    protein_properties['Turn'] = turn_list
    protein_properties['Sheet'] = sheet_list
    return protein_properties

def weighted_avg(property_series, corona_df):
    property_df = pd.DataFrame(index=property_series.index,
    columns= corona_df.columns)
```

```
    for column in corona_df.columns:
        perc_abun = corona_df[column].tolist()
        renorm_abun = [i/sum(perc_abun) for i in perc_abun]
        corona_df[column] = renorm_abun
    for protein in corona_df.index:
        property_val = property_series[protein]
        property_df.loc[protein] = corona_df.loc[protein]*property_val
    weighted_property = property_df.sum(axis=0)
    return weighted_property

base_file_name = 'PCF-SD-5502--02--2023_'
min_sample_ID = 1066959
max_sample_ID = 1066994
replicates = 3
corona_list = []
names_df = pd.read_excel('Sample Names.xlsx')
sample_names = names_df['Samples'].tolist()
for i in range(min_sample_ID, max_sample_ID + 1):
    corona_list.append(load_protein_corona(base_file_name + str(i) + '_PD2.xlsx'))
grouped_coronas = collate_corona_files(corona_list, 'Abundance/MW', sample_names)
grouped_coronas = grouped_coronas.sort_values(by = 'Description')
grouped_coronas.to_excel('Grouped Coronas.xlsx')

filtered_coronas = filter_proteins(grouped_coronas.copy(), 6.5*10**5)
filtered_coronas.to_excel('Filtered Coronas.xlsx')
average_corona = average_proteins(filtered_coronas, replicates, sample_names)
average_corona.to_excel('Averaged Coronas.xlsx')
proteins_heatmap(average_corona) # standard heatmap
#cluster_proteins(filtered_coronas) # hierarchical clustering
classified_df = classify_proteins(average_corona)
classified_df.to_excel('Protein Pie Charts.xlsx')
accession_list = filtered_coronas.index.tolist()
proteins = get_prot_sequences(accession_list)
sequences_df = pd.DataFrame(proteins)
prot_props = prot_analysis(proteins, filtered_coronas.index)
properties_of_interest = ['Charge at pH = 7.4', 'Aromaticity',
                          'Hydropathy', 'Helix', 'Turn', 'Sheet']
avg_property_df = pd.DataFrame(columns=properties_of_interest,
                               index=filtered_coronas.columns[1:])
for property_type in properties_of_interest:
    avg_property = weighted_avg(prot_props[property_type],
            filtered_coronas[filtered_coronas.columns[1:]])
    avg_property_df[property_type] = avg_property
avg_property_df.to_excel('Protein Properties.xlsx')
print('Done')
```

LNPs treatment in 16HBE14o- with R553X mutations

[0651] 16HBE14o- human bronchial epithelial cells with R553X mutation were maintained in complete growth media composed of Minimum Essential Medium (Gibco, Cat#11095-072) with 10% Fetal Bovine Serum (Gibco, Cat#26140-079) and 1% Penicillin/Streptomycin (Gibco, Cat#15140-122). Plates and flasks were coated with LHC basal medium (Gibco, Cat#12677-027) with 1.34 µL/mL Bovine serum albumin 7.5% (Gibco, Cat#15260-037), 10 µL/mL Bovine collagen solution, Type 1 (Advanced BioMatrix, Cat#5005-100mL), 10 µL/mL Fibronectin from human plasma (1mg/mL) (Thermofisher, Cat#33016-015) by 2h incubation at 37 °C followed by thorough removal of coating solution and storage at 4 °C up to three months. Cells were thawed into a T75 flask at a seeding density of 1 x 10^6 per flask in complete growth media and incubated at 37 °C for 3 days until confluency. Medium was changed three times a week. For the LNP-ABEs treatment, cells were seeded onto a 6-well plate with a seeding density of 3 x 10^5 cells/well in 2 mL of complete growth media. Cells were incubated overnight to reach 80% confluency and the media was replaced with fresh complete growth media before LNP-ABEs treatment. For the treatment, 100 µL of LNP-ABE were added to each well at 80% cell confluency stage and 48 h post-treatment, 1 mL of complete growth media was added to each well. 72 h post-treatment, cells were collected using TrypLE Express (Gibco, Cat# 12604-021).

LNP-ABE treatment in undifferentiated HBE cells with CF R553X/F508del mutation

[0652] Cystic fibrosis patient derived HBE cells with compound heterozygous mutation of R553X/F508del (passage 2, P2) were thawed and seeded at 1.3 x 10^5 cells/well on 6-well plates precoated with 3T3 conditioned media (Cell culture core, Rosalind Franklin University of Medicine and Science), with daily maintenance in BEGM Bronchial Epithelial Cell Growth Medium (Lonza, Cat# CC3170). At day 4, undifferentiated cells were treated with 100 µL of 1.4 µg/well of LNP-ABEs, with media change 48 h post-treatment. An untreated group was used as the negative control. Once confluent at day 6, cells were established on transwell inserts for cell differentiation (passage 3, P3) with seeding at 1.5 x 10^5 cells/insert (Corning, #3378; HTS Transwell®-24-well permeable support with 0.4 µm pore polyester membrane and 6.5 mm inserts) with complete growth medium on both apical and basal sides. After 24 hrs, the inserts were changed to a 2% UG Differentiating medium (Cell Culture Core, Rosalind Franklin University of Medicine and Science). After 96 h, cultures were brought to the Air-liquid interface (ALI). Cultures were maintained for 4 weeks with media change every other day. The

fully differentiated HBEs were finally detached from the inserts by adding ice-cold Accumax (Cat# 00-4666-56, Thermofisher) and incubating at 37 °C for 30 min for further analyses.

CFTR quantification using Jess™ capillary western blot

[0653] P3 R553X/F508del HBE cells were lysed immediately following TECC-24 functional analysis. After removing the media from the basolateral side, cells were washed with 100 µL of 1X DPBS at room temperature. 35 µL of RIPA buffer (Rockland Immunochemical, Cat# MB-077-0050) with 1% HALT (Thermofisher, Cat# 78442), 1% 0.5M EDTA (15575-038), 0.5% Universal Nuclease (Thermofisher, Cat# 88702) was then added directly to the apical side of the insert. After 30 min at 4 °C on a plate shaker, cell lysates were collected, and insoluble fractions were separated by centrifugation at 15,000 rpm for 5 minutes at 4 °C. Supernatant were transferred to new tubes and amount of protein in the cell lysates was measured using a bicinchoninic acid (BCA) assay (Thermofisher, Cat# 23227). The lysates were then diluted to a final concentration of 1.5 µg /µL in a 5x fluorescent master mix (ProteinSimple, EZ standard pack 1), and 3 µL was added per well to the JESS microplate. Polyclonal rabbit IgG anti-human CFTR antibody (Atlas, Cat# HPA021939) was used at 3 µg/mL and the monoclonal mouse IgG2A anti-Vinculin antibody (R&D systems, Cat# MAB6896) was used at 20 µg/mL concentration. For secondary antibodies (ProteinSimple, Anti-Rabbit detection module, Cat# DM-001, and Anti-Mouse detection module, Cat# DM-002), and chemiluminescence were used as per kit instructions. Additional reagents including Ab-diluent, washing buffer, and capillary cartridges were used from the 66-440 kDa Separation Module (SM-W004-1, ProteinSimple, Bio-Techne). The manufacturer's protocol was followed to perform the JESS™ capillary western blot instrument (ProteinSimple, Bio-Techne, Minneapolis, MN, USA). The results were analyzed using Compass for Simple Western software.

Measurement of CFTR function in fully differentiated HBE cells

[0654] Post-treatment, undifferentiated HBE R553X/F508del cells at P3 were subjected to differentiation for 4 weeks. Once differentiated, CFTR function in the HBE cells was measured as transepithelial chloride secretion using a Transepithelial Current Clamp (TECC) and a 24-well electrode manifold (EP Devices). 4 days prior to the small molecule treatment, selected

wells were washed with 3 mM DTT (Roche Diagnostics, Cat# 10197777001) for 30 mins at 37 °C. 24 h before the small molecule treatment, cells were washed with 1X DPBS (Gibco, Cat# 14190-144,) for 30 mins at 37 °C then incubated for 24 hrs with either Trikafta or control vehicle 0.2% DMSO (Fisher Scientific, Cat# BP231100). To prepare the plate for functional assay, differentiation media was replaced with HEPES buffered F-12 assay medium (pH 7.4) on both apical and basolateral sides. After an incubation of 45 minutes at 37 °C without CO_2, the plate was mounted onto a 37 °C heated platform, and transepithelial resistance (Rt) and voltage (Vt) were continuously measured using the TECC device. Baseline values were first measured for 30 minutes, then Rt and Vt were measured (1) for 15 minutes after apical addition of benzamil (Sigma, Cat# 2417) (6 μM final concentration); (2) for 30 minutes after simultaneous apical/basolateral addition of forskolin (Sigma, Cat# F6886) (10 μM final concentration)/VX-770 (Selleckchem chemicals, Cat# S1144) (1 μM final concentration); (3) and for 15 minutes after basolateral addition of bumetanide (Sigma, Cat# B3023) (20 μM final concentration). CFTR functional results are presented as equivalent chloride current (Ieq) which was calculated using Ohm's law, Ieq = Vt/Rt.

LNP-tdTom treatment in fully differentiated HBE cells with CF R553X/F508del mutation

[0655] Cystic fibrosis patient derived HBE cells with compound heterozygous mutation of R553X/F508del (passage 2, P2) were thawed and seeded in BEGM growth medium on flasks precoated with 3T3 conditioned media. Once confluent at day 5, undifferentiated cells were established on Falcon inserts with complete growth medium on both apical and basal sides. After reaching full confluency on inserts, cultures were then brought to the Air-liquid interface (ALI) in 2% UG Differentiating medium (Cell Culture Core, Rosalind Franklin University of Medicine and Science). Cultures were maintained for 23 days with media change every other day. Cells were then treated with 50 μL of LNP-tdTom (12 ug tdTom mRNA per well, 30:1 total lipid to mRNA weight ratio) either to the apical side in liquid bolus for 8 h or to the basolateral side containing 650 μL of differential media for 24h. An untreated group was used as the negative control. Cells were finally collected using Accumax (Sigma, #SCR006).

Antibody staining and analysis by flow cytometry of the treated differentiated HBE cells

[0656] Collected cells were resuspended using the LIVE/DEAD™ Fixable Scarlet (723) Viability Kit (Thermofisher, #L34987) and fixed using 4% PFA for 10 min at RT. Cells were then permeabilized by adding eBioscience™ Permeabilization Buffer (Life Technologies, #00-8333-56) for 10 min at RT. Fc Receptor blocker (Biolegend, Human TrueStain FcX, #422302) was then used to block the cells in staining buffer (permeabilization buffer + 0.5% BSA) for 30 minutes at RT. Cells were first incubated uteroglobin/SCGB1A1 Antibody (Novus Biologicals LLC #MAB4218) for 50 min at RT and goat anti-rat IgG with Alexa Fluor 405 (ABCAM #ab175671) on ice for 30 min. After washing, the cells were marked with a variety of primary antibodies, namely Alexa Fluor 647-conjugated Anti-Cytokeratin 5 antibody (Abcam #ab193895), Alexa Fluor 750-conjugated Acetyl--Tubulin (Lys40) (D20G3) XP Rabbit mAb (Cell Signaling Technology #87488S), Alex Fluor 488 conjugated Mucin 5AC [45M1] mouse mAb (Abcam # ab309610-100UL) for 50 min at RT. After PBS wash and resuspension in PBS 0.5% BSA, samples were subsequently analyzed using a Thermofisher Attune Cytpix flow cytometer. Finally, the data collected from the flow cytometer were processed and analyzed using Flowjo software (BD).

LNPs treatment in A-498, Huh-7 and 16HBE14o- cells

[0657] A-498 cells were obtained from ATCC. Huh-7 cells were a kind gift from Dr. Hao Zhu's lab. A-498, Huh-7, or 16HBE14o- with F508del mutation were cultured in white-bottom 96-well plates at a density of 1×10^4 cells per well the day before transfection. The white-bottom 96-well plates for 16HBE14o- were pre-coated by coating solution as previously described and all cells were cultured in the medium as recommended by the suppliers. SORT LNPs (5A2-SC8/DOPE/cholesterol/DMG-PEG/DOTAP=21.6:12:24:2.4:40 molar ratio, total lipids/mRNA = 20/1, wt/wt) and non-SORT LNPs (MC3/DSPC/cholesterol/DMG-PEG=50/10/38.5/1.5 molar ratio, total lipids/mRNA = 20/1, wt/wt) containing luciferase (*Luc*) mRNA were prepared by vertex mixing using published protocol (*22*).

[0658] For vitronectin pre-coating assay, SORT and non-SORT LNPs containing *Luc* mRNA were incubated with or without 0.25 g vitronectin/g total lipid of human vitronectin (Thermo Fisher, Cat# A14700) for 15 minutes at 37°C. Then the culture was treated with either native LNPs or vitronectin-coated LNPs (25 ng of *Luc* mRNA per well, n=4). After 24 h, *Luc* mRNA expression was quantified by ONE-Glo + Tox kits (Promega, Cat# E7120) 24 h after the treatment by following Promega's standard protocol.

[0659] For vitronectin competitive assay, cells were incubated with human vitronectin by replacing culture media with the vitronectin-contained medium at 0.2 μg vitronectin/mL concentration prior LNP treatment. After 10-min incubation, vitronectin-coated LNPs (0.25 g vitronectin/g total lipid) were added into each well with 25 ng *Luc* mRNA (n=4). Following 4 h co-incubation, each well was washed with PBS one time and replaced with the fresh medium without vitronectin. After 24 h, *Luc* mRNA expression was quantified by ONE-Glo + Tox kits (Promega, Cat# E7120) 24 h after the treatment by following Promega's standard protocol.

Generation of the partially humanized exon replacement R553X mice

[0660] To produce the partially humanized *R553X* mouse *Cftr* allele ($Cftr^{R553X}$) mouse exon 12 and flanking intronic sequences (130 bp upstream; 89 bp downstream) were replaced with the corresponding human sequence exon 12 containing the R553X mutation.

[0661] Candidate guide RNAs (gRNA) in the introns surrounding exon 12 of mouse *Cftr* were tested *in vitro* using Guide-It gRNA *in vitro* transcription and screening kit (Takara) and two active guides were chosen (5'-GGCACTTGAGTTTATATGAT-3' for intron 11 and 5'-ATCAATTCCAGAGACAGAAC-3' for intron 12). A targeting vector plasmid was constructed containing the human sequences and 1 kb arms homologous to the mouse genome. The gRNAs (5 ng/μL each; Synthego), targeting vector plasmid (1ng/μL; VectorBuilder) and Cas9 protein (10 ng/μL; PNABio) were injected in the pronucleus of C57BL/6J zygotes. Embryos were transferred to pseudo-pregnant females. Founders were identified by genotyping and sequencing for correct integration of human exon 12 sequence.

[0662] Mice homozygous for the R553X mutation were created by breeding heterozygous males and females. Genotyping was completed by PCR analysis using DNA extracts from ear biopsies. Genotyping was completed to distinguish between alleles of mouse exon 12 and human exon 12 (R553X). To detect the human exon 12 containing R553X allele (205 bp) primers P1 (5'- AGAAGGAAGATGTGCCTTTCA -3') and P2 (5'-CAAATGCTTGCTAGACCAATAATTAGT -3') were used. To detect the mouse exon 12 (WT) allele (312 bp) primers P3 (5'- TGGGCTTATGGGTAGTCTTTGA-3') and P4 (5'-CAGGAAGCAGAAGAGAAATGTGT -3') were used in a single reaction. Primers were selected to surrounding introns since human and mouse exon 12 are highly homologous. PCR reactions were completed for 40 cycles of 95 °C for 30 seconds, 55 °C for 30 seconds and 72 °C for 30 seconds and products were run out on 2% agarose gels. All mice were allowed

unrestricted access to water and solid chow (Envigo, Teklad S-2335 breeder diet, Cat# 7904). All animals were maintained on a 12-h light, 12-h dark schedule at a mean ambient temperature of 22 °C and were housed in standard polysulfone microisolator cages in ventilated units with corncob bedding.

Crypt harvest and intestinal organoid culture

[0663] Intestinal organoids were cultured similar to previously described methods (D. R. McHugh *et al.*, A G542X cystic fibrosis mouse model for examining nonsense mutation directed therapies. *Plos One* **13**, e0199573 (2018)). Mice were euthanized by CO_2 asphyxiation, and 20 cm of intestine measured from the stomach were removed. Fecal matter was flushed from the intestine with Ca^{2+}- and Mg^{2+}-free PBS, and the intestine was flayed using dissecting scissors. The villi were scraped from the small intestine using a microscope slide, and the intestine was cut into ~1 cm segments, which were suspended in 2 mM EDTA in Ca^{2+}- and Mg^{2+}-free PBS. The intestinal segments were incubated on a shaker for 30 minutes at room temperature. The segments were then vortexed at for 10 seconds, allowed to settle, and then the supernatant was removed and stored in a 10 cm dish. This process was repeated until four supernatant fractions were produced. The fractions were inspected under a microscope, and the fraction which was most enriched for crypts was passed through a 70 µm cell strainer. The crypts were pelleted at 1,000xG for 10 minutes, then resuspended in 1:1 mixture of Intesticult Organoid Growth Media (STEMCELL Technologies, Cat# # 06005) and MatriGel (Corning) at a concentration of 10 crypts/µL. The organoids were seeded to 12-well plates, with 70 µL Matrigel:OGM added to each well in 4-5 droplets. The plate was placed in a 37 °C/5% CO_2 incubator for 15 minutes to allow the MatriGel to harden. The MatriGel domes were then immersed in 1 mL OGM and returned to the 37 °C/5% CO_2 incubator. OGM was changed every 3-4 days, and the organoids were passaged once every 5-7 days.

R553X correction in intestinal organoids using LNP-ABE

[0664] Organoids were grown in Matrigel droplets in a 12 well plate to approximately 75% confluency with Mouse Intesticult OGM containing 10 µM Y-27632 (STEMCELL Technologies) and 5 µM CHIR 99021 (Sigma-Aldrich). Organoids were released from Matrigel using PBS and centrifugation. Pelleted organoids are resuspended in 1 mL of Acutase (Life

Technologies) and incubated at 37 °C for 5 minutes to digest organoids into single cells. Digestion was halted by quenching the Acutase with 2 mL of DMEM media containing 10% FBS. Single stem cells were then placed in an eppedorf tube with 200 µL of OGM containing 0.8 µg of total RNA (*ABE* mRNA:sgR553X=1:1, wt/wt) and incubated at 37 °C/5% CO_2 for 4 hours. Cells and media were then placed in one well of a Matrigel coated 96-well plate. Cells were grown at 37 °C/5% CO_2 for approximately 4-5 days until full organoids develop from the surviving single stem cells. Forskolin-induced swelling of the resulting intestinal organoids were carried out as previously described (D. R. McHugh *et al.*, A G542X cystic fibrosis mouse model for examining nonsense mutation directed therapies. *Plos One* **13**, e0199573 (2018); J. F. Dekkers *et al.*, A functional CFTR assay using primary cystic fibrosis intestinal organoids. *Nat. Med.* **19**, 939-945 (2013)), with small modifications. 200 µL OGM containing 20 µM forskolin was added to each well, creating a 10 µM final concentration. Kinetic brightfield images of FIS were acquired under live cell conditions with a Lionheart FX Automated Microscope (Biotek Instruments, Winooski, Vermont). After one hour of FIS, each organoid was scored as either corrected for CFTR activity, if the organoid swelled, or not corrected for CFTR activity, if the organoid did not swell. Eight 96 wells per treatment group were used for each experiment. DNA was isolated from each well for sequencing of the R553X locus.

Sanger sequencing analysis

[0665] Passive Cell lysis Buffer (Promega, Cat# E1941) with proteinase K (Thermal Fisher, Cat# EO0492) was used according to the manufacturer's recommendations to isolate genomic DNA of 16HBEge cells, $CFTR^{R553X/F508del}$ HBE cells through a PCR program (65 °C for 15 min, 95 °C for 10 min). R553X target sequence was amplified using KAPA polymerase (Roche, Cat# 50-196-5243) with the primers listed in **Table E7** following a PCR program (95 °C for 5 min; (95 °C for 30 s; 63 °C for 30 s; 72 °C for 30 s) for 35 cycles; 72 °C for 7 min and then keep at 4 °C). 40 ng of gDNA from intestinal organoids derived from R553X homozygous mice was used for the PCR reaction using a Phusion U Green Multiple PCR Master Mix (Thermo Fisher, Cat# F564S) with an amplification program (98 °C for 3 min; (98°C for 10 s; 58°C for 30 s; 72°C for 30 s) for 30 cycles; 72°C for 2 min). The PCR products were purified using Qiagen PCR purification kit (Qiagen, Cat# 28106), then sequenced by the McDermott Center Sequencing core facility at UTSW. The editing efficiency was determined by analyzing the

Sanger sequencing results with EditR (http://baseeditr.com/) (M. G. Kluesner *et al.*, EditR: A method to quantify base editing from Sanger sequencing. *CRISPR J.* **1**, 239-250 (2018)).

Targeted amplicon deep sequencing analysis

[0666] Deep amplicon sequencing was used to measure the on-target base editing efficiency in P3 CFTR$^{R553X/F508del}$ HBE culture and lungs from compound heterozygous R553X mice carrying human exon 12 containing the R553X mutation treated with LNP-ABE. Lung single cells were isolated from the snap frozen R553X mice, then NGFR$^+$ cells from bulk lung single cells were isolated from using magnetic cell separation as previously described. Genomic DNA was isolated from lung single cells and NGFR$^+$ cell from the bulk lung using NucleoSpin Tissue XS kits (MACHEREY-NAGEL, Cat# 740901.50). The on-target site for human R553X sequence was PCR amplified with primers listed in the supplementary **Table E7** with the addition of 8 bp barcodes on both ends. 40 ng of gDNA was used for the PCR reaction using a Phusion U Green Multiple PCR Master Mix (Thermo Fisher, Cat# F564S) with an amplification program (98 °C for 3 min; (98 °C for 10 s; 58 °C [R553X mouse]/63 °C [R553X HBE] for 30 s; 72 °C for 30 s) for 30 cycles; 72 °C for 2 min). PCR products were purified with Qiagen PCR purification kit (Qiagen, Cat# 28106) with 25 μL of DNase free water and quantified by Qubit dsDNA high-sensitivity assay (Invitrogen, Cat# Q33231). Targeted amplicon deep-sequencing library was then prepared and later sequenced by Novogene using Illumina NovaSeq 6000. After demultiplexing, amplicon sequencing data were analyzed with CRISPResso2 (https://crispresso.pinellolab.partners.org/) to determine the editing efficiency (K. Clement *et al.*, CRISPResso2 provides accurate and rapid genome editing sequence analysis. *Nat. Biotechnol.* **37**, 224-226 (2019)).

Display items

[0667] The images of lipid nanoparticles, human, mice, lungs, syringes, cells, proteins, plates, and pipettes (FIG. 17A, FIG. 17B, FIG. 18A, FIG. 20A, FIG. 23A, FIG. 29A, FIG. 31A, FIG. 31C, FIG. 36A, FIG. 36I, and FIG. 36N) were created with BioRender.com.

Statistical analyses

[0668] Statistical analyses were performed using Prism 9 (GraphPad Software, version 9.5.1). Data are presented as individual data points or mean +/- standard error of the mean (SEM). Statistical tests were performed in GraphPad Prism 9. A two-tailed unpaired t-test was used for comparison between the respective two groups, one-way ANOVA was used for comparison between three or more groups with one variable. P values less than 0.05 were considered statistically significant.

Supplementary Information

Additional background on basal cells and markers used in identification and characterization.

[0669] Basal cells are considered the tissue-specific stem cells in both mouse and human airway epithelium, given their capacity to self-renew and differentiation into various mature cell lineages including ciliated, secretory, goblet, and ionocytes (D. T. Montoro *et al.*, A revised airway epithelial hierarchy includes CFTR-expressing ionocytes. *Nature* **560**, 319-324 (2018); J. R. Rock, S. H. Randell, B. L. Hogan, Airway basal stem cells: a perspective on their roles in epithelial homeostasis and remodeling. *Dis. Models Mech.* **3**, 545-556 (2010); Y. Zhou *et al.*, Airway basal cells show regionally distinct potential to undergo metaplastic differentiation. *eLife* **11**, e80083 (2022); J. R. Rock *et al.*, Basal cells as stem cells of the mouse trachea and human airway epithelium. *Proc. Natl. Acad. Sci. USA* **106**, 12771-12775 (2009)). Basal cells in the mouse and human airway epithelium can be identified by the expression of nerve growth factor receptor (NGFR) (J. R. Rock *et al.*, Basal cells as stem cells of the mouse trachea and human airway epithelium. *Proc. Natl. Acad. Sci. USA* **106**, 12771-12775 (2009)) or cytoskeletal protein keratin 5 (KRT5) (W. Zuo *et al.*, p63+Krt5+ distal airway stem cells are essential for lung regeneration. *Nature* **517**, 616-620 (2015)). Lineage tracing and clonal growth studies have supported their roles in differentiation and regeneration (J. R. Rock, S. H. Randell, B. L. Hogan, Airway basal stem cells: a perspective on their roles in epithelial homeostasis and remodeling. *Dis. Models Mech.* **3**, 545-556 (2010); J. R. Rock *et al.*, Basal cells as stem cells of the mouse trachea and human airway epithelium. *Proc. Natl. Acad. Sci. USA* **106**, 12771-12775 (2009); W. Zuo *et al.*, p63+Krt5+ distal airway stem cells are essential for lung regeneration. *Nature* **517**, 616-620 (2015); Pooja *et al.*, Distal airway stem cells yield alveoli in vitro and during lung regeneration following H1N1 influenza infection. *Cell* **147**, 525-538 (2011)). When isolated, these cells (NGFR+, KRT5+) can grow in Matrigel, forming clonal structures with markers for ciliated and Clara cells (J. R. Rock *et al.*, Basal cells as stem cells of the mouse trachea and

human airway epithelium. *Proc. Natl. Acad. Sci. USA* **106**, 12771-12775 (2009)). This growth is supported by a medium with EGF, FGF, and bovine pituitary extract. Lineage tracing studies in mice using a CK5–CreER model show KRT5-positive basal cells developing into ciliated cells in the proximal airways under normal and post-sulfur dioxide injury conditions (J. R. Rock, S. H. Randell, B. L. Hogan, Airway basal stem cells: a perspective on their roles in epithelial homeostasis and remodeling. *Dis. Models Mech.* **3**, 545-556 (2010); J. R. Rock *et al.*, Basal cells as stem cells of the mouse trachea and human airway epithelium. *Proc. Natl. Acad. Sci. USA* **106**, 12771-12775 (2009)). More recently, KRT5-positive stem cells from bronchiolar regions have been observed to repair alveolar lung tissue post H1N1 infection, forming organized, spherical "pods" and expressing alveolar-specific proteins (W. Zuo *et al.*, p63+Krt5+ distal airway stem cells are essential for lung regeneration. *Nature* **517**, 616-620 (2015); Pooja *et al.*, Distal airway stem cells yield alveoli in vitro and during lung regeneration following H1N1 influenza infection. *Cell* **147**, 525-538 (2011)).

Additional information regarding Lung SORT LNP mediated CRISPR-Cas9 editing in the lungs.

[0670] Ai14 tdTomato reporter mice were analyzed following IV administration of Lung SORT LNPs encapsulating *Cas9* mRNA and sgTOM1 (B. T. Staahl *et al.*, Efficient genome editing in the mouse brain by local delivery of engineered Cas9 ribonucleoprotein complexes. *Nat. Biotechnol.* **35**, 431-434 (2017)) (2:1, wt:wt) (**Table E5**) (LNP-Cas9) (2 mg/kg per dose, 3 doses 7 days apart). LNP-Cas9 mediated in vivo editing yielded persistent tdTom expression across the lungs through the eight-month end point including >16% in whole lung cells, >40% in endothelial cells, >7% in immune cells, >25% in epithelial cells, >10% in stem cells (**FIG. 18**). In addition, analysis of lung immune cell types demonstrated that 22.6% of neutrophils, 41.7% of macrophages, 32.4% dendritic cells, 14.9% B cells, 14.9% $CD4^+$ T cells and 13.5% $CD8^+$ T cells were successfully edited (**FIG. 22**)

Additional information on VtnR expression in mouse tissues.

[0671] Previous work from other groups demonstrated preferential vascular expression of the vitronectin receptor (VtnR) in the lungs compared to the beds of other organs including the liver and the kidneys (B. Singh, C. Fu, J. Bhattacharya, Vascular expression of the αvβ3-integrin in lung and other organs. *Am. J. Physiol. Lung Cell Mol. Physiol.* **278**, L217-L226 (2000)).

Results of **FIG. 34A** confirmed that the lung is the most VtnR enriched organ (24.4%) among all tested organs (<4%). In non-endothelial cell populations, tdTom expression was enriched within the $VtnR^+$ fraction in heart (24.8% vs 2.9%), lung (23.3% vs 2.9%), liver (88.3% vs 52.4%), spleen (10.4% vs 4.6%), and kidney (24.6% vs 2.7%) (**FIG. 34C**) following LNP-Cre delivery.

Additional information on immune cell editing for CF.

[0672] CF is associated with neutrophil abnormality (L. M. Yonker *et al.*, Neutrophil dysfunction in cystic fibrosis. *J. Cyst. Fibros.* **20**, 1062-1071 (2021)) and macrophage dysfunction (J. L. Gillan *et al.*, CAGE sequencing reveals CFTR-dependent dysregulation of type I IFN signaling in activated cystic fibrosis macrophages. *Sci. Adv.* **9**, (2023)), causing chronic airway inflammation and a progressive decline in lung function. Flow cytometry analysis of a comprehensive immune cell panel was performed, revealing that 22.6% of neutrophils, 41.7% of macrophages, 32.4% of dendritic cells, 14.9% of B cells, 14.9% of $CD4^+$ T cells, and 13.5% of $CD8^+$ T cells were transfected by LNP-Cre in mouse lungs at tested dose (**FIG. 22**). Editing was also achieved in lung immune progenitor cells (**FIG. 33**).

Additional background on the HBE assay.

[0673] Unlike non-CF derived HBE cultures, fully differentiated CF HBE cultures display similar function characteristics associated with CF airway phenotype in vivo, including accumulation of thick sticky mucus and abnormal ion and fluid transport (D. M. Cholon, M. Gentzsch, Established and novel human translational models to advance cystic fibrosis research, drug discovery, and optimize CFTR-targeting therapeutics. *Curr. Opin. Pharmacol.* **64**, 102210 (2022)). By measuring CFTR-dependent current changes, the area under the curve (AUC) between forskolin and ivacaftor (VX-770) stimulation, one can quantify the functional restoration of CFTR. In this study, primary HBE culture carrying compound heterozygous mutations were used, one allele with R553X mutation and the other one with F508del mutation. Therefore, the baseline editing level of C% on the target position in untreated control is 50% (from the F508del allele). CFTR modulator Trikafta (elexacaftor/tezacaftor/ivacaftor, standard-of-care for CF persons with at least one F508del allele, or any of the other approved mutations (P. G. Middleton *et al.*, Elexacaftor–Tezacaftor–Ivacaftor for cystic fibrosis with a single

Phe508del allele. *New. Engl. J. Med.* **381**, 1809-1819 (2019))) was used to compare the therapeutic efficacy of LNP-ABE.

Additional information on the comparison of SORT LNP delivery to differentiated HBEs by basolateral and apical administration.

[0674] Additionally, the transfection efficiency of SORT LNPs delivering tdTomato mRNA (LNP-tdTom) in fully differentiated human bronchial epithelial (HBE) cells on trans-wells through basolateral administration was assessed (**FIG. 38**) to mimic in vivo systemic delivery to the lungs, as LNPs can reach the lung stem cells and mature epithelium through intravenous administration (IV) from blood side. Flow cytometry analysis showed that over 50% of basal cells, 36.8% of club cells, and 9.5% ciliated cells were transfected from the basolateral side.

Additional information on the functional organoid model.

[0675] In the forskolin-induced swelling (FIS) assay (D. R. McHugh *et al.*, A G542X cystic fibrosis mouse model for examining nonsense mutation directed therapies. *Plos One* **13**, e0199573 (2018); J. F. Dekkers *et al.*, A functional CFTR assay using primary cystic fibrosis intestinal organoids. *Nat. Med.* **19**, 939-945 (2013), forskolin activates CFTR, stimulates intracellular pathways, and phosphorylates CFTR to open the CFTR channel, permitting ion/water uptake and ultimately organoid swelling. Once the mutated CFTR gene is corrected, organoid swelling should be observed, while untreated organoids will remain at the baseline volume (**FIG. 36J**).

Additional information on Vitronectin-bound SORT LNP delivery.

[0676] Vitronectin is a protein secreted into the blood by the liver. Following intravenous administration of the LNPs, an interfacial layer of blood proteins, termed the protein corona, forms around the LNPs. Among the subset of proteins which highly bind to the surface of Lung SORT LNPs is vitronectin (**FIG. 30**). Functionally, vitronectin on the LNP surface may affect which cells can interact and take up the LNPs by binding to its cognate receptors (vitronectin receptor, VtnR) highly expressed by cells within the target organ.

Additional background information on CF gene therapy,

[0677] The first gene therapy clinical trial for CF took place in 1993 (A. L. Cooney, P. B. McCray, Jr., P. L. Sinn, Cystic fibrosis gene therapy: Looking back, looking forward. *Genes* **9**, 538 (2018)). Since then, numerous subsequent trials utilizing various viral vectors took place, but unfortunately there are still no FDA-approved gene therapies in CF. Advancement in viral vectors for gene therapy (A. L. Cooney, P. B. McCray, Jr., P. L. Sinn, Cystic fibrosis gene therapy: Looking back, looking forward. *Genes* **9**, 538 (2018)) and lung delivery (Z. Yan *et al.*, Recombinant adeno-associated virus-mediated editing of the G551D cystic fibrosis transmembrane conductance regulator mutation in ferret airway basal cells. *Hum. Gene Ther.* **33**, 1023-1036 (2022); M. K. Yanda *et al.*, Transduction of ferret surface and basal cells of airways, lung, liver, and pancreas via intratracheal or intravenous delivery of adeno-associated virus 1 or 6. *Hum. Gene Ther.* **34**, 1135-1144 (2023)) have continued to be made, with adeno-associated (AAV) virus and lentiviral vectors having been used in clinical trials for CF gene therapy. In one such ongoing clinical trial, AAV-based gene therapy 4D-710 is using an optimized AAV capsid for lung delivery. Due to the limited packaging capability of AAV (~4.6 kb), a transgene encoding human CFTR with a deletion in the regulatory domain [CFTRΔR] was constructed. Previous studies showed that AAV-mediated transgene expression of truncated CFTR leads to functional rescue of CFTR function in well differentiated CF airway epithelia, intestinal organoids, and mouse models (D. Vidović *et al.*, rAAV-CFTRΔR Rescues the Cystic Fibrosis Phenotype in Human Intestinal Organoids and Cystic Fibrosis Mice. *Am. J. Respir. Crit. Care Med.* **193**, 288-298 (2016); L. S. Ostedgaard *et al.*, CFTR with a partially deleted R domain corrects the cystic fibrosis chloride transport defect in human airway epithelia in vitro and in mouse nasal mucosa in vivo. *Proc. Natl. Acad. Sci. USA* **99**, 3093-3098 (2002)). Besides AAV (M. K. Yanda *et al.*, Transduction of ferret surface and basal cells of airways, lung, liver, and pancreas via intratracheal or intravenous delivery of adeno-associated virus 1 or 6. *Hum. Gene Ther.* **34**, 1135-1144 (2023)), lentiviral vectors also exhibit promise for CF gene therapy because of their larger packaging capability allowing expression of full-length human CFTR, their ability to integrate into the host genome facilitating durable gene expression, as well as their tolerability for repeat dosing. A trial using lentiviral vectors for CF gene therapy is currently in preparation (E. W. F. W. Alton *et al.*, Preparation for a first-in-man lentivirus trial in patients with cystic fibrosis. *Thorax* **72**, 137-147 (2017)). These advantages are associated with

the risk of gene disruption by unpredictable insertion of exogenous DNA. Collectively, these results highlight potential advantages of systemic delivery of therapeutic genome editors to treat multiple-organ disease, such as CF.

Sequences

#	SEQUENCE	ANNOTATION
1	AAGTAAAACCTCTACAAATG	sgTOM1
2	TTGCTCATTGACCTCCACTC	sgR553X
3	GGGAGACCCAAGCTGGCTAGCGTTTAAACTTCAGCTTGGCAATCCGGTAC TGTTGGTAAAGCCACCATGCAGAGAAGCCCCCTGGAAAAGGCCAGCGTGG TGAGCAAGCTGTTCTTCAGCTGGACCCGGCCCATCCTGCGGAAGGGCTAC AGACAGAGACTGGAACTGAGCGACATCTACCAGATCCCCAGCGTGGACAG CGCCGACAACCTGAGCGAGAAGCTGGAAAGAGAGTGGGACAGAGAGCTGG CCAGCAAGAAGAACCCCAAGCTGATCAACGCCCTGCGGCGGTGCTTCTTC TGGCGGTTCATGTTCTACGGCATCTTCCTGTACCTGGGCGAAGTGACCAA AGCCGTGCAGCCCCTGCTGCTGGGCAGAATCATCGCCAGCTACGACCCCG ACAACAAAGAGGAACGGAGCATCGCCATCTACCTCGGCATCGGCCTGTGC CTGCTGTTCATCGTCAGAACCCTGCTGCTGCACCCCGCCATCTTCGGACT GCACCACATCGGCATGCAGATGCGGATCGCCATGTTCAGCCTGATCTACA AGAAAACCCTGAAGCTGAGCAGCAGAGTGCTGGACAAGATCAGCATCGGA CAGCTGGTGAGCCTGCTGAGCAACAACCTGAACAAGTTCGACGAAGGCCT GGCCCTGGCCCACTTCGTGTGGATCGCCCCCCTGCAAGTGGCCCTGCTGA TGGGCCTGATCTGGGAACTGCTGCAGGCCAGCGCCTTCTGCGGACTGGGA TTCCTGATCGTGCTGGCCCTGTTCCAGGCCGGACTGGGGAGAATGATGAT GAAGTACCGGGACCAGAGAGCCGGCAAGATCAGCGAGAGACTGGTCATCA CCAGCGAGATGATCGAGAACATCCAGAGCGTGAAGGCCTACTGCTGGGAA GAGGCCATGGAAAAGATGATCGAGAACCTGCGGCAGACCGAGCTGAAGCT GACAAGAAAGGCCGCCTACGTGCGCTACTTCAACAGCAGCGCCTTCTTCT TCAGCGGCTTCTTCGTGGTGTTCCTGAGCGTGCTGCCCTACGCCCTGATC AAGGGCATCATCCTGAGAAAGATCTTCACCACCATCAGCTTCTGCATCGT GCTGCGGATGGCCGTGACCAGACAGTTCCCCTGGGCCGTGCAGACCTGGT ACGACAGCCTGGGCGCCATCAACAAGATCCAGGACTTCCTGCAGAAGCAA GAGTACAAGACCCTCGAGTACAACCTGACCACCACCGAGGTGGTCATGGA AAACGTGACCGCCTTCTGGGAGGAAGGCTTCGGCGAGCTGTTCGAGAAGG CCAAGCAGAACAACAACAACCGCAAGACCAGCAACGGCGACGACAGCCTG TTCTTCAGCAACTTCAGCCTGCTGGGGACCCCCGTGCTGAAGGACATCAA CTTCAAGATCGAGCGGGGACAGCTGCTGGCCGTGGCCGGAAGCACAGGCG CCGGAAAAACCAGCCTGCTCATGGTCATCATGGGCGAGCTGGAACCCAGC GAGGGCAAGATCAAGCACAGCGGCAGGATCAGCTTCTGCAGCCAGTTCAG CTGGATCATGCCCGGCACCATCAAAGAGAACATCATCTTCGGCGTGAGCT ACGACGAGTACAGATACCGCAGCGTGATCAAGGCCTGCCAGCTGGAAGAG GACATCAGCAAGTTCGCCGAGAAGGACAACATCGTGCTCGGCGAAGGCGG CATCACACTGAGCGGCGGACAGAGGGCCAGAATCAGCCTGGCCAGAGCCG TGTACAAGGACGCCGACCTGTACCTGCTGGACAGCCCCTTCGGCTACCTG GACGTGCTGACCGAGAAAGAGATCTTCGAGAGCTGCGTGTGCAAGCTGAT GGCCAACAAGACCCGGATCCTGGTCACCAGCAAGATGGAACACCTGAAGA AGGCCGACAAGATCCTGATCCTGCACGAGGGCAGCAGCTACTTCTACGGC	CFTR mRNA

#	SEQUENCE	ANNOTATION
	ACCTTCAGCGAGCTGCAGAACCTGCAGCCCGACTTCAGCAGCAAACTGAT	
	GGGCTGCGACAGCTTCGACCAGTTCAGCGCCGAGCGGAGAAACAGCATCC	
	TGACAGAGACACTGCACCGGTTCAGCCTGGAAGGCGACGCCCCCGTGAGC	
	TGGACCGAGACAAAGAAGCAGAGCTTCAAGCAGACCGGCGAGTTCGGCGA	
	GAAGCGGAAGAACAGCATCCTGAACCCCATCAACAGCATCCGGAAGTTCA	
	GCATCGTCCAGAAAACCCCCCTGCAGATGAACGGCATCGAAGAGGACAGC	
	GACGAGCCCCTGGAAAGACGGCTGAGCCTGGTGCCCGACAGCGAACAGGG	
	CGAAGCCATCCTGCCCCGGATCAGCGTGATCAGCACAGGCCCCACACTGC	
	AGGCCCGGAGAAGGCAGAGCGTGCTGAACCTGATGACCCACAGCGTGAAC	
	CAGGGACAGAACATCCACAGAAAGACCACCGCCAGCACACGGAAAGTGAG	
	CCTGGCCCCCCAGGCCAACCTGACTGAGCTGGACATCTACAGCAGACGGC	
	TGAGCCAAGAGACAGGCCTGGAAATCAGCGAGGAAATCAACGAAGAGGAC	
	CTGAAAGAGTGCTTCTTCGACGACATGGAAAGCATCCCCGCCGTGACAAC	
	CTGGAACACCTACCTGCGGTACATCACCGTGCACAAGAGCCTGATCTTCG	
	TGCTGATCTGGTGCCTCGTGATCTTCCTGGCCGAAGTGGCCGCCAGCCTG	
	GTGGTGCTGTGGCTGCTCGGAAACACCCCACTGCAGGACAAGGGCAACAG	
	CACCCACAGCCGGAACAACAGCTACGCCGTGATCATCACCAGCACCAGCA	
	GCTACTACGTGTTCTACATCTACGTGGGCGTCGCCGACACTCTGCTCGCC	
	ATGGGCTTCTTCAGAGGACTGCCCCTGGTGCACACCCTGATCACCGTGAG	
	CAAGATCCTGCACCACAAGATGCTGCACAGCGTCCTGCAGGCCCCCATGA	
	GCACACTGAACACCCTGAAAGCCGGCGGAATCCTGAACAGATTCAGCAAG	
	GACATCGCCATCCTGGACGACCTGCTGCCCCTGACCATCTTCGACTTCAT	
	CCAGCTGCTGCTGATCGTGATCGGCGCCATCGCCGTGGTGGCCGTGCTGC	
	AGCCCTACATCTTCGTGGCCACCGTGCCCGTGATCGTGGCCTTCATCATG	
	CTGCGGGCCTACTTCCTGCAGACCAGCCAGCAGCTGAAGCAGCTCGAGAG	
	CGAGGGCAGAAGCCCCATCTTCACCCACCTCGTGACCAGCCTGAAAGGCC	
	TGTGGACCCTGAGAGCCTTCGGCAGACAGCCCTACTTCGAGACACTGTTC	
	CACAAGGCCCTGAACCTGCACACCGCCAACTGGTTCCTGTACCTGAGCAC	
	CCTGCGGTGGTTCCAGATGAGGATCGAGATGATCTTCGTCATCTTCTTCA	
	TCGCCGTGACCTTCATCAGCATCCTCACCACTGGCGAAGGCGAGGGCAGA	
	GTGGGAATCATCCTGACCCTGGCCATGAACATCATGAGCACACTCCAGTG	
	GGCCGTGAACAGCAGCATCGACGTGGACAGCCTGATGCGGAGCGTGAGCC	
	GGGTGTTCAAGTTCATCGACATGCCCACAGAGGGCAAGCCCACCAAGAGC	
	ACCAAGCCCTACAAGAACGGCCAGCTGAGCAAAGTCATGATCATCGAGAA	
	CAGCCACGTCAAGAAGGACGACATCTGGCCCAGCGGAGGCCAGATGACCG	
	TGAAGGACCTGACCGCCAAGTACACCGAAGGCGGAAACGCCATCCTGGAA	
	AACATCAGCTTCAGCATCAGCCCCGGCCAGCGCGTGGGACTCCTGGGAAG	
	AACCGGAAGCGGCAAGAGCACTCTGCTGAGCGCCTTCCTGAGACTGCTGA	
	ACACCGAGGGCGAGATCCAGATCGACGGGGTGAGCTGGGACAGCATCACC	
	CTGCAACAATGGCGGAAGGCCTTCGGCGTGATCCCCCAGAAGGTGTTCAT	
	CTTCAGCGGCACGTTCCGGAAGAACCTGGACCCCTACGAGCAGTGGAGCG	
	ACCAAGAGATCTGGAAGGTGGCCGACGAAGTGGGACTGAGAAGCGTGATC	
	GAGCAGTTCCCCGGCAAGCTGGACTTCGTGCTGGTGGACGGCGGCTGCGT	
	GCTGAGCCACGGACACAAGCAGCTGATGTGCCTGGCCAGAAGCGTGCTGA	
	GCAAGGCCAAGATCCTGCTGCTCGACGAGCCCAGCGCCCACCTGGACCCC	
	GTGACCTACCAGATCATCCGGCGGACACTGAAGCAGGCCTTCGCCGACTG	
	CACCGTGATCCTGTGCGAGCACAGAATCGAGGCCATGCTGGAATGCCAGC	
	AGTTCCTGGTGATCGAAGAGAACAAAGTGCGGCAGTACGACAGCATCCAG	
	AAGCTGCTGAACGAGCGGAGCCTGTTCAGACAGGCCATCAGCCCCAGCGA	
	CAGAGTGAAGCTGTTCCCCCACCGGAACAGCAGCAAGTGCAAGAGCAAGC	

#	SEQUENCE	ANNOTATION
	CCCAGATCGCCGCCCTGAAAGAAGAAACCGAGGAAGAGGTGCAGGACACA CGGCTGTGAGAATTCtgcagAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAATTCG	

Claims

1. A method of treating a subject with cystic fibrosis, the method comprising administering to the subject a composition comprising a lipid nanoparticle (LNP) that comprises a gene editing system, wherein the gene editing system comprises:

    (i) a first nucleic acid encoding a base editor; and

    (ii) a second nucleic acid encoding a guide RNA (gRNA),

    and wherein the composition treats the cystic fibrosis in the subject.

2. A method of treating cystic fibrosis in a subject, the method comprising administering to the subject a composition comprising a lipid nanoparticle (LNP), wherein the LNP comprises (i) a first nucleic acid encoding a base editor; and (ii) a second nucleic acid encoding a guide RNA, wherein the first nucleic acid and the second nucleic acid are delivered to a lung cell in the subject.

3. The method of claim 1 or 2, wherein the *CFTR* gene of the subject comprises a R553X stop codon mutation.

4. The method of claim 3, wherein the administration of the composition results in an increase in the expression of the full-length cystic fibrosis transmembrane conductance regulator (CFTR) protein in the subject, as compared to a subject with cystic fibrosis and whose *CFTR* gene comprises a R553X stop codon mutation, and that is not administered the composition.

5. The method of claim 3, wherein the administration of the composition results in an increase in the function of the cystic fibrosis transmembrane conductance regulator (CFTR) protein in the subject, as compared to a subject with cystic fibrosis and whose *CFTR* gene comprises a R553X stop codon mutation, and that is not administered the composition.

6. The method of any of claims 1-5, wherein the nucleic acid encoding the base editor is RNA.

7. The method of any of claims 1-6, wherein the base editor is an adenine base editor (ABE).

8. The method of claim 7, wherein the ABE comprises a tRNA adenosine deaminase (TadA) protein, or a variant thereof, fused to a catalytically impaired Cas protein capable of binding to a specific nucleotide sequence.

9. The method of claim 7, wherein the ABE is ABE8e.

10. The method of any of claims 1-6, wherein the base editor is a cytosine base editor (CBE).

11. The method of claim 10, wherein the CBE comprises a cytidine deaminase protein, or a variant thereof, fused to a catalytically impaired Cas protein capable of binding to a specific nucleotide sequence.

12. The method of any of claims 7-11, wherein the ABE or the CBE further comprises a Cas9 enzyme that does not have any nuclease activity.

13. The method of any of claims 1-12, wherein the LNP comprises an ionizable cationic lipid, a zwitterionic phospholipid, a cholesterol, and a PEG lipid.

14. The method of any of claims 1-13, wherein the LNP comprises 5A2-SC8, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), cholesterol, DMG-PEG, and one or more selective organ targeting (SORT) molecules.

15. A method of delivering a gene editing system to a lung cell type in a subject, the method comprising administering to the subject a composition comprising a lipid nanoparticle (LNP) that comprises a gene editing system, wherein the gene editing system comprises (i) a first nucleic acid encoding an endonuclease or a base editor; and (ii) a second nucleic acid encoding a guide RNA (gRNA), and wherein the gene editing system is delivered to a lung cell type in a subject.

16. The method of claim 15, wherein the lung cell type is an endothelial cell or an epithelial cell.

17. The method of claim 15 or 16, wherein the lung cell type is an immune cell.

18. The method of any of claims 15-17, wherein the lung cell type is a stem cell.

19. The method of any of claims 15-18, wherein the endonuclease is a Cas nuclease of the CRISPR-Cas system.

20. The method of claim 19, wherein the Cas nuclease is a Cas9 nuclease, a Cas12 nuclease, or a Cas13 nuclease.

21. The method of any of claims 15-20, wherein the nucleic acid encoding the endonuclease is DNA.

22. The method of any of claims 15-20, wherein the nucleic acid encoding the endonuclease is RNA.

23. The method of any of claims 15-18, wherein the nucleic acid encoding the base editor is DNA.

24. The method of claim 15-18, wherein the nucleic acid encoding the base editor is RNA.

25. The method of any of claims 15-18, 23, and 24, wherein the base editor is an adenine base editor (ABE).

26. The method of claim 25, wherein the ABE comprises a tRNA adenosine deaminase (TadA) protein, or a variant thereof, fused to a catalytically impaired Cas protein capable of binding to a specific nucleotide sequence.

27. The method of claim 25, wherein the ABE is ABE8e.

28. The method of any of claim 15-18, 23, and 24, wherein the base editor is a cytosine base editor (CBE).

29. The method of claim 28, wherein the CBE comprises a cytidine deaminase protein, or a variant thereof, fused to a catalytically impaired Cas protein capable of binding to a specific nucleotide sequence.

30. The method of any of claims 15-18 and 23-29, wherein the ABE or the CBE further comprises a Cas9 enzyme that does not have any nuclease activity.

31. The method of any of claims 1-30, wherein the ratio of the first nucleic acid to the second nucleic acid is 1:1 on a molecule:molecule basis.

32. The method of any of claims 1-30, wherein the ratio of the first nucleic acid to the second nucleic acid is 1:1 on a weight basis.

33. The method of any of claims 1-30, wherein the ratio of the first nucleic acid to the second nucleic acid is 2:1 on a molecule:molecule basis.

34. The method of any of claims 1-30, wherein the ratio of the first nucleic acid to the second nucleic acid is 2:1 on a weight basis.

35. The method of any one of claims 15-34, wherein the LNP comprises an ionizable cationic lipid, a zwitterionic phospholipid, a cholesterol, and a PEG lipid.

36. The method of any one of claims 15-35, wherein the LNP comprises 5A2-SC8, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), cholesterol, DMG-PEG, and one or more selective organ targeting (SORT) molecules.

37. The method of claim 14 or 36, wherein the one or more SORT molecules comprises permanently positively charged moiety.

38. The method of claim 37, wherein the one or more SORT molecules is selected from the group consisting of 18:1 DOTMA (DOTMA); DORI, DC-6-14; 12:0 EPC (Chloride Salt); 14:0 EPC (Chloride Salt); 16:0 EPC (Chloride Salt); 18:0 EPC (Chloride Salt); 18:1 EPC (Chloride Salt); 16:0-18:1 EPC (Chloride Salt); 14:1 EPC (Triflate Salt); 18:0 DDAB (Dimethyldioctadecylammonium (Bromide Salt)); 14:0 TAP; 16:0 TAP; 18:0 TAP; 18:1 TAP (DOTAP); 18:1 TAP (DOTAP, MS Salt); 18:1 DODAP, or 18:1 PA (1,2-dioleoyl-sn-glycero-3-phosphate (sodium salt)) (18PA).

39. The method of claim 14 or 36, wherein the one or more SORT molecule comprises DOTAP (1,2-dioleoyl-3-trimethylammonium propane).

40. The method of claim 37 or 38, wherein the one or more SORT molecule comprises 18PA.

41. The method of claim 37 or 38, wherein the one or more SORT molecule comprises DODAP.

42. The method of claim 41, wherein the DODAP comprises about 20% molar ratio of the total lipids.

43. The method of claim 39, wherein the DOTAP comprises about 50% molar ratio of the total lipids.

44. The method of claim 40, wherein the 18PA comprises about 10% molar ratio of the total lipids.

45. The method of claim 37 or 38, wherein the one or more SORT molecule comprises DOTMA.

46. The method of claim 36, 37 or 38, wherein the LNP comprises a ratio of DOPE:DOTMA between 3:1 and 1:3.

47. The method of claim 46, wherein the ratio of DOPE:DOTMA is about 3:1.

48. The method of claim 46, wherein the ratio of DOPE:DOTMA is about 1:1.

49. The method of any of claims 14, 36-48, wherein the SORT molecule comprises from about 5% to about 60% molar percentage of the LNP.

50. The method of any of claims 14, 36-48, wherein the SORT molecule comprises about 40% molar percentage of the LNP.

51. The method of any of claims 14, 36-48, wherein the SORT molecule comprises about 50% molar percentage of the LNP.

52. The method of any of claims 1-51, wherein the LNP binds vitronectin.

53. The method of any one of claims 1-52, wherein the guide RNA comprises a circular RNA.

54. The method of any one of claims 1-52, wherein the guide RNA comprises a linear RNA.

55. The method of any of claims 1-52, wherein the guide RNA is a single guide RNA (sgRNA).

56. The method of any of claims 1-55, wherein the guide RNA comprises a target sequence that is complementary with a target sequence of a cystic fibrosis transmembrane conductance regulator (*CFTR*) gene.

57. The method of any of claims 1-56, wherein the nucleotide sequence of the guide RNA is AAGTAAAACCTCTACAAATG (SEQ ID NO: 1) or TTGCTCATTGACCTCCACTC (SEQ ID NO: 2).

58. The method of any one of claims 1 to 57, wherein the composition comprises a pharmaceutically acceptable carrier.

59. The method of any one of claims 1-58, wherein the composition is administered intravenously.

60. The method of any one of claims 1-59, wherein the subject is a human.

61. The method of any one of claims 15-60, wherein the subject has cystic fibrosis.

62. A method of modifying the nucleic acid sequence of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene in a lung cell type, wherein the *CFTR* gene comprises a R553X stop codon mutation, the method comprising:

    a. contacting the lung cell type with a composition comprising a lipid nanoparticle (LNP), wherein the LNP comprises (i) a first nucleic acid encoding a base editor; and (ii) a second nucleic acid encoding a guide RNA;
    b. determining the nucleic acid sequence of the *CFTR* gene in the lung cell type,

wherein the nucleic acid sequence of the *CFTR* gene in the lung cell type is modified to remove the R553X stop codon mutation.

63. The method of claim 62, wherein the modification comprises the replacing of the thymine at 1789 base in exon 11 of the *CFTR* gene with cytosine.

64. A method of increasing the expression of full-length cystic fibrosis transmembrane conductance regulator (CFTR) protein in a lung cell type, wherein a *CFTR* gene in the lung cell type comprises a R553X mutation, the method comprising

contacting the lung cell type with a composition comprising a lipid nanoparticle (LNP), wherein the LNP comprises (i) a first nucleic acid encoding a base editor; and (ii) a second nucleic acid encoding a guide RNA.

65. A method of modulating the activity of the cystic fibrosis transmembrane conductance regulator (CFTR) protein in a lung cell type, wherein the *CFTR* gene in the lung cell type comprises a R553X mutation, the method comprising

contacting the lung cell type with a composition comprising a lipid nanoparticle (LNP), wherein the LNP comprises (i) a first nucleic acid encoding a base editor; and (ii) a second nucleic acid encoding a guide RNA.

66. The method of claim 64, wherein expression of the CFTR protein is determined in a lung cell type in a subject, wherein the subject has been administered the composition, and wherein the expression is determined by one or more bioassays comprising sweat chloride concentration assay, β-adrenergic sweat assay, and nasal potential difference assay.

67. The method of claim 66, wherein expression of the CFTR protein is determined by analysis of chloride levels in the sweat of the subject.

68. The method of claim 66, wherein the chloride levels in the sweat of the subject after being administered the composition are decreased as compared to the chloride levels in the sweat of the subject before being administered the composition.

69. The method of claim 64, wherein the expression is measured using western blotting, immunoprecipitation, and anti-CFTR antibodies.

70. The method of claim 65, wherein the activity of the CFTR protein is increased in the lung cell type, as compared to a lung cell type comprising a *CFTR* gene comprising a R553X mutation, and that is not contacted with the composition.

71. The method of any one of claims 62-70 wherein the lung cell type is an endothelial cell or an epithelial cell.

72. The method of any one of claims 62-70, wherein the lung cell type is an immune cell.

73. The method of any one of claims 62-70, wherein the lung cell type is a stem cell.

74. The method of any one of claims 62-73, wherein the nucleic acid encoding the base editor is DNA.

75. The method of any one of claims 62-73, wherein the nucleic acid encoding the base editor is RNA.

76. The method of any one of claims 62-73, wherein the base editor is an adenine base editor (ABE).

77. The method of claim 76, wherein the ABE comprises a tRNA adenosine deaminase (TadA) protein, or a variant thereof, fused to a catalytically impaired Cas protein capable of binding to a specific nucleotide sequence.

78. The method of any one of claims 62-73, wherein the base editor is a cytosine base editor (CBE).

79. The method of claim 78, wherein the CBE comprises a cytidine deaminase protein, or a variant thereof, fused to a catalytically impaired Cas protein capable of binding to a specific nucleotide sequence.

80. The method of any of claims 76-79, wherein the ABE or the CBE further comprises a Cas9 enzyme that does not have any nuclease activity.

81. The method of any one of claims 62-80, wherein the ratio of the first nucleic acid to the second nucleic acid is 1:1 on a molecule:molecule basis.

82. The method of any one of claims 62-80, wherein the ratio of the first nucleic acid to the second nucleic acid is 1:1 on a weight basis.

83. The method of any one of claims 62-80, wherein the ratio of the first nucleic acid to the second nucleic acid is 2:1 molecule:molecule basis.

84. The method of any one of claims 62-80, wherein the ratio of the first nucleic acid to the second nucleic acid is 2:1 on a weight basis.

85. The method of any one of claims 62-84, wherein the LNP comprises an ionizable cationic lipid, a zwitterionic phospholipid, a cholesterol, and a PEG lipid.

86. The method of any one of claims 62-85, wherein the LNP comprises 5A2-SC8, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), cholesterol, DMG-PEG, and one or more selective organ targeting (SORT) molecules.

87. The method of claim 86, wherein the one or more SORT molecules comprises permanently positively charged moiety.

88. The method of claim 86 or 87, wherein the one or more SORT molecule is selected from the group consisting of 18:1 DOTMA (DOTMA); DORI, DC-6-14; 12:0 EPC (Chloride Salt); 14:0 EPC (Chloride Salt); 16:0 EPC (Chloride Salt); 18:0 EPC (Chloride Salt); 18:1 EPC (Chloride Salt); 16:0-18:1 EPC (Chloride Salt); 14:1 EPC (Triflate Salt); 18:0 DDAB (Dimethyldioctadecylammonium (Bromide Salt)); 14:0 TAP; 16:0 TAP; 18:0 TAP; 18:1 TAP (DOTAP); 18:1 TAP (DOTAP, MS Salt); 18:1 DODAP, or 18:1 PA (1,2-dioleoyl-sn-glycero-3-phosphate (sodium salt)) (18PA).

89. The method of any of claims 86-88, wherein the one or more SORT molecules comprises DOTAP (1,2-dioleoyl-3-trimethylammonium propane).

90. The method of any of claims 86-88, wherein the one or more SORT molecules comprises 18PA.

91. The method of any of claims 86-88, wherein the one or more SORT molecules comprises DODAP.

92. The method of claim 91, wherein the DODAP comprises about 20% molar ratio of the total lipids.

93. The method of claim 89, wherein the DOTAP comprises about 50% molar ratio of the total lipids.

94. The method of claim 90, wherein the 18PA comprises about 10% molar ratio of the total lipids.

95. The method of any of claims 86-88, wherein the SORT molecule comprises DOTMA.

96. The method of claim 86, 87 or 88, wherein the LNP comprises a ratio of DOPE:DOTMA of between 3:1 and 1:3.

97. The method of claim 96, wherein the ratio of DOPE:DOTMA is about 3:1.

98. The method of claim 96, wherein the ratio of DOPE:DOTMA is about 1:1.

99. The method of any of claims 86-97, wherein the SORT molecule comprises from about 5% to about 60% molar percentage of the LNP.

100. The method of any of claims 86-99, wherein the SORT molecule comprises about 40% molar percentage of the LNP.

101. The method of any of claims 86-99, wherein the SORT molecule comprises from 50% molar percentage of the LNP.

102. The method of any of claims 62-101, wherein the LNP binds vitronectin.

103. The method of any one of claims 62-102, wherein the guide RNA comprises a circular RNA.

104. The method of any one of claims 62-102, wherein the guide RNA comprises a linear RNA.

105. The method of any one of claims 62-102, wherein the guide RNA is a single guide RNA (sgRNA).

106. The method of any of claims 62-105, wherein the guide RNA comprises a target sequence that is complementary with a target sequence of a cystic fibrosis transmembrane conductance regulator (*CFTR*) gene.

107. The method of any of claims 62-106, wherein the nucleotide sequence of the guide RNA is AAGTAAAACCTCTACAAATG (SEQ ID NO: 1) or TTGCTCATTGACCTCCACTC (SEQ ID NO: 2).

108. The method of claim 5, wherein the function of the CFTR protein is determined by one or more bioassays comprising sweat chloride concentration assay, β-adrenergic sweat assay, and nasal potential difference assay.

109. The method of claim 108, wherein the function of the CFTR protein is determined by analysis of chloride levels in the sweat of the subject.

110. The method of claim 109, wherein the chloride levels in the sweat of the subject after being administered the composition are decreased as compared to the chloride levels in the subject before being administered the composition.

111. The method of any one of claims 62-110, wherein the composition comprises a pharmaceutically acceptable carrier.

112. The method of any one of claims 67-111, wherein the subject is a human.

113. The method of any one of claims 67-112, wherein the administration of the composition to the subject is by intravenous administration.

114. A method of restoring the function of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene in a subject with cystic fibrosis, the method comprising

administering to the subject a composition comprising a lipid nanoparticle (LNP), wherein the LNP comprises (i) a first nucleic acid encoding a base editor; and (ii) a second nucleic acid encoding a guide RNA.

115. The method of claim 114, wherein about 5% to about 95% of the function of the *CFTR* gene is restored.

116. The method of claim 114 or 115, wherein the restoring of the function of the *CFTR* gene is determined by the increase of CFTR protein expression.

117. The method of claim 116, wherein expression of the CFTR protein is determined by one or more bioassays comprising sweat chloride concentration assay, β-adrenergic sweat assay, and nasal potential difference assay.

118. The method of claim 116 or 117 wherein expression of the CFTR protein is determined by analysis of chloride levels in the sweat of the subject.

119. The method of claim 118, wherein chloride levels in the sweat of the subject after being administered the composition are decreased as compared to levels in a subject before being administered the composition.

120. The method of claim 116, wherein the expression is measured using western blotting, immunoprecipitation, and anti-CFTR antibodies.

121. The method of any one of claims 114-120, wherein the nucleic acid encoding the base editor is DNA.

122. The method of any one of claims 114-120, wherein the nucleic acid encoding the base editor is RNA.

123. The method of any one of claims 114-122, wherein the base editor is an adenine base editor (ABE).

124. The method of claim 123, wherein the base editor is ABE8e.

125. The method of claim 123, wherein the ABE comprises a tRNA adenosine deaminase (TadA) protein, or a variant thereof, fused to a catalytically impaired Cas protein capable of binding to a specific nucleotide sequence.

126. The method of any one of claims 114-122, wherein the base editor is a cytosine base editor (CBE).

127. The method of claim 123, wherein the CBE comprises a cytidine deaminase protein, or a variant thereof, fused to a catalytically impaired Cas protein capable of binding to a specific nucleotide sequence.

128. The method of claim 123-127, wherein the ABE or the CBE further comprises a Cas9 enzyme that does not have any nuclease activity.

129. The method of any one of claims 114-127, wherein the ratio of the first nucleic acid to the second nucleic acid is 1:1 on a molecule:molecule basis.

130. The method of any one of claims 114-127, wherein the ratio of the first nucleic acid to the second nucleic acid is 1:1 on a weight basis.

131. The method of any one of claims 114-130, wherein the ratio of the first nucleic acid to the second nucleic acid is 2:1 molecule:molecule basis.

132. The method of any one of claims 114-130, wherein the ratio of the first nucleic acid to the second nucleic acid is 2:1 on a weight basis.

133. The method of any one of claims 114-132, wherein the LNP comprises an ionizable cationic lipid, a zwitterionic phospholipid, a cholesterol, and a PEG lipid.

134. The method of any one of claims 114-133, wherein the LNP comprises 5A2-SC8, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), cholesterol, DMG-PEG, and one or more selective organ targeting (SORT) molecules.

135. The method of claim 133, wherein the one or more SORT molecules comprises permanently positively charged moiety.

136. The method of claim 134 or 135, wherein the one or more SORT molecule is selected from the group consisting of 18:1 DOTMA (DOTMA); DORI, DC-6-14; 12:0 EPC (Chloride Salt); 14:0 EPC (Chloride Salt); 16:0 EPC (Chloride Salt); 18:0 EPC (Chloride Salt); 18:1 EPC (Chloride Salt); 16:0-18:1 EPC (Chloride Salt); 14:1 EPC (Triflate Salt); 18:0 DDAB (Dimethyldioctadecylammonium (Bromide Salt)); 14:0 TAP; 16:0 TAP; 18:0 TAP; 18:1 TAP (DOTAP); 18:1 TAP (DOTAP, MS Salt); 18:1 DODAP, or 18:1 PA (1,2-dioleoyl-sn-glycero-3-phosphate (sodium salt)) (18PA).

137. The method of any of claims 134-136, wherein the one or more SORT molecules comprises DOTAP (1,2-dioleoyl-3-trimethylammonium propane).

138. The method of any of claims 134-136, wherein the one or more SORT molecules comprises 18PA.

139. The method of any of claims 134-136, wherein the one or more SORT molecules comprises DODAP.

140. The method of claim 139, wherein the DODAP comprises about 20% molar ratio of the total lipids.

141. The method of claim 137, wherein the DOTAP comprises about 50% molar ratio of the total lipids.

142. The method of claim 138, wherein the 18PA comprises about 10% molar ratio of the total lipids.

143. The method of any of claims 134-136, wherein the one or more SORT molecules comprises DOTMA.

144. The method of claim 134, 135, or 136, wherein the LNP comprises a ratio of DOPE:DOTMA of between 3:1 and 1:3.

145. The method of claim 144, wherein the ratio of DOPE:DOTMA is about 3:1.

146. The method of claim 144, wherein the ratio of DOPE:DOTMA is about 1:1.

147. The method of any of claims 115-146, wherein the one or more SORT molecules comprises from about 5% to about 60% molar percentage of the LNP.

148. The method of any of claims 134-146, wherein the one or more SORT molecules comprises about 40% molar percentage of the LNP.

149. The method of any of claims 134-146, wherein the SORT molecule comprises from 50% molar percentage of the LNP.

150. The method of any of claims 115-149, wherein the LNP binds vitronectin.

151. The method of any one of claims 115-150, wherein the guide RNA comprises a circular RNA.

152. The method of any one of claims 115-150, wherein the guide RNA comprises a linear RNA.

153. The method of any one of claims 115-150, wherein the guide RNA is a single guide RNA (sgRNA).

154. The method of any of claims 115-153, wherein the guide RNA comprises a target sequence that is complementary with a target sequence of a cystic fibrosis transmembrane conductance regulator (*CFTR*) gene.

155. The method of any of claims 112-151, wherein the nucleotide sequence of the guide RNA is AAGTAAAACCTCTACAAATG (SEQ ID NO: 1) or TTGCTCATTGACCTCCACTC (SEQ ID NO: 2).

156. The method of any one of claims 115-155, wherein the composition comprises a pharmaceutically acceptable carrier.

157. The method of any one of claims 115-156, wherein the subject is a human.

158. The method of any one of claims 115-157, wherein the administration of the composition to the subject is by intravenous administration.

159. The method of any of claims 1-158, wherein the LNP is localized to the lungs of the subject.

160. The method of any of claims 1-158, wherein the LNP is capable of delivering the first and second nucleic acids to the lungs of the subject.

161. A lung cell type comprising a modified cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, wherein the modification comprises the replacement of the thymine at 1789 base in exon 11 of the *CFTR* gene with cytosine.

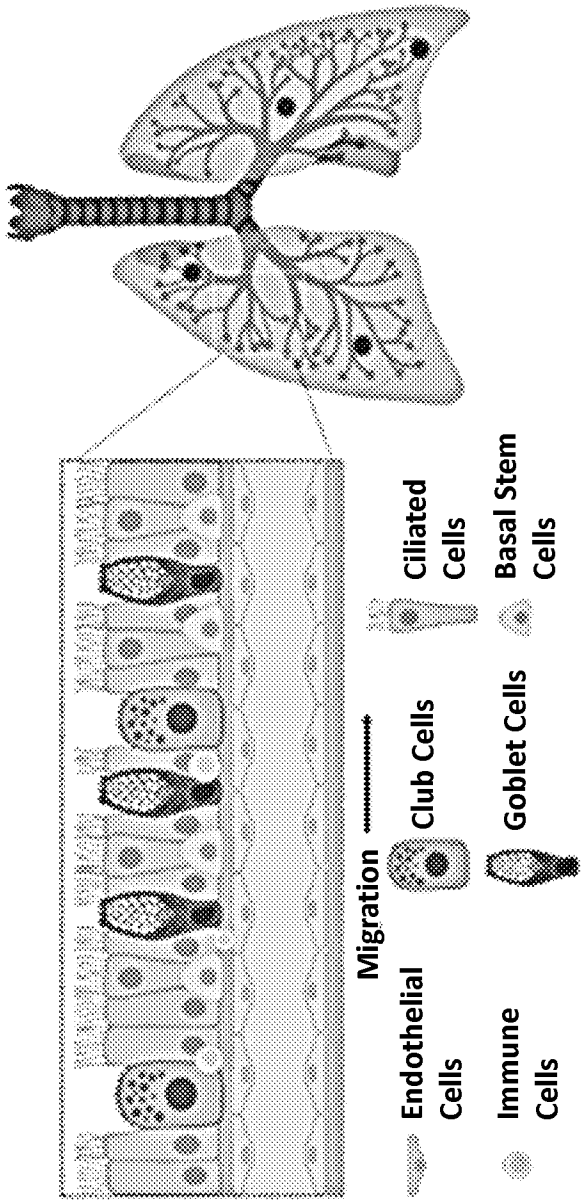

Migration
Endothelial Cells
Immune Cells
Club Cells
Goblet Cells
Ciliated Cells
Basal Stem Cells

FIG. 1A

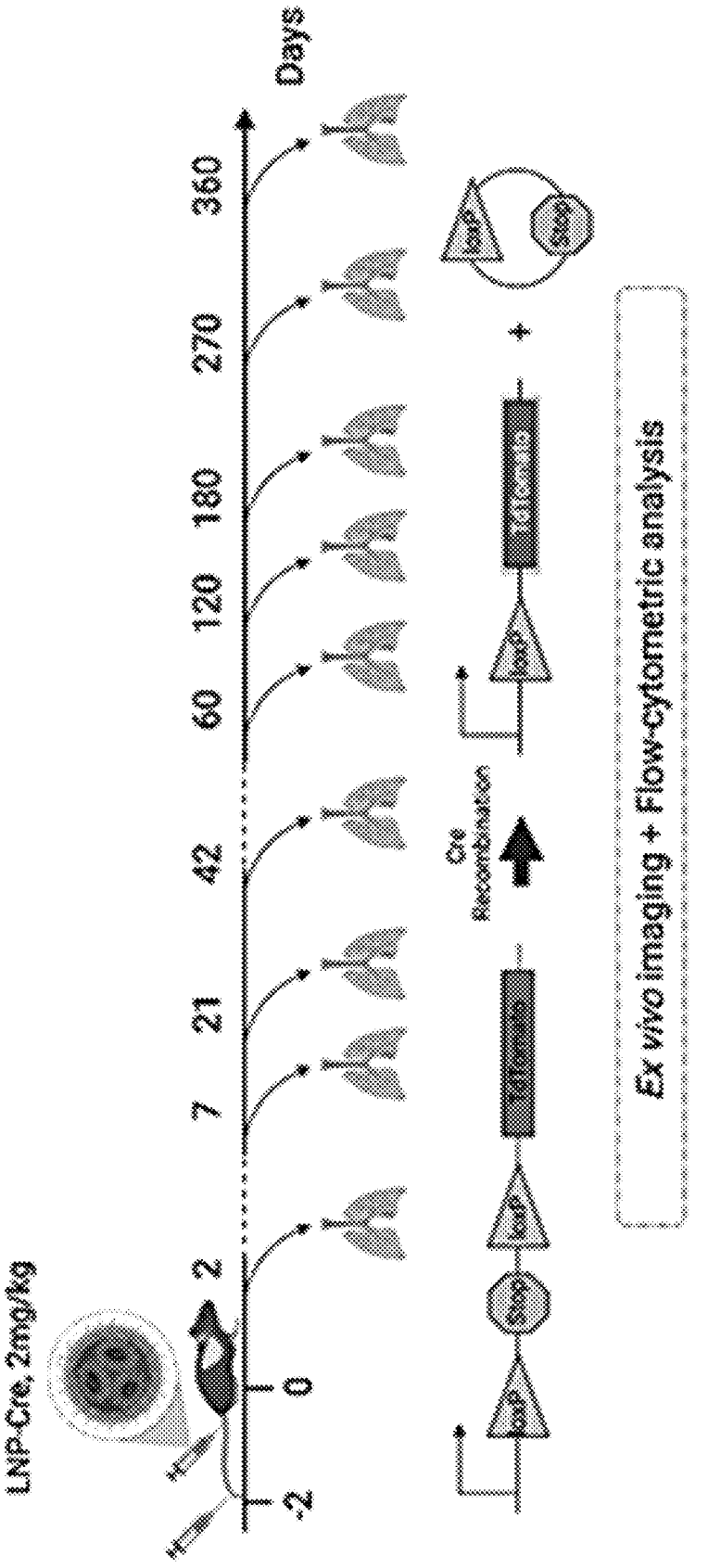

LNP-Cre, 2mg/kg
-2
0
2
7
21
42
60
120
180
270
360
Days
Cre Recombination
Ex vivo imaging + Flow-cytometric analysis

FIG. 1B

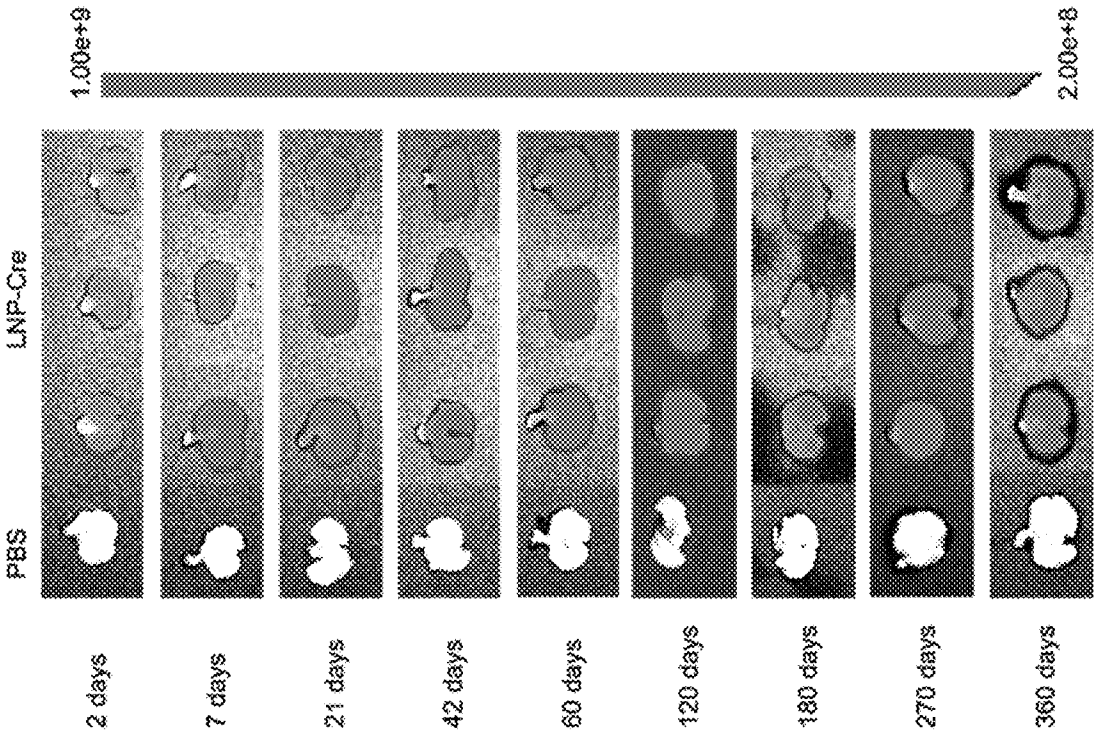

1.00e+9
2.00e+8
PBS
LNP-Cre
2 days
7 days
21 days
42 days
60 days
120 days
180 days
270 days
360 days

FIG. 1C

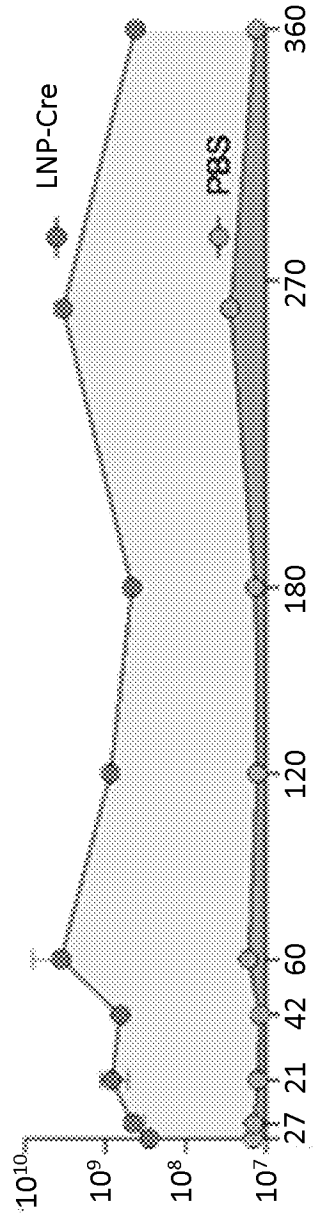

LNP-Cre
PBS
Average Radiance
[p/s/cm²/sr]
10¹⁰
10⁹
10⁸
10⁷
27
21
42
60
120
180
270
360
Days after injection

FIG. 1D

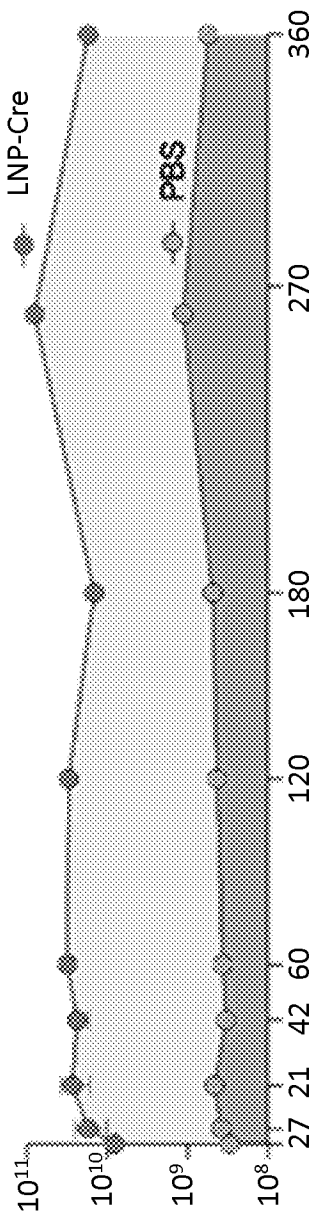

LNP-Cre
PBS
Total Flux
[p/s]
10¹¹
10¹⁰
10⁹
10⁸
27
21
42
60
120
180
270
360
Days after injection

FIG. 1E

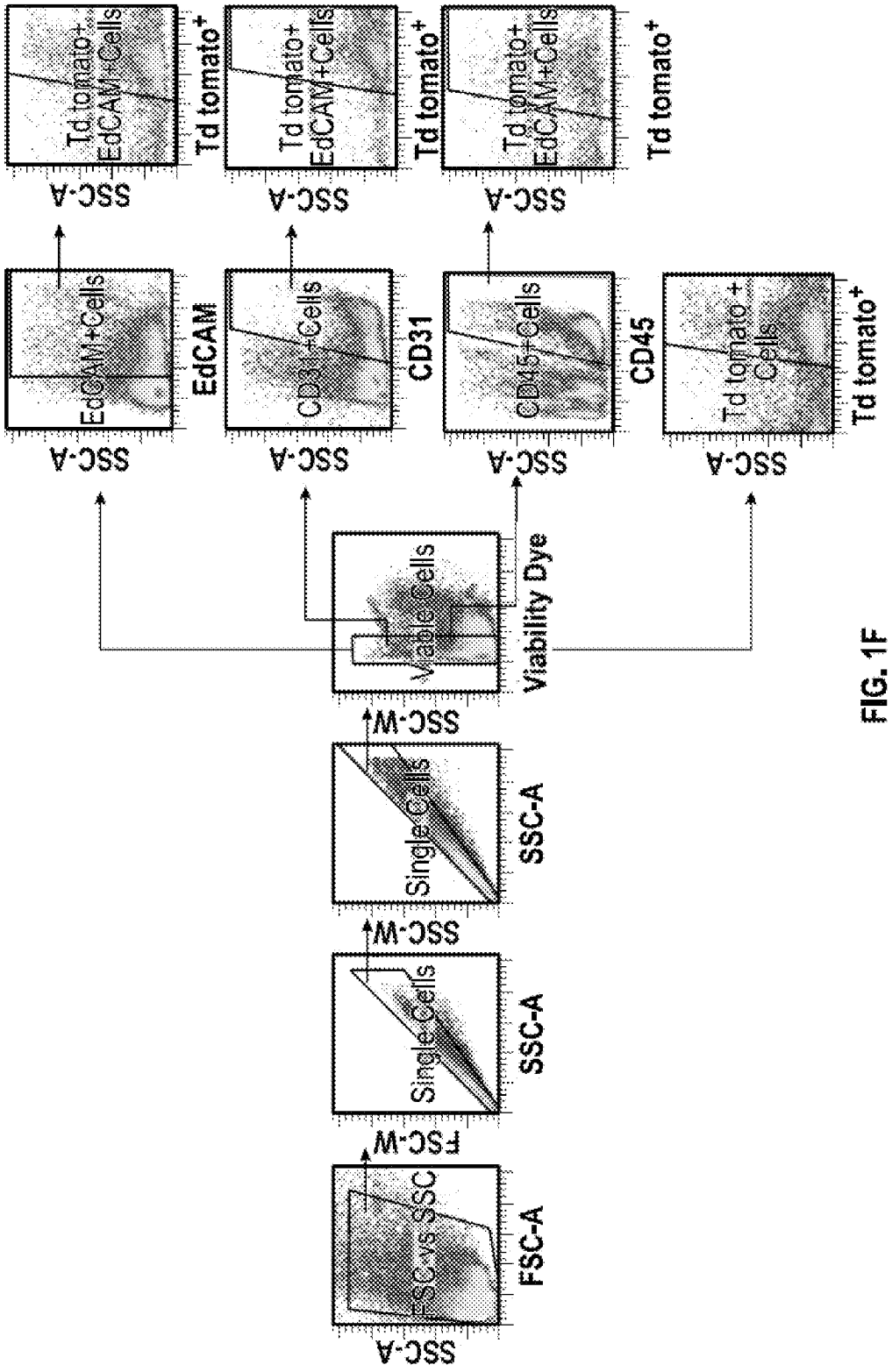

FSC-A
SSC-A
FSC vs SSC
FSC-W
SSC-A
Single Cells
SSC-W
SSC-A
Single Cells
SSC-W
Viability Dye
Viable Cells
SSC-A
EdCAM
EdCAM+Cells
SSC-A
CD31
CD31+Cells
SSC-A
CD45
CD45+Cells
SSC-A
Td tomato+
Td tomato+ Cells
SSC-A
Td tomato+
Td tomato+ EdCAM+Cells
SSC-A
Td tomato+
Td tomato+ EdCAM+Cells
SSC-A
Td tomato+
Td tomato+ EdCAM+Cells
SSC-A

FIG. 1F

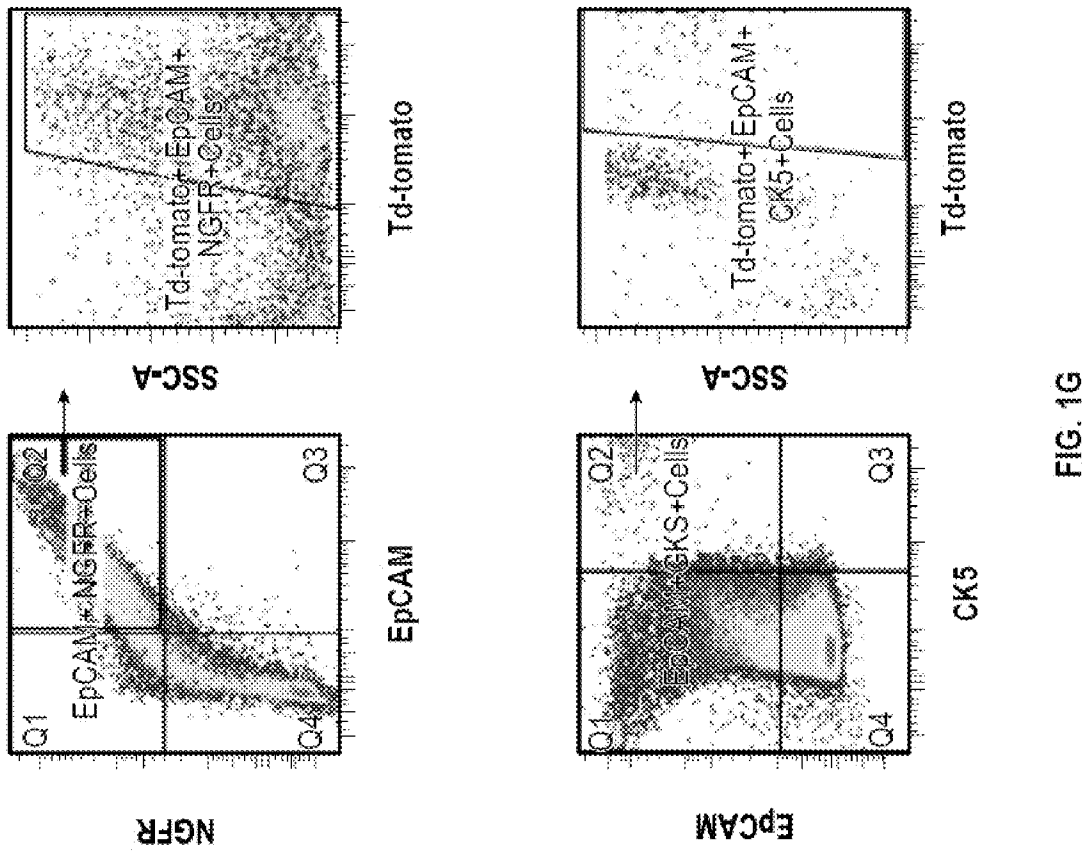

NGFR
EpCAM
Q1
Q2
Q3
Q4
EpCAM+NGFR+Cells
SSC-A
Td-tomato
Td-tomato+EpCAM+
NGFR+Cells
EpCAM
CK5
EpCAM+CK5+Cells
Td-tomato+EpCAM+
CK5+Cells

FIG. 1G

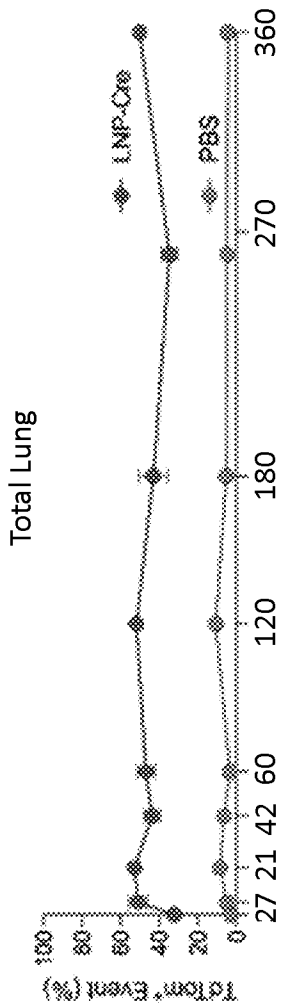


FIG. 1H

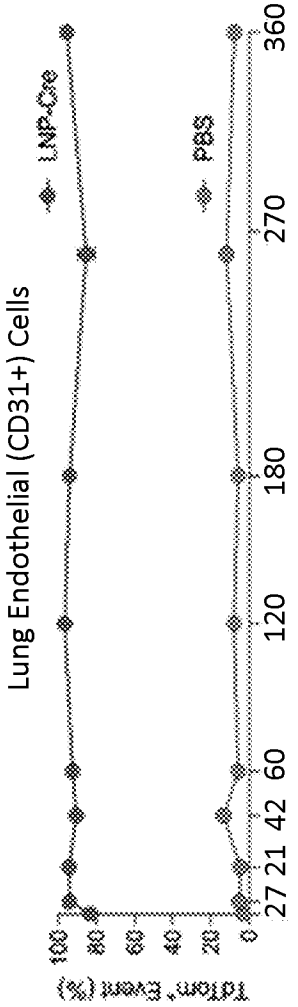


FIG. 1I

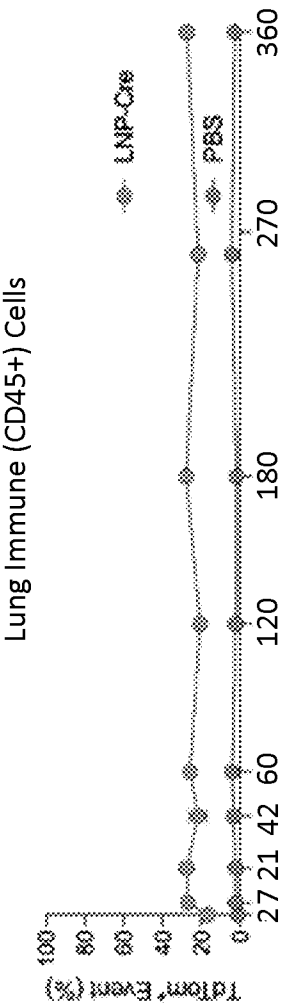


FIG. 1J

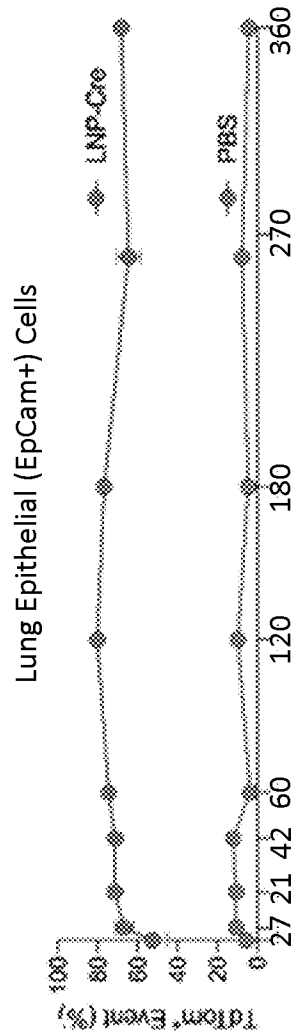


FIG. 1K

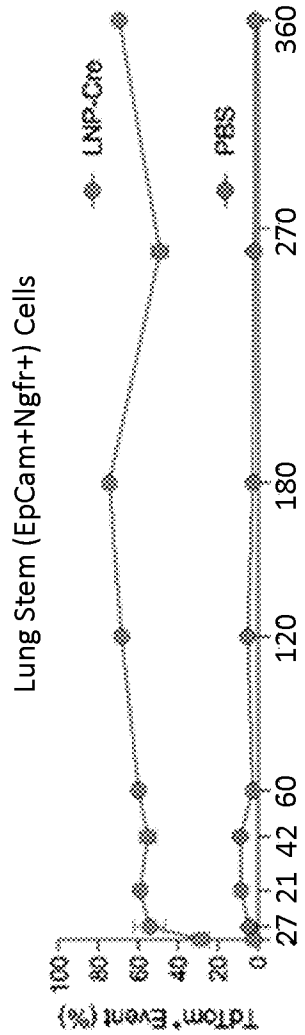


FIG. 1L

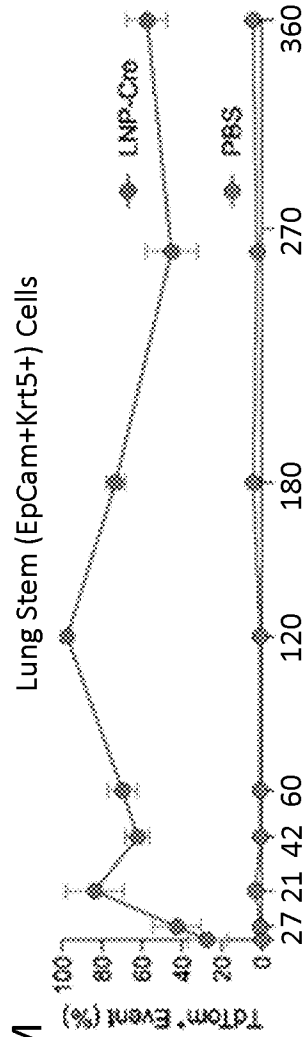


FIG. 1M

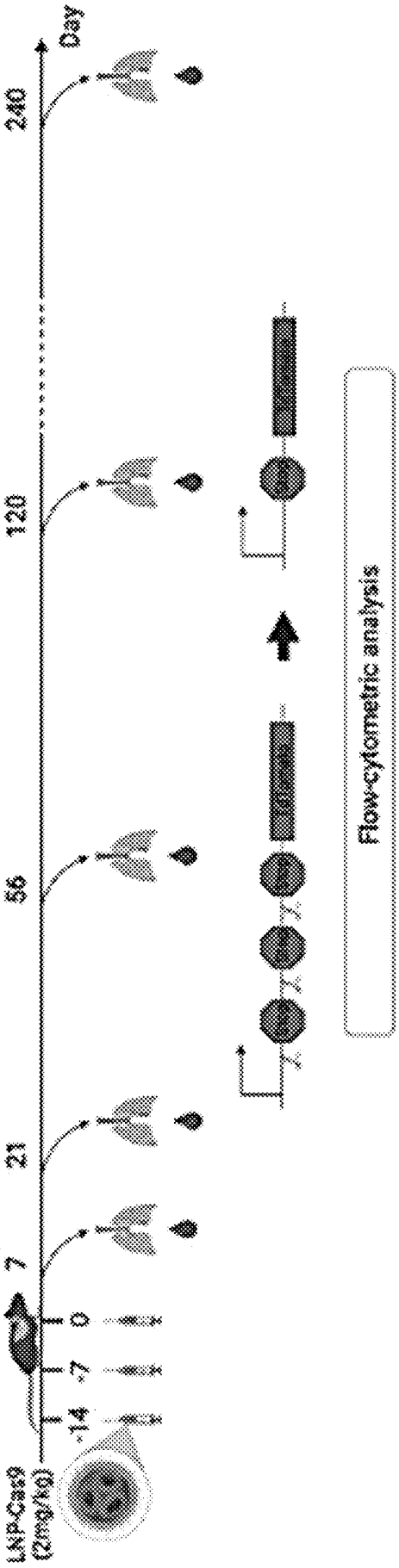

Day
240
120
56
21
7
0
-7
-14
LNP-Cas9
(2mg/kg)
Flow-cytometric analysis

FIG. 2A

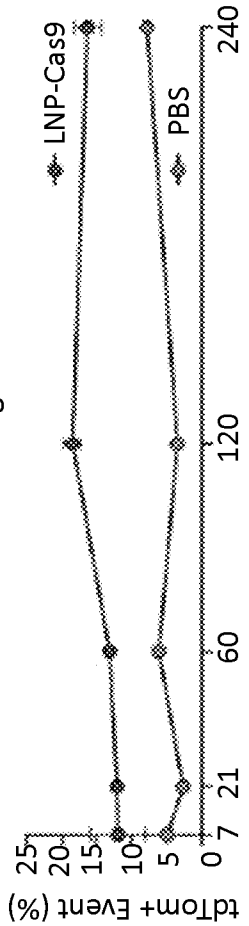


FIG. 2B

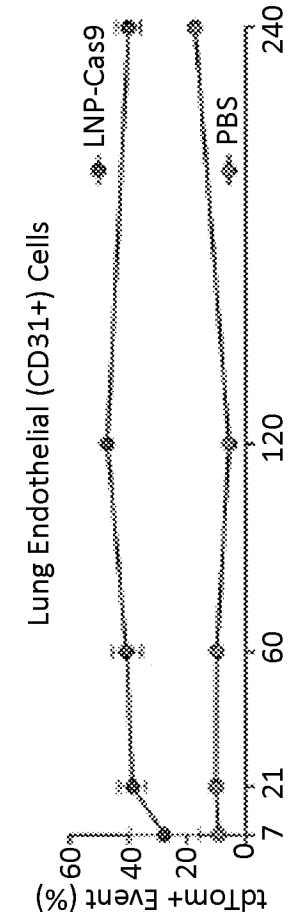


FIG. 2C

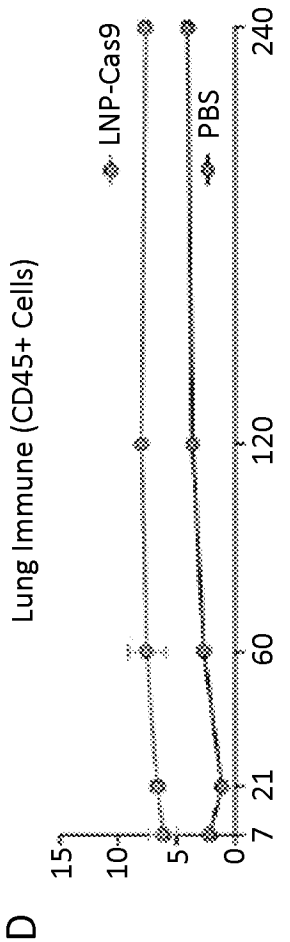


FIG. 2D

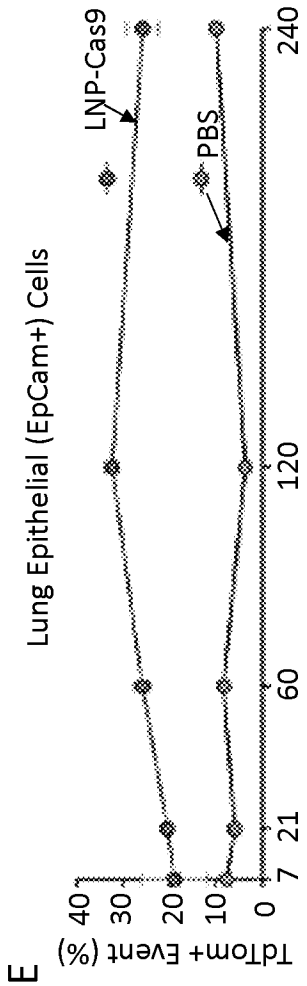


FIG. 2E

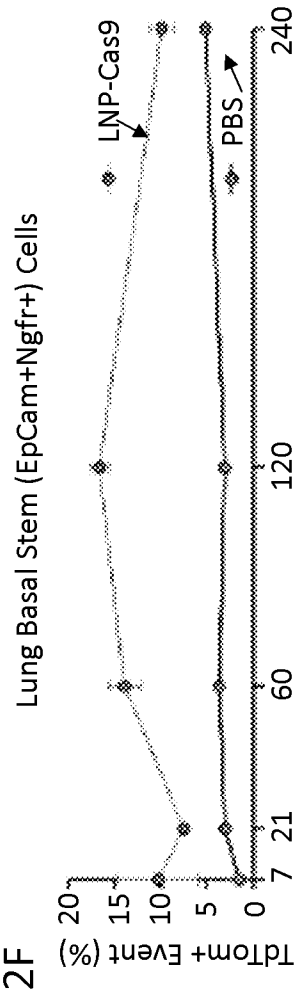


FIG. 2F

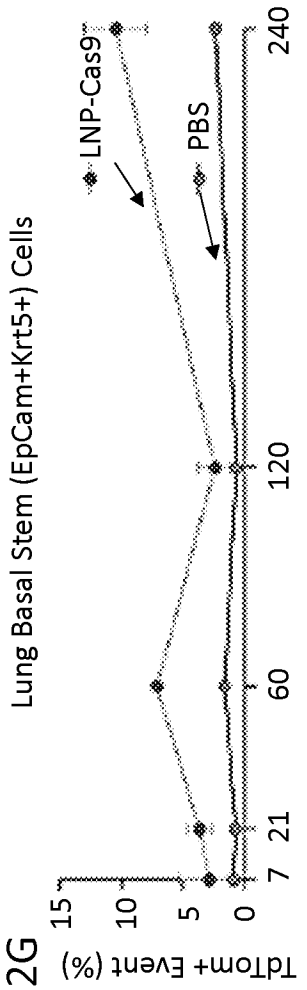


FIG. 2G

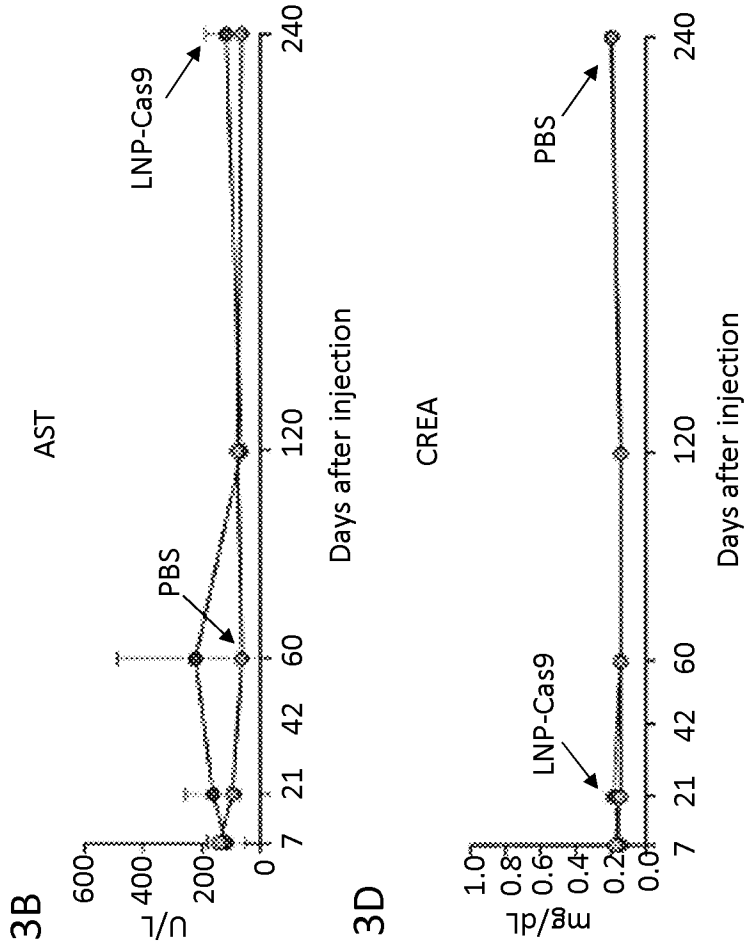


FIG. 3B

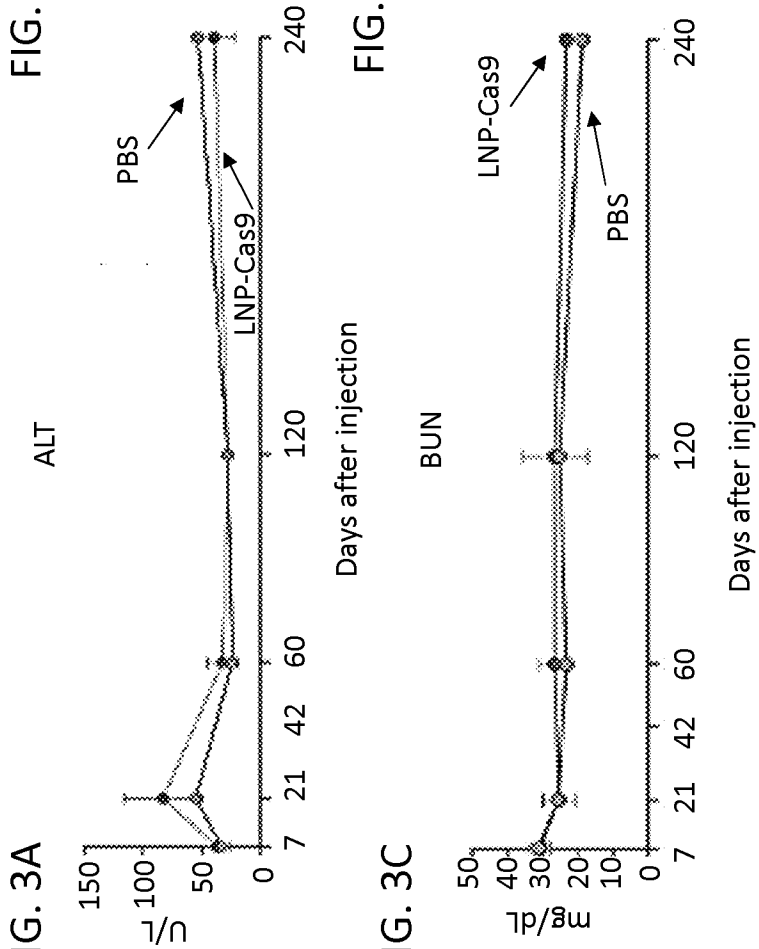


FIG. 3A

FIG. 3D

FIG. 3C

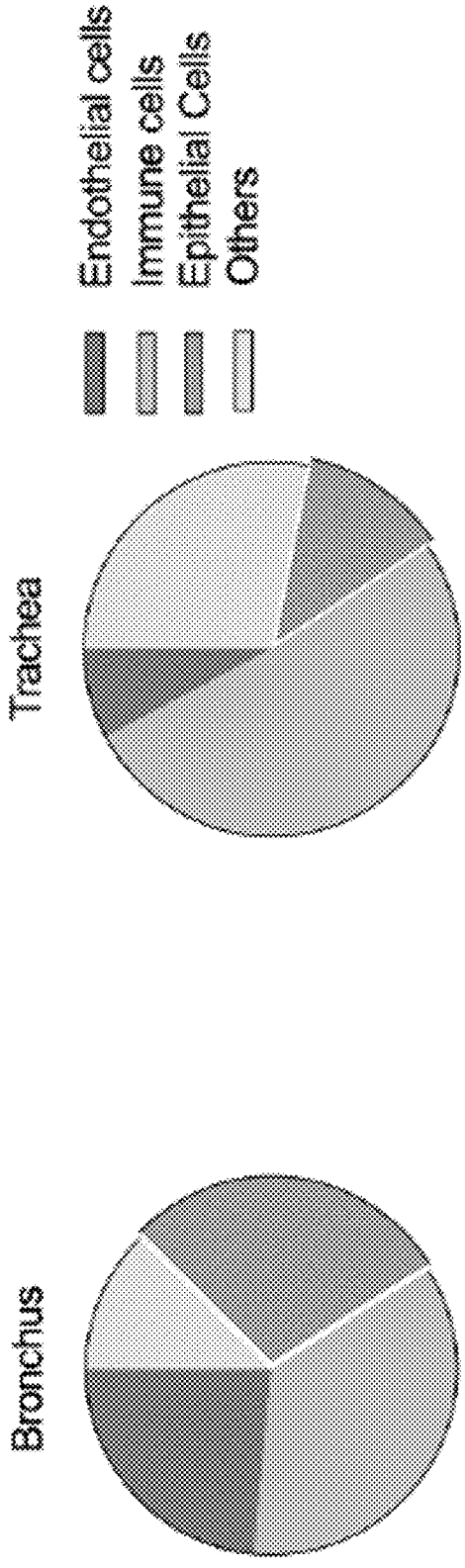

Endothelial cells
Immune cells
Epithelial Cells
Others
Trachea
Bronchus

FIG. 4A

FIG. 4B

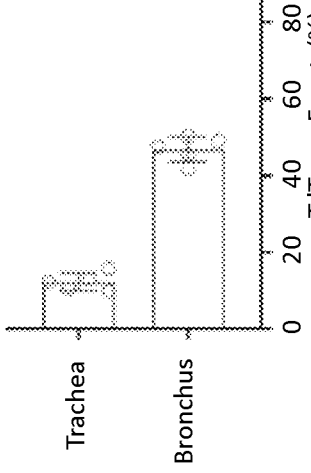


FIG. 4C

FIG. 4D

FIG. 4E

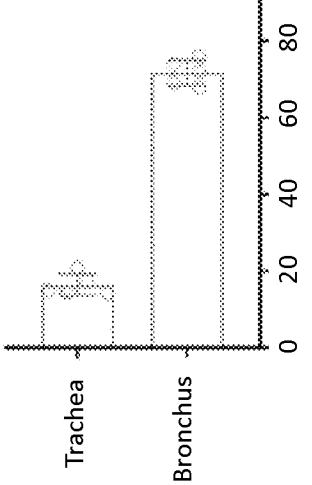


FIG. 4F

FIG. 4G

FIG. 5A

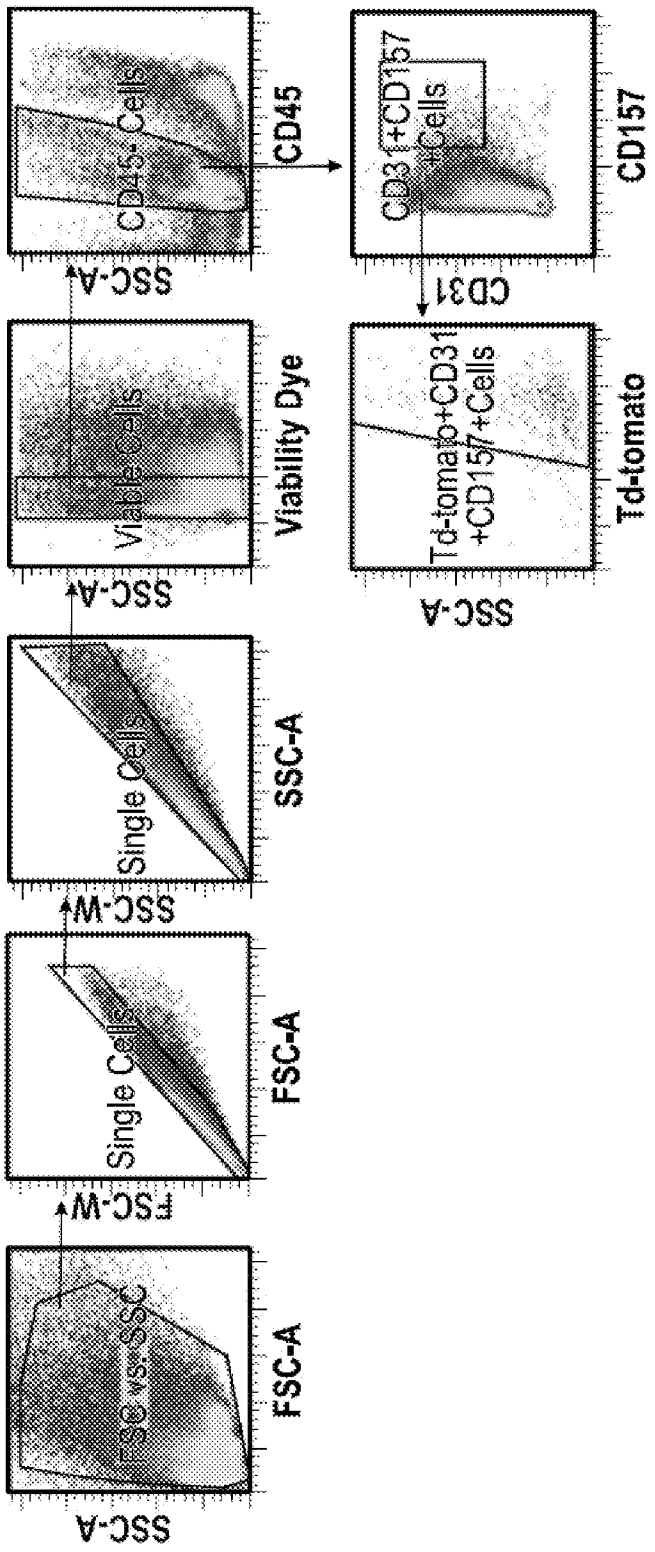

FSC-A
SSC-A
FSC vs. SSC
FSC-W
FSC-A
Single Cells
SSC-W
SSC-A
Single Cells
SSC-A
Viability Dye
Viable Cells
SSC-A
CD45
CD45+ Cells
CD31
CD157
CD31+CD157 +Cells
SSC-A
Td-tomato
Td-tomato+CD31 +CD157+Cells

FIG.5A

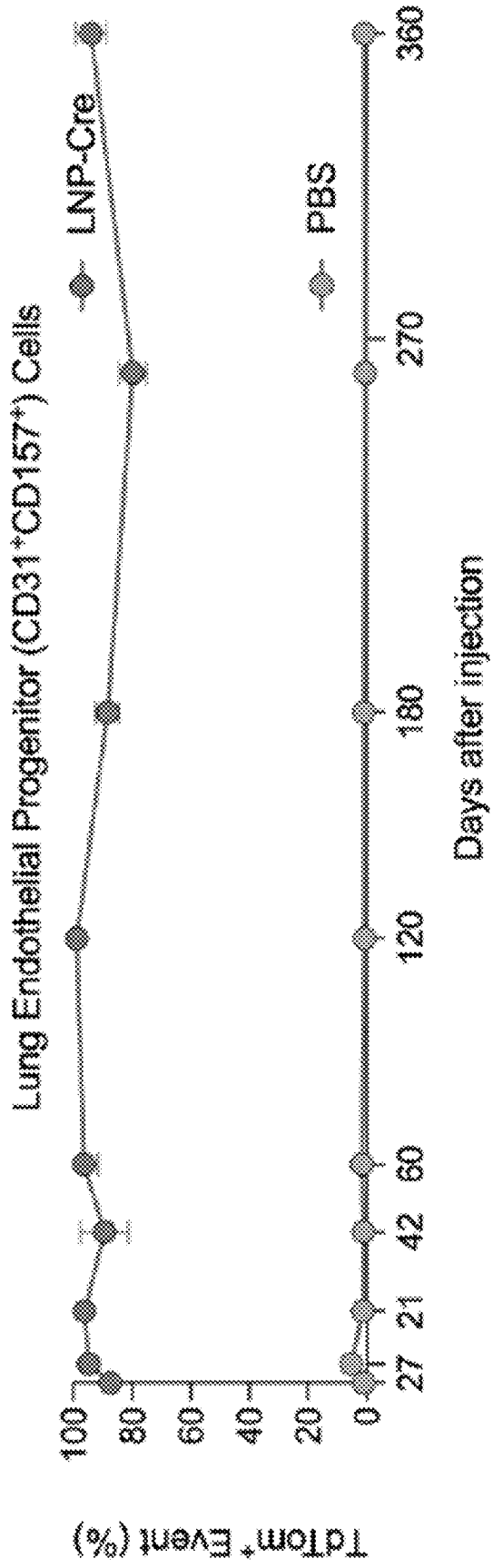

Lung Endothelial Progenitor (CD31+CD157+) Cells
LNP-Cre
PBS
tdTom+ Event (%)
100
80
60
40
20
0
27
21
42
60
120
180
270
360
Days after injection

FIG. 5B

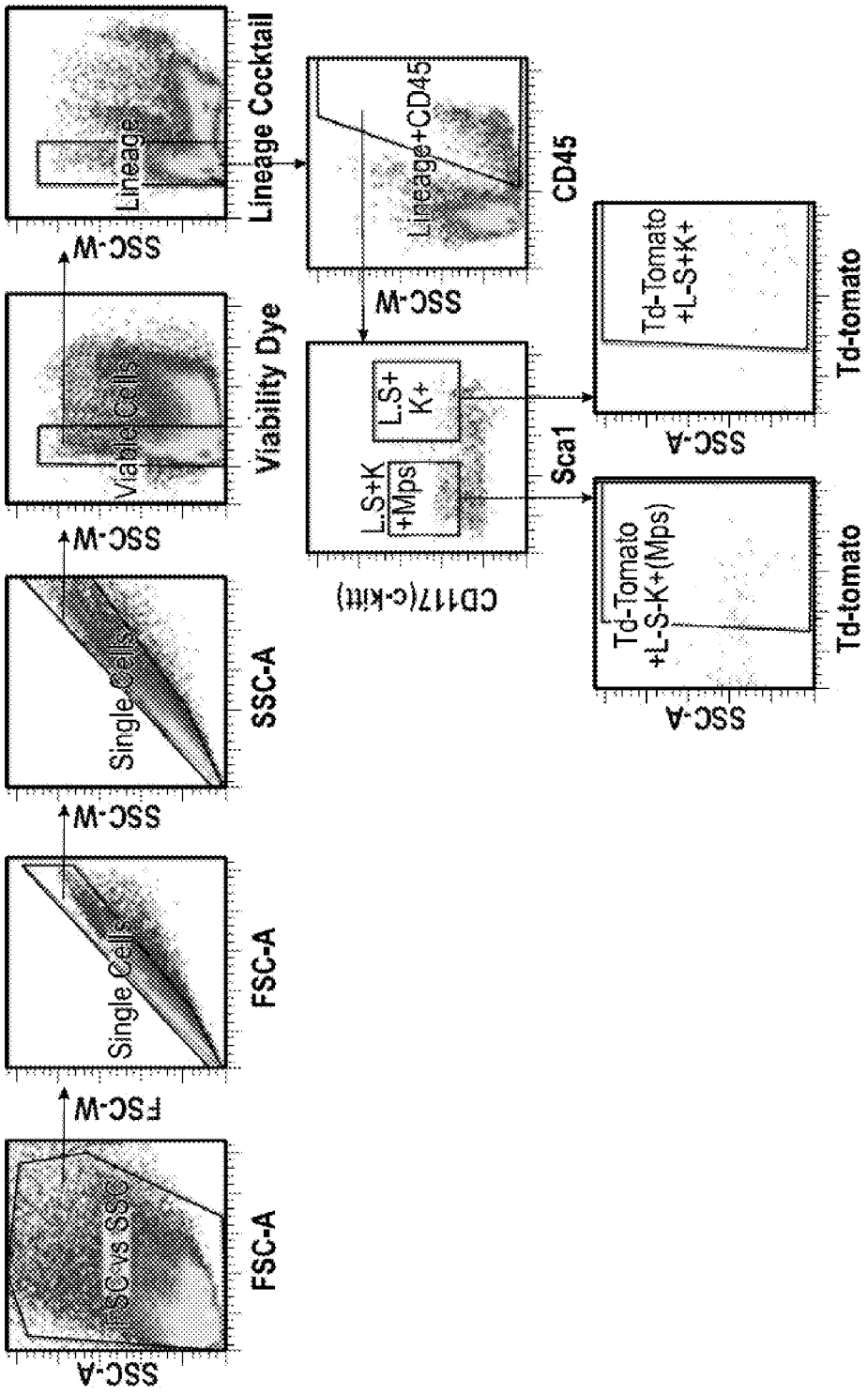

SSC-A
FSC-A
SC vs SSC
FSC-W
FSC-A
Single Cells
SSC-W
SSC-A
Single Cells
SSC-W
Viability Dye
Viable Cells
SSC-W
Lineage Cocktail
Lineage
SSC-W
CD45
Lineage+CD45
CD117(c-kit)
L.S+K +Mps
L.S+ K+
Sca1
SSC-A
Td-Tomato +L-S-K+(Mps)
Td-tomato
SSC-A
Td-Tomato +L-S+K+
Td-tomato

FIG. 6A

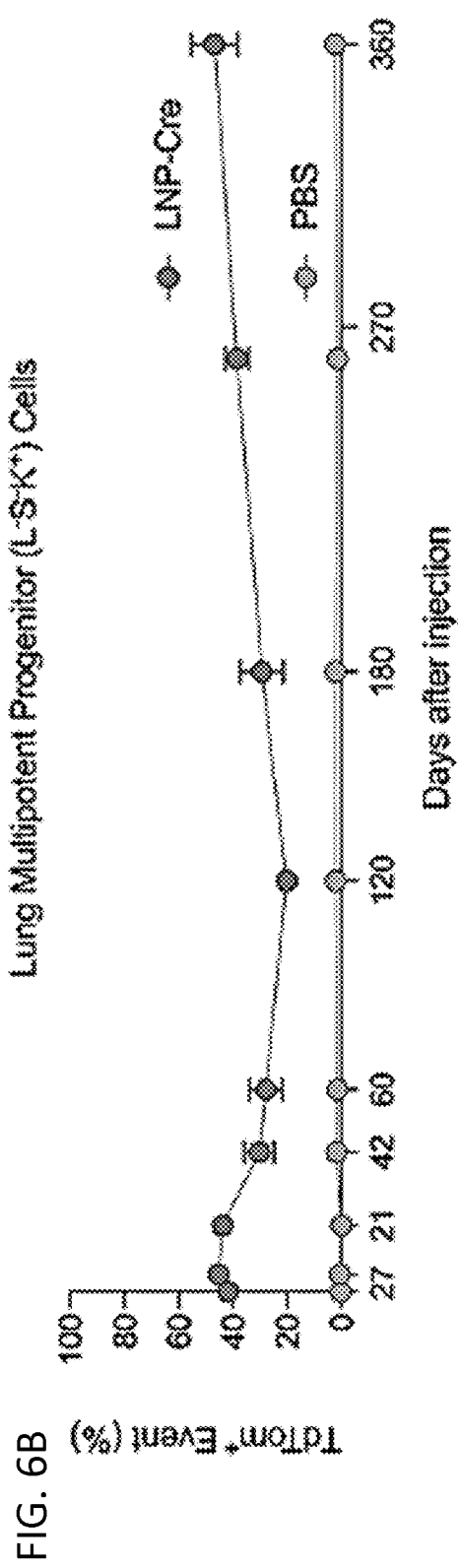

Lung Multipotent Progenitor (L-S+K+) Cells
LNP-Cre
PBS
tdTom+ Event (%)
100
80
60
40
20
0
27
21
42
60
120
180
270
360
Days after injection

FIG. 6B

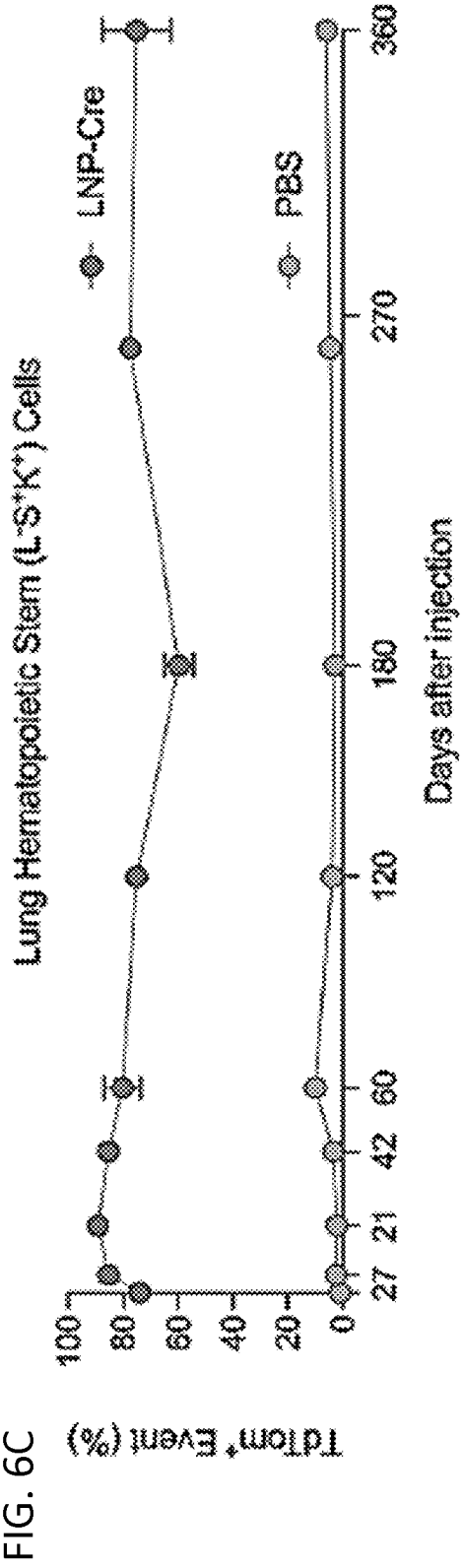

Lung Hematopoietic Stem (L-S+K+) Cells
LNP-Cre
PBS
tdTom+ Event (%)
100
80
60
40
20
0
27
21
42
60
120
180
270
360
Days after injection

FIG. 6C

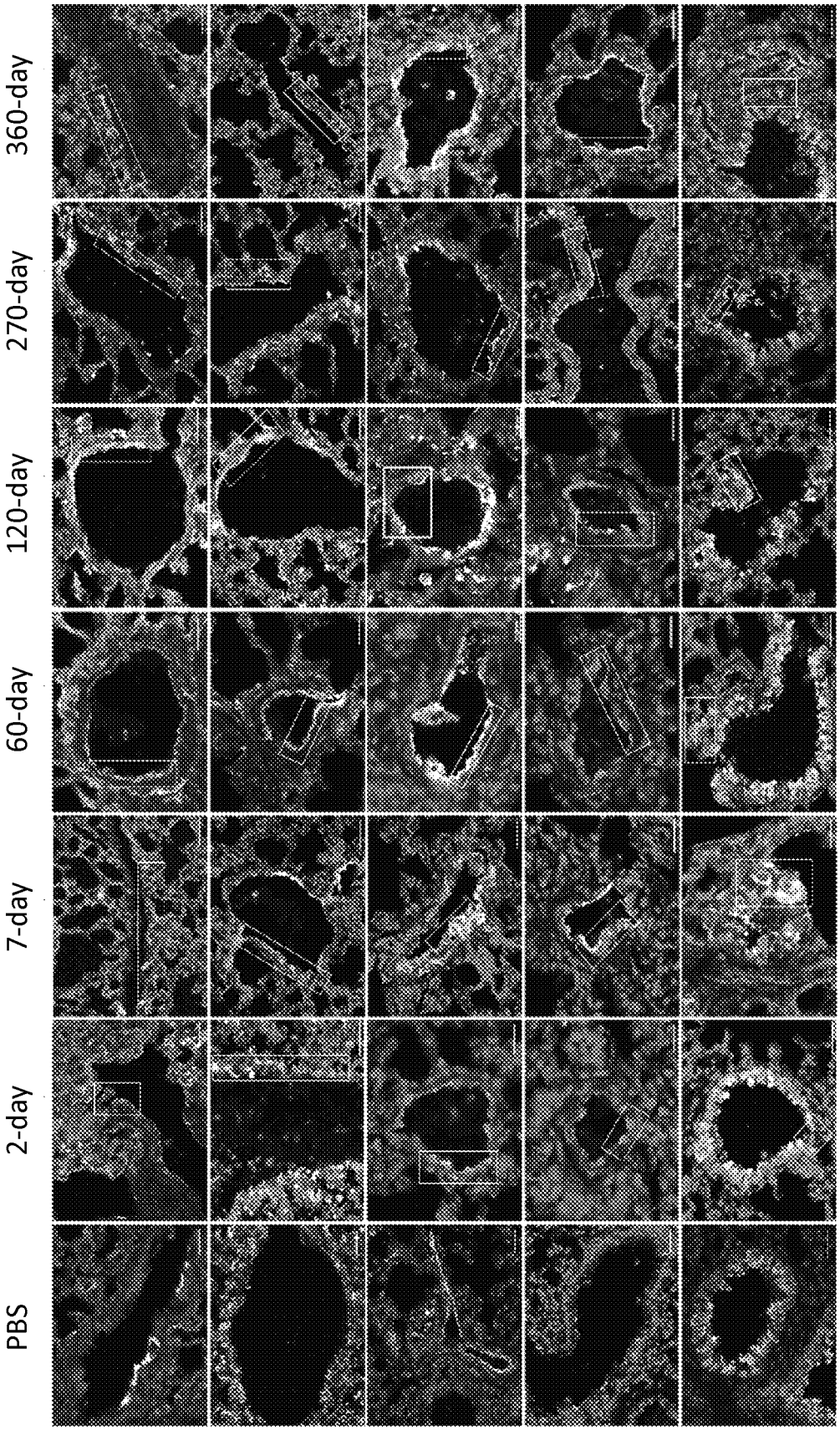

PBS
2-day
7-day
60-day
120-day
270-day
360-day
Club Cells (SCGB1A1)
Ciliated Cells (Tubulin)
Goblet Cells (MUC5AC)
AT2 Cells (ABCA3)
AT1 Cells (HOPX)

FIG. 7A

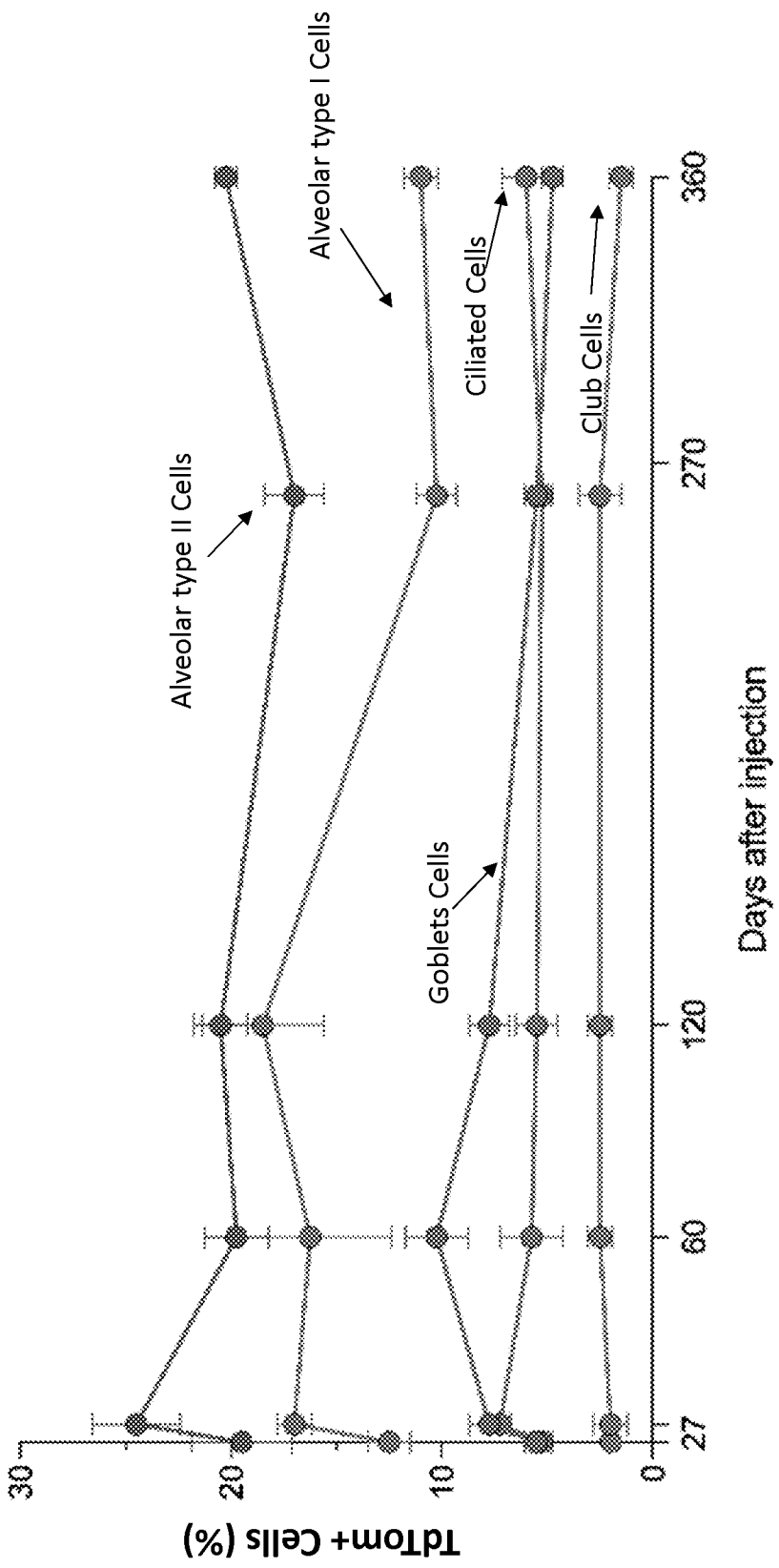

TdTom+ Cells (%)
30
20
10
0
27
60
120
270
360
Days after injection
Alveolar type II Cells
Alveolar type I Cells
Goblets Cells
Ciliated Cells
Club Cells

FIG. 7B

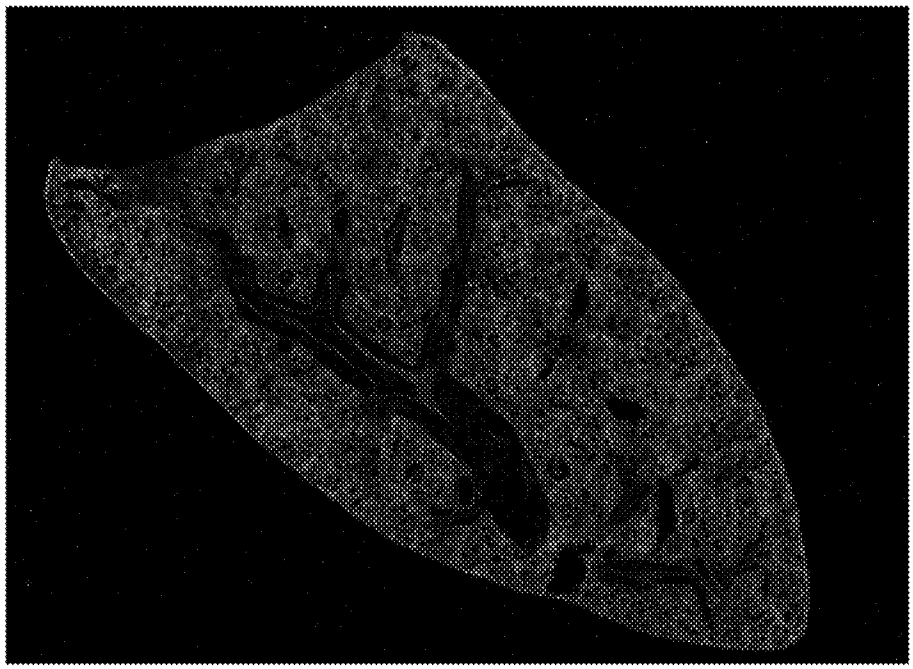


FIG. 7E

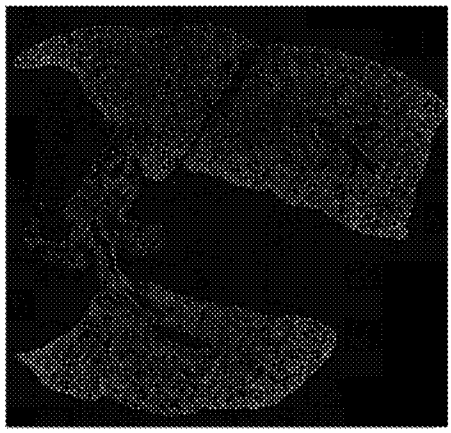


FIG. 7C

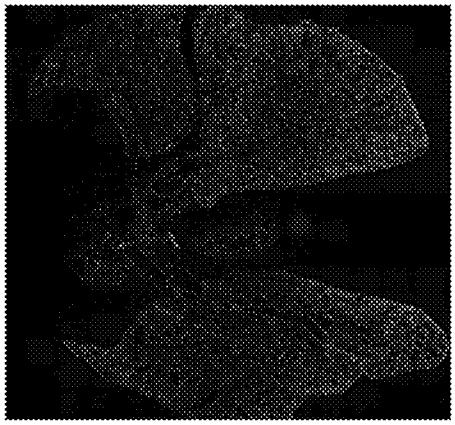


FIG. 7D

FIG. 8A

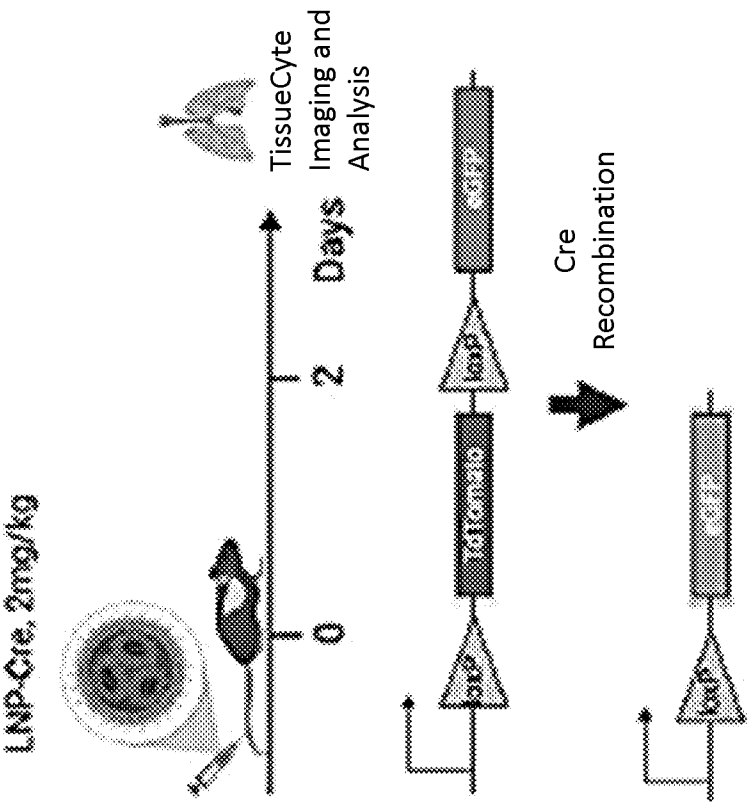
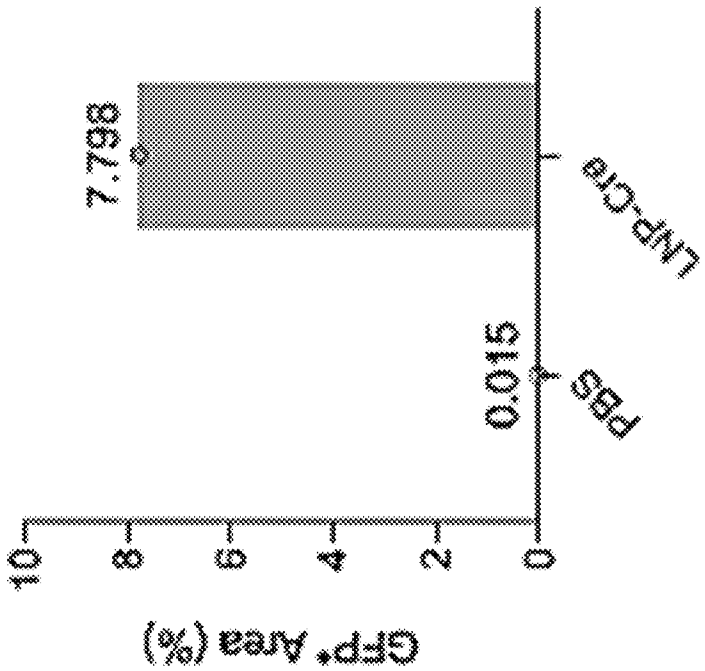

LNP-Cre, 2mg/kg
0
2
Days
TissueCyte Imaging and Analysis
loxP
tdTomato
loxP
Cre Recombination
GFP
loxP

FIG. 8B


GFP+ Area (%)
10
8
6
4
2
0
0.015
PBS
7.798
LNP-Cre

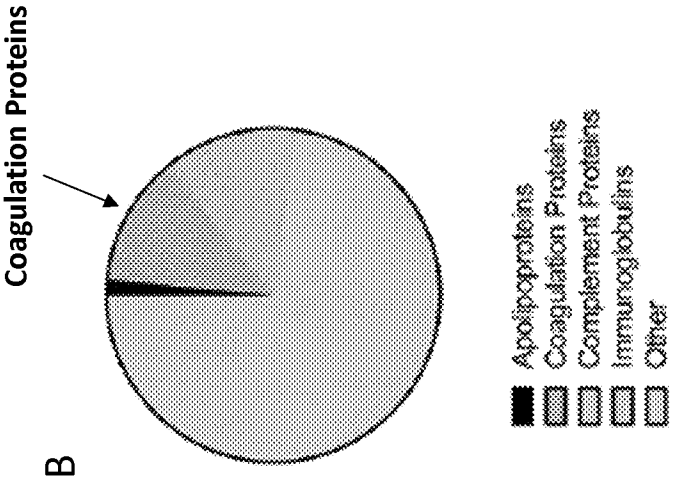

Coagulation Proteins
Apolipoproteins
Coagulation Proteins
Complement Proteins
Immunoglobulins
Other

FIG. 9B

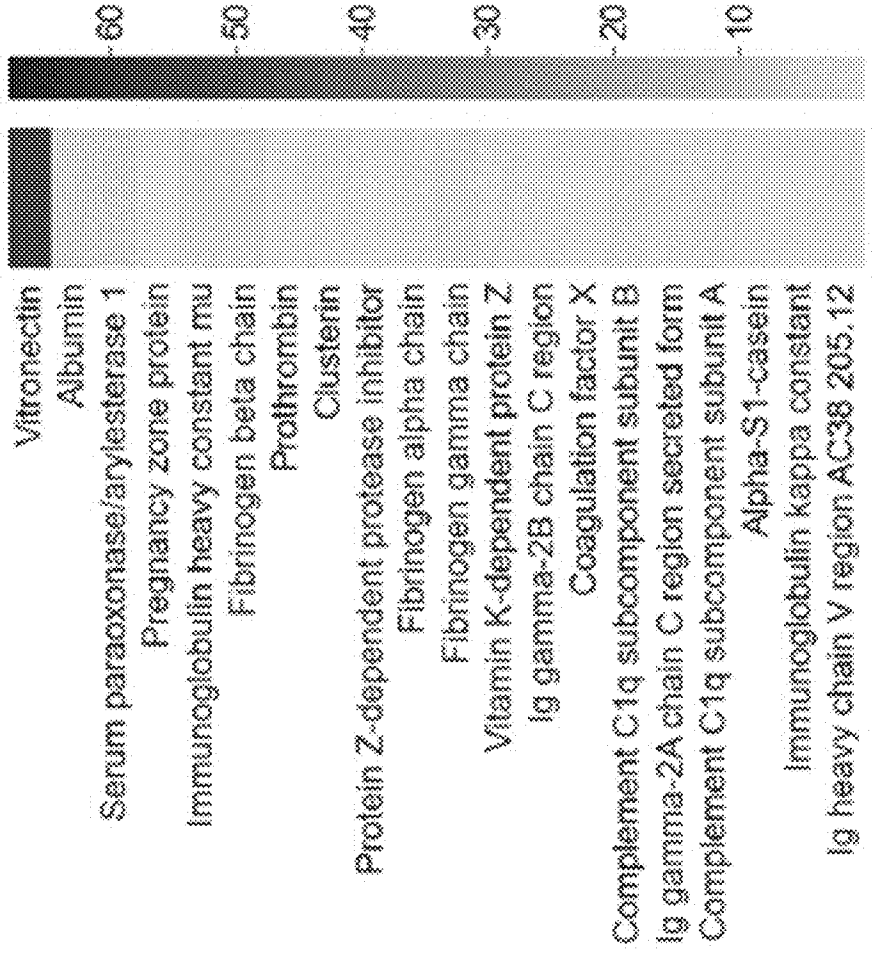

60
50
40
30
20
10
Vitronectin
Albumin
Serum paraoxonase/arylesterase 1
Pregnancy zone protein
Immunoglobulin heavy constant mu
Fibrinogen beta chain
Prothrombin
Clusterin
Protein Z-dependent protease inhibitor
Fibrinogen alpha chain
Fibrinogen gamma chain
Vitamin K-dependent protein Z
Ig gamma-2B chain C region
Coagulation factor X
Complement C1q subcomponent subunit B
Ig gamma-2A chain C region secreted form
Complement C1q subcomponent subunit A
Alpha-S1-casein
Immunoglobulin kappa constant
Ig heavy chain V region AC38 205.12

FIG. 9A

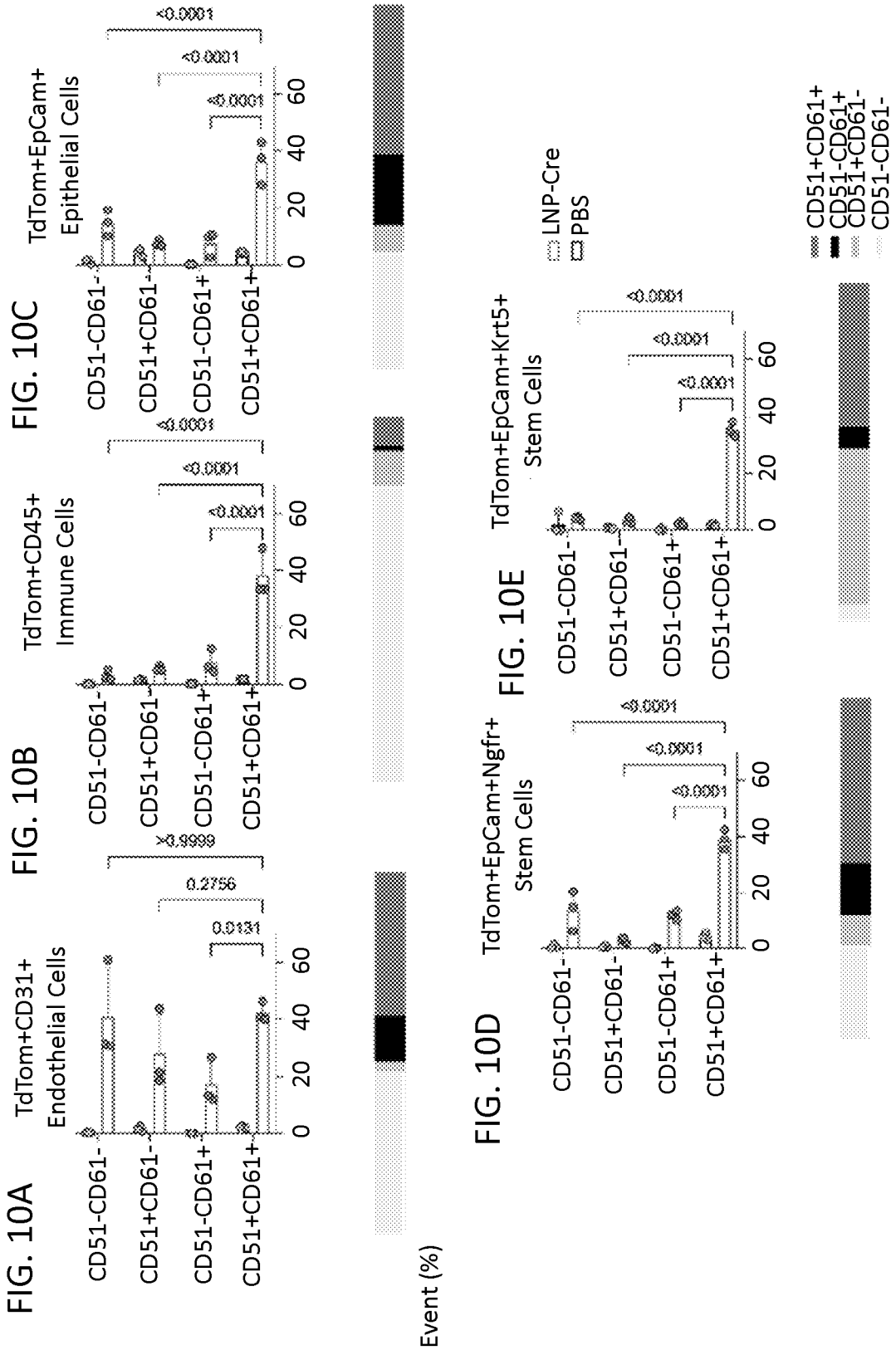


FIG. 10A
TdTom+CD31+ Endothelial Cells
FIG. 10B
TdTom+CD45+ Immune Cells
FIG. 10C
TdTom+EpCam+ Epithelial Cells
FIG. 10D
TdTom+EpCam+Ngfr+ Stem Cells
FIG. 10E
TdTom+EpCam+Krt5+ Stem Cells
CD51-CD61-
CD51+CD61-
CD51-CD61+
CD51+CD61+
Event (%)
0
20
40
60
<0.0001
>0.9999
0.2756
0.0131
LNP-Cre
PBS
CD51+CD61+
CD51-CD61+
CD51+CD61-
CD51-CD61-

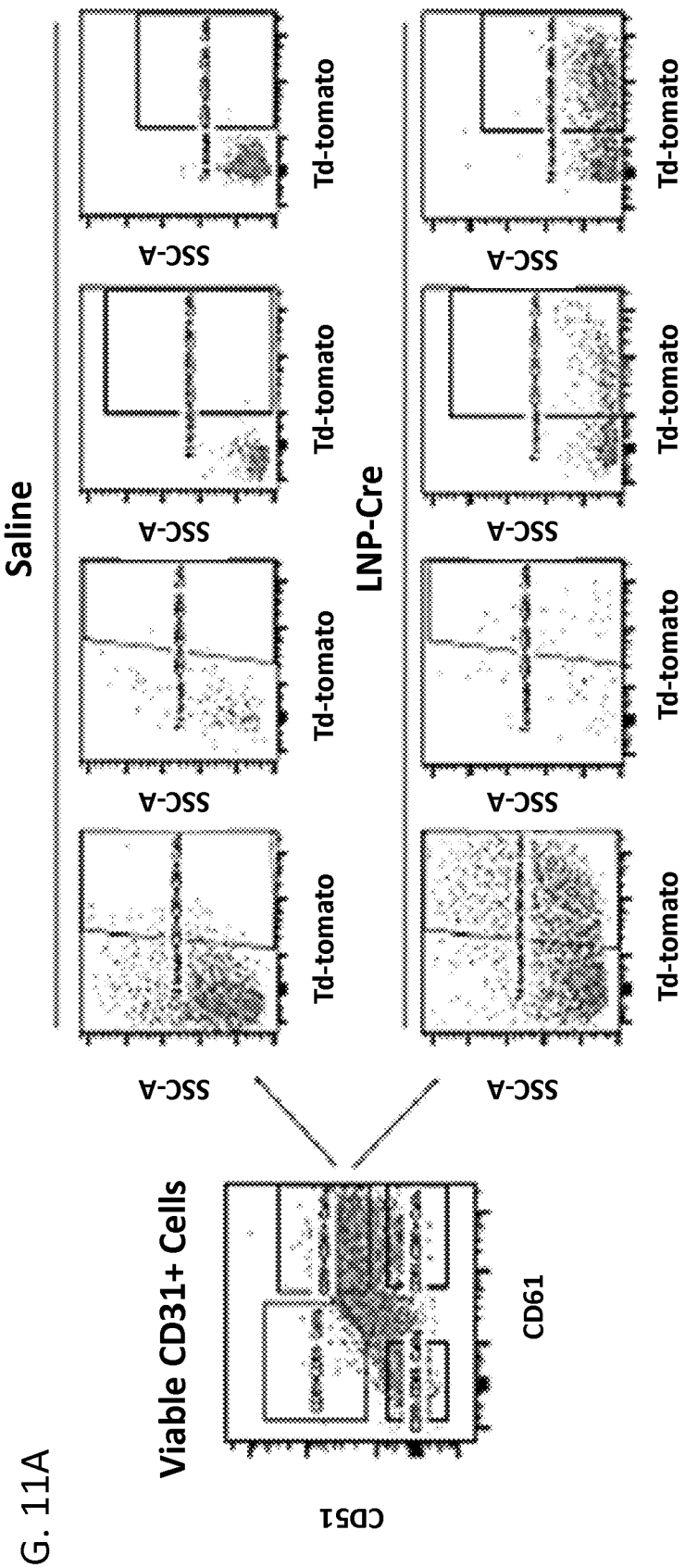


FIG. 11A

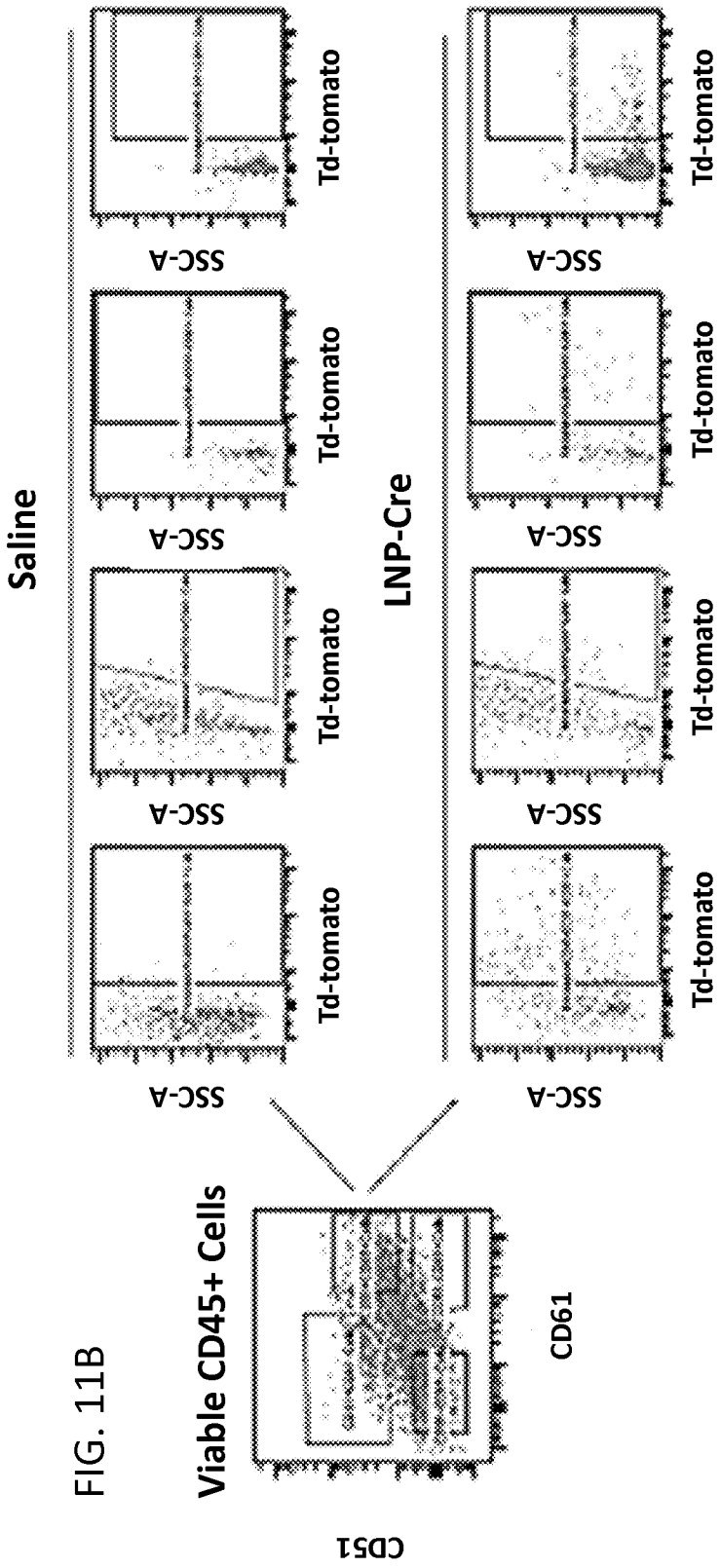


FIG. 11B

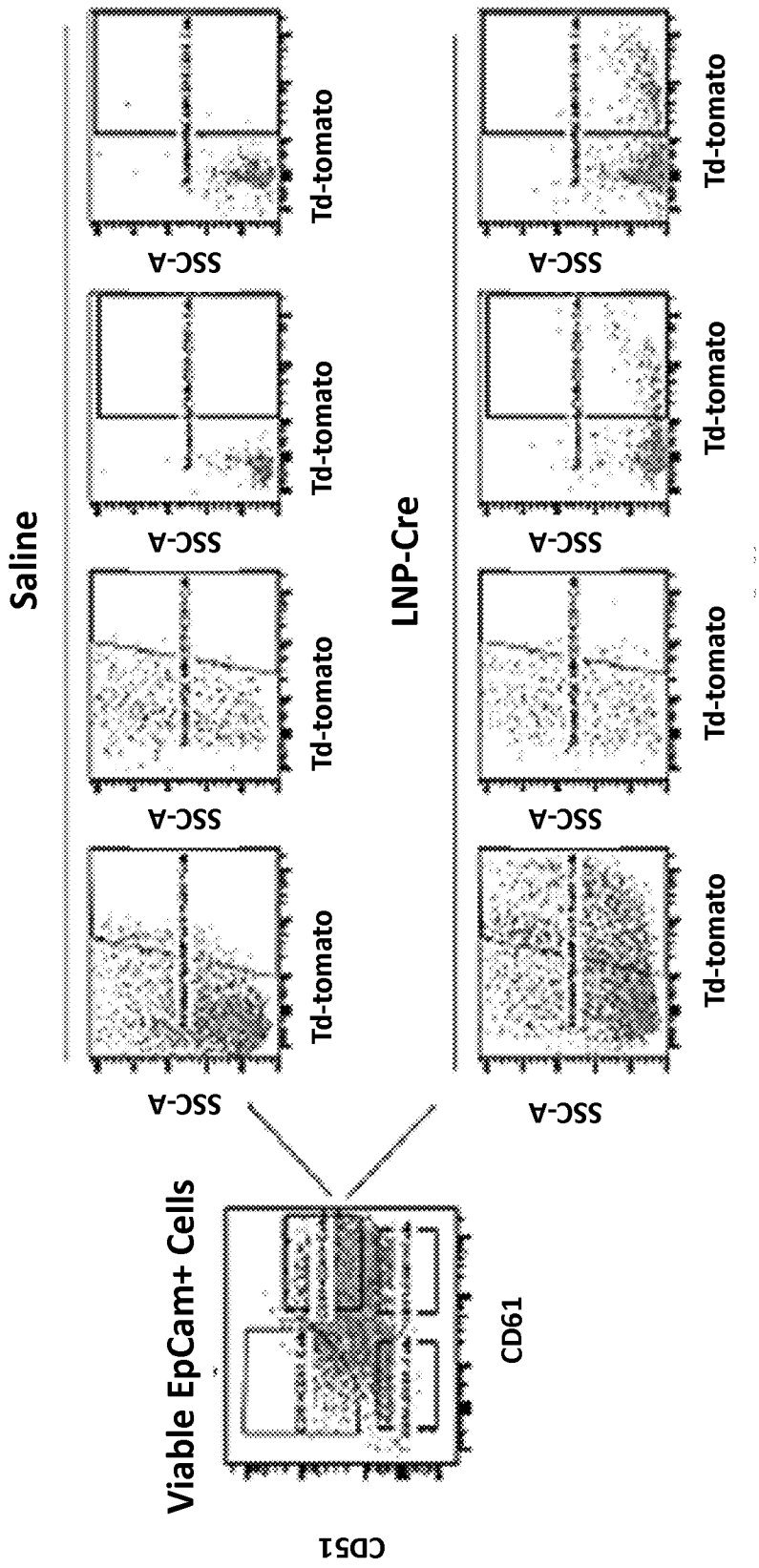


FIG. 11C

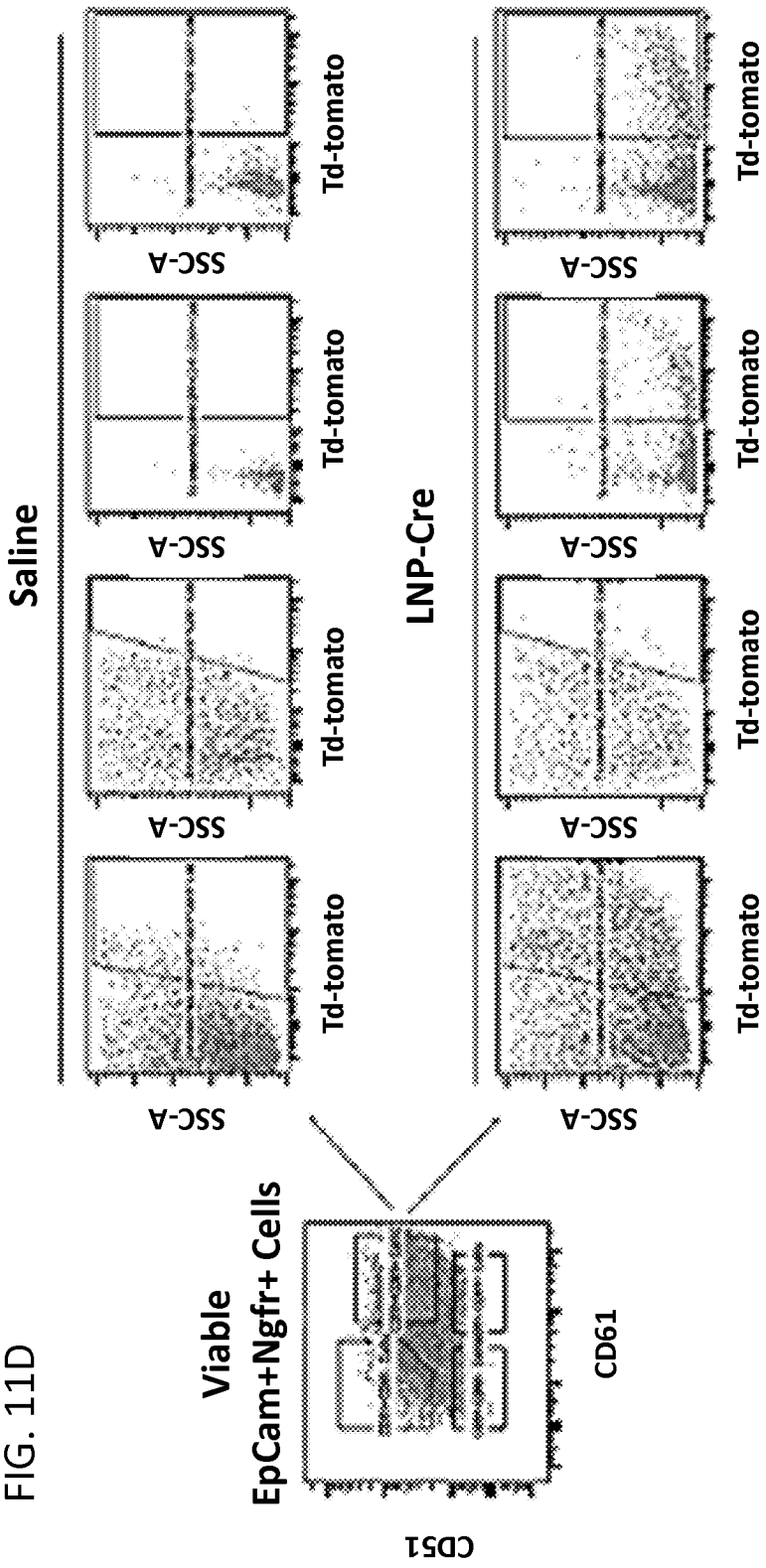

Saline
LNP-Cre
Td-tomato
SSC-A
Viable
EpCam+Ngfr+ Cells
CD61
CD51

FIG. 11D

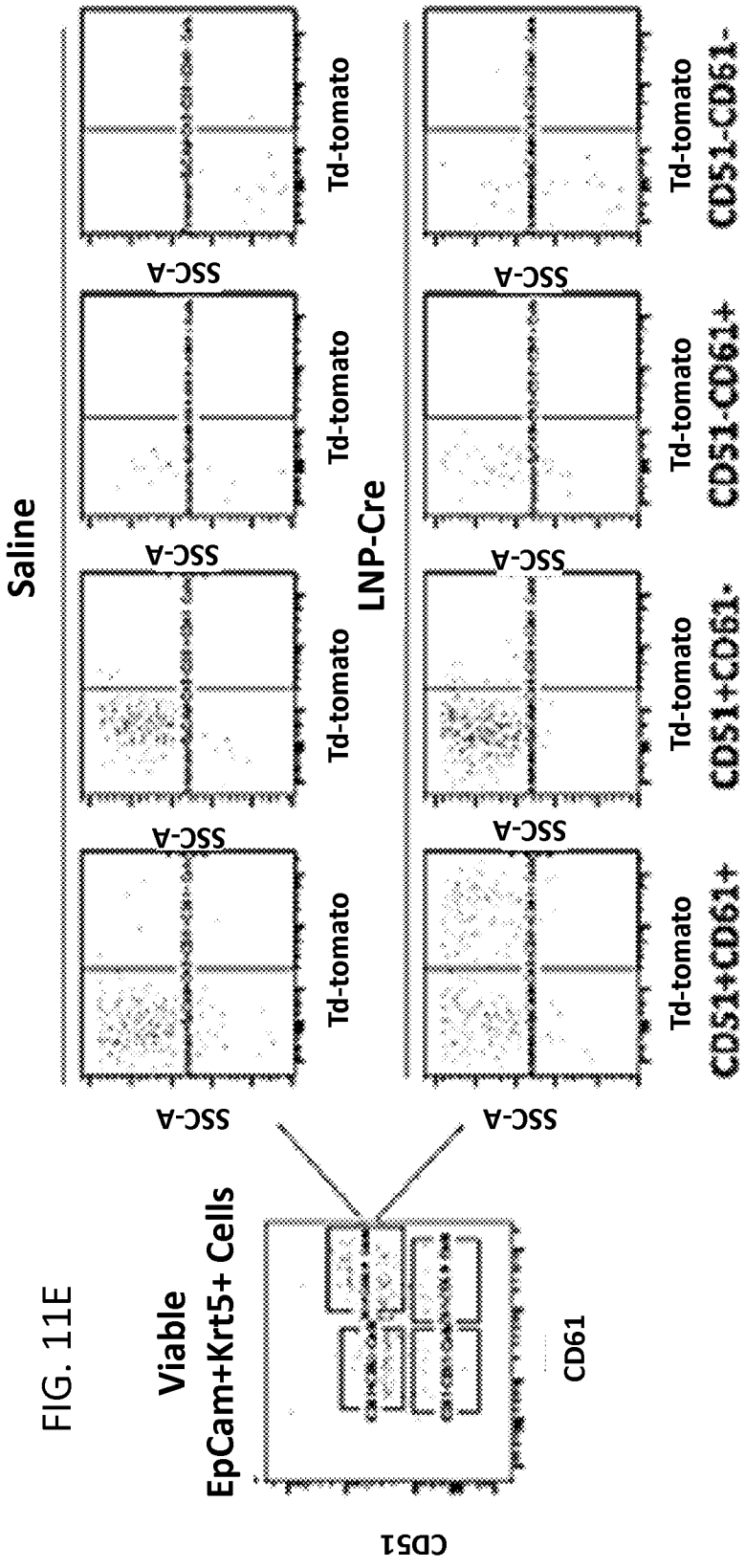

Saline
LNP-Cre
Viable
EpCam+Krt5+ Cells
CD51
CD61
SSC-A
Td-tomato
CD51+CD61+
CD51+CD61-
CD51-CD61+
CD51-CD61-

FIG. 11E

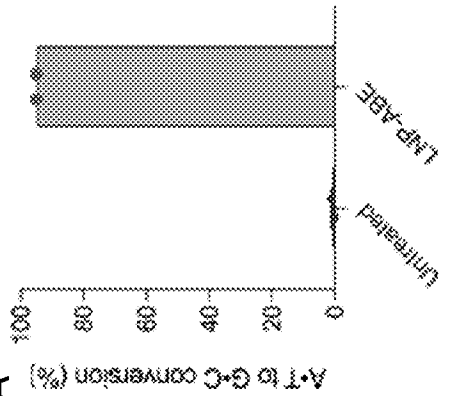


FIG. 12A

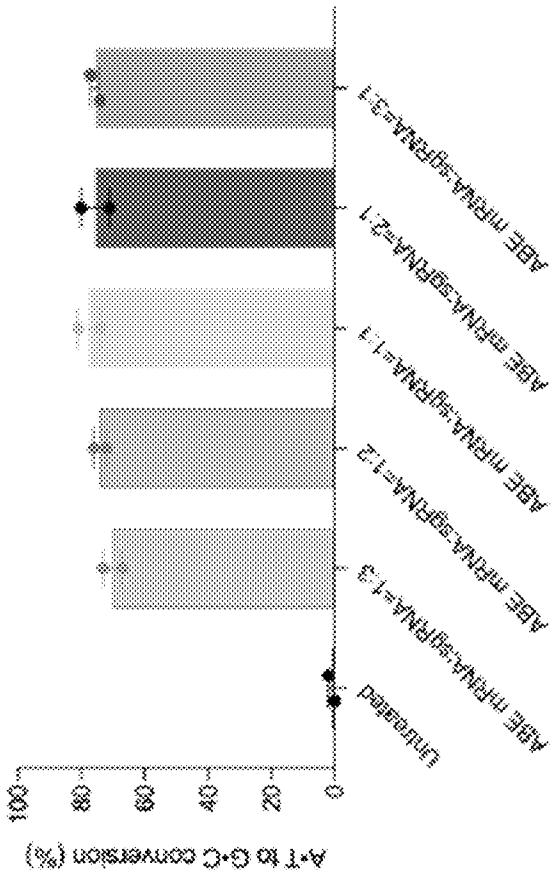
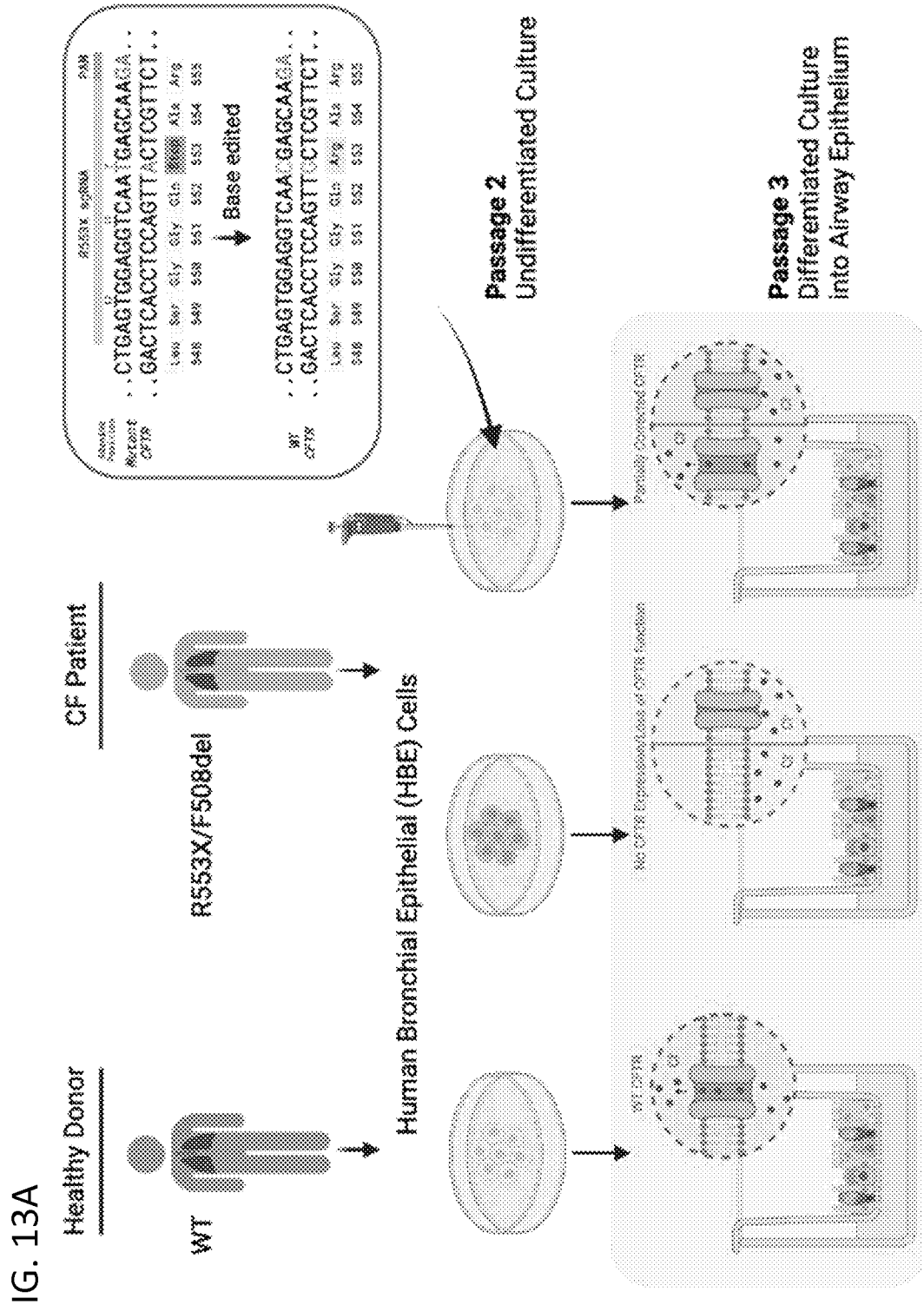


FIG. 12B

FIG. 13A


Healthy Donor
WT
CF Patient
R553X/F508del
Human Bronchial Epithelial (HBE) Cells
Base edited
Passage 2
Undifferentiated Culture
Passage 3
Differentiated Culture
into Airway Epithelium

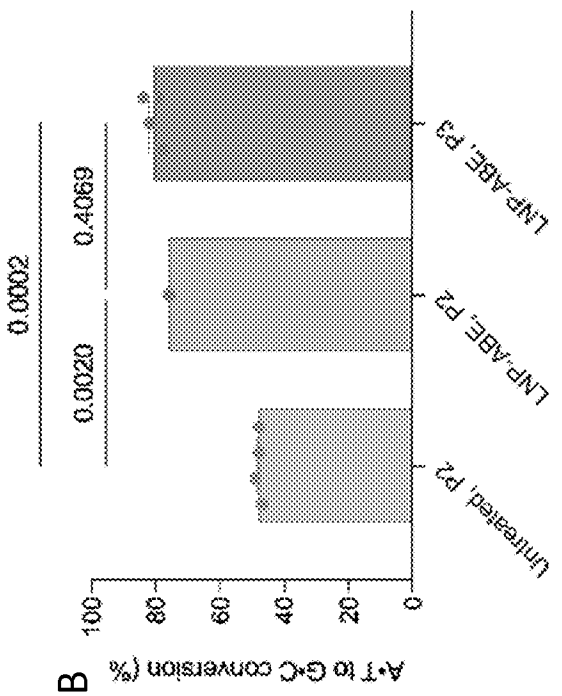


FIG. 13B

	T_7	T_{11}	T_{17}
A	0.0%	0.1%	0.0%
C	83.7%	14.5%	0.0%
G	0.0%	0.0%	0.0%
T	16.3%	85.4%	100.0%

FIG. 13C

FIG. 13D

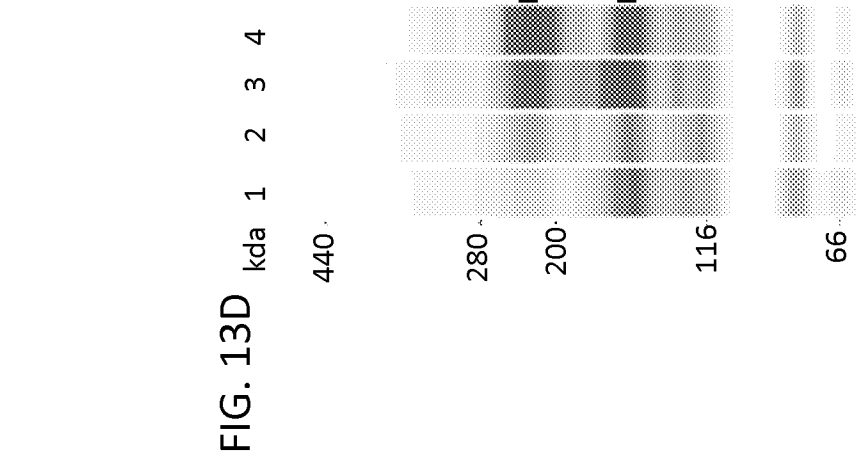


FIG. 13E

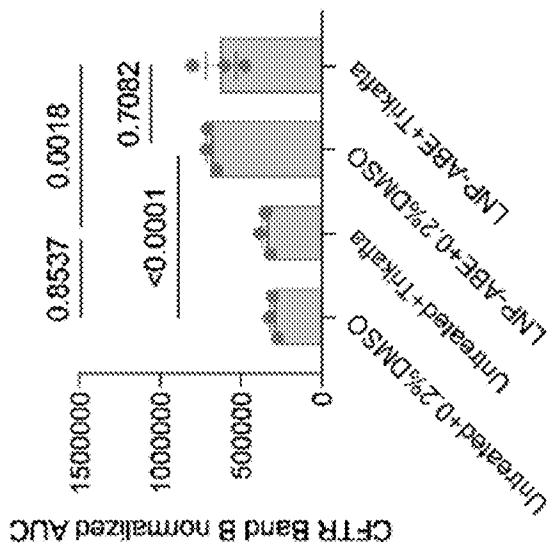
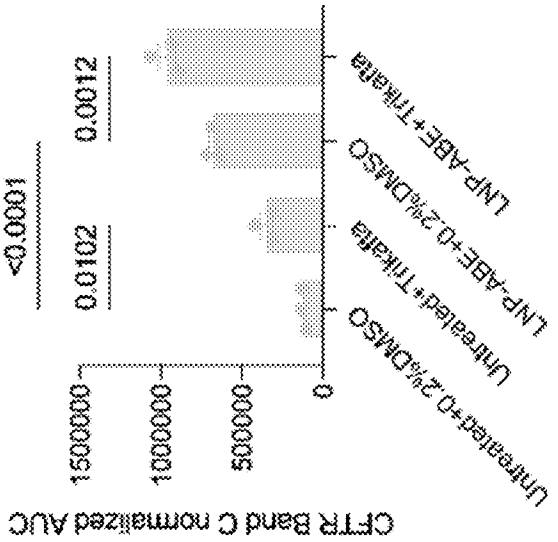


FIG. 13F



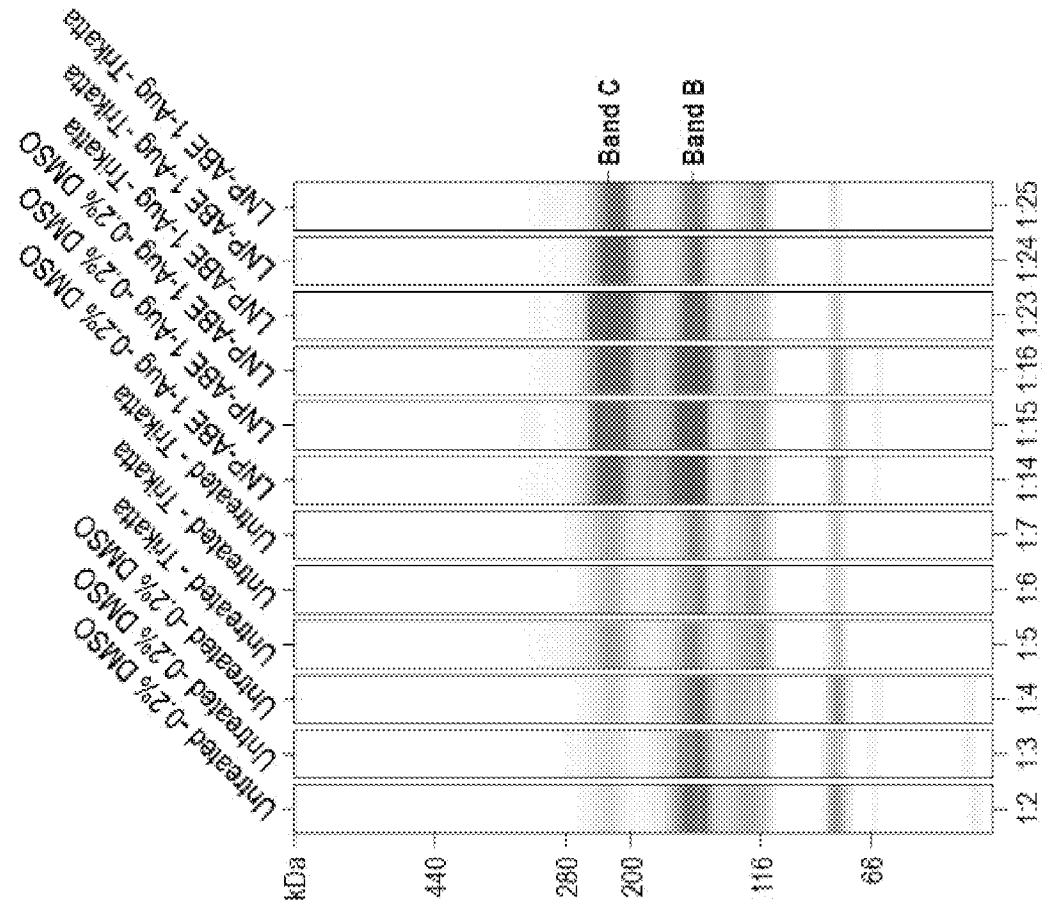

kDa
440
280
200
116
66
Band C
Band B
1:2
1:3
1:4
1:5
1:6
1:7
1:14
1:15
1:16
1:23
1:24
1:25
Untreated -0.2% DMSO
Untreated -0.2% DMSO
Untreated -0.2% DMSO
Untreated - Trikafta
Untreated - Trikafta
Untreated - Trikafta
LNP-ABE 1-Aug -0.2% DMSO
LNP-ABE 1-Aug -0.2% DMSO
LNP-ABE 1-Aug -0.2% DMSO
LNP-ABE 1-Aug - Trikafta
LNP-ABE 1-Aug - Trikafta
LNP-ABE 1-Aug - Trikafta

FIG. 14A

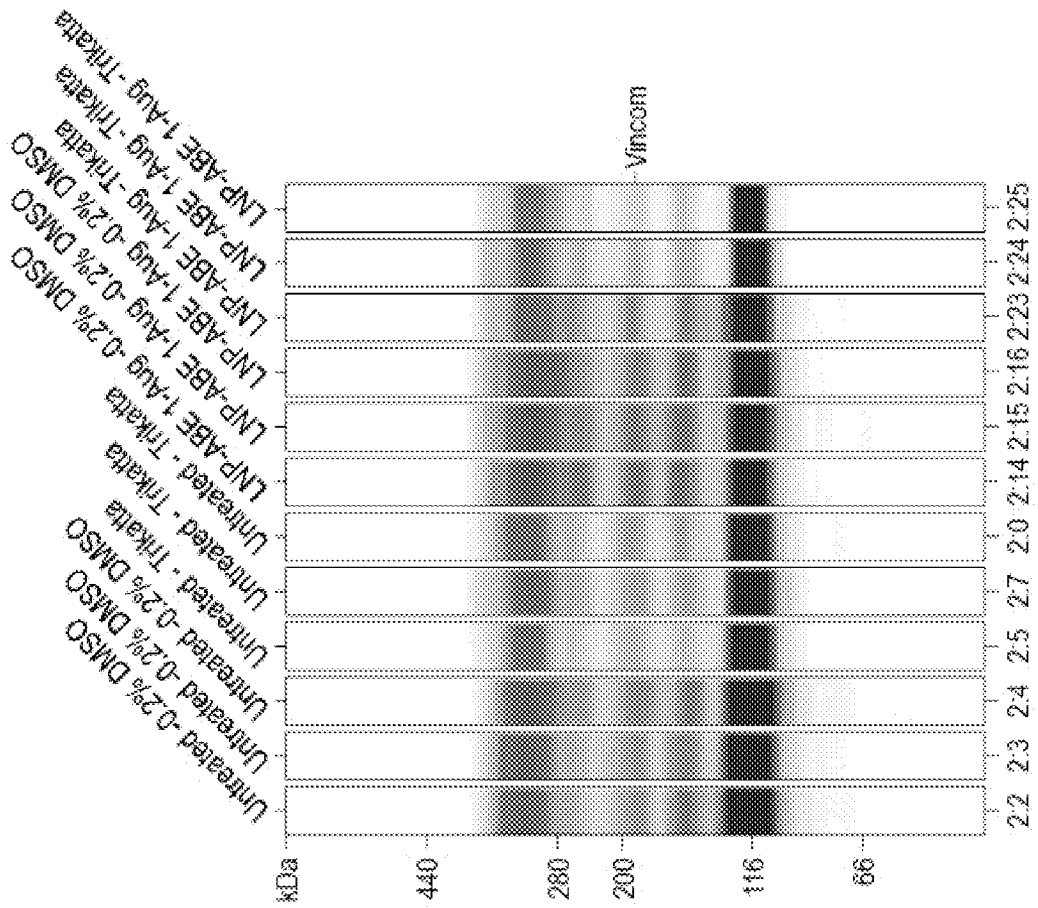

Untreated -0.2% DMSO
Untreated -0.2% DMSO
Untreated -0.2% DMSO
Untreated - Trikafta
Untreated - Trikafta
LNP-ABE 1-Aug -0.2% DMSO
LNP-ABE 1-Aug -0.2% DMSO
LNP-ABE 1-Aug - Trikafta
LNP-ABE 1-Aug - Trikafta
kDa
440
280
200
116
66
Vimcom
2-2 2-3 2-4 2-5 2-7 2-0 2-14 2-15 2-16 2-23 2-24 2-25

FIG. 14B

FIG. 15A

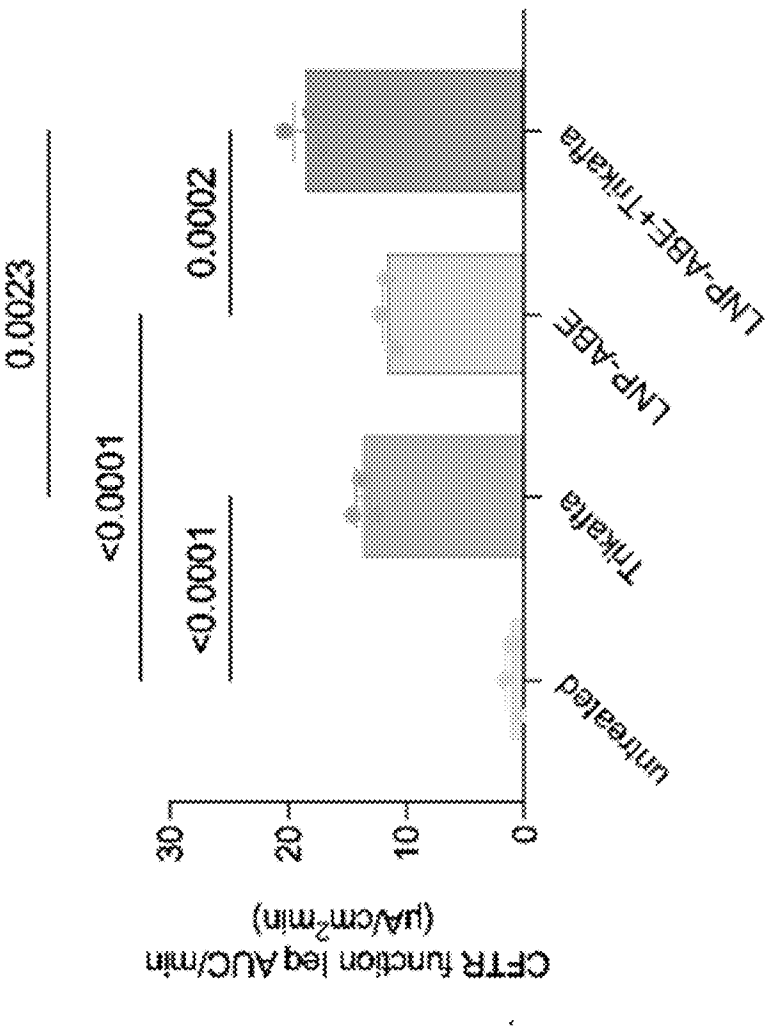


FIG. 15B

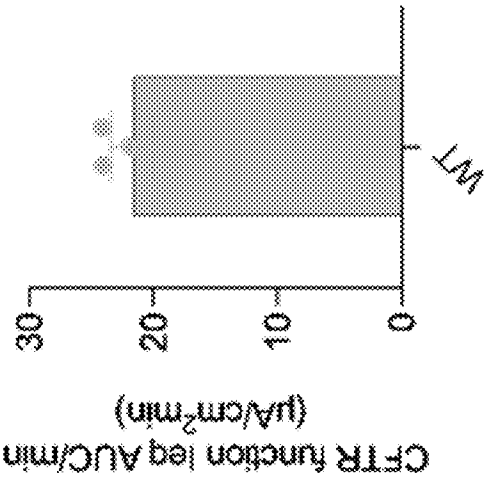


FIG. 16A

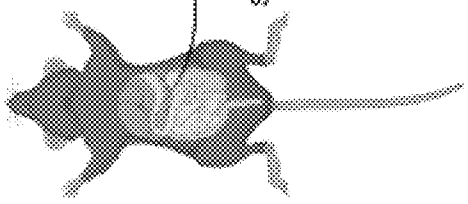


FIG. 16B

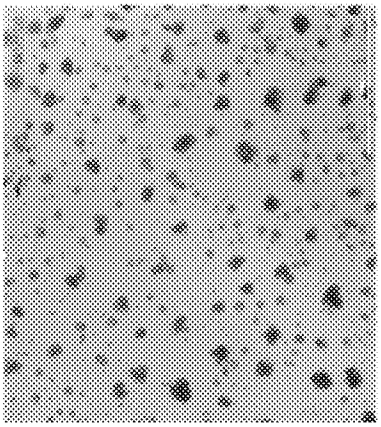


FIG. 16C

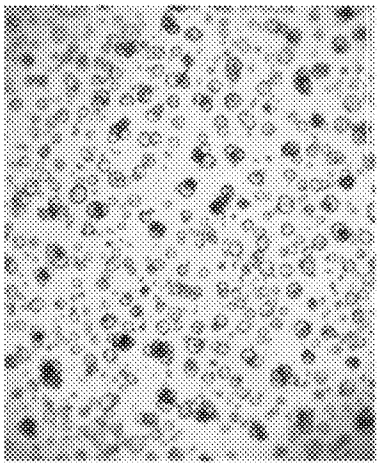


FIG. 16D

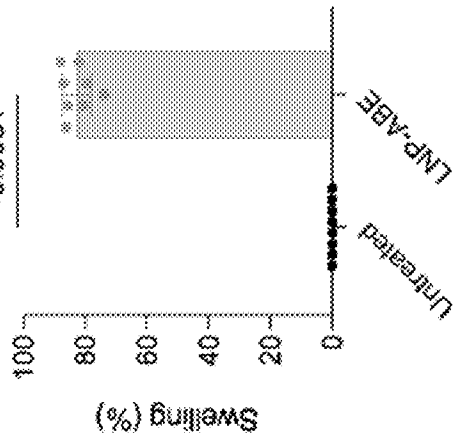


FIG. 16E

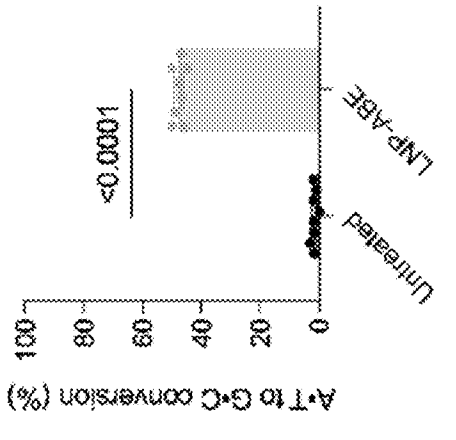


FIG. 16F

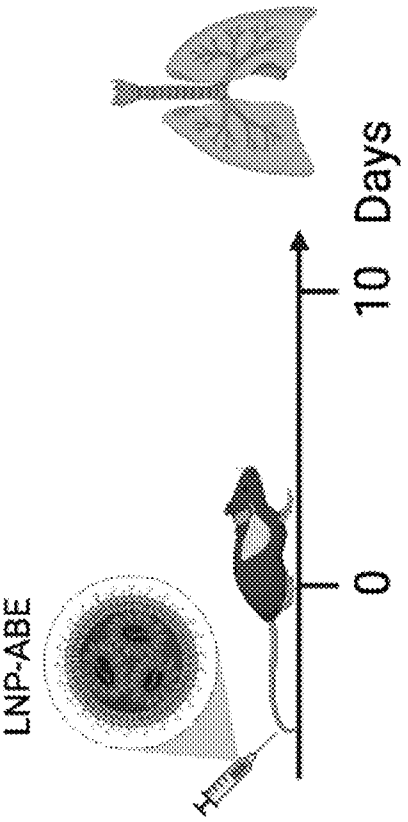


FIG. 16G

FIG. 17A

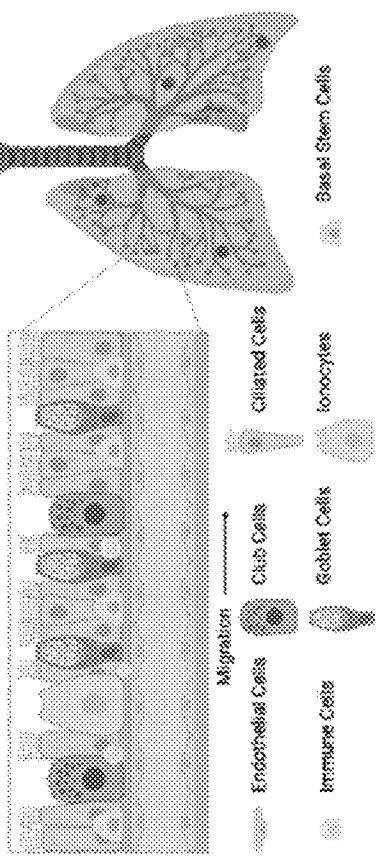


FIG. 17B

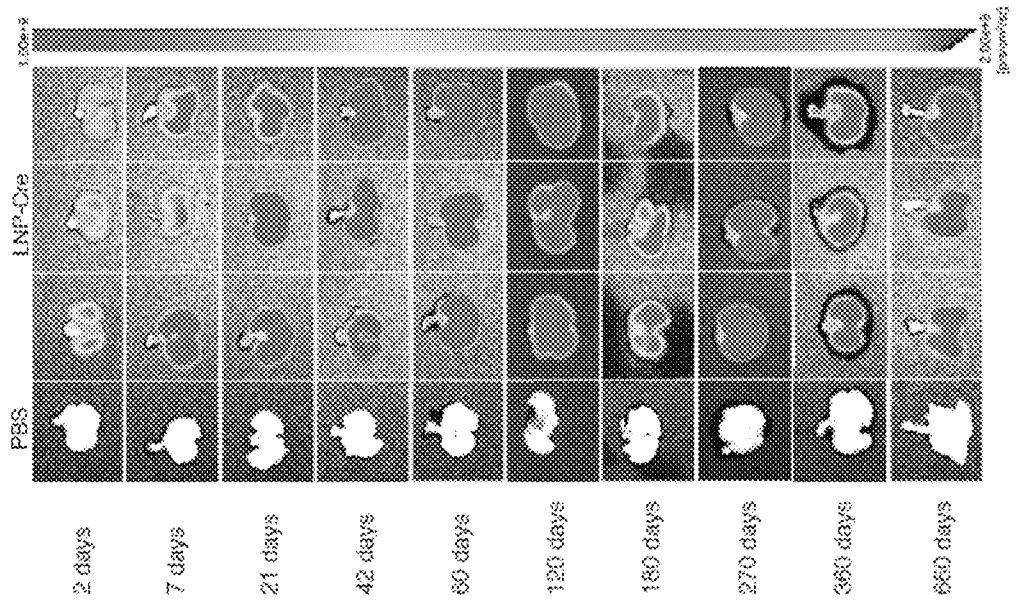

PBS
LNP-Cre
2 days
7 days
21 days
42 days
60 days
120 days
180 days
270 days
360 days
660 days

FIG. 17C

FIG. 17D

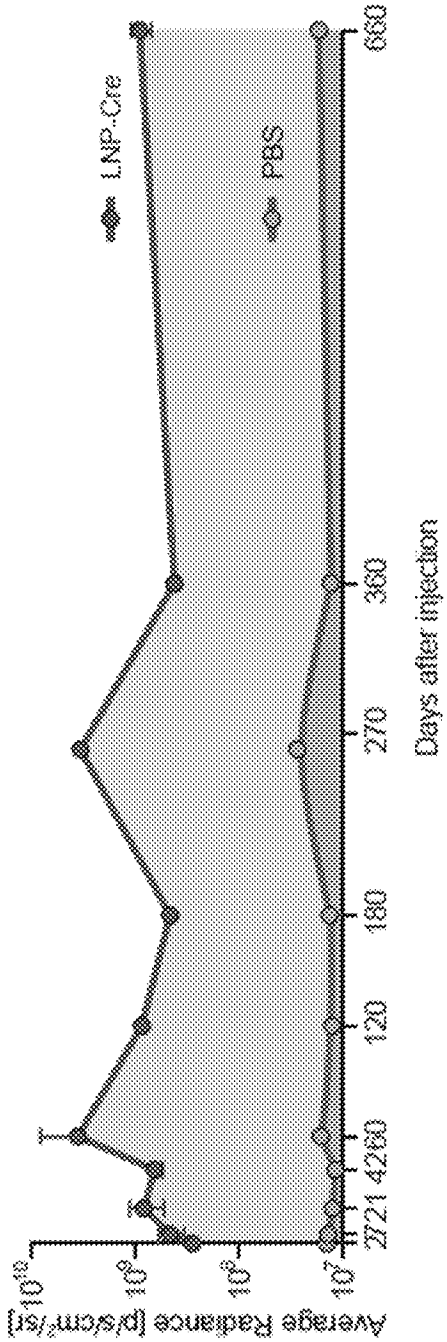
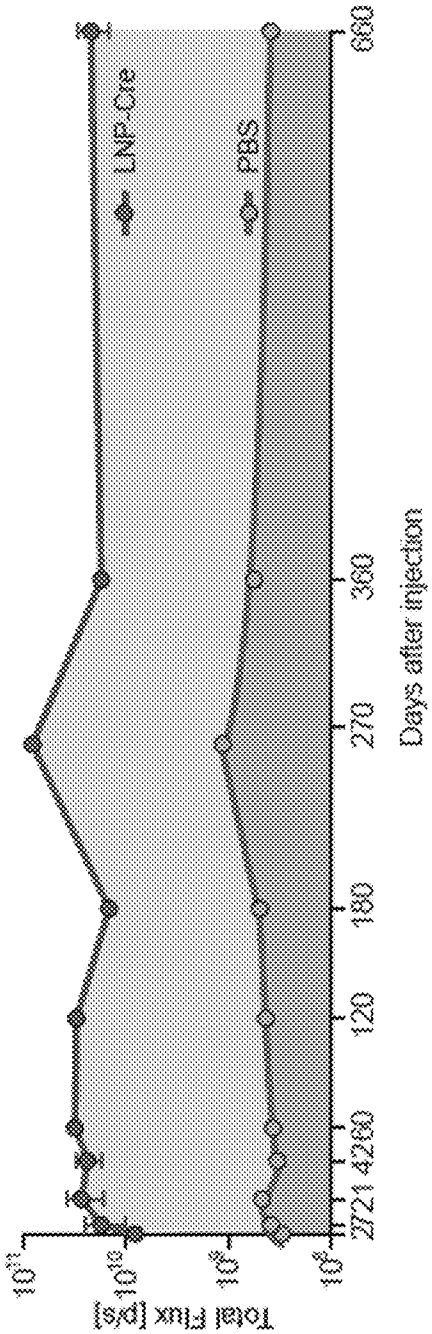


FIG. 17E



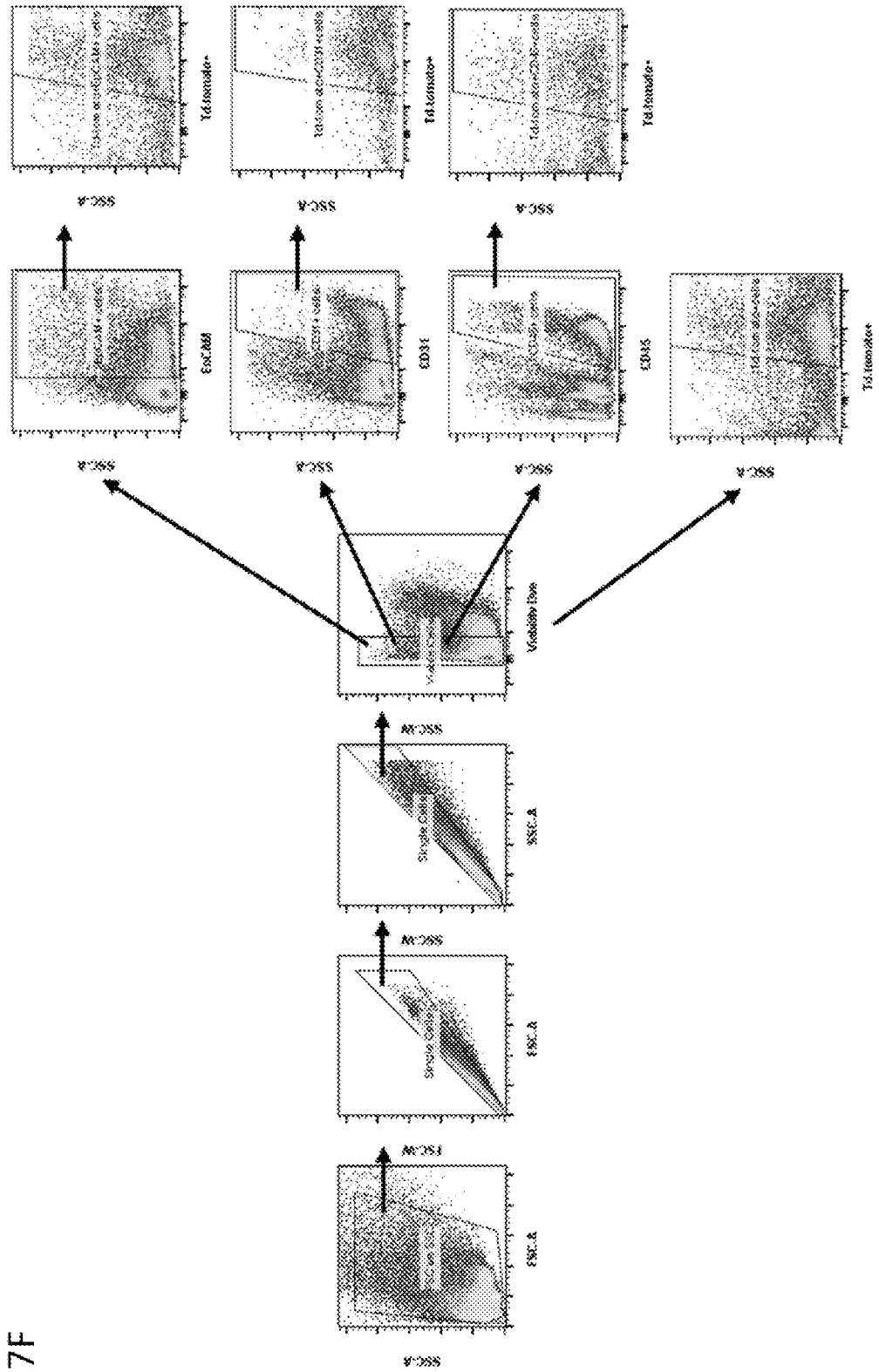


FIG. 17F

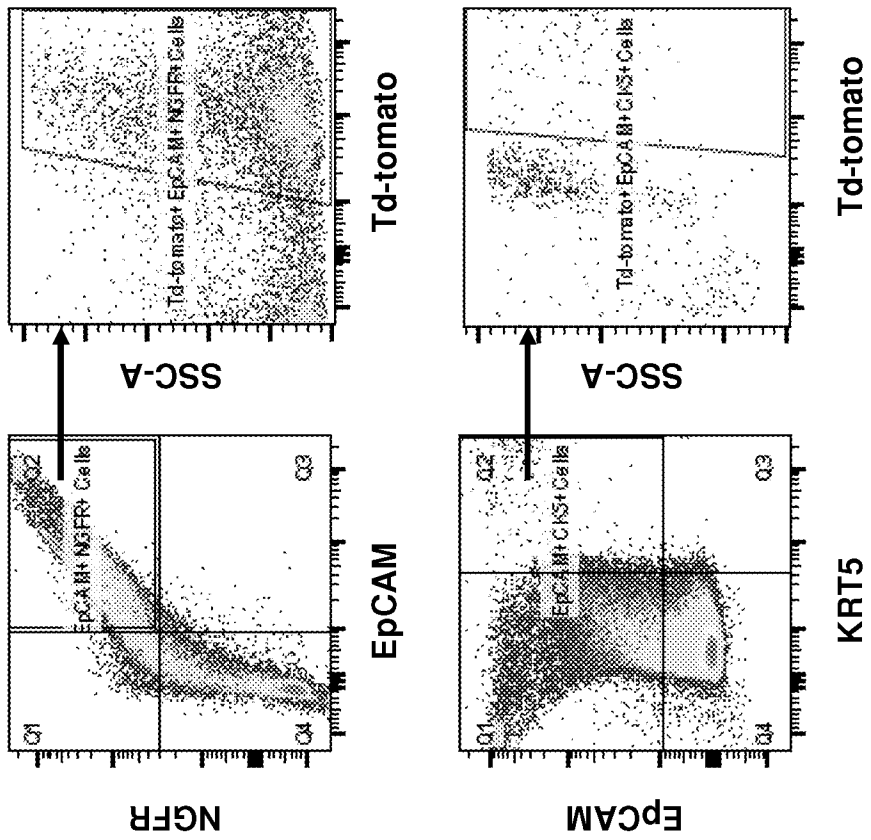

NGFR
EpCAM
Q1
Q2
Q3
Q4
EpCAM+ NGFR+ Cells
SSC-A
Td-tomato
Td-tomato+ EpCAM+ NGFR+ Cells
EpCAM
KRT5
EpCAM+ CK5+ Cells
Td-tomato+ EpCAM+ CK5+ Cells

FIG. 17G

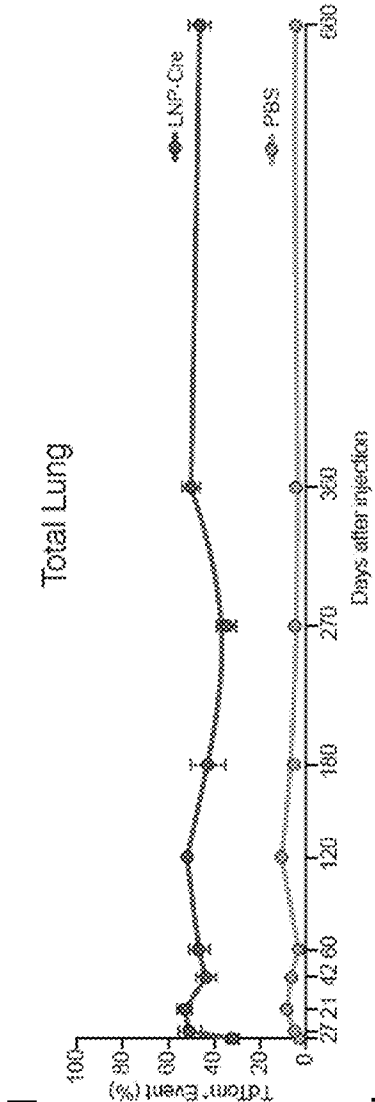


FIG. 17H

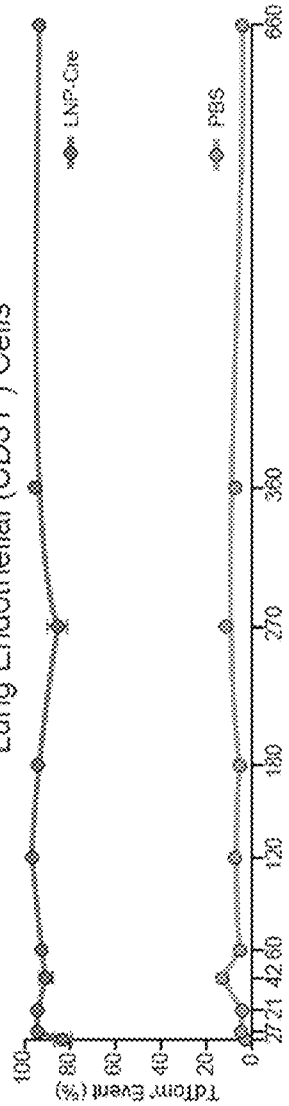


FIG. 17I

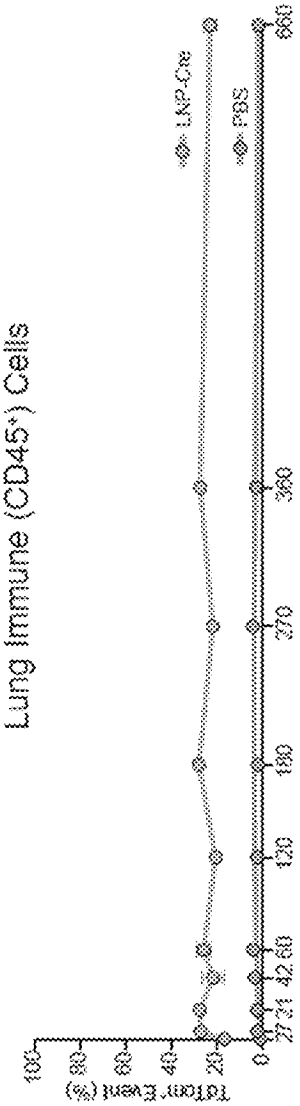


FIG. 17J

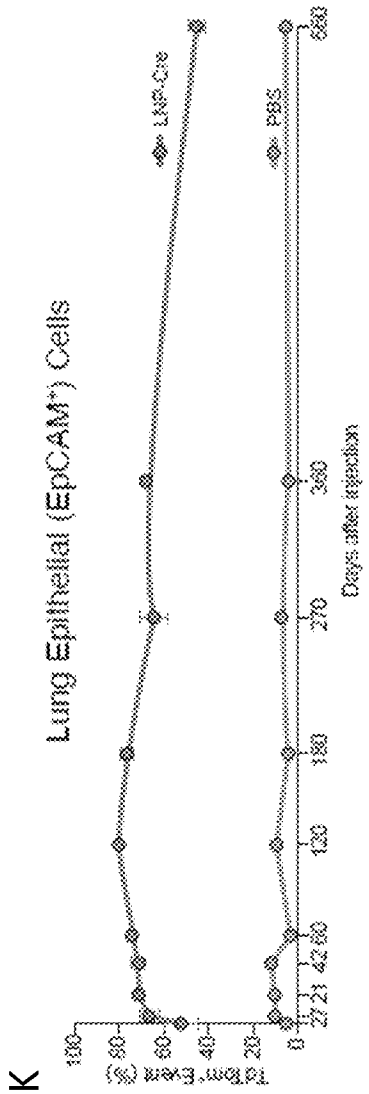


FIG. 17K

FIG. 17L

FIG. 17M

FIG. 18A

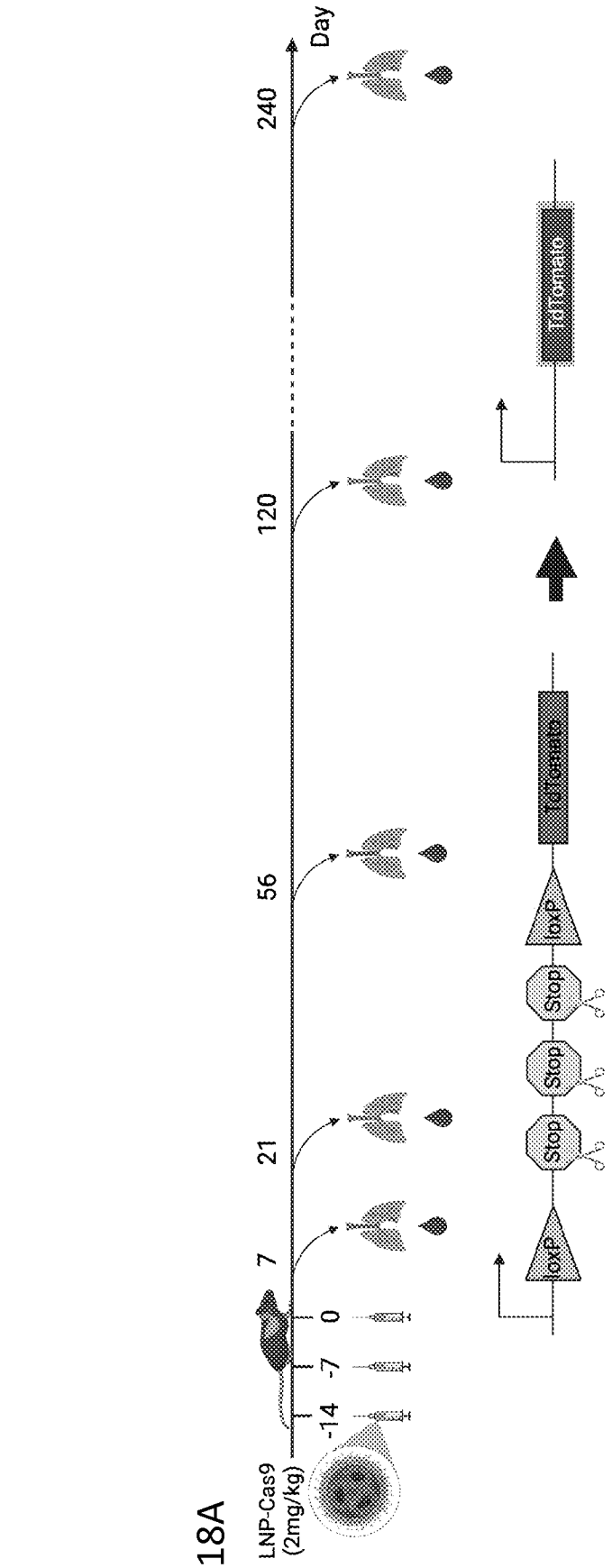


FIG. 18B

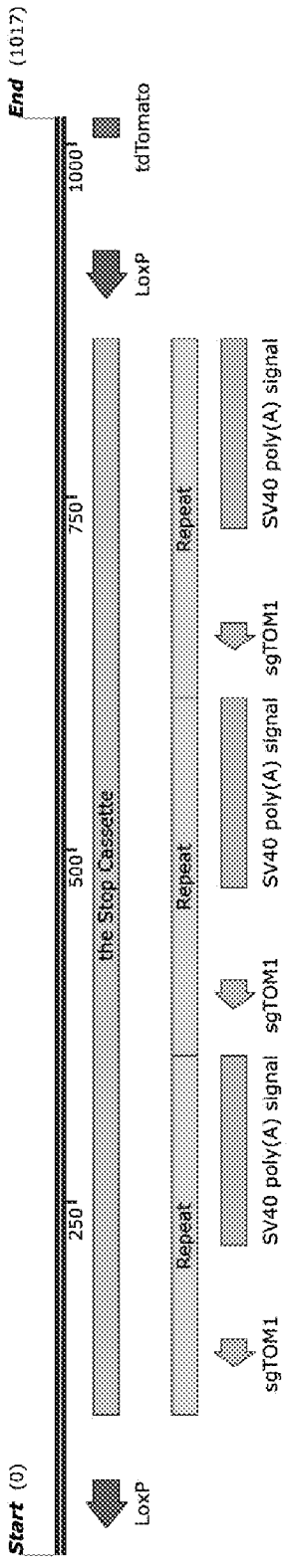


FIG. 18C

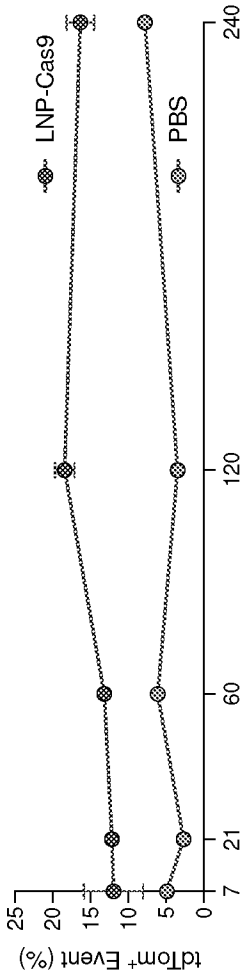


FIG. 18D

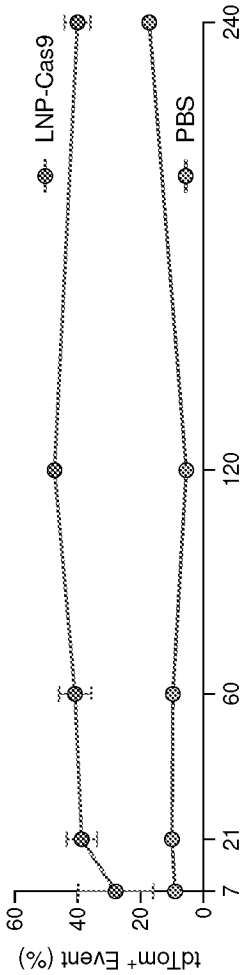


FIG. 18E

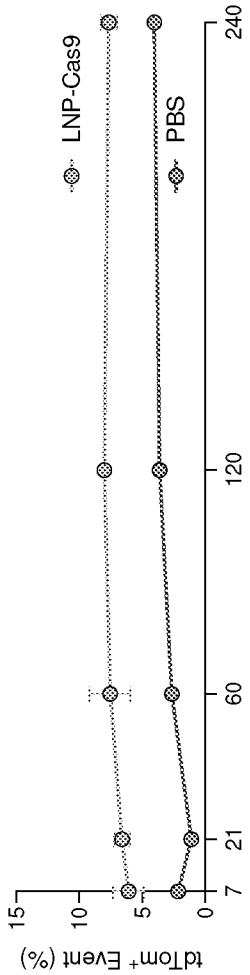


FIG. 18F

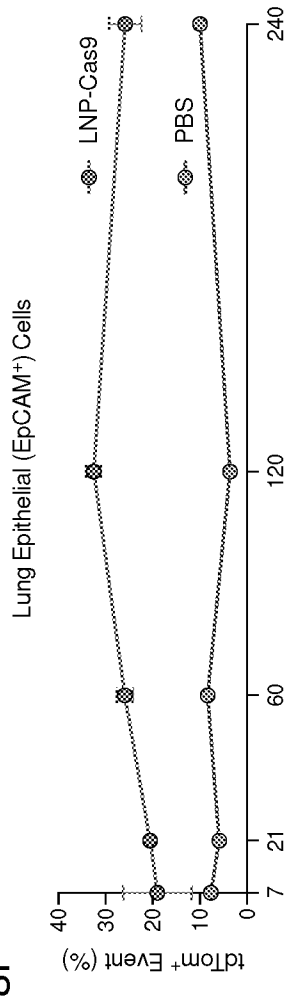


FIG. 18G

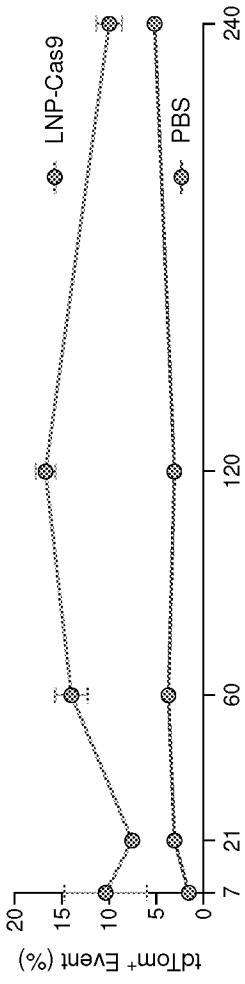


FIG. 18H

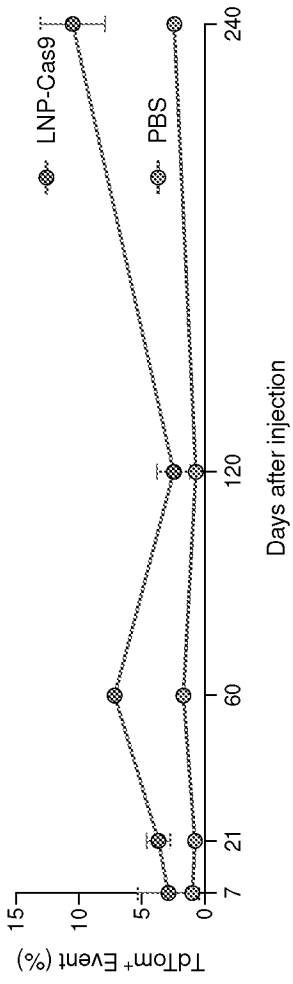


FIG. 19A

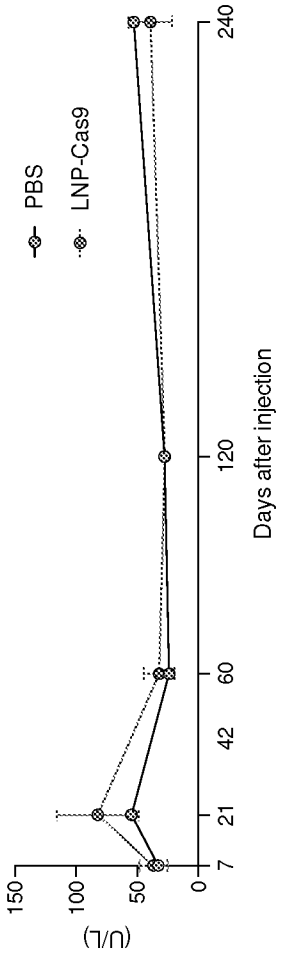


FIG. 19B

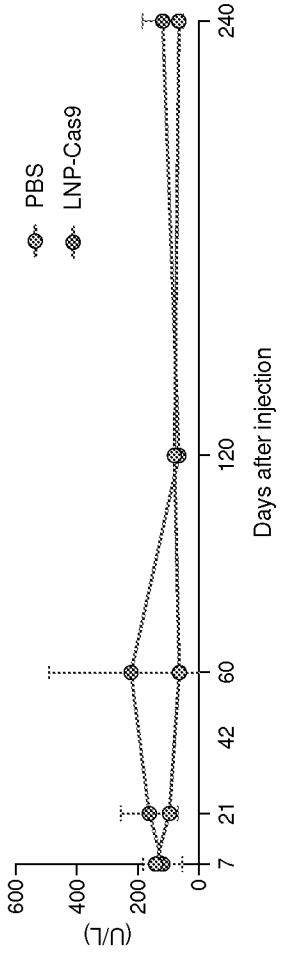


FIG. 19C

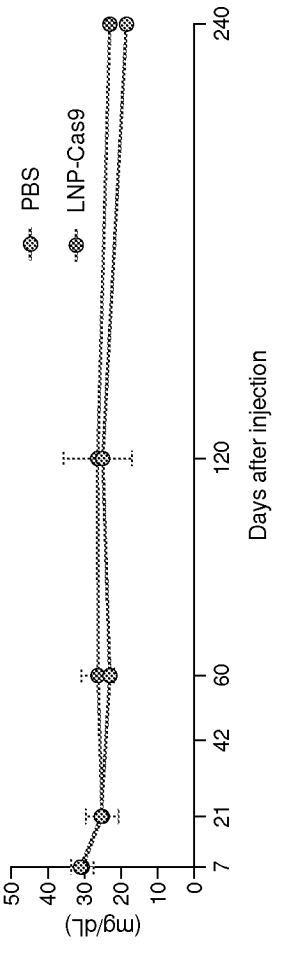
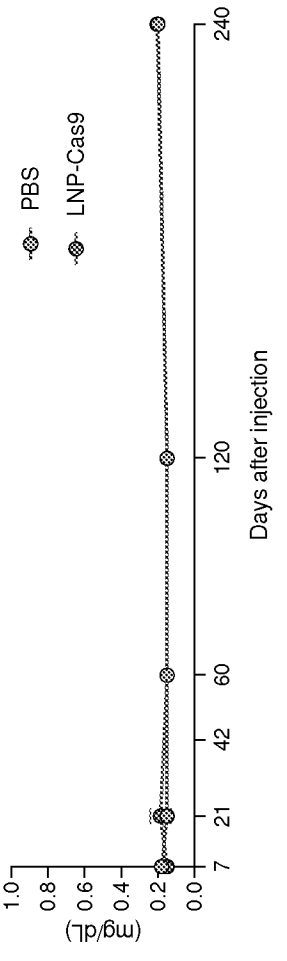


FIG. 19D



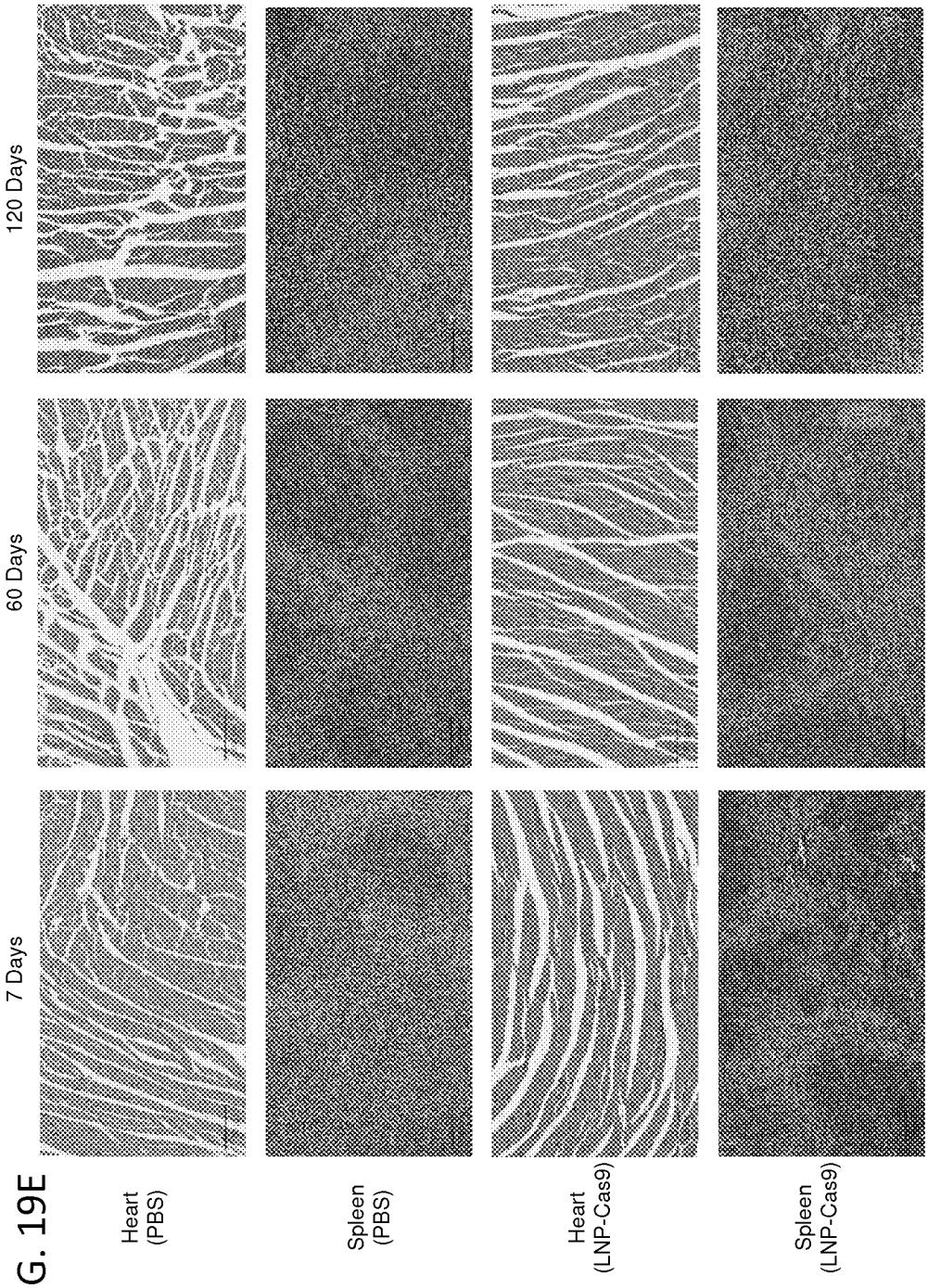

7 Days
60 Days
120 Days
Heart (PBS)
Spleen (PBS)
Heart (LNP-Cas9)
Spleen (LNP-Cas9)

FIG. 19E

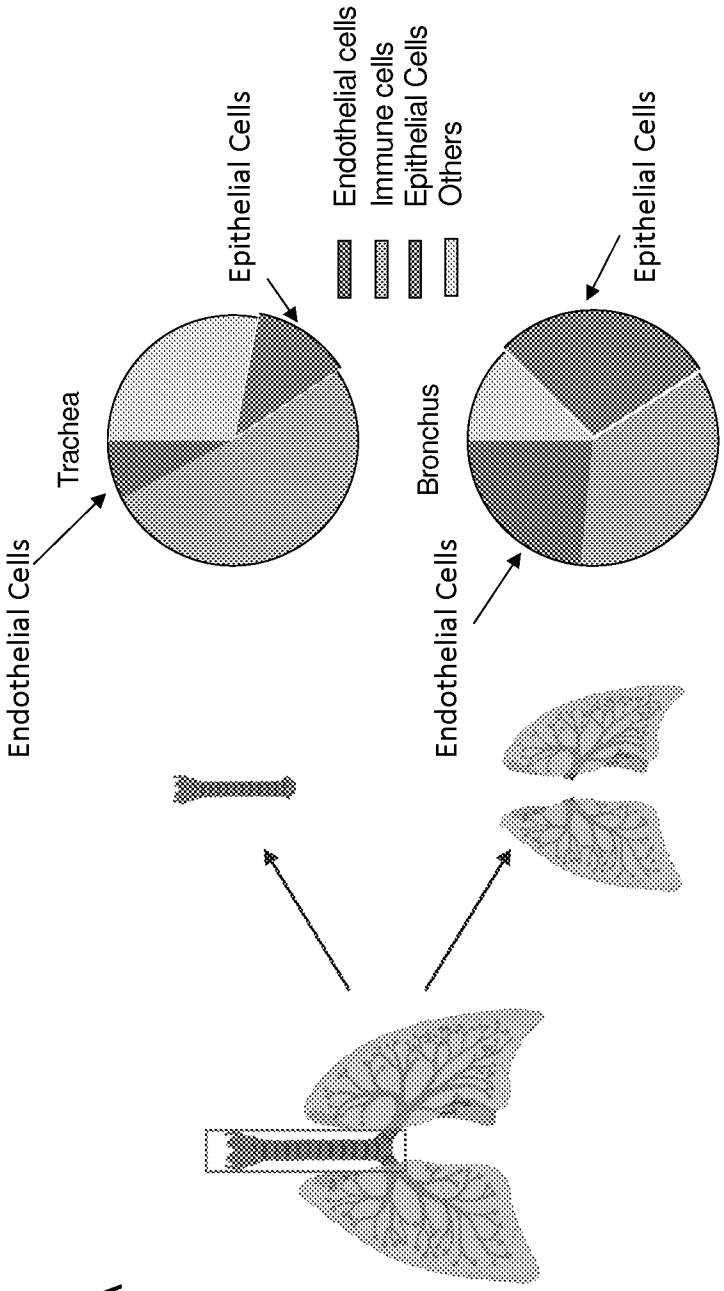

Endothelial cells
Immune cells
Epithelial Cells
Others
Epithelial Cells
Trachea
Endothelial Cells
Bronchus
Epithelial Cells
Endothelial Cells

FIG. 20A

FIG. 20B

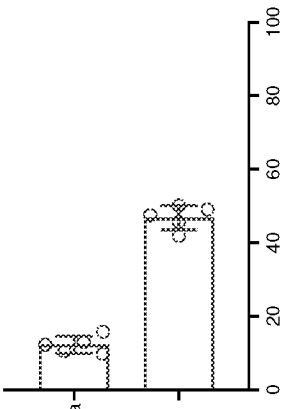


FIG. 20C

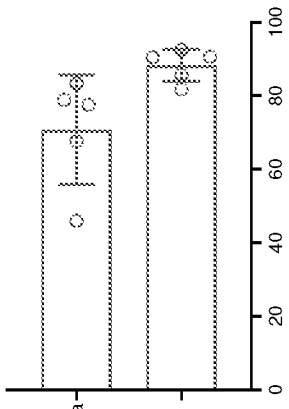


FIG. 20D

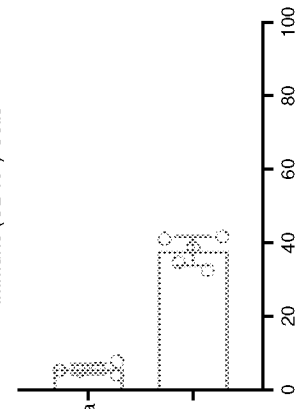


FIG. 20E

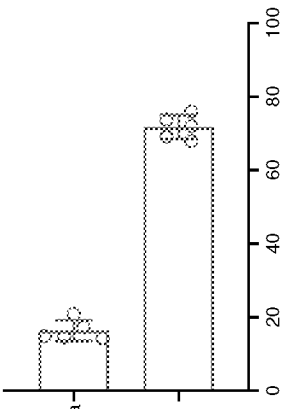


FIG. 20F

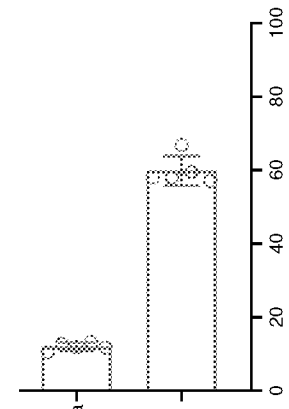
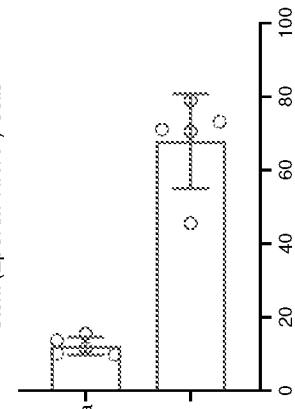


FIG. 20G



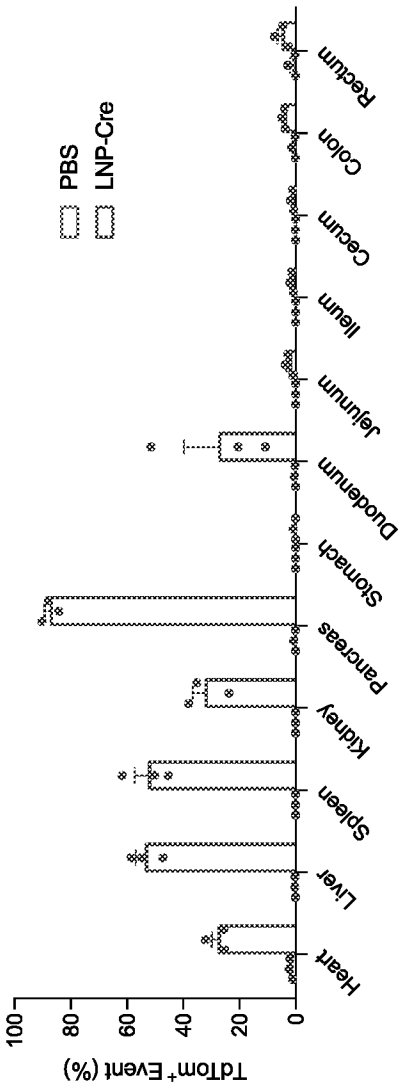


FIG. 21A

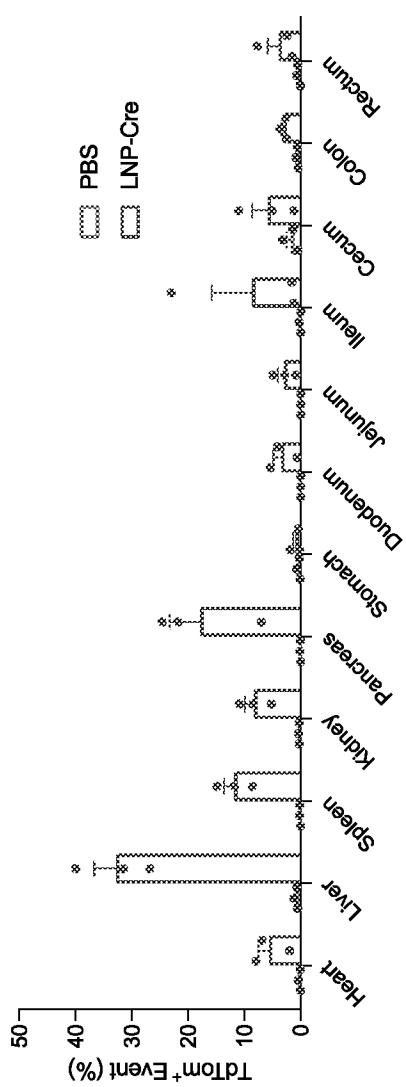
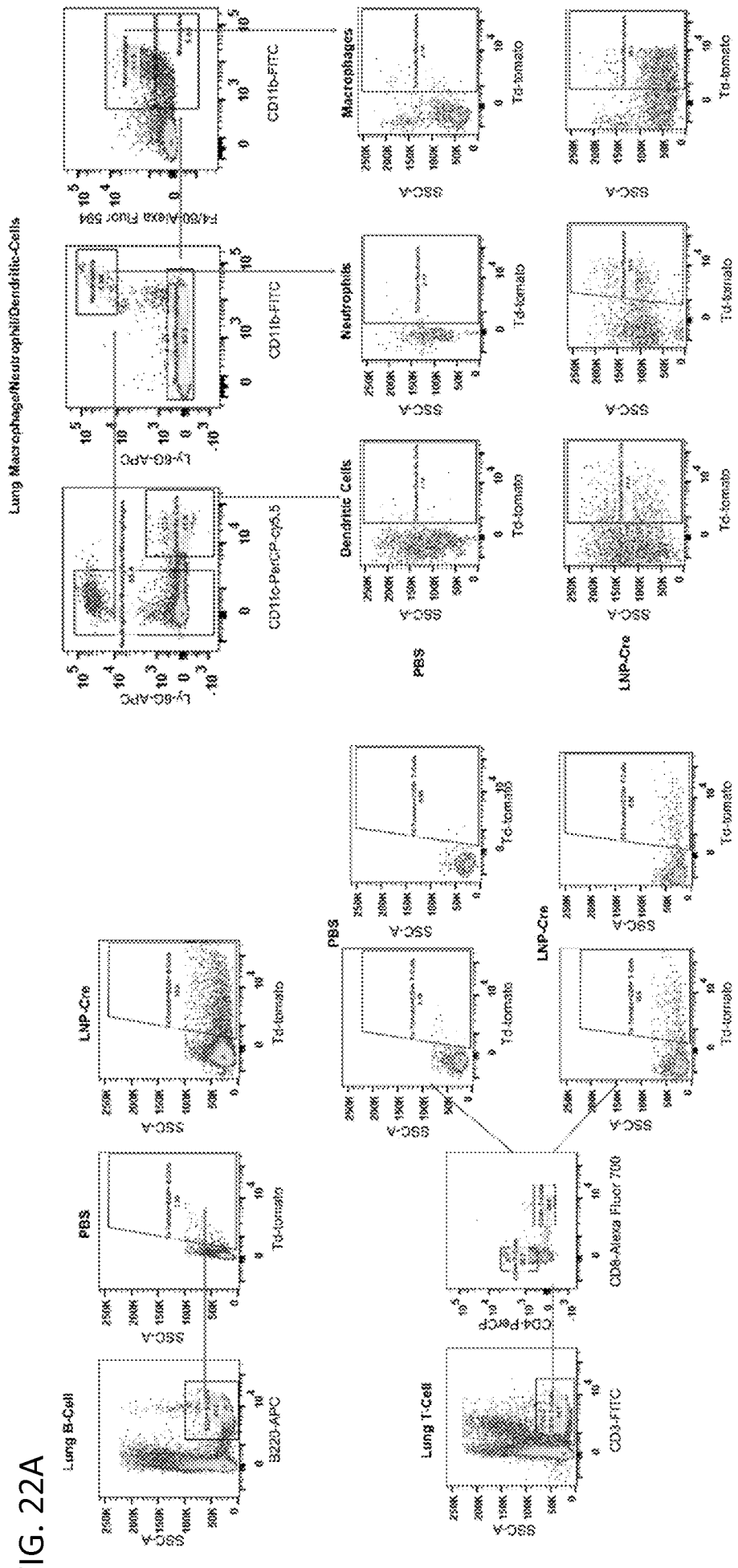


FIG. 21B

FIG. 22A


Lung Macrophage/Neutrophil/Dendritic-Cells
F4/80-Alexa Fluor 594
CD11b-FITC
Ly-6G-APC
CD11c-PerCP-cy5.5
Macrophages
Neutrophils
Dendritic Cells
SSC-A
Td-tomato
PBS
LNP-Cre
Lung B-Cell
B220-APC
Lung T-Cell
CD3-FITC
CD4-PerCP
CD8-Alexa Fluor 700

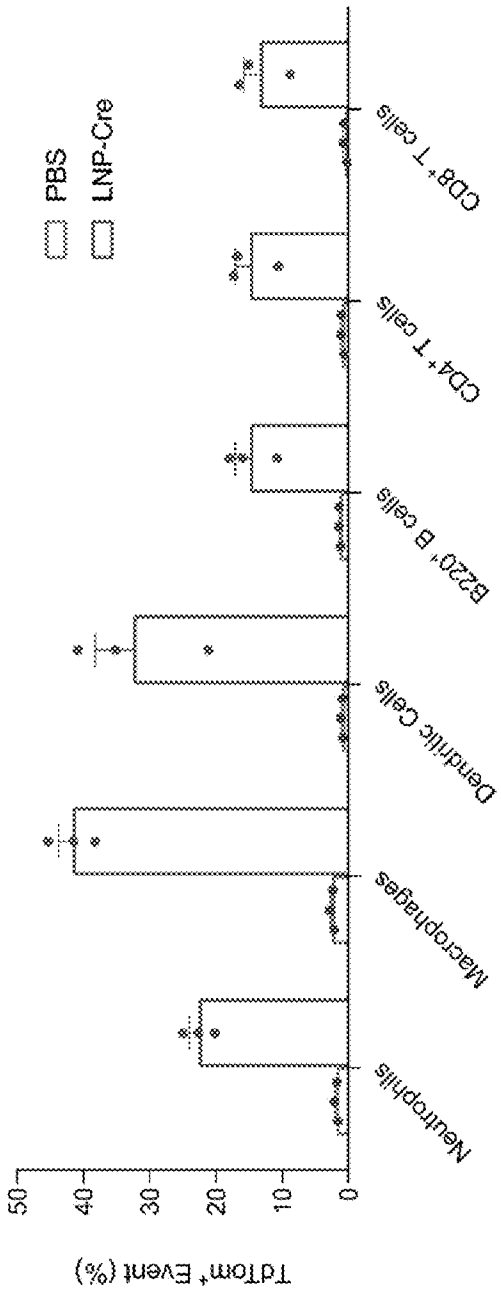

PBS
LNP-Cre
TdTom+ Event (%)
0
10
20
30
40
50
Neutrophils
Macrophages
Dendritic Cells
B220+ B cells
CD4+ T cells
CD8+ T cells

FIG. 22B

FIG. 23A

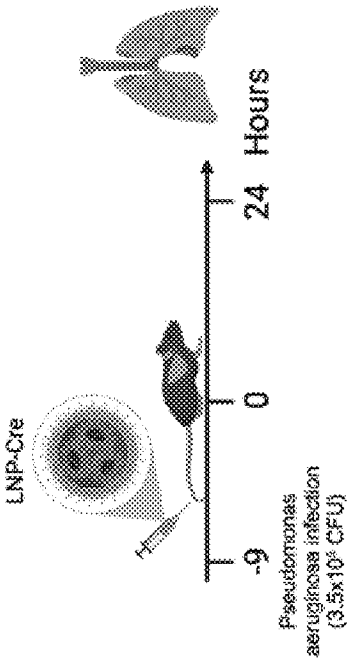


FIG. 23B

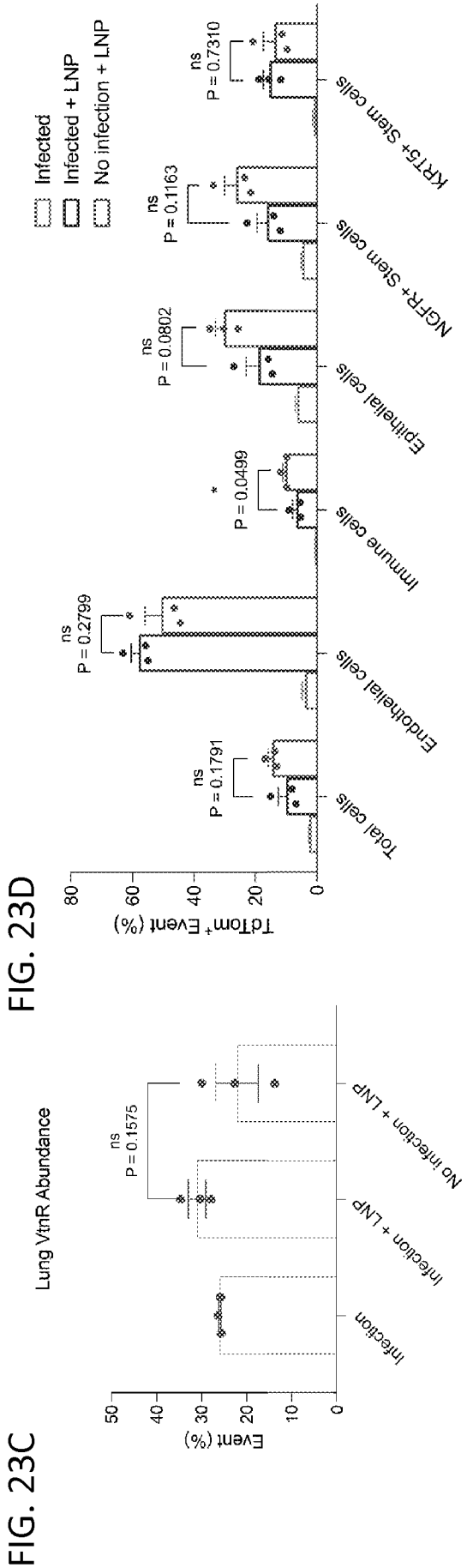


FIG. 23C

FIG. 23D

FIG. 24A

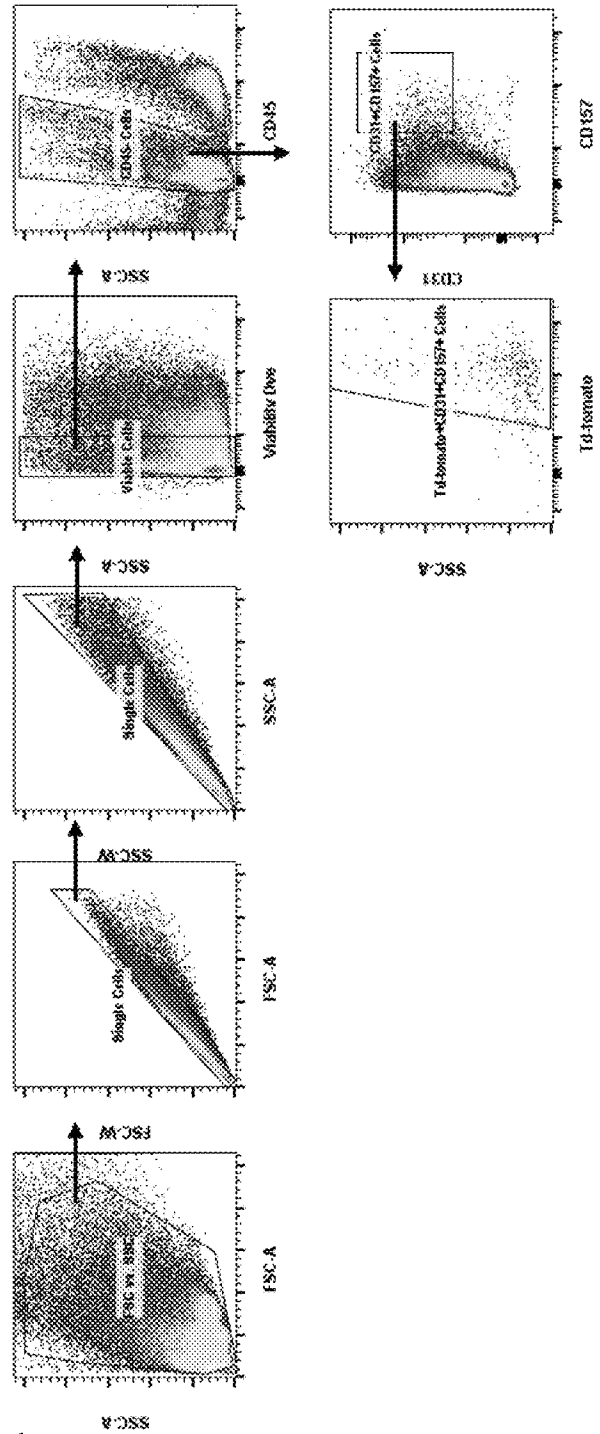
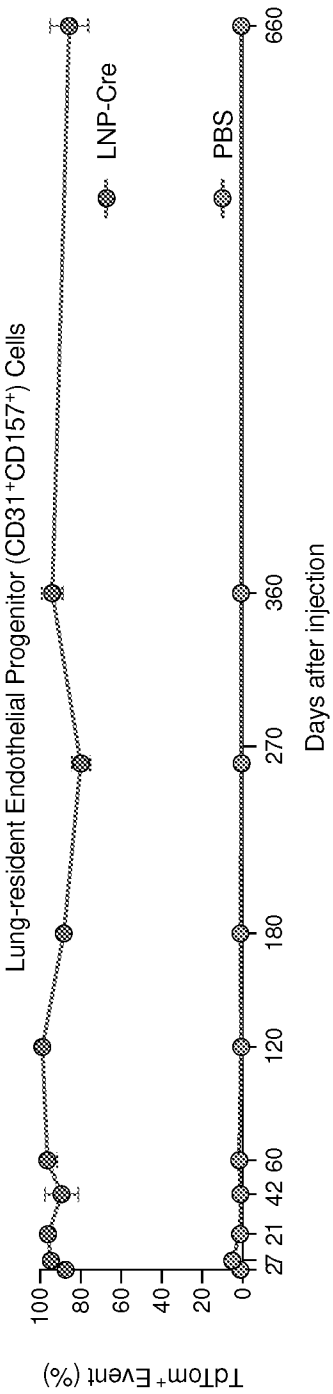


FIG. 24B



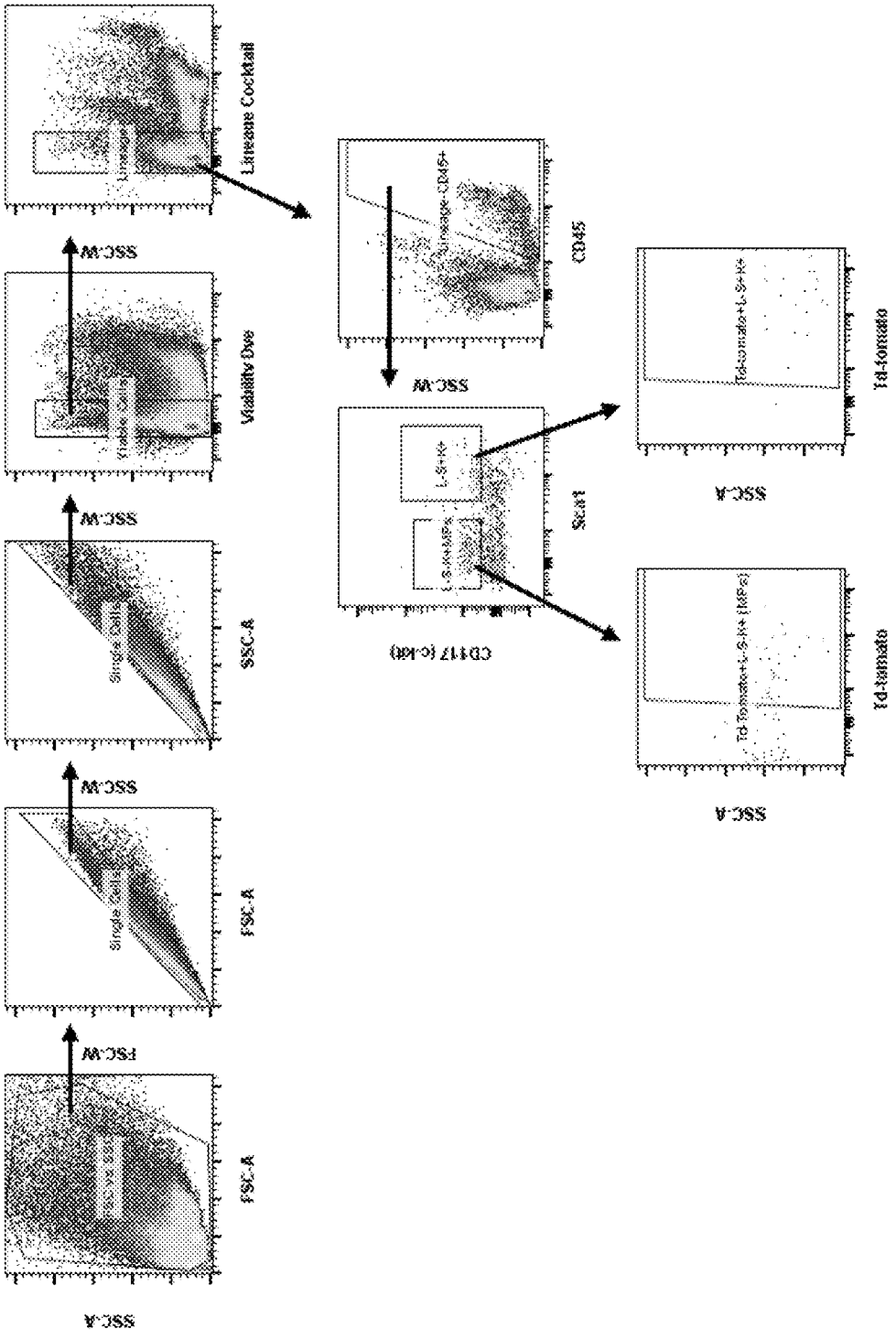

FSC-A
FSC-W
Single Cells
SSC-A
SSC-W
Viability Dye
Lineage Cocktail
CD45
CD117 (c-kit)
Scart
Td-tomato

FIG. 25A

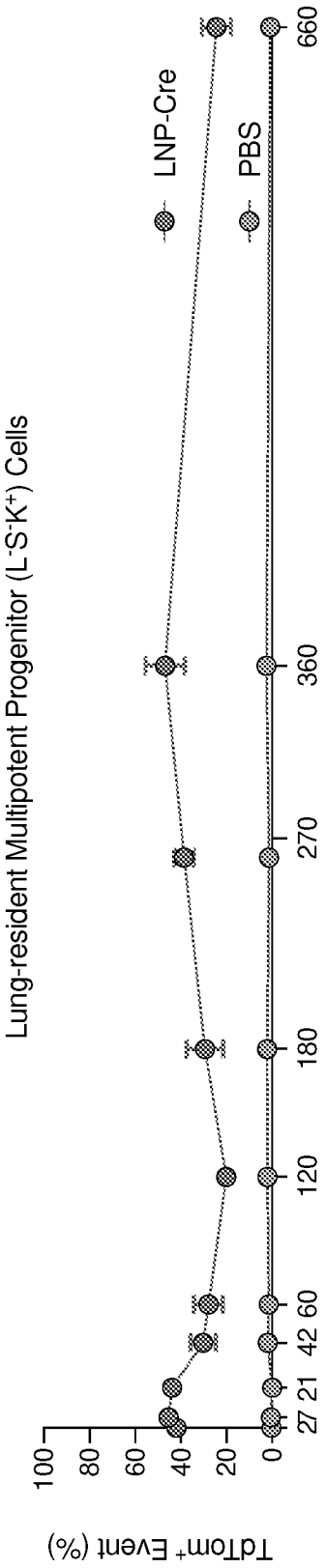


FIG. 25B

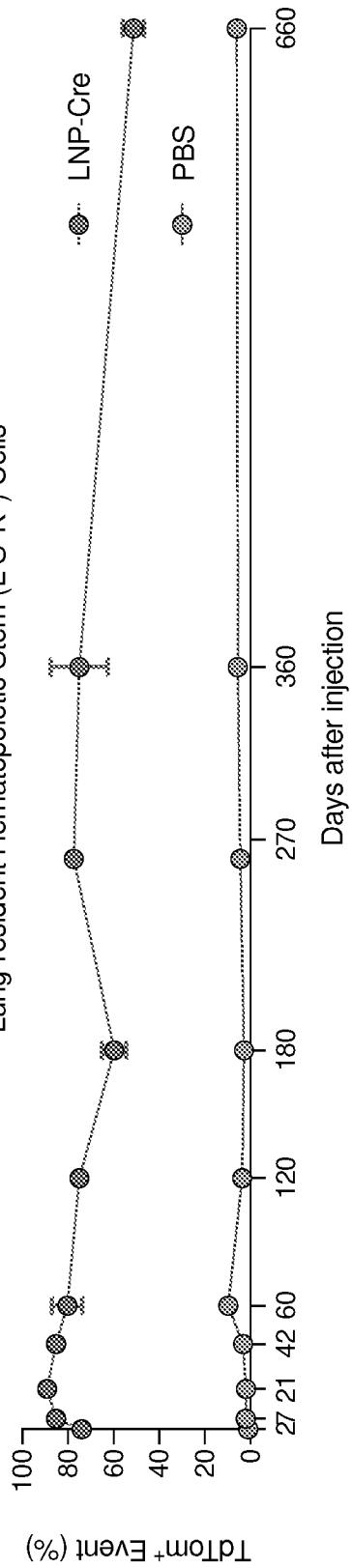


FIG. 25C

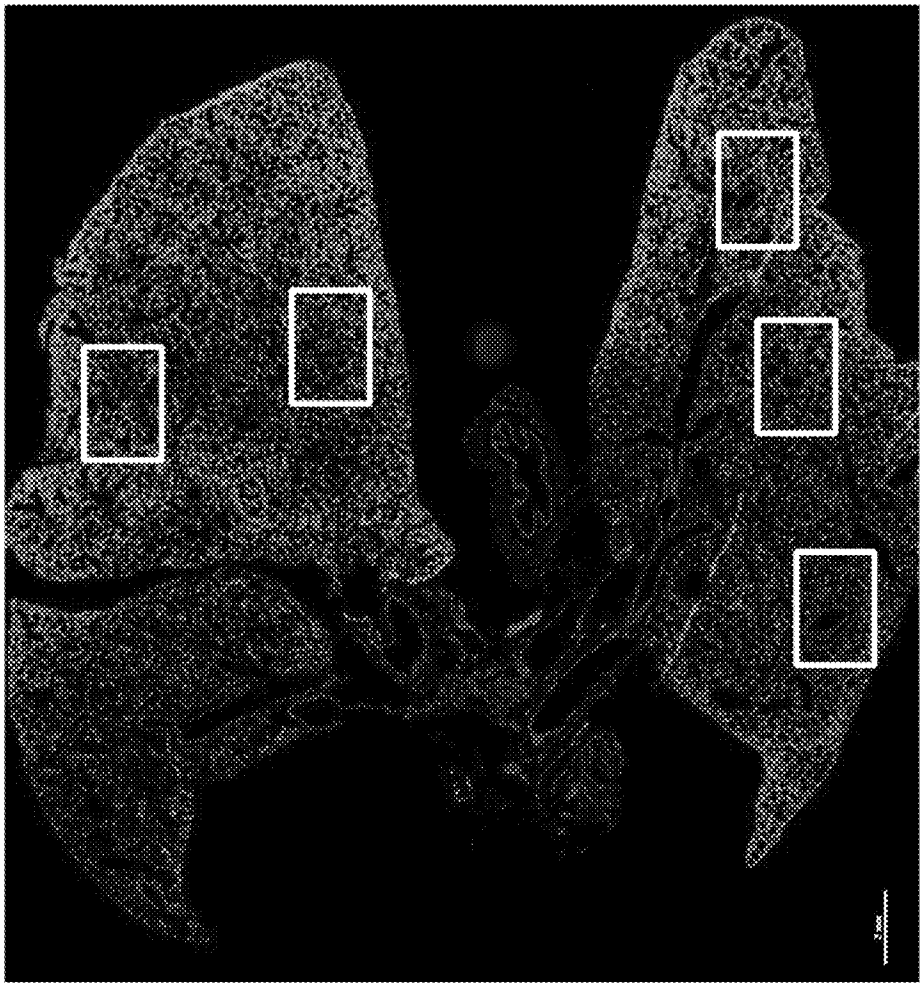


FIG. 26

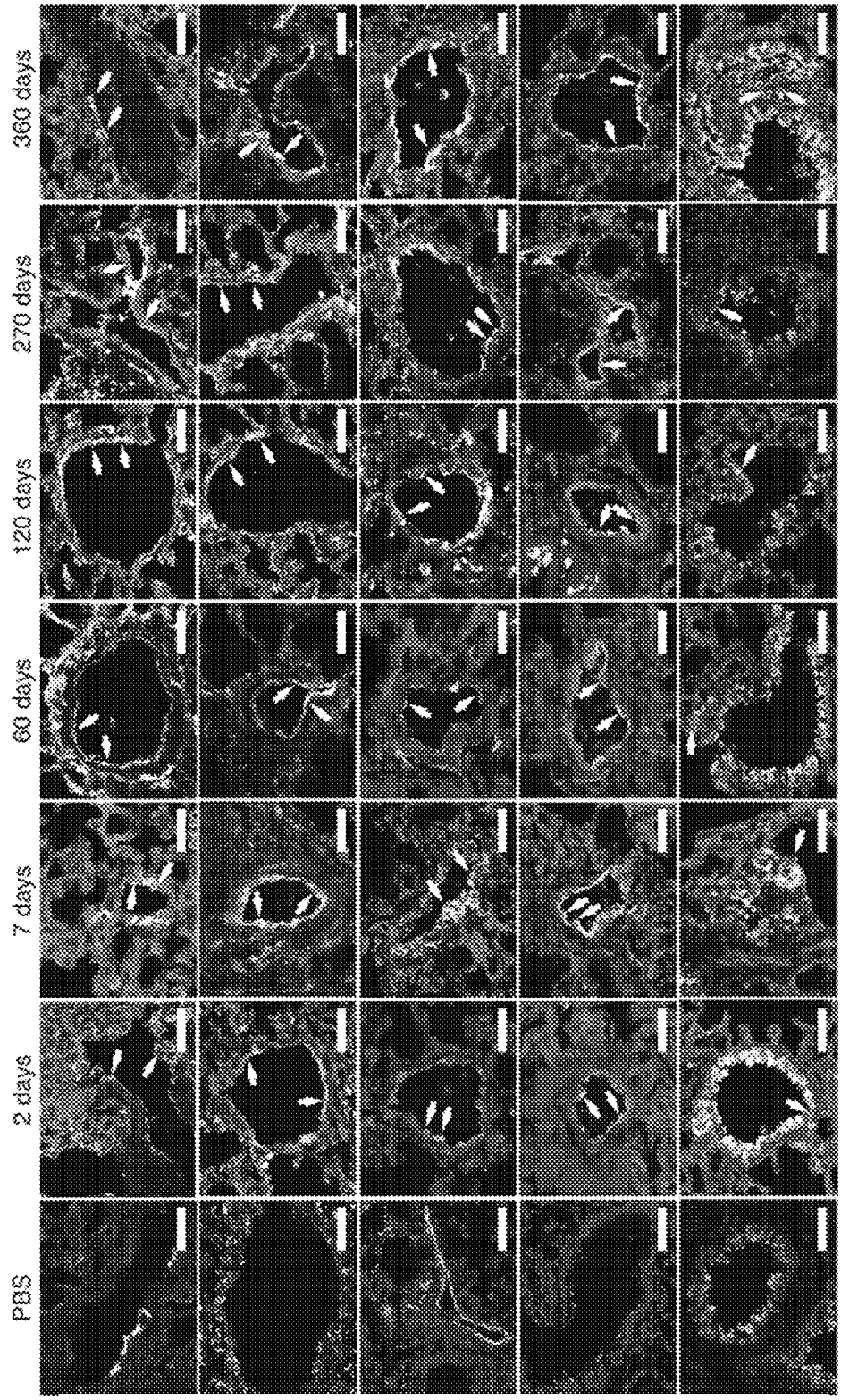

PBS
2 days
7 days
60 days
120 days
270 days
360 days
Club Cells (SCGB1A1)
Ciliated Cells (Tubulin)
Goblet Cells (MUC5AC)
AT2 Cells (ABCA3)
ATI Cells (HOPX)

FIG. 27A

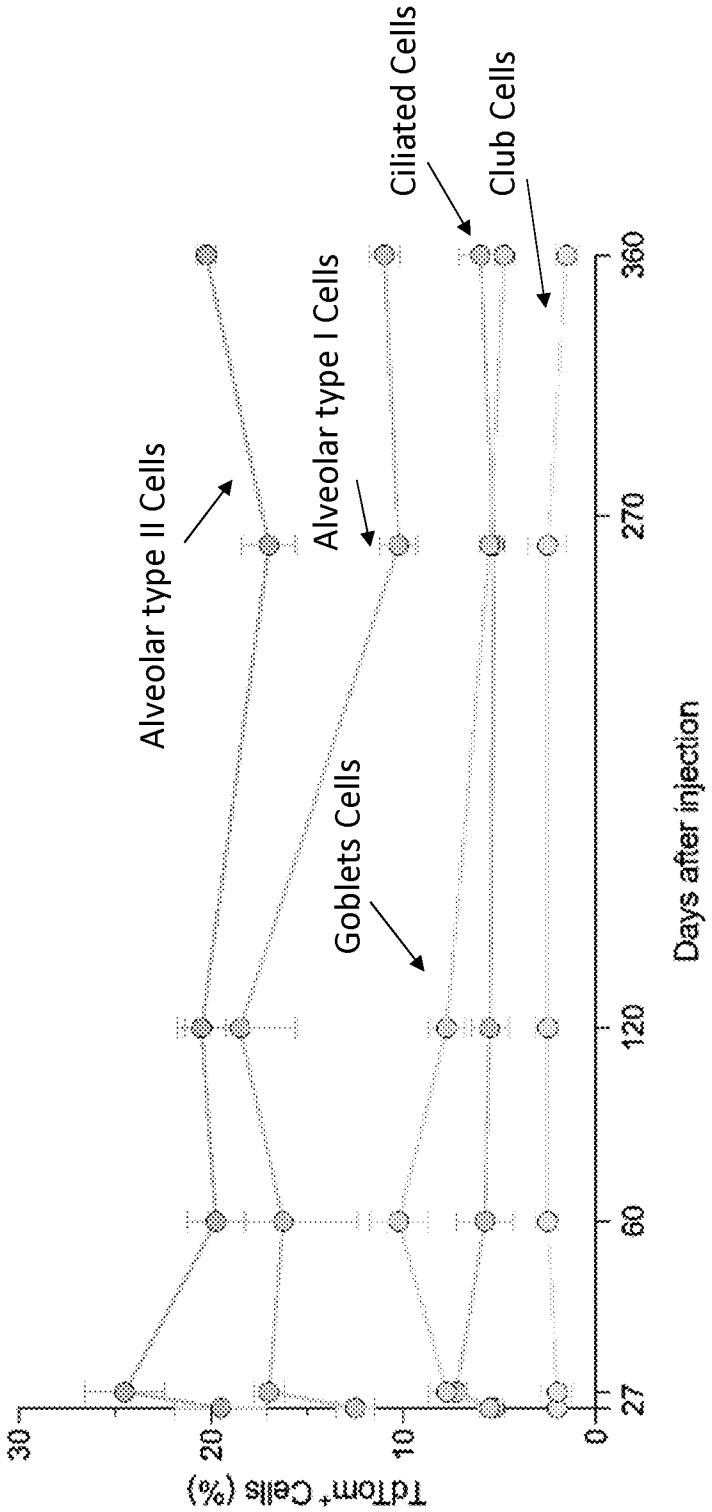

Alveolar type II Cells
Alveolar type I Cells
Goblets Cells
Ciliated Cells
Club Cells
tdTom+ Cells (%)
0
10
20
30
27
60
120
270
360
Days after injection

FIG. 27B

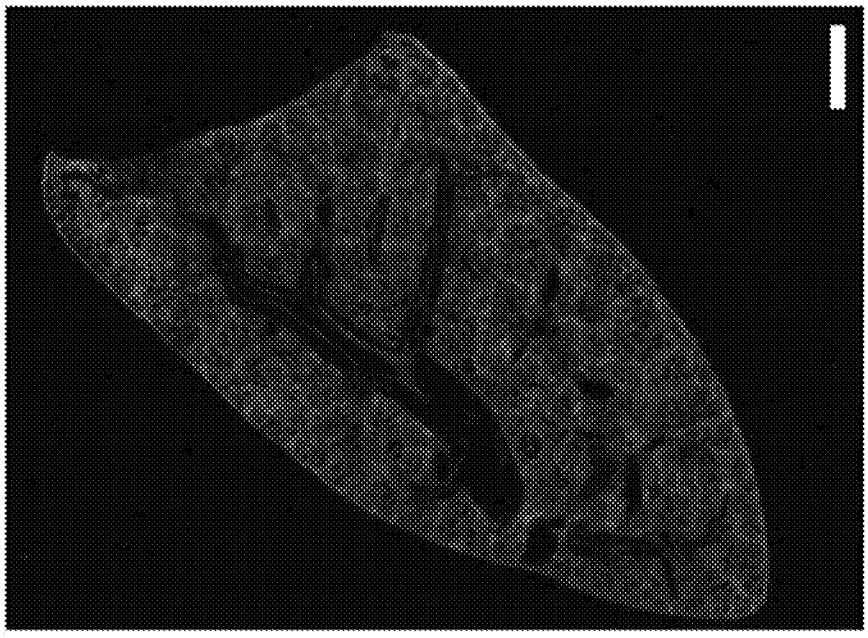


FIG. 27E

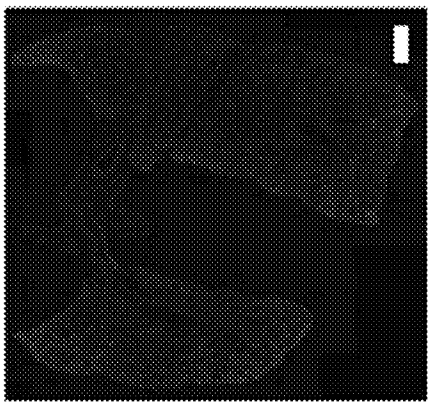


FIG. 27C

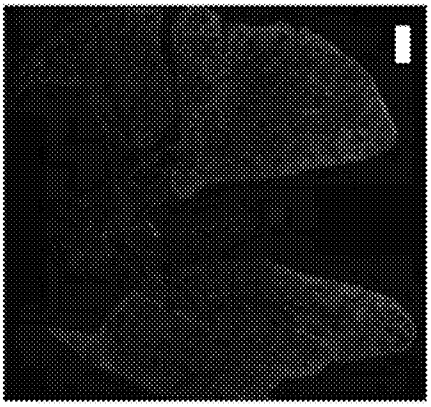


FIG. 27D

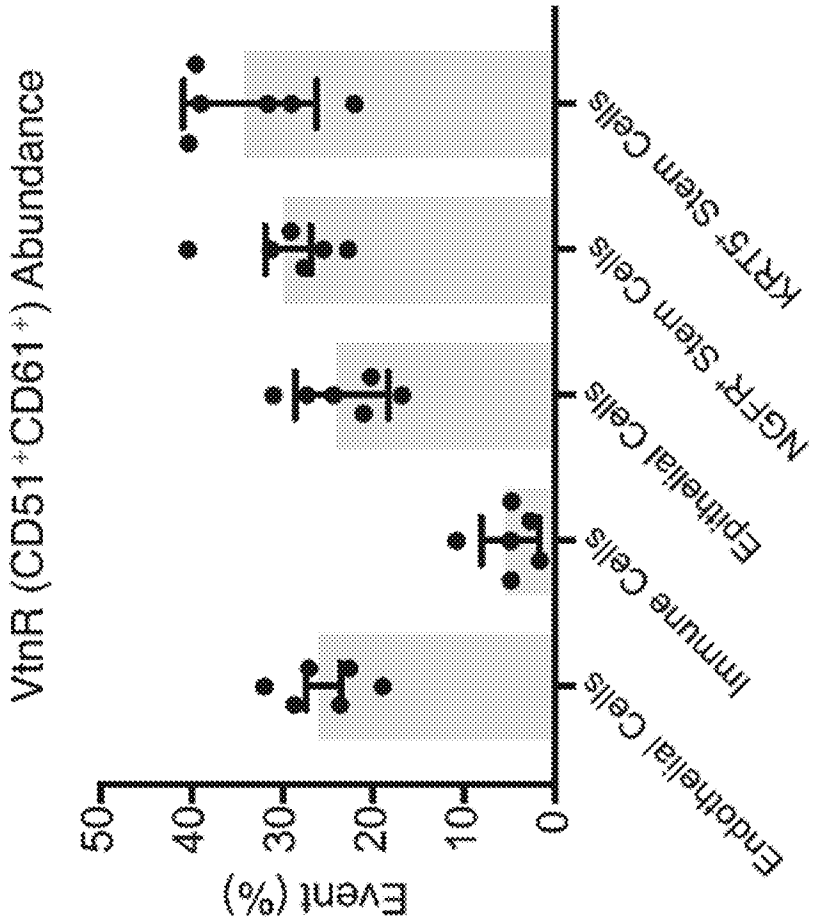

VtnR (CD51+CD61+) Abundance
Event (%)
0
10
20
30
40
50
Endothelial Cells
Immune Cells
Epithelial Cells
NGFR+ Stem Cells
KRT5+ Stem Cells

FIG. 27F

FIG. 27G

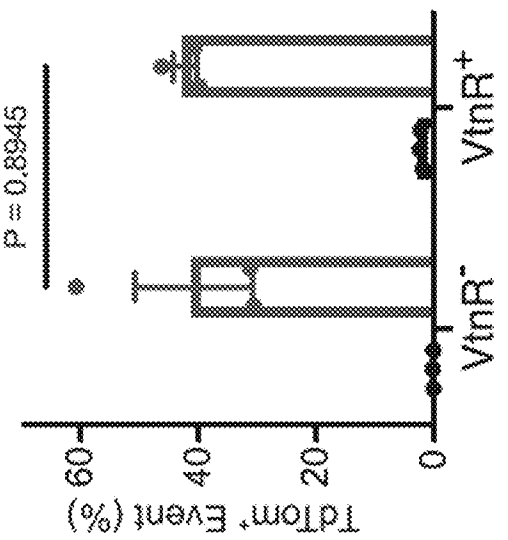


FIG. 27H

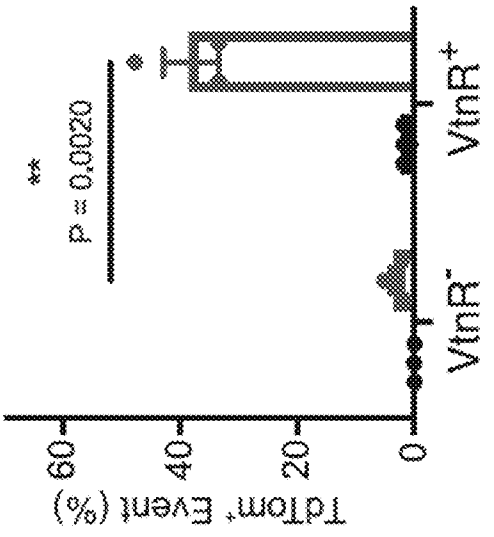


FIG. 27I

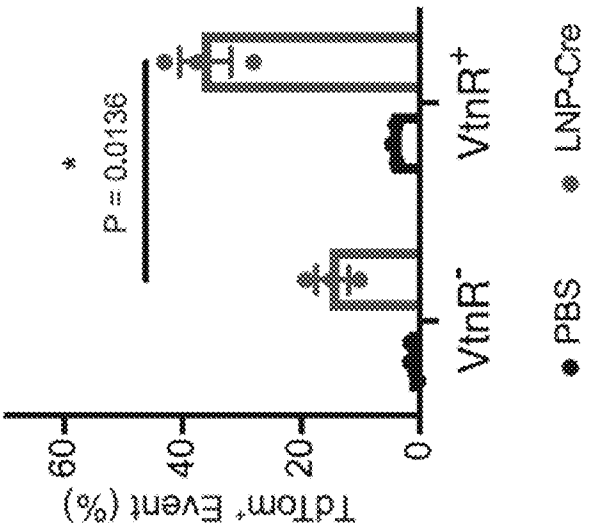


FIG. 27J

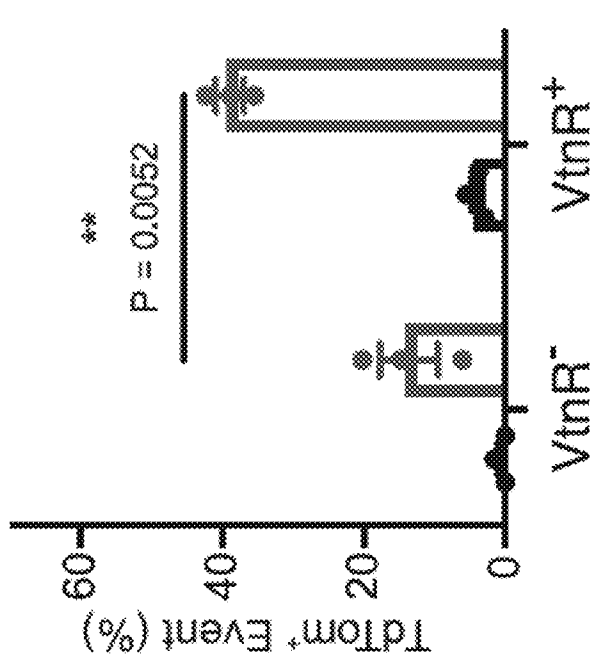
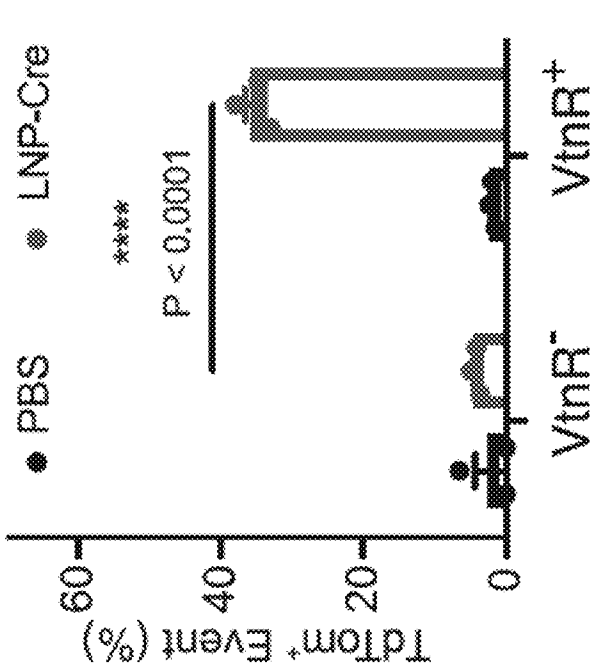


FIG. 27K



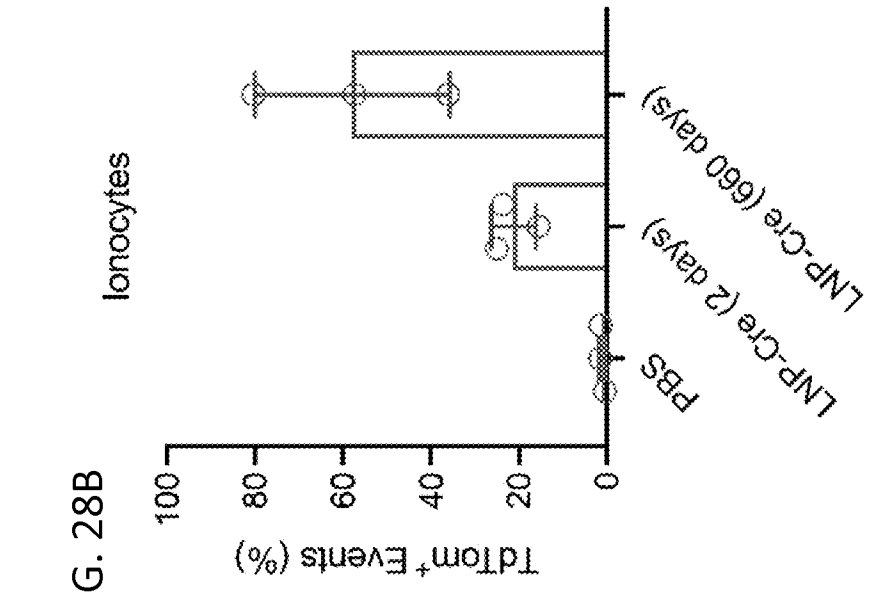


FIG. 28B

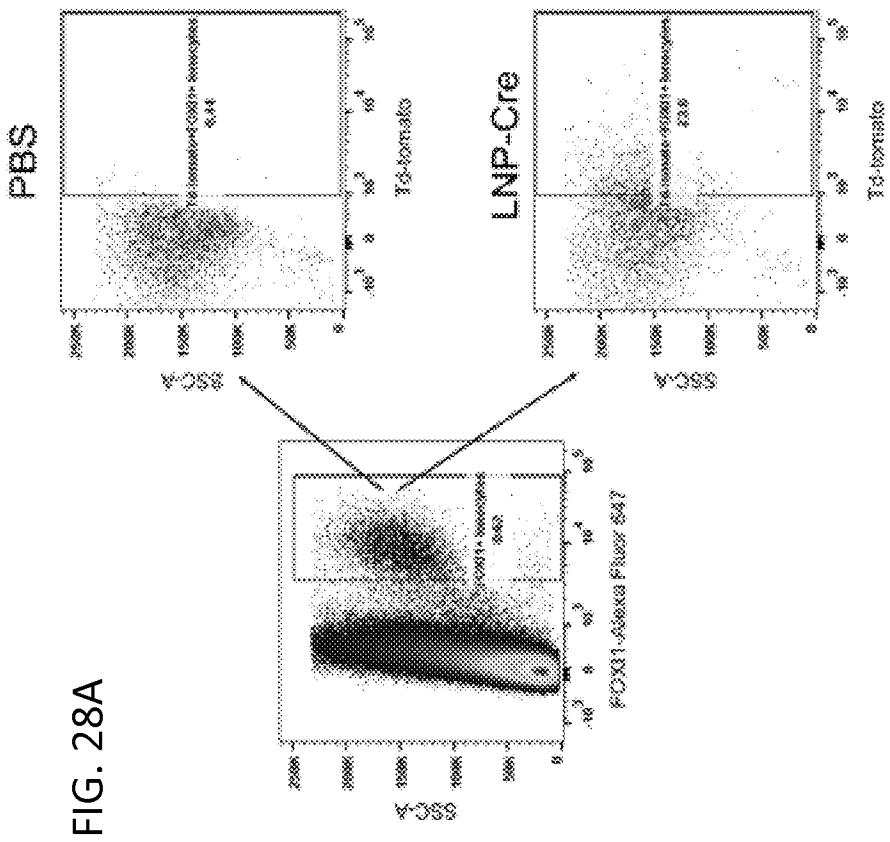


FIG. 28A

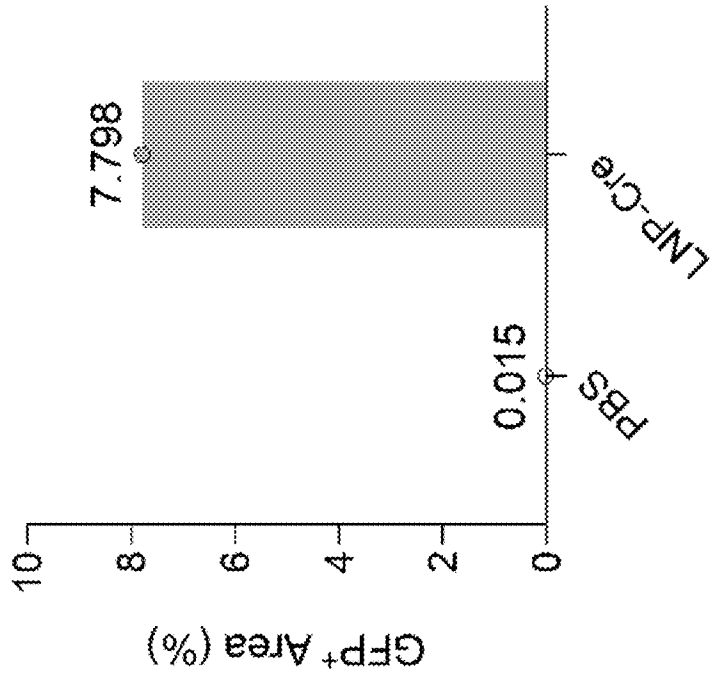


FIG. 29B

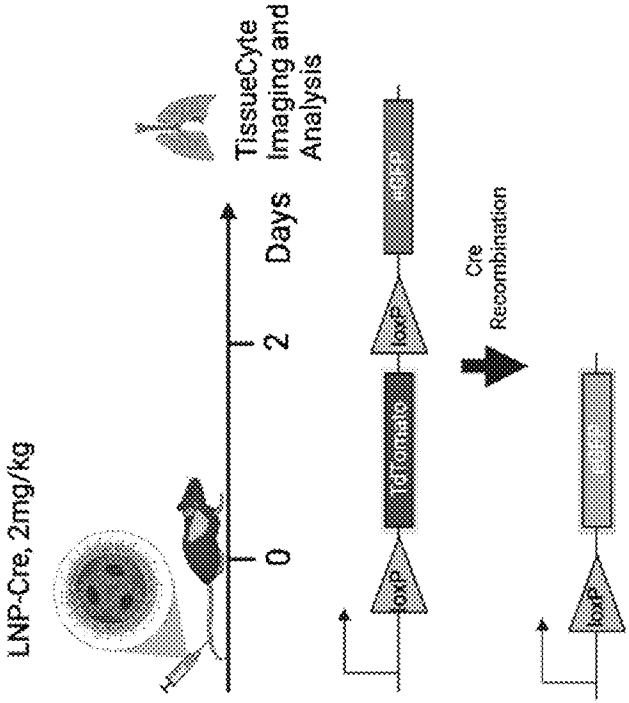


FIG. 29A

FIG. 30A

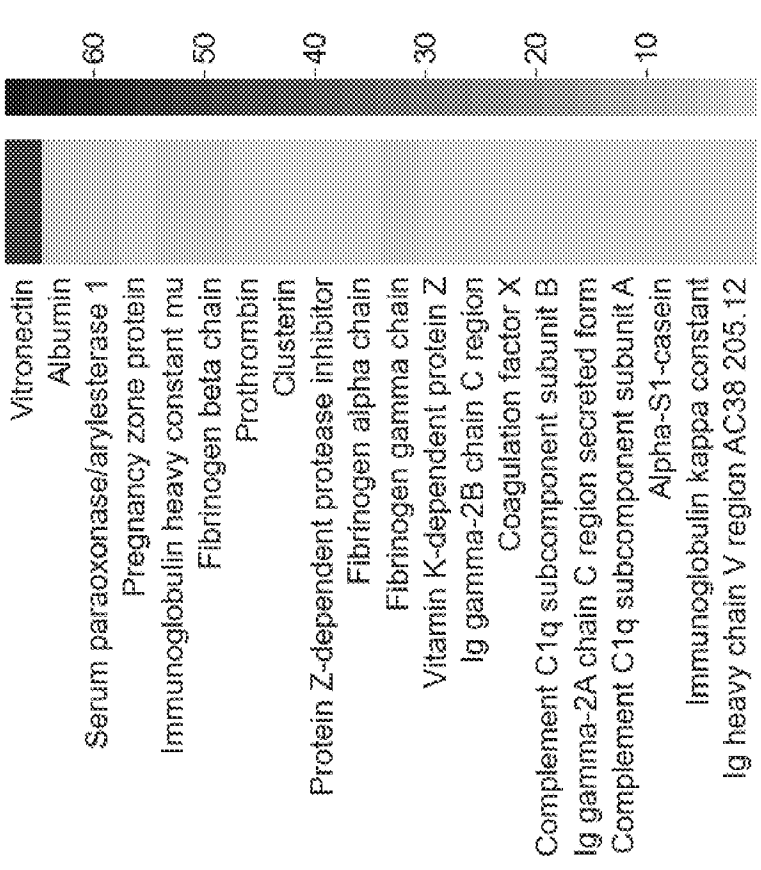


FIG. 30B

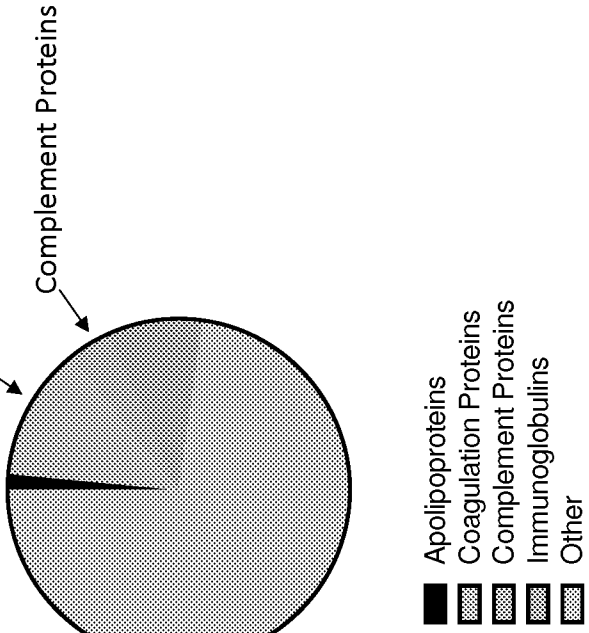


FIG. 31A

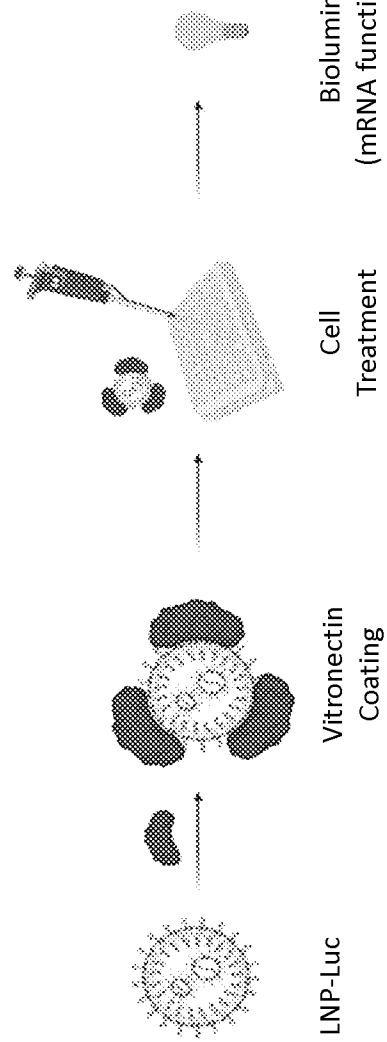


FIG. 31B

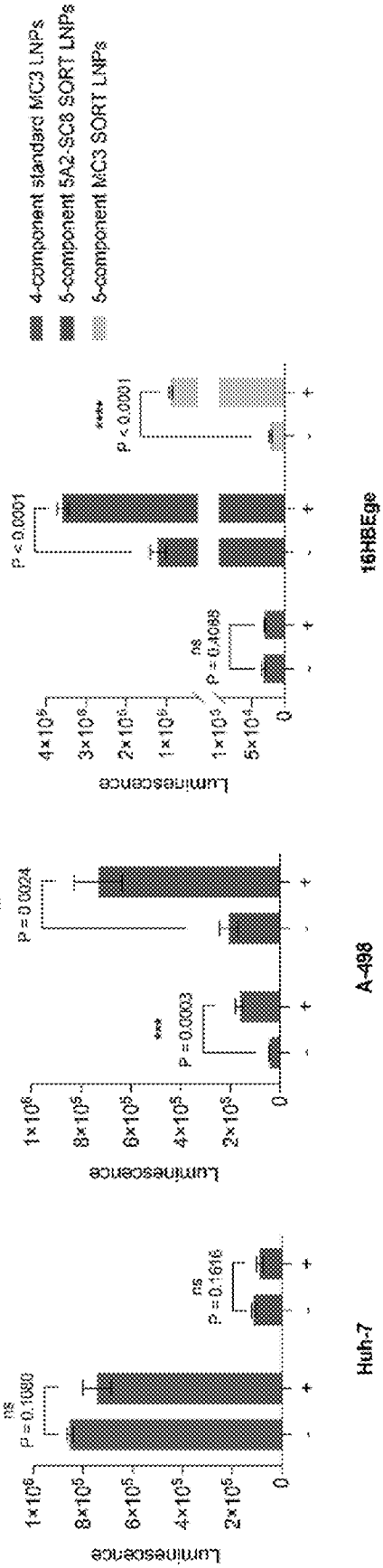


FIG. 31C

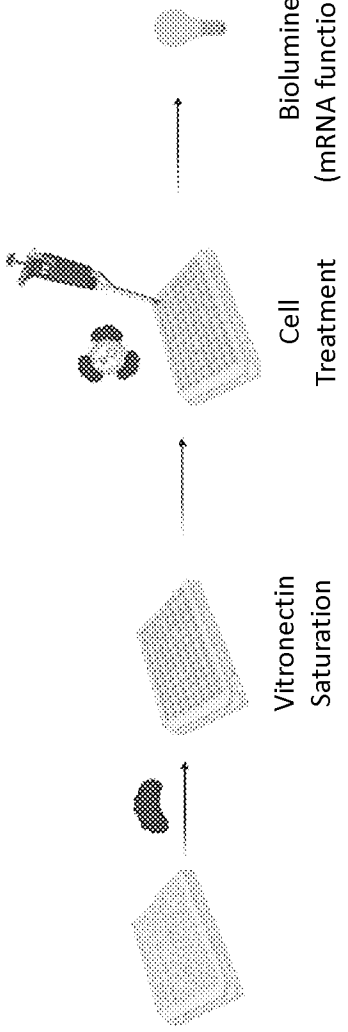


FIG. 31D

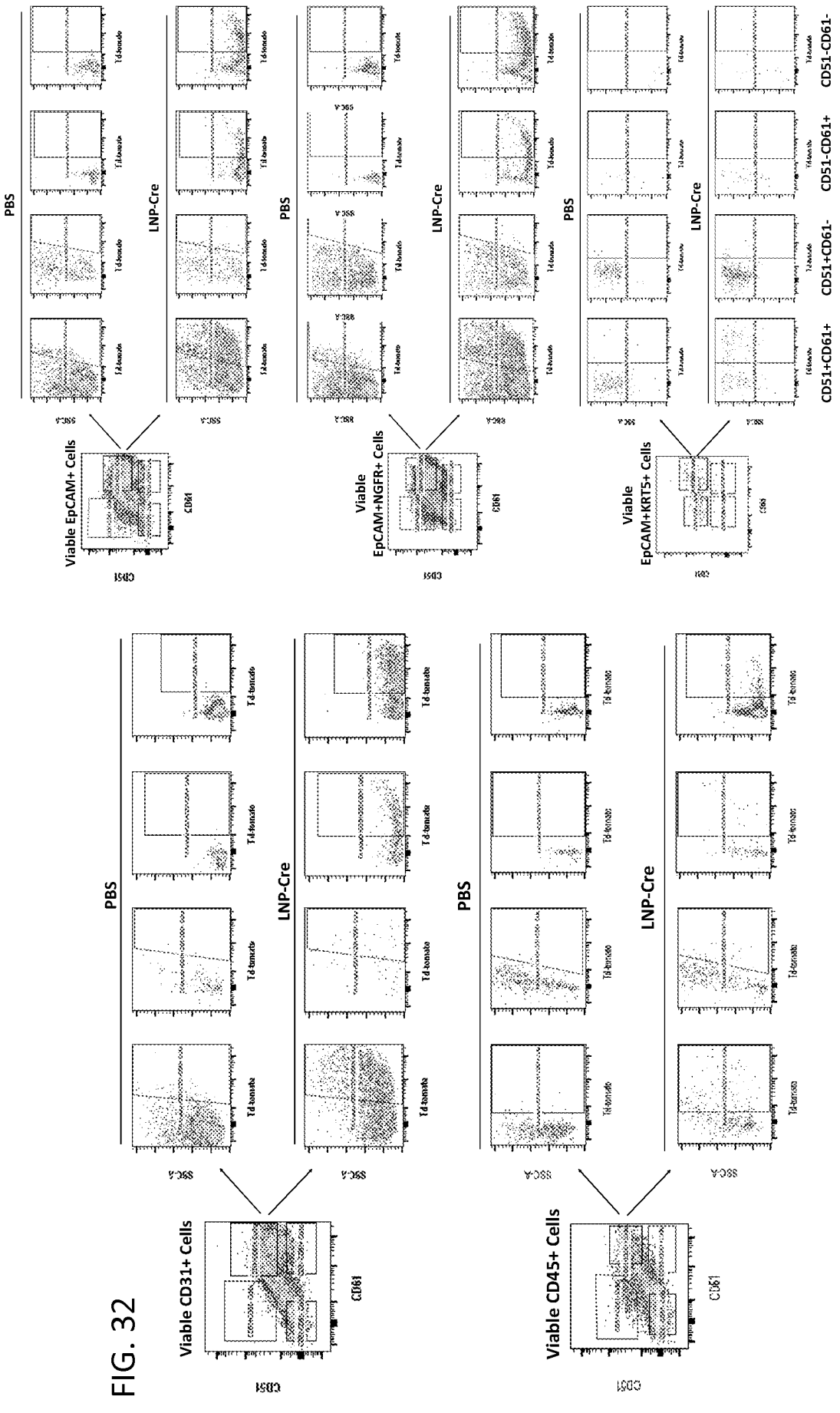

PBS
LNP-Cre
Viable CD31+ Cells
Viable CD45+ Cells
Viable EpCAM+ Cells
Viable EpCAM+NGFR+ Cells
Viable EpCAM+KRT5+ Cells
CD51+CD61+
CD51+CD61-
CD51-CD61+
CD51-CD61-
CD51
CD61
SSC-A
Td-tomato

FIG. 32

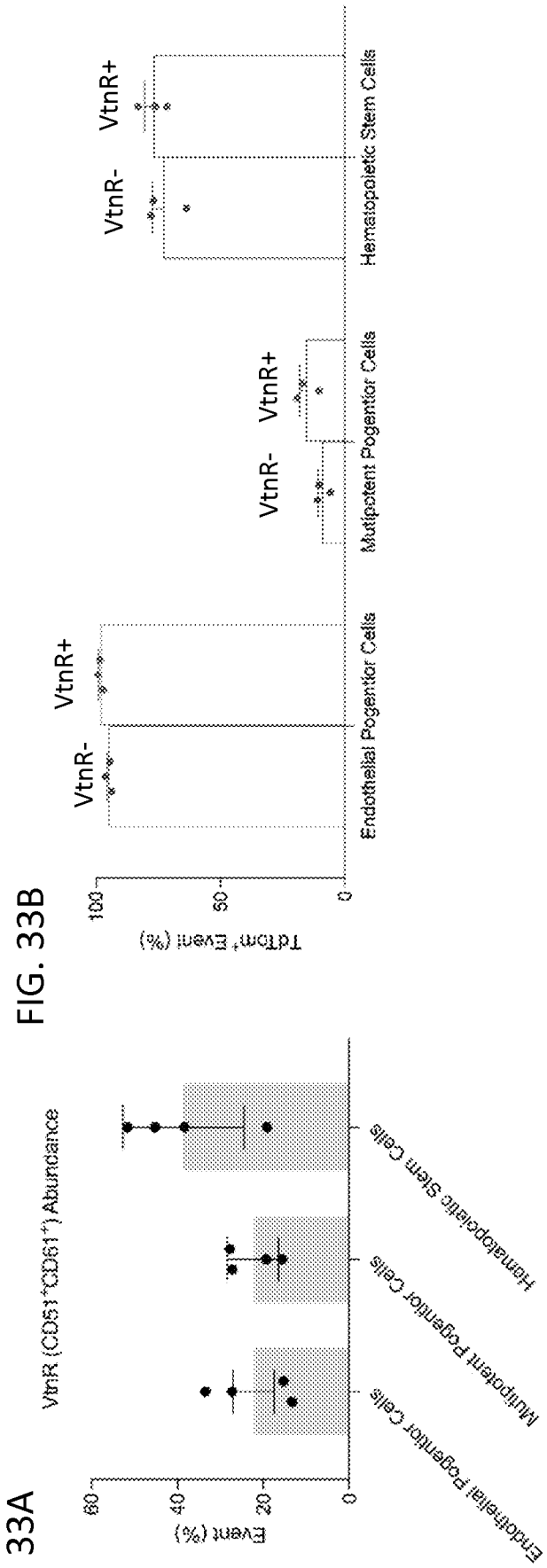


FIG. 33A

FIG. 33B

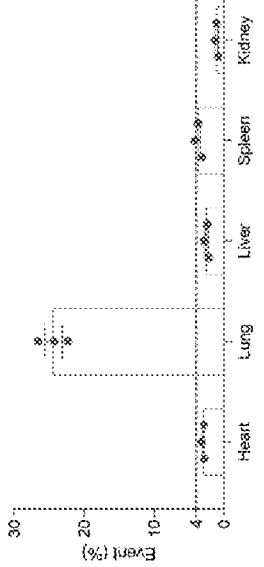


FIG. 34A

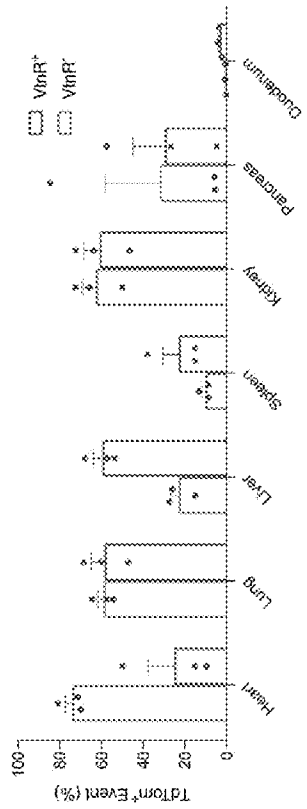


FIG. 34B

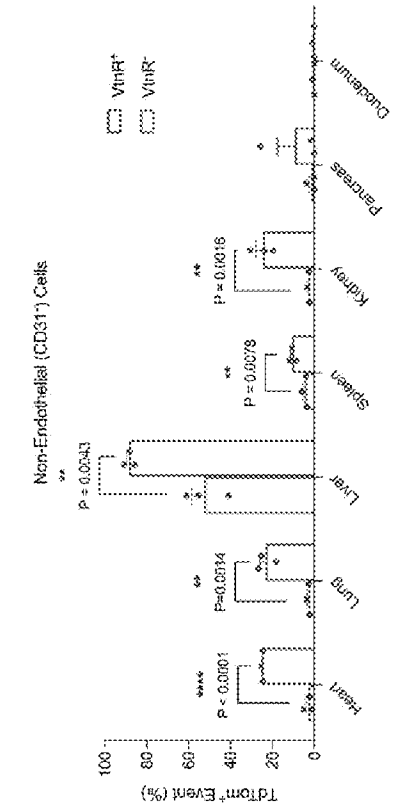


FIG. 34C

FIG. 35A

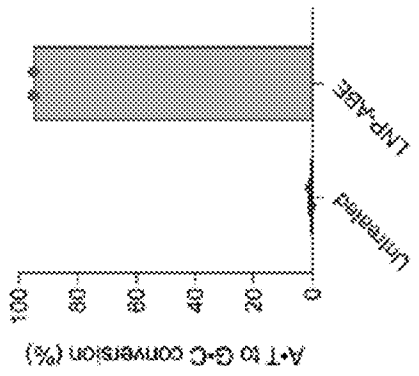


FIG. 35B

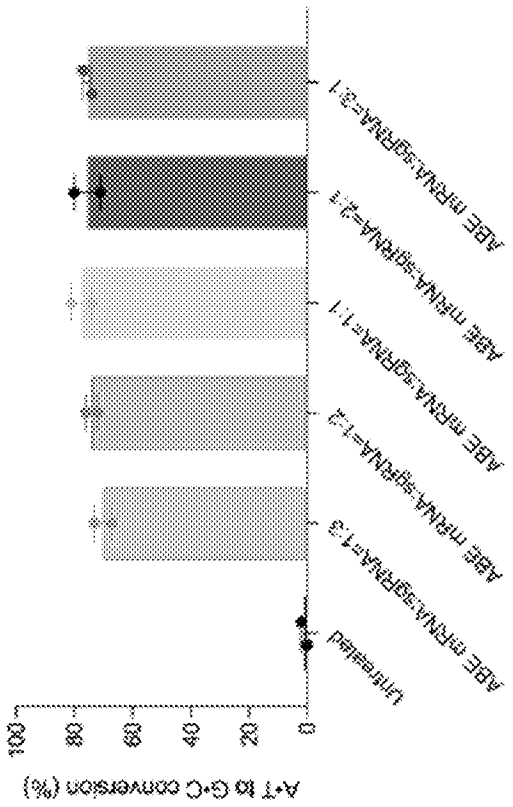
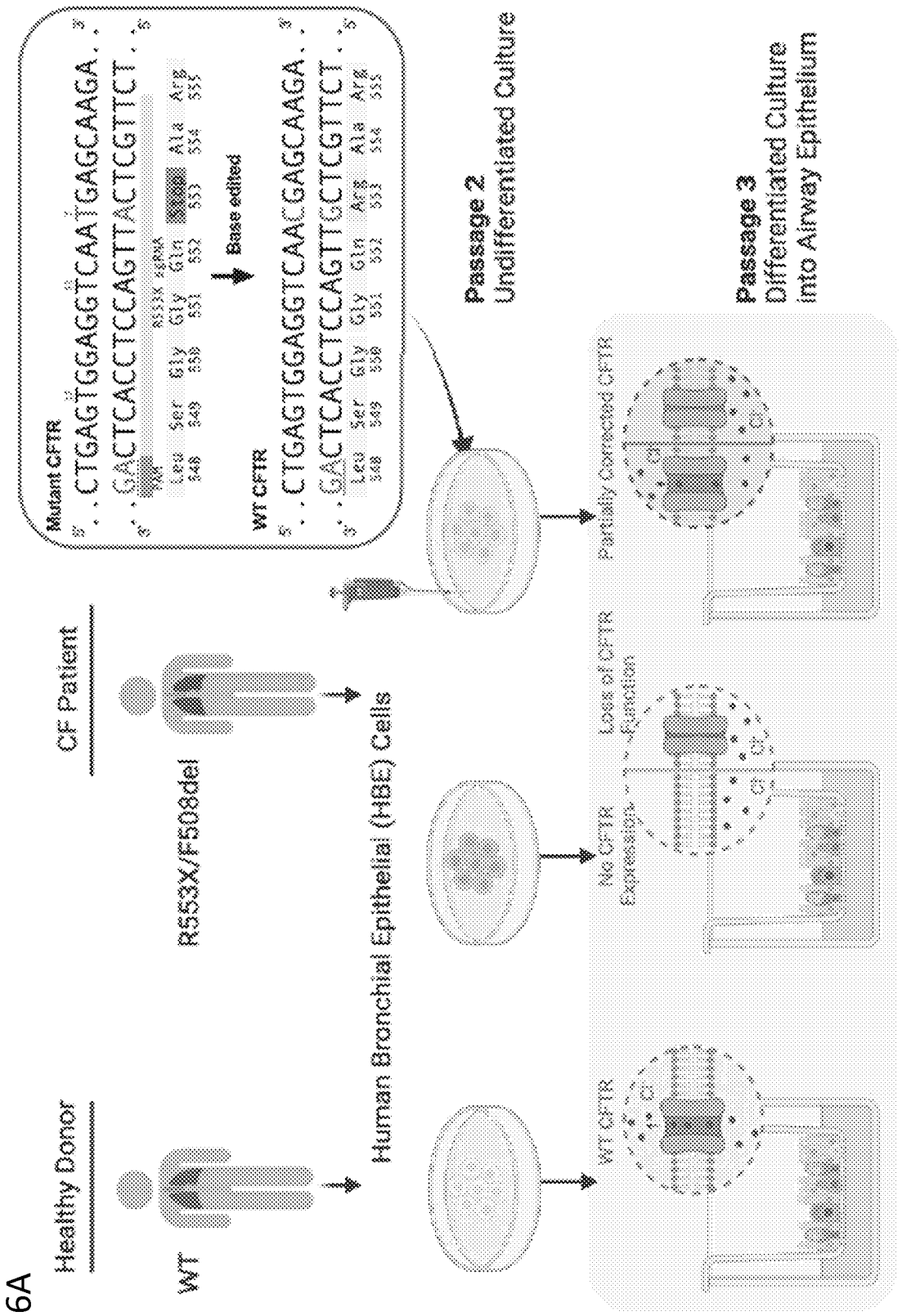


FIG. 36A


Healthy Donor
WT
CF Patient
R553X/F508del
Human Bronchial Epithelial (HBE) Cells
Mutant CFTR
WT CFTR
Base edited
Passage 2
Undifferentiated Culture
Passage 3
Differentiated Culture
into Airway Epithelium
WT CFTR
No CFTR Expression
Loss of CFTR Function
Partially Corrected CFTR

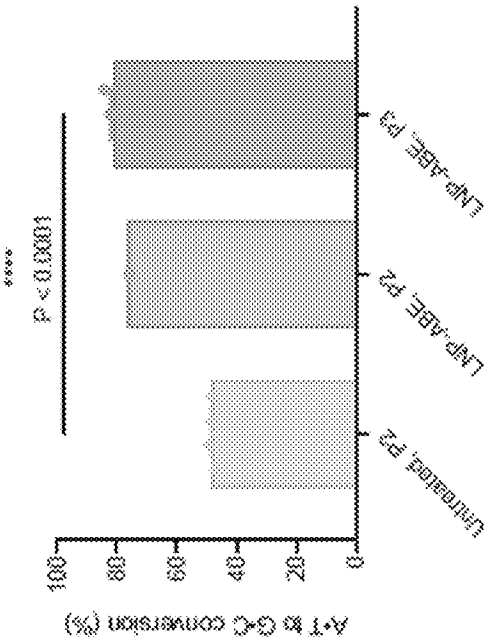


FIG. 36B

	T_7	T_{11}	T_{17}
A	0.0%	0.1%	0.0%
C	83.7%	14.5%	0.0%
G	0.0%	0.0%	0.0%
T	16.3%	85.4%	100.0%

FIG. 36C

FIG. 36D

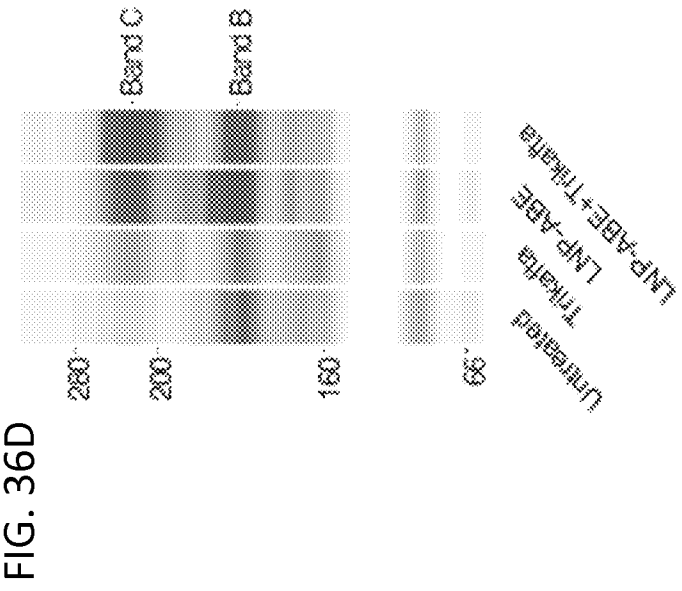


FIG. 36E

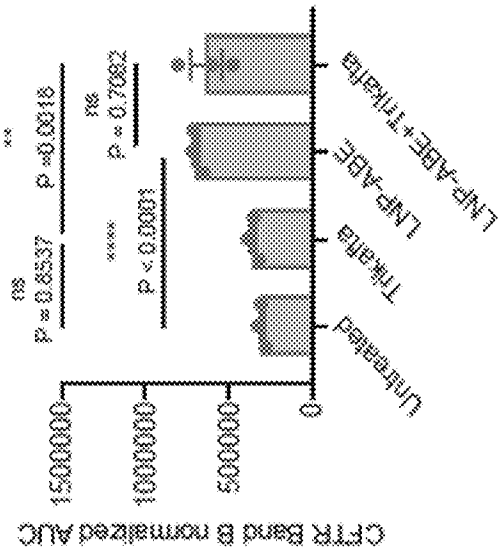


FIG. 36F

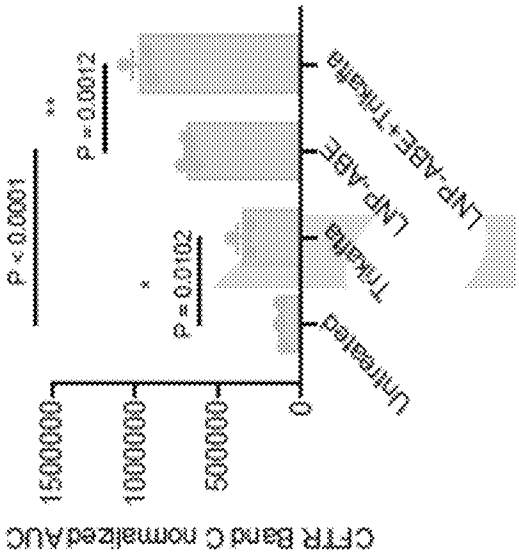


FIG. 36G

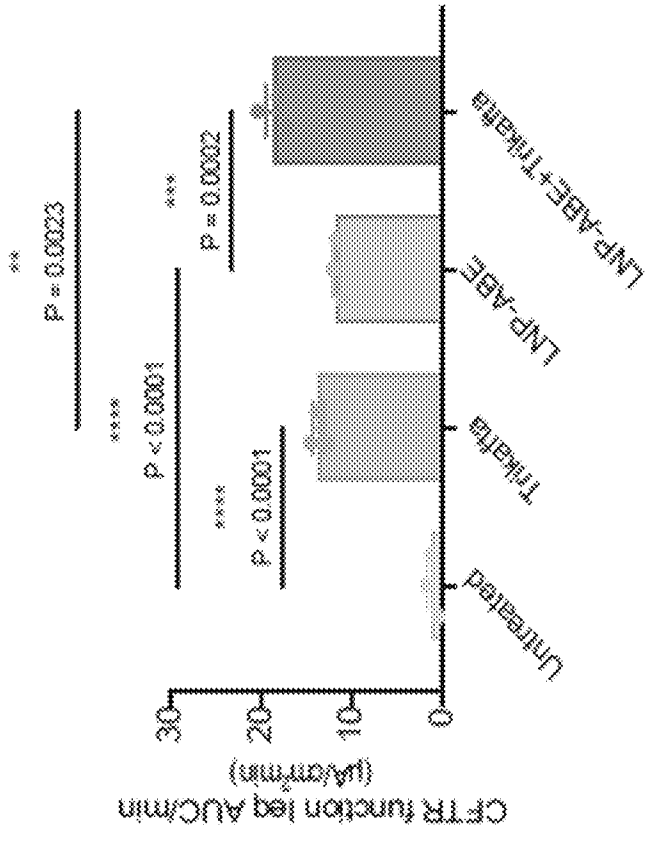


FIG. 36H

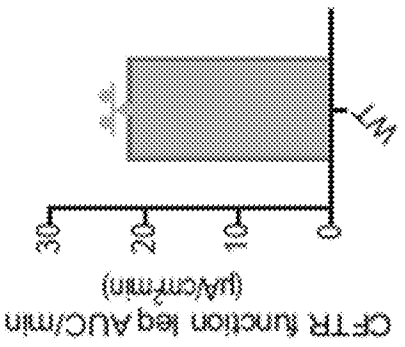


FIG. 36K

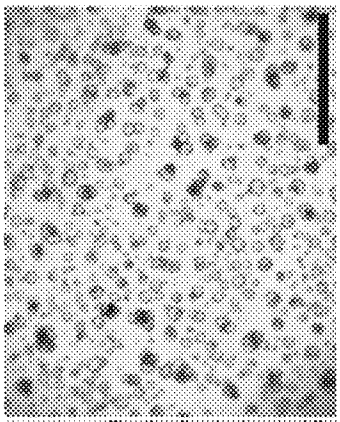


FIG. 36J

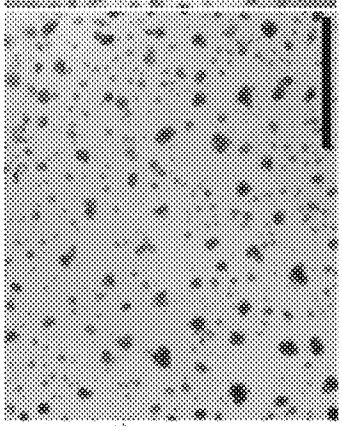


FIG. 36I

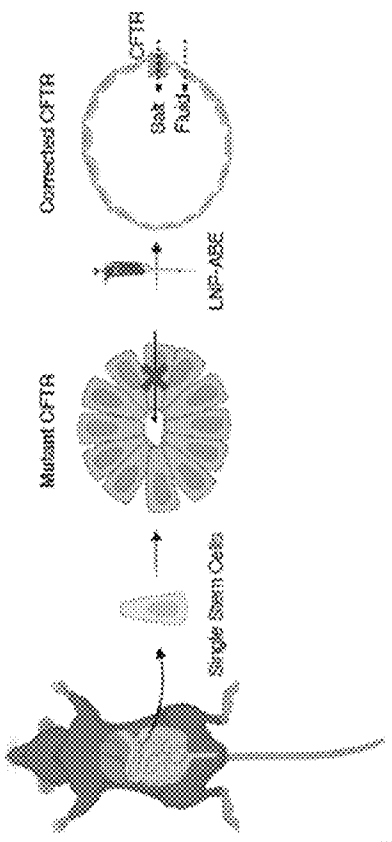


FIG. 36M

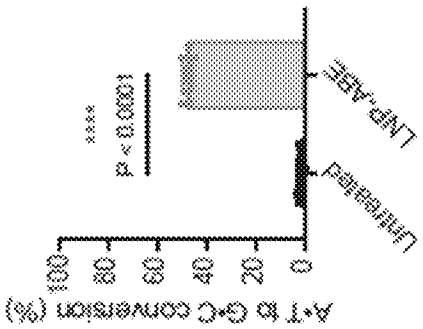


FIG. 36L

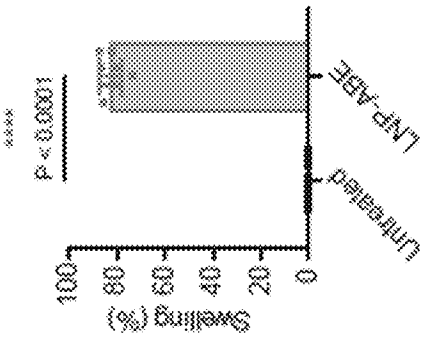


FIG. 36N

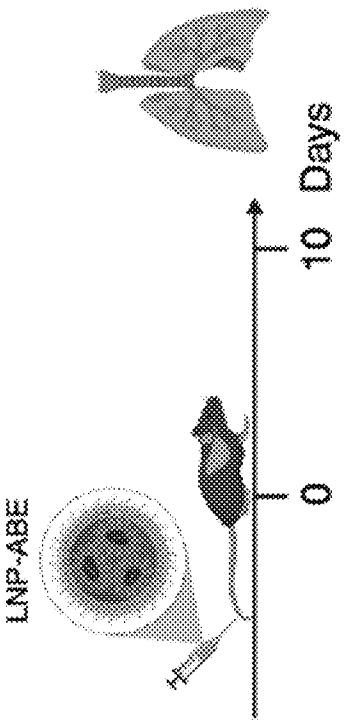


FIG. 36O

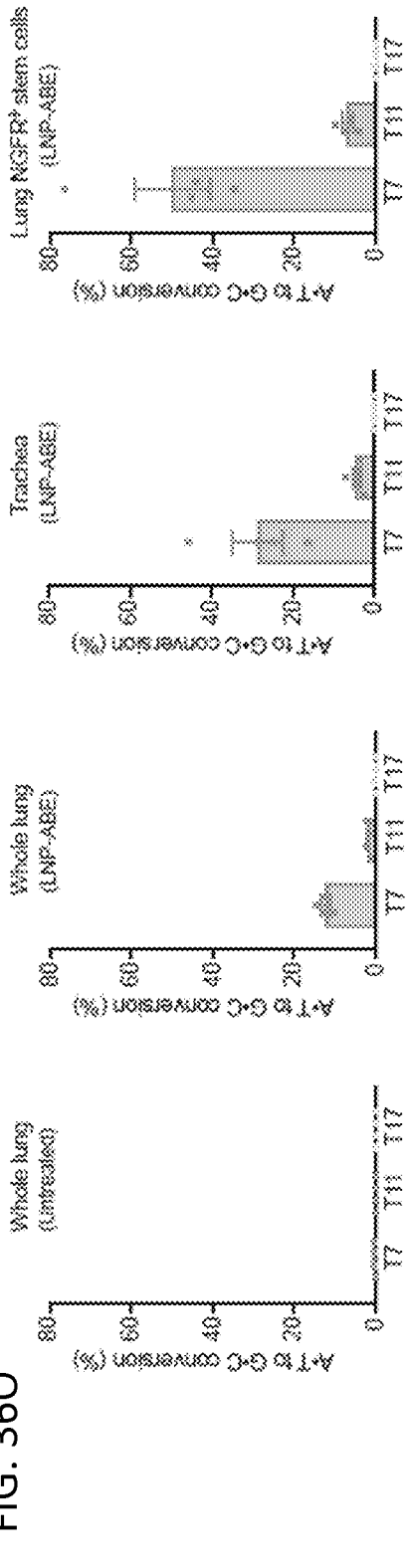


FIG. 37B

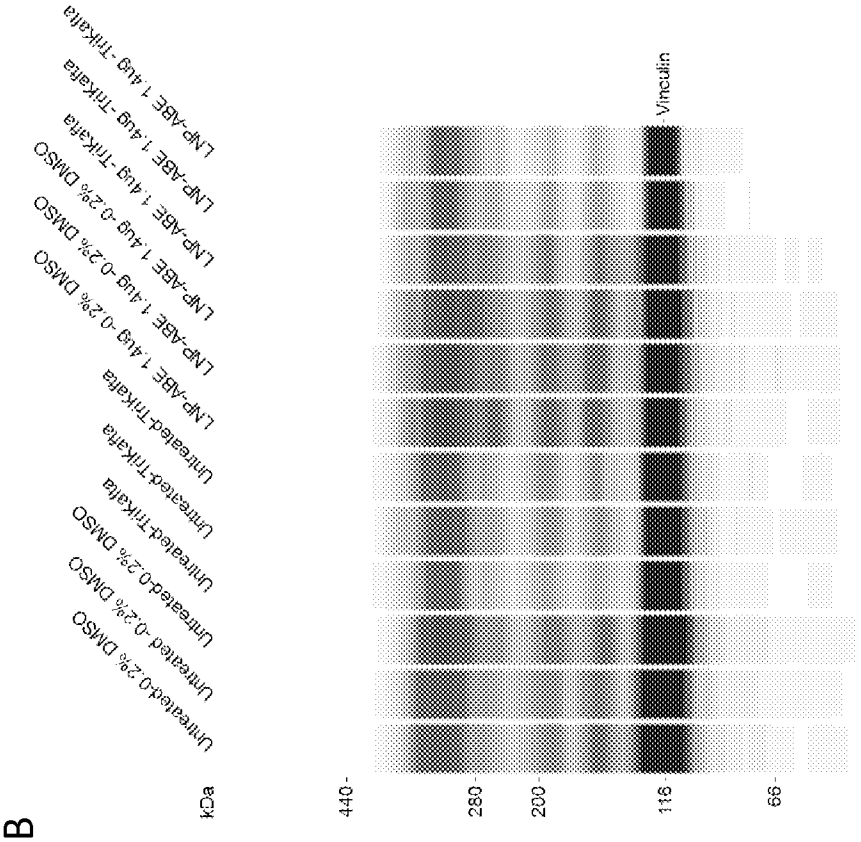


FIG. 37A

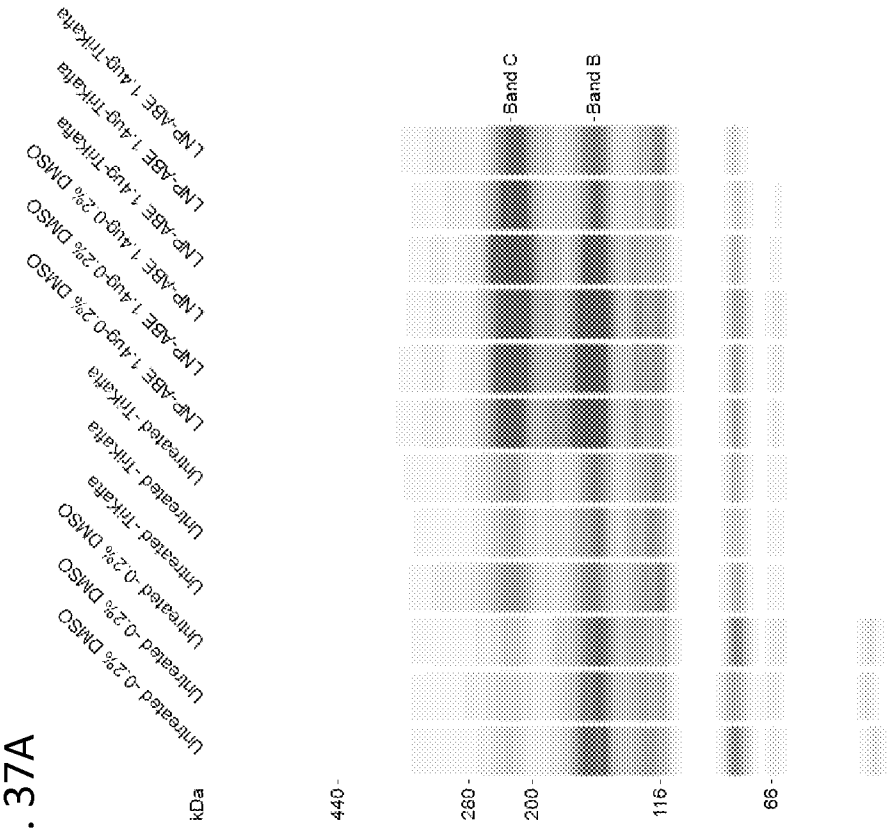


FIG. 38A

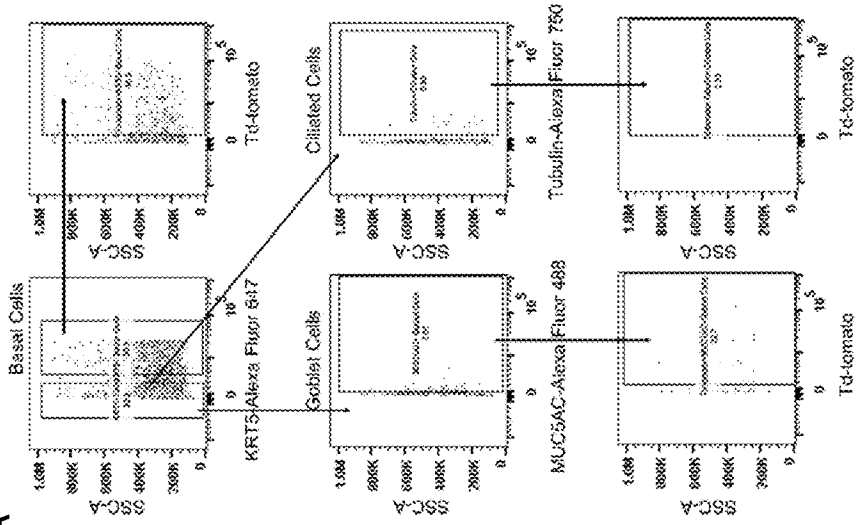
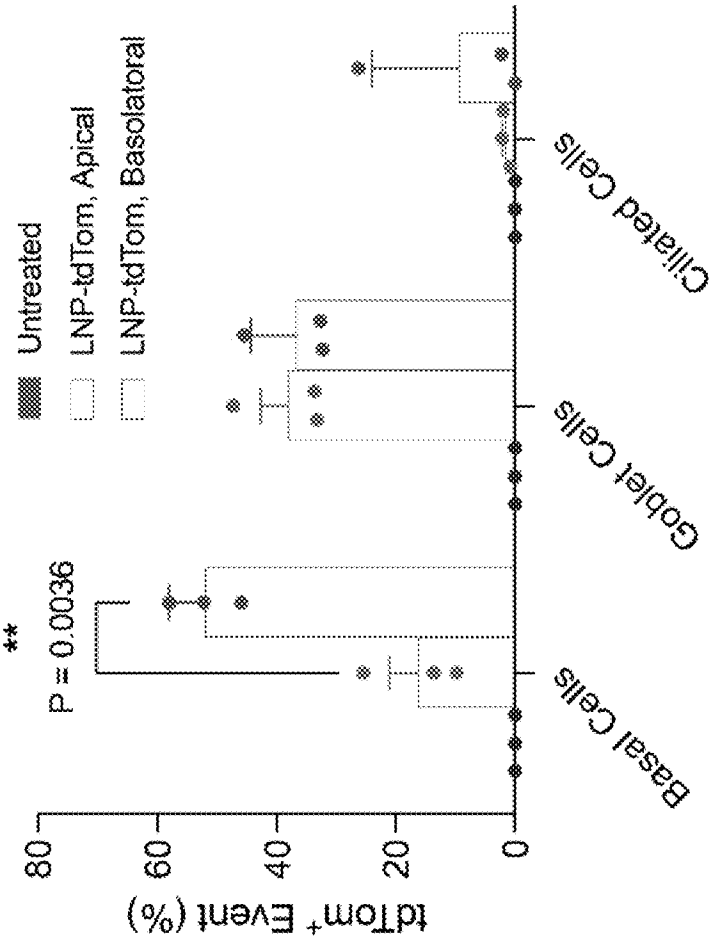


FIG. 38B



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2024/061579

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K48/00 C12N9/22 C12N15/88
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2022/216619 A1 (UNIV TEXAS [US]; RECODE THERAPEUTICS INC [US]) 13 October 2022 (2022-10-13)	15-20, 22, 35-39, 43, 49-52, 54-56, 58-61, 161
Y	claims 1-26; examples 1, 4-5	1-14,21, 23-33, 40-42, 44, 46-48, 53,57, 62-160

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 March 2025	01/04/2025

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Salminen, Aaro

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2024/061579

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WEI TUO ET AL: "Lung SORT LNPs enable precise homology-directed repair mediated CRISPR/Cas genome correction in cystic fibrosis models", NATURE COMMUNICATIONS, vol. 14, no. 1, 11 November 2023 (2023-11-11), XP093260752, UK ISSN: 2041-1723, DOI: 10.1038/s41467-023-42948-2 page 4, right-hand column, paragraph 4 - page 8, left-hand column, paragraph 3; figures 4-5 page 9, right-hand column, paragraph 1 page 10, left-hand column, paragraph 5 - right-hand column, paragraph 2	15-20, 22, 34-39, 43,45, 49,50, 52, 54-56, 58-61, 161

X	KULHANKOVA KATARINA ET AL: "Shuttle peptide delivers base editor RNPs to rhesus monkey airway epithelial cells in vivo", NATURE COMMUNICATIONS, vol. 14, no. 1, 5 December 2023 (2023-12-05), XP093260718, UK ISSN: 2041-1723, DOI: 10.1038/s41467-023-43904-w	161
Y	page 8, left-hand column, paragraph 2; figure 6	1-14,21, 23-33, 40-42, 44, 46-48, 53,57, 62-160

X	GEURTS MAARTEN H. ET AL: "CRISPR-Based Adenine Editors Correct Nonsense Mutations in a Cystic Fibrosis Organoid Biobank", CELL STEM CELL, vol. 26, no. 4, 2 April 2020 (2020-04-02), pages 503-510.e7, XP093260874, AMSTERDAM, NL ISSN: 1934-5909, DOI: 10.1016/j.stem.2020.01.019	161
Y	page 505, left-hand column, paragraph 3 - right-hand column, paragraph 2; figure 3	1-14,21, 23-33, 40-42, 44, 46-48, 53,57, 62-160

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2024/061579

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	US 2024/261440 A1 (WEI TUO [US] ET AL) 8 August 2024 (2024-08-08) claims 1-26; figures 1, 4	15-61, 161
X	CHENG QIANG ET AL: "Selective organ targeting (SORT) nanoparticles for tissue-specific mRNA delivery and CRISPR-Cas gene editing", NATURE NANOTECHNOLOGY, NATURE PUB. GROUP, INC, LONDON, vol. 15, no. 4, 1 April 2020 (2020-04-01), pages 313-320, XP037096153, ISSN: 1748-3387, DOI: 10.1038/S41565-020-0669-6 [retrieved on 2020-04-06] the whole document	161
X	WO 2022/204219 A1 (RECODE THERAPEUTICS INC [US]; UNIV TEXAS [US]) 29 September 2022 (2022-09-29) the whole document	161
X	WO 2020/051220 A1 (THE BOARD OF THE REGENTS OF THE UNIV OF TEXAS SYSTEM [US]) 12 March 2020 (2020-03-12) the whole document	161

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/061579

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2024/061579

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2022216619 A1	13-10-2022	AU 2022253004 A1	12-10-2023
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		US 2024207178 A1	27-06-2024
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		US 2024245618 A1	25-07-2024
		WO 2020051220 A1	12-03-2020