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(54) ICE-BASED LIPID NANOPARTICLE  
FORMULATIONS FOR DELIVERY OF mRNA

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(21) Appl. No.: 19/023,800

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- (60) Provisional application No. 62/464,330, filed on Feb. 27, 2017; provisional application No. 62/464,327, filed on Feb. 27, 2017; provisional application No. 62/421,021, filed on Nov. 11, 2016; provisional application No. 62/421,007, filed on Nov. 11, 2016; provisional application No. 62/420,428, filed on Nov. 10, 2016; provisional application No. 62/420,421, filed on Nov. 10, 2016.

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## ABSTRACT

The present invention provides, among other things, compositions and methods of formulating nucleic acid-containing nanoparticles comprising no more than three distinct lipids components, one distinct lipid component being a sterol-based cationic lipid. In some embodiments, the present invention provides compositions and methods in which the lipid nanoparticles further comprise helper lipids and PEG-modified lipids. The resulting formulation comprises a high encapsulation percentage for nucleic acids.

Specification includes a Sequence Listing.

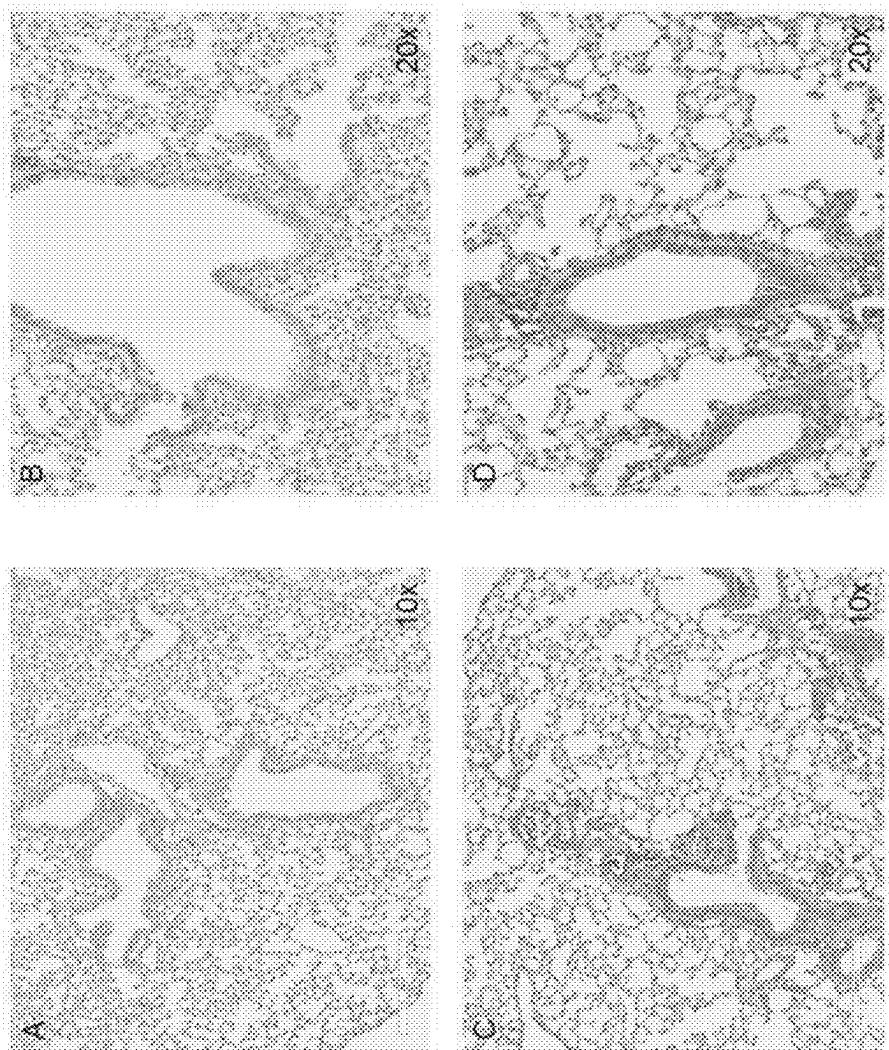


FIGURE 1

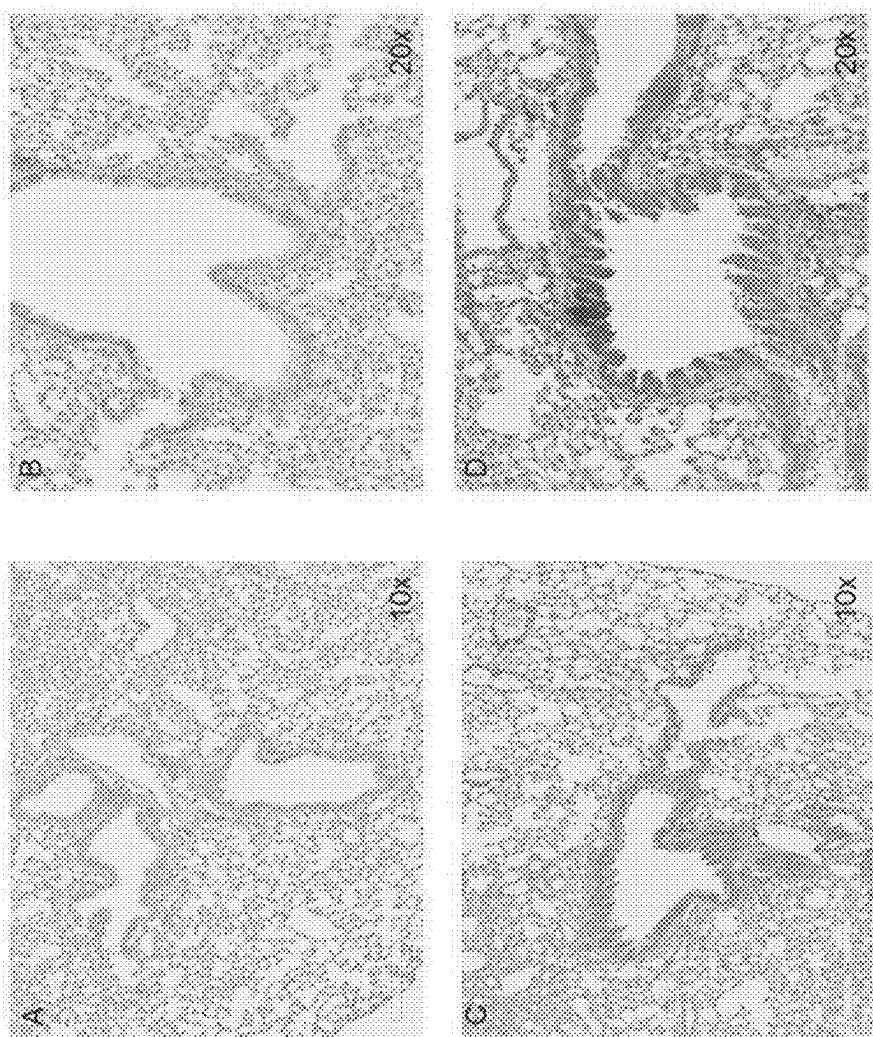


FIGURE 2

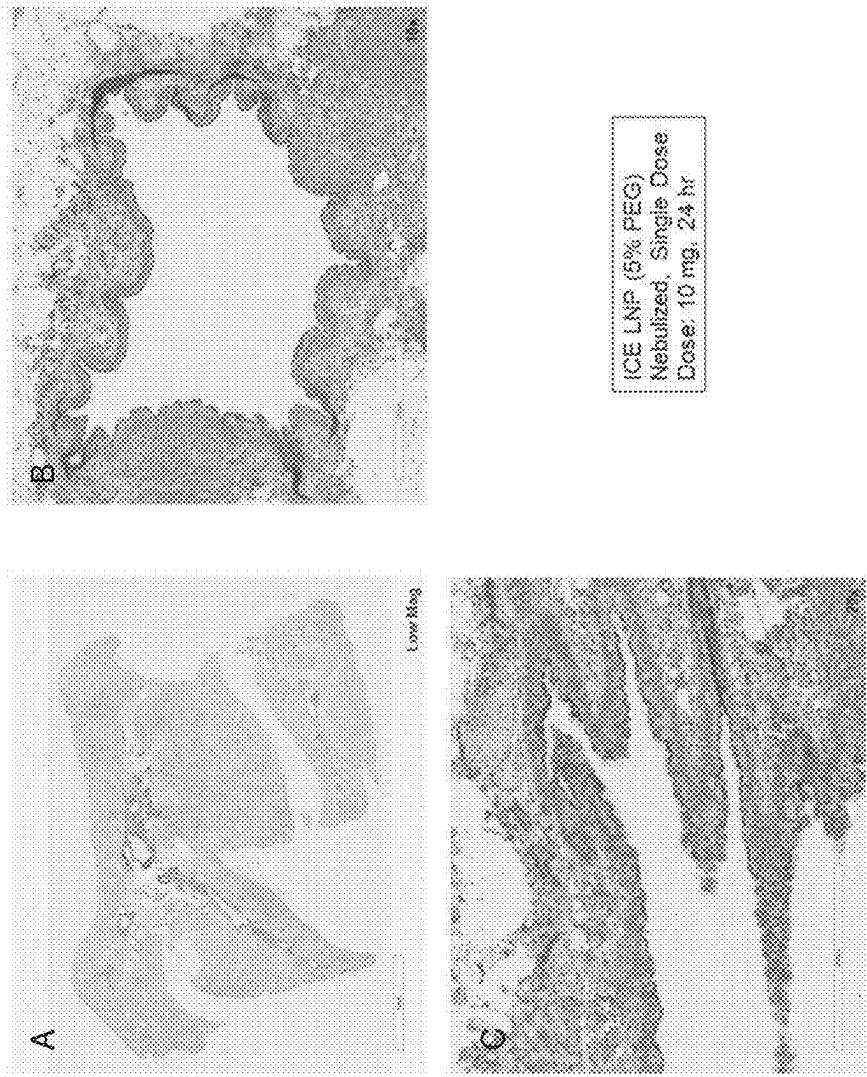
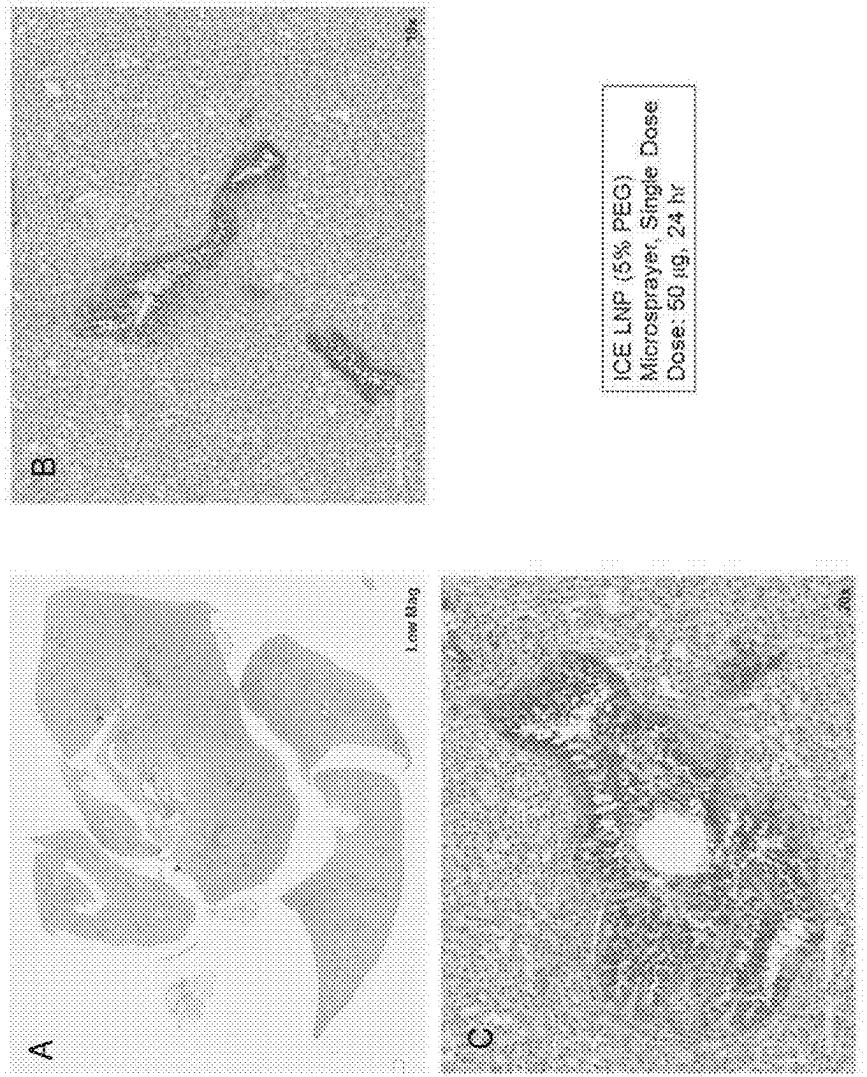


FIGURE 3



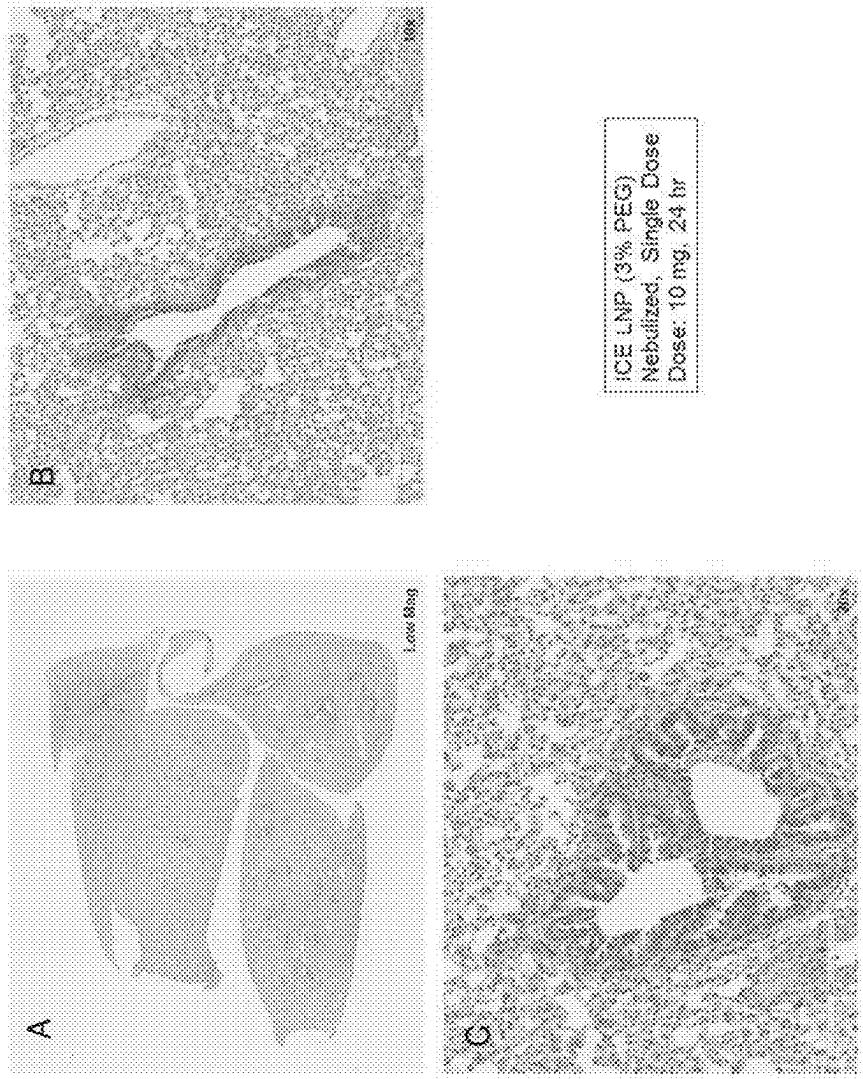


FIGURE 5

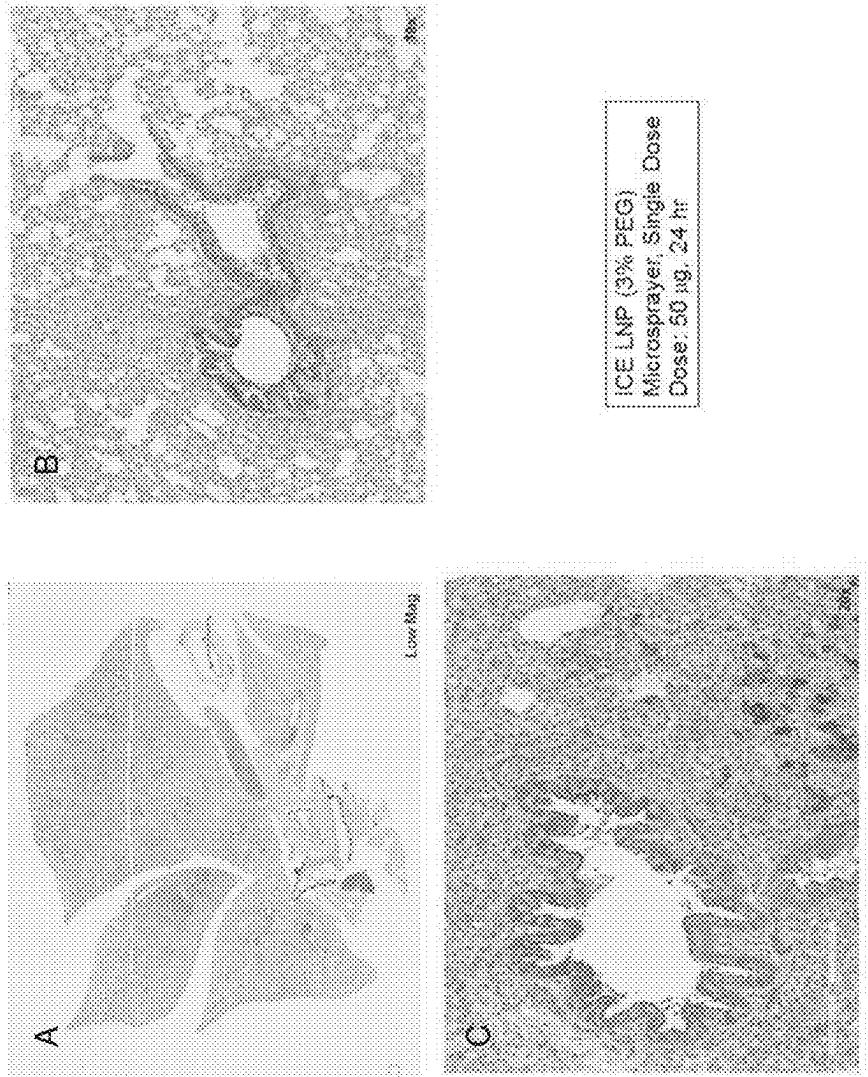


FIGURE 6

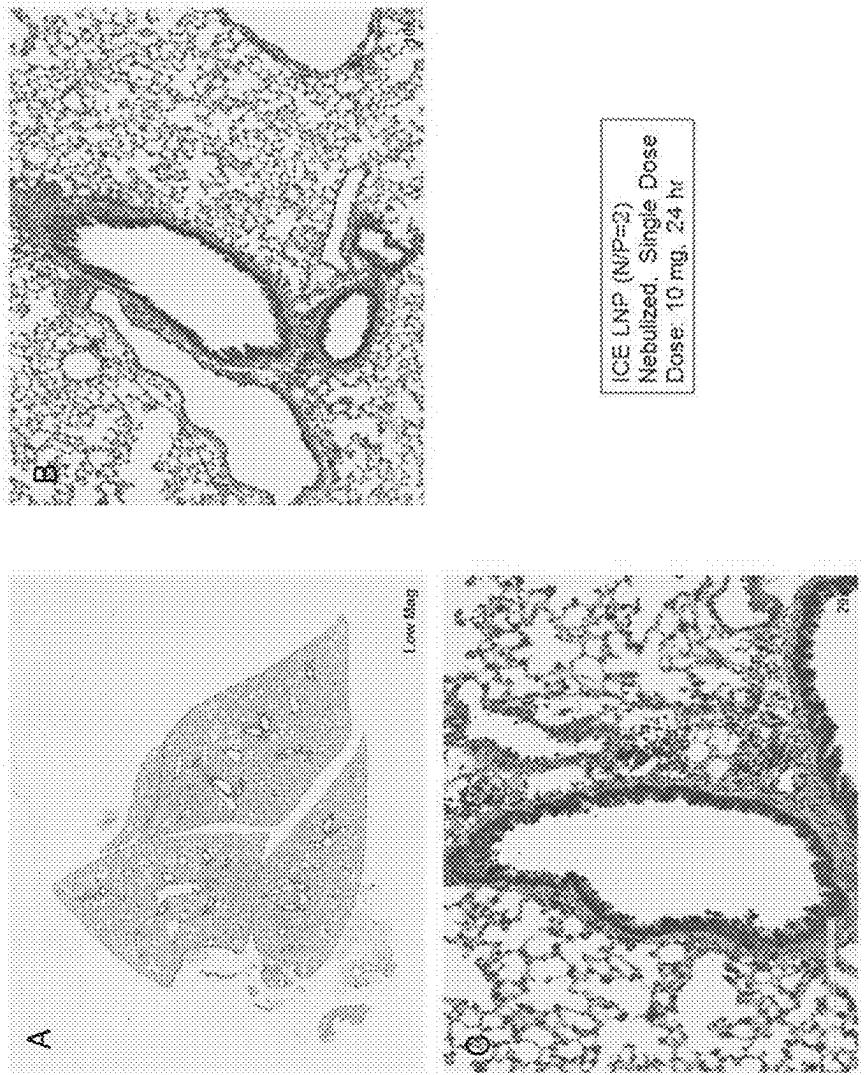


FIGURE 7

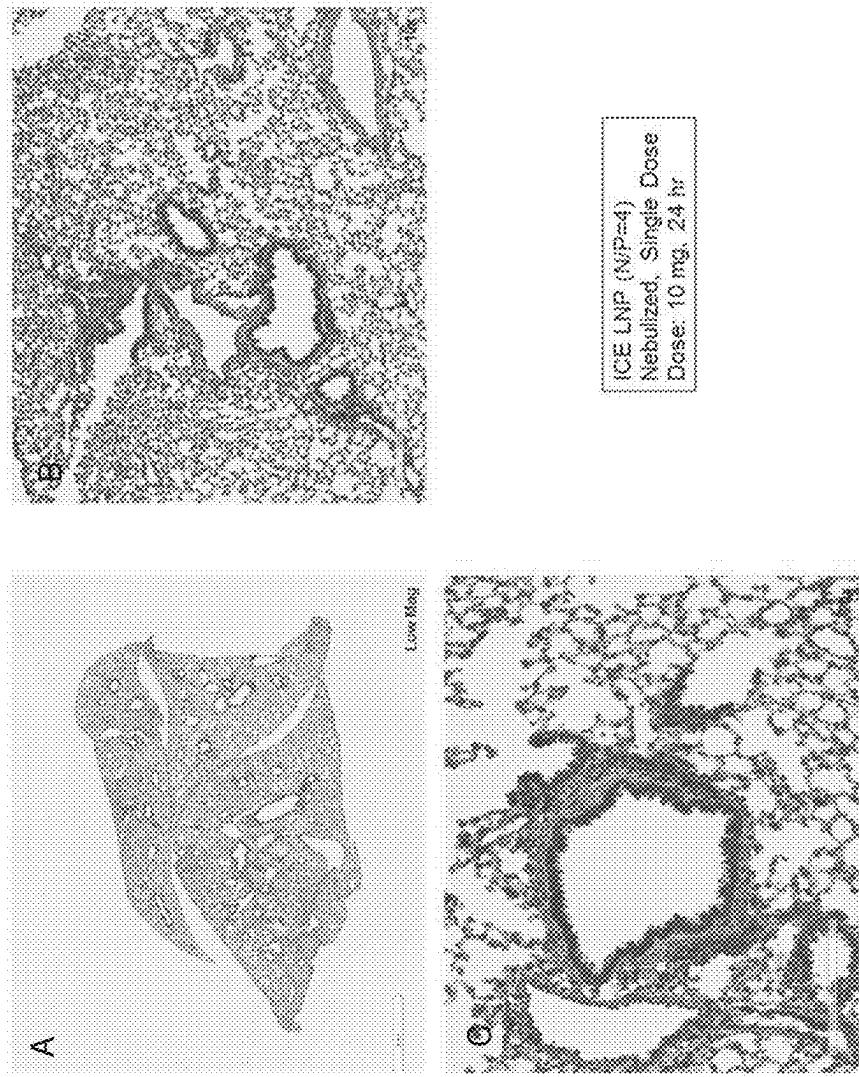


FIGURE 8

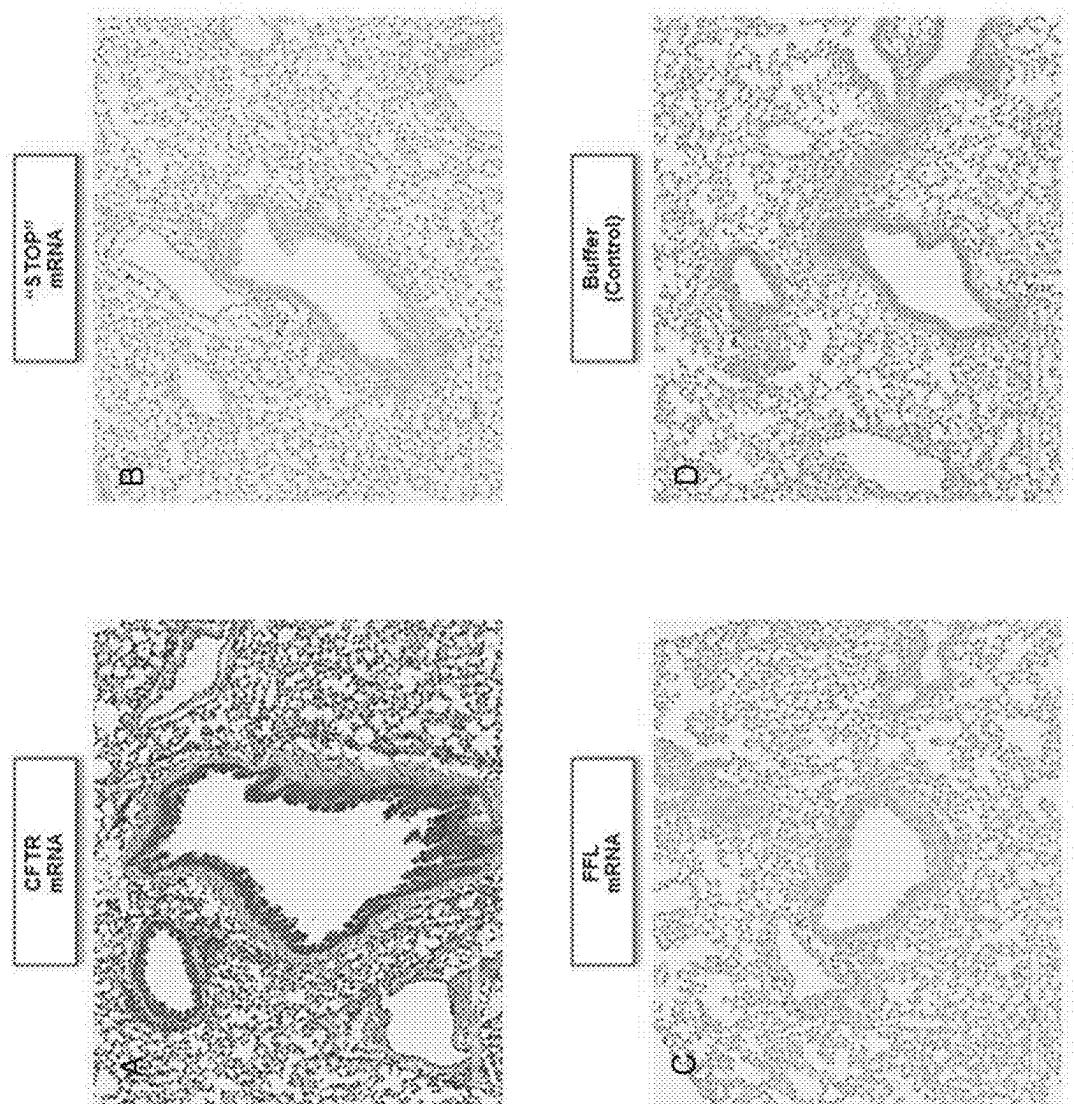


FIGURE 9

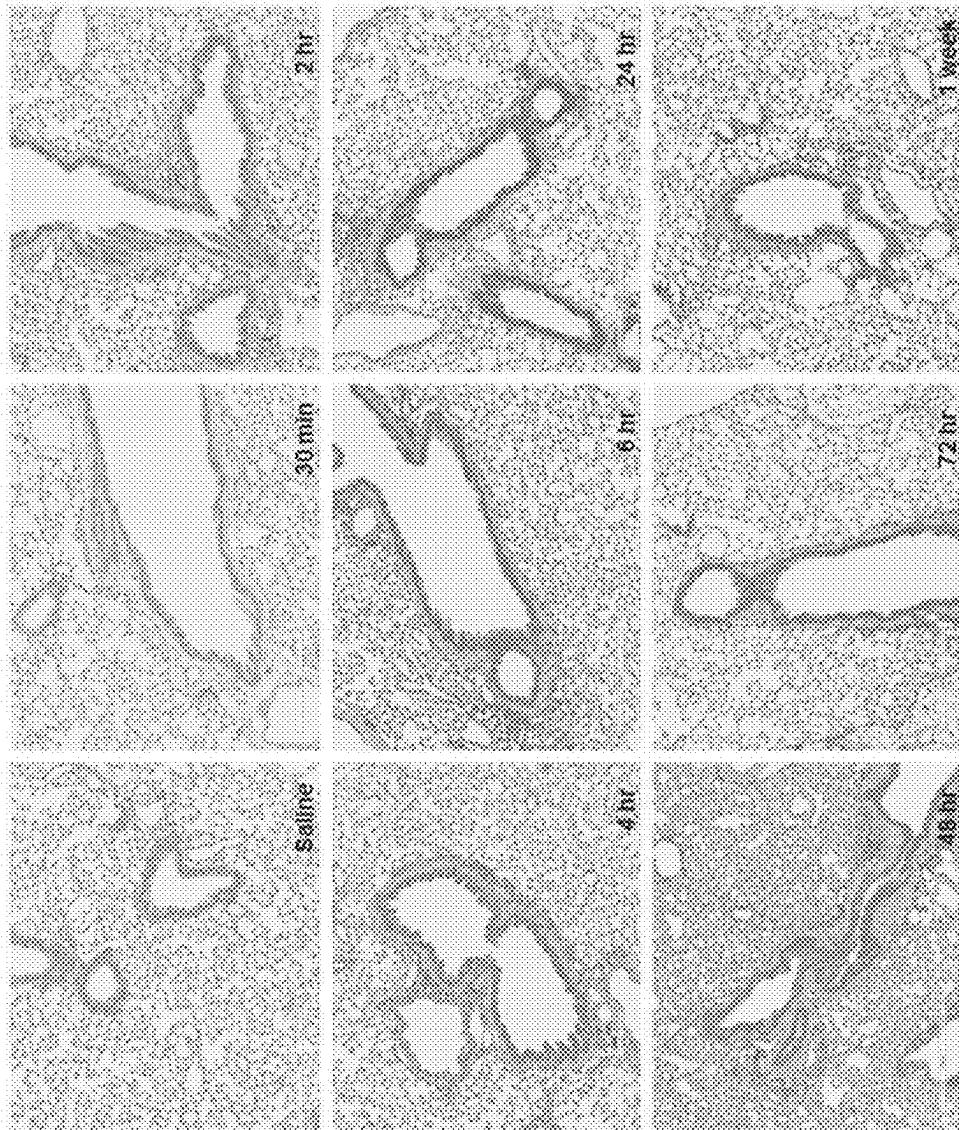


FIGURE 10

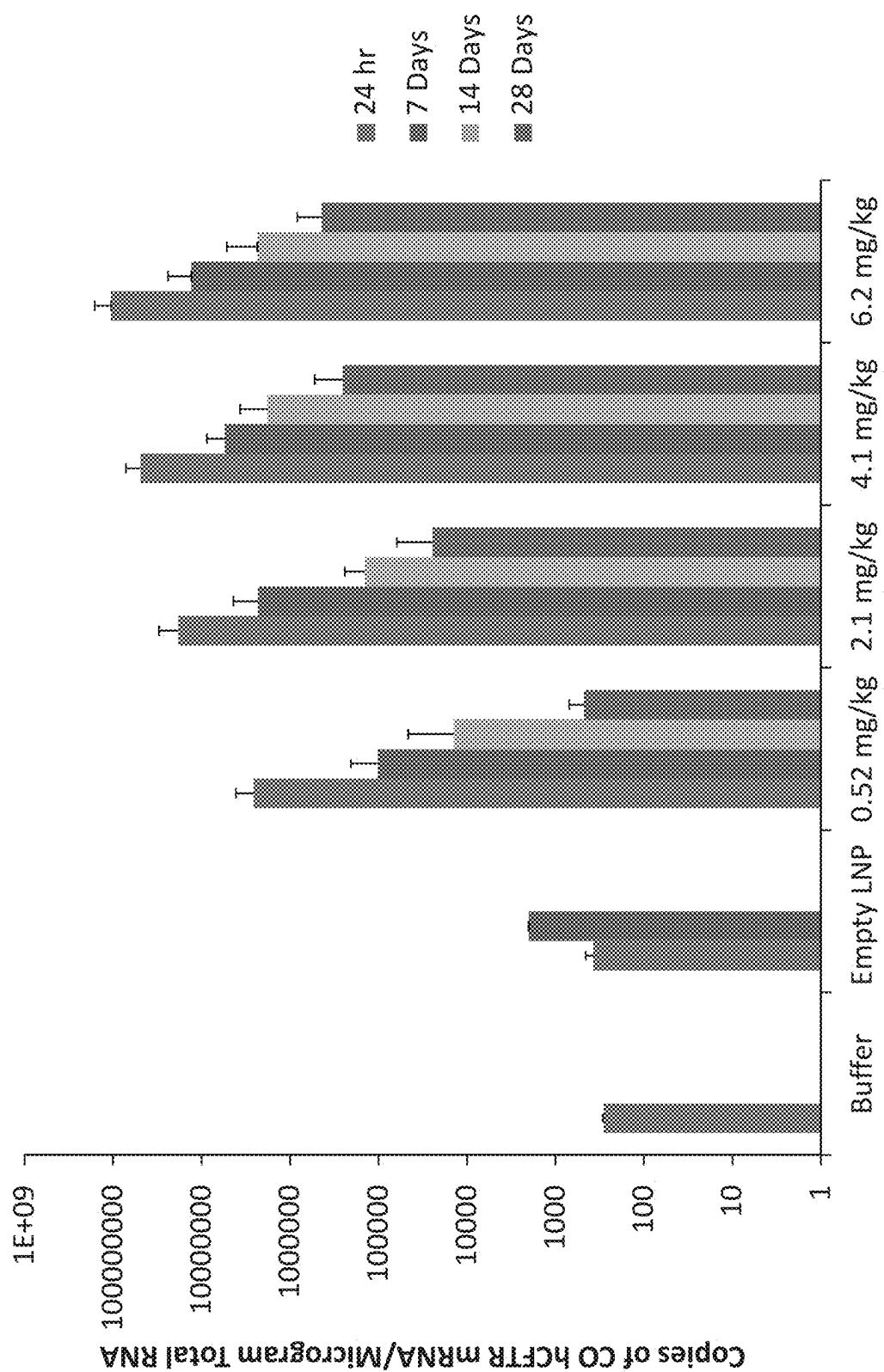


FIGURE 11

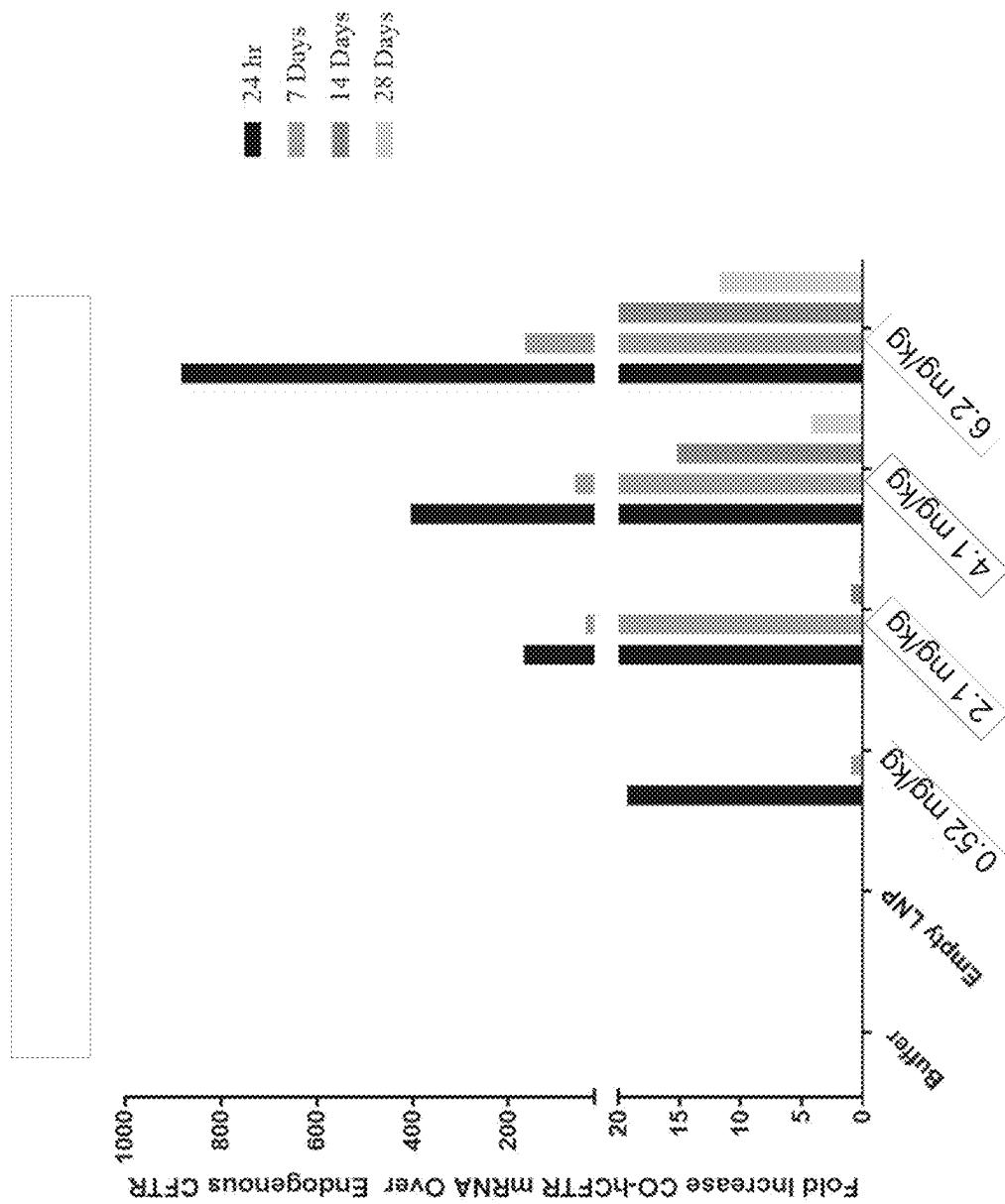


FIGURE 12

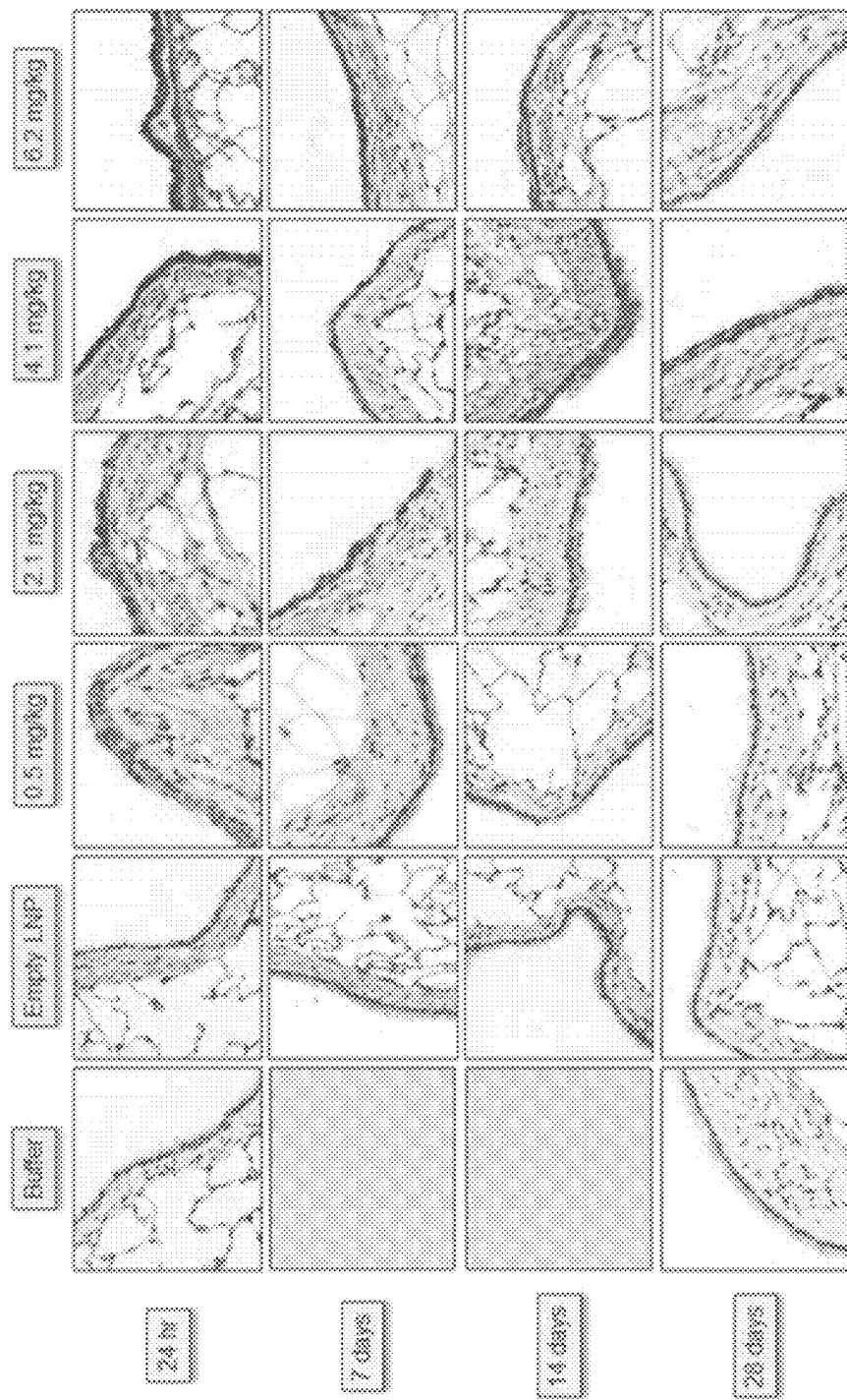


FIGURE 13

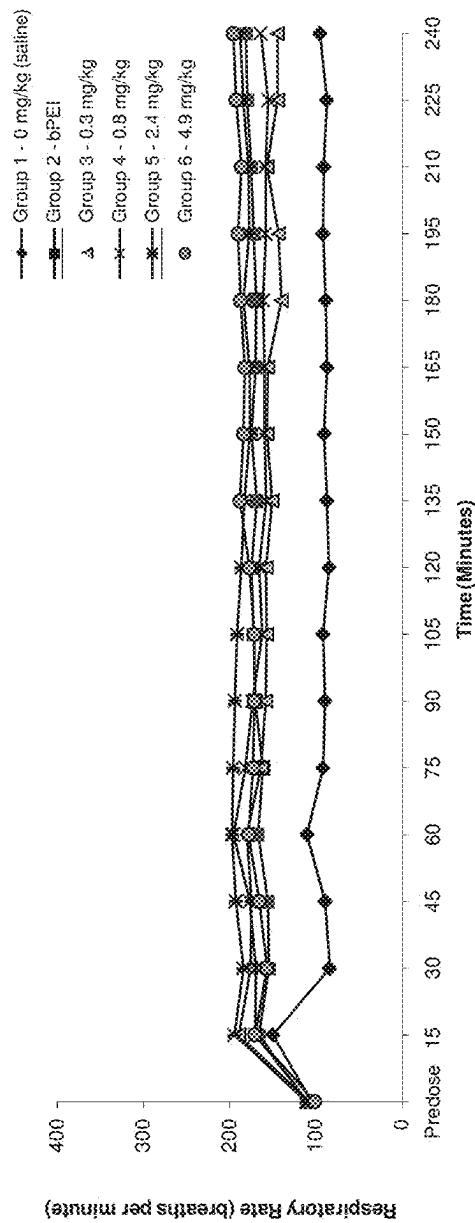


FIGURE 14A

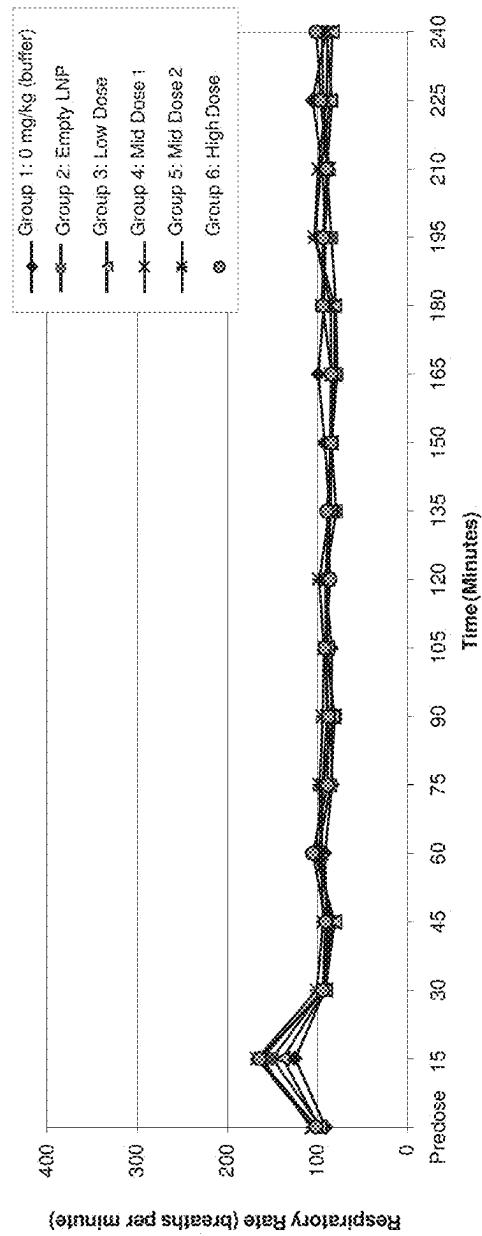


FIGURE 14B

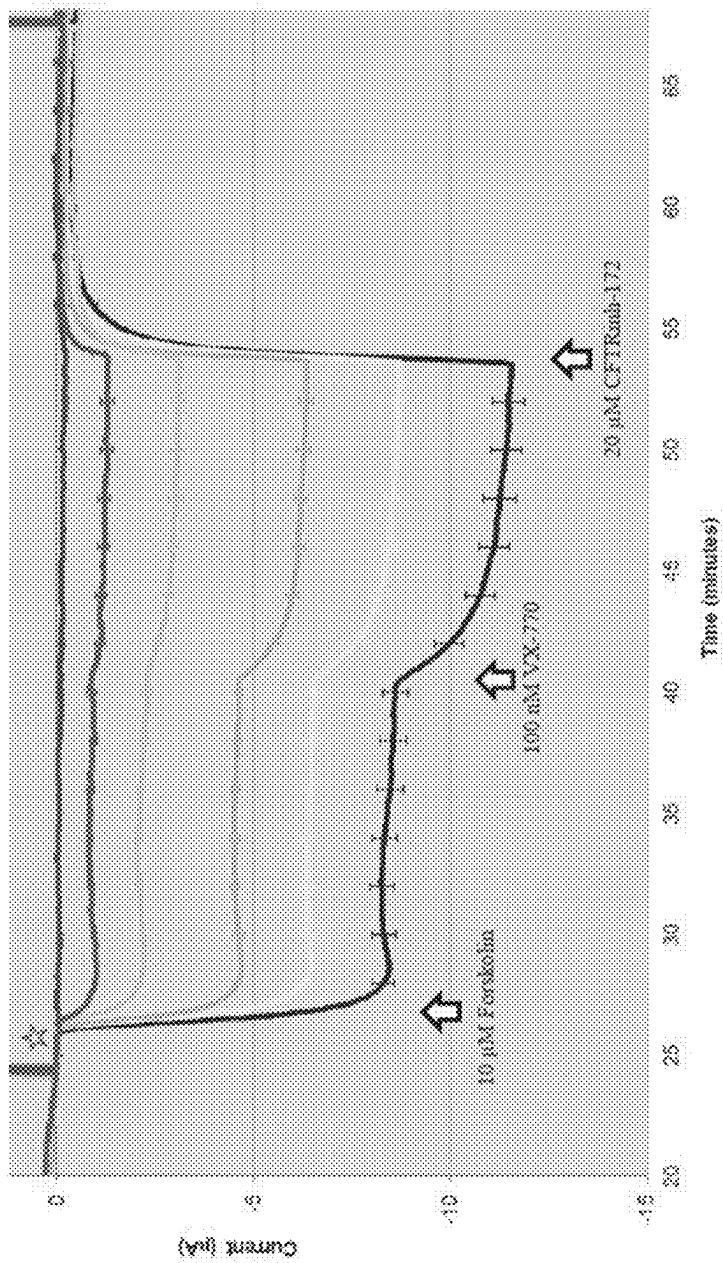


FIGURE 15

## ICE-BASED LIPID NANOPARTICLE FORMULATIONS FOR DELIVERY OF mRNA

### RELATED APPLICATIONS

[0001] This application is a continuation of U.S. patent application Ser. No. 17/239,131, filed Apr. 23, 2021, which is a divisional of U.S. patent application Ser. No. 16/599,928, filed Oct. 11, 2019, now U.S. Pat. No. 11,013,812, which is a divisional of U.S. patent application Ser. No. 15/809,605, filed Nov. 10, 2017, now U.S. Pat. No. 10,471,153, which claims priority to U.S. Provisional Patent Application Ser. Nos. 62/420,421, filed Nov. 10, 2016, 62/420,428, filed Nov. 10, 2016, 62/421,021, filed Nov. 11, 2016, 62/421,007, filed Nov. 11, 2016, 62/464,327, filed Feb. 27, 2017, and 62/464,330, filed Feb. 27, 2017, the entire disclosures of which are hereby incorporated herein by reference.

### SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML file, created on Jan. 15, 2025, is named 761236\_SA9-871DIV3CON\_ST26.xml and is 48,473 bytes in size.

### BACKGROUND

[0003] Nucleic acid-based technologies are increasingly important for various therapeutic applications including, but not limited to, messenger RNA therapy. Efforts to deliver nucleic acids have included the creation of compositions formulated to protect nucleic acids from degradation when delivered in vivo. One type of delivery vehicle for nucleic acids has been lipid nanoparticles. Important parameters to consider for the successful use of lipid nanoparticles as a delivery vehicle include lipid nanoparticle formation, physical properties of lipid components, nucleic acid encapsulation efficiencies, in vivo nucleic acid release potential, and lipid nanoparticle toxicity.

### SUMMARY OF THE INVENTION

[0004] This present invention offers a unique solution wherein sterol-based cationic lipid, helper lipid and PEG-modified lipid form a lipid nanoparticle formulation of RNA. This inventive three lipid component system upon formulation shows high RNA encapsulation efficiencies and successful efficacious delivery in vivo, particularly pulmonary delivery. Such formulation systems offer additional advantages of lower lipid load (compared to other conventional four lipid component systems) and higher tolerability/lower toxicity as the metabolized products of the cationic lipid (cholesterol derivative lipid) is cholesterol.

[0005] The invention is based, in part, on the surprising discovery that a three lipid component system based on sterol-based cationic lipids is unexpectedly effective in delivering mRNA and producing encoded protein or peptide in vivo, particularly in the lung. Indeed, prior to the present invention, cationic lipids have been extensively explored as an important component of liposomes used to encapsulate nucleic acids, including mRNA, for in vivo delivery. Due to the uniquely fragile and long structure of mRNA and the complicated in vivo translation process, cationic lipids used in the liposomes typically play two roles. First, cationic lipids promote interaction with negatively charged mRNA

during encapsulation, circulation and endocytosis, thereby capturing and protecting the mRNA. Then, once inside cytosol, cationic lipids need to be able to release the mRNA so that the mRNA can be translated to produce the encoded protein or peptide. Some cationic lipids, in particular, known as titratable cationic lipids, are particularly effective in delivering mRNA. Surprisingly, the present inventors found that liposomes comprising the sterol-based cationic lipids described herein can have an even higher encapsulation percentage for mRNA and can be even more effective in delivering various mRNA in vivo. Particularly, liposomes comprising the sterol-based cationic lipids described herein can be incredibly effective for pulmonary delivery of mRNA, and surprisingly successful for delivering mRNA via nebulization. Thus, the present inventors have demonstrated that the three lipid component system can be uniquely useful in delivering mRNA for highly efficient and sustained production of protein or peptide (e.g., therapeutic protein) in vivo, particularly in the pulmonary system. The present invention therefore permits an improved mRNA therapy that can significantly reduce the required amount of mRNA and associated lipids, administration frequency, and possible side effects, providing safer, more potent, and patient friendly mRNA therapy for various diseases.

[0006] In one aspect, the present invention provides methods of delivering nucleic acids in vivo comprising administering by pulmonary delivery to a subject in need of delivery a composition comprising nucleic acids; and lipid nanoparticles encapsulating the nucleic acids, wherein each individual lipid nanoparticle comprises no more than three distinct lipid components, one distinct lipid component being a sterol-based cationic lipid.

[0007] In another aspect, the present invention provides methods of delivering nucleic acids in vivo comprising administering by pulmonary delivery to a subject in need of delivery a composition comprising nucleic acids, wherein the nucleic acids encode a Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein; and lipid nanoparticles encapsulating the nucleic acids, wherein each individual lipid nanoparticle comprises no more than three distinct lipid components, one distinct lipid component being a sterol-based cationic lipid.

[0008] In some embodiments, the pulmonary delivery comprises nebulization. In some embodiments, said subject is a subject in need of delivery.

[0009] In some embodiments, the lipid nanoparticles have an encapsulation percentage for nucleic acids of at least 70% (e.g., at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%).

[0010] In some embodiments, the three distinct lipid components comprise one or both of helper lipids and PEG-modified lipids.

[0011] In some embodiments, the sterol-based cationic lipid has a structure according to Formula (A), [text missing or illegible when filed]

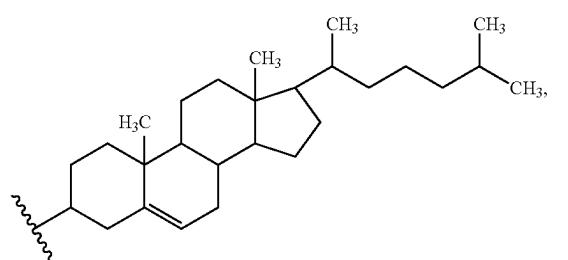
[0012] B-L<sup>1</sup>-S (Formula A), or a protonated form thereof, wherein B is a basic functional group wherein the protonated form has a pK<sub>a</sub> that is no more than about 8.0; L<sup>1</sup> is an optionally substituted linker group that is a C<sub>1</sub>-C<sub>20</sub> alkylene or a 2- to 20-membered heteroalkylene; and S is a sterol.

[0013] In some embodiments, B is an optionally substituted 5- or 6-membered nitrogen-containing heteroaryl. In

some embodiments, B is a group selected from pyrrolyl, imidazolyl, pyrazolyl, triazolyl, tetrazolyl, pyridyl, pyrimidyl, pyrazinyl, and pyridazinyl, each of which is optionally substituted. In some embodiments, B is a group selected from pyrrolyl, pyrazolyl, triazolyl, tetrazolyl, pyridyl, pyrimidyl, pyrazinyl, and pyridazinyl, each of which is optionally substituted. In some embodiments, B is a group selected from pyrrolyl, pyrazolyl, triazolyl, tetrazolyl, pyridyl, pyrimidyl, pyrazinyl, and pyridazinyl, each of which is optionally substituted.

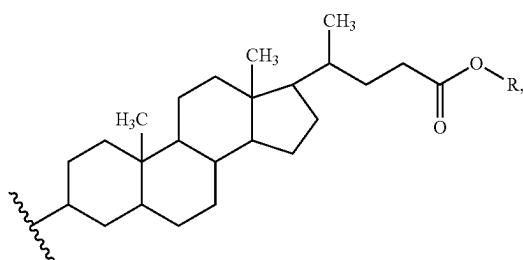
**[0014]** In some embodiments, L<sup>1</sup> is an optionally substituted linker group that is a C<sub>1</sub>-C<sub>20</sub> alkylene. In some embodiments, L<sup>1</sup> is an optionally substituted linker group that is a 2- to 20-membered heteroalkylene. In some embodiments, L<sup>1</sup> is a 2- to 20-membered heteroalkylene that is non-peptidic. In some embodiments, L<sup>1</sup> comprises a moiety that is an ester group, an amide group, a carbamate group, a carbonate group, or a urea group. In some embodiments, L<sup>1</sup> comprises a moiety that is an amide group, a carbamate group, a carbonate group, or a urea group. In some embodiments, L<sup>1</sup> comprises a moiety that is an amide group, a carbonate group, or a urea group. In some embodiments, L<sup>1</sup> is —X<sup>1</sup>—C(X<sup>3</sup>)—X<sup>2</sup>, —(C<sub>1</sub>-C<sub>19</sub> alkylene)-X<sup>1</sup>—C(X<sup>3</sup>)—X<sup>2</sup>, —X<sup>1</sup>—C(X<sup>3</sup>)—X<sup>2</sup>(C<sub>1</sub>-C<sub>19</sub> alkylene)-, —(C<sub>1</sub>-C<sub>19</sub> alkylene)-X<sup>1</sup>—, —X<sup>1</sup>—(C<sub>1</sub>-C<sub>19</sub> alkylene)-, wherein each X<sup>1</sup> and X<sup>2</sup> is independently a covalent bond, —O—, —S—, or —NH—; X<sup>3</sup> is independently =O, =S, or =NH; and wherein said C<sub>1</sub>-C<sub>19</sub> alkylene is optionally substituted. In some embodiments, L<sup>1</sup> does not comprise substituents having the structure —N(R')<sub>2</sub>, or a positively charged form thereof, wherein each R' is independently hydrogen or optionally substituted C<sub>1</sub>-C<sub>20</sub> alkyl.

**[0015]** In some embodiments, S is a zoosterol, or an oxidized or reduced form thereof. In some embodiments, S is a phytosterol, or an oxidized or reduced form thereof. In some embodiments, S is a synthetic sterol, or an oxidized or reduced form thereof. In some embodiments, S is a sterol selected from cholesterol, an oxidized form of cholesterol, a reduced form of cholesterol, alkyl lithocholate, stigmasterol, stigmastanol, campesterol, ergosterol, and sitosterol. In some embodiments, S is a sterol selected from an oxidized form of cholesterol, a reduced form of cholesterol, alkyl lithocholate, stigmasterol, stigmastanol, campesterol, ergosterol, and sitosterol. In some embodiments, S is a sterol selected from

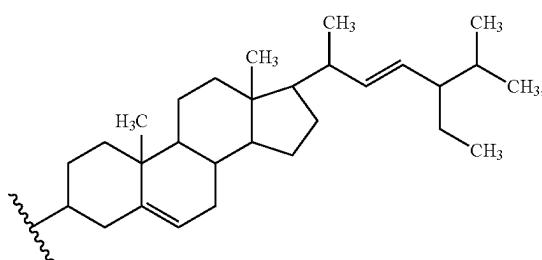


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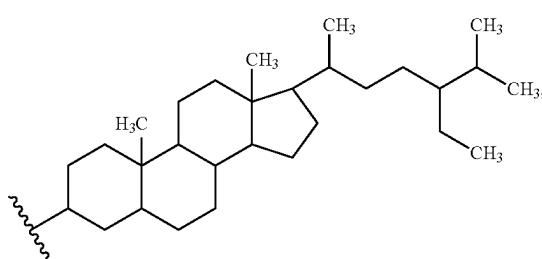
(alkyl lithocholate)



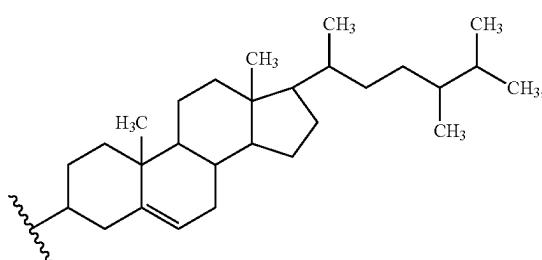
(stigmasterol)



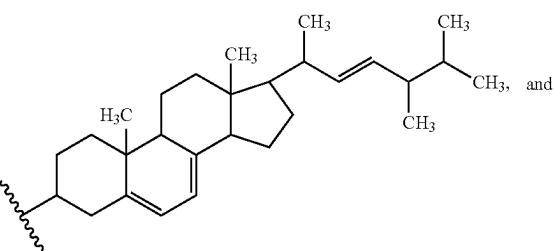
(stigmastanol)



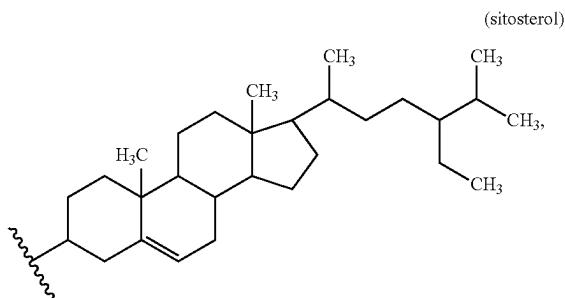
(campesterol)



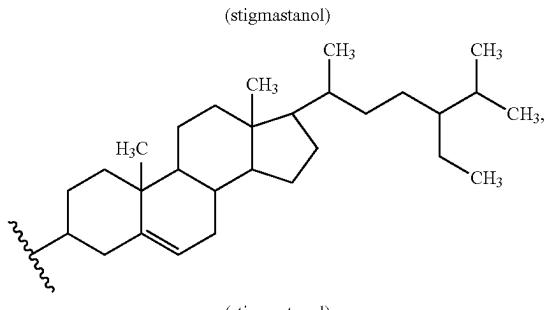
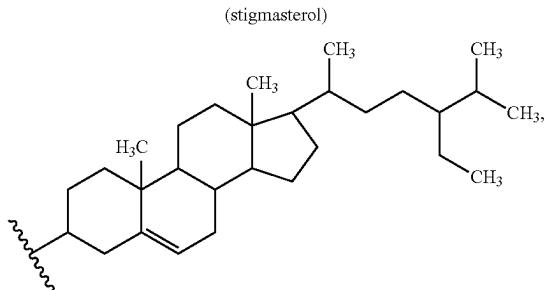
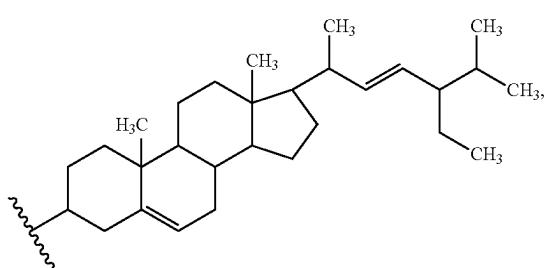
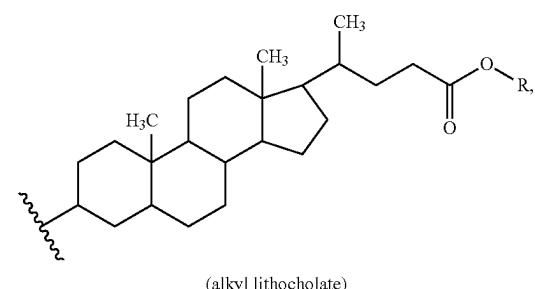
(ergosterol)



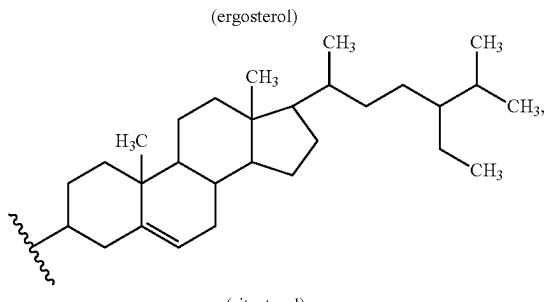
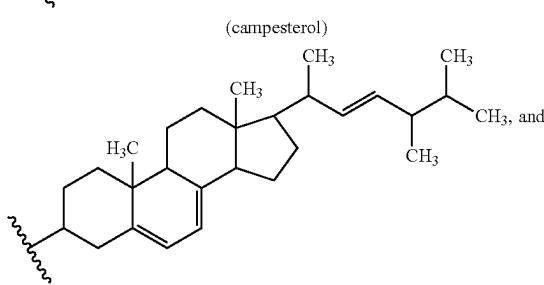
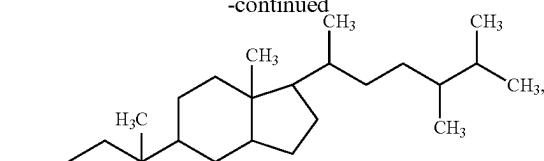
-continued



wherein R is optionally substituted C<sub>1</sub>-C<sub>20</sub> alkyl. In some embodiments, S is a sterol selected from



-continued



(sitosterol)

wherein R is optionally substituted C<sub>1</sub>-C<sub>20</sub> alkyl.

**[0016]** In some embodiments, the sterol-based cationic lipid comprises imidazole cholesterol ester (ICE). In some embodiments, the sterol-based cationic lipid does not comprise imidazole cholesterol ester (ICE).

**[0017]** In some embodiments, the nucleic acids are selected from DNA, siRNA, microRNA, and/or mRNA. In some embodiments, the nucleic acids are mRNA encoding a protein or a peptide. In some embodiments, the mRNA encoding a protein or a peptide is codon-optimized. In some embodiments, the mRNA comprises one or more modified nucleotides. In some embodiments, the mRNA comprises a modification of the 5' untranslated region of said mRNA. In some embodiments, said modification of the 5' untranslated region comprises the inclusion of a Cap1 structure. In some embodiments, the mRNA comprises a modification of the 3' untranslated region of said mRNA. In some embodiments, said modification of the 3' untranslated region comprises the inclusion of a poly A tail.

**[0018]** In some embodiments, the lipid nanoparticles have a size less than about 100 nm, 95 nm, 90 nm, 85 nm, 80 nm, 75 nm, 70 nm, 65 nm, 60 nm, 55 nm, 50 nm, 45 nm or 40 nm.

**[0019]** In another aspect, the present invention provides compositions formulated for nebulization comprising nucleic acids; and lipid nanoparticles encapsulating the nucleic acids, wherein each individual lipid nanoparticle comprises no more than three distinct lipid components, one distinct lipid component being a sterol-based cationic lipid, and further wherein the lipid nanoparticles have an encap-

sulation percentage for nucleic acids of at least 70% (e.g., at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%).

**[0020]** In another aspect, the present invention provides compositions comprising nucleic acids; and lipid nanoparticles encapsulating the nucleic acids, wherein each individual lipid nanoparticle comprises no more than three distinct lipid components, one distinct lipid component being a sterol-based cationic lipid, and further wherein the lipid nanoparticles have an encapsulation percentage for nucleic acids of at least 70% (e.g., at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%).

**[0021]** In some embodiments, the lipid nanoparticles have an encapsulation percentage for nucleic acids of at least 85%. In some embodiments, the lipid nanoparticles have an encapsulation percentage for nucleic acids of at least 90%.

**[0022]** In another aspect, the present invention provides compositions comprising mRNA encoding a Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein; and lipid nanoparticles encapsulating the mRNA, wherein each individual lipid nanoparticle comprises no more than three distinct lipid components, one distinct lipid component being a sterol-based cationic lipid, and further wherein the lipid nanoparticles have an encapsulation percentage for mRNA of at least 80%.

**[0023]** In some embodiments, the lipid nanoparticles have an encapsulation percentage for mRNA of at least 85% or at least 90%.

**[0024]** In another aspect, the present invention provides compositions comprising nucleic acids; and lipid nanoparticles encapsulating the nucleic acids, wherein each individual lipid nanoparticle comprises no more than three distinct lipid components, one distinct lipid component being a sterol-based cationic lipid, and further wherein the sterol-based cationic lipid constitutes no more than 70% of the total lipids (e.g., no more than 68%, no more than 67%, no more than 66%, no more than 65%, no more than 60%, no more than 55%, no more than 50%, no more than 45%, or no more than 40%).

**[0025]** In another aspect, the present invention provides compositions comprising mRNA encoding a Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein; and lipid nanoparticles encapsulating the mRNA, wherein each individual lipid nanoparticle comprises no more than three distinct lipid components, one distinct lipid component being a sterol-based cationic lipid, and further wherein the sterol-based cationic lipid constitutes no more than 70% of the total lipids (e.g., no more than 68%, no more than 67%, no more than 66%, no more than 65%, no more than 60%, no more than 55%, no more than 50%, no more than 45%, or no more than 40%).

**[0026]** In some embodiments, the sterol-based cationic lipid constitutes no more than 65% of the total lipids. In some embodiments, the sterol-based cationic lipid constitutes no more than 60% of the total lipids. In some embodiments, the three distinct lipid components comprise one or both of helper lipids and PEG-modified lipids.

**[0027]** In some embodiments, the sterol-based cationic lipid has a structure according to Formula (A), **[text missing or illegible when filed]**

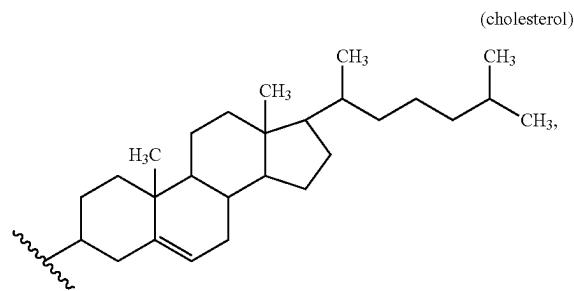
**[0028]** B-L<sup>1</sup>-S (Formula A), or a protonated form thereof, wherein B is a basic functional group wherein the protonated form has a pK<sub>a</sub> that is no more than

about 8.0; L<sup>1</sup> is an optionally substituted linker group that is a C<sub>1</sub>-C<sub>20</sub> alkylene or a 2- to 20-membered heteroalkylene; and S is a sterol.

**[0029]** In some embodiments, B is an optionally substituted 5- or 6-membered nitrogen-containing heteroaryl. In some embodiments, B is a group selected from pyrrolyl, imidazolyl, pyrazolyl, triazolyl, tetrazolyl, pyridyl, pyrimidyl, pyrazinyl, and pyridazinyl, each of which is optionally substituted. In some embodiments, B is a group selected from pyrrolyl, pyrazolyl, triazolyl, tetrazolyl, pyridyl, pyrimidyl, pyrazinyl, and pyridazinyl, each of which is optionally substituted. In some embodiments, B is a group selected from pyrrolyl, pyrazolyl, triazolyl, tetrazolyl, pyridyl, pyrimidyl, pyrazinyl, and pyridazinyl, each of which is optionally substituted.

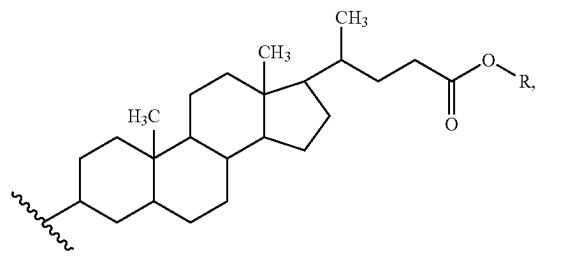
**[0030]** In some embodiments, L<sup>1</sup> is an optionally substituted linker group that is a C<sub>1</sub>-C<sub>20</sub> alkylene. In some embodiments, L<sup>1</sup> is an optionally substituted linker group that is a 2- to 20-membered heteroalkylene. In some embodiments, L<sup>1</sup> is a 2- to 20-membered heteroalkylene that is non-peptidic. In some embodiments, L<sup>1</sup> comprises a moiety that is an ester group, an amide group, a carbamate group, a carbonate group, or a urea group. In some embodiments, L<sup>1</sup> comprises a moiety that is an amide group, a carbamate group, a carbonate group, or a urea group. In some embodiments, L<sup>1</sup> comprises a moiety that is an amide group, a carbonate group, or a urea group. In some embodiments, L<sup>1</sup> is —X<sup>1</sup>—C(X<sup>3</sup>)—X<sup>2</sup>, —(C<sub>1</sub>-C<sub>19</sub> alkylene)-X<sup>1</sup>—C(X<sup>3</sup>)—X<sup>2</sup>, —X<sup>1</sup>—C(X<sup>3</sup>)—X<sup>2</sup>(C<sub>1</sub>-C<sub>19</sub> alkylene)-, —(C<sub>1</sub>-C<sub>19</sub> alkylene)-X<sup>1</sup>—, —X<sup>1</sup>—(C<sub>1</sub>-C<sub>19</sub> alkylene)-, wherein each X<sup>1</sup> and X<sup>2</sup> is independently a covalent bond, —O—, —S—, or —NH—; X<sup>3</sup> is independently =O, =S, or =NH; and wherein said C<sub>1</sub>-C<sub>19</sub> alkylene is optionally substituted. In some embodiments, L<sup>1</sup> does not comprise substituents having the structure —N(R')<sub>2</sub>, or a positively charged form thereof, wherein each R' is independently hydrogen or optionally substituted C<sub>1</sub>-C<sub>20</sub> alkyl.

**[0031]** In some embodiments, S is a zoosterol, or an oxidized or reduced form thereof. In some embodiments, S is a phytosterol, or an oxidized or reduced form thereof. In some embodiments, S is a synthetic sterol, or an oxidized or reduced form thereof. In some embodiments, S is a sterol selected from cholesterol, an oxidized form of cholesterol, a reduced form of cholesterol, alkyl lithocholate, stigmasterol, stigmastanol, campesterol, ergosterol, and sitosterol. In some embodiments, S is a sterol selected from an oxidized form of cholesterol, a reduced form of cholesterol, alkyl lithocholate, stigmasterol, stigmastanol, campesterol, ergosterol, and sitosterol. In some embodiments, S is a sterol selected from



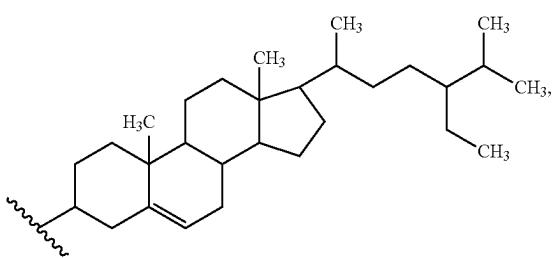
-continued

(alkyl lithocholate)

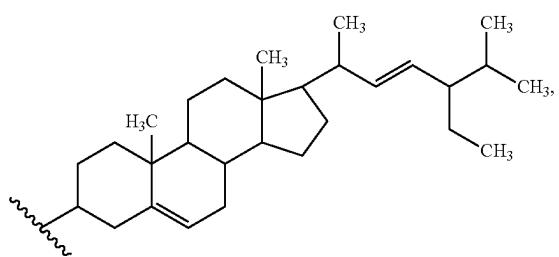


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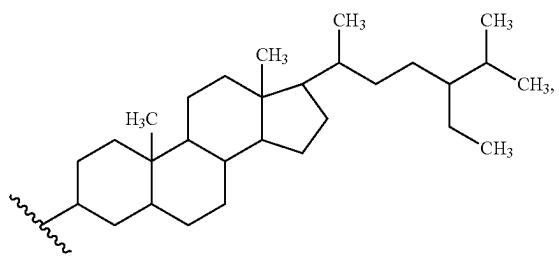
(sitosterol)



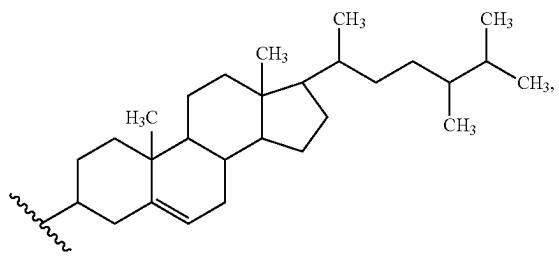
(stigmasterol)



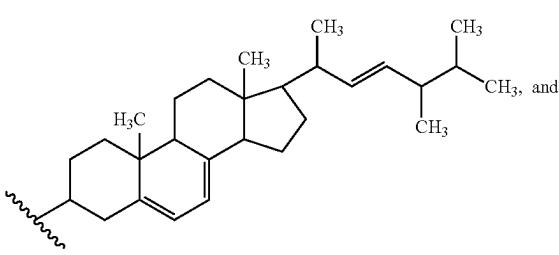
(stigmastanol)



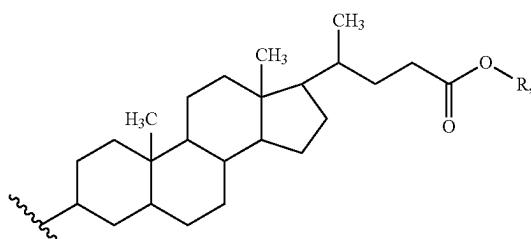
(campesterol)



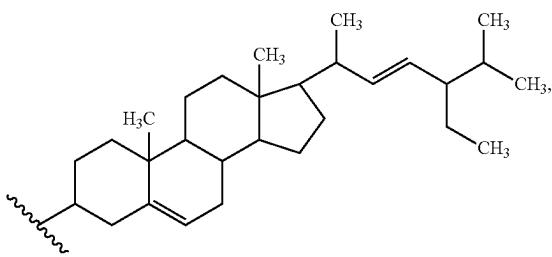
(ergosterol)



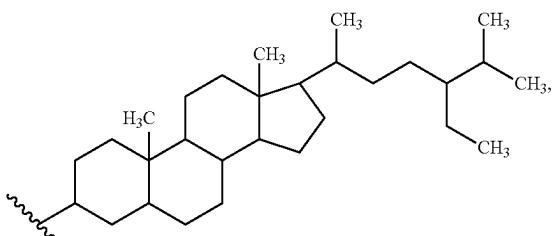
(alkyl lithocholate)



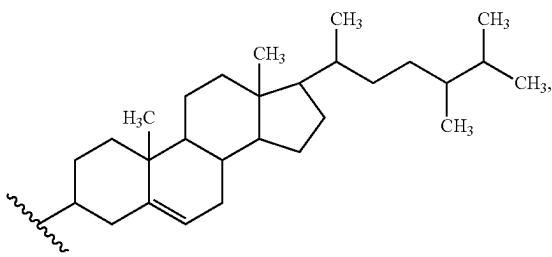
(stigmasterol)



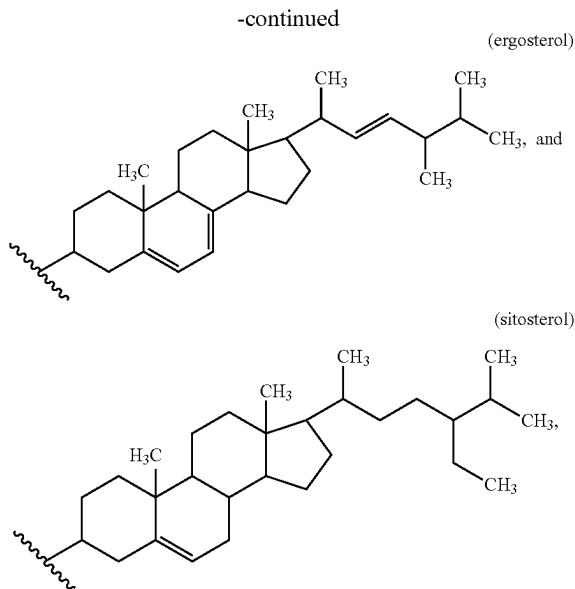
(stigmastanol)



(campesterol)



wherein R is optionally substituted C<sub>1</sub>-C<sub>20</sub> alkyl. In some embodiments, S is a sterol selected from



wherein R is optionally substituted C<sub>1</sub>-C<sub>20</sub> alkyl.

[0032] In some embodiments, the sterol-based cationic lipid comprises imidazole cholesterol ester (ICE). In some embodiments, the sterol-based cationic lipid does not comprise imidazole cholesterol ester (ICE).

[0033] In some embodiments, the nucleic acids are selected from DNA, siRNA, microRNA, and/or mRNA. In some embodiments, the nucleic acids are mRNA encoding a protein or a peptide. In some embodiments, the mRNA encoding a protein or a peptide is codon-optimized. In some embodiments, the mRNA comprises one or more modified nucleotides. In some embodiments, the mRNA comprises a modification of the 5' untranslated region of said mRNA. In some embodiments, said modification of the 5' untranslated region comprises the inclusion of a Cap1 structure. In some embodiments, the mRNA comprises a modification of the 3' untranslated region of said mRNA. In some embodiments, said modification of the 3' untranslated region comprises the inclusion of a poly A tail.

[0034] In some embodiments, the lipid nanoparticle has a size less than about 100 nm, 95 nm, 90 nm, 85 nm, 80 nm, 75 nm, 70 nm, 65 nm, 60 nm, 55 nm, 50 nm, 45 nm or 40 nm.

[0035] In some embodiments, the mRNA encoding a CFTR protein comprises SEQ ID NO: 1. In some embodiments, the mRNA encoding a CFTR protein comprises a polynucleotide sequence at least 70%, 75%, 80%, 85%, 90%, or 95% identical to SEQ ID NO: 1.

[0036] In some embodiments, the lipid nanoparticle is formed by mixing an mRNA solution and a lipid solution into a 20% ethanol. In some embodiments, the lipid nanoparticles are further purified by Tangential Flow Filtration.

[0037] In another aspect, the present invention provides methods of delivering mRNA in vivo comprising administering a composition to a subject. In some embodiments, said subject is a subject in need of delivery. In some embodiments, the composition is administered intravenously. In some embodiments, the composition is administered by pulmonary delivery. In some embodiments, the pulmonary delivery comprises nebulization.

[0038] In this application, the use of "or" means "and/or" unless stated otherwise. As used in this disclosure, the term "comprise" and variations of the term, such as "comprising" and "comprises," are not intended to exclude other additives, components, integers or steps. As used in this application, the terms "about" and "approximately" are used as equivalents. Both terms are meant to cover any normal fluctuations appreciated by one of ordinary skill in the relevant art.

[0039] Other features, objects, and advantages of the present invention are apparent in the detailed description, drawings and claims that follow. It should be understood, however, that the detailed description, the drawings, and the claims, while indicating embodiments of the present invention, are given by way of illustration only, not limitation. Various changes and modifications within the scope of the invention will become apparent to those skilled in the art.

#### BRIEF DESCRIPTION OF THE DRAWING

[0040] The drawings are for illustration purposes only, not for limitation.

[0041] FIG. 1 depicts exemplary immunohistochemical detection of human Cystic Fibrosis Transmembrane Conductance Regulator (hCFTR) protein in mouse lung 24 hours after treatment with ICE-based LNPs encapsulating codon-optimized hCFTR mRNA, B=Untreated mouse lung at 10× & 20× magnification, respectively. C, D=CO-hCFTR mRNA ICE LNP-treated mouse lung at 10× & 20× magnification, respectively.

[0042] FIG. 2 depicts exemplary immunohistochemical detection of human CFTR protein in mouse lung 24 hours after treatment with ICE-based LNPs encapsulating codon-optimized hCFTR. A, B=Untreated mouse lung at 10× & 20× magnification, respectively. C, D=CO-hCFTR mRNA ICE LNP-treated mouse lung at 10× & 20× magnification, respectively.

[0043] FIG. 3 depicts exemplary immunohistochemical detection of human Cystic Fibrosis Transmembrane Conductance Regulator (hCFTR) protein in the lungs of rats 24 hours after treatment with nebulized ICE-based lipid nanoparticles (LNPs) (5% PEG) encapsulating codon-optimized hCFTR mRNA.

[0044] FIG. 4 depicts exemplary immunohistochemical detection of hCFTR protein in the lungs of rats 24 hours after treatment with ICE-based LNPs (5% PEG) encapsulating codon-optimized hCFTR mRNA via intratracheal administration.

[0045] FIG. 5 depicts exemplary immunohistochemical detection of human CFTR protein in the lungs of rats 24 hours after treatment with nebulized ICE-based LNPs (3% PEG) encapsulating codon-optimized (CO) hCFTR mRNA.

[0046] FIG. 6 depicts exemplary immunohistochemical detection of human CFTR protein in the lungs of rats 24 hours after treatment with ICE-based LNPs (3% PEG) encapsulating CO-hCFTR mRNA via intratracheal (Microsprayer) administration.

[0047] FIG. 7 depicts exemplary immunohistochemical detection of human CFTR protein in the lungs of CFTR KO (knockout) mice 24 hours after treatment with nebulized ICE-based LNPs (N/P=2) encapsulating CO-hCFTR mRNA.

[0048] FIG. 8 depicts exemplary immunohistochemical detection of human CFTR protein in the lungs of CFTR KO mice 24 hours after treatment with nebulized ICE-based LNPs (N/P=4) encapsulating CO-hCFTR mRNA.

[0049] FIG. 9 depicts exemplary immunohistochemical detection of human CFTR protein in the lungs of wild-type mice 24 hours after treatment with nebulized ICE-based LNPs encapsulating various mRNA constructs including CFTR (CO-hCFTR mRNA), STOP (nonsense mutated CO-hCFTR mRNA unable to be translated into protein), and FFL (Firefly Luciferase mRNA).

[0050] FIG. 10 depicts exemplary immunohistochemical detection of human CFTR protein in the lungs of wild-type mice at various time points after a single exposure to nebulized ICE-based LNPs encapsulating CO-hCFTR mRNA.

[0051] FIG. 11 depicts an exemplary graph of the ratio of copies of CO-hCFTR mRNA per micrograms of total RNA in frozen lung sections from rats treated with either controls (buffer, Empty ICE LNP) or CO-hCFTR mRNA loaded ICE LNPs.

[0052] FIG. 12 depicts an exemplary graph of fold-increase of copies of CO-hCFTR over endogenous levels of CFTR mRNA in frozen lung sections of rats treated with either controls (buffer, Empty ICE LNP) or CO-hCFTR mRNA loaded ICE LNPs.

[0053] FIG. 13 depicts exemplary immunohistochemical detection of human CFTR protein in the lungs of rats after a single exposure to various doses of nebulized ICE-based LNPs encapsulating CO-hCFTR mRNA.

[0054] FIGS. 14A and 14B depict exemplary graphs of the effects of various dosages of hCFTR mRNA formulated with either branched PEI (FIG. 14A) or ICE (FIG. 14B) on the respiratory rate of rats relative to a control.

[0055] FIG. 15 depicts an exemplary graph showing a dose response of chloride-ion channel activity induced by hCFTR mRNA.

## DEFINITIONS

[0056] In order for the present invention to be more readily understood, certain terms are first defined below. Additional definitions for the following terms and other terms are set forth throughout the specification. The publications and other reference materials referenced herein to describe the background of the invention and to provide additional detail regarding its practice are hereby incorporated by reference.

[0057] Alkyl: As used herein, “alkyl” refers to a radical of a straight-chain or branched saturated hydrocarbon group having from 1 to 15 carbon atoms (“C<sub>1-15</sub> alkyl”). In some embodiments, an alkyl group has 1 to 3 carbon atoms (“C<sub>1-3</sub> alkyl”). Examples of C<sub>1-3</sub> alkyl groups include methyl (C<sub>1</sub>), ethyl (C<sub>2</sub>), n-propyl (C<sub>3</sub>), and isopropyl (C<sub>3</sub>). In some embodiments, an alkyl group has 8 to 12 carbon atoms (“C<sub>8-12</sub> alkyl”). Examples of C<sub>8-12</sub> alkyl groups include, without limitation, n-octyl (C<sub>8</sub>), n-nonyl (C<sub>9</sub>), n-decyl (C<sub>10</sub>), n-undecyl (C<sub>11</sub>), n-dodecyl (C<sub>12</sub>) and the like. The prefix “n-” (normal) refers to unbranched alkyl groups. For example, n-C<sub>8</sub> alkyl refers to —(CH<sub>2</sub>)<sub>7</sub>CH<sub>3</sub>, n-C<sub>10</sub> alkyl refers to —(CH<sub>2</sub>)<sub>9</sub>CH<sub>3</sub>, etc.

[0058] Amino acid: As used herein, term “amino acid,” in its broadest sense, refers to any compound and/or substance that can be incorporated into a polypeptide chain. In some embodiments, an amino acid has the general structure H<sub>2</sub>N—C(H)(R)—COHO. In some embodiments, an amino acid is a naturally occurring amino acid. In some embodiments, an amino acid is a synthetic amino acid; in some embodiments, an amino acid is a d-amino acid; in some

embodiments, an amino acid is an L-amino acid. “Standard amino acid” refers to any of the twenty standard L-amino acids commonly found in naturally occurring peptides. “Nonstandard amino acid” refers to any amino acid, other than the standard amino acids, regardless of whether it is prepared synthetically or obtained from a natural source. As used herein, “synthetic amino acid” encompasses chemically modified amino acids, including but not limited to salts, amino acid derivatives (such as amides), and/or substitutions. Amino acids, including carboxyl- and/or amino-terminal amino acids in peptides, can be modified by methylation, amidation, acetylation, protecting groups, and/or substitution with other chemical groups that can change the peptide’s circulating half-life without adversely affecting their activity. Amino acids may participate in a disulfide bond. Amino acids may comprise one or posttranslational modifications, such as association with one or more chemical entities (e.g., methyl groups, acetate groups, acetyl groups, phosphate groups, formyl moieties, isoprenoid groups, sulfate groups, polyethylene glycol moieties, lipid moieties, carbohydrate moieties, biotin moieties, etc.). The term “amino acid” is used interchangeably with “amino acid residue,” and may refer to a free amino acid and/or to an amino acid residue of a peptide. It will be apparent from the context in which the term is used whether it refers to a free amino acid or a residue of a peptide.

[0059] Animal: As used herein, the term “animal” refers to any member of the animal kingdom. In some embodiments, “animal” refers to humans, at any stage of development. In some embodiments, “animal” refers to non-human animals, at any stage of development. In certain embodiments, the non-human animal is a mammal (e.g., a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, and/or a pig). In some embodiments, animals include, but are not limited to, mammals, birds, reptiles, amphibians, fish, insects, and/or worms. In some embodiments, an animal may be a transgenic animal, genetically-engineered animal, and/or a clone.

[0060] Approximately or about: As used herein, the term “approximately” or “about,” as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain embodiments, the term “approximately” or “about” refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0061] Delivery: As used herein, the term “delivery” encompasses both local and systemic delivery. For example, delivery of mRNA encompasses situations in which an mRNA is delivered to a target tissue and the encoded protein is expressed and retained within the target tissue (also referred to as “local distribution” or “local delivery”), and situations in which an mRNA is delivered to a target tissue and the encoded protein is expressed and secreted into patient’s circulation system (e.g., serum) and systematically distributed and taken up by other tissues (also referred to as “systemic distribution” or “systemic delivery”).

[0062] Encapsulation: As used herein, the term “encapsulation,” or grammatical equivalent, refers to the process of confining an mRNA molecule within a nanoparticle.

[0063] Expression: As used herein, “expression” of a nucleic acid sequence refers to translation of an mRNA into a peptide, polypeptide, or protein, assembly of multiple polypeptides (e.g., heavy chain or light chain of antibody) into an intact protein (e.g., antibody) and/or post-translational modification of a polypeptide or fully assembled protein (e.g., antibody). In this application, the terms “expression” and “production,” and grammatical equivalent, are used inter-changeably.

[0064] Functional: As used herein, a “functional” biological molecule is a biological molecule in a form in which it exhibits a property and/or activity by which it is characterized.

[0065] Half-life: As used herein, the term “half-life” is the time required for a quantity such as nucleic acid or protein concentration or activity to fall to half of its value as measured at the beginning of a time period.

[0066] Improve, increase, or reduce: As used herein, the terms “improve,” “increase” or “reduce,” or grammatical equivalents, indicate values that are relative to a baseline measurement, such as a measurement in the same individual prior to initiation of the treatment described herein, or a measurement in a control subject (or multiple control subject) in the absence of the treatment described herein. A “control subject” is a subject afflicted with the same form of disease as the subject being treated, who is about the same age as the subject being treated.

[0067] Impurities: As used herein, the term “impurities” refers to substances inside a confined amount of liquid, gas, or solid, which differ from the chemical composition of the target material or compound. Impurities are also referred to as contaminants.

[0068] In Vitro: As used herein, the term “in vitro” refers to events that occur in an artificial environment, e.g., in a test tube or reaction vessel, in cell culture, etc., rather than within a multi-cellular organism.

[0069] In Vivo: As used herein, the term “in vivo” refers to events that occur within a multi-cellular organism, such as a human and a non-human animal. In the context of cell-based systems, the term may be used to refer to events that occur within a living cell (as opposed to, for example, in vitro systems).

[0070] Isolated: As used herein, the term “isolated” refers to a substance and/or entity that has been (1) separated from at least some of the components with which it was associated when initially produced (whether in nature and/or in an experimental setting), and/or (2) produced, prepared, and/or manufactured by the hand of man. Isolated substances and/or entities may be separated from about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% of the other components with which they were initially associated. In some embodiments, isolated agents are about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. As used herein, a substance is “pure” if it is substantially free of other components. As used herein, calculation of percent purity of isolated substances and/or entities should not include excipients (e.g., buffer, solvent, water, etc.).

[0071] Local distribution or delivery: As used herein, the terms “local distribution,” “local delivery,” or grammatical

equivalent, refer to tissue specific delivery or distribution. Typically, local distribution or delivery requires a protein or peptide encoded by mRNAs be translated and expressed intracellularly or with limited secretion that avoids entering the patient’s circulation system.

[0072] messenger RNA (mRNA): As used herein, the term “messenger RNA (mRNA)” refers to a polynucleotide that encodes at least one peptide, polypeptide or protein. mRNA as used herein encompasses both modified and unmodified RNA. mRNA may contain one or more coding and non-coding regions. mRNA can be purified from natural sources, produced using recombinant expression systems and optionally purified, chemically synthesized, etc. Where appropriate, e.g., in the case of chemically synthesized molecules, mRNA can comprise nucleoside analogs such as analogs having chemically modified bases or sugars, backbone modifications, etc. An mRNA sequence is presented in the 5’ to 3’ direction unless otherwise indicated. In some embodiments, an mRNA is or comprises natural nucleosides (e.g., adenosine, guanosine, cytidine, uridine); nucleoside analogs (e.g., 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, 5-methylcytidine, C-5 propynyl-cytidine, C-5 propynyl-uridine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5-propynyl-cytidine, C5-methylcytidine, 2-aminoadenosine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, 0(6)-methylguanine, and 2-thiocytidine); chemically modified bases; biologically modified bases (e.g., methylated bases); intercalated bases; modified sugars (e.g., 2'-fluororibose, ribose, 2'-deoxyribose, arabinose, and hexose); and/or modified phosphate groups (e.g., phosphorothioates and 5'-N-phosphoramidite linkages).

[0073] In some embodiments, the mRNA comprises one or more nonstandard nucleotide residues. The nonstandard nucleotide residues may include, e.g., 5-methyl-cytidine (“5mC”), pseudouridine (“ΨU”), and/or 2-thio-uridine (“2sU”). See, e.g., U.S. Pat. No. 8,278,036 or WO2011012316 for a discussion of such residues and their incorporation into mRNA. The mRNA may be RNA, which is defined as RNA in which 25% of U residues are 2-thio-uridine and 25% of C residues are 5-methylcytidine. Teachings for the use of RNA are disclosed US Patent Publication US 2012/0195936 and international publication WO 2011/012316, both of which are hereby incorporated by reference in their entirety. The presence of nonstandard nucleotide residues may render an mRNA more stable and/or less immunogenic than a control mRNA with the same sequence but containing only standard residues. In further embodiments, the mRNA may comprise one or more nonstandard nucleotide residues chosen from isocytosine, pseudoisocytosine, 5-bromouracil, 5-propynyluracil, 6-aminopurine, 2-aminopurine, inosine, diaminopurine and 2-chloro-6-aminopurine cytosine, as well as combinations of these modifications and other nucleobase modifications. Certain embodiments may further include additional modifications to the furanose ring or nucleobase. Additional modifications may include, for example, sugar modifications or substitutions (e.g., one or more of a 2'-O-alkyl modification, a locked nucleic acid (LNA)). In some embodiments, the RNAs may be complexed or hybridized with additional polynucleotides and/or peptide polynucleotides (PNA). In embodiments where the sugar modification is a 2'-O-alkyl modification, such modification may include, but are not

limited to a 2'-deoxy-2'-fluoro modification, a 2'-O-methyl modification, a 2'-O-methoxyethyl modification and a 2'-deoxy modification. In certain embodiments, any of these modifications may be present in 0-100% of the nucleotides—for example, more than 0%, 1%, 10%, 25%, 50%, 75%, 85%, 90%, 95%, or 100% of the constituent nucleotides individually or in combination.

[0074] Nucleic acid: As used herein, the term “nucleic acid,” in its broadest sense, refers to any compound and/or substance that is or can be incorporated into a polynucleotide chain. In some embodiments, a nucleic acid is a compound and/or substance that is or can be incorporated into a polynucleotide chain via a phosphodiester linkage. In some embodiments, “nucleic acid” refers to individual nucleic acid residues (e.g., nucleotides and/or nucleosides). In some embodiments, “nucleic acid” refers to a polynucleotide chain comprising individual nucleic acid residues. In some embodiments, “nucleic acid” encompasses RNA as well as single and/or double-stranded DNA and/or cDNA. Furthermore, the terms “nucleic acid,” “DNA,” “RNA,” and/or similar terms include nucleic acid analogs, i.e., analogs having other than a phosphodiester backbone. For example, the so-called “peptide nucleic acids,” which are known in the art and have peptide bonds instead of phosphodiester bonds in the backbone, are considered within the scope of the present invention. The term “nucleotide sequence encoding an amino acid sequence” includes all nucleotide sequences that are degenerate versions of each other and/or encode the same amino acid sequence. Nucleotide sequences that encode proteins and/or RNA may include introns. Nucleic acids can be purified from natural sources, produced using recombinant expression systems and optionally purified, chemically synthesized, etc. Where appropriate, e.g., in the case of chemically synthesized molecules, nucleic acids can comprise nucleoside analogs such as analogs having chemically modified bases or sugars, backbone modifications, etc. A nucleic acid sequence is presented in the 5' to 3' direction unless otherwise indicated. In some embodiments, a nucleic acid is or comprises natural nucleosides (e.g., adenosine, thymidine, guanosine, cytidine, uridine, deoxyadenosine, deoxythymidine, deoxyguanosine, and deoxycytidine); nucleoside analogs (e.g., 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, 5-methylcytidine, C-5 propynyl-cytidine, C-5 propynyl-uridine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5-propynyl-cytidine, C5-methylcytidine, 2-aminoadenosine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, 0(6)-methylguanine, and 2-thiocytidine); chemically modified bases; biologically modified bases (e.g., methylated bases); intercalated bases; modified sugars (e.g., 2'-fluororibose, ribose, 2'-deoxyribose, arabinose, and hexose); and/or modified phosphate groups (e.g., phosphorothioates and 5'-N-phosphoramidite linkages). In some embodiments, the present invention is specifically directed to “unmodified nucleic acids,” meaning nucleic acids (e.g., polynucleotides and residues, including nucleotides and/or nucleosides) that have not been chemically modified in order to facilitate or achieve delivery.

[0075] Patient: As used herein, the term “patient” or “subject” refers to any organism to which a provided composition may be administered, e.g., for experimental, diagnostic, prophylactic, cosmetic, and/or therapeutic purposes. Typical patients include animals (e.g., mammals such as

mice, rats, rabbits, non-human primates, and/or humans). In some embodiments, a patient is a human. A human includes pre and post-natal forms.

[0076] Pharmaceutically acceptable: The term “pharmaceutically acceptable” as used herein, refers to substances that, within the scope of sound medical judgment, are suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0077] Pharmaceutically acceptable salt: Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge et al., describes pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences* (1977) 66:1-19. Pharmaceutically acceptable salts of the compounds of this invention include those derived from suitable inorganic and organic acids and bases. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. Salts derived from appropriate bases include alkali metal, alkaline earth metal, ammonium and  $N^+(C_{1-4} \text{ alkyl})_4$  salts. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, non-toxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, sulfonate and aryl sulfonate. Further pharmaceutically acceptable salts include salts formed from the quaternization of an amine using an appropriate electrophile, e.g., an alkyl halide, to form a quaternized alkylated amino salt.

[0078] Subject: As used herein, the term “subject” refers to a human or any non-human animal (e.g., mouse, rat, rabbit, dog, cat, cattle, swine, sheep, horse or primate). A human includes pre- and post-natal forms. In many embodiments, a subject is a human being. A subject can be a patient, which refers to a human presenting to a medical provider for diagnosis or treatment of a disease. The term “subject” is used herein interchangeably with “individual” or “patient.” A subject can be afflicted with or is susceptible to a disease or disorder (e.g., cystic fibrosis) but may or may not display symptoms of the disease or disorder.

[0079] Substantially: As used herein, the term “substantially” refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will

understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term “substantially” is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

**[0080]** Systemic distribution or delivery: As used herein, the terms “systemic distribution,” “systemic delivery,” or grammatical equivalent, refer to a delivery or distribution mechanism or approach that affect the entire body or an entire organism. Typically, systemic distribution or delivery is accomplished via body’s circulation system, e.g., blood stream. Compared to the definition of “local distribution or delivery.”

**[0081]** Target tissues: As used herein, the term “target tissues” refers to any tissue that is affected by a disease to be treated. In some embodiments, target tissues include those tissues that display disease-associated pathology, symptom, or feature.

**[0082]** Transfer vehicle: In some embodiments, the transfer vehicle is a liposomal vesicle, or other means to facilitate the transfer of a nucleic acid to target cells and tissues. Suitable transfer vehicles include, but are not limited to, liposomes, nanoliposomes, ceramide-containing nanoliposomes, proteoliposomes, nanoparticulates, calcium phosphor-silicate nanoparticulates, calcium phosphate nanoparticulates, silicon dioxide nanoparticulates, nanocrystalline particulates, semiconductor nanoparticulates, poly(D-arginine), nanodendrimers, starch-based delivery systems, micelles, emulsions, niosomes, plasmids, viruses, calcium phosphate nucleotides, aptamers, peptides and other vectorial tags. Also contemplated is the use of bionanocapsules and other viral capsid proteins assemblies as a suitable transfer vehicle. (Hum. Gene Ther. 2008 September; 19(9): 887-95).

**[0083]** Therapeutically effective amount: As used herein, the term “therapeutically effective amount” of a therapeutic agent means an amount that is sufficient, when administered to a subject suffering from or susceptible to a disease, disorder, and/or condition, to treat, diagnose, prevent, and/or delay the onset of the symptom(s) of the disease, disorder, and/or condition. It will be appreciated by those of ordinary skill in the art that a therapeutically effective amount is typically administered via a dosing regimen comprising at least one unit dose.

**[0084]** Treating: As used herein, the term “treat,” “treatment,” or “treating” refers to any method used to partially or completely alleviate, ameliorate, relieve, inhibit, prevent, delay onset of, reduce severity of and/or reduce incidence of one or more symptoms or features of a particular disease, disorder, and/or condition. Treatment may be administered to a subject who does not exhibit signs of a disease and/or exhibits only early signs of the disease for the purpose of decreasing the risk of developing pathology associated with the disease.

**[0085]** Yield: As used herein, the term “yield” refers to the percentage of mRNA recovered after encapsulation as compared to the total mRNA as starting material. In some embodiments, the term “recovery” is used interchangeably with the term “yield”.

#### DETAILED DESCRIPTION

**[0086]** The present invention provides, among other things, methods and compositions for delivering mRNA in

vivo using improved liposomes incorporating a sterol-based cationic lipid, a helper lipid, and a PEG or PEG-modified lipid as described herein.

#### Lipid Nanoparticles

**[0087]** According to the present invention, suitable compositions described herein comprise nucleic acids; and lipid nanoparticles encapsulating the nucleic acids, wherein said lipid nanoparticles comprise distinct lipid components. In some embodiments, there are no more than three distinct lipid components and exemplary distinct lipid components include sterol-based cationic lipids, helper lipids (e.g., non-cationic lipids), and PEG-modified lipids.

**[0088]** In some embodiments, the lipid nanoparticles have an encapsulation percentage of nucleic acids that is at least about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95%.

**[0089]** In some embodiments, the percentage of lipid nanoparticles in a composition that encapsulate a nucleic acid is at least about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 85%, about 90%, or about 95%.

#### Sterol-Based Cationic Lipids

**[0090]** As used herein, the phrase “sterol-based lipid” or “sterol-based cationic lipid” refers to a cationic lipid comprising a steroid moiety. As used herein, the phrase “cationic lipid” refers to any of a number of lipid species that carry a net positive charge at a selected pH, such as physiological pH.

**[0091]** In embodiments, a sterol-based cationic lipid has a structure according to Formula (A), [text missing or illegible when filed]

**[0092]** B-L<sup>1</sup>-S (Formula A), or a protonated form thereof.

**[0093]** B is a basic functional group (e.g., wherein the protonated form has a pK<sub>a</sub> that is no more than about 8.0).

**[0094]** L<sup>1</sup> is an optionally substituted linker group (e.g., a C<sub>1</sub>-C<sub>20</sub> alkylene or a 2- to 20-membered heteroalkylene).

**[0095]** S is a sterol.

**[0096]** In some embodiments, B is a basic functional group wherein the protonated form has a pK<sub>a</sub> that is no more than about 9.0, about 8.5, about 8.0, about 7.5, or about 7.0. In embodiments, B is a basic functional group wherein the protonated form has a pK<sub>a</sub> that is no more than about 8.0 or about 7.5.

**[0097]** In some embodiments, B is a basic functional group wherein the protonated form has a pK<sub>a</sub> that is about 4.0 to about 9.0, about 4.0 to about 8.0, about 4.5 to about 8.0, about 5.0 to about 8.0, about 5.5 to about 8.0, or about 6.0 to about 8.0.

**[0098]** In some embodiments, B is an optionally substituted 5- or 6-membered nitrogen-containing heteroaryl.

**[0099]** In some embodiments, B is a group selected from pyrrolyl, imidazolyl, pyrazolyl, triazolyl, tetrazolyl, pyridyl, pyrimidyl, pyrazinyl, and pyridazinyl, each of which is optionally substituted. For any optionally substituted group described herein (e.g., a group that has 0, 1, 2, 3, or 4 independent substituent groups), exemplary substituent groups include but are not limited to: halogen (e.g., —F, —Cl, —Br, or —I), C<sub>1-20</sub> alkyl (e.g., a C<sub>1-6</sub> alkyl), C<sub>1-20</sub>

haloalkyl (e.g., a C<sub>1-6</sub> haloalkyl), —CN, —OH, —O(C<sub>1-20</sub> alkyl) (e.g., —O(C<sub>1-6</sub> alkyl)), —O(C<sub>1-20</sub> haloalkyl) (e.g., —O(C<sub>1-6</sub> haloalkyl)), —NH<sub>2</sub>, —NH(C<sub>1-6</sub> alkyl), and —NH(C<sub>1-6</sub> alkyl)<sub>2</sub>.

[0100] In some embodiments, B is a group selected from pyrrolyl, pyrazolyl, triazolyl, tetrazolyl, pyridyl, pyrimidyl, pyrazinyl, and pyridazinyl, each of which is optionally substituted.

[0101] In some embodiments, B is a group selected from pyrrolyl, pyrazolyl, triazolyl, tetrazolyl, pyrimidyl, pyrazinyl, and pyridazinyl, each of which is optionally substituted.

[0102] In some embodiments, L<sup>1</sup> is a linker group that is optionally substituted C<sub>1-C<sub>20</sub></sub> alkylene (e.g., an optionally substituted C<sub>1-C<sub>10</sub></sub> alkylene or an optionally substituted C<sub>1-C<sub>5</sub></sub> alkylene). As used herein, the term "alkylene" represents a saturated divalent straight or branched chain hydrocarbon group and is exemplified by methylene, ethylene, isopropylene and the like.

[0103] In some embodiments, L<sup>1</sup> is a linker group that is optionally substituted 2- to 20-membered heteroalkylene (e.g., an optionally substituted 2- to 12-membered heteroalkylene or an optionally substituted 2- to 6-membered heteroalkylene). As used herein, the term "heteroalkylene" refers to a divalent heteroalkyl group. The term "heteroalkyl group" refers to a stable straight or branched chain hydrocarbon radical, having the stated number of atoms, wherein the atoms are selected from carbon and from one to three heteroatoms selected from the group consisting of O, N, Si and S, and wherein the nitrogen and sulfur atoms can optionally be oxidized and the nitrogen heteroatom can optionally be quaternized. The heteroatom(s) O, N and S can be placed at any interior position of the heteroalkyl group.

[0104] In some embodiments, L<sup>1</sup> is a linker group that is optionally substituted 2- to 20-membered heteroalkylene, and wherein L<sup>1</sup> is non-peptidic (that is, a non-peptidic linker L<sup>1</sup> is one that does not comprise amino acid residues).

[0105] In some embodiments, L<sup>1</sup> is a linker group that comprises a moiety that is an ester group, an amide group, a carbamate group, a carbonate group, or a urea group.

[0106] In some embodiments, L<sup>1</sup> is a linker group that comprises a moiety that is an amide group, a carbamate group, a carbonate group, or a urea group.

[0107] In some embodiments, L<sup>1</sup> is a linker group that comprises a moiety that is an amide group, a carbonate group, or a urea group.

[0108] In some embodiments, L<sup>1</sup> is a linker group represented by a formula that is: —X<sup>1</sup>—C(X<sup>3</sup>)—X<sup>2</sup>, —(C<sub>1-C<sub>19</sub></sub> alkylene)—X<sup>1</sup>—C(X<sup>3</sup>)—X<sup>2</sup>, —X<sup>1</sup>—C(X<sup>3</sup>)—X<sup>2</sup>(C<sub>1-C<sub>19</sub></sub> alkylene)-, —(C<sub>1-C<sub>19</sub></sub> alkylene)—X<sup>1</sup>—, —X<sup>1</sup>—(C<sub>1-C<sub>19</sub></sub> alkylene)-.

[0109] Each X<sup>1</sup> and X<sup>2</sup> is independently, a covalent bond, —O—, —S—, or —NH—.

[0110] X<sup>3</sup> is independently =O, =S, or =NH.

[0111] The C<sub>1-C<sub>19</sub></sub> alkylene group (e.g., a C<sub>1-C<sub>5</sub></sub> alkylene or a C<sub>1-C<sub>10</sub></sub> alkylene group) is independently optionally substituted.

[0112] In some embodiments, L<sup>1</sup> is unsubstituted.

[0113] In some embodiments, L<sup>1</sup> does not comprise substituents having the structure —N(R<sup>1</sup>)<sub>2</sub>, or a positively charged form thereof, wherein each R<sup>1</sup> is independently hydrogen or optionally substituted C<sub>1-C<sub>20</sub></sub> alkyl.

[0114] In some embodiments, S is a zoosterol, or an oxidized or reduced form thereof. In embodiments, S is a

zoosterol. In embodiments, S is an oxidized form of a zoosterol. In embodiments, S is a reduced form of a zoosterol.

[0115] In some embodiments, S is a phytosterol, or an oxidized or reduced form thereof. In embodiments, S is a phytosterol. In embodiments, S is an oxidized form of a phytosterol. In embodiments, S is a reduced form of a phytosterol.

[0116] In some embodiments, S is a synthetic sterol (e.g., non-naturally occurring), or an oxidized or reduced form thereof. In embodiments, S is a synthetic sterol. In embodiments, S is an oxidized form of a synthetic sterol. In embodiments, S is a reduced form of a synthetic sterol.

[0117] In some embodiments, S is an oxidized form of a sterol as described herein.

[0118] In some embodiments, an oxidized form of a sterol is one in which the parent sterol has been modified to include further oxygen-containing groups. In embodiments, an oxidized form of a sterol includes one or more (e.g., 1, 2, 3, or 4) additional hydroxyl groups and/or carbonyl-containing groups (e.g., a ketone, an aldehyde, a carboxylic acid, or a carboxylic ester moiety) as compared to the parent sterol.

[0119] In some embodiments, an oxidized form of a sterol is one in which the parent sterol has been modified to include unsaturated carbon-carbon bonds (e.g., carbon-carbon double bonds). In embodiments, an oxidized form of a sterol includes one or more (e.g., 1, 2, or 3) additional carbon-carbon double bonds as compared to the parent sterol.

[0120] In some embodiments, S is a reduced form of a sterol as described herein.

[0121] In some embodiments, a reduced form of a sterol is one in which the parent sterol has been modified to include fewer oxygen-containing groups. In embodiments, a reduced form of a sterol includes a reduced number (e.g., 1, 2, 3, or 4 fewer moieties) of hydroxyl groups and/or carbonyl-containing groups (e.g., a ketone, an aldehyde, a carboxylic acid, or a carboxylic ester moiety) as compared to the parent sterol.

[0122] In some embodiments, a reduced form of a sterol is one in which the parent sterol has been modified to include fewer unsaturated carbon-carbon bonds (e.g., carbon-carbon double bonds). In embodiments, a reduced form of a sterol includes a reduced number (e.g., 1, 2, or 3 fewer) of carbon-carbon double bonds as compared to the parent sterol.

[0123] In some embodiments, S is a sterol that is cholesterol, alkyl lithocholate, stigmasterol, stigmastanol, campesterol, ergosterol, or sitosterol, or any oxidized or reduced form thereof.

[0124] In some embodiments, S is a sterol selected from cholesterol, an oxidized form of cholesterol, a reduced form of cholesterol, alkyl lithocholate, stigmasterol, stigmastanol, campesterol, ergosterol, and sitosterol.

[0125] In some embodiments, S is a sterol selected from an oxidized form of cholesterol, a reduced form of cholesterol, alkyl lithocholate, stigmasterol, stigmastanol, campesterol, ergosterol, and sitosterol.

[0126] In some embodiments, S is a sterol that is cholesterol, alkyl lithocholate, stigmasterol, stigmastanol, campesterol, ergosterol, or sitosterol.

[0127] In some embodiments, S is a sterol that is an oxidized form of: cholesterol, alkyl lithocholate, stigmasterol, stigmastanol, campesterol, ergosterol, or sitosterol.

**[0128]** In some embodiments, S is a sterol that is a reduced form of: cholesterol, alkyl lithocholate, stigmasterol, stigmastanol, campesterol, ergosterol, or sitosterol.

**[0129]** In some embodiments, S is a sterol that is cholesterol, an oxidized form of cholesterol, or a reduced form of cholesterol.

**[0130]** In some embodiments, S is a sterol that is alkyl lithocholate, an oxidized form of alkyl lithocholate, or a reduced form of alkyl lithocholate.

**[0131]** In some embodiments, S is a sterol that is stigmasterol, an oxidized form of stigmasterol, or a reduced form of stigmasterol.

**[0132]** In some embodiments, S is a sterol that is stigmastanol, an oxidized form of stigmastanol, or a reduced form of stigmastanol.

**[0133]** In some embodiments, S is a sterol that is campesterol, an oxidized form of campesterol, or a reduced form of campesterol.

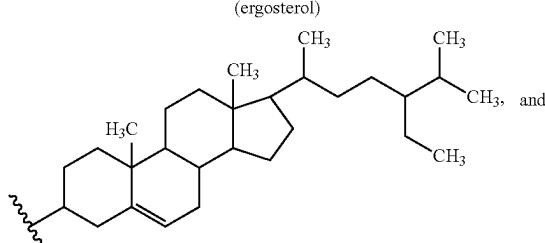
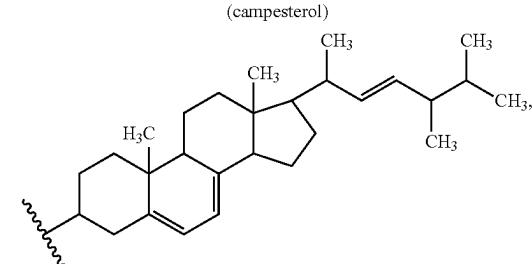
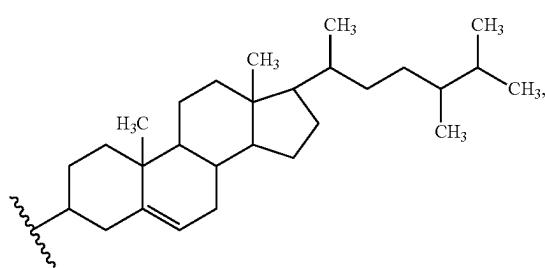
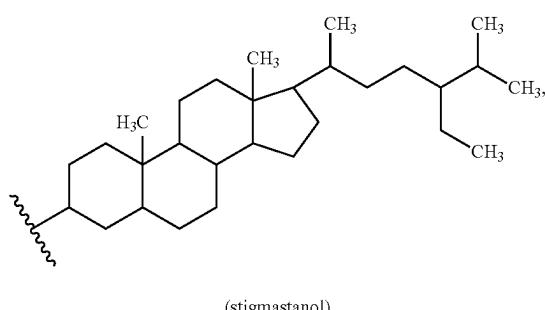
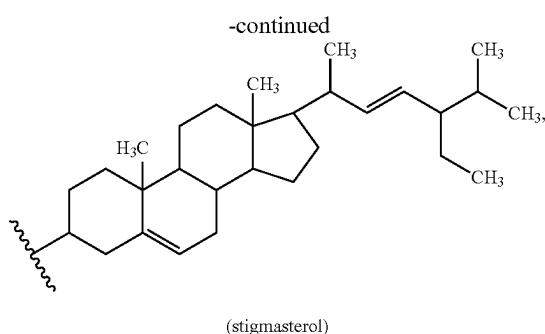
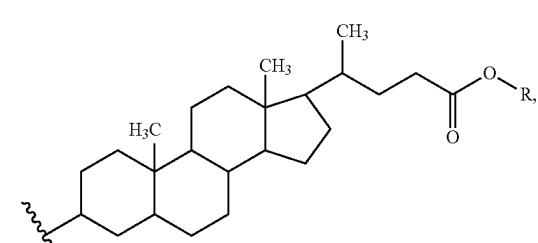
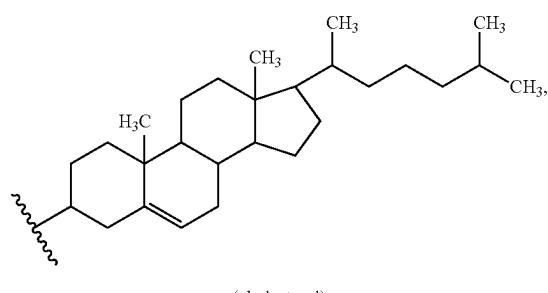
**[0134]** In some embodiments, S is a sterol that is ergosterol, an oxidized form of ergosterol, or a reduced form of ergosterol.

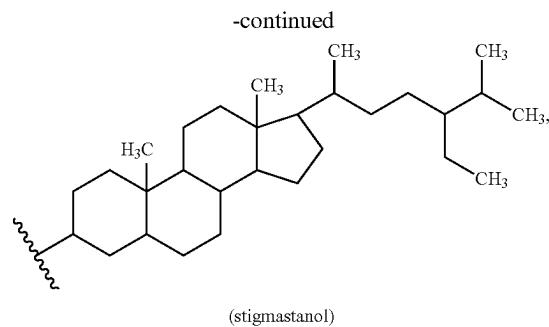
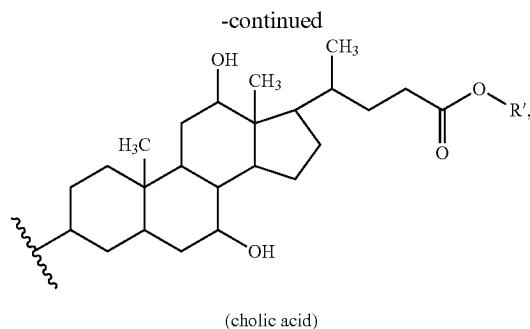
**[0135]** In some embodiments, S is a sterol that is sitosterol, an oxidized form of sitosterol, or a reduced form of sitosterol.

**[0136]** In some embodiments, S is a sterol that is a bile acid or an alkyl ester thereof, or an oxidized form thereof, or a reduced form thereof.

**[0137]** In some embodiments, S is a sterol that is cholic acid or an alkyl ester thereof, or an oxidized form thereof, or a reduced form thereof.

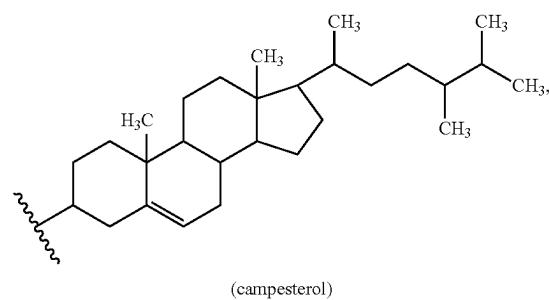
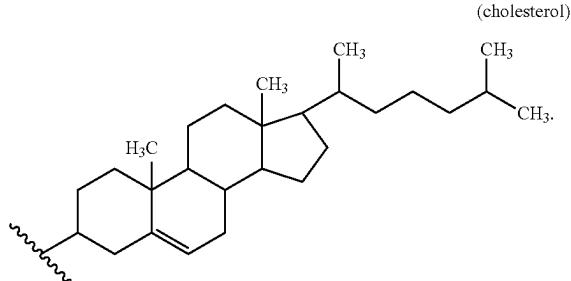
**[0138]** In some embodiments, S is a sterol selected from



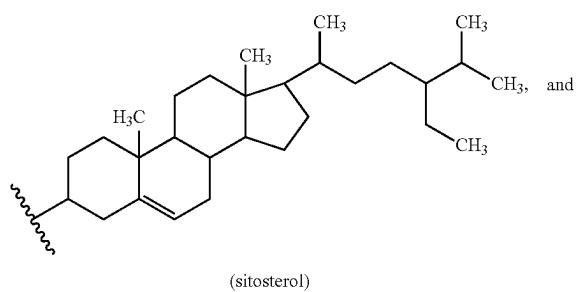
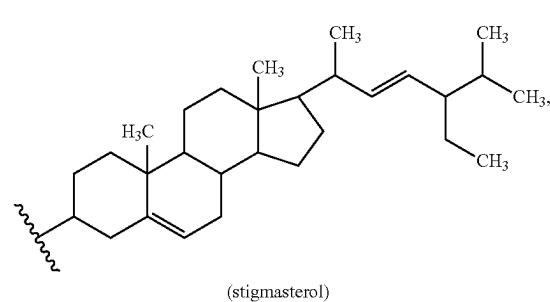
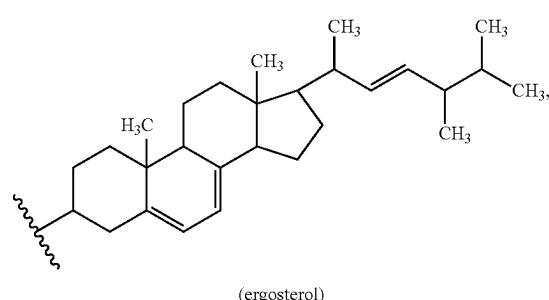
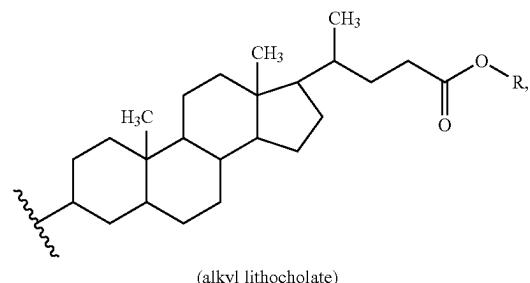


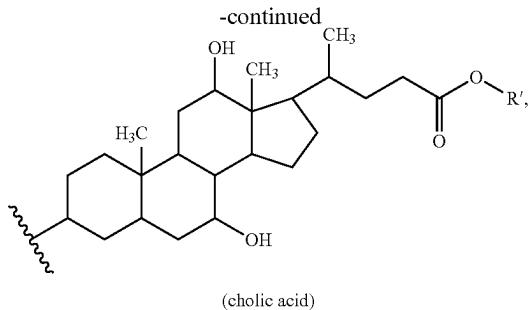
wherein R is optionally substituted C<sub>1</sub>-C<sub>20</sub> alkyl, and R' is H or optionally substituted C<sub>1</sub>-C<sub>20</sub> alkyl.

**[0139]** In some embodiments, S is a sterol that is



**[0140]** In some embodiments, S is a sterol selected from





wherein R is optionally substituted C<sub>1</sub>-C<sub>20</sub> alkyl, and R' is H or optionally substituted C<sub>1</sub>-C<sub>20</sub> alkyl.

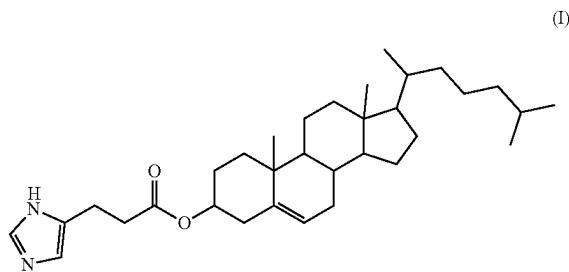
**[0141]** In some embodiments, a sterol-based cationic lipid comprises imidazole cholesterol ester (ICE).

**[0142]** In some embodiments, a sterol-based cationic lipid does not comprise imidazole cholesterol ester (ICE).

**[0143]** In some embodiments, a sterol-based cationic lipid is a cholesterol-based cationic lipid. Such cholesterol-based cationic lipids can be used, either alone or in combination with other lipids as described herein. Suitable cholesterol-based cationic lipids include, for example, DC-Chol (N,N-dimethyl-N-ethylcarboxamidocholesterol), 1,4-bis(3-N-oleylamino-propyl)piperazine (Gao, et al. Biochem. Biophys. Res. Comm. 179, 280 (1991); Wolf et al. BioTechniques 23, 139 (1997); U.S. Pat. No. 5,744,335).

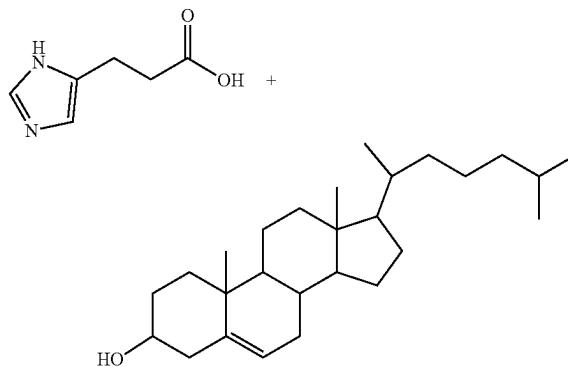
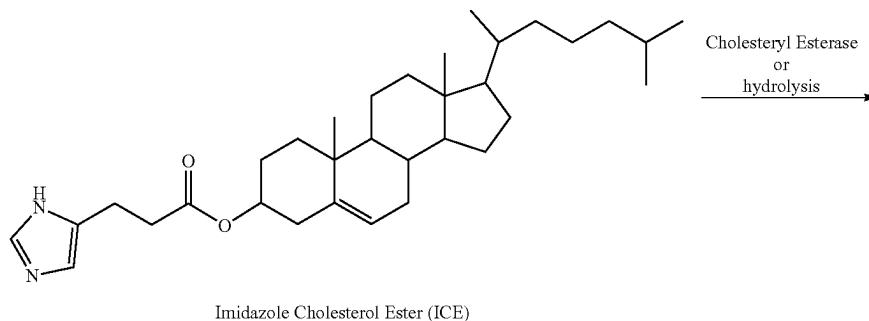
**[0144]** In embodiments, sterol-based cationic lipids are dialkylamino-, imidazole-, and guanidinium-containing sterol-based cationic lipids. For example, certain embodiments

are directed to a composition comprising one or more sterol-based cationic lipids comprising an imidazole, for example, the imidazole cholesterol ester or “ICE” lipid (3S, 10R, 13R, 17R)-10, 13-dimethyl-17-((R)-6-methylheptan-2-yl)-2, 3, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl 3-(1H-imidazol-4-yl)propanoate, as represented by structure (I) below. In certain embodiments, a lipid nanoparticle for delivery of RNA (e.g., mRNA) encoding a functional protein may comprise one or more imidazole-based cationic lipids, for example, the imidazole cholesterol ester or “ICE” lipid (3S, 10R, 13R, 17R)-10, 13-dimethyl-17-((R)-6-methylheptan-2-yl)-2, 3, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl 3-(1H-imidazol-4-yl)propanoate, as represented by structure (I).



**[0145]** An exemplary hydrolysis reaction of structure (I) is provided in Scheme 1.

Scheme 1



**[0146]** Without wishing to be bound by a particular theory, it is believed that the fusogenicity of the imidazole-based cationic lipid ICE is related to the endosomal disruption which is facilitated by the imidazole group, which has a lower pKa relative to traditional cationic lipids. The endosomal disruption in turn promotes osmotic swelling and the disruption of the liposomal membrane, followed by the transfection or intracellular release of the polynucleotide contents loaded or encapsulated therein into the target cell.

**[0147]** The imidazole-based cationic lipids are also characterized by their reduced toxicity relative to other cationic lipids. In some embodiments, one or more of the lipid nanoparticles which comprises the blended pharmaceutical composition comprise an imidazole-based cationic lipid such as ICE, to reduce the relative concentration of other more toxic cationic lipids in such blended pharmaceutical composition. The imidazole-based cationic lipids (e.g., ICE) may be used as the sole cationic lipid in one or more of the lipid nanoparticles that comprise the blended formulations, or alternatively may be combined with traditional cationic lipids (e.g., DOPE, DC-Chol), non-cationic lipids, PEG-modified lipids and/or helper lipids. The cationic lipid may comprise a molar ratio of about 1% to about 90%, about 2% to about 90%, about 5% to about 90%, about 10% to about 90%, about 15% to about 90%, about 20% to about 90%, about 30% to about 90%, about 40% to about 90%, about 50% to about 90%, about 60% to about 90%, about 70% to about 90%, about 80% to about 90%, about 1% to about 80%, about 1% to about 70%, about 1% to about 60%, about 1% to about 50%, about 1% to about 40%, about 1% to about 30%, about 1% to about 20%, about 1% to about 15%, about 1% to about 10%, about 2% to about 70%, about 5% to about 50%, about 10% to about 40% of the total lipid present in the lipid nanoparticle, or preferably about 20% to about 70% of the total lipid present in the lipid nanoparticle.

#### Ratio of Distinct Lipid Components

**[0148]** In embodiments where a lipid nanoparticle comprises three and no more than three distinct lipid components, the ratio of total lipid content (i.e., the ratio of lipid component (1): lipid component (2): lipid component (3)) can be represented as x:y:z, wherein

$$(y + z) = 100 - x.$$

**[0149]** In some embodiments, each of "x," "y," and "z" represents molar percentages of the three distinct lipid components, and the ratio is a molar ratio.

**[0150]** In some embodiments, each of "x," "y," and "z" represents weight percentages of the three distinct lipid components, and the ratio is a weight ratio.

**[0151]** In some embodiments, lipid component (1), represented by variable "x," is a sterol-based cationic lipid.

**[0152]** In some embodiments, lipid component (2), represented by variable "y," is a helper lipid.

**[0153]** In some embodiments, lipid component (3), represented by variable "z," is a PEG lipid.

**[0154]** In some embodiments, variable "x," representing the molar percentage of lipid component (1) (e.g., a sterol-based cationic lipid), is at least about 10%, about 20%, about

30%, about 40%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95%.

**[0155]** In some embodiments, variable "x," representing the molar percentage of lipid component (1) (e.g., a sterol-based cationic lipid), is no more than about 95%, about 90%, about 85%, about 80%, about 75%, about 70%, about 65%, about 60%, about 55%, about 50%, about 40%, about 30%, about 20%, or about 10%. In some embodiments, variable "x" is no more than about 65%, about 60%, about 55%, about 50%, or about 40%.

**[0156]** In some embodiments, variable "x," representing the molar percentage of lipid component (1) (e.g., a sterol-based cationic lipid), is: at least about 50% but less than about 95%; at least about 50% but less than about 90%; at least about 50% but less than about 85%; at least about 50% but less than about 80%; at least about 50% but less than about 75%; at least about 50% but less than about 70%; at least about 50% but less than about 65%; or at least about 50% but less than about 60%.

**[0157]** In some embodiments, variable "x," representing the weight percentage of lipid component (1) (e.g., a sterol-based cationic lipid), is at least about 10%, about 20%, about 30%, about 40%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95%.

**[0158]** In some embodiments, variable "x," representing the weight percentage of lipid component (1) (e.g., a sterol-based cationic lipid), is no more than about 95%, about 90%, about 85%, about 80%, about 75%, about 70%, about 65%, about 60%, about 55%, about 50%, about 40%, about 30%, about 20%, or about 10%.

**[0159]** In some embodiments, variable "x," representing the weight percentage of lipid component (1) (e.g., a sterol-based cationic lipid), is: at least about 50% but less than about 95%; at least about 50% but less than about 90%; at least about 50% but less than about 85%; at least about 50% but less than about 80%; at least about 50% but less than about 75%; at least about 50% but less than about 70%; at least about 50% but less than about 65%; or at least about 50% but less than about 60%.

**[0160]** In some embodiments, variable "z," representing the molar percentage of lipid component (3) (e.g., a PEG-modified lipid) is no more than about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, or 25%. In some embodiments, variable "z," representing the molar percentage of lipid component (3) (e.g., a PEG-modified lipid) is about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%. In embodiments, variable "z," representing the molar percentage of lipid component (3) (e.g., a PEG-modified lipid) is about 1% to about 10%, about 2% to about 10%, about 3% to about 10%, about 4% to about 10%, about 5% to about 10%, about 6% to about 10%, about 7% to about 10%, about 8% to about 10%, about 9% to about 10%, about 1% to about 9%, about 1% to about 8%, about 1% to about 7.5%, about 1% to about 7%, about 1% to about 6%, about 1% to about 5%, about 1% to about 4%, about 1% to about 3%, about 1% to about 2%, about 2.5% to about 10%, about 2.5% to about 7.5%, about 2.5% to about 5%, about 5% to about 7.5%, or about 5% to about 10%.

**[0161]** In some embodiments, variable "z," representing the weight percentage of lipid component (3) (e.g., a PEG-modified lipid) is no more than about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, or 25%. In some

embodiments, variable "z," representing the weight percentage of lipid component (3) (e.g., a PEG lipid) is about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%. In some embodiments, variable "z," representing the weight percentage of lipid component (3) (e.g., a PEG-modified lipid) is about 1% to about 10%, about 2% to about 10%, about 3% to about 10%, about 4% to about 10%, about 5% to about 10%, about 6% to about 10%, about 7% to about 10%, about 8% to about 10%, about 9% to about 10%, about 1% to about 9%, about 1% to about 8%, about 1% to about 7.5%, about 1% to about 7%, about 1% to about 6%, about 1% to about 5%, about 1% to about 4%, about 1% to about 3%, about 1% to about 2%, about 2.5% to about 10%, about 2.5% to about 7.5%, about 2.5% to about 5%, about 5% to about 7.5%, or about 5% to about 10%.

[0162] For compositions having three and only three distinct lipid components, variables "x," "y," and "z" may be in any combination so long as the total of the three variables sums to 100% of the total lipid content.

#### Other Lipid Components

[0163] The use of polyethylene glycol (PEG)-modified phospholipids and derivatized lipids such as derivatized ceramides (PEG-CER), including N-Octanoyl-Sphingosine-1-[Succinyl(Methoxy Polyethylene Glycol)-2000](C8 PEG-2000 ceramide) is also contemplated by the present invention, either alone or preferably in combination with other lipid formulations together which comprise the transfer vehicle (e.g., a lipid nanoparticle). Contemplated PEG-modified lipids include, but are not limited to, a polyethylene glycol chain of up to 5 kDa in length covalently attached to a lipid with alkyl chain(s) of C6-C20 length. The addition of such components may prevent complex aggregation and may also provide a means for increasing circulation lifetime and increasing the delivery of the lipid-nucleic acid composition to the target tissues, (Klibanov et al. (1990) FEBS Letters, 268 (1): 235-237), or they may be selected to rapidly exchange out of the formulation *in vivo* (see U.S. Pat. No. 5,885,613). Particularly useful exchangeable lipids are PEG-ceramides having shorter acyl chains (e.g., C14 or C18).

[0164] The PEG-modified phospholipid and derivitized lipids of the present invention may comprise a molar ratio from about 0% to about 20%, about 0.5% to about 20%, about 1% to about 20%, about 2% to about 20%, about 3% to about 20%, about 4% to about 20%, about 5% to about 20%, about 10% to about 20%, about 15% to about 20%, about 0% to about 15%, about 0% to about 10%, about 0% to about 5%, about 0% to about 4%, about 0% to about 3%, about 0% to about 220%, about 0% to about 1%, about 5% to about 15%, about 4% to about 10%, or about 2% of the total lipid present in the liposomal transfer vehicle.

[0165] The present invention also contemplates the use of non-cationic lipids. As used herein, the phrase "non-cationic lipid" refers to any neutral, zwitterionic or anionic lipid. As used herein, the phrase "anionic lipid" refers to any of a number of lipid species that carry a net negative charge at a selected pH, such as physiological pH. Non-cationic lipids include, but are not limited to, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoylphosphatidylethanolamine (DOPE), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleyl-phosphatidylethanolamine (POPE), dioleoyl-phospha-

tidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1-trans PE, 1-stearoyl-2-oleoyl-phosphatidylethanolamine (SOPE), cholesterol, or a mixture thereof. Such non-cationic lipids may be used alone, but are preferably used in combination with other excipients, for example, cationic lipids.

[0166] In some embodiments, cationic lipids that are not sterol-based cationic lipids can be used in liposomal compositions of the invention. Cationic lipids include, but are not limited to, N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), 5-carboxyspermylglycine-octadecylamide (DOGS), 2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminium (DOSPA), 1,2-Dioleoyl-3-Dimethylammonium-Propane (DODAP), 1,2-Dioleoyl-3-Trimethylammonium-Propane (DOTAP), 1,2-distearoyloxy-N,N-dimethyl-3-aminopropane (DSDMA), 1,2-dioleyloxy-N,N-dimethyl-3-aminopropane (DODMA), 1,2-dilinoleoyloxy-N,N-dimethyl-3-aminopropane (DLinDMA), 1,2-dilinolenyloxy-N,N-dimethyl-3-aminopropane (DLendDMA), N-dioleyl-N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide (DMRIE), 3-dimethylamino-2-(cholest-5-en-3-beta-oxybutan-4-oxy)-1-(*cis,cis*-9,12-octadecadienoxy)propane (CLinDMA), 2-[5'-(cholest-5-en-3-beta-oxy)-3'-oxapentenoxy]-3-dimethyl-1-(*cis,cis*-9',1-2'-octadecadienoxy)propane (CpLinDMA), N,N-dimethyl-3,4-dioleyloxybenzylamine (DMOBA), 1,2-N,N-dioleylcarbamyl-3-dimethylaminopropane (DOcarbDAP), 2,3-Dilinoleoyloxy-N,N-dimethylpropylamine (DLinDAP), 1,2-N,N'-Dilinoleylcarbamyl-3-dimethylaminopropane (DLincarbDAP), 1,2-Dilinoleoylcarbamyl-3-dimethylaminopropane or (DLinCDAP), 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA), 2,2-dilinoleyl-4-dimethylaminooethyl-[1,3]-dioxolane (DLin-K-XTC2-DMA), or mixtures thereof.

[0167] Additional cationic lipids include 2-((2,3-Bis((9Z, 12Z)-octadeca-9,12-dien-1-yloxy)propyl)disulfanyl)-N,N-dimylethanamine (further described in U.S. Provisional Application No: PCT/US2012/041663, filed Jun. 8, 2012, the entire teachings of which are incorporated herein by reference in their entirety), cleavable lipids, such as, for example, one or more cationic lipids that comprise a cleavable disulfide (S—S) functional group, as further described in U.S. International Application No: PCT/US2012/041663. In addition, several reagents are commercially available to enhance transfection efficacy. Suitable examples include LIPOFECTIN (DOTMA:DOPE) (Invitrogen, Carlsbad, Calif.), LIPOFECTAMINE (DOSPA:DOPE) (Invitrogen), LIPOFECTAMINE2000 (Invitrogen), FUGENE, TRANS-FECTAM (DOGS), and EFFECTENE.

#### Formation of Liposomes Encapsulating mRNA

[0168] The liposomal transfer vehicles for use in the present invention can be prepared by various techniques which are presently known in the art. Multi-lamellar vesicles (MLV) may be prepared conventional techniques, for example, by depositing a selected lipid on the inside wall of a suitable container or vessel by dissolving the lipid in an appropriate solvent, and then evaporating the solvent to leave a thin film on the inside of the vessel or by spray

drying. An aqueous phase may then be added to the vessel with a vortexing motion which results in the formation of MLVs. Uni-lamellar vesicles (ULV) can then be formed by homogenization, sonication or extrusion of the multi-lamellar vesicles. In addition, unilamellar vesicles can be formed by detergent removal techniques.

[0169] In certain embodiments of this invention, the compositions of the present invention comprise a transfer vehicle wherein the therapeutic RNA (e.g., mRNA encoding CFTR) is associated on both the surface of the transfer vehicle (e.g., a liposome) and encapsulated within the same transfer vehicle. For example, during preparation of the compositions of the present invention, cationic liposomal transfer vehicles may associate with the nucleic acids (e.g., mRNA) through electrostatic interactions with such therapeutic mRNA.

[0170] In some embodiments, the compositions and methods of the invention comprise mRNA encapsulated in a liposome. In some embodiments, the one or more mRNA species may be encapsulated in the same liposome. In some embodiments, the one or more mRNA species may be encapsulated in different liposomes. In some embodiments, the mRNA is encapsulated in one or more liposomes, which differ in their lipid composition, molar ratio of lipid components, size, charge (Zeta potential), targeting ligands and/or combinations thereof. In some embodiments, the one or more liposome may have a different composition of sterol-based cationic lipids, neutral lipid, PEG-modified lipid and/or combinations thereof. In some embodiments the one or more liposomes may have a different molar ratio of sterol-based cationic lipid, neutral lipid, and PEG-modified lipid used to create the liposome.

[0171] The process of incorporation of a desired mRNA into a liposome is often referred to as “loading”. Exemplary methods are described in Lasic, et al., FEBS Lett., 312: 255-258, 1992, which is incorporated herein by reference. The liposome-incorporated nucleic acids may be completely or partially located in the interior space of the liposome, within the bilayer membrane of the liposome, or associated with the exterior surface of the liposome membrane. The incorporation of a nucleic acid into liposomes is also referred to herein as “encapsulation” wherein the nucleic acid is entirely contained within the interior space of the liposome. The purpose of incorporating an mRNA into a transfer vehicle, such as a liposome, is often to protect the nucleic acid from an environment which may contain enzymes or chemicals that degrade nucleic acids and/or systems or receptors that cause the rapid excretion of the nucleic acids. Accordingly, in some embodiments, a suitable delivery vehicle is capable of enhancing the stability of the mRNA contained therein and/or facilitate the delivery of mRNA to the target cell or tissue.

[0172] Any desired lipids may be mixed at any ratios suitable for encapsulating mRNAs. In some embodiments, a suitable lipid solution contains a mixture of desired lipids including sterol-based cationic lipids, non-cationic lipids, and/or PEG-modified lipids. In some embodiments, a suitable lipid solution contain a mixture of desired lipids including one or more sterol-based cationic lipids, one or more helper lipids (e.g. non cationic lipids) and one or more PEG-modified lipids.

[0173] Exemplary combinations of sterol-based cationic lipids, non-cationic lipids, and PEG-modified lipids are described in the Examples section. For example, a suitable

lipid solution may contain ICE, DOPE and DMG-PEG2K. The selection of sterol-based cationic lipids, non-cationic lipids and/or PEG-modified lipids which comprise the lipid mixture as well as the relative molar ratio of such lipids to each other, is based upon the characteristics of the selected lipid(s) and the nature of the and the characteristics of the mRNA to be encapsulated. Additional considerations include, for example, the saturation of the alkyl chain, as well as the size, charge, pH, pKa, fusogenicity and toxicity of the selected lipid(s). Thus the molar ratios may be adjusted accordingly.

[0174] In some embodiments, a process for encapsulating mRNA in lipid nanoparticles comprises mixing an mRNA solution and a lipid solution, wherein the mRNA solution and/or the lipid solution are heated to a pre-determined temperature greater than ambient temperature prior to mixing to form lipid nanoparticles that encapsulate mRNA (see U.S. patent application Ser. No. 14/790,562 entitled “Encapsulation of messenger RNA”, filed Jul. 2, 2015 and its provisional U.S. patent application Ser. No. 62/020,163, filed Jul. 2, 2014, the disclosure of which are hereby incorporated in their entirety).

[0175] In some embodiments, a process for encapsulating mRNA in lipid nanoparticles comprises combining pre-formed lipid nanoparticles with mRNA (see U.S. Provisional Application Ser. No. 62/420,413, filed Nov. 10, 2016 and U.S. Provisional Application Ser. No. 62/580,155, filed Nov. 1, 2017, the disclosures of which are hereby incorporated by reference). In some embodiments, combining pre-formed lipid nanoparticles with mRNA results in lipid nanoparticles that show improved efficacy of intracellular delivery of the mRNA. In some embodiments, combining pre-formed lipid nanoparticles with mRNA results in very high encapsulation efficiencies of mRNA encapsulated in lipid nanoparticles (i.e., in the range of 90-95%). In some embodiments, combining pre-formed lipid nanoparticles with mRNA is achieved with pump systems which maintain the lipid/mRNA (N/P) ratio constant throughout the process and which also afford facile scale-up.

[0176] Suitable liposomes in accordance with the present invention may be made in various sizes. In some embodiments, provided liposomes may be made smaller than previously known mRNA encapsulating liposomes. In some embodiments, decreased size of liposomes is associated with more efficient delivery of mRNA. Selection of an appropriate liposome size may take into consideration the site of the target cell or tissue and to some extent the application for which the liposome is being made.

[0177] In some embodiments, an appropriate size of liposome is selected to facilitate systemic distribution of a protein or a peptide encoded by the mRNA. In some embodiments, it may be desirable to limit delivery of the mRNA to certain cells or tissues. For example, to target hepatocytes, a liposome may be sized such that its dimensions are smaller than the fenestrations of the endothelial layer lining hepatic sinusoids in the liver; in such cases the liposome could readily penetrate such endothelial fenestrations to reach the target hepatocytes.

[0178] Alternatively or additionally, a liposome may be sized such that the dimensions of the liposome are of a sufficient diameter to limit or expressly avoid distribution into certain cells or tissues. For example, a liposome may be sized such that its dimensions are larger than the fenestra-

tions of the endothelial layer lining hepatic sinusoids to thereby limit distribution of the liposomes to hepatocytes.

[0179] In some embodiments, the size of a liposome is determined by the length of the largest diameter of the liposome particle. In some embodiments, a suitable liposome has a size no greater than about 250 nm (e.g., no greater than about 225 nm, 200 nm, 175 nm, 150 nm, 125 nm, 100 nm, 75 nm, or 50 nm). In some embodiments, a suitable liposome has a size ranging from about 10-250 nm (e.g., ranging from about 10-225 nm, 10-200 nm, 10-175 nm, 10-150 nm, 10-125 nm, 10-100 nm, 10-75 nm, or 10-50 nm). In some embodiments, a suitable liposome has a size ranging from about 100-250 nm (e.g., ranging from about 100-225 nm, 100-200 nm, 100-175 nm, 100-150 nm). In some embodiments, a suitable liposome has a size ranging from about 10-100 nm (e.g., ranging from about 10-90 nm, 10-80 nm, 10-70 nm, 10-60 nm, or 10-50 nm). In a particular embodiment, a suitable liposome has a size less than about 100 nm. In some embodiments, majority of nanoparticles in a composition, i.e., greater than about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of the purified nanoparticles, have a size less than about 100 nm (e.g., less than about 95 nm, about 90 nm, about 85 nm, about 80 nm, about 75 nm, about 70 nm, about 65 nm, about 60 nm, about 55 nm, about 50 nm, about 45 nm, or about 40 nm). In some embodiments, substantially all of the purified nanoparticles have a size less than 100 nm (e.g., less than about 95 nm, about 90 nm, about 85 nm, about 80 nm, about 75 nm, about 70 nm, about 65 nm, about 60 nm, about 55 nm, about 50 nm, about 45 nm, or about 40 nm).

[0180] A variety of alternative methods known in the art are available for sizing of a population of liposomes. One such sizing method is described in U.S. Pat. No. 4,737,323, incorporated herein by reference. Sonicating a liposome suspension either by bath or probe sonication produces a progressive size reduction down to small ULV less than about 0.05 microns in diameter. Homogenization is another method that relies on shearing energy to fragment large liposomes into smaller ones. In a typical homogenization procedure, MLV are recirculated through a standard emulsion homogenizer until selected liposome sizes, typically between about 0.1 and 0.5 microns, are observed. The size of the liposomes may be determined by quasi-electric light scattering (QELS) as described in Bloomfield, Ann. Rev. Biophys. Bioeng., 10:421-150 (1981), incorporated herein by reference. Average liposome diameter may be reduced by sonication of formed liposomes. Intermittent sonication cycles may be alternated with QELS assessment to guide efficient liposome synthesis.

#### Purification

[0181] Typically, subsequent to formulation the lipid nanoparticles containing mRNA are purified and/or concentrated. Various purification methods may be used. In some embodiments, lipid nanoparticles are purified using Tangential Flow Filtration. Tangential flow filtration (TFF), also referred to as cross-flow filtration, is a type of filtration wherein the material to be filtered is passed tangentially across a filter rather than through it. In TFF, undesired permeate passes through the filter, while the desired retentate passes along the filter and is collected downstream. It is important to note that the desired material is typically

contained in the retentate in TFF, which is the opposite of what one normally encounters in traditional-dead end filtration.

[0182] Depending upon the material to be filtered, TFF is usually used for either microfiltration or ultrafiltration. Microfiltration is typically defined as instances where the filter has a pore size of between 0.05  $\mu\text{m}$  and 1.0  $\mu\text{m}$ , inclusive, while ultrafiltration typically involves filters with a pore size of less than 0.05  $\mu\text{m}$ . Pore size also determines the nominal molecular weight limits (NMWL), also referred to as the molecular weight cut off (MWCO) for a particular filter, with microfiltration membranes typically having NMWLs of greater than 1,000 kilodaltons (kDa) and ultrafiltration filters having NMWLs of between 1 kDa and 1,000 kDa.

[0183] A principal advantage of tangential flow filtration is that non-permeable particles that may aggregate in and block the filter (sometimes referred to as “filter cake”) during traditional “dead-end” filtration are instead carried along the surface of the filter. This advantage allows tangential flow filtration to be widely used in industrial processes requiring continuous operation since down time is significantly reduced because filters do not generally need to be removed and cleaned.

[0184] Tangential flow filtration can be used for several purposes including concentration and diafiltration, among others. Concentration is a process whereby solvent is removed from a solution while solute molecules are retained. In order to effectively concentrate a sample, a membrane having a NMWL or MWCO that is substantially lower than the molecular weight of the solute molecules to be retained is used. Generally, one of skill may select a filter having a NMWL or MWCO of three to six times below the molecular weight of the target molecule(s).

[0185] Diafiltration is a fractionation process whereby small undesired particles are passed through a filter while larger desired nanoparticles are maintained in the retentate without changing the concentration of those nanoparticles in solution. Diafiltration is often used to remove salts or reaction buffers from a solution. Diafiltration may be either continuous or discontinuous. In continuous diafiltration, a diafiltration solution is added to the sample feed at the same rate that filtrate is generated. In discontinuous diafiltration, the solution is first diluted and then concentrated back to the starting concentration. Discontinuous diafiltration may be repeated until a desired concentration of nanoparticles is reached.

[0186] Purified and/or concentrated lipid nanoparticles may be formulated in a desired buffer such as, for example, PBS.

[0187] Thus, the present invention provides a composition comprising purified nanoparticles described herein. In some embodiments, majority of purified nanoparticles in a composition, i.e., greater than about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of the purified nanoparticles, have a size less than about 100 nm (e.g., less than about 95 nm, about 90 nm, about 85 nm, about 80 nm, about 75 nm, about 70 nm, about 65 nm, about 60 nm, about 55 nm, about 50 nm, about 45 nm, or about 40 nm). In some embodiments, substantially all of the purified nanoparticles have a size less than 100 nm (e.g., less than about 95 nm, about 90 nm, about 85 nm, about 80 nm, about 75 nm, about 70 nm, about 65 nm, about 60 nm, about 55 nm, about 50 nm, about 45 nm, or about 40 nm).

**[0188]** In some embodiments, greater than about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% of the purified nanoparticles in a composition provided by the present invention have a size ranging from about 40-90 nm (e.g., about 40-85 nm, about 40-80 nm, about 40-75 nm, about 40-70 nm, about 40-65 nm, or about 40-60 nm). In some embodiments, substantially all of the purified nanoparticles have a size ranging from about 40-90 nm (e.g., about 40-85 nm, about 40-80 nm, about 40-75 nm, about 40-70 nm, about 40-65 nm, or about 40-60 nm).

**[0189]** In some embodiments, the dispersity, or measure of heterogeneity in size of molecules (PDI), of nanoparticles in a composition provided by the present invention is less than about 0.16 (e.g., less than about 0.15, 0.14, 0.13, 0.12, 0.11, 0.10, 0.09, or 0.08).

**[0190]** In some embodiments, greater than about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of the purified lipid nanoparticles in a composition provided by the present invention encapsulate an mRNA within each individual particle. In some embodiments, substantially all of the purified lipid nanoparticles in a composition encapsulate an mRNA within each individual particle.

**[0191]** In some embodiments, a composition according to the present invention contains at least about 1 mg, 5 mg, 10 mg, 100 mg, 500 mg, or 1000 mg of encapsulated mRNA. In some embodiments, a process according to the present invention results in greater than about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% recovery of mRNA.

#### Nucleic Acids and mRNA

**[0192]** The present invention can be used to deliver any type of nucleic acid. A nucleic acid may be selected from the group comprising (but not limited to) DNA, messenger RNA (mRNA), small nuclear RNA (snRNA), guide RNA (gRNA), CRISPR RNA (crRNA), long noncoding RNA (lncRNA), micro RNA (miRNA), small interfering RNA (siRNA), and short hairpin RNA (shRNA).

**[0193]** The present invention can be used to deliver any mRNA. mRNA is typically thought of as the type of RNA that carries information from DNA to the ribosome. Typically, in eukaryotic organisms, mRNA processing comprises the addition of a “cap” on the N-terminal (5') end, and a “tail” on the C-terminal (3') end. A typical cap is a 7-methylguanosine cap, which is a guanosine that is linked through a 5'-5'-triphosphate bond to the first transcribed nucleotide. The presence of the cap is important in providing resistance to nucleases found in most eukaryotic cells. The tail is typically a polyadenylation event whereby a polyadenylyl moiety is added to the 3' end of the mRNA molecule. The presence of this “tail” serves to protect the mRNA from

exonuclease degradation. Messenger RNA typically is translated by the ribosomes into a series of amino acids that make up a protein or a peptide.

**[0194]** Any mRNA capable of being translated into one or more peptides (e.g., antigens) or peptide fragments is contemplated as within the scope of the present invention. In some embodiments, an mRNA encodes one or more naturally occurring peptides. In some embodiments, an mRNA encodes one or more modified or non-natural peptides.

**[0195]** In some embodiments an mRNA encodes an intracellular protein or peptide. In some embodiments, an mRNA encodes a cytosolic protein or peptide. In some embodiments, an mRNA encodes a protein or peptide associated with the actin cytoskeleton. In some embodiments, an mRNA encodes a protein or peptide associated with the plasma membrane. In some specific embodiments, an mRNA encodes a transmembrane protein or peptide. In some specific embodiments an mRNA encodes an ion channel protein or peptide. In some embodiments, an mRNA encodes a perinuclear protein or peptide. In some embodiments, an mRNA encodes a nuclear protein or peptide. In some specific embodiments, an mRNA encodes a transcription factor. In some embodiments, an mRNA encodes a chaperone protein or peptide. In some embodiments, an mRNA encodes an intracellular enzyme (e.g., mRNA encoding an enzyme associated with urea cycle or lysosomal storage metabolic disorders). In some embodiments, an mRNA encodes a protein or peptide involved in cellular metabolism, DNA repair, transcription and/or translation. In some embodiments, an mRNA encodes an extracellular protein or peptide. In some embodiments, an mRNA encodes a protein or peptide associated with the extracellular matrix. In some embodiments an mRNA encodes a secreted protein or peptide. In specific embodiments, an mRNA used in the composition and methods of the invention may be used to express functional proteins or enzymes that are excreted or secreted by one or more target cells into the surrounding extracellular fluid (e.g., mRNA encoding hormones and/or neurotransmitters).

**[0196]** In some embodiments, the compositions and methods of the invention provide for delivery of mRNA encoding a secreted protein. In some embodiments, the compositions and methods of the invention provide for delivery of mRNA encoding one or more secreted proteins listed in Table 1; thus, compositions of the invention may comprise an mRNA encoding a protein listed in Table 1 (or a homolog thereof) along with other components set out herein, and methods of the invention may comprise preparing and/or administering a composition comprising an mRNA encoding a protein listed in Table 1 (or a homolog thereof) along with other components set out herein.

TABLE 1

Secreted Proteins		
Uniprot ID	Protein Name	Gene Name
A1E959	Odontogenic ameloblast-associated protein	ODAM
A1KZ92	Peroxidasin-like protein	PXDNL
A1L453	Serine protease 38	PRSS38
A1L4H1	Soluble scavenger receptor cysteine-rich domain-containing protein SSC5D	SSC5D
A2RUU4	Colipase-like protein 1	CLPSL1
A2VDF0	Fucose mutarotase	FUOM
A2VEC9	SCO-spondin	SSPO
A3KMH1	von Willebrand factor A domain-containing protein 8	VWA8

TABLE 1-continued

Secreted Proteins		
Uniprot ID	Protein Name	Gene Name
A4D0S4	Laminin subunit beta-4	LAMB4
A4D1T9	Probable inactive serine protease 37	PRSS37
A5D8T8	C-type lectin domain family 18 member A	CLEC18A
A6NC86	phospholipase A2 inhibitor and Ly6/PLAUR domain-containing protein	PINLYP
A6NCI4	von Willebrand factor A domain-containing protein 3A	VWA3A
A6ND01	Probable folate receptor delta	FOLR4
A6NDD2	Beta-defensin 108B-like	
A6NE02	BTB/POZ domain-containing protein 17	BTBD17
A6NEF6	Growth hormone 1	GH1
A6NF02	NPIP-like protein LOC730153	
A6NFB4	HCG1749481, isoform CRA_k	CSH1
A6NFZ4	Protein FAM24A	FAM24A
A6NG13	Glycosyltransferase 54 domain-containing protein	
A6NGN9	IgLON family member 5	IGLON5
A6NHN0	Otolin-1	OTOL1
A6NHN6	Nuclear pore complex-interacting protein-like 2	NPIPL2
A6NIT3	Leukocyte immunoglobulin-like receptor subfamily A member 5	LILRA5
A6NIT4	Chorionic somatomammotropin hormone 2 isoform 2	CSH2
A6NI69	IgA-inducing protein homolog	IGIP
A6NKQ9	Choriogonadotropin subunit beta variant 1	CGB1
A6NMZ7	Collagen alpha-6(VI) chain	COL6A6
A6NNS2	Dehydrogenase/reductase SDR family member 7C	DHRS7C
A6XGL2	Insulin A chain	INS
A8K0G1	Protein Wnt	WNT7B
A8K2U0	Alpha-2-macroglobulin-like protein 1	A2ML1
A8K7I4	Calcium-activated chloride channel regulator 1	CLCA1
A8MTL9	Serpin-like protein HMSD	HMSD
A8MV23	Serpin E3	SERPINE3
A8MZB6	Oocyte-secreted protein 1 homolog	OOSP1
A8TX70	Collagen alpha-5(VI) chain	COL6A5
B0ZBE8	Natriuretic peptide	NPPA
B1A4G9	Somatotropin	GH1
B1A4H2	HCG1749481, isoform CRA_d	CSH1
B1A4H9	Chorionic somatomammotropin hormone	CSH2
B1AJZ6	Protein Wnt	WNT4
B1AKI9	Isthmin-1	ISM1
B2RNN3	Complement C1q and tumor necrosis factor-related protein 9B	C1QTNF9B
B2RUY7	von Willebrand factor C domain-containing protein 2-like	VWC2L
B3GLJ2	Prostate and testis expressed protein 3	PATE3
B4DI03	SEC11-like 3 ( <i>S. cerevisiae</i> ), isoform CRA_a	SEC11L3
B4DJF9	Protein Wnt	WNT4
B4DUL4	SEC11-like 1 ( <i>S. cerevisiae</i> ), isoform CRA_d	SEC11L1
B5MCC8	Protein Wnt	WNT10B
B8A595	Protein Wnt	WNT7B
B8A597	Protein Wnt	WNT7B
B8A598	Protein Wnt	WNT7B
B9A064	Immunoglobulin lambda-like polypeptide 5	IGLL5
C9J3H3	Protein Wnt	WNT10B
C9J8I8	Protein Wnt	WNT5A
C9JAF2	Insulin-like growth factor II Ala-25 Del	IGF2
C9JC12	Protein Wnt	WNT10B
C9JL84	HERV-H LTR-associating protein 1	HHLA1
C9JNR5	Insulin A chain	INS
C9JUI2	Protein Wnt	WNT2
D6RF47	Protein Wnt	WNT8A
D6RF94	Protein Wnt	WNT8A
E2RYF7	Protein PBMUCL2	HCG22
E5RFR1	PENK(114-133)	PENK
E7EML9	Serine protease 44	PRSS44
E7EPC3	Protein Wnt	WNT9B
E7EVP0	Nociceptin	PNOC
E9PD02	Insulin-like growth factor I	IGF1
E9PH60	Protein Wnt	WNT16
E9PJL6	Protein Wnt	WNT11
F5GYM2	Protein Wnt	WNT5B
F5H034	Protein Wnt	WNT5B
F5H364	Protein Wnt	WNT5B
F5H7Q6	Protein Wnt	WNT5B
F8WCM5	Protein INS-IGF2	INS-IGF2
F8WDR1	Protein Wnt	WNT2

TABLE 1-continued

Secreted Proteins		
Uniprot ID	Protein Name	Gene Name
H0Y663	Protein Wnt	WNT4
H0YK72	Signal peptidase complex catalytic subunit SEC11A	SEC11A
H0YK83	Signal peptidase complex catalytic subunit SEC11A	SEC11A
H0YM39	Chorionic somatomammotropin hormone	CSH2
H0YMT7	Chorionic somatomammotropin hormone	CSH1
H0YN17	Chorionic somatomammotropin hormone	CSH2
H0YN45	Signal peptidase complex catalytic subunit SEC11A	SEC11A
H0YNG3	Signal peptidase complex catalytic subunit SEC11A	SEC11A
H0YNX5	Signal peptidase complex catalytic subunit SEC11A	SEC11A
H7BZB8	Protein Wnt	WNT10A
H9KV56	Choriogonadotropin subunit beta variant 2	CGB2
I3L0L8	Protein Wnt	WNT9B
J3KNZ1	Choriogonadotropin subunit beta variant 1	CGB1
J3KP00	Choriogonadotropin subunit beta	CGB7
J3QT02	Choriogonadotropin subunit beta variant 1	CGB1
O00175	C—C motif chemokine 24	CCL24
O00182	Galectin-9	LGALS9
O00187	Mannan-binding lectin serine protease 2	MASP2
O00230	Cortistatin	CORT
O00253	Agouti-related protein	AGRP
O00270	12-(S)-hydroxy-5,8,10,14-eicosatetraenoic acid receptor	GPR31
O00292	Left-right determination factor 2	LEFTY2
O00294	Tubby-related protein 1	TULP1
O00295	Tubby-related protein 2	TULP2
O00300	Tumor necrosis factor receptor superfamily member 11B	TNFRSF11B
O00339	Matrilin-2	MATN2
O00391	Sulfhydryl oxidase 1	QSOX1
O00468	Agrin	AGRN
O00515	Ladinin-1	LAD1
O00533	Processed neural cell adhesion molecule L1-like protein	CHL1
O00584	Ribonuclease T2	RNASET2
O00585	C—C motif chemokine 21	CCL21
O00602	Ficolin-1	FCN1
O00622	Protein CYR61	CYR61
O00626	MDC(5-69)	CCL22
O00634	Netrin-3	NTN3
O00744	Protein Wnt-10b	WNT10B
O00755	Protein Wnt-7a	WNT7A
O14498	Immunoglobulin superfamily containing leucine-rich repeat protein	ISLR
O14511	Pro-neuregulin-2, membrane-bound isoform	NRG2
O14594	Neurocan core protein	NCAN
O14625	C—X—C motif chemokine 11	CXCL11
O14638	Ectonucleotide pyrophosphatase/phosphodiesterase family member 3	ENPP3
O14656	Torsin-1A	TOR1A
O14657	Torsin-1B	TOR1B
O14786	Neuropilin-1	NRP1
O14788	Tumor necrosis factor ligand superfamily member 11, membrane form	TNFSF11
O14791	Apolipoprotein L1	APOL1
O14793	Growth/differentiation factor 8	MSTN
O14904	Protein Wnt-9a	WNT9A
O14905	Protein Wnt-9b	WNT9B
O14944	Proepiregulin	EREG
O14960	Leukocyte cell-derived chemotaxin-2	LECT2
O15018	Processed PDZ domain-containing protein 2	PDZD2
O15041	Semaphorin-3E	SEMA3E
O15072	A disintegrin and metalloproteinase with thrombospondin motifs 3	ADAMTS3
O15123	Angiopoietin-2	ANGPT2
O15130	Neuropeptide FF	NPFF
O15197	Ephrin type-B receptor 6	EPHB6
O15204	ADAM DEC1	ADAMDEC1
O15230	Laminin subunit alpha-5	LAMA5
O15232	Matrilin-3	MATN3
O15240	Neuroendocrine regulatory peptide-1	VGF
O15263	Beta-defensin 4A	DEFB4A
O15335	Chondroadherin	CHAD
O15393	Transmembrane protease serine 2 catalytic chain	TMPRSS2
O15444	C—C motif chemokine 25	CCL25
O15467	C—C motif chemokine 16	CCL16
O15496	Group 10 secretory phospholipase A2	PLA2G10

TABLE 1-continued

Secreted Proteins		
Uniprot ID	Protein Name	Gene Name
O15520	Fibroblast growth factor 10	FGF10
O15537	Retinoschisin	RS1
O43157	Plexin-B1	PLXNB1
O43184	Disintegrin and metalloproteinase domain-containing protein 12	ADAM12
O43240	Kallikrein-10	KLK10
O43278	Kunitz-type protease inhibitor 1	SPINT1
O43320	Fibroblast growth factor 16	FGF16
O43323	Desert hedgehog protein C-product	DHH
O43405	Cochlin	COCH
O43508	Tumor necrosis factor ligand superfamily member 12, membrane form	TNFSF12
O43555	Progesterone receptor-binding protein 2	GNRH2
O43557	Tumor necrosis factor ligand superfamily member 14, soluble form	TNFSF14
O43692	Peptidase inhibitor 15	PI15
O43699	Sialic acid-binding Ig-like lectin 6	SIGLEC6
O43820	Hyaluronidase-3	HYAL3
O43827	Angiopoietin-related protein 7	ANGPTL7
O43852	Calumenin	CALU
O43854	EGF-like repeat and discoidin I-like domain-containing protein 3	EDIL3
O43866	CD5 antigen-like	CD5L
O43897	Tolloid-like protein 1	TLL1
O43915	Vascular endothelial growth factor D	FIGF
O43927	C—X—C motif chemokine 13	CXCL13
O60218	Aldo-keto reductase family 1 member B10	AKR1B10
O60235	Transmembrane protease serine 11D	TMPRSS11D
O60258	Fibroblast growth factor 17	FGF17
O60259	Kallikrein-8	KLK8
O60383	Growth/differentiation factor 9	GDF9
O60469	Down syndrome cell adhesion molecule	DSCAM
O60542	Persephin	PSPN
O60565	Gremlin-1	GREM1
O60575	Serine protease inhibitor Kazal-type 4	SPINK4
O60676	Cystatin-8	CST8
O60687	Sushi repeat-containing protein SRPX2	SRPX2
O60844	Zymogen granule membrane protein 16	ZG16
O60882	Matrix metalloproteinase-20	MMP20
O60938	Keratocan	KERA
O75015	Low affinity immunoglobulin gamma Fc region receptor III-B	FCGR3B
O75077	Disintegrin and metalloproteinase domain-containing protein 23	ADAM23
O75093	Slit homolog 1 protein	SLIT1
O75094	Slit homolog 3 protein	SLIT3
O75095	Multiple epidermal growth factor-like domains protein 6	MEGF6
O75173	A disintegrin and metalloproteinase with thrombospondin motifs 4	ADAMTS4
O75200	Nuclear pore complex-interacting protein-like 1	NPIPL1
O75339	Cartilage intermediate layer protein 1 C1	CILP
O75354	Ectonucleoside triphosphate diphosphohydrolase 6	ENTPD6
O75386	Tubby-related protein 3	TULP3
O75398	Deformed epidermal autoregulatory factor 1 homolog	DEAF1
O75443	Alpha-tectorin	TECTA
O75445	Usherin	USH2A
O75462	Cytokine receptor-like factor 1	CRLF1
O75487	Glypican-4	GPC4
O75493	Carbonic anhydrase-related protein 11	CA11
O75594	Peptidoglycan recognition protein 1	PGLYRP1
O75596	C-type lectin domain family 3 member A	CLEC3A
O75610	Left-right determination factor 1	LEFTY1
O75629	Protein CREG1	CREG1
O75636	Ficolin-3	FCN3
O75711	Scrapie-responsive protein 1	SCRG1
O75715	Epididymal secretory glutathione peroxidase	GPX5
O75718	Cartilage-associated protein	CRTAP
O75829	Chondrosurfactant protein	LECT1
O75830	Serpin I2	SERPINI2
O75882	Attractin	ATRN
O75888	Tumor necrosis factor ligand superfamily member 13	TNFSF13
O75900	Matrix metalloproteinase-23	MMP23A
O75951	Lysozyme-like protein 6	LYZL6

TABLE 1-continued

Secreted Proteins		
Uniprot ID	Protein Name	Gene Name
O75973	C1q-related factor	C1QL1
O76038	Secretagogin	SCGN
O76061	Stanniocalcin-2	STC2
O76076	WNT1-inducible-signaling pathway protein 2	WISP2
O76093	Fibroblast growth factor 18	FGF18
O76096	Cystatin-F	CST7
O94769	Extracellular matrix protein 2	ECM2
O94813	Slit homolog 2 protein C-product	SLIT2
O94907	Dickkopf-related protein 1	DKK1
O94919	Endonuclease domain-containing 1 protein	ENDOD1
O94964	N-terminal form	SOGA1
O95025	Semaphorin-3D	SEMA3D
O95084	Serine protease 23	PRSS23
O95150	Tumor necrosis factor ligand superfamily member 15	TNFSF15
O95156	Neurexophilin-2	NXPH2
O95157	Neurexophilin-3	NXPH3
O95158	Neurexophilin-4	NXPH4
O95388	WNT1-inducible-signaling pathway protein 1	WISP1
O95389	WNT1-inducible-signaling pathway protein 3	WISP3
O95390	Growth/differentiation factor 11	GDF11
O95393	Bone morphogenetic protein 10	BMP 10
O95399	Urotensin-2	UTS2
O95407	Tumor necrosis factor receptor superfamily member 6B	TNFRSF6B
O95428	Papilin	PAPLN
O95445	Apolipoprotein M	APOM
O95450	A disintegrin and metalloproteinase with thrombospondin motifs 2	ADAMTS2
O95460	Matriulin-4	MATN4
O95467	LHAL tetrapeptide	GNAS
O95631	Netrin-1	NTN1
O95633	Follistatin-related protein 3	FSTL3
O95711	Lymphocyte antigen 86	LY86
O95715	C—X—C motif chemokine 14	CXCL14
O95750	Fibroblast growth factor 19	FGF19
O95760	Interleukin-33	IL33
O95813	Cerberus	CER1
O95841	Angiopoietin-related protein 1	ANGPTL1
O95897	Noelin-2	OLFM2
O95925	Eppin	EPPIN
O95965	Integrin beta-like protein 1	ITGBL1
O95967	EGF-containing fibulin-like extracellular matrix protein 2	EFEMP2
O95968	Secretoglobin family 1D member 1	SCGB1D1
O95969	Secretoglobin family 1D member 2	SCGB1D2
O95970	Leucine-rich glioma-inactivated protein 1	LGI1
O95972	Bone morphogenetic protein 15	BMP15
O95994	Anterior gradient protein 2 homolog	AGR2
O95998	Interleukin-18-binding protein	IL18BP
O96009	Napsin-A	NAPSA
O96014	Protein Wnt-11	WNT11
P00450	Ceruloplasmin	CP
P00451	Factor VIIIa light chain	F8
P00488	Coagulation factor XIII A chain	F13A1
P00533	Epidermal growth factor receptor	EGFR
P00709	Alpha-lactalbumin	LALBA
P00734	Prothrombin	F2
P00738	Haptoglobin beta chain	HP
P00739	Haptoglobin-related protein	HPR
P00740	Coagulation factor IXa heavy chain	F9
P00742	Factor X heavy chain	F10
P00746	Complement factor D	CFD
P00747	Plasmin light chain B	PLG
P00748	Coagulation factor XIIa light chain	F12
P00749	Urokinase-type plasminogen activator long chain A	PLAU
P00750	Tissue-type plasminogen activator	PLAT
P00751	Complement factor B Ba fragment	CFB
P00797	Renin	REN
P00973	2'-5'-oligoadenylate synthase 1	OAS1
P00995	Pancreatic secretory trypsin inhibitor	SPINK1
P01008	Antithrombin-III	SERPINC1
P01009	Alpha-1-antitrypsin	SERPINA1
P01011	Alpha-1-antichymotrypsin His-Pro-less	SERPINA3
P01019	Angiotensin-1	AGT
P01023	Alpha-2-macroglobulin	A2M

TABLE 1-continued

Secreted Proteins		
Uniprot ID	Protein Name	Gene Name
P01024	Acylation stimulating protein	C3
P01031	Complement C5 beta chain	C5
P01033	Metalloproteinase inhibitor 1	TIMP1
P01034	Cystatin-C	CST3
P01036	Cystatin-S	CST4
P01037	Cystatin-SN	CST1
P01042	Kininogen-1 light chain	KNG1
P01127	Platelet-derived growth factor subunit B	PDGFB
P01135	Transforming growth factor alpha	TGFA
P01137	Transforming growth factor beta-1	TGFB1
P01138	Beta-nerve growth factor	NGF
P01148	Gonadoliberin-1	GNRH1
P01160	Atrial natriuretic factor	NPPA
P01178	Oxytocin	OXT
P01185	Vasopressin-neurophysin 2-copeptin	AVP
P01189	Corticotropin	POMC
P01210	PENK(237-258)	PENK
P01213	Alpha-neoendorphin	PDYN
P01215	Glycoprotein hormones alpha chain	CGA
P01222	Thyrotropin subunit beta	TSHB
P01225	Follitropin subunit beta	FSHB
P01229	Lutropin subunit beta	LHB
P01233	Choriogonadotropin subunit beta	CGB8
P01236	Prolactin	PRL
P01241	Somatotropin	GH1
P01242	Growth hormone variant	GH2
P01243	Chorionic somatomammotropin hormone	CSH2
P01258	Katacalcin	CALCA
P01266	Thyroglobulin	TG
P01270	Parathyroid hormone	PTH
P01275	Glucagon	GCG
P01282	Intestinal peptide PHM-27	VIP
P01286	Somatoliberin	GHRH
P01298	Pancreatic prohormone	PPY
P01303	C-flanking peptide of NPY	NPY
P01308	Insulin	INS
P01344	Insulin-like growth factor II	IGF2
P01350	Big gastrin	GAST
P01374	Lymphotxin-alpha	LTA
P01375	C-domain 1	TNF
P01562	Interferon alpha-1/13	IFNA1
P01563	Interferon alpha-2	IFNA2
P01566	Interferon alpha-10	IFNA10
P01567	Interferon alpha-7	IFNA7
P01568	Interferon alpha-21	IFNA21
P01569	Interferon alpha-5	IFNA5
P01570	Interferon alpha-14	IFNA14
P01571	Interferon alpha-17	IFNA17
P01574	Interferon beta	IFNB1
P01579	Interferon gamma	IFNG
P01583	Interleukin-1 alpha	IL1A
P01584	Interleukin-1 beta	IL1B
P01588	Erythropoietin	EPO
P01591	Immunoglobulin J chain	IGJ
P01732	T-cell surface glycoprotein CD8 alpha chain	CD8A
P01833	Polymeric immunoglobulin receptor	PIGR
P01857	Ig gamma-1 chain C region	IGHG1
P01859	Ig gamma-2 chain C region	IGHG2
P01860	Ig gamma-3 chain C region	IGHG3
P01861	Ig gamma-4 chain C region	IGHG4
P01871	Ig mu chain C region	IGHM
P01880	Ig delta chain C region	IGHD
P02452	Collagen alpha-1(I) chain	COL1A1
P02458	Chondrocalcin	COL2A1
P02461	Collagen alpha-1(III) chain	COL3A1
P02462	Collagen alpha-1(IV) chain	COL4A1
P02647	Apolipoprotein A-I	APOA1
P02649	Apolipoprotein E	APOE
P02652	Apolipoprotein A-II	APOA2
P02654	Apolipoprotein C-I	APOC1
P02655	Apolipoprotein C-II	APOC2
P02656	Apolipoprotein C-III	APOC3
P02671	Fibrinogen alpha chain	FGA

TABLE 1-continued

Secreted Proteins		
Uniprot ID	Protein Name	Gene Name
P02675	Fibrinopeptide B	FGB
P02679	Fibrinogen gamma chain	FGG
P02741	C-reactive protein	CRP
P02743	Serum amyloid P-component(1-203)	APCS
P02745	Complement C1q subcomponent subunit A	C1QA
P02746	Complement C1q subcomponent subunit B	C1QB
P02747	Complement C1q subcomponent subunit C	C1QC
P02748	Complement component C9b	C9
P02749	Beta-2-glycoprotein 1	APOH
P02750	Leucine-rich alpha-2-glycoprotein	LRG1
P02751	Ugl-Y2	FN1
P02753	Retinol-binding protein 4	RBP4
P02760	Trypsinogen	AMBP
P02763	Alpha-1-acid glycoprotein 1	ORM1
P02765	Alpha-2-HS-glycoprotein chain A	AHSG
P02766	Transthyretin	TTR
P02768	Serum albumin	ALB
P02771	Alpha-fetoprotein	AFP
P02774	Vitamin D-binding protein	GC
P02775	Connective tissue-activating peptide III	PPBP
P02776	Platelet factor 4	PF4
P02778	CXCL10(1-73)	CXCL10
P02786	Transferrin receptor protein 1	TFRC
P02787	Serotransferrin	TF
P02788	Lactoferrin-C	LTF
P02790	Hemopexin	HPX
P02808	Statherin	STATH
P02810	Salivary acidic proline-rich phosphoprotein 1/2	PRH2
P02812	Basic salivary proline-rich protein 2	PRB2
P02814	Peptide D1A	SMR3B
P02818	Osteocalcin	BGLAP
P03950	Angiogenin	ANG
P03951	Coagulation factor XIa heavy chain	F11
P03952	Plasma kallikrein	KLKB1
P03956	27 kDa interstitial collagenase	MMP1
P03971	Muellerian-inhibiting factor	AMH
P03973	Antileukoproteinase	SLPI
P04003	C4b-binding protein alpha chain	C4BPA
P04004	Somatomedin-B	VTN
P04054	Phospholipase A2	PLA2G1B
P04085	Platelet-derived growth factor subunit A	PDGFA
P04090	Relaxin A chain	RLN2
P04114	Apolipoprotein B-100	APOB
P04118	Colipase	CLPS
P04141	Granulocyte-macrophage colony-stimulating factor	CSF2
P04155	Trefoil factor 1	TFF1
P04180	Phosphatidylcholine-sterol acyltransferase	LCAT
P04196	Histidine-rich glycoprotein	HRG
P04217	Alpha-1B-glycoprotein	A1BG
P04275	von Willebrand antigen 2	VWF
P04278	Sex hormone-binding globulin	SHBG
P04279	Alpha-inhibin-31	SEMG1
P04280	Basic salivary proline-rich protein 1	PRB1
P04628	Proto-oncogene Wnt-1	WNT1
P04745	Alpha-amylase 1	AMY1A
P04746	Pancreatic alpha-amylase	AMY2A
P04808	Prorelaxin H1	RLN1
P05000	Interferon omega-1	IFNW1
P05013	Interferon alpha-6	IFNA6
P05014	Interferon alpha-4	IFNA4
P05015	Interferon alpha-16	IFNA16
P05019	Insulin-like growth factor I	IGF1
P05060	GAWK peptide	CHGB
P05090	Apolipoprotein D	APOD
P05109	Protein S100-A8	S100A8
P05111	Inhibin alpha chain	INHA
P05112	Interleukin-4	IL4
P05113	Interleukin-5	IL5
P05120	Plasminogen activator inhibitor 2	SERPINB2
P05121	Plasminogen activator inhibitor 1	SERPINE1
P05154	Plasma serine protease inhibitor	SERPINAS
P05155	Plasma protease C1 inhibitor	SERPING1
P05156	Complement factor I heavy chain	CFI

TABLE 1-continued

Secreted Proteins		
Uniprot ID	Protein Name	Gene Name
P05160	Coagulation factor XIII B chain	F13B
P05161	Ubiquitin-like protein ISG15	ISG15
P05230	Fibroblast growth factor 1	FGF1
P05231	Interleukin-6	IL6
P05305	Big endothelin-1	EDN1
P05408	C-terminal peptide	SCG5
P05451	Lithostathine-1-alpha	REG1A
P05452	Tetranectin	CLEC3B
P05543	Thyroxine-binding globulin	SERPINA7
P05814	Beta-casein	CSN2
P05997	Collagen alpha-2(V) chain	COL5A2
P06276	Cholinesterase	BCHE
P06307	Cholecystokinin-12	CCK
P06396	Gelsolin	GSN
P06681	Complement C2	C2
P06702	Protein S100-A9	S100A9
P06727	Apolipoprotein A-IV	APOA4
P06734	Low affinity immunoglobulin epsilon Fc receptor soluble form	FCER2
P06744	Glucose-6-phosphate isomerase	GPI
P06850	Corticoliberin	CRH
P06858	Lipoprotein lipase	LPL
P06881	Calcitonin gene-related peptide 1	CALCA
P07093	Glia-derived nexin	SERPINE2
P07098	Gastric triacylglycerol lipase	LIPF
P07225	Vitamin K-dependent protein S	PROS1
P07237	Protein disulfide-isomerase	P4HB
P07288	Prostate-specific antigen	KLK3
P07306	Asialoglycoprotein receptor 1	ASGR1
P07355	Annexin A2	ANXA2
P07357	Complement component C8 alpha chain	C8A
P07358	Complement component C8 beta chain	C8B
P07360	Complement component C8 gamma chain	C8G
P07477	Alpha-trypsin chain 2	PRSS1
P07478	Trypsin-2	PRSS2
P07492	Neuromedin-C	GRP
P07498	Kappa-casein	CSN3
P07585	Decorin	DCN
P07911	Uromodulin	UMOD
P07942	Laminin subunit beta-1	LAMB1
P07988	Pulmonary surfactant-associated protein B	SFTPB
P07998	Ribonuclease pancreatic	RNASE1
P08118	Beta-microseminoprotein	MSMB
P08123	Collagen alpha-2(I) chain	COL1A2
P08185	Corticosteroid-binding globulin	SERPINA6
P08217	Chymotrypsin-like elastase family member 2A	CELA2A
P08218	Chymotrypsin-like elastase family member 2B	CELA2B
P08253	72 kDa type IV collagenase	MMP2
P08254	Stromelysin-1	MMP3
P08294	Extracellular superoxide dismutase [Cu—Zn]	SOD3
P08476	Inhibin beta A chain	INHBA
P08493	Matrix Gla protein	MGP
P08572	Collagen alpha-2(IV) chain	COL4A2
P08581	Hepatocyte growth factor receptor	MET
P08603	Complement factor H	CFH
P08620	Fibroblast growth factor 4	FGF4
P08637	Low affinity immunoglobulin gamma Fc region receptor III-A	FCGR3A
P08697	Alpha-2-antiplasmin	SERPINF2
P08700	Interleukin-3	IL3
P08709	Coagulation factor VII	F7
P08833	Insulin-like growth factor-binding protein 1	IGFBP1
P08887	Interleukin-6 receptor subunit alpha	IL6R
P08949	Neuromedin-B-32	NMB
P08F94	Fibrocystin	PKHD1
P09038	Fibroblast growth factor 2	FGF2
P09228	Cystatin-SA	CST2
P09237	Matrilysin	MMP7
P09238	Stromelysin-2	MMP10
P09341	Growth-regulated alpha protein	CXCL1
P09382	Galectin-1	LGALS1
P09466	Glycodelin	PAEP
P09486	SPARC	SPARC

TABLE 1-continued

Secreted Proteins		
Uniprot ID	Protein Name	Gene Name
P09529	Inhibin beta B chain	INHBB
P09544	Protein Wnt-2	WNT2
P09603	Processed macrophage colony-stimulating factor 1	CSF1
P09681	Gastric inhibitory polypeptide	GIP
P09683	Secretin	SCT
P09919	Granulocyte colony-stimulating factor	CSF3
POC091	FRAS1-related extracellular matrix protein 3	FREM3
POCOL4	C4d-A	C4A
POCOL5	Complement C4-B alpha chain	C4B
POCOP6	Neuropeptide S	NPS
POC7L1	Serine protease inhibitor Kazal-type 8	SPINK8
POC862	Complement C1q and tumor necrosis factor-related protein 9A	C1QTNF9
POC8F1	Prostate and testis expressed protein 4	PATE4
POCG01	Gastrokine-3	GKN3P
POCG36	Cryptic family protein 1B	CFC1B
POCG37	Cryptic protein	CFC1
POCJ68	Humanin-like protein 1	MTRNR2L1
POCJ69	Humanin-like protein 2	MTRNR2L2
POCJ70	Humanin-like protein 3	MTRNR2L3
POCJ71	Humanin-like protein 4	MTRNR2L4
POCJ72	Humanin-like protein 5	MTRNR2L5
POCJ73	Humanin-like protein 6	MTRNR2L6
POCJ74	Humanin-like protein 7	MTRNR2L7
POCJ75	Humanin-like protein 8	MTRNR2L8
POCJ76	Humanin-like protein 9	MTRNR2L9
POCJ77	Humanin-like protein 10	MTRNR2L10
PODJD7	Pepsin A-4	PGA4
POJD8	Pepsin A-3	PGA3
POJD9	Pepsin A-5	PGA5
PODJ18	Amyloid protein A	SAA1
PODJ19	Serum amyloid A-2 protein	SAA2
P10082	Peptide YY(3-36)	PPY
P10092	Calcitonin gene-related peptide 2	CALCB
P10124	Serglycin	SRGN
P10145	MDNCF-a	IL8
P10147	MIP-1-alpha(4-69)	CCL3
P10163	Peptide P-D	PRB4
P10451	Osteopontin	SPP1
P10599	Thioredoxin	TXN
P10600	Transforming growth factor beta-3	TGFB3
P10643	Complement component C7	C7
P10645	Vasostatin-2	CHGA
P10646	Tissue factor pathway inhibitor	TFPI
P10720	Platelet factor 4 variant(4-74)	PF4V1
P10745	Retinol-binding protein 3	RBP3
P10767	Fibroblast growth factor 6	FGF6
P10909	Clusterin alpha chain	CLU
P10912	Growth hormone receptor	GHR
P10915	Hyaluronan and proteoglycan link protein 1	HAPLN1
P10966	T-cell surface glycoprotein CD8 beta chain	CD8B
P10997	Islet amyloid polypeptide	IAPP
P11047	Laminin subunit gamma-1	LAMC1
P11150	Hepatic triacylglycerol lipase	LIPC
P11226	Mannose-binding protein C	MBL2
P11464	Pregnancy-specific beta-1-glycoprotein 1	PSG1
P11465	Pregnancy-specific beta-1-glycoprotein 2	PSG2
P11487	Fibroblast growth factor 3	FGF3
P11597	Cholesteryl ester transfer protein	CETP
P11684	Uteroglobin	SCGB1A1
P11686	Pulmonary surfactant-associated protein C	SFTPC
P12034	Fibroblast growth factor 5	FGF5
P12107	Collagen alpha-1(XI) chain	COL11A1
P12109	Collagen alpha-1(VI) chain	COL6A1
P12110	Collagen alpha-2(VI) chain	COL6A2
P12111	Collagen alpha-3(VI) chain	COL6A3
P12259	Coagulation factor V	F5
P12272	PTHRP[1-36]	PTHLH
P12273	Prolactin-inducible protein	PIP
P12544	Granzyme A	GZMA
P12643	Bone morphogenetic protein 2	BMP2
P12644	Bone morphogenetic protein 4	BMP4
P12645	Bone morphogenetic protein 3	BMP3

TABLE 1-continued

Secreted Proteins		
Uniprot ID	Protein Name	Gene Name
P12724	Eosinophil cationic protein	RNASE3
P12821	Angiotensin-converting enzyme, soluble form	ACE
P12838	Neutrophil defensin 4	DEFA4
P12872	Motilin	MLN
P13232	Interleukin-7	IL7
P13236	C—C motif chemokine 4	CCL4
P13284	Gamma-interferon-inducible lysosomal thiol reductase	IFI30
P13500	C—C motif chemokine 2	CCL2
P13501	C—C motif chemokine 5	CCL5
P13521	Secretogranin-2	SCG2
P13591	Neural cell adhesion molecule 1	NCAM1
P13611	Versican core protein	VCAN
P13671	Complement component C6	C6
P13688	Carcinoembryonic antigen-related cell adhesion molecule 1	CEACAM1
P13725	Oncostatin-M	OSM
P13726	Tissue factor	F3
P13727	Eosinophil granule major basic protein	PRG2
P13942	Collagen alpha-2(XI) chain	COL11A2
P13987	CD59 glycoprotein	CD59
P14138	Endothelin-3	EDN3
P14174	Macrophage migration inhibitory factor	MIF
P14207	Folate receptor beta	FOLR2
P14222	Perforin-1	PRF1
P14543	Nidogen-1	NID1
P14555	Phospholipase A2, membrane associated	PLA2G2A
P14625	Endoplasmin	HSP90B1
P14735	Insulin-degrading enzyme	IDE
P14778	Interleukin-1 receptor type 1, soluble form	IL1R1
P14780	82 kDa matrix metalloproteinase-9	MMP9
P15018	Leukemia inhibitory factor	LIF
P15085	Carboxypeptidase A1	CPA1
P15086	Carboxypeptidase B	CPB1
P15151	Poliovirus receptor	PVR
P15169	Carboxypeptidase N catalytic chain	CPN1
P15248	Interleukin-9	IL9
P15291	N-acetyllactosamine synthase	B4GALT1
P15309	PAPG9	ACPP
P15328	Folate receptor alpha	FOLR1
P15374	Ubiquitin carboxyl-terminal hydrolase isozyme L3	UCHL3
P15502	Elastin	ELN
P15509	Granulocyte-macrophage colony-stimulating factor receptor subunit alpha	CSF2RA
P15515	Histatin-1	HTN1
P15516	His3-(31-51)-peptide	HTN3
P15692	Vascular endothelial growth factor A	VEGFA
P15814	Immunoglobulin lambda-like polypeptide 1	IGLL1
P15907	Beta-galactoside alpha-2,6-sialyltransferase 1	ST6GAL1
P15941	Mucin-1 subunit beta	MUC1
P16035	Metalloproteinase inhibitor 2	TIMP2
P16112	Aggrecan core protein 2	ACAN
P16233	Pancreatic triacylglycerol lipase	PNLIP
P16442	Histo-blood group ABO system transferase	ABO
P16471	Prolactin receptor	PRLR
P16562	Cysteine-rich secretory protein 2	CRISP2
P16619	C—C motif chemokine 3-like 1	CCL3L1
P16860	BNP(3-29)	NPPB
P16870	Carboxypeptidase E	CPE
P16871	Interleukin-7 receptor subunit alpha	IL7R
P17213	Bactericidal permeability-increasing protein	BPI
P17538	Chymotrypsinogen B	CTRBL1
P17931	Galectin-3	LGALS3
P17936	Insulin-like growth factor-binding protein 3	IGFBP3
P17948	Vascular endothelial growth factor receptor 1	FLT1
P18065	Insulin-like growth factor-binding protein 2	IGFBP2
P18075	Bone morphogenetic protein 7	BMP7
P18428	Lipopolysaccharide-binding protein	LBP
P18509	PACAP-related peptide	ADCYAP1
P18510	Interleukin-1 receptor antagonist protein	IL1RN
P18827	Syndecan-1	SDC1
P19021	Peptidylglycine alpha-hydroxylating monooxygenase	PAM
P19235	Erythropoietin receptor	EPOR

TABLE 1-continued

Secreted Proteins		
Uniprot ID	Protein Name	Gene Name
P19438	Tumor necrosis factor-binding protein 1	TNFRSF1A
P19652	Alpha-1-acid glycoprotein 2	ORM2
P19801	Amiloride-sensitive amino oxidase [copper-containing]	ABP1
P19823	Inter-alpha-trypsin inhibitor heavy chain H2	ITIH2
P19827	Inter-alpha-trypsin inhibitor heavy chain H1	ITIH1
P19835	Bile salt-activated lipase	CEL
P19875	C—X—C motif chemokine 2	CXCL2
P19876	C—X—C motif chemokine 3	CXCL3
P19883	Follistatin	FST
P19957	Elafin	PI3
P19961	Alpha-amylase 2B	AMY2B
P20061	Transcobalamin-1	TCN1
P20062	Transcobalamin-2	TCN2
P20142	Gastricsin	PGC
P20155	Serine protease inhibitor Kazal-type 2	SPINK2
P20231	Tryptase beta-2	TPSB2
P20333	Tumor necrosis factor receptor superfamily member 1B	TNFRSF1B
P20366	Substance P	TAC1
P20382	Melanin-concentrating hormone	PMCH
P20396	Thyroliberin	TRH
P20742	Pregnancy zone protein	PZP
P20774	Mimecan	OGN
P20783	Neurotrophin-3	NTF3
P20800	Endothelin-2	EDN2
P20809	Interleukin-11	IL11
P20827	Ephrin-A1	EFNA1
P20849	Collagen alpha-1(IX) chain	COL9A1
P20851	C4b-binding protein beta chain	C4BPB
P20908	Collagen alpha-1(V) chain	COL5A1
P21128	Poly(U)-specific endoribonuclease	ENDOU
P21246	Pleiotrophin	PTN
P21583	Kit ligand	KITLG
P21741	Midkine	MDK
P21754	Zona pellucida sperm-binding protein 3	ZP3
P21781	Fibroblast growth factor 7	FGF7
P21802	Fibroblast growth factor receptor 2	FGFR2
P21810	Biglycan	BGN
P21815	Bone sialoprotein 2	IBSP
P21860	Receptor tyrosine-protein kinase erbB-3	ERBB3
P21941	Cartilage matrix protein	MATN1
P22003	Bone morphogenetic protein 5	BMP5
P22004	Bone morphogenetic protein 6	BMP6
P22079	Lactoperoxidase	LPO
P22105	Tenascin-X	TNXB
P22301	Interleukin-10	IL10
P22303	Acetylcholinesterase	ACHE
P22352	Glutathione peroxidase 3	GPX3
P22362	C—C motif chemokine 1	CCL1
P22455	Fibroblast growth factor receptor 4	FGFR4
P22466	Galanin message-associated peptide	GAL
P22692	Insulin-like growth factor-binding protein 4	IGFBP4
P22749	Granulysin	GNLY
P22792	Carboxypeptidase N subunit 2	CPN2
P22891	Vitamin K-dependent protein Z	PROZ
P22894	Neutrophil collagenase	MMP8
P23142	Fibulin-1	FBLN1
P23280	Carbonic anhydrase 6	CA6
P23352	Anosmin-1	KAL1
P23435	Cerebellin-1	CBLN1
P23560	Brain-derived neurotrophic factor	BDNF
P23582	C-type natriuretic peptide	NPPC
P23946	Chymase	CMA1
P24043	Laminin subunit alpha-2	LAMA2
P24071	Immunoglobulin alpha Fc receptor	FCAR
P24347	Stromelysin-3	MMP11
P24387	Corticotropin-releasing factor-binding protein	CRHBP
P24592	Insulin-like growth factor-binding protein 6	IGFBP6
P24593	Insulin-like growth factor-binding protein 5	IGFBP5
P24821	Tenascin	TNC
P24855	Deoxyribonuclease-1	DNASE1
P25067	Collagen alpha-2(VIII) chain	COL8A2
P25311	Zinc-alpha-2-glycoprotein	AZGP1
P25391	Laminin subunit alpha-1	LAMA1

TABLE 1-continued

Secreted Proteins		
Uniprot ID	Protein Name	Gene Name
P25445	Tumor necrosis factor receptor superfamily member 6	FAS
P25940	Collagen alpha-3(V) chain	COL5A3
P25942	Tumor necrosis factor receptor superfamily member 5	CD40
P26022	Pentraxin-related protein PTX3	PTX3
P26927	Hepatocyte growth factor-like protein beta chain	MST1
P27169	Serum paraoxonase/arylesterase 1	PON1
P27352	Gastric intrinsic factor	GIF
P27487	Dipeptidyl peptidase 4 membrane form	DPP4
P27539	Embryonic growth/differentiation factor 1	GDF1
P27658	Vastatin	COL8A1
P27797	Calreticulin	CALR
P27918	Properdin	CFP
P28039	Acyloxyacyl hydrolase	AOAH
P28300	Protein-lysine 6-oxidase	LOX
P28325	Cystatin-D	CST5
P28799	Granulin-1	GRN
P29122	Proprotein convertase subtilisin/kexin type 6	PCSK6
P29279	Connective tissue growth factor	CTGF
P29320	Ephrin type-A receptor 3	EPHA3
P29400	Collagen alpha-5(IV) chain	COL4A5
P29459	Interleukin-12 subunit alpha	IL12A
P29460	Interleukin-12 subunit beta	IL12B
P29508	Serpin B3	SERPINB3
P29622	Kallistatin	SERPINA4
P29965	CD40 ligand, soluble form	CD40LG
P30990	Neurotensin/neuromedin N	NTS
P31025	Lipocalin-1	LCN1
P31151	Protein S100-A7	S100A7
P31371	Fibroblast growth factor 9	FGF9
P31431	Syndecan-4	SDC4
P31947	14-3-3 protein sigma	SFN
P32455	Interferon-induced guanylate-binding protein 1	GBP1
P32881	Interferon alpha-8	IFNA8
P34096	Ribonuclease 4	RNASE4
P34130	Neurotrophin-4	NTF4
P34820	Bone morphogenetic protein 8B	BMP8B
P35030	Trypsin-3	PRSS3
P35052	Secreted glycan-1	GPC1
P35070	Betaacellulin	BTC
P35225	Interleukin-13	IL13
P35247	Pulmonary surfactant-associated protein D	SFTPD
P35318	ADM	ADM
P35542	Serum amyloid A-4 protein	SAA4
P35555	Fibrillin-1	FBN1
P35556	Fibrillin-2	FBN2
P35625	Metalloproteinase inhibitor 3	TIMP3
P35858	Insulin-like growth factor-binding protein complex acid labile subunit	IGFALS
P35916	Vascular endothelial growth factor receptor 3	FLT4
P35968	Vascular endothelial growth factor receptor 2	KDR
P36222	Chitinase-3-like protein 1	CHI3L1
P36952	Serpin B5	SERPINB5
P36955	Pigment epithelium-derived factor	SERPINF1
P36980	Complement factor H-related protein 2	CFHR2
P39059	Collagen alpha-1(XV) chain	COL15A1
P39060	Collagen alpha-1(XVIII) chain	COL18A1
P39877	Calcium-dependent phospholipase A2	PLA2G5
P39900	Macrophage metalloelastase	MMP12
P39905	Glial cell line-derived neurotrophic factor	GDNF
P40225	Thrombopoietin	THPO
P40967	M-alpha	PMEL
P41159	Leptin	LEP
P41221	Protein Wnt-5a	WNT5A
P41222	Prostaglandin-H2 D-isomerase	PTGDS
P41271	Neuroblastoma suppressor of tumorigenicity 1	NBL1
P41439	Folate receptor gamma	FOLR3
P42127	Agouti-signaling protein	ASIP
P42702	Leukemia inhibitory factor receptor	LIFR
P42830	ENA-78(9-78)	CXCL5
P43026	Growth/differentiation factor 5	GDF5
P43251	Biotinidase	BTD
P43652	Afamin	AFM
P45452	Collagenase 3	MMP13

TABLE 1-continued

Secreted Proteins		
Uniprot ID	Protein Name	Gene Name
P47710	Casoxin-D	CSN1S1
P47929	Galectin-7	LGALS7B
P47972	Neuronal pentraxin-2	NPTX2
P47989	Xanthine oxidase	XDH
P47992	Lymphotactin	XCL1
P48023	Tumor necrosis factor ligand superfamily member 6, membrane form	FASLG
P48052	Carboxypeptidase A2	CPA2
P48061	Stromal cell-derived factor 1	CXCL12
P48304	Lithostathine-1-beta	REG1B
P48307	Tissue factor pathway inhibitor 2	TFPI2
P48357	Leptin receptor	LEPR
P48594	Serpin B4	SERPINB4
P48645	Neuromedin-U-25	NMU
P48740	Mannan-binding lectin serine protease 1	MASP1
P48745	Protein NOV homolog	NOV
P48960	CD97 antigen subunit beta	CD97
P49223	Kunitz-type protease inhibitor 3	SPINT3
P49747	Cartilage oligomeric matrix protein	COMP
P49763	Placenta growth factor	PGF
P49765	Vascular endothelial growth factor B	VEGFB
P49767	Vascular endothelial growth factor C	VEGFC
P49771	Fms-related tyrosine kinase 3 ligand	FLT3LG
P49862	Kallikrein-7	KLK7
P49863	Granzyme K	GZMK
P49908	Selenoprotein P	SEPP1
P49913	Antibacterial protein FALL-39	CAMP
P50607	Tubby protein homolog	TUB
P51124	Granzyme M	GZMM
P51512	Matrix metalloproteinase-16	MMP16
P51654	Glypican-3	GPC3
P51671	Eotaxin	CCL11
P51884	Lumican	LUM
P51888	Prolargin	PRELP
P52798	Ephrin-A4	EFNA4
P52823	Stanniocalcin-1	STC1
P53420	Collagen alpha-4(IV) chain	COL4A4
P53621	Coatomer subunit alpha	COPA
P54108	Cysteine-rich secretory protein 3	CRISP3
P54315	Pancreatic lipase-related protein 1	PNLIPRP1
P54317	Pancreatic lipase-related protein 2	PNLIPRP2
P54793	Arylsulfatase F	ARSF
P55000	Secreted Ly-6/uPAR-related protein 1	SLURP1
P55001	Microfibrillar-associated protein 2	MFAP2
P55056	Apolipoprotein C-IV	APOC4
P55058	Phospholipid transfer protein	PLTP
P55075	Fibroblast growth factor 8	FGF8
P55081	Microfibrillar-associated protein 1	MFAP1
P55083	Microfibril-associated glycoprotein 4	MFAP4
P55107	Bone morphogenetic protein 3B	GDF10
P55145	Mesencephalic astrocyte-derived neurotrophic factor	MANF
P55259	Pancreatic secretory granule membrane major glycoprotein GP2	GP2
P55268	Laminin subunit beta-2	LAMB2
P55773	CCL23(30-99)	CCL23
P55774	C—C motif chemokine 18	CCL18
P55789	FAD-linked sulfhydryl oxidase ALR	GFER
P56703	Proto-oncogene Wnt-3	WNT3
P56704	Protein Wnt-3a	WNT3A
P56705	Protein Wnt-4	WNT4
P56706	Protein Wnt-7b	WNT7B
P56730	Neurotrypsin	PRSS12
P56851	Epididymal secretory protein E3-beta	EDDM3B
P56975	Neuregulin-3	NRG3
P58062	Serine protease inhibitor Kazal-type 7	SPINK7
P58215	Lysyl oxidase homolog 3	LOXL3
P58294	Prokineticin-1	PROK1
P58335	Anthrax toxin receptor 2	ANTXR2
P58397	A disintegrin and metalloproteinase with thrombospondin motifs 12	ADAMTS12

TABLE 1-continued

Secreted Proteins		
Uniprot ID	Protein Name	Gene Name
P58417	Neurexophilin-1	NXPH1
P58499	Protein FAM3B	FAM3B
P59510	A disintegrin and metalloproteinase with thrombospondin motifs 20	ADAMTS20
P59665	Neutrophil defensin 1	DEFA1B
P59666	Neutrophil defensin 3	DEFA3
P59796	Glutathione peroxidase 6	GPX6
P59826	BPI fold-containing family B member 3	BPIFB3
P59827	BPI fold-containing family B member 4	BPIFB4
P59861	Beta-defensin 131	DEFB131
P60022	Beta-defensin 1	DEFB1
P60153	Inactive ribonuclease-like protein 9	RNASE9
P60827	Complement C1q tumor necrosis factor-related protein 8	C1QTNF8
P60852	Zona pellucida sperm-binding protein 1	ZP1
P60985	Keratinocyte differentiation-associated protein	KRTDAP
P61109	Kidney androgen-regulated protein	KAP
P61278	Somatostatin-14	SST
P61366	Osteocrin	OSTN
P61626	Lysozyme C	LYZ
P61769	Beta-2-microglobulin	B2M
P61812	Transforming growth factor beta-2	TGFB2
P61916	Epididymal secretory protein E1	NPC2
P62502	Epididymal-specific lipocalin-6	LCN6
P62937	Peptidyl-prolyl cis-trans isomerase A	PPIA
P67809	Nuclease-sensitive element-binding protein 1	YBX1
P67812	Signal peptidase complex catalytic subunit SEC11A	SEC11A
P78310	Coxsackievirus and adenovirus receptor	CXADR
P78333	Secreted glycan-5	GPC5
P78380	Oxidized low-density lipoprotein receptor 1	OLR1
P78423	Processed fractalkine	CX3CL1
P78509	Reelin	RELN
P78556	CCL20(2-70)	CCL20
P80075	MCP-2(6-76)	CCL8
P80098	C—C motif chemokine 7	CCL7
P80108	Phosphatidylinositol-glycan-specific phospholipase D	GPLD1
P80162	C—X—C motif chemokine 6	CXCL6
P80188	Neutrophil gelatinase-associated lipocalin	LCN2
P80303	Nucleobindin-2	NUCB2
P80511	Calctermuin	S100A12
P81172	Hepcidin-25	HAMP
P81277	Prolactin-releasing peptide	PRLH
P81534	Beta-defensin 103	DEFB103A
P81605	Dermcidin	DCD
P82279	Protein crumbs homolog 1	CRB1
P82987	ADAMTS-like protein 3	ADAMTSL3
P83105	Serine protease HTRA4	HTRA4
P83110	Serine protease HTRA3	HTRA3
P83859	Orexigenic neuropeptide QRFP	QRFP
P98088	Mucin-5AC	MUC5AC
P98095	Fibulin-2	FBLN2
P98160	Basement membrane-specific heparan sulfate proteoglycan core protein	HSPG2
P98173	Protein FAM3A	FAM3A
Q00604	Norrin	NDP
Q00796	Sorbitol dehydrogenase	SORD
Q00887	Pregnancy-specific beta-1-glycoprotein 9	PSG9
Q00888	Pregnancy-specific beta-1-glycoprotein 4	PSG4
Q00889	Pregnancy-specific beta-1-glycoprotein 6	PSG6
Q01523	HDS(56-94)	DEFA5
Q01524	Defensin-6	DEFA6
Q01955	Collagen alpha-3(IV) chain	COL4A3
Q02297	Pro-neuregulin-1, membrane-bound isoform	NRG1
Q02325	Plasminogen-like protein B	PLGLB1
Q02383	Semenogelin-2	SEMG2
Q02388	Collagen alpha-1(VII) chain	COL7A1
Q02505	Mucin-3A	MUC3A
Q02509	Otoconin-90	OC90
Q02747	Guanylin	GUCA2A
Q02763	Angiopoietin-1 receptor	TEK
Q02817	Mucin-2	MUC2
Q02985	Complement factor H-related protein 3	CFHR3
Q03167	Transforming growth factor beta receptor type 3	TGFBR3
Q03403	Trefoil factor 2	TFF2

TABLE 1-continued

Secreted Proteins		
Uniprot ID	Protein Name	Gene Name
Q03405	Urokinase plasminogen activator surface receptor	PLAUR
Q03591	Complement factor H-related protein 1	CFHR1
Q03692	Collagen alpha-1(X) chain	COL10A1
Q04118	Basic salivary proline-rich protein 3	PRB3
Q04756	Hepatocyte growth factor activator short chain	HGFAC
Q04900	Sialomucin core protein 24	CD164
Q05315	Eosinophil lysophospholipase	CLC
Q05707	Collagen alpha-1(IV) chain	COL14A1
Q05996	Processed zona pellucida sperm-binding protein 2	ZP2
Q06033	Inter-alpha-trypsin inhibitor heavy chain H3	ITIH3
Q06141	Regenerating islet-derived protein 3-alpha	REG3A
Q06828	Fibromodulin	FMOD
Q07092	Collagen alpha-1(XVI) chain	COL16A1
Q07325	C—X—C motif chemokine 9	CXCL9
Q07507	Dermatopontin	DPT
Q075Z2	Binder of sperm protein homolog 1	BSPH1
Q07654	Trefoil factor 3	TFF3
Q07699	Sodium channel subunit beta-1	SCN1B
Q08345	Epithelial discoidin domain-containing receptor 1	DDR1
Q08380	Galectin-3-binding protein	LGALS3BP
Q08397	Lysyl oxidase homolog 1	LOXL1
Q08431	Lactadherin	MFGE8
Q08629	Testican-1	SPOCK1
Q08648	Sperm-associated antigen 11B	SPAG11B
Q08830	Fibrinogen-like protein 1	FGL1
Q10471	Polypeptide N-acetylgalactosaminyltransferase 2	GALNT2
Q10472	Polypeptide N-acetylgalactosaminyltransferase 1	GALNT1
Q11201	CMP-N-acetyleneuraminate-beta-galactosamide-alpha-2,3-sialyltransferase 1	ST3GAL1
Q11203	CMP-N-acetyleneuraminate-beta-1,4-galactoside alpha-2,3-sialyltransferase	ST3GAL3
Q11206	CMP-N-acetyleneuraminate-beta-galactosamide-alpha-2,3-sialyltransferase 4	ST3GAL4
Q12794	Hyaluronidase-1	HYAL1
Q12805	EGF-containing fibulin-like extracellular matrix protein 1	EFEMP1
Q12836	Zona pellucida sperm-binding protein 4	ZP4
Q12841	Follistatin-related protein 1	FSTL1
Q12904	Aminoacyl tRNA synthase complex-interacting multifunctional protein 1	AIMP1
Q13018	Soluble secretory phospholipase A2 receptor	PLA2R1
Q13072	B melanoma antigen 1	BAGE
Q13093	Platelet-activating factor acetylhydrolase	PLA2G7
Q13103	Secreted phosphoprotein 24	SPP2
Q13162	Peroxiredoxin-4	PRDX4
Q13201	Platelet glycoprotein Ia*	MMRN1
Q13214	Semaphorin-3B	SEMA3B
Q13219	Pappalysin-1	PAPPA
Q13231	Chitotriosidase-1	CHIT1
Q13253	Noggin	NOG
Q13261	Interleukin-15 receptor subunit alpha	IL15RA
Q13275	Semaphorin-3F	SEMA3F
Q13291	Signaling lymphocytic activation molecule	SLAMF1
Q13316	Dentin matrix acidic phosphoprotein 1	DMP1
Q13361	Microfibrillar-associated protein 5	MFAP5
Q13410	Butyrophilin subfamily 1 member A1	BTN1A1
Q13421	Mesothelin, cleaved form	MSLN
Q13429	Insulin-like growth factor I	IGF-I
Q13443	Disintegrin and metalloproteinase domain-containing protein 9	ADAM9
Q13519	Neuropeptide 1	PNOC
Q13751	Laminin subunit beta-3	LAMB3
Q13753	Laminin subunit gamma-2	LAMC2
Q13790	Apolipoprotein F	APOF
Q13822	Ectonucleotide pyrophosphatase/phosphodiesterase family member 2	ENPP2
Q14031	Collagen alpha-6(IV) chain	COL4A6
Q14050	Collagen alpha-3(IX) chain	COL9A3
Q14055	Collagen alpha-2(IX) chain	COL9A2
Q14112	Nidogen-2	NID2
Q14114	Low-density lipoprotein receptor-related protein 8	LRP8
Q14118	Dystroglycan	DAG1
Q14314	Fibroleukin	FGL2

TABLE 1-continued

Secreted Proteins		
Uniprot ID	Protein Name	Gene Name
Q14393	Growth arrest-specific protein 6	GAS6
Q14406	Chorionic somatomammotropin hormone-like 1	CSHL1
Q14507	Epididymal secretory protein E3-alpha	EDDM3A
Q14508	WAP four-disulfide core domain protein 2	WFDC2
Q14512	Fibroblast growth factor-binding protein 1	FGFBP1
Q14515	SPARC-like protein 1	SPARCL1
Q14520	Hyaluronan-binding protein 2 27 kDa light chain	HABP2
Q14563	Semaphorin-3A	SEMA3A
Q14623	Indian hedgehog protein	IHH
Q14624	Inter-alpha-trypsin inhibitor heavy chain H4	ITIH4
Q14667	UPF0378 protein KIAA0100	KIAA0100
Q14703	Membrane-bound transcription factor site-1 protease	MBTPS1
Q14766	Latent-transforming growth factor beta-binding protein 1	LTBP1
Q14767	Latent-transforming growth factor beta-binding protein 2	LTBP2
Q14773	Intercellular adhesion molecule 4	ICAM4
Q14993	Collagen alpha-1(XIX) chain	COL19A1
Q14CN2	Calcium-activated chloride channel regulator 4, 110 kDa form	CLCA4
Q15046	Lysine-tRNA ligase	KARS
Q15063	Periostin	POSTN
Q15109	Advanced glycosylation end product-specific receptor	AGER
Q15113	Procollagen C-endopeptidase enhancer 1	PCOLCE
Q15166	Serum paraoxonase/lactonase 3	PON3
Q15195	Plasminogen-like protein A	PLGLA
Q15198	Platelet-derived growth factor receptor-like protein	PDGFRL
Q15223	Poliovirus receptor-related protein 1	PVRL1
Q15238	Pregnancy-specific beta-1-glycoprotein 5	PSG5
Q15363	Transmembrane emp24 domain-containing protein 2	TMED2
Q15375	Ephrin type-A receptor 7	EPHA7
Q15389	Angiopoietin-1	ANGPT1
Q15465	Sonic hedgehog protein	SHH
Q15485	Ficolin-2	FCN2
Q15517	Corneodesmosin	CDSN
Q15582	Transforming growth factor-beta-induced protein ig-h3	TGFBI
Q15661	Trypsin alpha/beta-1	TPSAB1
Q15726	Metastatin	KISS1
Q15782	Chitinase-3-like protein 2	CHI3L2
Q15828	Cystatin-M	CST6
Q15846	Clusterin-like protein 1	CLUL1
Q15848	Adiponectin	ADIPOQ
Q16206	Protein disulfide-thiol oxidoreductase	ENOX2
Q16270	Insulin-like growth factor-binding protein 7	IGFBP7
Q16363	Laminin subunit alpha-4	LAMA4
Q16378	Proline-rich protein 4	PRR4
Q16557	Pregnancy-specific beta-1-glycoprotein 3	PSG3
Q16568	CART(42-89)	CARTPT
Q16610	Extracellular matrix protein 1	ECM1
Q16619	Cardiotrophin-1	CTF1
Q16623	Syntaxin-1A	STX1A
Q16627	HCC-1(9-74)	CCL14
Q16651	Prostasin light chain	PRSS8
Q16661	Guanylate cyclase C-activating peptide 2	GUCA2B
Q16663	CCL15(29-92)	CCL15
Q16674	Melanoma-derived growth regulatory protein	MIA
Q16769	Glutaminyl-peptide cyclotransferase	QPCT
Q16787	Laminin subunit alpha-3	LAMA3
Q16842	CMP-N-acetylneuraminate-beta-galactosamide-alpha-2,3-sialyltransferase 2	ST3GAL2
Q17RR3	Pancreatic lipase-related protein 3	PNLIPRP3
Q17RW2	Collagen alpha-1(XXIV) chain	COL24A1
Q17RY6	Lymphocyte antigen 6K	LY6K
Q1L6U9	Prostate-associated microseminoprotein	MSMP
Q1W4C9	Serine protease inhibitor Kazal-type 13	SPINK13
Q1ZYL8	Izumo sperm-egg fusion protein 4	IZUMO4
Q29960	HLA class I histocompatibility antigen, Cw-16 alpha chain	HLA-C
Q2l0M5	R-spondin-4	RSPO4
Q2L4Q9	Serine protease 53	PRSS53
Q2MKA7	R-spondin-1	RSPO1
Q2MV58	Tectonic-1	TCTN1
Q2TAL6	Brorin	VWC2
Q2UY09	Collagen alpha-1(XXVIII) chain	COL28A1

TABLE 1-continued

Secreted Proteins		
Uniprot ID	Protein Name	Gene Name
Q2VPA4	Complement component receptor 1-like protein	CR1L
Q2WEN9	Carcinoembryonic antigen-related cell adhesion molecule 16	CEACAM16
Q30KP8	Beta-defensin 136	DEFB136
Q30KP9	Beta-defensin 135	DEFB135
Q30KQ1	Beta-defensin 133	DEFB133
Q30KQ2	Beta-defensin 130	DEFB130
Q30KQ4	Beta-defensin 116	DEFB116
Q30KQ5	Beta-defensin 115	DEFB115
Q30KQ6	Beta-defensin 114	DEFB114
Q30KQ7	Beta-defensin 113	DEFB113
Q30KQ8	Beta-defensin 112	DEFB112
Q30KQ9	Beta-defensin 110	DEFB110
Q30KR1	Beta-defensin 109	DEFB109P1
Q32P28	Prolyl 3-hydroxylase 1	LEPRE1
Q3B7J2	Glucose-fructose oxidoreductase domain-containing protein 2	GFOD2
Q3SY79	Protein Wnt	WNT3A
Q3T906	N-acetylglucosamine-1-phosphotransferase subunits alpha/beta	GNPTAB
Q495T6	Membrane metallo-endopeptidase-like 1	MMEL1
Q49AH0	Cerebral dopamine neurotrophic factor	CDNF
Q4G0G5	Secretoglobin family 2B member 2	SCGB2B2
Q4GOM1	Protein FAM132B	FAM132B
Q4LDE5	Sushi, von Willebrand factor type A, EGF and pentraxin domain-containing protein 1	SVEP1
Q4QY38	Beta-defensin 134	DEFB134
Q4VAJ4	Protein Wnt	WNT10B
Q4W5P6	Protein TMEM155	TMEM155
Q4ZHG4	Fibronectin type III domain-containing protein 1	FNDC1
Q53H76	Phospholipase A1 member A	PLA1A
Q53RD9	Fibulin-7	FBLN7
Q53S33	BolA-like protein 3	BOLA3
Q5BLP8	Neuropeptide-like protein C4orf48	C4orf48
Q5DT21	Serine protease inhibitor Kazal-type 9	SPINK9
Q5EBL8	PDZ domain-containing protein 11	PDZD11
Q5FYB0	Arylsulfatase J	ARSJ
Q5FYB1	Arylsulfatase I	ARSI
Q5GAN3	Ribonuclease-like protein 13	RNASE13
Q5GAN4	Ribonuclease-like protein 12	RNASE12
Q5GAN6	Ribonuclease-like protein 10	RNASE10
Q5GFL6	von Willebrand factor A domain-containing protein 2	VWA2
Q5HRA3	Neuromedin-S	NMS
Q5H8C1	FRAS1-related extracellular matrix protein 1	FREM1
Q5IJ48	Protein crumbs homolog 2	CRB2
Q5J5C9	Beta-defensin 121	DEFB121
Q5JS37	NHL repeat-containing protein 3	NHLRC3
Q5JTB6	Placenta-specific protein 9	PLAC9
Q5JU69	Torsin-2A	TOR2A
Q5JXM2	Methyltransferase-like protein 24	METTL24
Q5IZY3	Ephrin type-A receptor 10	EPHA10
Q5K4E3	Polyserase-2	PRSS36
Q5SRR4	Lymphocyte antigen 6 complex locus protein G5c	LY6G5C
Q5T1H1	Protein eyes shu homolog	EYS
Q5T4F7	Secreted frizzled-related protein 5	SFRP5
Q5T4W7	Artemin	ARTN
Q5T7M4	Protein FAM132A	FAM132A
Q5TEH8	Protein Wnt	WNT2B
Q5TIE3	von Willebrand factor A domain-containing protein 5B1	VWA5B1
Q5UCC4	ER membrane protein complex subunit 10	EMC10
Q5VST6	Abhydrolase domain-containing protein FAM108B1	FAM108B1
Q5VTL7	Fibronectin type III domain-containing protein 7	FNDC7
Q5VUM1	UPF0369 protein C6orf57	C6orf57
Q5VV43	Dyslexia-associated protein KIAA0319	KIAA0319
Q5VWW1	Complement C1q-like protein 3	C1QL3
Q5VXJ9	Lipase member N	LIPN
Q5VXJ0	Lipase member K	LIPK
Q5VXM1	CUB domain-containing protein 2	CDCP2
Q5VYX0	Renalase	RNLS
Q5VYY2	Lipase member M	LIPM
Q5W186	Cystatin-9	CST9
Q5W5W9	Regulated endocrine-specific protein 18	RESP18
Q5XG92	Carboxylesterase 4A	CES4A
Q63HQ2	Pikachurin	EGFLAM

TABLE 1-continued

Secreted Proteins		
Uniprot ID	Protein Name	Gene Name
Q641Q3	Meteorin-like protein	METRNL
Q66K79	Carboxypeptidase Z	CPZ
Q685J3	Mucin-17	MUC17
Q68BL7	Olfactomedin-like protein 2A	OLFML2A
Q68BL8	Olfactomedin-like protein 2B	OLFML2B
Q68DV7	E3 ubiquitin-protein ligase RNF43	RNF43
Q6B9Z1	Insulin growth factor-like family member 4	IGFL4
Q6BAA4	Fc receptor-like B	FCRLB
Q6E0U4	Dermokine	DMKN
Q6EMK4	Vasorin	VASN
Q6FHJ7	Secreted frizzled-related protein 4	SFRP4
Q6GPI1	Chymotrypsin B2 chain B	CTRB2
Q6GTS8	Probable Carboxypeptidase PM20D1	PM20D1
Q6H9L7	Isthmin-2	ISM2
Q6IE36	Ovostatin homolog 2	OVOS2
Q6IE37	Ovostatin homolog 1	OVOS1
Q6IE38	Serine protease inhibitor Kazal-type 14	SPINK14
Q6ISS4	Leukocyte-associated immunoglobulin-like receptor 2	LAIR2
Q6JVE5	Epididymal-specific lipocalin-12	LCN12
Q6JVE6	Epididymal-specific lipocalin-10	LCN10
Q6JVE9	Epididymal-specific lipocalin-8	LCN8
Q6KF10	Growth/differentiation factor 6	GDF6
Q6MZB2	Follistatin-related protein 4	FSTL4
Q6NSX1	Coiled-coil domain-containing protein 70	CCDC70
Q6NT32	Carboxylesterase 5A	CES5A
Q6NT52	Choriogonadotropin subunit beta variant 2	CGB2
Q6NU16	Chondroactherin-like protein	CHADL
Q6NUJ1	Saposin A-like	PSAPL1
Q6P093	Arylacetamide deacetylase-like 2	AADAACL2
Q6P4A8	Phospholipase B-like 1	PLBD1
Q6P5S2	UPF0762 protein C6orf58	C6orf58
Q6P988	Protein notum homolog	NOTUM
Q6PCB0	von Willebrand factor A domain-containing protein 1	VWA1
Q6PDA7	Sperm-associated antigen 11A	SPAG11A
Q6PEW0	Inactive serine protease 54	PRSS54
Q6PEZ8	Podocan-like protein 1	PODNL1
Q6PKH6	Dehydrogenase/reductase SDR family member 4-like 2	DHRS4L2
Q6Q788	Apolipoprotein A-V	APOA5
Q6SPF0	Atherin	SAMD1
Q6UDR6	Kunitz-type protease inhibitor 4	SPINT4
Q6URK8	Testis, prostate and placenta-expressed protein	TEPP
Q6UW01	Cerebellin-3	CBLN3
Q6UW10	Surfactant-associated protein 2	SFTA2
Q6UW15	Regenerating islet-derived protein 3-gamma	REG3G
Q6UW32	Insulin growth factor-like family member 1	IGFL1
Q6UW78	UPF0723 protein C11orf83	C11orf83
Q6UW88	Epigen	EPGN
Q6UWE3	Colipase-like protein 2	CLPSL2
Q6UWF7	NXPE family member 4	NXPE4
Q6UWF9	Protein FAM180A	FAM180A
Q6UWM5	GLIPR1-like protein 1	GLIPR1L1
Q6UWN8	Serine protease inhibitor Kazal-type 6	SPINK6
Q6UWP2	Dehydrogenase/reductase SDR family member 11	DHRS11
Q6UWP8	Suprabasin	SBSN
Q6UWQ5	Lysozyme-like protein 1	LYZL1
Q6UWQ7	Insulin growth factor-like family member 2	IGFL2
Q6UWR7	Ectonucleotide pyrophosphatase/phosphodiesterase family member 6 soluble form	ENPP6
Q6UWT2	Adropin	ENHO
Q6UWU2	Beta-galactosidase-1-like protein	GLB1L
Q6UWW0	Lipocalin-15	LCN15
Q6UWX4	HHIP-like protein 2	HHIPL2
Q6UWY0	Arylsulfatase K	ARSK
Q6UWY2	Serine protease 57	PRSS57
Q6UWY5	Olfactomedin-like protein 1	OLFML1
Q6UX06	Olfactomedin-4	OLFML4
Q6UX07	Dehydrogenase/reductase SDR family member 13	DHRS13
Q6UX39	Amelotin	AMTN
Q6UX46	Protein FAM150B	FAM150B
Q6UX73	UPF0764 protein C16orf89	C16orf89
Q6UXB0	Protein FAM131A	FAM131A
Q6UXB1	Insulin growth factor-like family member 3	IGFL3
Q6UXB2	VEGF co-regulated chemokine 1	CXCL17

TABLE 1-continued

Secreted Proteins		
Uniprot ID	Protein Name	Gene Name
Q6UXF7	C-type lectin domain family 18 member B	CLEC18B
Q6UXH0	Hepatocellular carcinoma-associated protein TD26	C19orf80
Q6UXH1	Cysteine-rich with EGF-like domain protein 2	CRELD2
Q6UXH8	Collagen and calcium-binding EGF domain-containing protein 1	CCBE1
Q6UXH9	Inactive serine protease PAMR1	PAMR1
Q6UXI7	Vitrin	VIT
Q6UXI9	Nephronectin	NPNT
Q6UXN2	Trem-like transcript 4 protein	TREML4
Q6UXS0	C-type lectin domain family 19 member A	CLEC19A
Q6UXT8	Protein FAM150A	FAM150A
Q6UXT9	Abhydrolase domain-containing protein 15	ABHD15
Q6UXV4	Apolipoprotein O-like	APOOL
Q6UXX5	Inter-alpha-trypsin inhibitor heavy chain H6	ITIH6
Q6UXX9	R-spondin-2	RSPO2
Q6UY14	ADAMTS-like protein 4	ADAMTSL4
Q6UY27	Prostate and testis expressed protein 2	PATE2
Q6W4X9	Mucin-6	MUC6
Q6WN34	Chordin-like protein 2	CHRDL2
Q6WRI0	Immunoglobulin superfamily member 10	IGSF10
Q6X4U4	Sclerostin domain-containing protein 1	SOSTDC1
Q6X784	Zona pellucida-binding protein 2	ZPBP2
Q6XE38	Secretoglobin family 1D member 4	SCGB1D4
Q6XPR3	Repetin	RPTN
Q6XZB0	Lipase member I	LIPI
Q6ZMM2	ADAMTS-like protein 5	ADAMTSL5
Q6ZMP0	Thrombospondin type-1 domain-containing protein 4	THSD4
Q6ZNF0	Iron/zinc purple acid phosphatase-like protein	PAPL
Q6ZRI0	Otegelin	OTOG
Q6ZRP7	Sulfhydryl oxidase 2	QSOX2
Q6ZWJ8	Kielin/chordin-like protein	KCP
Q75N90	Fibrillin-3	FBN3
Q76510	Urotensin-2B	UTS2D
Q76B58	Protein FAM5C	FAM5C
Q76LX8	A disintegrin and metalloproteinase with thrombospondin motifs 13	ADAMTS13
Q76M96	Coiled-coil domain-containing protein 80	CCDC80
Q7L1S5	Carbohydrate sulfotransferase 9	CHST9
Q7L513	Fc receptor-like A	FCRLA
Q7L8A9	Vasohibin-1	VASH1
Q7RTM1	Otopetrin-1	OTOP1
Q7RTW8	Otoancorin	OTOA
Q7RTY5	Serine protease 48	PRSS48
Q7RTY7	Ovochymase-1	OVCH1
Q7RTZ1	Ovochymase-2	OVCH2
Q7Z304	MAM domain-containing protein 2	MAMDC2
Q7Z3S9	Notch homolog 2 N-terminal-like protein	NOTCH2NL
Q7Z4H4	Intermedin-short	ADM2
Q7Z4P5	Growth/differentiation factor 7	GDF7
Q7Z4R8	UPF0669 protein C6orf120	C6orf120
Q7Z4W2	Lysozyme-like protein 2	LYZL2
Q7Z5A4	Serine protease 42	PRSS42
Q7Z5A7	Protein FAM19A5	FAM19A5
Q7Z5A8	Protein FAM19A3	FAM19A3
Q7Z5A9	Protein FAM19A1	FAM19A1
Q7Z5J1	Hydroxysteroid 11-beta-dehydrogenase 1-like protein	HSD11B1
Q7Z5L0	Vitelline membrane outer layer protein 1 homolog	VMO1
Q7Z5L3	Complement C1q-like protein 2	C1QL2
Q7Z5L7	Podocan	PODN
Q7Z5P4	17-beta-hydroxysteroid dehydrogenase 13	HSD17B13
Q7Z5P9	Mucin-19	MUC19
Q7Z5Y6	Bone morphogenetic protein 8A	BMP8A
Q7Z7B7	Beta-defensin 132	DEFB132
Q7Z7B8	Beta-defensin 128	DEFB128
Q7ZTC8	Transcription initiation factor TFIID subunit 8	TAF8
Q7ZTH5	Transmembrane emp24 domain-containing protein 4	TMED4
Q86SG7	Lysozyme g-like protein 2	LYG2
Q86SI9	Protein CEI	C5orf38
Q86TE4	Leucine zipper protein 2	LUZP2
Q86TH1	ADAMTS-like protein 2	ADAMTSL2
Q86U17	Serpin A11	SERPINA11
Q86UU9	Endokinin-A	TAC4

TABLE 1-continued

Secreted Proteins		
Uniprot ID	Protein Name	Gene Name
Q86UW8	Hyaluronan and proteoglycan link protein 4	HAPLN4
Q86UX2	Inter-alpha-trypsin inhibitor heavy chain H5	ITIH5
Q86V24	Adiponectin receptor protein 2	ADIPOR2
Q86VB7	Soluble CD163	CD163
Q86VR8	Four-jointed box protein 1	FJX1
Q86WD7	Serpin A9	SERPINA9
Q86WN2	Interferon epsilon	IFNE
Q86WS3	Placenta-specific 1-like protein	PLAC1L
Q86X52	Chondroitin sulfate synthase 1	CHSY1
Q86XP6	Gastrokine-2	GKN2
Q86XS5	Angiopoietin-related protein 5	ANGPTL5
Q86Y27	B melanoma antigen 5	BAGE5
Q86Y28	B melanoma antigen 4	BAGE4
Q86Y29	B melanoma antigen 3	BAGE3
Q86Y30	B melanoma antigen 2	BAGE2
Q86Y38	Xylosyltransferase 1	XYLT1
Q86Y78	Ly6/PLAUR domain-containing protein 6	LYPD6
Q86YD3	Transmembrane protein 25	TMEM25
Q86YJ6	Threonine synthase-like 2	THNSL2
Q86YW7	Glycoprotein hormone beta-5	GPHB5
Q86Z23	Complement C1q-like protein 4	C1QL4
Q8IU57	Interleukin-28 receptor subunit alpha	IL28RA
Q8IUA0	WAP four-disulfide core domain protein 8	WFDC8
Q8IUB2	WAP four-disulfide core domain protein 3	WFDC3
Q8IUB3	Protein WFDC10B	WFDC10B
Q8IUB5	WAP four-disulfide core domain protein 13	WFDC13
Q8IUH2	Protein CREG2	CREG2
Q8IUK5	Plexin domain-containing protein 1	PLXDC1
Q8IUL8	Cartilage intermediate layer protein 2 C2	CILP2
Q8IUX7	Adipocyte enhancer-binding protein 1	AEBP1
Q8IUX8	Epidermal growth factor-like protein 6	EGFL6
Q8IVL8	Carboxypeptidase O	CPO
Q8IVN8	Somatomedin-B and thrombospondin type-1 domain-containing protein	SBSPON
Q8IVW8	Protein spinster homolog 2	SPNS2
Q8IW75	Serpin A12	SERPINA12
Q8IW92	Beta-galactosidase-1-like protein 2	GLB1L2
Q8IWL1	Pulmonary surfactant-associated protein A2	SFTPA2
Q8IWL2	Pulmonary surfactant-associated protein A1	SFTPA1
Q8IWV2	Contactin-4	CNTN4
Q8IWY4	Signal peptide, CUB and EGF-like domain-containing protein 1	SCUBE1
Q8IX30	Signal peptide, CUB and EGF-like domain-containing protein 3	SCUBE3
Q8IXA5	Sperm acrosome membrane-associated protein 3, membrane form	SPACA3
Q8IXB1	DnaJ homolog subfamily C member 10	DNAJC10
Q8IXL6	Extracellular serine/threonine protein kinase Fam20C	FAM20C
Q8IYD9	Lung adenoma susceptibility protein 2	LAS2
Q8IYP2	Serine protease 58	PRSS58
Q8IYS5	Osteoclast-associated immunoglobulin-like receptor	OSCAR
Q8IZC6	Collagen alpha-1(XVII) chain	COL27A1
Q8IZJ3	C3 and PZP-like alpha-2-macroglobulin domain-containing protein 8	CPAMD8
Q8IZN7	Beta-defensin 107	DEFB107B
Q8NOV4	Leucine-rich repeat LGI family member 2	LGI2
Q8N104	Beta-defensin 106	DEFB106B
Q8N119	Matrix metalloproteinase-21	MMP21
Q8N129	Protein canopy homolog 4	CNPY4
Q8N135	Leucine-rich repeat LGI family member 4	LGI4
Q8N145	Leucine-rich repeat LGI family member 3	LGI3
Q8N158	Glypican-2	GPC2
Q8N1E2	Lysozyme g-like protein 1	LYG1
Q8N2E2	von Willebrand factor D and EGF domain-containing protein	VWDE
Q8N2E6	Prosalusin	TOR2A
Q8N2S1	Latent-transforming growth factor beta-binding protein 4	LTBP4
Q8N302	Angiogenic factor with G patch and FHA domains 1	AGGF1
Q8N307	Mucin-20	MUC20
Q8N323	NXPE family member 1	NXPE1
Q8N387	Mucin-15	MUC15
Q8N3Z0	Inactive serine protease 35	PRSS35
Q8N436	Inactive carboxypeptidase-like protein X2	CPXM2
Q8N474	Secreted frizzled-related protein 1	SFRP1

TABLE 1-continued

Secreted Proteins		
Uniprot ID	Protein Name	Gene Name
Q8N475	Follistatin-related protein 5	FSTL5
Q8N4F0	BPI fold-containing family B member 2	BPIFB2
Q8N4T0	Carboxypeptidase A6	CPA6
Q8N5W8	Protein FAM24B	FAM24B
Q8N687	Beta-defensin 125	DEFB125
Q8N688	Beta-defensin 123	DEFB123
Q8N690	Beta-defensin 119	DEFB119
Q8N6C5	Immunoglobulin superfamily member 1	IGSF1
Q8N6C8	Leukocyte immunoglobulin-like receptor subfamily A member 3	LILRA3
Q8N6G6	ADAMTS-like protein 1	ADAMTSL1
Q8N6Y2	Leucine-rich repeat-containing protein 17	LRRC17
Q8N729	Neuropeptide W-23	NPW
Q8N8U9	BMP-binding endothelial regulator protein	BMPER
Q8N907	DAN domain family member 5	DAND5
Q8NAT1	Glycosyltransferase-like domain-containing protein 2	GTDC2
Q8NAU1	Fibronectin type III domain-containing protein 5	FNDC5
Q8NB37	Parkinson disease 7 domain-containing protein 1	PDDC1
Q8NB13	Draxin	DRAVIN
Q8NBM8	Prenylcysteine oxidase-like	PCYOX1L
Q8NBP7	Proprotein convertase subtilisin/kexin type 9	PCSK9
Q8NBQ5	Estradiol 17-beta-dehydrogenase 11	HSD17B11
Q8NBV8	Synaptotagmin-8	SYT8
Q8NC3	Group XV phospholipase A2	PLA2G15
Q8NCF0	C-type lectin domain family 18 member C	CLEC18C
Q8NCW5	NAD(P)H-hydrate epimerase	APOA1BP
Q8ND2	Hemicentin-2	HMCN2
Q8NDX9	Lymphocyte antigen 6 complex locus protein G5b	LY6G5B
Q8NDZ4	Deleted in autism protein 1	C3orf58
Q8NEB7	Acrosin-binding protein	ACRBP
Q8NES8	Beta-defensin 124	DEFB124
Q8NET1	Beta-defensin 108B	DEFB108B
Q8NEX5	Protein WFDC9	WFDC9
Q8NEX6	Protein WFDC11	WFDC11
Q8NF86	Serine protease 33	PRSS33
Q8NFM7	Interleukin-17 receptor D	IL17RD
Q8NFQ5	BPI fold-containing family B member 6	BPIFB6
Q8NFQ6	BPI fold-containing family C protein	BPIFC
Q8NFW4	Follicular dendritic cell secreted peptide	FDCSP
Q8NFW1	Collagen alpha-1(XXII) chain	COL22A1
Q8NG35	Beta-defensin 105	DEFB105B
Q8NG41	Neuropeptide B-23	NPB
Q8NHW6	Otospiralin	OTOS
Q8NI99	Angiopoietin-related protein 6	ANGPTL6
Q8TAA1	Probable ribonuclease 11	RNASE11
Q8TAG5	V-set and transmembrane domain-containing protein 2A	VSTM2A
Q8TAL6	Fin bud initiation factor homolog	FIBIN
Q8TAT2	Fibroblast growth factor-binding protein 3	FGFBP3
Q8TAX7	Mucin-7	MUC7
Q8TB22	Spermatogenesis-associated protein 20	SPATA20
Q8TB73	Protein NDNF	NDNF
Q8TB96	T-cell immunomodulatory protein	ITFG1
Q8TC92	Protein disulfide-thiol oxidoreductase	ENOX1
Q8TCV5	WAP four-disulfide core domain protein 5	WFDC5
Q8TD06	Anterior gradient protein 3 homolog	AGR3
Q8TD33	Secretoglobin family 1C member 1	SCGB1C1
Q8TD46	Cell surface glycoprotein CD200 receptor 1	CD200R1
Q8TDE3	Ribonuclease 8	RNASE8
Q8TDF5	Neuropilin and tolloid-like protein 1	NETO1
Q8TDL5	BPI fold-containing family B member 1	BPIFB1
Q8TE56	A disintegrin and metalloproteinase with thrombospondin motifs 17	ADAMTS17
Q8TE57	A disintegrin and metalloproteinase with thrombospondin motifs 16	ADAMTS16
Q8TE58	A disintegrin and metalloproteinase with thrombospondin motifs 15	ADAMTS15
Q8TE59	A disintegrin and metalloproteinase with thrombospondin motifs 19	ADAMTS19
Q8TE60	A disintegrin and metalloproteinase with thrombospondin motifs 18	ADAMTS18
Q8TE99	Acid phosphatase-like protein 2	ACPL2
Q8TER0	Sushi, nidogen and EGF-like domain-containing protein 1	SNED1

TABLE 1-continued

Secreted Proteins		
Uniprot ID	Protein Name	Gene Name
Q8TEU8	WAP, kazal, immunoglobulin, kunitz and NTR domain-containing protein 2	WFIKKN2
Q8WTQ1	Beta-defensin 104	DEFB104B
Q8WTR8	Netrin-5	NTN5
Q8WTU2	Scavenger receptor cysteine-rich domain-containing group B protein	SRCRB4D
Q8WU66	Protein TSPEAR	TSPEAR
Q8WUA8	Tsukushin	TSKU
Q8WUF8	Protein FAM172A	FAM172A
Q8WUJ1	Neuferricin	CYB5D2
Q8WUY1	UPF0670 protein THEM6	THEM6
Q8WVN6	Secreted and transmembrane protein 1	SECTM1
Q8WVQ1	Soluble calcium-activated nucleotidase 1	CANT1
Q8WWA0	Intelectin-1	ITLN1
Q8WWG1	Neuregulin-4	NRG4
Q8WWQ2	Inactive heparanase-2	HPSE2
Q8WWU7	Intelectin-2	ITLN2
Q8WWY7	WAP four-disulfide core domain protein 12	WFDC12
Q8WWY8	Lipase member H	LIPH
Q8WWZ8	Oncoprotein-induced transcript 3 protein	OIT3
Q8WX39	Epididymal-specific lipocalin-9	LCN9
Q8WXA2	Prostate and testis expressed protein 1	PATE1
Q8WXD2	Secretogranin-3	SCG3
Q8WXF3	Relaxin-3 A chain	RLN3
Q8WXI7	Mucin-16	MUC16
Q8WXQ8	Carboxypeptidase A5	CPA5
Q8WXS8	A disintegrin and metalloproteinase with thrombospondin motifs 14	ADAMTS14
Q92484	Acid sphingomyelinase-like phosphodiesterase 3a	SMPDL3A
Q92485	Acid sphingomyelinase-like phosphodiesterase 3b	SMPDL3B
Q92496	Complement factor H-related protein 4	CFHR4
Q92520	Protein FAM3C	FAM3C
Q92563	Testican-2	SPOCK2
Q92583	C—C motif chemokine 17	CCL17
Q92626	Peroxidasin homolog	PXDN
Q92743	Serine protease HTRA1	HTRA1
Q92752	Tenascin-R	TNR
Q92765	Secreted frizzled-related protein 3	FRZB
Q92819	Hyaluronan synthase 2	HAS2
Q92820	Gamma-glutamyl hydrolase	GGH
Q92824	Proprotein convertase subtilisin/kexin type 5	PCSK5
Q92832	Protein kinase C-binding protein NELL1	NELL1
Q92838	Ectodysplasin-A, membrane form	EDA
Q92874	Deoxyribonuclease-1-like 2	DNASE1L2
Q92876	Kallikrein-6	KLK6
Q92913	Fibroblast growth factor 13	FGF13
Q92954	Proteoglycan 4 C-terminal part	PRG4
Q93038	Tumor necrosis factor receptor superfamily member 25	TNFRSF25
Q93091	Ribonuclease K6	RNASE6
Q93097	Protein Wnt-2b	WNT2B
Q93098	Protein Wnt-8b	WNT8B
Q95460	Major histocompatibility complex class I-related gene protein	MR1
Q969D9	Thymic stromal lymphopoietin	TSLP
Q969E1	Liver-expressed antimicrobial peptide 2	LEAP2
Q969H8	UPF0556 protein C19orf10	C19orf10
Q969Y0	NXPE family member 3	NXPE3
Q96A54	Adiponectin receptor protein 1	ADIPOR1
Q96A83	Collagen alpha-1(XXVI) chain	EMID2
Q96A84	EMI domain-containing protein 1	EMID1
Q96A98	Tuberoinfundibular peptide of 39 residues	PTH2
Q96A99	Pentraxin-4	PTX4
Q96BH3	Epididymal sperm-binding protein 1	ELSPBP1
Q96BQ1	Protein FAM3D	FAM3D
Q96CG8	Collagen triple helix repeat-containing protein 1	CTHRC1
Q96DA0	Zymogen granule protein 16 homolog B	ZG16B
Q96DN2	von Willebrand factor C and EGF domain-containing protein	VWCE
Q96DR5	BPI fold-containing family A member 2	BPIFA2
Q96DR8	Mucin-like protein 1	MUCL1
Q96DX4	RING finger and SPRY domain-containing protein 1	RSPRY1
Q96EE4	Coiled-coil domain-containing protein 126	CCDC126

TABLE 1-continued

Secreted Proteins		
Uniprot ID	Protein Name	Gene Name
Q96GS6	Abhydrolase domain-containing protein FAM108A1	FAM108A1
Q96GW7	Brevican core protein	BCAN
Q96HF1	Secreted frizzled-related protein 2	SFRP2
Q96I82	Kazal-type serine protease inhibitor domain-containing protein 1	KAZALD1
Q96ID5	Immunoglobulin superfamily member 21	IGSF21
Q96II8	Leucine-rich repeat and calponin homology domain-containing protein 3	LRCH3
Q96IY4	Carboxypeptidase B2	CPB2
Q96JB6	Lysyl oxidase homolog 4	LOXL4
Q96JK4	HHIP-like protein 1	HHIPL1
Q96KN2	Beta-Ala-His dipeptidase	CNDP1
Q96KW9	Protein SPACA7	SPACA7
Q96KX0	Lysozyme-like protein 4	LYZL4
Q96L15	Ecto-ADP-ribosyltransferase 5	ART5
Q96LB8	Peptidoglycan recognition protein 4	PGLYRP4
Q96LB9	Peptidoglycan recognition protein 3	PGLYRP3
Q96LC7	Sialic acid-binding Ig-like lectin 10	SIGLEC10
Q96LR4	Protein FAM19A4	FAM19A4
Q96MK3	Protein FAM20A	FAM20A
Q96MS3	Glycosyltransferase 1 domain-containing protein 1	GLT1D1
Q96NY8	Processed poliovirus receptor-related protein 4	PVRL4
Q96NZ8	WAP, kazal, immunoglobulin, kunitz and NTR domain-containing protein 1	WF1KKN1
Q96NZ9	Proline-rich acidic protein 1	PRAP1
Q96P44	Collagen alpha-1(XXI) chain	COL21A1
Q96PB7	Noelin-3	OLFM3
Q96PC5	Melanoma inhibitory activity protein 2	MIA2
Q96PD5	N-acetylglucosaminyl-L-alanine amidase	PGLYRP2
Q96PH6	Beta-defensin 118	DEFB118
Q96PL1	Secretoglobin family 3A member 2	SCGB3A2
Q96PL2	Beta-tectorin	TECTB
Q96QH8	Sperm acrosome-associated protein 5	SPACA5
Q96QR1	Secretoglobin family 3A member 1	SCGB3A1
Q96QU1	Protocadherin-15	PCDH15
Q96QV1	Hedgehog-interacting protein	HHIP
Q96RW7	Hemicentin-1	HMCN1
Q96S42	Nodal homolog	NODAL
Q96S86	Hyaluronan and proteoglycan link protein 3	HAPLN3
Q96SL4	Glutathione peroxidase 7	GPX7
Q96SM3	Probable carboxypeptidase X1	CPXM1
Q96T91	Glycoprotein hormone alpha-2	GPHA2
Q99062	Granulocyte colony-stimulating factor receptor	CSF3R
Q99102	Mucin-4 alpha chain	MUC4
Q99217	Amelogenin, X isoform	AMELX
Q99218	Amelogenin, Y isoform	AMELY
Q99435	Protein kinase C-binding protein NELL2	NELL2
Q99470	Stromal cell-derived factor 2	SDF2
Q99542	Matrix metalloproteinase-19	MMP19
Q99574	Neuroserpin	SERPINI1
Q99584	Protein S100-A13	S100A13
Q99616	C—C motif chemokine 13	CCL13
Q99645	Epiphycan	EPYC
Q99674	Cell growth regulator with EF hand domain protein 1	CGREF1
Q99715	Collagen alpha-1(XII) chain	COL12A1
Q99727	Metalloproteinase inhibitor 4	TIMP4
Q99731	C—C motif chemokine 19	CCL19
Q99748	Neurturin	NRTN
Q99935	Proline-rich protein 1	PROL1
Q99942	E3 ubiquitin-protein ligase RNF5	RNF5
Q99944	Epidermal growth factor-like protein 8	EGFL8
Q99954	Submaxillary gland androgen-regulated protein 3A	SMR3A
Q99969	Retinoic acid receptor responder protein 2	RARRES2
Q99972	Myocilin	MYOC
Q99983	Osteomodulin	OMD
Q99985	Semaphorin-3C	SEMA3C
Q99988	Growth/differentiation factor 15	GDF15
Q9BPW4	Apolipoprotein L4	APOL4
Q9BQ08	Resistin-like beta	RETNLB
Q9BQ16	Testican-3	SPOCK3
Q9BQ51	Programmed cell death 1 ligand 2	PDCD1LG2
Q9BQB4	Sclerostin	SOST
Q9BQI4	Coiled-coil domain-containing protein 3	CCDC3

TABLE 1-continued

Secreted Proteins		
Uniprot ID	Protein Name	Gene Name
Q9BQP9	BPI fold-containing family A member 3	BPIFA3
Q9BQR3	Serine protease 27	PRSS27
Q9BQY6	WAP four-disulfide core domain protein 6	WFDC6
Q9BRR6	ADP-dependent glucokinase	ADPGK
Q9BS86	Zona pellucida-binding protein 1	ZPBP
Q9BSG0	Protease-associated domain-containing protein 1	PRADC1
Q9BSG5	Retbindin	RTBDN
Q9BT30	Probable alpha-ketoglutarate-dependent dioxygenase ABH7	ALKBH7
Q9BT56	Speixin	C12orfG9
Q9BT67	NEDD4 family-interacting protein 1	NDFIP1
Q9BTY2	Plasma alpha-L-fucosidase	FUCA2
Q9BU40	Chordin-like protein 1	CHRDL1
Q9BUD6	Spondin-2	SPON2
Q9BUN1	Protein MENT	MENT
Q9BUR5	Apolipoprotein O	APOO
Q9BV94	ER degradation-enhancing alpha-mannosidase-like 2	EDEM2
Q9BWP8	Collectin-11	COLEC11
Q9BWS9	Chitinase domain-containing protein 1	CHID1
Q9BX67	Junctional adhesion molecule C	JAM3
Q9BX93	Group XIIIB secretory phospholipase A2-like protein	PLA2G12B
Q9BXI9	Complement C1q tumor necrosis factor-related protein 6	C1QTNF6
Q9BXJ0	Complement C1q tumor necrosis factor-related protein 5	C1QTNF5
Q9BXJ1	Complement C1q tumor necrosis factor-related protein 1	C1QTNF1
Q9BXJ2	Complement C1q tumor necrosis factor-related protein 7	C1QTNF7
Q9BXJ3	Complement C1q tumor necrosis factor-related protein 4	C1QTNF4
Q9BXJ4	Complement C1q tumor necrosis factor-related protein 3	C1QTNF3
Q9BXJ5	Complement C1q tumor necrosis factor-related protein 2	C1QTNF2
Q9BXN1	Asporin	ASPN
Q9BXP8	Pappalysin-2	PAPPA2
Q9BXR6	Complement factor H-related protein 5	CFHR5
Q9BXS0	Collagen alpha-1(XXV) chain	COL25A1
Q9BXX0	EMILIN-2	EMILIN2
Q9BXY4	R-spondin-3	RSPO3
Q9BY15	EGF-like module-containing mucin-like hormone receptor-like 3 subunit beta	EMR3
Q9BY50	Signal peptidase complex catalytic subunit SEC11C	SEC11C
Q9BY76	Angiopoietin-related protein 4	ANGPTL4
Q9BYF1	Processed angiotensin-converting enzyme 2	ACE2
Q9BYJ0	Fibroblast growth factor-binding protein 2	FGFBP2
Q9BYW3	Beta-defensin 126	DEFB126
Q9BYX4	Interferon-induced helicase C domain-containing protein 1	IFIH1
Q9BYZ8	Regenerating islet-derived protein 4	REG4
Q9BZ76	Contactin-associated protein-like 3	CNTNAP3
Q9BZG9	Ly-6/neurotoxin-like protein 1	LYNX1
Q9BZJ3	Tryptase delta	TPSD1
Q9BZM1	Group XIIA secretory phospholipase A2	PLA2G12A
Q9BZM2	Group IIF secretory phospholipase A2	PLA2G2F
Q9BZM5	NKG2D ligand 2	ULBP2
Q9BZP6	Acidic mammalian chitinase	CHIA
Q9BZZ2	Sialoadhesin	SIGLEC1
Q9C0B6	Protein FAM5B	FAM5B
Q9GZM7	Tubulointerstitial nephritis antigen-like	TINAGL1
Q9GZN4	Brain-specific serine protease 4	PRSS22
Q9GZP0	Platelet-derived growth factor D, receptor-binding form	PDGFD
Q9GZT5	Protein Wnt-10a	WNT10A
Q9GZU5	Nyctalopin	NYX
Q9GZV7	Hyaluronan and proteoglycan link protein 2	HAPLN2
Q9GZV9	Fibroblast growth factor 23	FGF23
Q9GZX9	Twisted gastrulation protein homolog 1	TWSG1
Q9GZZ7	GDNF family receptor alpha-4	GFRA4
Q9GZZ8	Extracellular glycoprotein lacritin	LACRT
Q9H0B8	Cysteine-rich secretory protein LCCL domain-containing 2	CRISPLD2
Q9H106	Signal-regulatory protein delta	SIRPD
Q9H114	Cystatin-like 1	CSTL1
Q9H173	Nucleotide exchange factor SIL1	SIL1
Q9H1E1	Ribonuclease 7	RNASE7
Q9H1F0	WAP four-disulfide core domain protein 10A	WFDC10A
Q9H1J5	Protein Wnt-8a	WNT8A
Q9H1J7	Protein Wnt-5b	WNT5B
Q9H1M3	Beta-defensin 129	DEFB129
Q9H1M4	Beta-defensin 127	DEFB127

TABLE 1-continued

Secreted Proteins		
Uniprot ID	Protein Name	Gene Name
Q9H1Z8	Augurin	C2orf40
Q9H239	Matrix metalloproteinase-28	MMP28
Q9H2A7	C—X—C motif chemokine 16	CXCL16
Q9H2A9	Carbohydrate sulfotransferase 8	CHST8
Q9H2R5	Kallikrein-15	KLK15
Q9H2X0	Chordin	CHRD
Q9H2X3	C-type lectin domain family 4 member M	CLEC4M
Q9H306	Matrix metalloproteinase-27	MMP27
Q9H324	A disintegrin and metalloproteinase with thrombospondin motifs 10	ADAMTS10
Q9H336	Cysteine-rich secretory protein LCCL domain-containing 1	CRISPLD1
Q9H3E2	Sorting nexin-25	SNX25
Q9H3R2	Mucin-13	MUC13
Q9H3U7	SPARC-related modular calcium-binding protein 2	SMOC2
Q9H3Y0	Peptidase inhibitor R3HDML	R3HDML
Q9H4A4	Aminopeptidase B	RNPEP
Q9H4F8	SPARC-related modular calcium-binding protein 1	SMOC1
Q9H4G1	Cystatin-9-like	CST9L
Q9H5V8	CUB domain-containing protein 1	CDCP1
Q9H6B9	Epoxide hydrolase 3	EPHX3
Q9H6E4	Coiled-coil domain-containing protein 134	CCDC134
Q9H741	UPF0454 protein C12orf49	C12orf49
Q9H772	Gremlin-2	GREM2
Q9H7Y0	Deleted in autism-related protein 1	CXorf36
Q9H8L6	Multimerin-2	MMRN2
Q9H9S5	Fukutin-related protein	FKRP
Q9HAT2	Sialate O-acetylerase	SIAE
Q9HB40	Retinoid-inducible serine carboxypeptidase	SCPEP1
Q9HB63	Netrin-4	NTN4
Q9HBj0	Placenta-specific protein 1	PLAC1
Q9HC23	Prokineticin-2	PROK2
Q9HC57	WAP four-disulfide core domain protein 1	WFDC1
Q9HC73	Cytokine receptor-like factor 2	CRLF2
Q9HC84	Mucin-5B	MUC5B
Q9HCB6	Spondin-1	SPON1
Q9HCQ7	Neuropeptide NPSF	NPVF
Q9HCT0	Fibroblast growth factor 22	FGF22
Q9HD89	Resistin	RETN
Q9NNX1	Tuftelin	TUFT1
Q9NNX6	CD209 antigen	CD209
Q9NP55	BPI fold-containing family A member 1	BPIFA1
Q9NP70	Ameloblastin	AMBN
Q9NP95	Fibroblast growth factor 20	FGF20
Q9NP99	Triggering receptor expressed on myeloid cells 1	TREM1
Q9NPA2	Matrix metalloproteinase-25	MMP25
Q9NPE2	Neugrin	NGRN
Q9NPH0	Lysophosphatidic acid phosphatase type 6	ACP6
Q9NPH6	Odorant-binding protein 2b	OBP2B
Q9NQ30	Endothelial cell-specific molecule 1	ESM1
Q9NQ36	Signal peptide, CUB and EGF-like domain-containing protein 2	SCUBE2
Q9NQ38	Serine protease inhibitor Kazal-type 5	SPINK5
Q9NQ76	Matrix extracellular phosphoglycoprotein	MEPE
Q9NQ79	Cartilage acidic protein 1	CRTAC1
Q9NR16	Scavenger receptor cysteine-rich type 1 protein M160	CD163L1
Q9NR23	Growth/differentiation factor 3	GDF3
Q9NR71	Neutral ceramidase	ASAH2
Q9NR99	Matrix-remodeling-associated protein 5	MXRA5
Q9NRA1	Platelet-derived growth factor C	PDGFC
Q9NRC9	Otoraplin	OTOR
Q9NRE1	Matrix metalloproteinase-26	MMP26
Q9NRJ3	C—C motif chemokine 28	CCL28
Q9NRM1	Enamelin	ENAM
Q9NRN5	Olfactomedin-like protein 3	OLFML3
Q9NRR1	Cytokine-like protein 1	CYTL1
Q9NS15	Latent-transforming growth factor beta-binding protein 3	LTBP3
Q9NS62	Thrombospondin type-1 domain-containing protein 1	THSD1
Q9NS71	Gastrokine-1	GKN1
Q9NS98	Semaphorin-3G	SEMA3G
Q9NSA1	Fibroblast growth factor 21	FGF21
Q9NT22	EMILIN-3	EMILIN3
Q9NTU7	Cerebellin-4	CBLN4
Q9NVR0	Kelch-like protein 11	KLHL11

TABLE 1-continued

Secreted Proteins		
Uniprot ID	Protein Name	Gene Name
Q9NWH7	Spermatogenesis-associated protein 6	SPATA6
Q9NXC2	Glucose-fructose oxidoreductase domain-containing protein 1	GFOD1
Q9NY56	Odorant-binding protein 2a	OBP2A
Q9NY84	Vascular non-inflammatory molecule 3	VNN3
Q9NZ20	Group 3 secretory phospholipase A2	PLA2G3
Q9NZC2	Triggering receptor expressed on myeloid cells 2	TREM2
Q9NZK5	Adenosine deaminase CECR1	CECR1
Q9NZK7	Group II E secretory phospholipase A2	PLA2G2E
Q9NZP8	Complement C1r subcomponent-like protein	C1RL
Q9NZV1	Cysteine-rich motor neuron 1 protein	CRIM1
Q9NZW4	Dentin sialoprotein	DSPP
Q9P0G3	Kallikrein-14	KLK14
Q9P0W0	Interferon kappa	IFNK
Q9P218	Collagen alpha-1(XX) chain	COL20A1
Q9P2C4	Transmembrane protein 181	TMEM181
Q9P2K2	Thioredoxin domain-containing protein 16	TXND16
Q9P2N4	A disintegrin and metalloproteinase with thrombospondin motifs 9	ADAMTS9
Q9UBC7	Galanin-like peptide	GALP
Q9UBD3	Cytokine SCM-1 beta	XCL2
Q9UBD9	Cardiotrophin-like cytokine factor 1	CLCF1
Q9UBM4	Opticin	OPTC
Q9UBP4	Dickkopf-related protein 3	DKK3
Q9UBQ6	Exostosin-like 2	EXTL2
Q9UBR5	Chemokine-like factor	CKLF
Q9UBS5	Gamma-aminobutyric acid type B receptor subunit 1	GABBR1
Q9UBT3	Dickkopf-related protein 4 short form	DKK4
Q9UBU2	Dickkopf-related protein 2	DKK2
Q9UBU3	Ghrelin-28	GHRL
Q9UBV4	Protein Wnt-16	WNT16
Q9UBX5	Fibulin-5	FBLN5
Q9UBX7	Kallikrein-11	KLK11
Q9UEF7	Klotho	KL
Q9UFP1	Protein FAM198A	FAM198A
Q9UGM3	Deleted in malignant brain tumors 1 protein	DMBT1
Q9UGM5	Fetuin-B	FETUB
Q9UGP8	Translocation protein SEC63 homolog	SEC63
Q9UHF0	Neurokinin-B	TAC3
Q9UHF1	Epidermal growth factor-like protein 7	EGFL7
Q9UHG2	ProSAAS	PCSK1N
Q9UH18	A disintegrin and metalloproteinase with thrombospondin motifs 1	ADAMTS1
Q9UHL4	Dipeptidyl peptidase 2	DPP7
Q9UI42	Carboxypeptidase A4	CPA4
Q9UIG4	Psoriasis susceptibility 1 candidate gene 2 protein	PSORS1C2
Q9UIK5	Tomoregulin-2	TMEFF2
Q9UIQ6	Leucyl-cysteinyl aminopeptidase, pregnancy serum form	LNPEP
Q9UIA9	Ectonucleotide pyrophosphatase/phosphodiesterase family member 5	ENPP5
Q9UJH8	Meteorin	METRN
Q9UJJ9	N-acetylglucosamine-1-photransferase subunit gamma	GNPTG
Q9UJW2	Tubulointerstitial nephritis antigen	TINAG
Q9UK05	Growth/differentiation factor 2	GDF2
Q9UK55	Protein Z-dependent protease inhibitor	SERPINA10
Q9UK85	Dickkopf-like protein 1	DKKL1
Q9UKJ1	Paired immunoglobulin-like type 2 receptor alpha	PILRA
Q9UKP4	A disintegrin and metalloproteinase with thrombospondin motifs 7	ADAMTS7
Q9UKP5	A disintegrin and metalloproteinase with thrombospondin motifs 6	ADAMTS6
Q9UKQ2	Disintegrin and metalloproteinase domain-containing protein 28	ADAM28
Q9UKQ9	Kallikrein-9	KLK9
Q9UKR0	Kallikrein-12	KLK12
Q9UKR3	Kallikrein-13	KLK13
Q9UKU9	Angiopoietin-related protein 2	ANGPTL2
Q9UKZ9	Procollagen C-endopeptidase enhancer 2	PCOLCE2
Q9UL52	Transmembrane protease serine 11E non-catalytic chain	TMPRSS11E
Q9ULC0	Endomucin	EMCN
Q9ULI3	Protein HEG homolog 1	HEG1
Q9ULZ1	Apelin-13	APLN
Q9ULZ9	Matrix metalloproteinase-17	MMP17
Q9UM21	Alpha-1,3-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyltransferase A soluble form	MGAT4A

TABLE 1-continued

Secreted Proteins		
Uniprot ID	Protein Name	Gene Name
Q9UM22	Mammalian ependymin-related protein 1	EPDR1
Q9UM73	ALK tyrosine kinase receptor	ALK
Q9UMD9	97 kDa linear IgA disease antigen	COL17A1
Q9UMX5	Neudesin	NENF
Q9UN73	Protocadherin alpha-6	PCDHA6
Q9UNA0	A disintegrin and metalloproteinase with thrombospondin motifs 5	ADAMTS5
Q9UNI1	Chymotrypsin-like elastase family member 1	CELA1
Q9UNK4	Group IID secretory phospholipase A2	PLA2G2D
Q9UP79	A disintegrin and metalloproteinase with thrombospondin motifs 8	ADAMTS8
Q9UPZ6	Thrombospondin type-1 domain-containing protein 7A	THSD7A
Q9UQ72	Pregnancy-specific beta-1-glycoprotein 11	PSG11
Q9UQ74	Pregnancy-specific beta-1-glycoprotein 8	PSG8
Q9UQC9	Calcium-activated chloride channel regulator 2	CLCA2
Q9UQE7	Structural maintenance of chromosomes protein 3	SMC3
Q9UQP3	Tenascin-N	TNN
Q9Y223	UDP-N-acetylglucosamine 2-epimerase	GNE
Q9Y240	C-type lectin domain family 11 member A	CLEC11A
Q9Y251	Heparanase 8 kDa subunit	HPSE
Q9Y258	C—C motif chemokine 26	CCL26
Q9Y264	Angiopoietin-4	ANGPT4
Q9Y275	Tumor necrosis factor ligand superfamily member 13b, membrane form	TNFSF13B
Q9Y287	BRI2 intracellular domain	ITM2B
Q9Y2E5	Epididymis-specific alpha-mannosidase	MAN2B2
Q9Y334	von Willebrand factor A domain-containing protein 7	VWA7
Q9Y337	Kallikrein-5	KLK5
Q9Y3B3	Transmembrane emp24 domain-containing protein 7	TMED7
Q9Y3E2	BolA-like protein 1	BOLA1
Q9Y426	C2 domain-containing protein 2	C2CD2
Q9Y4K0	Lysyl oxidase homolog 2	LOXL2
Q9Y4X3	C—C motif chemokine 27	CCL27
Q9Y5C1	Angiopoietin-related protein 3	ANGPTL3
Q9Y5I2	Protocadherin alpha-10	PCDHA10
Q9Y5I3	Protocadherin alpha-1	PCDHA1
Q9Y5K2	Kallikrein-4	KLK4
Q9Y5L2	Hypoxia-inducible lipid droplet-associated protein	HILPDA
Q9Y5Q5	Atrial natriuretic peptide-converting enzyme	CORIN
Q9Y5R2	Matrix metalloproteinase-24	MMP24
Q9Y5U5	Tumor necrosis factor receptor superfamily member 18	TNFRSF18
Q9Y5W5	Wnt inhibitory factor 1	WIF1
Q9Y5X9	Endothelial lipase	LIPG
Q9Y625	Secreted glycan-6	GPC6
Q9Y646	Carboxypeptidase Q	CPQ
Q9Y6C2	EMILIN-1	EMILIN1
Q9Y6F9	Protein Wnt-6	WNT6
Q9Y6I9	Testis-expressed sequence 264 protein	TEX264
Q9Y6L7	Toll-like protein 2	TLL2
Q9Y6N3	Calcium-activated chloride channel regulator family member 3	CLCA3P
Q9Y6N6	Laminin subunit gamma-3	LAMC3
Q9Y6R7	IgGFc-binding protein	FCGBP
Q9Y6Y9	Lymphocyte antigen 96	LY96
Q9Y6Z7	Collectin-10	COLEC10

[0197] In some embodiments, the compositions and methods of the invention provide for the delivery of one or more mRNAs encoding one or more additional exemplary proteins listed in Table 2; thus, compositions of the invention may comprise an mRNA encoding a protein listed in Table 2 (or a homolog thereof) along with other components set

out herein, and methods of the invention may comprise preparing and/or administering a composition comprising an mRNA encoding a protein chosen from the proteins listed in Table 2 (or a homolog thereof) along with other components set out herein.

TABLE 2

Additional Exemplary Proteins		
Uniprot ID	Protein Name	Gene Name
A6NGW2	Putative stereocilin-like protein	STRCP1
A6NIE9	Putative serine protease 29	PRSS29P
A6NJ16	Putative V-set and immunoglobulin	IGHV4OR15-8

TABLE 2-continued

Additional Exemplary Proteins		
Uniprot ID	Protein Name	Gene Name
	domain-containing-like protein IGHV4OR15-8	
A6NJS3	Putative V-set and immunoglobulin domain-containing-like protein IGHV1OR21-1	IGHV1OR21-1
A6NMY6	Putative annexin A2-like protein	ANXA2P2
A8MT79	Putative zinc-alpha-2-glycoprotein-like 1	
A8MWS1	Putative killer cell immunoglobulin-like receptor like protein KIR3DP1	KIR3DP1
A8MXU0	Putative beta-defensin 108A	DEFB108P1
C9JUS6	Putative adrenomedullin-5-like protein	ADM5
POC7V7	Putative signal peptidase complex catalytic subunit SEC11B	SEC11B
POC854	Putative cat eye syndrome critical region protein 9	CECR9
Q13046	Putative pregnancy-specific beta-1-glycoprotein 7	PSG7
Q16609	Putative apolipoprotein(a)-like protein 2	LPAL2
Q2TV78	Putative macrophage-stimulating protein MSTP9	MST1P9
Q5JQD4	Putative peptide YY-3	PYY3
Q5R387	Putative inactive group IIC secretory phospholipase A2	PLA2G2C
Q5VSP4	Putative lipocalin 1-like protein 1	LCN1P1
Q5W188	Putative cystatin-9-like protein CST9LP1	CST9LP1
Q6UXR4	Putative serpin A13	SERPINA13P
Q86SH4	Putative testis-specific prion protein	PRNT
Q86YQ2	Putative latherin	LATH
Q8IVG9	Putative humanin peptide	MT-RNR2
Q8NHM4	Putative trypsin-6	TRY6
Q8NHW4	C—C motif chemokine 4-like	CCL4L2
Q9H7L2	Putative killer cell immunoglobulin-like receptor-like protein KIR3DX1	KIR3DX1
Q9NRI6	Putative peptide YY-2	PYY2
Q9UF72	Putative TP73 antisense gene protein 1	TP73-AS1
Q9UKY3	Putative inactive carboxylesterase 4	CES1P1

**[0198]** The Uniprot IDs set forth in Table 1 and Table 2 refer to the human versions the listed proteins and the sequences of each are available from the Uniprot database. Sequences of the listed proteins are also generally available for various animals, including various mammals and animals of veterinary or industrial interest. Accordingly, in some embodiments, compositions and methods of the invention provide for the delivery of one or more mRNAs encoding one or more proteins chosen from mammalian homologs or homologs from an animal of veterinary or industrial interest of the secreted proteins listed in Table 1 or Table 2; thus, compositions of the invention may comprise an mRNA encoding a protein chosen from mammalian homologs or homologs from an animal of veterinary or industrial interest of a protein listed in Table 1 or Table 2 along with other components set out herein, and methods of the invention may comprise preparing and/or administering a composition comprising an mRNA encoding a protein chosen from mammalian homologs or homologs from an animal of veterinary or industrial interest of a protein listed in Table 1 or Table 2 along with other components set out herein. In some embodiments, mammalian homologs are chosen from mouse, rat, hamster, gerbil, horse, pig, cow, llama, alpaca, mink, dog, cat, ferret, sheep, goat, or camel homologs. In some embodiments, the animal of veterinary or industrial interest is chosen from the mammals listed above and/or chicken, duck, turkey, salmon, catfish, or tilapia.

**[0199]** In embodiments, the compositions and methods of the invention provide for the delivery of mRNA encoding a lysosomal protein chosen from Table 3. In some embodiments, the compositions and methods of the invention provide for the delivery of one or more mRNAs encoding one or more lysosomal and/or related proteins listed in Table 3; thus, compositions of the invention may comprise an mRNA encoding a protein listed in Table 3 (or a homolog thereof) along with other components set out herein, and methods of the invention may comprise preparing and/or administering a composition comprising an mRNA encoding a protein chosen from the proteins listed in Table 3 (or a homolog thereof) along with other components set out herein.

TABLE 3

Lysosomal and Related Proteins
$\alpha$ -fucosidase
$\alpha$ -galactosidase
$\alpha$ -glucosidase
$\alpha$ -Iduronidase
$\alpha$ -mannosidase
$\alpha$ -N-acetylgalactosaminidase ( $\alpha$ -galactosidase B)
$\beta$ -galactosidase
$\beta$ -glucuronidase
$\beta$ -hexosaminidase
$\beta$ -mannosidase

TABLE 3-continued

Lysosomal and Related Proteins
3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) lyase
3-methylcrotonyl-CoA carboxylase
3-O-sulfogalactosyl cerebroside sulfatase (arylsulfatase A)
acetyl-CoA transferase
acid alpha-glucosidase
acid ceramidase
acid lipase
acid phosphatase
acid sphingomyelinase
alpha-galactosidase A
arylsulfatase A
beta-galactosidase
beta-glucocerebrosidase
beta-hexosaminidase
biotinidase
cathepsin A
cathepsin K
CLN3
CLN5
CLN6
CLN8
CLN9
cystine transporter (cystinosin)
cytosolic protein beta3A subunit of the adaptor protein-3 complex, AP3
formyl-Glycine generating enzyme (FGE)
galactocerebrosidase
galactose-1-phosphate uridylyltransferase (GALT)
galactose 6-sulfatase
(also known as N-acetylgalactosamine-6-sulfatase)
glucocerebrosidase
glucuronate sulfatase
glucuronidase
glycoprotein cleaving enzymes
glycosaminoglycan cleaving enzymes
glycosylasparaginase (aspartylglucosaminidase)
GM2-AP
Heparan-alpha-glucosaminide N-acetyltransferase (HGSNAT, TMEM76)
Heparan sulfatase
hexosaminidase A lysosomal proteases methylmalonyl-CoA mutase
hyaluronidase
Iduronate sulfatase
LAMP-2
lysosomal $\alpha$ -mannosidase
Lysosomal p40 (C2orf18)
Major facilitator superfamily domain containing 8 protein (MFSD8 or CLN7)
N-acetylgalactosamine 4-sulfatase
N-acetyl glucosamine 6-sulfatase
N-acetyl glucosaminidase

TABLE 3-continued

Lysosomal and Related Proteins
N-acetylglucosamine-1-phosphate transferase
NPC1
NPC2
palmitoyl-protein thioesterase
palmitoyl-protein thioesterase (CLN1)
Saposin A (Sphingolipid activator protein A)
Saposin B (Sphingolipid activator protein B)
Saposin C (Sphingolipid activator protein C)
Saposin D (Sphingolipid activator protein D)
sialic acid transporter (sialin)
sialidase
Sialin
sulfatase
Transmembrane protein 74 (TMEM74)
tripeptidyl-peptidase
tripeptidyl-peptidase I (CLN2)
UDP-N-acetylglucosamine-phosphotransferase

[0200] Information regarding lysosomal proteins is available from Lubke et al., "Proteomics of the Lysosome," *Biochim Biophys Acta.* (2009) 1793: 625-635. In some embodiments, the protein listed in Table 3 and encoded by mRNA in the compositions and methods of the invention is a human protein. Sequences of the listed proteins are also available for various animals, including various mammals and animals of veterinary or industrial interest as described above.

[0201] In some embodiments, the compositions and methods of the invention provide for the delivery of mRNA encoding a therapeutic protein (e.g., cytosolic, transmembrane or secreted) such as those listed in Table 4. In some embodiments, the compositions and methods of the invention provide for the delivery of an mRNA encoding a therapeutic protein useful in treating a disease or disorder (i.e., indication) listed in Table 4; thus, compositions of the invention may comprise an mRNA encoding a therapeutic protein listed or not listed in Table 4 (or a homolog thereof, as discussed below) along with other components set out herein for treating a disease or disorder (i.e., indication) listed in Table 4, and methods of the invention may comprise preparing and/or administering a composition comprising an mRNA encoding a such a protein (or a homolog thereof, as discussed below) along with other components set out herein for treatment of a disease or disorder listed in Table 4.

TABLE 4

Exemplary Indications and Related Proteins	
Indication	Therapeutic Protein
3-Methylcrotonyl-CoA carboxylase deficiency	Methylcrotonyl-CoA carboxylase
3-Methylglutaconic aciduria	Methylglutaconyl-CoA hydratase
Actinic keratoses	
Acute intermittent porphyria	Porphobilinogen deaminase
Acute lymphocytic leukemia	
Acute myeloid leukemia	
Addison's disease	
Adenosine deaminase deficiency	Adenosine deaminase
Adrenoleukodystrophy	ABCD1
Adrenomyeloneuropathy	
AIDS/HIV	
Alcohol use disorders	
Alkaptonuria	Homogentisate 1,2-dioxygenase
Allergic asthma	Anti-IgE mAb
Allergies (dermatitis, rhinitis)	
Alopecia areata	

TABLE 4-continued

Exemplary Indications and Related Proteins	
Indication	Therapeutic Protein
Alpers' disease	POLG
Alpers-Huttenlocher syndrome	
Alpha 1-antitrypsin deficiency	Alpha 1 protease inhibitor
Alpha-mannosidosis	Alpha-D-mannosidase
Alport syndrome	
Alzheimer's disease	
Amyloid light-chain amyloidosis	
Amyotrophic lateral sclerosis (ALS)	
Anemia	Erythropoietin
Aortic valve stenosis	
Argininemia	Arginase
Argininosuccinic acidemia	Argininosuccinate lyase
Arrhythmogenic right ventricular dysplasia	
Autism	
Autosomal dominant and recessive progressive external ophthalmoplegia with mitochondrial DNA deletions	
Autosomal recessive polycystic kidney disease	ARPKD
Bacterial infections	
Basal cell carcinoma	
Batten disease	Battenin + others
B-cell chronic lymphocytic leukemia	
Becker muscular dystrophy	Dystrophin
Beta-thalassemia	Beta globin
Binge eating disorder	
Bipolar disorder	
Bladder cancer	
Blepharospasm, Cervical dystonia, Chronic migraine, more	Botulinum toxin
Bronchiolitis obliterans	
Brugada syndrome	
Buerger's disease	
CACNA1A	
CACNB4-related Episodic Ataxia Type 2	
Cancer and depression	
Cancer and sexual dysfunction	
Cancer in pregnancy	
Carbamylphosphate synthetase deficiency	Carbamylphosphate synthetase
Carcinoma of the gallbladder	
Cardiomyopathy (diabetic)	
Cardiomyopathy (hypertrophic)	
Carnitine uptake defect	SLC22A5
Catecholaminergic polymorphic ventricular tachycardia	
CDKL5-related Atypical Rett Syndrome	
Celiac disease	
Cellulitis	
Cerebrovascular disease	
Cervix uteri cancer	
Chronic fatigue syndrome	
Chronic graft versus host disease	
Chronic idiopathic urticaria	
Chronic immune thrombocytopenia	Thrombopoietin
Chronic kidney disease	
Chronic liver disease	
Chronic lymphocytic leukemia	
Chronic myeloid leukemia	
Chronic pancreatitis	
Cirrhosis of the liver	
Citrullinemia, type I	Argininosuccinate synthase
Classic Rett Syndrome	
Classical galactosemia	Galactose-1-phosphate uridylyltransferase
<i>Clostridium difficile</i> associated diarrhea	
Clotting disorders	
COAD/COPD	
Cocaine addiction	
COL4A5-related disorders	
Cold contact urticaria	
Contraception, female	
Coronary artery diseases	
Corpus uteri cancer	
Corticobasal degeneration	

TABLE 4-continued

Exemplary Indications and Related Proteins	
Indication	Therapeutic Protein
Crigler-Najjar syndrome	UDP-glucuronosyltransferase
Critical limb ischemia	
CTNS-related cystinosis	
Cutaneous lupus erythematosus	
Cutaneous neuroendocrine carcinoma (Merkel Cell)	
Cystic fibrosis	CFTR
Cystic fibrosis	Deoxyribonuclease I
Cystinosis	Cystinosin
Cystinuria	SLC7A9
Dementia (Lewy body)	
Depression	
Diabetic foot infections	
Diabetic foot ulcer	
Diabetic peripheral neuropathy	
Diabetic ulcers	
Diarrhoeal diseases	
Diffuse large B-cell lymphoma	
DiGeorge syndrome	
Diverticulitis	
Drug use disorders	
Duchenne muscular dystrophy	Dystrophin
Dysarthria	
Dyskinesia (levodopa-induced)	
Early-onset autosomal dominant Alzheimer's disease	
Eczema	
Ehlers-Danlos syndrome, type 1	
EIF2B1	
EIF2B2	
EIF2B3	
EIF2B4	
EIF2B5-related childhood ataxia with central nervous system hypomyelination/vanishing white matter	
Eosinophilic esophagitis	
Epilepsy	
Erectile dysfunction	
Erythropoietic protoporphyrin	Ferrochelatase
Esophageal carcinoma	
Essential tremor	
Fabry disease	Alpha galactosidase
Familial adenomatous polyposis	APC
Familial chylomicronemia	Lipoprotein lipase
Familial dysbetalipoproteinemia	Apolipoprotein E
Familial isolated dilated cardiomyopathy	
Familial mediterranean fever	Pyrin (MEFV)
Familial melanoma	
Female infertility	
Female sexual dysfunction	Follicle stimulating hormone
Fibromyalgia	
FMR1-related disorders	
Fracture healing	
Fragile X Premature Ovarian Failure Syndrome	
Fragile X syndrome	FMRP
Fragile X-Associated Tremor/Ataxia Syndrome	
Friedreich's ataxia	
Frontotemporal dementia	
Fryns syndrome	
Galactocerebrosidase deficiencies	
GALE deficiency	Galactose epimerase
GALK deficiency	Galactokinase
GALT-related galactosemia	
Gastric cancer	
Gastrosophageal reflux disease	
Gaucher disease	Glucocerebrosidase
Gilbert syndrome	UDP-glucuronosyltransferase
Glioblastoma multiforme	
Glomerulonephritis	
Glutaric acidemia, type I	Glutaryl-CoA dehydrogenase
GM2 gangliosidosis	HEXA, HEXB
Gout	Urate oxidase
Graft versus host disease	

TABLE 4-continued

Exemplary Indications and Related Proteins	
Indication	Therapeutic Protein
Growth hormone deficiency	Growth hormone 1/Growth hormone 2
Head and neck cancer, Metastatic colorectal cancer	Anti-EGFr mAb
Hearing loss, adult onset	
Heart failure	
Hemachromatosis	HFE protein
Hemifacial spasm	
Hemolytic uremic syndrome	Anti-complement factor C5 mAb
Hemophilia A	Factor VIII
Hemophilia A, Hemophilia B	Factor VII
Hemophilia B	Factor IX
Hepatitis B, Hepatitis C	Interferon alpha
HER2+ breast cancer, gastric cancer	Anti-HER2 mAb
Heredity angioedema	C1 esterase inhibitor
Heredity hemorrhagic telangiectasia	
Heredity hemorrhagic telangiectasia (AT)	
Heredity spherocytosis	
Hidradenitis suppurativa	
Homocystinuria	Cystathionine beta-synthase
Homozygous familial hypercholesterolemia	LDL receptor
Hunter syndrome (MPS II)	Iduronate-2-sulfatase
Huntington disease	Huntingtin
Hurler syndrome (MPS I)	Alpha-L iduronidase
Hydrocephalus	
Hyperalgesia	
Hyperbilirubinemia	
Hyperhidrosis	
Hyperlipidemia	
Hypermethioninemia	Methionine adenosyltransferase
Hyperoxaluria, type I	Serine-pyruvate aminotransferase
Hypertension	
Hyperuricemia	
Hyponatremia	
Hypoparathyroidism	Parathyroid hormone
Hypophosphatasia	TNSALP
Idiopathic pulmonary fibrosis	
Iminoglycinuria	
Immunoglobulin deficiency	Immunoglobulin
Infection (adenovirus)	
Infection (anthrax prophylaxis)	
Infection (BK virus)	
Infection ( <i>Clostridium difficile</i> prophylaxis)	
Infection (Dengue fever prophylaxis)	
Infection (Epstein-Barr virus)	
Infection (Hepatitis-D)	
Infection (Lyme disease prophylaxis)	
Infection (Smallpox virus)	
Infectious diseases vaccines	Infectious antigen
Inflammatory heart diseases	
Insomnia	
Interstitial cystitis	
Iron-deficiency anaemia	
Irritable bowel disease	
Ischaemic heart disease	
Isovaleric aciduria	Isovaleric acid CoA dehydrogenase deficiency
Jansky-Bielschowsky disease	
Juvenile Batten disease	
Juvenile Neuronal Ceroid Lipofuscinosis (JNCL)	
Juvenile rheumatoid arthritis	TNF-alpha inhibitors
Kennedy's disease (SBMA)	
Keratoconus	
Krabbe disease	Galactocerebrosidase
Leber's hereditary optic neuropathy	NADH dehydrogenase
Leiomyosarcoma	
Lennox-Gastaut syndrome	
Lesch-Nyhan syndrome	Hypoxanthine phosphoribosyltransferase 1
Leukaemia	
Li-Fraumeni syndrome	TP53
Lipoma	
Liposarcoma	
Liver cancer	

TABLE 4-continued

Exemplary Indications and Related Proteins	
Indication	Therapeutic Protein
Long-chain 3-OH acyl-CoA dehydrogenase deficiency	Long-chain-3-hydroxyacyl-CoA dehydrogenase
Lower respiratory infections	
Lysosomal acid lipase deficiency	Lysosomal acid lipase
Macular degeneration	
Major depressive disorder	
Malignant fibrous histiocytoma	
Mantle cell lymphoma	
Maple syrup urine disease	3-methyl-2-oxobutanoate dehydrogenase
Marfan syndrome	FBN1
Maroteaux-Lamy syndrome (MPS VI)	N-acetylgalactosamine 4-sulfatase
Mastocytosis	
McArdle disease	Muscle glycogen phosphorylase
MECP2-related disorders	
MECP2-related Severe Neonatal Encephalopathy	
Medium-chain acyl-CoA dehydrogenase deficiency	Acyl-CoA dehydrogenase
Melanoma	Anti-CTLA4 mAb
Metachromatic leukodystrophy	Arylsulfatase A
Metastatic colorectal cancer, NSCLC, others	Anti-VEGF mAb
Methylmalonyl-CoA mutase deficiency	Methylmalonyl-CoA mutase
Migraine	
Mitochondrial oxidative phosphorylation disorders	
Morquio syndrome, type A (MPS IVA)	Galactose 6-sulfate sulfatase
Morquio syndrome, type B (MPS IVB)	Beta-galactosidase
Mouth and oropharynx cancers	
Multiple carboxylase deficiency	Biotin-methylcrotonoyl-CoA-carboxylase ligase
Multiple myeloma	
Multiple sclerosis	Anti-VLA-4 mAb
Multiple sclerosis	Interferon beta
Multiple system atrophy	
Myasthenia gravis	
Myelofibrosis	
Narcolepsy	
Neonatal bronchopulmonary dysplasia	
Neonatal infections	
Nephritis and nephrosis	
Neurofibromatosis, type 1	NF-1
Neuronal ceroid lipofuscinoses-related diseases	
Neutropenia	
Niemann Pick disease, type A/B	G-CSF
Niemann Pick disease, type C	SMPD1
Niemann-Pick disease Type C1	NPC1
Nocturia	
Non-alcoholic fatty liver disease	
Non-Hodgkin lymphoma	Anti-CD20 mAb
Non-small cell lung cancer	
Notch-3 related cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)	
Obesity	
Ophthalmoparesis	
Opioid induced constipation	
Ornithine transcarbamylase deficiency	Ornithine transcarbamylase
Osteoarthritis	
Osteopetrosis	
Osteoporosis	Anti-RANKL mAb
Ovarian cancer	
Paget disease of bone	Sequestosome 1
Pain	
Pancreatic carcinoma	
Panic disorder	
Parkinson disease	
Paroxysmal nocturnal hemoglobinuria	Anti-complement factor C5 Mab
<i>Pediculosis capitis</i> (head lice)	
Pelizaeus-Merzbacher disease	
Pemphigus vulgaris	
Peptic ulcer disease	
Peripheral neuropathy	
Peyronie's disease	
Phenylketonuria	Phenylalanine hydroxylase
Pneumococcal infection prophylaxis	
POLG-related sensory ataxic neuropathy	
Polycystic kidney disease	
Polycystic ovary syndrome	

TABLE 4-continued

Exemplary Indications and Related Proteins	
Indication	Therapeutic Protein
Polycythaemia vera	
Polymerase G-related disorders	
Polymorphous light eruption	
Pompe disease	Alpha glucosidase
Porphyria cutanea tarda	Uroporphyrinogen decarboxylase
Post herpetic neuralgia	
Post-organ transplant	
Pouchitis	
PPM-X Syndrome	
Prader-Willi syndrome	
Preeclampsia	
Premature ejaculation	
Prematurity and low birth weight	
Primary ciliary dyskinesia	
Primary glomerular diseases	
Primary humoral immune deficiencies (e.g., CVID)	Immunoglobulin
Proctitis	
Progressive multifocal leukoencephalopathy	
Progressive supranuclear palsy	
Propionic acidemia	Propionyl-CoA carboxylase
Prostate cancer	
Psoriasis	Anti-IL-12 & IL-23 mAb
Psoriatic arthritis	TNF-alpha inhibitors
PTT-1	
Pulmonary arterial hypertension	
Pulmonary arterial hypertension	
Raynaud's phenomenon	
Refractive errors	
Renal cell carcinoma	
Restless leg syndrome	
Retinitis pigmentosa	
Rheumatic heart disease	
Rheumatoid arthritis	Anti-interleukin-6 (IL-6) mAb
Rheumatoid arthritis	T-cell costimulation blocker
Rheumatoid arthritis	TNF-alpha inhibitor
Romano-Ward syndrome	
Rosacea	
Sanfilippo syndrome, type A (MPS IIIA)	Heparan N-sulfatase
Sanfilippo syndrome, type B (MPS IIIB)	N-acetyl-alpha-D-glucosaminidase
Santavuori-Haltia disease	
Schizophrenia	
Schnitzler syndrome	
Scleroderma	
SCN1A	
SCN1B-related seizure disorders	
Short-chain acyl-CoA dehydrogenase deficiency	Butyryl-CoA dehydrogenase
Sickle cell disease	Hemoglobin
SLC3A1-related disorders	
Small cell lung cancer	
SMN-1-related spinal muscular atrophy (SMA)	
Spinal muscular atrophy	Survival motor neuron protein
Squamous cell carcinoma of head and neck	
Stickler syndrome	
Stomach cancer	
Stroke prophylaxis	
Synovial sarcoma	
Systemic lupus erythematosus	Anti-BAFF
Systemic sclerosis	
Tetrahydrobiopterin-deficient hyperphenylalaninemia	Tetrahydrobiopterin
Thromboangiitis obliterans	
Thrombotic disorders	
Thyroid cancer	
TPP1 deficiencies	
Trachea, bronchus, lung cancers	
Tricuspid atresia	
TSC1	
TSC2-related tuberous sclerosis	

TABLE 4-continued

Exemplary Indications and Related Proteins	
Indication	Therapeutic Protein
Type 2 diabetes mellitus	Glucagon-like peptide 1 (GLP-1) agonist
Type 2 diabetes mellitus	Insulin
Tyrosinemia, type I	Fumarylacetoacetate
Ulcerative colitis	
Uterine fibroids	
Varicose veins	
Venous thromboembolism	
Very long-chain acyl-CoA dehydrogenase deficiency	Long-chain-acyl-CoA dehydrogenase
von Gierke's disease	Glucose-6-phosphatase
Von Hippel-Lindau disease	pVHL
Wegener granulomatosis	
Wilson disease	Wilson disease protein
X-Linked adrenal hypoplasia	
X-linked adrenoleukodystrophy	
X-linked agammaglobulinemia	Bruton's tyrosine kinase

[0202] In some embodiments, the present invention is used to prevent, treat and/or cure a subject affected with a disease or disorder listed or associated with the proteins listed in Tables 1, 2, 3 or 4. In some embodiments, an mRNA encodes one or more of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), argininosuccinate syn-

thetase (ASS1), Factor IX, survival motor neuron 1 (SMN1), or phenylalanine hydroxylase (PAH).

[0203] In some embodiments, an mRNA encoding any one of the proteins listed in Tables 1, 2, 3 or 4 is codon-optimized. In one embodiment, an mRNA encoding CFTR is codon-optimized.

TABLE 5

Human CFTR	
Codon-Optimized Human CFTR mRNA coding sequence	AUGCAACGCUCUCUCUUAGAAAAGGCCUCGGUGGUGCUAACGUUU CUCUCUGGGAUCAGCCAUCCUAGGAAAGGGACAGAGAACUCGCCUC AACCUGUCGAGAAGCUCGAGAGAAUGGGACAGAGAACUCGCCUC AAAGAAAGAACCGGAAGCUGAAUUAUGCGCUUAGCGGGUGCUUUU UGCGGUUCAUGUUCACGGCAUCUCCUACCCUACUCCGGAGAGGU CAAGGCGUGCAGCCCCUGUUGCGUCCGGACGAUUAUGCCUACU ACCCGACAACAAGGAAGAAGAACAGCAUCGCUAACUACUUGGGCA GGCUGUGCCUGCUUUUACUGCGGACCCUCUUGGUUGCAUCUGC UAUUUCGGCGUGCAUCACAUUUGCGAUGAGAAGAAUUGCCAUG UUUUCUCUGAUCAAAAGAAAACUGAAGCUCUCGAGCCGGUGGU UGACAAGAAUUCCAUCGGCAGCUCGUGUCCCUCUCCAAACAAUC UGAACAGUUCGACAGGGGCCUCCUGGCCACUUCUGUGGGGAUC GCCCUUCUGCAAGGGCGCUUCUGAUGGGCCUGACUCCGGAGCUG GCAAGGCCGGCAUUCUGUGGGCUUGGAUUCUGAUCGUGCGGCC UGUCCAGGGCGACUGGGGGCGGAUGAUGAUGAAGUACAGGGACCA GAGAGCGGAAGAGUUUCCGAACCGCUGGUGAUACUUCCGGAUA AUCGAAAACAUCAGCAGUGAAGGCCUACUGCUGGGAGAGGCCAU GGAAAAGAUGAUUGAAAACUCCGGCAAACCGACUGAAGCUGAC GCAAGGCCGGCUUACUGCGCUUUUACACUUCGUGCCUUUUCUUC UCCGGGUUCUUCUGUGGUUUCUCCUGCGCUCCCCUACGCCUUGA UAAGGGAAUCAUCAGCAAGGAAGAUCUUCUACCCACAUUUCUUG UCGUGCCUGCAUGGCCUGACCCGGCAGUUCCAUGGGCGUGCC ACUUGGUACGACUCCUGGGAGCCAUUAACAAGAUCCAGGACUUC UCAAAAGCAGGAGUACAAGACCCUCGAGUACAAACCGACUACUAC AGGUUCGUGAUGGAAACGUCACCGCCGGGGGGGGGGGGGGGG CGAACUGUUCGAGAAGGCCAAGCAGAACACAAACCCGAAGACCU CGAACGGUGACGACUCCCUUUCUUCUACACUUCAGCCUGCGCC ACGCCCGUGCUGAAGGACAUUAACUUCAGACUAGCAGGAGAGG CCUGGCCGGUGCCGGGAUCGACCGGAGCAGAAAGACUUCUGCG UGGUCAUGGGAGAGCUUGAACUAGCAGGGAAAGAUCAAGCA CUCCGGCCGAUCAGCUUCUGUAGCAGUUUUCUGGAUAGGCC GAACCAUUAAGGAAAACAUCAUUUCGGCGUGGUCCUACGAUGA CGCUACGGGUCCGUGAUCAGACUAGCCUGCCAGCUGGAAGAGG AAAGGUUCGCGAGAAGAUACACUUCGUGUGGGCGAAGGGGG ACCUUGUCGGGGGGCCAGCGGGCUAGAAUCUCGCGUGGCCAG GUUAAGGACCCGACUGUAUCUCCUGGACUCCCCCUUCGGAUACC UGGACGUCCUGACCGAAAAGGAGAACUUCGGAUCUGCUGCGUG GCUGAUGGCUAACAGACUCCUGUGACCUCCAAAUGGAGC ACCUUGAAGGAGACAAGACUUCUGAUUUCUGCAUGAGGGGUCC UACUUUACGGCACCUUCUGGAGUUGCAGAACUUCGAGCCGAC CUCAUCGAGCUGAUGGGUUGCGACAGCUUCGACAGCAGUUC CCCG

TABLE 5-continued

TABLE 5-continued

Human CFTR

TABLE 5-continued

TABLE 5-continued

Human CFTR	
	AGGACUCCCACUCGUUCACAGCUUAUCACUGUCUCCAAGAAUUCUCC ACCAUAGAUGCUCAUAGCGUACUGCAGGCCAUUCGUCCACCUUG AAUACGCUCAAGGGGGAGGUUUUAAAUCGCUUCUCAAAGAUA UUGCAAUUUUUGGAUGACCUUCUGCCCCUGACGAUCUUCGACUUCAUC CAGUUGUJCGUGCUGAUJUUGGGCUAUUGCAGUAGUCGCUGUCC UCCAGCCUUACAUUUUUGCGCACGGGUCCGGUGAUCGUGGCCUUU AUCAUUGCUGCGGGCCUAUUUCUUGCAGACGUACAGCAGCUUAAGCA ACUGGAGCUGAAGGGAGGUCCUAUCUUACGCAUCUUGUGACCA GUUUGAAGGGAUUGUGGACGUUJUGCGCCUJUUGGCAGGCAGCCUAC UUUGAACACUGUUCACAAAGCUGAAUCUCCAUACGGCAAAUUG GUUUGUUAUUUUGAGUACCCUCCGAUGGUUUUAGAUGCGCAUUGAG AUGAUUUUUGUGAUCUUCUUUAUCGCGUGACUUUAUCUCCAUU UGACCACGGGAGAGGGCAGGGACGGGUCCAUUAUCUGACACUC GCCAUGAACUUAUGAGCACUUUGCAGUGGGCAGUGAACAGCUCGA UUGAUGGGAUGCCUGAUGAGGUCCUUUCGAGGGCUCUUUAGU CAUCAGACAUGCCAGGGAAAAGCCACAAAAAGUACGAAACCCU AUAAGAAUGGGCAAUUAGAGUAAGGUAAUGAUCAUCGAGAACAGU CGUGAAGAAGGAUGACAUCUGGCCUAGCGGGGUCCAGAUGACCG AAGGACCUGACGGCAAAAUACACCGAGGGAGGGAACGCAAUCCUUGA AAACACUUCGUUCAGCAUUAAGCCCGGUCAAGCUGUGGGGUUGCUCG GGAGGACCGGGUCAAGGAAAUCGACGUUGCUGUCGGCCUUCUUGAG ACUUCUGAAUACAGGGGAGUAGUCCAGAUCGACGGGUUUCGUGG GAUAGCAUCACCUUGCAGCAGGGCGAAAGCGUUUUGGAGUAAUCC CCAAAAGGUUUUUAUUCUAGCGGAACCUCCGAAAGAAUCUGCAUC CUUAGAACAGGGUGCAGAUCAAGAGAUUUGGAAAGUCGGGACGA GGUUGGCUUCGGAGUGUAAUCGAGCAGGUUUCGGAAAACUCGAC UUUGUCCUUGUGAUGAGGGGAUGCGUCCUGUGCGAUGGGCACAAGC AGCUCAUGUGCCUGGCCGAUCCGUCCUCUAAAAGCGAAAUUUCU CUCUUGGAUGAACCUUCGGCCCAUCUGGACCCGUAAUCAGAU CAUCAGAGGACACUUAAGCAGCGUUUGCCGACUGCAGGUUUC UCUGUGAGCAUCGUAAUCGAGGCCAUGCUCGAAUGCCAGCAUUU GUCAUCGAAGAGAAUAGGUCCGCCAGUACGACUCCAUCCAGAAGC GUUUAUGAGAGAAUUGUUCGGCAGGGGAUUUCACCAUCCGAA GGGUGAAACUUUUUCCACACAGAAAUCGUCAAGUGCAAGUCAA ACCGCAGAUCGCGGCCUUGAAAAGAGACUGAAGAAGAAGUCAA GACACGCGUCUUUA (SEQ ID NO: 3)
Human CFTR Protein Sequence	MQRSPLEKASVVKSLFFSWTRPILRKGYRQRLELSDIYQIPSVDSADNLSEK LEREWDRRELASKNPKLINALRRCFFWRPMFYGIFLYLGEVTKAQPLL GRIIASYDPDNKEERSIAIYLGIGLCLLFIVRTLLLHPAIFGLHHIGMQRIA MFSLIYKTKTLKLSRVLDDKISIGQLVSSLNNLNKFDEGLALAHFVWIAPLQ VALLMGLIWELLQASAFCGLGFLIVLALPQAGLGRIVIMMKYRDQRACKIS ERLVITSEMIENIQSVKAYCWEEMEKMIEENLRQTELKLTRKAAYVRYFN SSAFFFGFVVFLSVPYALIKGILRKIFTISFCIVLRAVTRQFPWAVQT WYDSLGAINKIQDFLQKQEYKTLEYNLTTEVVMENVTAFWEEGFELFE KAKQNRRNRTSNGDLSFSNFSLLGTPVLDKINFKIERGQLLAVAGSTG AGKTSLLMVIVIGELEPSEGKIKHSGRISFCQSFWIMP GTIKE NI IFGVSYDE YRYRSVIKACQLEEDISKFAEKDNIVLGEGGITLSGGQRARISLARAVYKD ADLYLLDPSFGYLDVLTEKEIFESCVKLMANKTRILVTSKMEHLKKADKI LILHEGSSYFYGTFSELQNLQPDFSSKLMGCDSFQFSAERRNSILTETLHR FSLEGDAPVSWTETKKQSFQKTGEFGEKRKNSILNPINSIRKFSIVQKTPQ MNGIEEDSDEPLERRLSLVPDSEQGEAILPRISVISTGPTLQARRQSVLN MTHSVNQGQNIHRKTTA STRKVSLAPQANLT EIDYSRRLSQETGLEISEEI NEEDLKEPQFDDMESIPAVTTWNTYLRYITVHKSLIFVLIWCLVIFLAEVAA SLVVLWLGNTPQDKGNSTHSRNNSYAVITSTSSYYVFYIYVGVADTLL AMGFFRGLPLVHTLITVSKILHHKMLHSVLQAPMSTLNTLKAGGI LNRFSK DIAILDLLPLTIFDPIQLLLIVIGAIAVVAVLPYIFVATVPVIVAFIMLRAY FLQTSQQLQKLESEGRSPPIFTHLVTSLKGLWTLRAFGRQPYFETLFHKALN LHTANWFLYLTSLRWFQMRRIEMIFVIFIATFISI LTTGEGEGRVGIILTLA MNEVISTLQWAVNSSIDVDSLIVIRVSRSRVEKFIDMPTEGKPTKSTKPYKNGQ LSKVMI IENSHVKDDIWPSSQQMTVKDLTAKYTECGNAILENISFSISPGQ RVGLLGRGTGSGKSTLLSAFLRLLNTEGEI QIDGVWSWD SITLQQWRKAFGVIP QKVFIFSGTFRKNLDPEQWSDQEIWKVADEVGLRSVIEQFPGKLD FVVLVD GGCVLSHGHKQLMCLARSVLSKAKILLDEPSAHDLPVTQIIRRTLQKAF ADCTVILCEHRIEAMLECQQFLVIEENKVRQYDSIQKLLNERSLFRQAI DRVKLFFHRNSSKCKSKPQIAALKETEEEVQDTRL (SEQ ID NO: 4)

Exemplary Codon-Optimized Human Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) mRNAs

Construct design:

[0204] X—Coding Sequence—Y

5' and 3' UTR Sequences:

X (5' UTR Sequence) =  
(SEQ ID NO: 5)

GGACAGAUCGCCUGGAGACGCCAUCCACGCUGUUUUGACCUCAUAGAA  
GACACCGGGACCGAUCAGCCUCCGGCCGGAACGGUGCAUUGGAAC  
GCGGAUCCCCGUGCCAAGAGUGACUCACCGUCCUUGACAGC

Y (3' UTR Sequence) =  
(SEQ ID NO: 6)

CGGGUGGCAUCCCUGUGACCCCUCCCCAGUGCCUCUCCUGGCCUGG  
AAGUUGCACUCCAGUGCCCACCAAGCCUUGUCCUAUAAAUAAGUUG  
CAUCAAGCU  
OR

(SEQ ID NO: 7)

GGGUGGCAUCCCUGUGACCCCUCCCCAGUGCCUCUCCUGGCCUGGAAG  
UUGCCACUCCAGUGCCCACCAAGCCUUGUCCUAUAAAUAAGUUGCAU  
CAAAGCU

[0205] In one embodiment, a codon-optimized human CFTR mRNA sequence includes SEQ ID NO: 1.

[0206] In one embodiment, a full-length codon-optimized human CFTR mRNA sequence is:

(SEQ ID NO: 8)

GGACAGAUCGCCUGGAGACGCCAUCCACGCUGUUUUGACCUCAUAGAA  
GACACCGGGACCGAUCAGCCUCCGGCCGGAACGGUGCAUUGGAAC  
GCGGAUCCCCGUGCCAAGAGUGACUCACCGUCCUUGACACGAUGCAAC  
GCUCUCCUCUUGAAAAGGCCUCGGUGGUGUCCAAGCUCUUCUUCUGUG  
GACUAGACCCAUCUGAGAAAAGGGGUACAGACAGCGCUUUGGAGCUGUCC  
GAUAUCUAUCAAAUCCCUUCCUGGGACUCCCGGGACAACCUGUCCGAGA  
AGCUCGAGAGAGAAUGGGACAGAGAACUCGCCUCAAAAAGAAGAACCGAA  
GCUGAUAAAUGCGCUUAGCGGUGCUUUUUCUGCGGUUCAUGUUCAC  
GGCAUCUUCUCUACUCCUGGGAGAGGUACCAAGGCCGUGCAGCCCGU  
UGCGUGGGACGGAAUUAUGCCUCCUACGACCCGACAACAAGGAAGAAAG  
AAGCAUCGCUAUCUACUUGGGCAUCGGUCUGGCCUGCUUUUCAUCGUC  
CGGACCCUCUUGUUGCAUCCUGCUAUUUUCGCCUGCAUCACAUUGCA  
UGCAGAUGAGAAAUGCAGUUGUCCUGAUCUACAAGAAAACUCUGAA  
GCUCUCGAGCCCGUGCUUGACAAGAUUCCAUCGCCAGCUCGUGUCC  
CUGCUCUCCAACAUCAUGAACAGUUCGACGAGGGCCUGCCUGGCC  
ACUUCGUGUGGAUCGCCUCUGCAAGUGGCCUUCUGAUGGCCUGAU  
CUGGGAGCUGCUGCAAGCCUCGGCAUUCUGUGGGCUUGGAUUCUGAUC  
GUGCUGGCACUGUUCAGGCCAGUGGGCGGAUGAUGAAGUAC  
GGGACCAAGAGGCCGAAAGAUUCCGAACGGCUGGUGAUCACUUCGGA

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AAUGAUCGAAAACAUCAGUCAGUGAAGGCCUACUGCUGGGAGAGGCC  
AUGGAAAAGAUGAUUGAAAACCUCGGCAAACCGAGCUGAAGCUGACCC  
GCAAGGCCGCUACGUGCGCUUUUCAACUCGUCCGCUUUUCUUCUC  
CGGGUUUCUGUGGUUUUCUCCUGCUCCCUACGCCUGAUUAAG  
GGAAUCAUCCUCAGGAAGAUCUUCACCAUUCUUCUGUAUCGUGC  
UCCGCAUGGCCGUGACCCGGCAGUUCCAAGGGCCUGCAGACUUGGUA  
CGACUCCUUGGGAGCCAUAAGAUCAGGACUUCUCAAAAGCAG  
GAGUACAAGACCCUCGAGUACAACCUGACUACUACCGAGGUGCUGAUGG  
AAAACGUCACGCCUUUJGGAGGAGGGAUUUGGCGAACGUUCGAGAA  
GGCCAAGCAGAACAAACAACACCGAACACCUCGAAACGGUGACGAUCC  
CUCUUCUUUCAACUUCAGCCUGCCGGACGCCUGCUGAAGGACA  
UUAACUUCAGAUCGAAAGAGGACAGCUCCUGCGGUGGCCGAUCGAC  
CGGAGCCGAAAGACUUCUCCUGCUGAUGGUGAUCAUGGGAGAGCUUGAA  
CCUAGCGAGGGAAAGAUCAAGCACUCCGGCCCAUCAGCUUCUGUAGCC  
AGUUUUCCUGGAUCAUGCCGGAACCAUAAGAAAACAUCUUCUCCGG  
CGUGUCCUACGAUGAAUACCGCUACCGGUCCUGAUCAAAGCCUGCCAG  
CUGGAAGAGGAAUUAAGUUCGCGGAGAAAGAUAAACAUUCGUGCUGG  
GCGAAGGGGUAAUACCUCUGCGGGGCCAGCGGGCAUGAAUCUGCU  
GGCCAGAGCCGUGUAUAGGACGCCGACCUGUAUCUCCUGGACUCCCC  
UUCGGAUACCUGGACGUCCUGACCGAAAAGGAGAUUCUUCGAAUCUGCG  
UGUGCAAGCUGAUGGCUAACAGACUCGCAUCCUCUGGACCUCCAAA  
GGAGCACCUAGAAGCCAGACAAGAUUCUGAUUCUCCUAGAGGGGUCC  
UCCUACUUUACGGCACCUUCUGGAGUGCAGAACUUCGAGCCGACU  
UCUCAUCGAAGCUGAUGGGUUGCGACAGCUUCGACCAGUUCUCCGCGA  
AAGAAGAACUCGAUCCUGACCGAAAACCUUCGACCCGACUUCUUGGAA  
GGCGACCCCCUGUGUCAUGGACCGAGACUAAGAAGCAGAGCUUCAAGC  
AGACCGGGAAUUCGGGAAAGAGGAAGAACAGCAUCUUGAACCCAU  
UAACUCCAUCCGCAAGUUCUAAUCGUGCAAAGACGCCACUGCAGAUG  
AACGGCAUUGAGGAGGACUCCGACGAAACCCUUGAGAGGGCGCUGUCC  
UGGUGCCGGACAGCGACAGGGAGAAGCCAUCCUGGUCCGGAUUUCGU  
GAUCUCCACUGGUCCGACGCCUAGCCGGCGCAGGUCCGUGCUG  
AACCGUGACCCACAGCGUGAACCGAGGGCAAAACAUUCACCGCAAGA  
CUACCGCAUCCACCCGAAAGGUCCUGGCCUCAAGCGAAUCUUC  
CGAGCUCGACAUCUACUCCCGAGACUGUCGACGAAACCGGGCUCGAA  
AUUUCGAGAAAUAUCAAGAGGAGGAUCUGAAAGAGUGCUUCUUCGACG  
AUAUGGAGUGCAUCCCGCCUGACGACUUGGAACACUUAUCUGCGUA  
CAUCACUGUGCAAGUCAUUGAUUCUGUGCCUGAUUUGGUGCCUGGUG  
AUUUUCUGGCCAGGUUCGCGGCCUACUGGUGGUGGUCCUGGUUGG  
GAAACACGCCUCUGCAAGACAAGGGAAACUCCACGCACUCGAGAAACAA

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CAGCUAUGCCGUGAUUAUCACUUCACCUCUCCUUAAUACGUGUUUCUAC  
 AUCUACGUCGGAGGGCGGAUACCCUGCUCGCAGAUGGGUUUCUUCAGAG  
 GACUGCCGUGGUCCACACCUUGAUCACCGUCAGCAAGAUUCUUCACCA  
 CAAGAUGUUGCAUAGCUGUGCAGGCCCCAUGUCCACCCUCAACACU  
 CUGAAGGCCGGAGGCAUUCUGAACAGAUUCUCCAAGGACAUCGCUAUCC  
 UGGACGAUCUCCUGCCGCUUACCAUCUUGACUUCAUCCAGCUGCUGCU  
 GAUCGUGAUUGGAGCAAUCGAGUGGGGGCGUGCUGCAGCCUUACAUU  
 UUCGUGGCCACUGUGCCGUCAUUGUGGCGUCAUCAUGCUGCGGGCCU  
 ACUUCUCCAAACCAGCAGCAGCUGAAGCACUGGAAUCGGAGGGACG  
 AUCCCCCAUCUUCACUCACCUUGUGACGUCGUUGAAGGGACUGUGGAC  
 CUCCGGGCUUUCGGACGGCAGCCUACUUCGAAACCCUUCACCAAGG  
 CCCUGAACCUCCACACGCCAAUUGGUUCCUGUACCUUGGUACCCUGCG  
 GUGGUCCAGAUGCAGCAUCGAGAUGAUUUUCGUCAUCUUCUCAUCGCG  
 GUCAUUCUCAUCAGCAUCCUGACUACGGAGGGAGGGACGGCG  
 GAAUAAAUCUGACCCUCGCCAUGAACAUUAUGAGCACCCUGCAGUGGGC  
 AGUGAACAGCUCGAUCGACGUGGACAGCCUGAUGCGAAGCGUCAGCCGC  
 GUGUCAAGUUCAUCGACAUGCCUACUGAGGGAAACCCACAUAGUCCA  
 CUAAGCCCACAAAAAUGGCCAGCUGAGCAAGGUCAUGAUCAUCGAAAA  
 CUCCCACGUGAAGAGGACGAUAAAUGGCCUCCGGAGGUCAAAUAGACC  
 GUGAAGGACCUGACCGCAAAGUACACCGAGGGAGGAACGCCAUUCUCG  
 AAAACAUCAUCUUCUCCAUUUCGCCGGACAGCGGGUCCGGCUUCUCCG  
 GCGGACCGGUUCCGGAAAGUCAACUCUGCUCUGCGUUUCCUCCGGCUG  
 CUGAAUACCGAGGGGAAACCAAAUUGACGGCGUGCUUUGGGAUUCC  
 UUACUCUGCAGCAGUGCGGAAGGCCUUCGGCGUGAUCCCCAGAGGU  
 GUUCAUCUUCUGGGUACCUUCCGGAAGAACCUUGGAUCCUACGAGCAG  
 UGGAGCGACCAAGAAACUGGAAGGUCGCCACGAGGUCGGCUCGCGCU  
 CCGUGAUUGAACAUUUCCUGGAAAGCUGGACUUCUGCUGCUGCGACGG  
 GGGAUUGUGGUCCUGCGCACGGACAUAGCAGCUAUGUGCCUCGACGG  
 UCCGUGCUCUCCAAGGCCAAGAACUUCUGCUGCUGGACGAACCUUCGG  
 ACCUGGAUCCGGUCACCUUCCAGAUCAUCAGGAGGACCCUGAAGCAGGC  
 CUUUGCCGAUUGCACCGUGAUUCUCUGCGAGCACCGCAUCGAGGCCAUG  
 CUGGAGGCCAGCAGUCCUGGUCAUCGAGGAGAACAGGUCCGCAAG  
 ACGACUCCAUUAAAAGCUCCUCAACGAGCGGUCGUGUUCAGACAAGC  
 UAUUUCACCGUCCGAUAGAGUGAACGCUUCCCGCAUCGGAACAGCUA  
 AAUGGCAAAUCGAAGGCCAGAUCGCGCCUUGAAGGAAGAGACUGAGG  
 AAGAGGUGCAGGACACCCGGUUUACGGGUGGCAUCCCUGUGACCCU  
 CCCAGUGCCUCUCCUGGCCUUGGAAGUUGCCACUCCAGUGGCCACCG  
 CCUUGGUCAUAAAAGUUGCAUCAAGCU

[0207] In one embodiment, another full-length codon-optimized human CFTR mRNA sequence is:

(SEQ ID NO: 9)  
 GGACAGAUCGCCUGGAGACGCCAUCCACGCUGUUUGACCUCCAUAGAA  
 GACACGGGGACCGAUCAGCCUCCGCGCCGGAACGGUGCAUUGGAAC  
 GCGGAUUCCCCGUGCCAAGAGUGACUACCGUCCUUGACACGAUGCAAC  
 GCUCUCUCCUUGAAAAGGCCUGGGUGGUCCAAAGCUCUUCUUCUGUG  
 GACUAGACCAUCCUGAGAAAGGGGUACAGACAGCGCUUGGAGCUGUCC  
 GAUAUCUAAUCCCUUCUGUGGACUCCCGGACAACCCUGUCCGAGA  
 AGCUCGAGAGAGAAUGGGACAGAGAACUGCCUAAAGAAGAACCCGAA  
 GCUGAUAAAUGCGCUUAGCGGUGGUUUUUCUGGCGGUCAUGUUCUAC  
 GGCAUCUUCUCCUACCUCCUGGGAGAGGUACCAAGGCCGUGCAGCCCUGU  
 UGCUGGGACGGAUUAUJGCCUUCACGACCCGACAACAAGGAAGAACAG  
 AAGCAUCGCUAUCUACUUGGGCAUCGGCUGUGCCUGCUUUUCAUCGUC  
 CGGACCCUCUUGUUGCAUCCUGCUUUCGGCCUGCAUCACAUUGGCA  
 UGCAGAUGAGAAUUGCCAUGUUUUCUGAUCAUACAAGAAAACUCUGAA  
 GCUCUCGAGCCGCGUGGUACAGAGAACGGCCAGCUCGUGUCC  
 CUGCUCUCCAAACAUUGCAACAGUUCAGCAGGGCUCGCCUUGGCC  
 ACUUCGUGGGAUCGCCUUCUGCAAGUGGCGUUCUGAUGGGCCUGAU  
 CUGGGAGCUGCGAACCCUCGGCAUUCUGUGGGCUUGGAUUCUGAU  
 GUGCUGGACUGUUCCAGGGCGACUGGGGGCGAUGAUGAUGAAGUACA  
 GGGACCAAGAGGCCGAAAGAUUCCGACAGCUCGGCUGGUACUUCGGA  
 AAUGAUCGAAAACAUCCAGCAGUGAAGGCCUACUGCUGGGAAAGAGGC  
 AUGGAAAAGAUGAUUGAAAACCUCCGCAAACCGAGCUGAAGCUGACCC  
 GCAAGGCCGCUACGUGCGCUUUUCAACUCGUCCGCUUUCUUCUUC  
 CGGUUCUUCUGGGGUUUCUCUGGUCCUACGCCUGAUUAAG  
 GGAAUCAUCCUCAAGGAAGAUUCUACCCACCAUUCUUCUGAUUCGUC  
 UCCGCAUGGCCUGACCCGGCAGUUCCAUGGGCGUGCAGACUUGGUA  
 CGACUCCUCCUGGGAGCCAUAACAGAACCCAGGACUUCUCCUCAAAAGCAG  
 GAGUACAAAGACCCUCUGAGUACACCCUGACUACCCAGGGUGCUGAUGG  
 AAAACGUACCGCCUUUJUGGGAGGAGGAUUUGGCGAACUGUUCGAGAA  
 GGCCAAGCAGAACACAAACCGCAAGACCUUCGAAACGGUGACGACUCC  
 CUCUUCUUUCAAACUUCAGCCUGCCUCCGUGCAGGCCUGCAGAAGGACA  
 UUAACUCAAGAUCGAAAGAGGACAGCUCUCCUGCGGUGGCGGAUCGAC  
 CGGAGCCGGAAAGACUUCCCUGCGUGAUGGUGAUCAUGGGAGAGCUUGAA  
 CCUAGCGAGGGAAAGAUCAAGCACUCCGGCGCAUCAGCUUCUGUAGCC  
 AGUUUUCUGGAUCAUGCCCGAACCAUAAGGAAAACAUCAUUCUUCGG  
 CGUGUCCUACGAUGAAUACCGCUACCCGGCGUGAUAAGAACAUUCGUG  
 CUGGAAGAGGAUAAAAGUUCGCGGAGAAAGAUAAACAUCGUGCUG  
 GCGAAGGGGUAAUACCUUUGCGGGGGCCAGCGGGCUAGAAUCUCGCU

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GGCCAGAGCCGUGUAUAAGGACGCCGACCUGUAUCUCCUGGACUCCCC  
 UUCGGAUACCUGGACGUCCUGACCGAAAAGGAGAUCUUCGAAUCGUGCG  
 UGUGCAAGCUGAUGGCUAACAAAGACUCGCAUCCUCGUGACCUCCAAA  
 GGAGCACCGUGAAGAAGGGAGACAAGAAUCUGAUUCUGCAUGAGGGGUCC  
 UCCUACUUUUACGGCACCUUCUCGGAGUUGCAGAACUUGCAGCCCCACU  
 UCUCAUCGAAGCUGAUGGGUUGCGAGACGUUCGACAGGUUCCGCCGA  
 AAGAAGGAACUCGAUCCUGACGGAAACCUUGCACCGCUUCUUGGAA  
 GGCAGCAGCCCCUGUGUCAUGGACCGAGACUAAGAACAGAGCUCAAGC  
 AGACCGGGAAUUCGGGAAAGAGGAAGAACAGCAUCUAGAACCCAU  
 UAACUCCAUCCCAAGUUCUCAUCUGCAGAAAGACGCCACUGCAGAUG  
 AACGGCAUUGAGGAGGACUCCGACGAACCCCUUGAGAGGGGCCUGUCC  
 UGGUGCCGGACAGCAGGGAGAACCCAUCUCCUGCCUCCGGAUUUCGU  
 GAUCUCCACUGGUCCGACGCUCCAAGCCGGGGCGAGUCCGUGCUG  
 AACCGUGAUGACCCACAGCGUGAACCGAGGGCAAAACAUUACCGCAAGA  
 CUACCGCAUCCACCCGGAAAGGUUCCUGGCCACCUAGCGAAUUCUAC  
 CGAGCUCGACAUCUACUCCCGAGACUGUCGCAGGAACCGGGCUCGAA  
 AUUUCGAAAGAAUACACGAGGAGGAUCUGAAAGAGUGCUUUCUGACG  
 AU AUGGAGUCGAUACCGCCGUGACGACUUGGAACACUUAUCUGCGUA  
 CAUCACUGUGACAAGUCAUUGAUUUCUGCUGGUUUGGUGCCUGGUG  
 AUUUCUCCUGCCGAGGUUCGCGGCCUACUGGGUGUCCUGGUUGG  
 GAAACACGCCUCUGCAAGACAAGGGAAACUCCACGCACUCGAGAAACAA  
 CACCUAUCCGUGAUUAUCACUUCACCUCUCCUUAUACGUGUUCUAC  
 AUCUACGUCGGAGUGGCGGAUACCUUCGUCGCGAUGGGGUUUCUACAG  
 GACUGCCGUGGUCCACACCUUGAUACCGUCAGCAAGAUUUCUACCA  
 CAAGAUGUUGCAUAGCGUGCGUGCCAGGGCCCAUGGUCCACCCUCAAC  
 CUGAAGGCCGGAGGCAUUCUGAACAGAUUCUCCAAGGACAUCCGUAUCC  
 UGGACGAUCUCCUGCCGCUUACCAUCUUCUGACUCAUCCAGCUGCUGCU  
 GAUCGUGAUUGGAGCAAUCGCAUGGGUGGCGUGCUGCAGCCUUA  
 UUCGUGGCCACUGUGCCGUCAUUGUGGCGUUCAUCAUCGUGCGGGCC  
 ACUUCUCCAAACCCAGCAGCUGAAGAACUGGAUCCGAGGGACG  
 AUCCCCCAUCUUCACUCACCUUUGUGACGUCGUUGAAGGGACUGGGAC  
 CUCCGGGCUUUCGGACGGCAGCCUACUUCGAAACCCUCUUCACAGG  
 CCCUGAACCUCCACACGCCAAUUGGUUCUGUACCGUCCACCCUGCG  
 GUGGUUCCAGAUGCGCAUCGAGAUGAUUUUCGUCAUCUUCUACGCG  
 GUCACAUUCAUCAGCAUCCUGACUACCGGAGAGGGAGAGGGACGGG  
 GAAUAAUCCUGACCCUCGCCAUGAACAUUAUGAGCACCCUGCAGUGGG  
 AGUGAACAGCUCGAUCGACGUGGACAGCCUGAUGCGAAGCGUCAUC  
 GUGUUCAGUUCUACGACAUGCCUACUGAGGGAAAACCCACUAAGUCCA  
 CUAAGCCUACAAAAAUGGCCAGCUGAGCAAGGUCAUGAUCAUCGAAA

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CUCCCACGUGAAGAAGGACGAUAAAUGGCCUCCGGAGGUAAAUGACCC  
 GUGAAGGACCGUGACCGCAAAGUACACCGAGGGAGGAACGCCAUUCUG  
 AAAACAUACAGCUUCUCCAUUUCGCCGGACAGCGGGUCGGCCUUCUG  
 GCGGACCGGUUCCGGGAAAGUCAACUCUGCUGCGCUUUCUCCGGCUG  
 CUGAAUACCGAGGGGGAAUCCAAUUGACGGCGUGUUCUGGGAUUCCA  
 UUACUCUGCAGCAGGGCGGAAGGCCUUCGGCGUGAUCCCCCAGAAGGU  
 GUCAUCUUCUGGGUACCUUCGGGAAAGAACUGGUACCUUACGAGCAG  
 UGGAGCGACCAAGAAAUCUGGAAGGUUCGCCACGAGGUCCUGCGCU  
 CCGUGAUUGAACAAUUCUGGGAAAGCUGGUUCUGCUGCGACGG  
 GGGAUUGGUCCUGUCGACGGACAUAGCAGCUCAUGUGGUCCUGCAGGG  
 UCCGUGCUCUCCAAGGCCAGAACUUCUGCUGCUGGUAGAACUUCGG  
 ACCUGGAUCGGUCCACCUACCAGAACUACAGGAGGACCCUGAAGCAGGC  
 CUUUGCCGAUUGCACCGUGAUUCUCCUGCGAGCACCGCAUCGAGGG  
 CUGGAGUGCCAGCAGUUCUGGUCAUCGAGGAGAACAAAGGUCCGAAU  
 ACGACUCCAUUCAAAAGCUCCUACCGAGCGGUCGUUCAGACAGC  
 UAUUUCACCGUCCGAUAGAGUGAAGCUCUUCGGCAUCGGAACAGCU  
 AAGUGCAAACUGAAGCCGAGACUGCGACGCCUUGAAGGAAGAGACUGAG  
 AAGAGGUGCAGGACACCCGGUUAAGGGUGGCAUCCUGUGACCCUC  
 CCCAGUGCCUCUCCUGGCCUGGAAGUUGGCCACUCCAGUGGCCAC  
 CUUGGUCCUAUAAAAGUUGCAUCAAAGCU

[0208] In one embodiment, a codon-optimized human CFTR mRNA sequence includes SEQ ID NO: 2.

[0209] In one embodiment, a full-length codon-optimized human CFTR mRNA sequence is:

(SEQ ID NO: 10)  
 GGACAGAUCCGUGGAGACGCCAUCCACGCUGUUUUGACCUCAUAGAA  
 GACACGGGACCGAUCCAGCCUCCGGGGGGACGGUGCAUUGGAAC  
 GCGGAUUCCCCGUGCCAAGAGUGACUACCGGUCCUUGACAGCAUGCAGC  
 GGUCCCCGUCGAAAAGCCAGUGUGGUCCAAACUCUUCUUCUCAUG  
 GACUCGCCUACCUUAGAAAGGGGUACGGCAGGGGUAGUUGU  
 GACAUUACAGAACUCCUCGGUAGAUUCGGGGAUACCUUCUGGAGA  
 AGCUCGAACGGAAUUGGGACCGCGAACUCGCGUCAAGAAAACCGAA  
 GCUCAUACGCACUGAGAAGGUGCUUCUUCUGGCGGUUCAUGUUC  
 GGUACUUCUUCGGUACUCCUGGGAGGUACAAAAGCAGUCCACCC  
 UGUUGGUUGCAUUAUCGCCUCGUACGACCCGAUAACAAAGAAGAAC  
 GAGCAUCGCGAUCUACCUCCGGGAUCGGACUGGUUUGGUUCAUC  
 AGAACACUUUUGUUGCAUCCAGCAACUUCGCCUCCAUACACGG  
 UGCAGAUCGCGAAUCGCUAUGGUUAGCUUGAUCUACAAAAGACAC  
 ACUCUCUGCGGGGUUGGUAGAUAGAUUCCAUCGGUAGUUGGUU  
 CUAAGCCUACAAAAAUGGCCAGCUGAGCAAGGUCAUGAUCAUCGAAA

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CUGCUUAGUAAAACCUCACAAAAUUCGAUGAGGGACUGGCGCUGGCAC  
AUUUCGUGUGGAUUGCCCCGUUGCAAGUCGCCUUUUGUGGCCUGGGAUUUCGUAU  
UUGGGAGCUGUGCCAGGCAUCGCCUUUUGUGGCCUGGGAUUUCGUAU  
GUGUUGGCAUUGUUUCAGGCUGGGCUUGGGCGGAUGAUGAAGUAUC  
GCGACCAGAGAGCGGGUAAAUCUCGGAAAGACUCGUCAUCUUCGGA  
AAUGAUCGAAAACAUCAGCUGGUAAAGCCUAAUUGCUGGGAAAGAACGU  
AUGGAGAAGAUGAUUGAAAACCUCCGCAAACUGAGCUGAACUGACCC  
GCAAGGCGCGUAGUCGGUAAUUCGCUAAUCGUAGCGUUCUUCUUUUC  
CGGGUUCUUCGUUGUCUUUCUCGGUUUUGCCUUAUGCCUUGAUUAAG  
GGGAUUAUCCUCCGCAAGAUUUUACACACGAAUUCGUUCUGCAUUGUA  
UGCGCAUUGCAGUGACACGGCAUUUCGGUGGGCCUGCGAGACAGUGGA  
UGACUCGCUUGGAGCGAUCAACAAAUCGAAGACUUCUUGCAAAAGCAA  
GAGUACAAGACCCUGGAGUACAAUCUACUACUACGGAGGUAGUAUAGG  
AGAAUGUGACGGCUUUUUGGGAAAGAGGGUUUUGGAGAACUGUUUUGAGAA  
AGCAAAGCAGAAAACAACAACCGCAAGACCUAAAUGGGACGAAUUC  
CUGUUUUUCUGAACUUCUCCUGCUCGGAACACCGUGUUGAAGGACA  
UCAAUUUAAGAUGAGAGGGACAGCUUCUGCGGUAGCGGGAGAC  
UGGUGCGGGAAAACUAGCCUCUUGAUGGUGAUUAUGGGGAGCUUGAG  
CCCAGCGAGGGAAAGAUAAAACACUCCGGCGUAUCUCAUUCUGUAGCC  
AGUUUCAUGGAUCAUGCCCGAACAUAAAAGAGAACAUCAUUUCGG  
AGUAUCCUAUGAUGAGUACCGAACAGAUCGGUCAUUAAGGCUGGCCAG  
UUGGAAGAGGACAUUUCUAAGUUCGGCGAGAACAGGUAACAUUCGUUUGG  
GAGAAGGGGUAAAACAUUUGUCGGGAGGGCAGCGAGCGCGGAUCAGCC  
CCGGAGAGCGGUAAACAAAAGAUGCAGAUUUGUAUCUGCUUGAUUCACCG  
UUUGGAUCCUCGACGUUAUGACAGAAAAAGAAAUCUUCGAGUGCG  
UGUGUAACUUAUGGCUAAAAGACGAGAACUCCUGGUGACAUCAAAA  
GGAACACCUCUAAAAGCGGACAAGAACUCCUGAUCUCCACGAGAAC  
UCCUACUUUACGGCACUUUCUCAGAGUUGCAAAACUUGCAGCCGGACU  
UCUCAAGCAAACUCAUGGGUGUGACUCAUUCGACCGAGUACCGCGG  
ACGGCGGAACUCGAUCUUGACGGAAACGCUGCACCGAUUCUGCUUGAG  
GGUGAUGCCCGGUAUUCGUGGAGCGAGACAAAAGAGCAGUGCUUUAAGC  
AGACAGGGAAAUGGUGAGAAAAGAACAGUAUCUUGAUUAUCCU  
UAACUCAUUUCGCAAGUUCUCAUUCGUCCAGAAAACUCCACUGCAGA  
AAUGGAAUUGAAGAGGAUUCGGACGAACCCUGGAGCGCAGGUAGCC  
UCGUGCCGGAUUUCGAGCAAGGGAGGCCAUUCUCCCGGUUUUCGGU  
GAUUUCAACCGACCUACACUUCAGGCAGGGCAAGGCAAUUCGUGCUC  
AACCUCAUGACGCAUCUGGUAAAACAGGGCAAAACAUUCACCGCAAA  
CGACGGCCUACGAGAAAAGUGUCACUUGCACCCAGGGCAUUUGAG  
UGAACUCGACAUUCAGCGUAGGCCUUUCGCAAGAAAACCGGACUUGAG

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AUACAGCGAAGAAAUCAAUGAAGAAGAUUUGAAAGAGUGUUUCUUUGAAG  
ACAUGGAAUCAAUCCCAGCGGUGACAACGUGGAACACAUACUUGCGUUA  
CAUCACGGUGCAAAAGGUCCUUGAUUUUCGUCCUCAUCUGGUGUCUGUG  
AUCUUUCUCGCGUGAGGUGCAGCGUCACUUGUGGUCCUCUGGCUGCUUG  
GUAAAACGCCUUGCAAGACAAAGGCAAUUCUACACACUCAAGAAACAA  
UUCCUAUGCCGUGUAUUAUCACUUUCUACAAGCUCGUAAUACGUGUUUUAC  
AUCUACGUAGGAGUGGCCGACACUCUGCGUGGAUGGGGUUCUUCGAG  
GACUCCCACUCGUUCAACACGCUUAAUCACUGUCUCCAAGAUUCUCCACCA  
UAAGAUGCUUCAUAGCGUACUGCAGGCUCCCAUGGUCCACCUUGAAUACG  
CUAAGGCGGGAGGUAUUUUGAAUCGUUCUCAAAAGAUUAUGCAAUU  
UGGAUGACCUUCUGCCCCUGACGAUCUUCGACUUCAUCCAGUUGUUGCU  
GAUCGUGAUUAGGGCUAUUGCAGUAGUGCUGGUCCAGCCUUAACUU  
UUUGUCGCACCGUUCGGUGAUCGUGGCCGUUUAUCUGCUGCGGGCC  
AUUUCUUGCAGACGUACACGCAGCUUAAAGCAACUGGAGUCUGAAGGGAG  
GUCGCCAUUCUUUACGCAUCUUGUGACCAGUUUGAAGGGAUUUGUGGACG  
UUGCGGCCUUUUGGCAGGCAGCCUACUUUGAACACUGUUCCACAAAG  
CGCGUAAUCUCCAUACGGCAAAUUGGUUUUUGUAUUUGAGUACCCUCCG  
AUGGUUCAGAUGCGCAUUGAGAUGAUUUUUGUGAUUCUUCUUUAUCGCG  
GUGACUUUUAUCUCCAUUCUGACCACGGGAGGGCGAGGGACGGGUCC  
GUAAAUCUGACACUCGCCAUGAACAUUAUGAGCACUUUGCAGGGC  
AGUGAACAGCUCGAUUGAUGUGGAUAGCCUGAUGAGGUCCGUUUCGGAG  
GUCUUUAAGUCAUCGACAUGCCGACGGAGGGAAAGCCCACAAAAAGUA  
CGAAACCCUUAAGAAUGGCCAUUUGAGUAAAGGUUAAGUCAUCGAGAA  
CAGUACGUGAAGAAGGAUGACAUUCUGGCCUAGCGGGGUCAAGAUGACC  
GUGAAGGACCUACGGCAAAUACACCAGGGAGGGAAACGCCAUUCUUG  
AAAACAUCUCGUUCAGCAUUAAGCCCCGGUCAGCGUGUGGGGUUGCUGG  
GAGGACCGGUCCAGGAAAUCGACGUUGCGUGCCUUCUUGAGACUU  
CUGAAUACAGAGGGUGAGAUCAGAUCGACGGCGUUUCGUGGGAUAGCA  
UCACCUUCGAGCAGUGGCCGAAAGCGUUUUGAGUAAUCUCCCAAAAGGU  
CUUUAUCUUUAGCGGAACCUUCGAAAGAAUCGACGUCCUUAUGAACAG  
UGGUCAAGAUCAAGAGAUUUGGAAAGUCGCGGACGAGGUUGGCCUUCGGA  
GUGUAAUCGAGCAGGUUCGGGAAAACUGACGUUUGUCCUUGUAGAUGG  
GGGAUGCGUCCUGCGCAUGGGCACAAGCAGCUAUGUGCCUGCGCGA  
UCCGUCCUCUCAAGCGAAAUUCUUCUUGGAUGAACCUUCGGGCC  
AUCUGGACCCGGUAACGUACAGAUCAGAUCAGAAGGACACUUAAGCAGGC  
GUUUGCCGACUGCACGGUGAUUCUCUGUGAGCAUCGUACUGAGGCCAUG  
CUCGAAUCCAGCAUUUCUUGUCAUCGAAGAGAAAUAAGGUCCGCG  
ACGACUCCAUCAGAAGCUGCUUAAUGAGAGAUCAUUGUUCGGCAGGC  
GAUUCACCAUCAGAAGCUGCUUAAUGAGAGAUCAUUGUUCGGCAGGC

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AAGUGCAAGUCCAAACCGCAGAUUCGCGGCCUUAGAAAAGAGACUGAAG  
AAGAAGUUCAAGACACGCGUCUUUAACGGGUGGCAUCCUGUGACCCCC  
CCCCAGUGCCUCUCCUGGCCUUGGAAGUUGCCACUCCAGUGCCCACAG  
CCUUGGUCCUAAUAAAAGUUGCAUCAAGCU

[0210] In one embodiment, another full-length codon-optimized human CFTR mRNA sequence is:

(SEQ ID NO: 11)  
GGACAGAUCGCCUGGAGACGCCAUCCACGCCUGUUUUGACCUCCAUAGAA  
GACACCGGGACCGAUCGCCUCCGGCCGGAACGGUGCAUUGGAAC  
GCGGAUUCCCCUGCCAAGAGUGACUCACCGGUCCUUGACACGAUGCAGC  
GGUCCCCGCUKGAAAAGGCCAGUGUGUGUCAAACUCUUCUUCUCAUG  
GACUCGGCCUAUCCUUAAGAAAGGGGUUAUCGGCAGAGGCUUGAGUUGUC  
GACAUCUACCAAGAUCCCCUGGUAGAUUCGGCGGAUACCUCUCGGAGA  
AGCUCGAACGGGAAUGGGACCGCGAACUCGGCUCUAAGAAAAACCGAA  
GCUCAUCAACGCACUGAGAAGGGUGCUUCUUCUGGCGGUUCAUGUUCAC  
GGUAUCUUCUUGUAUCUGGGAGGUACAAAAGCAGGUCAACCCCGU  
UGUUGGGUCGCAUUAUCGCCUUGUACGACCCGAUAACAAAGAAGAACG  
GAGCAUCGCGAUCUACCUCCGGGAUCGGACUGGUUUCUUCUACUGUC  
AGAACACUUUUGUUGCAUCAGCAAUUCGCCUCCAUCAACUGGUA  
UGCAGAUGCAGAACUGCUAUGUUUAGCUUGAUCAACAAAAGCACUGAA  
ACUCUCGUCCGGGUUGGUAAGAUUUUCAUCGGUCAGUUGGUUGGU  
CUGCUUAGUAAAACCUCAACAAUUCGAUGAGGGACUGGCGCUGGCAC  
AUUUCGUUGUGGAUUGCCCGUUGCAAGUGCCUUUUGAUUGGCCUUAU  
UUGGGAGCUGUUGCAGGCAUCUGCCUUUUGGGCCUUGGAAUUCUGAUU  
GUGUUGGCAUUGUUUUCAGGCUUGGGCUUGGGCGGAUGAUGAUGAAGUAUC  
GCGACCAGAGAGCGGGAAAAUCUGGAAGACUCGUCAUCACUUCGGA  
AAUGAUCGAAAACAUCAGUGGUCAAAGGCCAUUGCUGGGAGAACGU  
AUGGAGAAGAUGAUUGAAAACCUCCGCCAAACUGAGCUGAACUGACCC  
GCAAGGGCGCGUUAUGUCCGGUAAAUCUUCGUCAGCUGUUCUUCUUUC  
CGGUUCUUCGUUGUCUUUCUCUGGUUUGCCUUUAGCCUUGAUUAAG  
GGGUAAAUCUCCCGCAAGAUUUUCCACAGCAUUCGUUCUGCAUUGUAU  
UGCGCAUGGCAGUGACACGGCAUUUUCGUGGGCGUGCAGACAUGGUA  
UGACUCGUUCUGGAGCGAUCAACAAAUCGAAGACUUCUUCUGCAAAAGCAA  
GAGUACAAGACCCUGGAGUACAAUCUACUACUACGGAGGUAGUAUUGG  
AGAAUGUGACGGCUUUUUGGGAGAGGGUUUUGGAGAACUGUUUUGAGAA  
AGCAAACAGAAUACAACAACCGCAAGACCUAAAUGGGACGUAUCC  
CUGUUUUUCUGCAACUUCUCCUGGUACACCCGUGUUGAAGGACA  
UCAAUUCAAGAUUGAGAGGGACAGCUUCUGCGGUAGCGGGAGCAGC  
UGGUUGCGGGAAAACUAGCCUUCUGGUAGGGAGUAAAUGGGGGACGUUGG

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GUGACUUUUAUCUCCAUUUGACCACGGAGAGGGCGAGGGACGGGUUG
GUAAAUCUGACACUCGCCAUGAACAUUAUGAGCACUUUGCAGUGGGC
AGUGAACAGCUCGAUUGAUGUGGAUAGCCUGAUGAGGUCCGUUUCGAGG
GCUUUUAGUUCAUCGACAUGCCGACGGAGGGAAAGCCCACAAAAAGUA
CGAAACCCUUAAGAAUGGGCAUUGAGUAAGGUAAUGAUCAUCGAGGA
CAGUCACGUGAAGAAGGAUGACAUCCUGGCCUAGCGGGGUUGAGACC
GUGAAGGACCUGACGGAAAAUACACCAGGGAGGGACGCAAUCCUUG
AAAACAUUCGUUCAGCAUUAAGCCCCGGUCAGCGUGUGGGGUUGCUCGG
GAGGACCGGGUGCAGGAAAUCGACGUUGCUGUGCCUUCUUGAGACUU
CUGAAUACAGAGGGUGAGAUCCAGAACGACGGCGUUUCGUGGGAUAGCA
UCACCUUGCAGCAGUGGCGGAAAGCGUUUGGAGUAUACCCCCAAAAGGU
CUUUAUCUUUAGCGGAACCUUCCGAAAGAACUUCGAUCCUUAUGAACAG
UGGCAGAUCAAGAGAUUUGGAAAGUCGCGGACGAGGUUGGCCUUCGGA
GUGUAUCGAGCAGUUUCCGGAAAACUCGACUUUGUCCUUGAGAUGG
GGGAUGCGUCCUGUCGCAUGGGCACAAGCAGCUAUGUGCCUGCGCGA
UCCGUCCUCUCAAAGCGAAAAAUUCUUCUUGGAUGAACCUUCGGCC
AUCUGGACCCGUAACGUUAUCAGAUCAUCAGAACAGCACUUAAGCAGGC
GUUUGCCGACUGCACGGUGAUUCUGUGAGCAUCGUAUCGAGGCCAUG
CUCGAAUGCAGCAUUUCUUGUCAUCGAAGAGAAUAGGUCCGCGAGU
ACGACUCCAUCAGAACGUGCUUAAUGAGAGAUCAUUGUUCGGCAGGC
GAUUCACCAUCCGAUAGGGUGAAACUUUUCACACAGAAAUUCGUCG
AAGUGCAAGUCAAACCGCAGAUCGCGGCCUUGAAAAGAAGAGACUGAAG
AAGAAGUUCAGACACCGCUCUAAAAGGGUGGCAUCCUGUGACCCUC
CCCAGUGCCUCUCCUGGCCUUGGAAGUUGCCACUCCAGUGGCCACCAGC
CUUGGUCCUAAAAGUUGCAUCAAAGCU

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**[0211]** SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11 include 5' and 3' untranslated regions framing a codon-optimized hCFTR-encoding mRNA.

**[0212]** In some embodiments, a suitable mRNA sequence may be an mRNA sequence a homolog or an analog of human CFTR (hCFTR) protein. For example, a homolog or an analog of hCFTR protein may be a modified hCFTR protein containing one or more amino acid substitutions, deletions, and/or insertions as compared to a wild-type or naturally-occurring hCFTR protein while retaining substantial hCFTR protein activity. In some embodiments, an mRNA suitable for the present invention encodes an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more homologous to SEQ ID NO: 4. In some embodiments, an mRNA suitable for the present invention encodes a protein substantially identical to hCFTR protein. In some embodiments, an mRNA suitable for the present invention encodes an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO: 4. In some embodiments, an mRNA suitable for the present invention encodes a fragment or a portion of

hCFTR protein. In some embodiments, an mRNA suitable for the present invention encodes a fragment or a portion of hCFTR protein, wherein the fragment or portion of the protein still maintains CFTR activity similar to that of the wild-type protein. In some embodiments, an mRNA suitable for the present invention has a nucleotide sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical SEQ ID NO: 1, SEQ ID NO: 8 or SEQ ID NO: 9. In some embodiments, an mRNA suitable for the present invention has a nucleotide sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical SEQ ID NO: 2, SEQ ID NO: 10 or SEQ ID NO: 11. In some embodiments, an mRNA suitable for the present invention comprises a nucleotide sequence identical to SEQ ID NO: 1. In some embodiments, an mRNA suitable for the present invention comprises a nucleotide sequence identical to SEQ ID NO: 8. In some embodiments, an mRNA suitable for the present invention comprises a nucleotide sequence identical to SEQ ID NO: 9. In some embodiments, a suitable mRNA encodes a fusion protein comprising a full length, fragment or portion of an hCFTR protein fused to another protein (e.g., an N or C terminal fusion). In some embodiments, the protein fused to the mRNA encoding a full length, fragment or portion of an hCFTR protein encodes a signal or a cellular targeting sequence.

**[0213]** mRNAs according to the present invention may be synthesized according to any of a variety of known methods. For example, mRNAs according to the present invention may be synthesized via *in vitro* transcription (IVT). Briefly, IVT is typically performed with a linear or circular DNA template containing a promoter, a pool of ribonucleotide triphosphates, a buffer system that may include DTT and magnesium ions, and an appropriate RNA polymerase (e.g., T3, T7 or SP6 RNA polymerase), DNase I, pyrophosphatase, and/or RNase inhibitor. The exact conditions will vary according to the specific application.

#### Non-Coding Regions

**[0214]** In some embodiments, mRNAs include a 5' and/or 3' untranslated region. In some embodiments, a 5' untranslated region includes one or more elements that affect an mRNA's stability or translation, for example, an iron responsive element. In some embodiments, a 5' untranslated region may be between about 50 and 500 nucleotides in length.

**[0215]** In some embodiments, a 3' untranslated region includes one or more of a polyadenylation signal, a binding site for proteins that affect an mRNA's stability or location in a cell, or one or more binding sites for miRNAs. In some embodiments, a 3' untranslated region may be between 50 and 500 nucleotides in length or longer.

**[0216]** Exemplary 3' and/or 5' UTR sequences can be derived from mRNA molecules which are stable (e.g., globin, actin, GAPDH, tubulin, histone, or citric acid cycle enzymes) to increase the stability of the sense mRNA molecule. For example, a 5' UTR sequence may include a partial sequence of a CMV immediate-early 1 (IE1) gene, or a fragment thereof to improve the nuclease resistance and/or improve the half-life of the polynucleotide. Also contemplated is the inclusion of a sequence encoding human growth hormone (hGH), or a fragment thereof to the 3' end or untranslated region of the polynucleotide (e.g., mRNA) to

further stabilize the polynucleotide. Generally, these modifications improve the stability and/or pharmacokinetic properties (e.g., half-life) of the polynucleotide relative to their unmodified counterparts, and include, for example modifications made to improve such polynucleotides' resistance to *in vivo* nuclease digestion.

[0217] According to various embodiments, any size mRNA may be encapsulated by provided liposomes. In some embodiments, the provided liposomes may encapsulate mRNA of greater than about 0.5 kb, 1 kb, 1.5 kb, 2 kb, 2.5 kb, 3 kb, 3.5 kb, 4 kb, 4.5 kb, or 5 kb in length.

[0218] Typically, mRNA synthesis includes the addition of a "cap" on the N-terminal (5') end, and a "tail" on the C-terminal (3') end. The presence of the cap is important in providing resistance to nucleases found in most eukaryotic cells. The presence of a "tail" serves to protect the mRNA from exonuclease degradation.

[0219] Thus, in some embodiments, mRNAs (e.g., mRNAs encoding CFTR) include a 5' cap structure. A 5' cap is typically added as follows: first, an RNA terminal phosphatase removes one of the terminal phosphate groups from the 5' nucleotide, leaving two terminal phosphates; guanosine triphosphate (GTP) is then added to the terminal phosphates via a guanylyl transferase, producing a 5'5' triphosphate linkage; and the 7-nitrogen of guanine is then methylated by a methyltransferase. Examples of cap structures include, but are not limited to, m7G(5')ppp(5')(A,G(5')ppp(5')A and G(5')ppp(5')G. Additional cap structures are described in published US Application No. US 2016/0032356 and U.S. Provisional Application 62/464,327, filed Feb. 27, 2017, which are incorporated herein by reference.

[0220] In some embodiments, the cap is a Cap0 structure. Cap0 structures lack a 2'-O-methyl residue of the ribose attached to bases 1 and 2. In some embodiments, the cap is a Cap1 structure. Cap1 structures have a 2'-O-methyl residue at base 2. In some embodiments, the cap is a Cap2 structure. Cap2 structures have a 2'-O-methyl residue attached to both bases 2 and 3.

[0221] In some embodiments, mRNAs (e.g., mRNAs encoding CFTR) include a 3' tail structure. Typically, a tail structure includes a poly(A) and/or poly(C) tail. A poly-A or poly-C tail on the 3' terminus of mRNA typically includes at least 50 adenosine or cytosine nucleotides, at least 150 adenosine or cytosine nucleotides, at least 200 adenosine or cytosine nucleotides, at least 250 adenosine or cytosine nucleotides, at least 300 adenosine or cytosine nucleotides, at least 350 adenosine or cytosine nucleotides, at least 400 adenosine or cytosine nucleotides, at least 450 adenosine or cytosine nucleotides, at least 500 adenosine or cytosine nucleotides, at least 550 adenosine or cytosine nucleotides, at least 600 adenosine or cytosine nucleotides, at least 650 adenosine or cytosine nucleotides, at least 700 adenosine or cytosine nucleotides, at least 750 adenosine or cytosine nucleotides, at least 800 adenosine or cytosine nucleotides, at least 850 adenosine or cytosine nucleotides, at least 900 adenosine or cytosine nucleotides, at least 950 adenosine or cytosine nucleotides, or at least 1 kb adenosine or cytosine nucleotides, respectively. In some embodiments, a poly-A or poly-C tail may be about 10 to 800 adenosine or cytosine nucleotides (e.g., about 10 to 200 adenosine or cytosine nucleotides, about 10 to 300 adenosine or cytosine nucleotides, about 10 to 400 adenosine or cytosine nucleotides, about 10 to 500 adenosine or cytosine nucleotides, about 10 to 550 adenosine or cytosine nucleotides, about 10 to 600

adenosine or cytosine nucleotides, about 50 to 600 adenosine or cytosine nucleotides, about 100 to 600 adenosine or cytosine nucleotides, about 150 to 600 adenosine or cytosine nucleotides, about 200 to 600 adenosine or cytosine nucleotides, about 250 to 600 adenosine or cytosine nucleotides, about 300 to 600 adenosine or cytosine nucleotides, about 350 to 600 adenosine or cytosine nucleotides, about 400 to 600 adenosine or cytosine nucleotides, about 450 to 600 adenosine or cytosine nucleotides, about 500 to 600 adenosine or cytosine nucleotides, about 10 to 150 adenosine or cytosine nucleotides, about 20 to 100 adenosine or cytosine nucleotides, about 20 to 70 adenosine or cytosine nucleotides, or about 20 to 60 adenosine or cytosine nucleotides) respectively. In some embodiments, a tail structure includes a combination of poly(A) and poly(C) tails with various lengths described herein. In some embodiments, a tail structure includes at least 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 94%, 95%, 96%, 97%, 98%, or 99% adenosine nucleotides. In some embodiments, a tail structure includes at least 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 94%, 95%, 96%, 97%, 98%, or 99% cytosine nucleotides.

#### Modified mRNA

[0222] In some embodiments, mRNA according to the present invention may be synthesized as unmodified or modified mRNA. Typically, mRNAs are modified to enhance stability. Modifications of mRNA can include, for example, modifications of the nucleotides of the RNA. A modified mRNA according to the invention can thus include, for example, backbone modifications, sugar modifications or base modifications. In some embodiments, mRNAs may be synthesized from naturally occurring nucleotides and/or nucleotide analogues (modified nucleotides) including, but not limited to, purines (adenine (A), guanine (G)) or pyrimidines (thymine (T), cytosine (C), uracil (U)), and as modified nucleotides analogues or derivatives of purines and pyrimidines, such as e.g. 1-methyl-adenine, 2-methyl-adenine, 2-methylthio-N-6-isopentenyl-adenine, N6-methyl-adenine, N6-isopentenyl-adenine, 2-thio-cytosine, 3-methyl-cytosine, 4-acetyl-cytosine, 5-methyl-cytosine, 2,6-diaminopurine, 1-methyl-guanine, 2-methyl-guanine, 2,2-dimethyl-guanine, 7-methyl-guanine, inosine, 1-methyl-inosine, pseudouracil (5-uracil), dihydro-uracil, 2-thio-uracil, 4-thio-uracil, 5-carboxymethylaminomethyl-2-thio-uracil, 5-(carboxyhydroxymethyl)-uracil, 5-fluoro-uracil, 5-bromo-uracil, 5-carboxymethylaminomethyl-uracil, 5-methyl-2-thio-uracil, 5-methyl-uracil, N-uracil-5-oxy-acetic acid methyl ester, 5-methylaminomethyl-uracil, 5-methoxyaminomethyl-2-thio-uracil, 5'-methoxycarbonyl-methyl-uracil, 5-methoxy-uracil, uracil-5-oxyacetic acid (v), 1-methyl-pseudouracil, queosine, .beta.-D-mannosyl-queosine, wybutoxosine, and phosphoramidates, phosphorothioates, peptide nucleotides, methylphosphonates, 7-deazaguanosine, 5-methylcytosine and inosine. The preparation of such analogues is known to a person skilled in the art e.g., from the U.S. Pat. Nos. 4,373,071, 4,401,796, 4,415,732, 4,458,066, 4,500,707, 4,668,777, 4,973,679, 5,047,524, 5,132,418, 5,153,319, 5,262,530 and 5,700,642, the disclosures of which are incorporated by reference in their entirety.

[0223] In some embodiments, mRNAs (e.g., mRNAs encoding CFTR) may contain RNA backbone modifications. Typically, a backbone modification is a modification in which the phosphates of the backbone of the nucleotides

contained in the RNA are modified chemically. Exemplary backbone modifications typically include, but are not limited to, modifications from the group consisting of methylphosphonates, methylphosphoramides, phosphoramides, phosphorothioates (e.g., cytidine 5'-O-(1-thiophosphate)), boranophosphates, positively charged guanidinium groups etc., which means by replacing the phosphodiester linkage by other anionic, cationic or neutral groups.

[0224] In some embodiments, mRNAs (e.g., mRNAs encoding CFTR) may contain sugar modifications. A typical sugar modification is a chemical modification of the sugar of the nucleotides it contains including, but not limited to, sugar modifications chosen from the group consisting of 2'-deoxy-2'-fluoro-oligoribonucleotide (2'-fluoro-2'-deoxycytidine 5'-triphosphate, 2'-fluoro-2'-deoxyuridine 5'-triphosphate), 2'-deoxy-2'-deamino-oligoribonucleotide (2'-amino-2'-deoxycytidine 5'-triphosphate, 2'-amino-2'-deoxyuridine 5'-triphosphate), 2'-O-alkyloligoribonucleotide, 2'-deoxy-2'-C-alkyloligoribonucleotide (2'-O-methylcytidine 5'-triphosphate, 2'-methyluridine 5'-triphosphate), 2'-C-alkyloligoribonucleotide, and isomers thereof (2'-aracytidine 5'-triphosphate, 2'-arauridine 5'-triphosphate), azidotriphosphates (2'-azido-2'-deoxycytidine 5'-triphosphate, 2'-azido-2'-deoxyuridine 5'-triphosphate) or 4'-thio-substituted ribonucleotides.

[0225] In some embodiments, mRNAs (e.g., mRNAs encoding CFTR) may contain modifications of the bases of the nucleotides (base modifications). A modified nucleotide which contains a base modification is also called a base-modified nucleotide. Examples of such base-modified nucleotides include, but are not limited to, 2-amino-6-chloropurine riboside 5'-triphosphate, 2-aminoadenosine 5'-triphosphate, 2-thiocytidine 5'-triphosphate, 2-thiouridine 5'-triphosphate, 4-thiouridine 5'-triphosphate, 5-aminoallylcytidine 5'-triphosphate, 5-aminoallyluridine 5'-triphosphate, 5-bromocytidine 5'-triphosphate, 5-bromouridine 5'-triphosphate, 5-iodocytidine 5'-triphosphate, 5-iodouridine 5'-triphosphate, 5-methylcytidine 5'-triphosphate, 5-methyluridine 5'-triphosphate, 6-azacytidine 5'-triphosphate, 6-azauridine 5'-triphosphate, 6-chloropurine riboside 5'-triphosphate, 7-deazaadenosine 5'-triphosphate, 7-deazaguanosine 5'-triphosphate, 8-azaadenosine 5'-triphosphate, 8-azidoadenosine 5'-triphosphate, benzimidazole riboside 5'-triphosphate, N1-methyladenosine 5'-triphosphate, N1-methylguanosine 5'-triphosphate, N6-methyladenosine 5'-triphosphate, N6-methylguanosine 5'-triphosphate, pseudouridine 5'-triphosphate, puromycin 5'-triphosphate or xanthosine 5'-triphosphate.

#### Pharmaceutical Compositions

[0226] To facilitate expression of mRNA in vivo, delivery vehicles such as liposomes can be formulated in combination with one or more additional nucleic acids, carriers, targeting ligands or stabilizing reagents, or in pharmaceutical compositions where it is mixed with suitable excipients. Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, Pa., latest edition.

[0227] Provided liposomally-encapsulated or associated mRNAs, and compositions containing the same, may be administered and dosed in accordance with current medical practice, taking into account the clinical condition of the subject, the site and method of administration, the schedul-

ing of administration, the subject's age, sex, body weight and other factors relevant to clinicians of ordinary skill in the art. The "effective amount" for the purposes herein may be determined by such relevant considerations as are known to those of ordinary skill in experimental clinical research, pharmacological, clinical and medical arts. In some embodiments, the amount administered is effective to achieve at least some stabilization, improvement or elimination of symptoms and other indicators as are selected as appropriate measures of disease progress, regression or improvement by those of skill in the art. For example, a suitable amount and dosing regimen is one that causes at least transient protein (e.g., enzyme) production.

[0228] Suitable routes of administration include, for example, oral, rectal, vaginal, transmucosal, pulmonary including intratracheal or inhaled, or intestinal administration; parenteral delivery, including intradermal, transdermal (topical), intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, and/or intranasal administration.

[0229] Alternately or additionally, liposomally encapsulated mRNAs and compositions of the invention may be administered in a local rather than systemic manner, for example, via injection of the pharmaceutical composition directly into a targeted tissue, preferably in a sustained release formulation. Local delivery can be affected in various ways, depending on the tissue to be targeted. For example, aerosols containing compositions of the present invention can be inhaled (for nasal, tracheal, or bronchial delivery); compositions of the present invention can be injected into the site of injury, disease manifestation, or pain, for example; compositions can be provided in lozenges for oral, tracheal, or esophageal application; can be supplied in liquid, tablet or capsule form for administration to the stomach or intestines, can be supplied in suppository form for rectal or vaginal application; or can even be delivered to the eye by use of creams, drops, or even injection. Formulations containing provided compositions complexed with therapeutic molecules or ligands can even be surgically administered, for example in association with a polymer or other structure or substance that can allow the compositions to diffuse from the site of implantation to surrounding cells. Alternatively, they can be applied surgically without the use of polymers or supports.

[0230] In some embodiments, provided liposomes and/or compositions are formulated such that they are suitable for extended-release of the mRNA contained therein. Such extended-release compositions may be conveniently administered to a subject at extended dosing intervals. For example, in one embodiment, the compositions of the present invention are administered to a subject twice day, daily or every other day. In a preferred embodiment, the compositions of the present invention are administered to a subject twice a week, once a week, every ten days, every two weeks, every three weeks, or more preferably every four weeks, once a month, every six weeks, every eight weeks, every other month, every three months, every four months, every six months, every eight months, every nine months or annually. Also contemplated are compositions and liposomes which are formulated for depot administration (e.g., intramuscularly, subcutaneously, intravitrally) to either deliver or release mRNA over extended periods of time.

Preferably, the extended-release means employed are combined with modifications made to the mRNA to enhance stability.

[0231] Also contemplated herein are lyophilized pharmaceutical compositions comprising one or more of the liposomes disclosed herein and related methods for the use of such compositions as disclosed for example, in U.S. Provisional Application No. 61/494,882, filed Jun. 8, 2011, the teachings of which are incorporated herein by reference in their entirety. For example, lyophilized pharmaceutical compositions according to the invention may be reconstituted prior to administration or can be reconstituted *in vivo*. For example, a lyophilized pharmaceutical composition can be formulated in an appropriate dosage form (e.g., an intradermal dosage form such as a disk, rod or membrane) and administered such that the dosage form is rehydrated over time *in vivo* by the individual's bodily fluids.

[0232] Provided liposomes and compositions may be administered to any desired tissue. In some embodiments, the mRNA delivered by provided liposomes or compositions is expressed in the tissue in which the liposomes and/or compositions were administered. In some embodiments, the mRNA delivered is expressed in a tissue different from the tissue in which the liposomes and/or compositions were administered. Exemplary tissues in which delivered mRNA may be delivered and/or expressed include, but are not limited to the lungs, liver, kidney, heart, spleen, serum, brain, skeletal muscle, lymph nodes, skin, and/or cerebrospinal fluid.

[0233] In some embodiments, a target tissue is lung. In some embodiments, a target tissue is the upper (i.e., superior) lobe of the right or left lung. In some embodiments, a target tissue is the lower (i.e., inferior) lobe of the right or left lung. In some embodiments, a target tissue is the middle lobe of the right lung.

[0234] In some embodiments, a target tissue is the apical segment of the right lung or the apicoposterior segment of the left lung. In some embodiments, a target tissue is the posterior segment of the right lung. In some embodiments, a target tissue is the anterior segment of the right or left lung. In some embodiments, a target tissue is the superior segment of the right or left lung. In some embodiments, a target tissue is the lateral basal segment of the right or left lung. In some embodiments, a target tissue is the anterior basal segment of the right lung. In some embodiments, a target tissue is the anteromedial basal segment of the left lung. In some embodiments, a target tissue is the lateral segment of the right lung. In some embodiments, a target tissue is the medial segment of the right lung. In some embodiments, a target tissue is the superior lingular segment of the left lung. In some embodiments, a target tissue is the inferior lingular segment of the left lung. In some embodiments, a target tissue is the posterior basal segment of the right or left lung. In some embodiments, a target tissue is the medial basal segment of the right lung.

[0235] In particular embodiments, a target tissue is epithelial cells in the lung. In some embodiments, a target tissue is smooth muscle cells in the lung. In some embodiment, a target tissue is pancreatic duct epithelial cells. In some embodiment, a target tissue is bile-duct epithelial cells. In some embodiment, a target tissue is epithelial cells of the salivary glands. In some embodiment, a target tissue is renal epithelial cells. In some embodiment, a target tissue is beta-S

cells in sweat gland secretory coils of sweat glands. In some embodiment, a target tissue is epithelial cells of the reproductive tract.

[0236] According to various embodiments, the timing of expression of delivered mRNAs can be tuned to suit a particular medical need. In some embodiments, the expression of the protein encoded by delivered mRNA is detectable 1, 2, 3, 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66, and/or 72 hours in serum or target tissues after a single administration of provided liposomes or compositions. In some embodiments, the expression of the protein encoded by the mRNA is detectable 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, and/or 7 days in serum or target tissues after a single administration of provided liposomes or compositions. In some embodiments, the expression of the protein encoded by the mRNA is detectable 1 week, 2 weeks, 3 weeks, and/or 4 weeks in serum or target tissues after a single administration of provided liposomes or compositions. In some embodiments, the expression of the protein encoded by the mRNA is detectable after a month or longer after a single administration of provided liposomes or compositions.

[0237] In some embodiments, mRNA (e.g., encoding CFTR protein) in a formulation as provided herein (e.g. encapsulated in a lipid nanoparticle consisting of 3 distinct lipid components, one of which is a sterol-based cationic lipid) delivered to the lung (e.g., by nebulization) is expressed in lung tissue for at least 7 days, at least 14 days, at least 21 days, or at least 28 days. In some embodiments, mRNA (e.g., encoding CFTR protein) in a formulation as provided herein (e.g. encapsulated in a lipid nanoparticle consisting of 3 distinct lipid components, one of which is a sterol-based cationic lipid) delivered to the lung (e.g., by nebulization) is expressed in lung tissue for up to 7 days, up to 14 days, up to 21 days, or up to 28 days. In some embodiments, a protein (e.g., CFTR) encoded by an mRNA (e.g., encoding CFTR protein) in a formulation as provided herein (e.g. encapsulated in a lipid nanoparticle consisting of 3 distinct lipid components, one of which is a sterol-based cationic lipid) delivered to the lung (e.g., by nebulization) is expressed in lung tissue for at least 7 days, at least 14 days, at least 21 days, or at least 28 days. In some embodiments, a protein (e.g., CFTR) encoded by an mRNA (e.g., encoding CFTR protein) in a formulation as provided herein (e.g. encapsulated in a lipid nanoparticle consisting of 3 distinct lipid components, one of which is a sterol-based cationic lipid) delivered to the lung (e.g., by nebulization) is expressed in lung tissue for up to 7 days, up to 14 days, up to 21 days, or up to 28 days.

[0238] The present invention can be used to deliver mRNA at various doses. In some embodiments, an mRNA is administered at a dose ranging from about 0.1-5.0 mg/kg body weight, for example about 0.1-4.5, 0.1-4.0, 0.1-3.5, 0.1-3.0, 0.1-2.5, 0.1-2.0, 0.1-1.5, 0.1-1.0, 0.1-0.5, 0.1-0.3, 0.3-5.0, 0.3-4.5, 0.3-4.0, 0.3-3.5, 0.3-3.0, 0.3-2.5, 0.3-2.0, 0.3-1.5, 0.3-1.0, 0.3-0.5, 0.5-5.0, 0.5-4.5, 0.5-4.0, 0.5-3.5, 0.5-3.0, 0.5-2.5, 0.5-2.0, 0.5-1.5, or 0.5-1.0 mg/kg body weight. In some embodiments, an mRNA is administered at a dose of or less than about 5.0, 4.5, 4.0, 3.5, 3.0, 2.5, 2.0, 1.5, 1.0, 0.8, 0.6, 0.5, 0.4, 0.3, 0.2, or 0.1 mg/kg body weight.

## EXAMPLES

[0239] While certain compounds, compositions and methods of the present invention have been described with

specificity in accordance with certain embodiments, the following examples serve only to illustrate the invention and are not intended to limit the same. Lipid Materials

**[0240]** The formulations described herein included a multi-component lipid mixture of varying ratios employing cationic lipids, helper lipids and PEG-modified lipids designed to encapsulate various nucleic acid-based materials. Cationic lipids can include (but not exclusively) sterol-based cationic lipids, ICE (imidazole cholesterol ester), 1,2-dioleoyl-3-dimethylammonium-propane (DODAP), 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), 1,2-Dioleyloxy-3-dimethylaminopropane (DODMA), 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA), C12-200, DLinSSDMA, Target 24, etc. Helper lipids can include (but not exclusively) DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine), DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine), DOPE (1,2-dioleyl-sn-glycero-3-phosphoethanolamine), DPPE (1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine), DMPE (1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine), DOPG (2-dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol)), cholesterol, etc. The PEG-modified lipids can include (but not exclusively) a poly(ethylene) glycol chain of up to 5 kDa in length covalently attached to a lipid with alkyl chain(s) of C<sub>6</sub>-C<sub>20</sub> length.

**[0241]** N/P ratio is the molar ratios of the nitrogen (amine) groups of cationic carriers to those of the phosphate ones of mRNA.

#### Messenger RNA Material

**[0242]** The formulations described herein included messenger RNA (mRNA). mRNA can (but not exclusively) encode human Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) and Firefly Luciferase (FFL). Exemplary mRNA coding sequences as disclosed herein include SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3. An exemplary 5' UTR mRNA sequence as disclosed herein is SEQ ID NO: 5. Exemplary 3' UTR mRNA sequences as disclosed herein are SEQ ID NO: 6 and SEQ ID NO: 7. Exemplary full length mRNA sequences (i.e., a coding sequence plus a 5' UTR and a 3' UTR) as disclosed herein include SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11.

#### Exemplary LNP Formulations Comprising Different Cationic Lipids

**[0243]** In some embodiments, one particular application of the novel lipid composition comprising a sterol-based cationic lipid, a helper lipid, and a PEG or PEG-modified lipid is pulmonary delivery of mRNA, such as CFTR mRNA. Lipid nanoparticle formulations composed of ICE lipid, DOPE lipid and DMG-PEG lipid (molar ratio of 60:35:5) exhibit high percent encapsulation values for mRNA, such as CFTR mRNA (>80%), as determined by fluorescence-based detection of mRNA. As shown in Table 5, this particular formulation composition is superior because conventional pH titratable cationic lipids with DOPE and DMG-PEG 2K do not show such high percent encapsulation.

TABLE 5

pH titratable Cationic Lipid	% mRNA encapsulation
ICE	90
DODMA	51
DODAP	49
C12-200	39
DLinSSDMA	57
Target 24	59

**[0244]** All of these exemplary formulations were prepared as 5 mg mRNA LNP preparations.

#### Formulation Example #1 (ICE Lipid)

**[0245]** Aliquots of 10 mg/mL ethanolic solutions of ICE, DOPE and DMG-PEG2K (molar ratio of 60:35:5) were mixed and diluted with ethanol to 15 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of CFTR mRNA was prepared from a 1 mg/mL stock solution so as to have a final volume of 60 mL.

**[0246]** In this process, the lipids dissolved in ethanol and the mRNA dissolved in citrate buffer were mixed using a pump system. The instantaneous mixing of the two streams resulted in formation of lipid nanoparticles, which was a self-assembly process driven by electrostatic attraction and van der wall forces. The resultant formulation mixture was in citrate buffer containing 20% ethanol. The formulation was subjected to a buffer exchange and the resultant formulation was adjusted to the desired mRNA concentration and stored frozen until further use. Final concentration: 0.5 mg/mL CFTR mRNA (encapsulated). % mRNA Encapsulation: 90%. Size: 60 nm. PDI: 0.14.

#### Formulation Example #2 (DODMA Lipid)

**[0247]** Aliquots of 10 mg/mL ethanolic solutions of DODMA, DOPE and DMG-PEG2K (molar ratio of 60:35:5) were mixed and diluted with ethanol to 15 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of CFTR mRNA was prepared from a 1 mg/mL stock solution so as to have a final volume of 60 mL. In this process, the lipids dissolved in ethanol and the mRNA dissolved in citrate buffer were mixed using a pump system. The instantaneous mixing of the two streams resulted in formation of lipid nanoparticles, which was a self-assembly process driven by electrostatic attraction and van der wall forces. The resultant formulation mixture was in citrate buffer containing 20% ethanol. The formulation was subjected to a buffer exchange and the resultant formulation was adjusted to the desired mRNA concentration and stored frozen until further use. Final concentration: 0.5 mg/mL CFTR mRNA (encapsulated). % mRNA Encapsulation: 51%. Size: 62 nm. PDI: 0.18.

#### Formulation Example #3 (DODAP Lipid)

**[0248]** Aliquots of 10 mg/mL ethanolic solutions of DODAP, DOPE and DMG-PEG2K (molar ratio of 60:35:5) were mixed and diluted with ethanol to 15 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150

mM NaCl, pH 4.5) of CFTR mRNA was prepared from a 1 mg/mL stock solution so as to have a final volume of 60 mL. In this process, the lipids dissolved in ethanol and the mRNA dissolved in citrate buffer were mixed using a pump system. The instantaneous mixing of the two streams resulted in formation of lipid nanoparticles, which was a self-assembly process driven by electrostatic attraction and van der wall forces. The resultant formulation mixture was in citrate buffer containing 20% ethanol. The formulation was subjected to a buffer exchange and the resultant formulation was adjusted to the desired mRNA concentration and stored frozen until further use. Final concentration: 0.5 mg/mL CFTR mRNA (encapsulated). % mRNA Encapsulation: 49%. Size: 78 nm. PDI: 0.19.

#### Formulation Example #4 (C12-200 Lipid)

**[0249]** Aliquots of 10 mg/mL ethanolic solutions of C12-200, DOPE and DMG-PEG2K (molar ratio of 60:35:5) were mixed and diluted with ethanol to 15 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of CFTR mRNA was prepared from a 1 mg/mL stock solution so as to have a final volume of 60 mL.

**[0250]** In this process, the lipids dissolved in ethanol and the mRNA dissolved in citrate buffer were mixed using a pump system. The instantaneous mixing of the two streams resulted in formation of lipid nanoparticles, which was a self-assembly process driven by electrostatic attraction and van der wall forces. The resultant formulation mixture was in citrate buffer containing 20% ethanol. The formulation was subjected to a buffer exchange and the resultant formulation was adjusted to the desired mRNA concentration and stored frozen until further use. Final concentration: 0.5 mg/mL CFTR mRNA (encapsulated). % mRNA Encapsulation: 39%. Size: 98 nm. PDI: 0.22.

#### Formulation Example #5 (DLin-SS-DMA Lipid)

**[0251]** Aliquots of 10 mg/mL ethanolic solutions of DLin-SS-DMA, DOPE and DMG-PEG2K (molar ratio of 60:35:5) were mixed and diluted with ethanol to 15 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of CFTR mRNA was prepared from a 1 mg/mL stock solution so as to have a final volume of 60 mL.

**[0252]** In this process, the lipids dissolved in ethanol and the mRNA dissolved in citrate buffer were mixed using a pump system. The instantaneous mixing of the two streams resulted in formation of lipid nanoparticles, which was a self-assembly process driven by electrostatic attraction and van der wall forces. The resultant formulation mixture was in citrate buffer containing 20% ethanol. The formulation was subjected to a buffer exchange and the resultant formulation was adjusted to the desired mRNA concentration and stored frozen until further use. Final concentration: 0.5 mg/mL CFTR mRNA (encapsulated). % mRNA Encapsulation: 57%.

#### Formulation Example #6 (Target 24 Lipid)

**[0253]** Aliquots of 10 mg/mL ethanolic solutions of Target 24 (3-(5-(bis(2-hydroxydodecyl)amino)pentan-2-yl)-6-(5-(2-hydroxydodecyl)(2-hydroxyundecyl)amino)pentan-2-yl)-1,4-dioxane-2,5-dione), DOPE and DMG-PEG2K (molar ratio of 60:35:5) were mixed and diluted with ethanol to 15 mL final volume. Separately, an aqueous buffered solu-

tion (10 mM citrate/150 mM NaCl, pH 4.5) of CFTR mRNA was prepared from a 1 mg/mL stock solution so as to have a final volume of 60 mL.

**[0254]** In this process, the lipids dissolved in ethanol and the mRNA dissolved in citrate buffer were mixed using a pump system. The instantaneous mixing of the two streams resulted in formation of lipid nanoparticles, which was a self-assembly process driven by electrostatic attraction and van der wall forces. The resultant formulation mixture was in citrate buffer containing 20% ethanol. The formulation was subjected to a buffer exchange and the resultant formulation was adjusted to the desired mRNA concentration and stored frozen until further use. Final concentration: 0.5 mg/mL CFTR mRNA (encapsulated). % mRNA Encapsulation: 59%. Size: 92 nm. PDI: 0.24.

### Exemplary ICE Formulation Protocols

#### A. Formulation Example—1 mg Scale, N/P=4

**[0255]** Aliquots of 10 mg/mL ethanolic solutions of ICE, DOPE, cholesterol and DMG-PEG2K (molar ratio of 60:35:5) were mixed and diluted with ethanol to 3 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of CFTR mRNA was prepared from a 1 mg/mL stock solution. The lipid solution was injected rapidly into the aqueous mRNA solution and shaken to yield a final suspension in 20% ethanol. The resulting nanoparticle suspension was filtered, diafiltrated, concentrated and stored frozen until further use. Final concentration of the formulation was determined at 0.5 mg/mL CFTR mRNA (encapsulated). The formulation had about 89% mRNA encapsulation (89% of the lipid nanoparticles contained mRNA) and the lipid nanoparticles had an average size of around 67 nm with polydispersibility index (PDI) of 0.19.

#### B. Formulation Example—15 mg Scale, N/P=2

**[0256]** Aliquots of 10 mg/mL ethanolic solutions of ICE, DOPE and DMG-PEG2K (molar ratio of 60:35:5) were mixed and diluted with ethanol to 45 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of CFTR mRNA was prepared from a 1 mg/mL stock solution so as to have a final volume of 180 mL.

**[0257]** In this process, the lipids dissolved in ethanol and the mRNA dissolved in citrate buffer were mixed using a pump system. The instantaneous mixing of the two streams resulted in formation of lipid nanoparticles, which was a self-assembly process driven by electrostatic attraction and van der Waals forces. The resultant formulation mixture was in citrate buffer containing 20% ethanol. The formulation was subjected to a buffer exchange and the resultant formulation was adjusted to the desired mRNA concentration and stored frozen until further use. Final concentration of the formulation was determined at 0.5 mg/mL CFTR mRNA (encapsulated). The lipid nanoparticles had an average size of around 70 nm with PDI of 0.17.

#### C. Formulation Example—15 mg Scale, N/P=4

**[0258]** Aliquots of 10 mg/mL ethanolic solutions of ICE, DOPE and DMG-PEG2K (molar ratio of 60:35:5) were mixed and diluted with ethanol to 45 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150

mM NaCl, pH 4.5) of CFTR mRNA was prepared from a 1 mg/mL stock solution so as to have a final volume of 180 mL.

[0259] In this process, the lipids dissolved in ethanol and the mRNA dissolved in citrate buffer were mixed using a pump system. The instantaneous mixing of the two streams resulted in formation of lipid nanoparticles. The resultant formulation mixture was in citrate buffer containing 20% ethanol. The formulation was subjected to a buffer exchange and the resultant formulation was adjusted to the desired mRNA concentration and stored frozen until further use. Final concentration of the formulation was determined at 0.5 mg/mL CFTR mRNA (encapsulated). The formulation had about 85% mRNA encapsulation and the lipid nanoparticles had an average size of around 65 nm with PDI of 0.13.

#### D. Formulation Example—30 mg Scale, N/P=4

[0260] Aliquots of 10 mg/mL ethanolic solutions of ICE, DOPE and DMG-PEG2K (molar ratio of 60:35:5) were mixed and diluted with ethanol to 90 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of CFTR mRNA was prepared from a 1 mg/mL stock solution so as to have a final volume of 360 mL.

[0261] In this process, the lipids dissolved in ethanol and the mRNA dissolved in citrate buffer were mixed using a pump system. The instantaneous mixing of the two streams resulted in formation of lipid nanoparticles. The resultant formulation mixture was in citrate buffer containing 20% ethanol. The formulation was subjected to a buffer exchange using TFF system and the resultant formulation was adjusted to the desired mRNA concentration and stored frozen until further use. Final concentration of the formulation was determined at 0.5 mg/mL CFTR mRNA (encapsulated). The formulation had about 86% of mRNA encapsulation and the lipid nanoparticles had an average size of around 89 nm with PDI of 0.12.

#### E. Formulation Example—30 mg Scale, 3% PEG, N/P=4

[0262] Aliquots of 10 mg/mL ethanolic solutions of ICE, DOPE and DMG-PEG2K (molar ratio of 60:37:3) were mixed and diluted with ethanol to 90 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of CFTR mRNA was prepared from a 1 mg/mL stock solution so as to have a final volume of 360 mL.

[0263] In this process, the lipids dissolved in ethanol and the mRNA dissolved in citrate buffer were mixed using a pump system. The instantaneous mixing of the two streams resulted in formation of lipid nanoparticles. The resultant formulation mixture was in citrate buffer containing 20% ethanol. The formulation was subjected to a buffer exchange using TFF system and the resultant formulation was adjusted to the desired mRNA concentration and stored frozen until further use. Final concentration of the formulation was determined at 0.5 mg/mL CFTR mRNA (encapsulated). The lipid nanoparticles had an average size of around 94 nm with PDI of 0.15.

#### F. Formulation Example—60 mg Scale, N/P=4

[0264] Aliquots of 10 mg/mL ethanolic solutions of ICE, DOPE and DMG-PEG2K (molar ratio of 60:35:5) were

mixed and diluted with ethanol to 180 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of CFTR mRNA was prepared from a 1 mg/mL stock solution so as to have a final volume of 720 mL.

[0265] In this process, the lipids dissolved in ethanol and the mRNA dissolved in citrate buffer were mixed using a pump system. The instantaneous mixing of the two streams resulted in formation of lipid nanoparticles. The resultant formulation mixture was in citrate buffer containing 20% ethanol. The formulation was subjected to a buffer exchange using TFF system and the resultant formulation was adjusted to the desired mRNA concentration and stored frozen until further use. Final concentration of the formulation was determined at 0.5 mg/mL CFTR STOP mRNA (encapsulated). The formulation had about 97% mRNA encapsulation and the lipid nanoparticles had an average size of around 67 nm with PDI of 0.12.

#### G. Formulation Example—3.5 Gram Scale, N/P=4

[0266] Aliquots of 10 mg/mL ethanolic solutions of ICE, DOPE and DMG-PEG2K (molar ratio of 60:35:5) were mixed and diluted with ethanol to 42 L final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of CFTR mRNA was prepared from a 1 mg/mL stock solution so as to have a final volume of 42 L.

[0267] In this process, the lipids dissolved in ethanol and the mRNA dissolved in citrate buffer were mixed using a pump system. The instantaneous mixing of the two streams resulted in formation of lipid nanoparticles. The resultant formulation mixture was in citrate buffer containing 20% ethanol. The formulation was subjected to a buffer exchange using TFF system and the resultant formulation is adjusted to the desired mRNA concentration and stored frozen until further use. Final concentration of the formulation was determined at 0.5 mg/mL CFTR mRNA (encapsulated). The formulation had about 91% mRNA encapsulation and the lipid nanoparticles had an average size of around 50 nm with PDI of 0.17.

#### H. Formulation Example—200 mg Scale, N/P=4

[0268] Aliquots of 10 mg/mL ethanolic solutions of ICE, DOPE and DMG-PEG2K (molar ratio of 60:35:5) were mixed and diluted with ethanol to 600 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of FFL mRNA was prepared from a 1 mg/mL stock solution so as to have a final volume of 2.4 L.

[0269] In this process, the lipids dissolved in ethanol and the mRNA dissolved in citrate buffer were mixed using a pump system. The instantaneous mixing of the two streams results in formation of lipid nanoparticles. The resultant formulation mixture is in citrate buffer containing 20% ethanol. The formulation is subjected to a buffer exchange using TFF system and the resultant formulation was adjusted to the desired mRNA concentration and stored frozen until further use. Final concentration of the formulation was determined at 0.5 mg/mL CFTR mRNA (encapsulated). The formulation had about 95% mRNA encapsulation and the lipid nanoparticles had an average size of around 59 nm with PDI of 0.15.

#### I. Formulation Example—200 mg Scale, N/P=4

[0270] Aliquots of 10 mg/mL ethanolic solutions of ICE, DOPE and DMG-PEG2K (molar ratio of 60:35:5) were

mixed and diluted with ethanol to 600 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of hCFTR-STOP mRNA was prepared from a 1 mg/mL stock solution so as to have a final volume of 2.4 L.

[0271] In this process, the lipids dissolved in ethanol and the mRNA dissolved in citrate buffer were mixed using a pump system. The instantaneous mixing of the two streams resulted in formation of lipid nanoparticles. The resultant formulation mixture was in citrate buffer containing 20% ethanol. The formulation was subjected to a buffer exchange using TFF system and the resultant formulation is adjusted to the desired mRNA concentration and stored frozen until further use. Final concentration of the formulation was determined at 0.5 mg/mL CFTR STOP mRNA (encapsulated). The formulation had about 88% mRNA encapsulation and the lipid nanoparticles had an average size of around 57 nm with PDI of 0.18.

#### Pulmonary Delivery of ICE LNPs

[0272] Several exemplary studies were performed to demonstrate successful mRNA delivery and subsequent human CFTR protein production from ICE-based LNPs encapsulating codon-optimized hCFTR mRNA. Immunohistochemical analysis was performed on all lung sections using an anti-human CFTR antibody to specifically detect human CFTR protein within the lungs of treated animals.

#### Example 1. In Vivo Expression of hCFTR in Rat Lungs after Intratracheal Administration

[0273] FIG. 1 shows exemplary immunohistochemical detection of hCFTR protein in mice lungs 24 hours after pulmonary delivery of hCFTR mRNA lipid nanoparticles prepared by the process described above (Formulation Example #1).

[0274] Mice were administered LNP formulations containing hCFTR mRNA via microsprayer (intratracheal aerosol). The LNP formulations were made using ICE lipid as the cationic lipid. The fixed lung tissues from these mice were analyzed for the presence of hCFTR protein by immunohistochemical staining.

[0275] Protein was detected throughout the entire lung including both the bronchial epithelial cells and the alveolar regions. Positive (brown) staining was observed in all mRNA lipid nanoparticle test article groups, as compared to the lack of brown staining in the lungs of untreated control mice. Panel A depicts untreated mouse lung at 10 $\times$  magnification. Panel B depicts untreated mouse lung at 20 $\times$  magnification. Panel C depicts codon-optimized hCFTR (CO-hCFTR) mRNA ICE LNP-treated mouse lung at 10 $\times$  magnification. Panel D depicts CO-hCFTR mRNA ICE LNP-treated mouse lung at 20 $\times$  magnification.

#### Example 2. In Vivo Expression of hCFTR in Rat Lungs after Nebulization Administration

[0276] FIG. 2 shows exemplary immunohistochemical detection of hCFTR protein in mice lungs 24 hours after pulmonary delivery of hCFTR mRNA lipid nanoparticles prepared by the process described above (Formulation Example #1).

[0277] Mice were administered, via nebulization, LNP formulations containing hCFTR mRNA. The LNP formulations were made using ICE lipid as the cationic lipid. The

fixed lung tissues from these mice were analyzed for the presence of hCFTR protein by immunohistochemical staining.

[0278] Protein was detected throughout the entire lung including both the bronchial epithelial cells and the alveolar regions. Positive (brown) staining was observed in all mRNA lipid nanoparticle test article groups, as compared to the lack of brown staining in the lungs of untreated control mice. Panel A depicts untreated mouse lung at 10 $\times$  magnification. Panel B depicts untreated mouse lung at 20 $\times$  magnification. Panel C depicts codon-optimized hCFTR (CO-hCFTR) mRNA ICE LNP-treated mouse lung at 10 $\times$  magnification. Panel D depicts CO-hCFTR mRNA ICE LNP-treated mouse lung at 20 $\times$  magnification.

#### Example 3. In Vivo Expression of hCFTR in Rat Lungs (10 mg, 5% PEG)

[0279] FIG. 3 shows exemplary immunohistochemical analysis of hCFTR protein in rat lungs 24 hours after pulmonary delivery of codon-optimized hCFTR (CO-hCFTR) mRNA lipid nanoparticles prepared by the process described above using ICE-based lipid nanoparticles.

[0280] In some representative studies, rats were placed in an aerosol chamber which allowed for full motion of the rats. An aerosol was produced via nebulization of 10 mg (as measured by encapsulated mRNA) CO-hCFTR mRNA encapsulated in ICE LNPs (containing 5% [mol] of PEG-modified lipid) which filled the chamber containing the rats. The rats were exposed for a given period of time with during which the aerosol was freely taken into the lungs via normal breathing. After the exposure, the rats were removed and placed back into their cages. The rats were then sacrificed 24 hours after exposure and the lungs were harvested (a portion was snap frozen and a separate portion was fixed in 10% NBF and embedded in paraffin) for analysis. The fixed lung tissues from these rats were analyzed for the presence of hCFTR protein by immunohistochemical staining.

[0281] Protein was detected throughout the entire lung, including the bronchial epithelial cells and the alveolar regions, as shown in FIG. 3. Positive (brown) staining was observed in the mRNA lipid nanoparticle test article group. Panel A depicts the CO-hCFTR mRNA ICE LNP-treated rat lung at a low magnification. Panel B depicts CO-hCFTR mRNA ICE LNP-treated rat lung at 10 $\times$  magnification. Panel C depicts CO-hCFTR mRNA ICE LNP-treated rat lung at 20 $\times$  magnification.

#### Example 4. In Vivo Expression of hCFTR in Rat Lungs (50 $\mu$ g mRNA, 5% PEG)

[0282] FIG. 4 shows exemplary immunohistochemical analysis of hCFTR protein in rat lungs 24 hours after pulmonary delivery of CO-hCFTR mRNA lipid nanoparticles prepared by the process described above using ICE-based lipid nanoparticles.

[0283] In some representative studies, rats were exposed to CO-hCFTR mRNA ICE LNPs using direct instillation via a MicroSprayer apparatus. An aerosol was produced via a MicroSprayer apparatus during intratracheal administration of 50  $\mu$ g CO-hCFTR mRNA encapsulated ICE LNPs (containing 5% [mol] of PEG-modified lipid) in rats. The rats were then sacrificed 24 hours after exposure and the lungs were harvested (a portion was snap frozen and a separate portion was fixed in 10% NBF and embedded in paraffin) for

analysis. The fixed lung tissues from these rats were analyzed for the presence of hCFTR protein by immunohistochemical staining.

[0284] Protein was detected throughout the entire lung, including the bronchial epithelial cells and the alveolar regions, as shown in FIG. 4. Positive (brown) staining was observed in the mRNA lipid nanoparticle test article group. Panel A depicts the CO-hCFTR mRNA ICE LNP-treated rat lung at a low magnification. Panel B depicts CO-hCFTR mRNA ICE LNP-treated rat lung at 10 $\times$  magnification. Panel C depicts CO-hCFTR mRNA ICE LNP-treated rat lung at 20 $\times$  magnification.

**Example 5. In Vivo Expression of hCFTR in Rat Lungs (10 mg, 3% PEG)**

[0285] FIG. 5 shows exemplary immunohistochemical analysis of hCFTR protein in rat lungs 24 hours after pulmonary delivery of CO-hCFTR mRNA lipid nanoparticles prepared by the process described above using ICE-based lipid nanoparticles.

[0286] In some representative studies, rats were placed in an aerosol chamber which allowed for full motion of the rats. An aerosol was produced via nebulization of 10 mg CO-hCFTR mRNA encapsulated in ICE LNPs (containing 3% [mol] of PEG-modified lipid) which filled the chamber containing the rats. The rats were exposed for a given period of time during which the aerosol was freely taken into the lungs via normal breathing. After the exposure, the rats were removed and placed back into their cages. The rats were then sacrificed 24 hours after exposure and the lungs were harvested (a portion was snap frozen and a separate portion was fixed in 10% NBF and embedded in paraffin) for analysis. The fixed lung tissues from these rats were analyzed for the presence of hCFTR protein by immunohistochemical staining.

[0287] Protein was detected throughout the entire lung, including the bronchial epithelial cells and the alveolar regions, as shown in FIG. 5. Positive (brown) staining was observed in the mRNA lipid nanoparticle test article group. Panel A depicts the CO-hCFTR mRNA ICE LNP-treated rat lung at a low magnification. Panel B depicts CO-hCFTR mRNA ICE LNP-treated rat lung at 10 $\times$  magnification. Panel C depicts CO-hCFTR mRNA ICE LNP-treated rat lung at 20 $\times$  magnification.

**Example 6. In Vivo Expression of hCFTR in Rat Lungs (50  $\mu$ g mRNA, 3% PEG)**

[0288] FIG. 6 shows exemplary immunohistochemical analysis of hCFTR protein in rat lungs 24 hours after pulmonary delivery of CO-hCFTR mRNA lipid nanoparticles prepared by the process described above using ICE-based lipid nanoparticles.

[0289] In some representative studies, rats were exposed to CO-hCFTR mRNA ICE LNPs using direct instillation via a MicroSprayer apparatus. An aerosol was produced via a MicroSprayer apparatus during intratracheal administration of 50  $\mu$ g CO-hCFTR mRNA encapsulated ICE LNPs (containing 3% [mol] of PEG-modified lipid) in rats. The rats were then sacrificed 24 hours after exposure and the lungs were harvested (a portion was snap frozen and a separate portion was fixed in 10% NBF and embedded in paraffin) for

analysis. The fixed lung tissues from these rats were analyzed for the presence of hCFTR protein by immunohistochemical staining.

[0290] Protein was detected throughout the entire lung, including the bronchial epithelial cells and the alveolar regions, as shown in FIG. 6. Positive (brown) staining was observed in the mRNA lipid nanoparticle test article group. Panel A depicts the CO-hCFTR mRNA ICE LNP-treated rat lung at a low magnification. Panel B depicts CO-hCFTR mRNA ICE LNP-treated rat lung at 10 $\times$  magnification. Panel C depicts CO-hCFTR mRNA ICE LNP-treated rat lung at 20 $\times$  magnification.

**Example 7. In Vivo Expression of hCFTR in Mouse Lungs (10 mg, N/P=2)**

[0291] FIG. 7 shows exemplary immunohistochemical analysis of hCFTR protein in CFTR KO mouse lungs 24 hours after pulmonary delivery of CO-hCFTR mRNA lipid nanoparticles prepared by the process described above using ICE-based lipid nanoparticles.

[0292] In some representative studies, mice were placed in an aerosol chamber which allowed for full motion of the mice. An aerosol was produced via nebulization of 10 mg CO-hCFTR mRNA encapsulated in ICE LNPs (N/P=2) which filled the chamber containing the mice. The mice were exposed for a given period of time with which the aerosol was freely taken into the lungs via normal breathing. After the exposure, the mice were removed and placed back into their cages. The mice were then sacrificed 24 hours after exposure and the lungs were harvested (a portion was snap frozen and a separate portion was fixed in 10% NBF and embedded in paraffin) for analysis. The fixed lung tissues from these mice were analyzed for the presence of hCFTR protein by immunohistochemical staining.

[0293] Protein was detected throughout the entire lung, including the bronchial epithelial cells and the alveolar regions, as shown in FIG. 7. Positive (brown) staining was observed in the mRNA lipid nanoparticle test article group. Panel A depicts the CO-hCFTR mRNA ICE LNP-treated mouse lung at a low magnification. Panel B depicts CO-hCFTR mRNA ICE LNP-treated mouse lung at 10 $\times$  magnification. Panel C depicts CO-hCFTR mRNA ICE LNP-treated mouse lung at 20 $\times$  magnification.

**Example 8. In Vivo Expression of hCFTR in Mouse Lungs (10 mg, N/P=4)**

[0294] FIG. 8 shows exemplary immunohistochemical analysis of hCFTR protein in CFTR KO mouse lungs 24 hours after pulmonary delivery of CO-hCFTR mRNA lipid nanoparticles prepared by the process described above using ICE-based lipid nanoparticles.

[0295] In some representative studies, mice were placed in an aerosol chamber which allowed for full motion of the mice. An aerosol was produced via nebulization of 10 mg CO-hCFTR mRNA encapsulated in ICE LNPs (N/P=4) which filled the chamber containing the mice. The mice were exposed for a given period of time with which the aerosol was freely taken into the lungs via normal breathing. After the exposure, the mice were removed and placed back into their cages. The mice were then sacrificed 24 hours after exposure and the lungs were harvested (a portion was snap frozen and a separate portion was fixed in 10% NBF and embedded in paraffin) for analysis. The fixed lung tissues

from these mice were analyzed for the presence of hCFTR protein by immunohistochemical staining.

[0296] Protein was detected throughout the entire lung, including the bronchial epithelial cells and the alveolar regions, as shown in FIG. 8. Positive (brown) staining was observed in the mRNA lipid nanoparticle test article group. Panel A depicts the CO-hCFTR mRNA ICE LNP-treated mouse lung at a low magnification. Panel B depicts CO-hCFTR mRNA ICE LNP-treated mouse lung at 10 $\times$  magnification. Panel C depicts CO-hCFTR mRNA ICE LNP-treated mouse lung at 20 $\times$  magnification. Widespread distribution of hCFTR protein is observed in both example 5 (N/P=2) and example 6 (N/P=4).

#### Example 9. In Vivo Expression of hCFTR in Mouse Lungs with Different Formulations

[0297] FIG. 9 shows exemplary immunohistochemical analysis of hCFTR protein in wild-type mouse lungs 24 hours after pulmonary delivery of CO-hCFTR mRNA lipid nanoparticles prepared by the process described above using ICE-based lipid nanoparticles.

[0298] In some representative studies, several groups of wild-type mice were separated and placed in individual aerosol chambers. Each group was treated with a different formulation. The formulations consisted of ICE-based LNPs (N/P=4) encapsulating the following mRNA constructs: 1) CO-hCFTR mRNA ICE LNP; 2) CO-hCFTR “STOP” mRNA (nonsense mutated CO-hCFTR mRNA unable to translate protein) ICE LNP; 3) FFL (Firefly Luciferase) mRNA ICE LNP; and 4) Buffer (Vehicle).

[0299] In some embodiments, mice were placed in an aerosol chamber which allowed for full motion of the mice. An aerosol was produced via nebulizer of CO-hCFTR mRNA encapsulated in ICE LNPs (N/P=4) which filled the chamber containing the mice. The mice were exposed for a total of 50 mg (shown in FIG. 7) aerosolized CO-hCFTR mRNA (as measured by encapsulated mRNA) for a given period of time with which the aerosol was freely taken into the lungs via normal breathing. After the exposure, the mice were removed and placed back into their cages. The mice were then sacrificed 24 hours after exposure and the lungs were harvested (a portion was snap frozen and a separate portion was fixed in 10% NBF and embedded in paraffin) for analysis. The fixed lung tissues from these mice were analyzed for the presence of hCFTR protein by immunohistochemical staining.

[0300] In FIG. 9, positive (brown) staining was observed only in the CO-hCFTR mRNA LNP test article group. The first panel depicts the CO-hCFTR mRNA ICE LNP-treated mouse lung showing widespread distribution of human CFTR protein observed throughout the entire lung, including both bronchial epithelial and alveolar regions. The second panel depicts CO-hCFTR “STOP” mRNA ICE LNP-treated mouse lung with no positive (brown) staining observed. The third panel depicts FFL mRNA ICE LNP-treated mouse lung with no positive (brown) staining observed. The fourth panel depicts buffer treated mouse lung with no positive (brown) staining observed.

#### Example 10. In Vivo Expression of hCFTR in Mouse Lungs at Different Time Points

[0301] FIG. 10 shows exemplary immunohistochemical analysis of hCFTR protein in wild-type mouse lungs at

predetermined time points after pulmonary delivery of CO-hCFTR mRNA lipid nanoparticles prepared by the process described above using ICE-based lipid nanoparticles.

[0302] In some representative studies, the pharmacokinetic behavior of hCFTR protein after treatment of CO-hCFTR mRNA encapsulated ICE LNPs was performed in CFTR KO mice. In some embodiments, mice were placed in an aerosol chamber which allowed for full motion of the mice. An aerosol was produced via nebulization of CO-hCFTR mRNA encapsulated in ICE LNPs (N/P=4) which filled the chamber containing the mice. The mice were exposed to 10 mg aerosolized CO-hCFTR mRNA for a given period of time during which the aerosol was freely taken into the lungs via normal breathing. After the exposure, the mice were removed and placed back into their cages. Selected cohorts of treated mice were sacrificed at pre-determined time points after aerosol exposure. The time range for sacrificing the mice post-administration ranged from 30 minutes to one week, specifically, 30 minutes, 2 hours, 4 hours, 6 hours, 24 hours, 48 hours, 72 hours and 1 week as shown in FIG. 10. The mouse lungs were harvested (a portion was snap frozen and a separate portion was fixed in 10% NBF and embedded in paraffin) for analysis. The fixed lung tissues from these mice were analyzed for the presence of hCFTR protein by immunohistochemical staining.

[0303] As shown in FIG. 10, protein or positive (brown) staining was detected throughout the entire lung, including both the bronchial epithelial cells and the alveolar regions, in the hCFTR mRNA treated mouse groups sacrificed more than 30 minutes after administration. The most positive or brown staining was observed at 24 hours, 48 hours and 72 hours after hCFTR mRNA delivery. No positive (brown) staining was observed in the saline treated control group.

#### Example 11. In Vivo Expression of hCFTR in Rat Lungs at Different Dose Levels

[0304] In some representative studies, rats were placed in an aerosol chamber which allowed for full motion of the rats. An aerosol was produced via nebulization of CO-hCFTR mRNA encapsulated in ICE LNPs which filled the chamber containing the rats. The rats were exposed for a given period of time with which the aerosol was freely taken into the lungs via normal breathing. Several groups were dosed at different dose levels as following: 1) Buffer; 2) Empty ICE LNP (~8.0 mg/kg equivalent); 3) ICE LNP (0.5 mg/kg); 4) ICE LNP (2.1 mg/kg); 5) ICE LNP (4.1 mg/kg); and 6) ICE LNP (6.2 mg/kg).

[0305] After administration, the rats were removed and placed back into their cages. Furthermore, each group of rats was split into selected cohorts that were sacrificed at different predetermined time points post-administration. The time range for sacrificing the rats post-administration was 24 hours to 28 days, specifically 24 hours, 7 days, 14 days and 28 days.

[0306] Quantitative PCR, which measures copies of CO-hCFTR mRNA per total RNA, was performed on snap frozen lungs of all treated rats in each group. The results are represented in FIG. 11, showing the ratio of copies of CO-hCFTR mRNA per micrograms of total RNA. Results were analyzed across the different doses administered and at the selected sacrifice time points for each dose level. Background levels of CO-hCFTR mRNA are indicated by the control groups’ (Buffer and Empty LNP) values. Copies of

CO-hCFTR mRNA per total RNA in frozen lung sections of rats increased as the dose level increased. At the same dose level, copies of CO-hCFTR mRNA per total RNA in frozen lung sections of rats decreased as the sacrifice time post-administration increased.

**[0307]** FIG. 12 shows a comparison of the levels of copies of exogenous CO-hCFTR mRNA per copy of endogenous CFTR mRNA. The fold-increase or ratio of copies of CO-hCFTR mRNA per copy of endogenous CFTR mRNA in frozen lung sections of rats was assessed and plotted as a function of dose and sacrifice time. The data was also obtained using Quantitative PCR. Fold-increase of copies of CO-hCFTR mRNA over endogenous levels of CFTR mRNA increased as the dose level increased. At the same mRNA dosage, fold-increase of copies of CO-hCFTR mRNA over endogenous levels of CFTR mRNA decreased as the sacrifice time post-administration increased.

[0308] Immunohistochemical analysis of the rat lungs was also performed and is shown in FIG. 13. The fixed lung tissues from these rats were analyzed for the presence of hCFTR protein by immunohistochemical staining. A dose-dependent increase in hCFTR protein was observed as determined by positive (brown) staining throughout the entire lung, including both bronchial epithelial and alveolar regions. A decrease in hCFTR protein was observed as determined by positive (brown) staining as the time after the single dose of administration increased beyond 24 hours. However, detectable levels of hCFTR protein were observed 28 days after a single exposure of hCFTR mRNA ICE LNPs. No positive (brown) staining was observed in the saline treated control group or in the empty LNP group.

### Example 12. In Vivo Effect of hCFTR Formulations on Respiration

[0309] In some representative studies, rats were exposed to a single dose of hCFTR mRNA via inhalation. The

hCFTR mRNA was formulated with either ICE as described above or with branched PEI (bPEI). As shown in FIG. 14A, the various dosages of hCFTR mRNA formulated with bPEI caused an adverse respiratory rate increase relative to a saline control in the rats studied. When the rats were sacrificed, it was also found that the weight of the lungs was also increased. By comparison, as shown in FIG. 14B, the various dosages of hCFTR mRNA formulated with ICE did not cause an increase in respiratory rate relative to a buffer control in the rats studied. There was also no increase in the weight of the rat lungs observed when the rats were sacrificed.

### Example 13. In Vitro Expression and Activity of hCFTR at Different Dose Levels

[0310] In some representative experiments, hCFTR mRNA was transfected into cultured Fischer rat thyroid cells using Lipofectamine according to standard procedures. As is shown in FIG. 15, there was a dose response of chloride-ion channel activity induced by hCFTR mRNA. This indicates that the hCFTR mRNA produced active CFTR protein in the transfected cells.

EQUIVALENTS

[0311] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. The scope of the present invention is not intended to be limited to the above Description, but rather is as set forth in the following claims:

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**1-88.** (canceled)

**89.** A method of delivering messenger RNAs (mRNAs) encoding a protein or a peptide *in vivo* comprising administering by pulmonary delivery to a subject in need of delivery a composition comprising:

the mRNAs; and

lipid nanoparticles encapsulating the mRNAs, wherein each individual lipid nanoparticle comprises no more than three distinct lipid components, wherein the three lipid components are a non-cationic lipid, a PEG-modified lipid, and a sterol-based cationic lipid, wherein the sterol-based cationic lipid has a structure according to Formula (A),



or a protonated form thereof, wherein:

B is a basic functional group selected from pyrrolyl, pyrazolyl, triazolyl, tetrazolyl, pyridyl, pyrimidyl, pyrazinyl, and pyridazinyl wherein the protonated form has a pKa that is no more than about 8.0;

L<sup>1</sup> is an optionally substituted linker group; and

S is a sterol.

**90.** The method of claim 89, wherein the mRNAs are codon-optimized and/or comprise one or more modified nucleotides.

**91.** The method of claim 89, wherein the lipid nanoparticles have a size less than about 100 nm.

**92.** The method of claim 89, wherein the lipid nanoparticles have a lipid/mRNA (N/P) ratio of 2 or 4.

**93.** The method of claim 89, wherein the molar percentage of the PEG-modified lipid in the lipid nanoparticles is no more than 5%.

**94.** The method of claim 89, wherein the molar percentage of the sterol-based cationic lipid in the lipid nanoparticles is no more than 70%.

**95.** The method of claim 89, wherein the molar percentage of the sterol-based cationic lipid in the lipid nanoparticles is at least 40%.

**96.** The method of claim 89, wherein the composition is administered intravenously, or by pulmonary delivery, wherein the pulmonary delivery comprises nebulization.

**97.** A composition comprising:

messenger RNAs (mRNAs) encoding a protein or a peptide; and

lipid nanoparticles encapsulating the mRNAs, wherein each individual lipid nanoparticle comprises no more than three distinct lipid components, wherein the three lipid components are a non-cationic lipid, a PEG-modified lipid, and a sterol-based cationic lipid, wherein the sterol-based cationic lipid has a structure according to Formula (A),



or a protonated form thereof, wherein:

B is a basic functional group selected from pyrrolyl, pyrazolyl, triazolyl, tetrazolyl, pyridyl, pyrimidyl, pyrazinyl, and pyridazinyl wherein the protonated form has a pKa that is no more than about 8.0;

L<sup>1</sup> is an optionally substituted linker group; and

S is a sterol,

further wherein the lipid nanoparticles have an encapsulation percentage for mRNAs of at least 70%.

**98.** The composition of claim 97, wherein the sterol-based cationic lipid constitutes no more than 70% of the total lipids.

**99.** The composition of claim 97, wherein L<sup>1</sup> is an optionally substituted linker group that is a C<sub>1</sub>-C<sub>20</sub> alkylene or a 2- to 20-membered heteroalkylene.

**100.** The composition of claim 97, wherein L<sup>1</sup> does not comprise substituents having the structure —N(R')<sub>2</sub>, or a

positively charged form thereof, wherein each R<sup>t</sup> is independently hydrogen or optionally substituted C<sub>1</sub>-C<sub>20</sub> alkyl.

**101.** The composition of claim 97, wherein S is a sterol selected from a zoosterol, or an oxidized or reduced form thereof, a phytosterol, or an oxidized or reduced form thereof, a synthetic sterol, or an oxidized or reduced form thereof, cholesterol, an oxidized form of cholesterol, a reduced form of cholesterol, alkyl lithocholate, stigmastanol, stigmastanol, campesterol, ergosterol, and sitosterol.

**102.** The composition of claim 97, wherein the mRNAs are codon-optimized and/or comprise one or more modified nucleotides.

**103.** The composition of claim 97, wherein the lipid nanoparticles have a lipid/mRNA (N/P) ratio of 2 or 4.

**104.** The composition of claim 97, wherein the molar percentage of the PEG-modified lipid in the lipid nanoparticles is no more than 5%.

**105.** The composition of claim 97, wherein the molar percentage of the sterol-based cationic lipid in the lipid nanoparticles is no more than 70%.

**106.** The composition of claim 97, wherein the molar percentage of the sterol-based cationic lipid in the lipid nanoparticles is at least 40%.

**107.** A composition formulated for nebulization comprising:

messenger RNAs (mRNAs) encoding a protein or a peptide; and

lipid nanoparticles encapsulating the mRNAs, wherein each individual lipid nanoparticle comprises no more than three distinct lipid components, wherein the three lipid components are a non-cationic lipid, a PEG-modified lipid, and a sterol-based cationic lipid, wherein the sterol-based cationic lipid has a structure according to Formula (A),

B-L<sup>1</sup>-S

(Formula A),

or a protonated form thereof, wherein:

B is a basic functional group selected from pyrrolyl, pyrazolyl, triazolyl, tetrazolyl, pyridyl, pyrimidyl, pyrazinyl, and pyridazinyl wherein the protonated form has a pKa that is no more than about 8.0;

L<sup>1</sup> is an optionally substituted linker group; and

S is a sterol, and

further wherein the lipid nanoparticles have an encapsulation percentage for mRNAs of at least 70%.

**108.** The composition of claim 107, wherein the mRNAs are codon-optimized and/or comprise one or more modified nucleotides.

\* \* \* \* \*