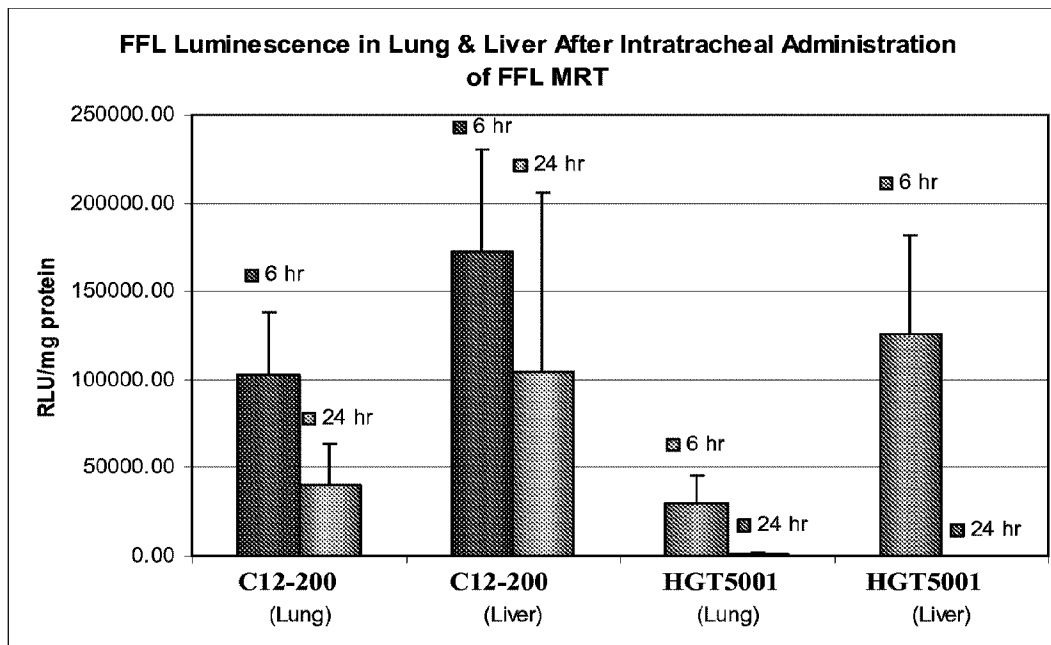




(86) Date de dépôt PCT/PCT Filing Date: 2013/06/07
(87) Date publication PCT/PCT Publication Date: 2013/12/12
(45) Date de délivrance/Issue Date: 2022/12/13
(85) Entrée phase nationale/National Entry: 2014/12/05
(86) N° demande PCT/PCT Application No.: US 2013/044771
(87) N° publication PCT/PCT Publication No.: 2013/185069
(30) Priorité/Priority: 2012/06/08 (US61/657,452)

(51) Cl.Int./Int.Cl. *A61K 9/72* (2006.01),
A61K 9/127 (2006.01)
(72) Inventeurs/Inventors:
HEARTLEIN, MICHAEL, US;
GUILD, BRAYDON CHARLES, US;
DEROSA, FRANK, US;
RUDOLPH, CARSTEN, DE;
PLANK, CHRISTIAN, DE
(73) Propriétaires/Owners:
ETHRIS GMBH, DE;
TRANSLATE BIO, INC., US
(74) Agent: FASKEN MARTINEAU DUMOULIN LLP

(54) Titre : ADMINISTRATION PULMONAIRE D'ARNM A DES CELLULES CIBLES AUTRES QUE PULMONAIRES
(54) Title: PULMONARY DELIVERY OF MRNA TO NON-LUNG TARGET CELLS



(57) Abrégé/Abstract:

Compositions comprising mRNA formulated for pulmonary administration and related methods for delivery of the mRNA and/or encoded protein to a non-lung cell or tissue. The compositions and methods may be used to prevent or ameliorate the symptoms of diseases associated with the mRNA encoded protein.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau(10) International Publication Number
WO 2013/185069 A1(43) International Publication Date
12 December 2013 (12.12.2013)

- (51) **International Patent Classification:**
A61K 48/00 (2006.01) A61K 9/00 (2006.01)
- (21) **International Application Number:**
PCT/US2013/044771
- (22) **International Filing Date:**
7 June 2013 (07.06.2013)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
61/657,452 8 June 2012 (08.06.2012) US
- (71) **Applicants:** SHIRE HUMAN GENETIC THERAPIES, INC. [US/US]; 300 Shire Way, Lexington, Massachusetts 02421 (US). ETHRIS GMBH [DE/DE]; Lochhamerstr. 11, 82152 Martinsried (DE).
- (72) **Inventors:** HEARTLEIN, Michael; Boxborough, Massachusetts (US). GUILD, Braydon Charles; Concord, Massachusetts (US). DEROSA, Frank; Chelmsford, Massachusetts (US). RUDOLPH, Carsten; Martinsried (DE). PLANK, Christian; Martinsried (DE).
- (74) **Agents:** CHEN, Fangli et al.; Choate, Hall & Stewart LLP, Two International Place, Boston, Massachusetts 02110 (US).

(81) **Designated States** (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

[Continued on next page]

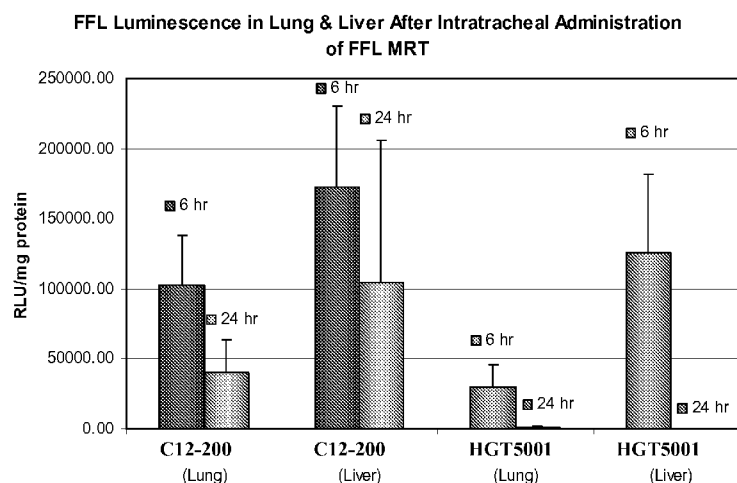
(54) **Title:** PULMONARY DELIVERY OF MRNA TO NON-LUNG TARGET CELLS

Figure 12

(57) **Abstract:** Compositions comprising mRNA formulated for pulmonary administration and related methods for delivery of the mRNA and/or encoded protein to a non-lung cell or tissue. The compositions and methods may be used to prevent or ameliorate the symptoms of diseases associated with the mRNA encoded protein.

WO 2013/185069 A1



— with sequence listing part of description (Rule 5.2(a))

PULMONARY DELIVERY OF mRNA TO NON-LUNG TARGET CELLS**RELATED APPLICATIONS****[0001]****BACKGROUND**

[0002] Conventional gene therapy involves the use of DNA for insertion of desired genetic information into host cells. The DNA introduced into the cell is usually integrated into the genome of one or more transfected cells, allowing for long-lasting action of the introduced genetic material in the host. While there may be substantial benefits to such sustained action, integration of exogenous DNA into a host genome may also have many deleterious effects. For example, it is possible that the introduced DNA will be inserted into an intact gene, resulting in a mutation which impedes or even totally eliminates the function of the endogenous gene. Thus, gene therapy with DNA may result in the impairment of a vital genetic function in the treated host, such as e.g., elimination or deleteriously reduced production of an essential enzyme or interruption of a gene critical for the regulation of cell growth, resulting in unregulated or cancerous cell proliferation. In addition, with conventional DNA based gene therapy it is necessary for effective expression of the desired gene product to include a strong promoter sequence, which again may lead to undesirable changes in the regulation of normal gene expression in the cell. It is also possible that the DNA based genetic material will result in the induction of undesired anti-DNA antibodies, which in turn, may trigger a possibly fatal immune response.

[0003] In contrast to DNA, the use of RNA as a gene therapy agent is substantially safer because (1) RNA does not involve the risk of being stably integrated into the genome of the transfected cell, thus eliminating the concern that the introduced genetic material will disrupt the normal functioning of an essential gene, or cause a mutation that results in deleterious or oncogenic effects; (2) extraneous promoter sequences are not required for effective translation of the encoded protein, again avoiding possible deleterious side effects; (3) in contrast to plasmid DNA (pDNA), messenger RNA (mRNA) is devoid of

immunogenic CpG motifs so that anti-RNA antibodies are not generated; and (4) any deleterious effects that do result from mRNA based on gene therapy would be of limited duration due to the relatively short half-life of RNA. Moreover in many applications, the transient nature of mRNA transfer to cells, i.e., wherein the duration of any therapeutic effect is limited by the life span of the mRNA and the protein product in the cells, is more desirable than the potentially longer lasting effect achieved using DNA based gene therapy. In addition, it is not necessary for mRNA to enter the nucleus to perform its function, thus avoiding a major barrier to DNA based gene therapy.

[0004] One reason that mRNA based gene therapy has not been used more in the past is that mRNA is far less stable than DNA, especially when it reaches the cytoplasm of a cell and is exposed to degrading enzymes. The presence of a hydroxyl group on the second carbon of the sugar moiety in mRNA causes steric hinderance that prevents the mRNA from forming the more stable double helix structure of DNA and thus makes the mRNA more prone to hydrolytic degradation. As a result, until recently, it was widely believed that mRNA was too labile to withstand transfection protocols.

[0005] Advances in RNA stabilizing modifications have sparked more interest in the use of mRNA in place of plasmid DNA in gene therapy. Yet, in spite of increased stability of modified mRNA, delivery of mRNA to cells in vivo in a manner allowing for therapeutic levels of protein production is still a challenge, particularly for mRNA encoding full length proteins. Some success has been achieved using viral vectors to introduce mRNA into a host, however mRNA transfection using viral vectors can result in an adverse immune response. In some circumstances, the viral vector may even integrate into the host genome. In addition, production of clinical grade viral vectors is also expensive and time consuming. Targeting delivery of the introduced genetic material using viral vectors can also be difficult to control.

[0006] Non-viral delivery of mRNA can be achieved using injection of naked nucleic acids, polyplexes, lipoplexes or liposome entrapped mRNA, biolistic delivery via gene gun, particulate carrier mediated delivery, and electroporation. Non-viral transfection or delivery vehicles are generally less-toxic, less immunogenic, and easier and less expensive to prepare than viral vectors for delivery of mRNA. Certain delivery vehicles, such as cationic lipid or polymer delivery vehicles may also help protect the transfected mRNA from endogenous RNases.

[0007] Liposomal delivery of nucleic acids has been employed as a means of effectuating the site-specific delivery of encapsulated plasmid DNA, antisense oligonucleotides, short interfering RNA and microRNA-based therapies. However the efficient, therapeutically effective, delivery of mRNAs to targeted cells and tissues, as well as the subsequent transfection of such targeted cells and tissues remains a technical challenge, particularly for delivery of mRNAs encoding full length proteins. It is important to design liposomal delivery systems that provide sufficient stability to reach desired target cells and the ability to efficiently release their encapsulated materials to such target cells to allow for translation of functional protein at therapeutically effective levels.

[0008] Many cationic lipids that are employed to construct such liposomal-based delivery vehicles are toxic to the targeted cells when used to deliver therapeutically effective amounts of the encapsulated agent. Accordingly, the toxicity associated with cationic lipid represents a significant obstacle to their general use as non-viral delivery vehicles, particularly in the quantities necessary to successfully deliver therapeutically effective amounts of mRNA to target cells.

[0009] To date, significant progress using mRNA gene therapy has been made in applications, particularly for which low levels of translation has not been a limiting factor, such as immunization with mRNA encoding antigens. Clinical trials involving vaccination against tumor antigens by intradermal injection of naked or protamine-complexed mRNA have demonstrated feasibility, lack of toxicity, and promising results. X. Su et al., *Mol. Pharmaceutics* 8:774-787 (2011). However, low levels of translation can restrict the exploitation of mRNA based gene therapy in other applications which require higher levels of sustained stability of the mRNA encoded protein to exert a prolonged biological or therapeutic effect.

[0010] In addition, because mRNA gene therapy benefits are relatively transient as compared to DNA based gene therapy, repeated administration, and typically by injection, are often required to provide long term effects. Thus, more efficient transfection in vivo and the ability to deliver mRNA noninvasively and/or to mucosal sites would improve the prospects for successful application of mRNA gene therapy.

SUMMARY

[0011] The present invention encompasses the surprising discovery that nanoparticle based formulations of mRNA are able to translocate following pulmonary delivery, i.e., move intact by either active or passive means from the lung to the systemic blood supply and subsequently to be deposited in different non-lung cells or tissues, such as, e.g., the liver. This translocation of the nanoparticle comprising an mRNA encoding a therapeutic protein, such as, e.g., beta-galactosidase, constitutes non-invasive systemic delivery of an active pharmaceutical ingredient beyond the lung to result in the production of a functional protein to systemically accessible non-lung cells or tissues.

[0012] Thus, the present invention provides methods for delivery of mRNA gene therapeutic agents using non-invasive pulmonary administrations. Among other things, the present invention provides for the delivery of mRNA encoding a protein, which can be used in a method for treating and/or preventing a disease. In one particular aspect, the invention provides a method for delivery of messenger RNA (mRNA) to non-lung cell or tissue comprising administering to the lung a composition comprising mRNA encoding a protein and a lipid carrier vehicle, wherein the administering to the lung results in the delivery of the mRNA and/or the protein to a non-lung cell or tissue.

[0013] In another aspect, the invention provides a method for delivery of a therapeutic protein to non-lung cell or tissue in a subject comprising administering to the lung a composition comprising mRNA encoding a therapeutic protein and a lipid carrier vehicle, such that the therapeutic protein is delivered to a non-lung cell or tissue.

[0014] In another aspect, the invention provides a method of inducing the production of a therapeutic protein in a non-lung cell or tissue in a subject comprising administering to the lung a composition comprising mRNA encoding a therapeutic protein and a lipid carrier vehicle.

[0015] In another aspect, the invention provides a method of treating a disease or disorder comprising administering to the lung a composition comprising mRNA encoding a therapeutic protein and a lipid carrier vehicle, wherein the administration to the lung results in the delivery of the therapeutic protein to a non-lung cell or tissue affected by the disease or disorder.

[0015a] According to one particular aspect, the invention relates to the use of a composition comprising mRNA encoding a protein and a lipid carrier vehicle comprising one or more PEG-modified lipids formulated for pulmonary delivery of mRNA, wherein administering to the lung delivers mRNA to a non-lung cell or tissue, and wherein the protein encoded by the mRNA is detectable in the non-lung cell or tissue for at least 6 hours following delivery to the lung.

[0015b] According to another particular aspect, the invention relates to a composition for pulmonary delivery of messenger RNA (mRNA) comprising mRNA encoding a protein and a lipid carrier vehicle, wherein the composition is formulated for pulmonary delivery to a non-lung cell or tissue and is formulated for an administration to the lung by nebulization, wherein the protein encoded by the mRNA is detectable in the non-lung cell or tissue for at least 6 hours following administration to the lung.

[0015c] According to another particular aspect, the invention relates to the use of a composition comprising mRNA encoding a protein and a lipid carrier vehicle comprising one or more PEG-modified lipids for delivering mRNA to a non-lung cell or tissue by nebulization delivers mRNA, and wherein the protein encoded by the mRNA is detectable in the non-lung cell or tissue for at least 6 hours following delivery to the lung.

[0015d] According to another particular aspect, the invention relates to a composition for pulmonary delivery of messenger RNA (mRNA) to a non-lung cell or tissue comprising mRNA encoding a protein and a lipid carrier vehicle comprising one or more PEG-modified lipids, wherein the composition is formulated for pulmonary delivery and administration to the lung, wherein the protein encoded by the mRNA is detectable in the non-lung cell or tissue for at least 6 hours following administration to the lung.

[0015e] According to another particular aspect, the invention relates to a composition for pulmonary delivery of messenger RNA (mRNA) to a non-lung cell or tissue comprising mRNA encoding a protein and a lipid carrier vehicle, wherein the composition is formulated for pulmonary delivery and administration to the lung by nebulization, wherein the protein encoded by the mRNA is detectable in the non-lung cell or tissue for at least 6 hours following administration to the lung.

[0016] In another aspect, the invention provides a composition for pulmonary delivery of messenger RNA (mRNA) comprising mRNA encoding a protein and a lipid carrier vehicle, wherein the composition is formulated such that once administered to the lung, it results in delivery of the mRNA and/or the protein to a non-lung cell or tissue.

[0017] In some embodiments, the composition is administered to the lung by aerosolization. In some embodiments, the composition is delivered to the lung by intratracheal aerosolization. In some embodiments, the composition is administered by nebulization. In some embodiments, the composition is administered to the lung by instillation. In some specific embodiments, the composition is administered to the lung of a subject using a device selected from the group consisting of a metered dose inhaler, jet-nebulizer, ultrasonic nebulizer, dry-powder-inhaler, propellant-based inhaler or an insufflator.

[0018] In some embodiments, the mRNA comprises a plurality of mRNA species, encoding one or more proteins. In some embodiments, the mRNA comprises at least two mRNA species, each encoding a different protein. In some embodiments, the mRNA encodes a full length protein. In some embodiments, the mRNA encodes a truncated version of a naturally occurring full length protein. In some embodiments, the mRNA encodes one or more proteins in a single transcript. In some embodiments, the mRNA encodes a chimeric protein, in which one or more protein sequences which are not naturally associated with the native protein are linked by a peptide bond in the resulting chimeric protein during expression. In some embodiments, an mRNA suitable for the present invention has a length of or greater than about 0.5kb, 1kb, 1.5kb, 2.0kb, 2.5kb, 3.0kb, 3.5kb, 4.0kb, 4.5kb, or 5.0kb.

[0019] In some embodiments, the mRNA encodes an intracellular protein. In some embodiments, the mRNA encodes a cytosolic protein. In some embodiments, the mRNA encodes a protein associated with the actin cytoskeleton. In some embodiments, the mRNA encodes a protein associated with the plasma membrane. In some specific embodiments, the mRNA encodes a transmembrane protein. In some specific embodiments, the mRNA encodes an ion channel protein. In some embodiments, the mRNA encodes a perinuclear protein. In some embodiments, the mRNA encodes a nuclear protein. In some specific embodiments, the mRNA encodes a transcription factor. In some embodiments, the mRNA encodes a chaperone protein. In some embodiments, the mRNA encodes an intracellular enzyme. In some embodiments, the mRNA encodes a protein involved in cellular metabolism, DNA repair, transcription and/or translation. In some embodiments, the mRNA

encodes an extracellular protein. In some embodiments, the mRNA encodes a protein associated with the extracellular matrix. In some embodiments the mRNA encodes a secreted protein.

[0020] In some embodiments, the mRNA encodes a protein (i.e. therapeutic protein), listed in Table 1, 2, 3 or 4. In some specific embodiments, the protein is selected from the group consisting of alpha galactosidase, erythropoietin, α 1-antitrypsin, carboxypeptidase N, alpha-L-iduronidase, iduronate-2-sulfatase, N-acetylglucosamine-1-phosphate transferase, N-acetylglucosaminidase, lysosomal acid lipase, arylsulfatase-A alpha-glucosaminide acetyltransferase, N-acetylglucosamine 6-sulfatase, N-acetylgalactosamine-4-sulfatase, beta-glucosidase, galactose-6-sulfate sulfatase, beta-galactosidase, beta-glucuronidase, glucocerebrosidase, heparan sulfamidase, hyaluronidase, galactocerebrosidase, human growth hormone, ornithine transcarbamylase (OTC), carbamyl phosphate synthetase-1 (CPS1), argininosuccinate synthetase-1 (ASS1), argininosuccinate lyase (ASL), arginase-1 (ARG1), cystic fibrosis transmembrane conductance regulator (CFTR), Factor VII, Factor VIII, Factor IX, heparan-N-sulfatase, and combinations thereof. In some specific embodiments, the protein is an intracellular or transmembrane protein selected from the group consisting of ornithine transcarbamylase (OTC), carbamyl phosphate synthetase-1 (CPS1), argininosuccinate synthetase-1 (ASS1), argininosuccinate lyase (ASL), arginase-1 (ARG1), cystic fibrosis transmembrane conductance regulator (CFTR), and combinations thereof.

[0021] In some embodiments, the mRNA encodes a protein that is associated with a disease or disorder (i.e., indication) listed in Table 4. In some embodiments, the protein for use in the method, is selected based on its ability to prevent, treat and/or cure a subject affected with a disease or disorder (i.e., indication) listed in Table 4. In specific embodiments, the disease or disorder is selected from the group consisting of SMN1-related spinal muscular atrophy (SMA), amyotrophic lateral sclerosis (ALS), GALT-related galactosemia, Cystic Fibrosis (CF), SLC3A1-related disorders, cystinuria, COL4A5-related disorders, Alport syndrome, galactocerebrosidase deficiencies, X-linked adrenoleukodystrophy, adrenomyeloneuropathy, Friedreich's ataxia, Pelizaeus-Merzbacher disease, TSC1 or TSC2-related tuberous sclerosis, Sanfilippo B syndrome (MPS IIIB), CTNS-related cystinosis, the FMR1-related disorders, include Fragile X syndrome, Fragile X-Associated Tremor/Ataxia Syndrome, Fragile X Premature Ovarian Failure Syndrome, Prader-Willi syndrome, Fabry disease, hereditary hemorrhagic telangiectasia (AT), Niemann-

Pick disease Type C1, neuronal ceroid lipofuscinoses-related diseases, Juvenile Neuronal Ceroid Lipofuscinosis (JNCL), Juvenile Batten disease, Santavuori-Haltia disease, Jansky-Bielschowsky disease, PTT-1 deficiency, TPP1 deficiency, EIF2B1, EIF2B2, EIF2B3, EIF2B4 and EIF2B5-related childhood ataxia with central nervous system hypomyelination/vanishing white matter, CACNA1A and CACNB4-related Episodic Ataxia Type 2, the MECP2-related disorders, Classic Rett Syndrome, MECP2-related Severe Neonatal Encephalopathy, PPM-X Syndrome, CDKL5-related Atypical Rett Syndrome, Kennedy's disease (SBMA), Notch-3 related cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), SCN1A and SCN1B-related seizure disorders, Polymerase G-related disorders, Alpers-Huttenlocher syndrome, POLG-related sensory ataxic neuropathy, dysarthria, ophthalmoparesis, autosomal dominant and recessive progressive external ophthalmoplegia with mitochondrial DNA deletions, X-Linked adrenal hypoplasia, X-linked agammaglobulinemia, Wilson's disease, and blood clotting disorders.

[0022] In some embodiments, following delivery to the lung, the mRNA and/or protein is delivered to a non-lung tissue. In some embodiments, the non-lung tissue comprises any organ and/or organ system of the body, excluding the lungs. In some specific embodiments, the non-lung tissue is selected from the group consisting of heart, liver, spleen, kidneys, skeletal muscle, lymph nodes, brain skin, cerebrospinal fluid, plasma and combinations thereof. In some specific embodiments, the non-lung tissue is liver. In some specific embodiments, the non-lung tissue is heart. In some specific embodiments, the non-lung tissue is spleen.

[0023] In some embodiments, following delivery to the lung, the mRNA and/or protein is delivered to a non-lung cell. In some embodiments, the non-lung cell is selected from the group consisting of hepatocytes, epithelial cells, hematopoietic cells, epithelial cells, endothelial cells, bone cells, stem cells, mesenchymal cells, neural cells, cardiac cells, adipocytes, vascular smooth muscle cells, cardiomyocytes, skeletal muscle cells, beta cells, pituitary cells, synovial lining cells, ovarian cells, testicular cells, fibroblasts, B cells, T cells, reticulocytes, leukocytes, granulocytes, tumor cells, macrophages, neutrophils, antigen presenting cells (dendritic cells), fibroblasts and combination thereof. In some specific embodiments, the non-lung cell is a hepatocyte.

[0024] In some embodiments, the mRNA and/or protein is detectable in the non-lung cell and/or tissue for at least about 4 hours, 6 hours, 8 hours, 10 hours, 12 hours, 18 hours, 24 hours, or more following the administration to the lung. In some embodiments, the mRNA and/or protein is detectable in the non-lung cell and/or tissue for at least about 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days or 10 days following the administration to the lung. In some embodiments, the mRNA and/or protein is detectable in the non-lung cell and/or tissue for at least about 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, or 6 weeks following administration to the lung. In some embodiments, the mRNA is detected using a methods selected the group consisting of in-situ hybridization, RT-PCR, Real-Time RT-PCR, Northern Blot, nuclease protection assay and combinations thereof. In some embodiments, the protein is detected using a methods selected from the group consisting of Western Blot, ELISA, immunoprecipitation, BCA assay, immunohistochemistry and combinations thereof.

[0025] In some embodiments, the mRNA is delivered at an amount greater than about 0.5 mg/kg (e.g., greater than about 1.0 mg/kg, 1.5 mg/kg, 2.0 mg/kg, 2.5 mg/kg, 3.0 mg/kg, 4.0 mg/kg, 5.0 mg/kg, 6.0 mg/kg, 7.0 mg/kg, 8.0 mg/kg, 9.0 mg/kg, or 10.0 mg/kg) body weight of mRNA per dose. In some embodiments, the mRNA is delivered at an amount ranging from about 0.1-100 mg/kg (e.g., about 0.1-90 mg/kg, 0.1-80 mg/kg, 0.1-70 mg/kg, 0.1-60 mg/kg, 0.1-50 mg/kg, 0.1-40 mg/kg, 0.1-30 mg/kg, 0.1-20 mg/kg, 0.1-10 mg/kg) body weight of mRNA per dose. In some embodiments, the mRNA is delivered at an amount of or greater than about 1 mg, 5 mg, 10 mg, 15 mg, 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, 50 mg, 55 mg, 60 mg, 65 mg, 70 mg, 75 mg, 80 mg, 85 mg, 90 mg, 95 mg, 100 mg, 150 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, 450 mg, or 500 mg per dose.

[0026] In some embodiments, the mRNA is encapsulated in a single lipid carrier vehicle. In some embodiments, the mRNA is encapsulated in one or more lipid carrier vehicles. In some embodiments, the mRNA is encapsulated in one or more lipid carrier vehicles, which differ in their lipid composition, molar ratio of lipid components, size, charge (Zeta potential), targeting ligands and combinations thereof.

[0027] In some embodiments, the lipid carrier vehicle is a liposome. In some embodiments, the liposome comprises one or more cationic lipids, one or more non-cationic lipids, one or more cholesterol-based lipids and one or more PEG-modified lipids. In some embodiments, the one or more cationic lipid is an ionizable lipid. In some embodiments, the

one or more cationic lipid is a cleavable lipid. In some embodiments, the one or more cationic lipid is a cholesterol-derived cationic lipid. In some embodiments, the one or more cationic lipids are selected from C12-200, HGT4003, HGT5000, HGT5001, RE-1, RE-2, RE-3, ICE, GL-67, DLinKC2-DMA, DODAP, DODMA, DLinDMA, CLinDMA and combinations thereof.

[0028] In some embodiments, the composition further comprises a pulmonary surfactant. In some embodiments, the composition is formulated as respirable particles. In some embodiments, the respirable particles have a size less than about 500 μ m (e.g., less than about 450 μ m, 400 μ m, 350 μ m, 300 μ m, 250 μ m, 200 μ m, 150 μ m, 100 μ m, or 50 μ m). In some embodiments, the composition is formulated as a nebulizable lipid. In some embodiments, the composition is formulated as a dry powder.

[0029] In various embodiments, the invention also provides a composition comprising mRNA encoding a protein and a lipid carrier vehicle as described herein for use in a method of delivery of messenger RNA (mRNA) to a non-lung cell or tissue, wherein the method comprises a step of administering the composition to the lung of a subject and further wherein the administering to the lung results in the delivery of the mRNA and/or protein to the non-lung cell or tissue.

[0030] In various embodiments, the invention provides a composition comprising mRNA encoding a protein and a lipid carrier vehicle as described herein for use in a method for delivery of therapeutic protein to a non-lung cell or tissue, wherein the method comprises a step of administering the composition to the lung of a subject.[0031]

[0032] In various embodiments, the invention provides a composition comprising mRNA encoding a protein and a lipid carrier vehicle as described herein for use in a method for inducing the production of a protein in a non-lung cell or tissue, wherein the method comprises a step of administering the composition to the lung.

[0033] In various embodiments, the invention provides a composition comprising mRNA encoding a protein and a lipid carrier vehicle as described herein for use in treating a disease or disorder, wherein the method comprises a step of administering the composition to the lung and further wherein the administering to the lung results in the delivery of mRNA and/or protein to a non-lung cell or tissue affected by the disease or disorder.[0034]

[0035] As used in this application, the terms “about” and “approximately” are used as equivalents. Any numerals used in this application with or without about/approximately are meant to cover any normal fluctuations appreciated by one of ordinary skill in the relevant art.

[0036] Other features, objects, and advantages of the present invention are apparent in the detailed description that follows. It should be understood, however, that the detailed description, while indicating embodiments of the present invention, is 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 from the detailed description.

BRIEF DESCRIPTION OF THE FIGURES

[0037] **Figure 1** shows bioluminescence Imaging (BLI) of mice at 6 hrs post intratracheal (IT) spray application. (A) Panels 1, 2, 3 show protein production in mice treated with naked FFL mRNA compared with protein production in mice treated with FFL mRNA in C12-200:DOPE: Cholesterol: DMG-PEG2000 (40:30:25:5) nanoparticles (NPs) in panels 4, 5, 6. (B) Panels 1, 2 (naked modified FFL mRNA) compared to panels 3, 4, 5 (modified FFL mRNA in C12-200 based NPs).

[0038] **Figure 2** shows BLI at 6 hrs post IT spray application using BLI. Panels 1, 2, 3 (FFL mRNA in C12-200 based NPs) compared to panels 4, 5, 6 (modified FFL mRNA in C12-200 based NPs).

[0039] **Figure 3** shows BLI images of mice post IT spray application. (A) All mice (FFL mRNA in C12-200 based NPs); panels 1, 2, 3 after 24 hours compared to panels 4, 5, 6 after 6 hours. (B) All mice (modified FFL mRNA in C12-200 based NPs); panels 1, 2 after 24 hours compared to panels 3, 4, 5 after 6 hours. (C) Panels 1, 2, (FFL mRNA in C12-200 based NPs) compared to panels 3, 4, 5 (modified FFL mRNA in C12-200 based NPs) after 24 hours.

[0040] **Figure 4** shows BLI images of mice treated with naked FFL mRNA at 24 hrs post applications. Panels 1, 2 (24 hours after first application); panels 3, 4 (24 hours after second application); panels 5, 6 (24 hours after third application).

[0041] **Figure 5** shows BLI images of mice treated with naked modified FFL mRNA at 24 hrs post application. Panels (24 hours after first application); panels 3, 4 (24 hours after second application); panels 5, 6 (24 hours after third application).

[0042] **Figure 6** shows BLI images of mice at 6 hours post IT spray application. (A) Panels 1, 2 (FFL mRNA in C12-200 based NPs (10 µg/mouse)); panels 3, 4 (FFL mRNA in C12-200 based NPs (5 µg/mouse)). (B) Panel 1 (modified FFL mRNA in C12-200 based NPs (10 µg/mouse)); panels 2, 3 (modified FFL mRNA in C12-200 based NPs (5 µg/mouse)).

[0043] **Figure 7** shows BLI images of mice at 6 hrs and 24 post IT spray application at doses of 10 µg/mouse. (A) All mice (FFL mRNA in C12-200 based NPs); panels 1, 2 (24 hours); panels 3, 4 (6 hours). (B) All mice (modified FFL mRNA in C12-200 based NPs); panel 1 (24 hours); panel 2 (6 hours). (C) Comparison of panels 1, 2 (FFL mRNA in C12-200 based NPs) with panel 3 (modified FFL mRNA in C12-200 based NPs) at 24 hrs post IT spray.

[0044] **Figure 8** shows biodistribution of FFL and modified FFL mRNA in C12-200 based NPs at 5 or 10 µg/mouse doses post IT spray.

[0045] **Figure 9** shows BLI images of mice at 6 hrs post IT spray application. Panels 1, 2, 3 (modified FFL mRNA in C12-200 based NPs); panels 4, 5 (modified FFL mRNA in HGT5001 based NPs)

[0046] **Figure 10** shows BLI images of mice at 6 hrs (panels 1, 2) and 24 hrs (panels 3, 4) post IT spray application of modified FFL mRNA in HGT5001:DOPE:Cholesterol:DMG-PEG2000 (40:20:35:5) nanoparticles at doses of 10µg/mouse.

[0047] **Figure 11** shows BLI image of mice at 24 hrs post IT spray application. (A) Mice with fur removed (modified FFL mRNA in C12-200 based NPs). (B) Mice with fur intact (modified FFL mRNA in C12-200 based NPs).

[0048] **Figure 12** shows FFL luminescence detected in lung, liver of mice after a single, intravenous injection treatment of mRNA-encapsulated lipid nanoparticles formulations, C12-200:DOPE:Cholesterol:DMG-PEG2000 (40:30:25:5) and HGT5001:DOPE:Cholesterol: DMG-PEG2000 (40:20:35:5). Mice were sacrificed at 6 hr and 24 hr post-administration.

[0049] **Figure 13** shows BLI images of mice 6 hours post nebulization with modified FFL mRNA (panels 1, 2) and FFL mRNA (panels 3, 4) in PEI based NPs.

DEFINITIONS

[0050] 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.

[0051] *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 $\text{H}_2\text{N}-\text{C}(\text{H})(\text{R})-\text{COOH}$. 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 carboxy- 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.

[0052] *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.

[0053] *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).

[0054] *Dosing regimen:* A “dosing regimen” (or “therapeutic regimen”), as that term is used herein, is a set of unit doses (typically more than one) that are administered individually to a subject, typically separated by periods of time. In some embodiments, a given therapeutic agent has a recommended dosing regimen, which may involve one or more doses. In some embodiments, a dosing regimen comprises a plurality of doses each of which are separated from one another by a time period of the same length; in some embodiments, a dosing regime comprises a plurality of doses and at least two different time periods separating individual doses.

[0055] *Expression:* As used herein, “expression” of a nucleic acid sequence refers to one or more of the following events: (1) production of an RNA template from a DNA sequence (*e.g.*, by transcription); (2) processing of an RNA transcript (*e.g.*, by splicing, editing, 5’ cap formation, and/or 3’ end formation); (3) translation of an RNA into a polypeptide or protein; and/or (4) post-translational modification of a polypeptide or protein. In this application, the terms “expression” and “production,” and grammatical equivalent, are used inter-changeably.

[0056] *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.

[0057] *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.

[0058] *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).

[0059] *messenger RNA (mRNA)*: As used herein, the term “messenger RNA (mRNA)” refers to a polynucleotide that encodes at least one polypeptide. mRNA as used herein encompasses both modified and unmodified RNA. mRNA may contain one or more coding and non-coding regions.

[0060] *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

analogues such as analogues 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 analogues (*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, O(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.

[0061] *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.

[0062] *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.

[0063] *Polypeptide:* As used herein, a "polypeptide", generally speaking, is a string of at least two amino acids attached to one another by a peptide bond. In some embodiments, a polypeptide may include at least 3-5 amino acids, each of which is attached to others by way of at least one peptide bond. Those of ordinary skill in the art will appreciate that

polypeptides sometimes include “non-natural” amino acids or other entities that nonetheless are capable of integrating into a polypeptide chain, optionally.

[0064] *Protein:* As used herein, the term “protein” or “therapeutic protein” refers to a polypeptide (*i.e.*, a string of at least two amino acids linked to one another by peptide bonds). Proteins may include moieties other than amino acids (*e.g.*, may be glycoproteins, proteoglycans, *etc.*) and/or may be otherwise processed or modified. Those of ordinary skill in the art will appreciate that a “protein” can be a complete polypeptide chain as produced by a cell (with or without a signal sequence), or can be a characteristic portion thereof. Those of ordinary skill will appreciate that a protein can sometimes include more than one polypeptide chain, for example linked by one or more disulfide bonds or associated by other means. Polypeptides may contain l-amino acids, d-amino acids, or both and may contain any of a variety of amino acid modifications or analogs known in the art. Useful modifications include, *e.g.*, terminal acetylation, amidation, methylation, *etc.* In some embodiments, proteins may comprise natural amino acids, non-natural amino acids, synthetic amino acids, and combinations thereof. The term “peptide” is generally used to refer to a polypeptide having a length of less than about 100 amino acids, less than about 50 amino acids, less than 20 amino acids, or less than 10 amino acids. In some embodiments, proteins are antibodies, antibody fragments, biologically active portions thereof, and/or characteristic portions thereof.

[0065] *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 but may or may not display symptoms of the disease or disorder.

[0066] *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.

[0067] *Treatment:* As used herein, the term “treatment” (also “treat” or “treating”) refers to any administration of a substance (e.g., provided compositions) that partially or completely alleviates, ameliorates, relieves, inhibits, delays onset of, reduces severity of, and/or reduces incidence of one or more symptoms, features, and/or causes of a particular disease, disorder, and/or condition (e.g., influenza). Such treatment may be of a subject who does not exhibit signs of the relevant disease, disorder and/or condition and/or of a subject who exhibits only early signs of the disease, disorder, and/or condition. Alternatively or additionally, such treatment may be of a subject who exhibits one or more established signs of the relevant disease, disorder and/or condition. In some embodiments, treatment may be of a subject who has been diagnosed as suffering from the relevant disease, disorder, and/or condition. In some embodiments, treatment may be of a subject known to have one or more susceptibility factors that are statistically correlated with increased risk of development of the relevant disease, disorder, and/or condition.

DETAILED DESCRIPTION

[0068] The present invention provides, among other things, methods and compositions for systemic delivery of mRNA and/or its protein product based on pulmonary delivery. In some embodiments, the present invention provides a method of administering a composition comprising mRNA and a lipid carrier vehicle to the lungs of a subject, for delivery of the mRNA and/or protein to non-lung cells and tissues. In some embodiments, mRNA encoding a single protein are delivered. In some embodiments one or more mRNA species encoding one or more proteins are delivered. In some embodiments, the mRNA is delivered using a single lipid carrier vehicle (e.g. liposome or lipid-derived nanoparticle). In some embodiments the mRNA is delivered using a one or more lipid carrier vehicles.

[0069] Various aspects of the invention are described in detail in the following sections. The use of sections is not meant to limit the invention. Each section can apply to any aspect of the invention. In this application, the use of “or” means “and/or” unless stated otherwise.

mRNA and mRNA Synthesis

[0070] 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.

[0071] In some embodiments, for the preparation of mRNA according to the invention, a DNA template is transcribed *in vitro*. A suitable DNA template typically has a promoter, for example a T3, T7 or SP6 promoter, for *in vitro* transcription, followed by desired nucleotide sequence for a desired mRNA and a termination signal.

[0072] Desired mRNA sequence according to the invention may be determined and incorporated into a DNA template using standard methods. For example, starting from a desired amino acid sequence a virtual reverse translation is carried out based on the degenerated genetic code. Optimization algorithms may then be used for selection of suitable codons. Typically, the G/C content can be optimized to achieve the highest possible G/C content on one hand, taking into the best possible account the frequency of the tRNAs according to codon usage on the other hand. The optimized RNA sequence can be established and displayed, for example, with the aid of an appropriate display device and compared with the original (wild-type) sequence. A secondary structure can also be analyzed to calculate stabilizing and destabilizing properties or, respectively, regions of the RNA.

[0073] mRNA according to the present invention may be synthesized as unmodified or modified mRNA. In some embodiments, the mRNA may include one or more chemical or structural modifications to abrogate mRNA interaction with toll-like receptors TLR3, TLR7, TLR8, and retinoid-inducible gene I (RIG-I) to reduce immunogenicity as well as improve stability of the mRNA.

[0074] For example, in certain embodiments, the mRNA may be modified as described in U.S. patent publication 2009/0286852, to comprise one or more pseudouridine residues. Kormann et al., *Nature Biotechnology* 29(2):154-157 (2011) describe replacement of uridine and cytidine with 2-thiouridine and 5-

methylcytidine to synergistically decrease mRNA binding to pattern recognition receptors TLR3, TLR7, TLR8, and RIG-I and increase stability of the mRNA. See EP2459231. In yet other embodiments, the mRNA may be modified to reduce immunogenicity as described in European Application EP10742089.

[0075] In other embodiments, modifications of mRNA can include, for example, modifications of the nucleotides of the RNA. An modified mRNA according to the invention can thus include, for example, backbone modifications, sugar modifications or base modifications. In some embodiments, mRNAs encoding a protein of interest 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-oxyacetic acid methyl ester, 5-methylaminomethyl-uracil, 5-methoxyaminomethyl-2-thio-uracil, 5'-methoxycarbonylmethyl-uracil, 5-methoxy-uracil, uracil-5-oxyacetic acid methyl ester, 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. No. 4,373,071, U.S. Pat. No. 4,401,796, U.S. Pat. No. 4,415,732, U.S. Pat. No. 4,458,066, U.S. Pat. No. 4,500,707, U.S. Pat. No. 4,668,777, U.S. Pat. No. 4,973,679, U.S. Pat. No. 5,047,524, U.S. Pat. No. 5,132,418, U.S. Pat. No. 5,153,319, U.S. Pat. Nos. 5,262,530 and 5,700,642.

See G. Tavernier et al, *J. Controlled Release* 150:238-247 (2011) and WO 2010/053572. See also US 2009/0286852 providing an extensive list of modified nucleosides, at ¶¶55, and 68-75 and WO 2008/052770 describing numerous mRNA modifications for increasing mRNA stability and protein production.

[0076] In some embodiments, mRNAs 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, methylphosphoramidates, phosphoramidates, 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.

[0077] In some embodiments, mRNAs 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'-deamine-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), or azidotriphosphates (2'-azido-2'-deoxycytidine 5'-triphosphate, 2'-azido-2'-deoxyuridine 5'-triphosphate).

[0078] In some embodiments, mRNAs 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, O6-methylguanosine

5'-triphosphate, pseudouridine 5'-triphosphate, puromycin 5'-triphosphate or xanthosine 5'-triphosphate.

[0079] In certain embodiments, stabilizing modifications may be made to either or both the 3' and 5' ends of the mRNA and include, e.g., end capping, polyA tail, replacement of unstable non-coding sequences (such as adenylate uridylylate rich elements (AREs) or addition or 3' or 5' untranslated sequences from stable mRNA (such as, e.g., β -globin, actin, GAPDH, tubulin, histone, or citric acid cycle enzyme mRNA). Stabilizing modifications may also be made within the mRNA, and include, e.g., codon optimization and/or modification of the Kozak sequence, and/or incorporation of modified nucleosides (such as, e.g., pyrrolo-pyrimidine, C5-iodouridine, 2-amino adenosine, and 2-thiothymidine). In certain embodiments, the modified mRNA used in the methods and compositions of the invention include a 5' untranslated sequence from CMV immediate-early 1 (IE1) gene:

XCAGAUCCGCGGAGACGCCAUCCACGCUGUUUUGACCUCCAUAGAAGACACC
GGGACCGAUCCAGCCUCCGCGGCCGGAACGGUGCAUUGGAACGCGGAUCCCC
CGUGCCAAGAGUGACUCACCGUCCUUGACACG, wherein X, if present is GGA (SEQ
ID NO:1), or a sequence that is at least 90% or at least 95% identical to SEQ ID NO:1, or a
and/or a 3' untranslated sequence from human growth hormone (hGH) gene:

CGGGUGGCAUCCCUGUGACCCCUCCCCAGUGCCUCUCCUGGCCCUGGAAGUUG
CCACUCCAGUGCCCACCGCCUUGUCCUAAUAAAAUUAAGUUGCAUC (SEQ ID
NO:2), or a sequence that is at least 90% or at least 95% identical to SEQ ID NO:2, to
improve the nuclease resistance and/or improve the half-life of the mRNA. In addition to
increasing the stability of the mRNA polynucleotide sequence, it has been surprisingly
discovered the inclusion of the untranslated sequence of CMV immediate-early 1 (IE1) gene
and/or the untranslated sequence from the hGH gene further enhances the translation of the
mRNA.

[0080] 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.

[0081] Thus, in some embodiments, mRNAs of the current invention 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 5'5'5 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.

[0082] In some embodiments, mRNAs of the current invention include a 3' poly(A) tail structure. A poly-A tail on the 3' terminus of mRNA typically includes about 10 to 300 adenosine nucleotides (e.g., about 10 to 200 adenosine nucleotides, about 10 to 150 adenosine nucleotides, about 10 to 100 adenosine nucleotides, about 20 to 70 adenosine nucleotides, or about 20 to 60 adenosine nucleotides). In some embodiments, mRNAs of the current invention include a 3' poly(C) tail structure. A suitable poly-C tail on the 3' terminus of mRNA typically include about 10 to 200 cytosine nucleotides (e.g., about 10 to 150 cytosine nucleotides, about 10 to 100 cytosine nucleotides, about 20 to 70 cytosine nucleotides, about 20 to 60 cytosine nucleotides, or about 10 to 40 cytosine nucleotides). The poly-C tail may be added to the poly-A tail or may substitute the poly-A tail.

[0083] In some embodiments, mRNAs of the current invention 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.

[0084] 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 of 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.

Proteins encoded by mRNAs

[0085] The mRNAs used in the compositions and methods of the invention may be used to express full length, truncated, native or modified protein for delivery to non-lung tissue and cells. In some embodiments, the mRNA comprises at least one mRNA species encoding a protein (i.e. a therapeutic protein). In some embodiments, the mRNA comprises a plurality of mRNA species, encoding one or more gene products. In some embodiments, the

mRNA comprises at least two mRNA species, each encoding a different gene product. In some embodiments, the mRNA encodes a full length protein. In some embodiments, the mRNA encode a truncated version of the naturally occurring full length protein. In some embodiments, the mRNA encode one or more truncated protein from different gene products in a single transcript. In some embodiments, the mRNA encodes a chimeric protein, in which one or more protein sequences which are not naturally associated with the native protein are linked by a peptide bond in the resulting chimeric protein during expression. In some embodiments, the mRNA may be used to express a partial or full length protein comprising cellular activity at a level equal to or greater than that of the native protein. In some embodiments, the mRNA may be used to express a partial or full length protein with cellular activity at a level equal to or less than that of the native protein.

[0086] In some embodiments the mRNA encodes an intracellular protein. In some embodiments, the mRNA encodes a cytosolic protein. In some embodiments, the mRNA encodes a protein associated with the actin cytoskeleton. In some embodiments, the mRNA encodes a protein associated with the plasma membrane. In some specific embodiments, the mRNA encodes a transmembrane protein. In some specific embodiments the mRNA encodes an ion channel protein. In some embodiments, the mRNA encodes a perinuclear protein. In some embodiments, the mRNA encodes a nuclear protein. In some specific embodiments, the mRNA encodes a transcription factor. In some embodiments, the mRNA encodes a chaperone protein. In some embodiments, the mRNA encodes an intracellular enzyme (e.g., mRNA encoding an enzyme associated with urea cycle or lysosomal storage metabolic disorders). In some embodiments, the mRNA encodes a protein involved in cellular metabolism, DNA repair, transcription and/or translation. In some embodiments, the mRNA encodes an extracellular protein. In some embodiments, the mRNA encodes a protein associated with the extracellular matrix. In some embodiments the mRNA encodes a secreted protein. In specific embodiments, the 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 neurotransmitters)

[0087] 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, as discussed below) 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, as discussed below) 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
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
A6NC14	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
A6NI73	Leukocyte immunoglobulin-like receptor subfamily A member 5	LILRA5
A6NIT4	Chorionic somatomammotropin hormone 2 isoform 2	CSH2

A6NJ69	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
A8MZH6	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 (S. cerevisiae), isoform CRA_a	SEC11L3
B4DJF9	Protein Wnt	WNT4
B4DUL4	SEC11-like 1 (S. cerevisiae), 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
C9JCI2	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
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
H0YNA5	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
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	Progonadoliberein-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
O75973	C1q-related factor	C1QL1
O76038	Secretagogen	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	BMP10
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	Matrilin-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
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	Gonadoliberein-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	Somatoliberein	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	Lymphotoxin-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
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	Trypstatin	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	Antileukoprotease	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	SERPINA5
P05155	Plasma protease C1 inhibitor	SERPING1
P05156	Complement factor I heavy chain	CFI
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
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
P0C091	FRAS1-related extracellular matrix protein 3	FREM3
P0C0L4	C4d-A	C4A
P0C0L5	Complement C4-B alpha chain	C4B
P0C0P6	Neuropeptide S	NPS
P0C7L1	Serine protease inhibitor Kazal-type 8	SPINK8
P0C862	Complement C1q and tumor necrosis factor-related protein 9A	C1QTNF9
P0C8F1	Prostate and testis expressed protein 4	PATE4
P0CG01	Gastroke-3	GKN3P
P0CG36	Cryptic family protein 1B	CFC1B
P0CG37	Cryptic protein	CFC1
P0CJ68	Humanin-like protein 1	MTRNR2L1
P0CJ69	Humanin-like protein 2	MTRNR2L2
P0CJ70	Humanin-like protein 3	MTRNR2L3
P0CJ71	Humanin-like protein 4	MTRNR2L4

P0CJ72	Humanin-like protein 5	MTRNR2L5
P0CJ73	Humanin-like protein 6	MTRNR2L6
P0CJ74	Humanin-like protein 7	MTRNR2L7
P0CJ75	Humanin-like protein 8	MTRNR2L8
P0CJ76	Humanin-like protein 9	MTRNR2L9
P0CJ77	Humanin-like protein 10	MTRNR2L10
P0DJD7	Pepsin A-4	PGA4
P0DJD8	Pepsin A-3	PGA3
P0DJD9	Pepsin A-5	PGA5
P0DJI8	Amyloid protein A	SAA1
P0DJI9	Serum amyloid A-2 protein	SAA2
P10082	Peptide YY(3-36)	PYY
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
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	PAPf39	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	CTRB1
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	IGFBP2

	2	
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
P19438	Tumor necrosis factor-binding protein 1	TNFRSF1A
P19652	Alpha-1-acid glycoprotein 2	ORM2
P19801	Amiloride-sensitive amine 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	Gastriecin	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
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 glypican-1	GPC1
P35070	Betacellulin	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
P47710	Casoxin-D	CSN1S1

P47929	Galectin-7	LGALS7B
P47972	Neuronal pentraxin-2	NPTX2
P47989	Xanthine oxidase	XDH
P47992	Lymphotoctin	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
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 glypican-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	Calcitermin	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	HD5(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
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(XIV) 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-acetylneuraminate-beta-galactosamide-alpha-2,3-sialyltransferase 1	ST3GAL1
Q11203	CMP-N-acetylneuraminate-beta-1,4-galactoside alpha-2,3-sialyltransferase	ST3GAL3
Q11206	CMP-N-acetylneuraminate-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
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	CLCA4

	regulator 4, 110 kDa form	
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	Tryptase alpha/beta-1	TPSAB1
Q15726	Metastin	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
Q2I0M5	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
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

Q4G0M1	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
Q5H8A3	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
Q5JZY3	Ephrin type-A receptor 10	EPHA10
Q5K4E3	Polyserase-2	PRSS36
Q5SRR4	Lymphocyte antigen 6 complex locus protein G5c	LY6G5C
Q5T1H1	Protein eyes shut 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
Q5VXI9	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
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
Q6MZW2	Follistatin-related protein 4	FSTL4
Q6NSX1	Coiled-coil domain-containing protein 70	CCDC70
Q6NT32	Carboxylesterase 5A	CES5A

Q6NT52	Choriogonadotropin subunit beta variant 2	CGB2
Q6NUI6	Chondroadherin-like protein	CHADL
Q6NUJ1	Saposin A-like	PSAPL1
Q6P093	Arylacetamide deacetylase-like 2	AADACL2
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	OLFM4
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
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	RSP02
Q6UY14	ADAMTS-like protein 4	ADAMTSL4
Q6UY27	Prostate and testis expressed protein 2	PATE2
Q6W4X9	Mucin-6	MUC6
Q6WN34	Chordin-like protein 2	CHRD2
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	Otogelin	OTOG
Q6ZRP7	Sulfhydryl oxidase 2	QSOX2
Q6ZWJ8	Kielin/chordin-like protein	KCP
Q75N90	Fibrillin-3	FBN3
Q765I0	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	HSD11B1L
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

Q7Z7C8	Transcription initiation factor TFIID subunit 8	TAF8
Q7Z7H5	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
Q86UW8	Hyaluronan and proteoglycan link protein 4	HAPLN4
Q86UX2	Inter-alpha-trypsin inhibitor heavy chain H5	ITI15
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	Gastrophilin-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
Q8I WV2	Contactin-4	CNTN4
Q8I WY4	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(XXVII) chain	COL27A1
Q8IZJ3	C3 and PZP-like alpha-2-macroglobulin domain-containing protein 8	CPAMD8
Q8IZN7	Beta-defensin 107	DEFB107B
Q8N0V4	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	Prosalsin	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
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
Q8NBI3	Draxin	DRAXIN
Q8NBM8	Prenylcysteine oxidase-like	PCYOX1L
Q8NBP7	Proprotein convertase subtilisin/kexin type 9	PCSK9
Q8NBQ5	Estradiol 17-beta-dehydrogenase 11	HSD17B11
Q8NBV8	Synaptotagmin-8	SYT8
Q8NCC3	Group XV phospholipase A2	PLA2G15
Q8NCF0	C-type lectin domain family 18 member C	CLEC18C
Q8NCW5	NAD(P)H-hydrate epimerase	APOA1BP
Q8NDA2	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
Q8NFK5	BPI fold-containing family B member 6	BPIFB6
Q8NFK6	BPI fold-containing family C protein	BPIFC
Q8NFU4	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	Secretoglobulin 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
Q8TEU8	WAP, kazal, immunoglobulin, kunitz and	WFIKKN2

	NTR domain-containing protein 2	
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
Q8WWQ1	Soluble calcium-activated nucleotidase 1	CANT1
Q8WWA0	Intellectin-1	ITLN1
Q8WWG1	Neuregulin-4	NRG4
Q8WWQ2	Inactive heparanase-2	HPSE2
Q8WWU7	Intellectin-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	PCSK5

	type 5	
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
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	LRCH3

	homology domain-containing protein 3	
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	WFIKKN1
Q96NZ9	Proline-rich acidic protein 1	PRAP1
Q96P44	Collagen alpha-1(XI) chain	COL21A1
Q96PB7	Noelin-3	OLFM3
Q96PC5	Melanoma inhibitory activity protein 2	MIA2
Q96PD5	N-acetylmuramoyl-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	Epiphyean	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
Q9BQ14	Coiled-coil domain-containing protein 3	CCDC3
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	Spexin	C12orf39
Q9BT67	NEDD4 family-interacting protein 1	NDFIP1
Q9BTY2	Plasma alpha-L-fucosidase	FUCA2
Q9BU40	Chordin-like protein 1	CHRD1

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 XIIB 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	Trypsin 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
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-acetyltransferase	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	SCUBE2

	domain-containing protein 2	
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	Gastrophilin-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
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 IIE 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	TXNDC16
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
Q9UHI8	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-cystinyl aminopeptidase, pregnancy serum form	LNPEP
Q9UJA9	Ectonucleotide pyrophosphatase/phosphodiesterase family member 5	ENPP5
Q9UJH8	Meteorin	METRIN
Q9UJJ9	N-acetylglucosamine-1-phosphotransferase 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
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 glypican-6	GPC6
Q9Y646	Carboxypeptidase Q	CPQ
Q9Y6C2	EMILIN-1	EMILIN1
Q9Y6F9	Protein Wnt-6	WNT6
Q9Y6I9	Testis-expressed sequence 264 protein	TEX264
Q9Y6L7	Tolloid-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
--------	--------------	---------

[0088] The Uniprot IDs set forth in Table 1 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; 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 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 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.

[0089] 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, as discussed below) 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, as discussed below) 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 domain-containing-like protein	IGHV4OR15-8

	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
P0C7V7	Putative signal peptidase complex catalytic subunit SEC11B	SEC11B
P0C854	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

[0090] The Uniprot IDs set forth in Table 2 refer to the human versions the listed putative proteins and the sequences of each are available from the Uniprot database. Sequences of the listed proteins are also 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 a protein chosen from mammalian homologs or homologs from an

animal of veterinary or industrial interest of a protein listed in 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 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 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.

[0091] 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, as discussed below) 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, as discussed below) along with other components set out herein.

Table 3. Lysosomal and Related Proteins

α -fucosidase
α -galactosidase
α -glucosidase
α -Iduronidase
α -mannosidase
α -N-acetylgalactosaminidase (α -galactosidase B)
β -galactosidase
β -glucuronidase
β -hexosaminidase
β -mannosidase

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-sulfate 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 α -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
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

[0092] 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. Accordingly, in some embodiments, compositions and methods of the invention provide for the delivery of one or more mRNAs encoding a protein chosen from mammalian homologs or homologs from an animal of veterinary or industrial interest of a protein listed in Table 3; 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 3 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 S3 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.

[0093] 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	Methylcrotonoyl-CoA carboxylase
3-Methylglutaconic aciduria	Methylglutaconyl-CoA hydratase
Actinic keratosis	
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	
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
Clostridium difficile associated diarrhea	
Clotting disorders	
COAD/COPD	
Cocaine addiction	
COL4A5-related disorders	
Cold contact urticaria	
Contraception, female	
Coronary artery diseases	
Corpus uteri cancer	
Corticobasal degeneration	
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 protoporphyria	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	Follicle stimulating hormone
Female sexual dysfunction	
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	
Gastroesophageal 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	
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
Hereditary angioedema	C1 esterase inhibitor
Hereditary hemorrhagic telangiectasia	
Hereditary hemorrhagic telangiectasia (AT)	
Hereditary 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
Hydrolethals	
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 (Clostridium difficile 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	
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	G-CSF
Niemann Pick disease, type A / B	SMPD1
Niemann Pick disease, type C	NPC1
Niemann-Pick disease Type C1	
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
Pediculosis capitis (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	
Polycythemia 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	
TPPI deficiencies	
Trachea, bronchus, lung cancers	
Tricuspid atresia	
TSC1	
TSC2-related tuberous sclerosis	
Type 2 diabetes mellitus	Glucagon-like peptide 1 (GLP-1) agonist
Type 2 diabetes mellitus	Insulin
Tyrosinemia, type I	Fumarylacetoacetase
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

[0094] In some embodiments, one or more therapeutic proteins of the current invention are selected from Table 1, 2, 3 or 4. In some specific embodiments, one or more therapeutic proteins are selected from the group consisting of alpha galactosidase, erythropoietin, α 1-antitrypsin, carboxypeptidase N, alpha-L-iduronidase, iduronate-2-sulfatase, N-acetylglucosamine-1-phosphate transferase, N-acetylglucosaminidase, lysosomal acid lipase, arylsulfatase-A alpha-glucosaminide acetyltransferase, N-acetylglucosamine 6-sulfatase, N-acetylgalactosamine-4-sulfatase, beta-glucosidase, galactose-6-sulfate sulfatase, beta-galactosidase, beta-glucuronidase, glucocerebrosidase, heparan sulfamidase, hyaluronidase, galactocerebrosidase, human growth hormone, ornithine transcarbamylase (OTC), carbamyl phosphate synthetase-1 (CPS1), argininosuccinate synthetase-1 (ASS1), argininosuccinate lyase (ASL), arginase-1 (ARG1), cystic fibrosis transmembrane conductance regulator (CFTR), Factor VII, Factor VIII, Factor IX, heparan-N-sulfatase, and combinations thereof.

[0095] 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. Diseases or disorders for which the compositions and methods of the invention may be employed include, but are not limited to, disorders such as SMN1-related spinal muscular atrophy (SMA), amyotrophic lateral sclerosis (ALS), GALT-related galactosemia, Cystic Fibrosis (CF), SLC3A1-related disorders, cystinuria, COL4A5-related disorders, Alport syndrome, galactocerebrosidase deficiencies, X-linked adrenoleukodystrophy, adrenomyeloneuropathy, Friedreich's ataxia, Pelizaeus-Merzbacher disease, TSC1 or TSC2-related tuberous sclerosis, Sanfilippo B syndrome (MPS IIIB), CTNS-related cystinosis, the FMR1-related disorders, include Fragile X syndrome, Fragile X-Associated Tremor/Ataxia Syndrome, Fragile X Premature Ovarian Failure Syndrome, Prader-Willi syndrome, Fabry disease, hereditary hemorrhagic telangiectasia (AT), Niemann-Pick disease Type C1, neuronal ceroid lipofuscinoses-related diseases, Juvenile Neuronal Ceroid Lipofuscinosis (JNCL), Juvenile Batten disease, Santavuori-Haltia disease, Jansky-Bielschowsky disease, PTT-1 deficiency, TPP1 deficiency, EIF2B1, EIF2B2, EIF2B3, EIF2B4 and EIF2B5-related childhood ataxia with central nervous system hypomyelination/vanishing white matter, CACNA1A and CACNB4-related Episodic Ataxia Type 2, the MECP2-related disorders, Classic Rett Syndrome, MECP2-related Severe Neonatal Encephalopathy, PPM-X Syndrome, CDKL5-related Atypical Rett Syndrome, Kennedy's disease (SBMA), Notch-3 related cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), SCN1A and SCN1B-related seizure disorders, Polymerase G-related disorders, Alpers-Huttenlocher syndrome, POLG-related sensory ataxic neuropathy, dysarthria, ophthalmoparesis, autosomal dominant and recessive progressive external ophthalmoplegia with mitochondrial DNA deletions, X-Linked adrenal hypoplasia, X-linked agammaglobulinemia, Wilson's disease, and blood clotting disorders.

[0096] In certain embodiments, the mRNA used in the compositions and methods of the invention may encode an antibody. In some embodiments, the mRNA may encode a protein that is made up of subunits that are encoded by more than one gene. For example, the protein may be a heterodimer, wherein each chain or subunit of the is encoded by a separate gene. Alternatively, a single mRNA may be engineered to encode more than one subunit. In one embodiment, the mRNA may encode full length antibodies (both heavy and light chains

of the variable and constant regions) or fragments of antibodies (e.g. Fab, Fv, or a single chain Fv (scFv) to confer immunity to a subject.

[0097] As used herein, the term “heavy chain” encompasses all types of naturally-occurring heavy chains of different classes of immunoglobulins, including but not limited to, IgM(μ), IgD(δ), IgG(γ), IgA(α), and IgE(ϵ), and biologically active variants thereof. Typically, a heavy chain according to the present invention contains the N-terminal variable region responsible for antigen recognition, typically including CDR 1, CDR 2 and CDR 3, separated by four framework regions (FR1, FR2, FR2, and FR4). Typically, the N-terminal variable region contains about 100 to 110 or more amino acids. In some embodiments, a heavy chain according to the present invention contains one or more of constant domains (e.g., C_H1, C_H2, and/or C_H3). In some embodiments, an mRNA encoding a heavy chain of an antibody is of or greater than 0.3 kb, 0.5 kb, 0.75 kb, 1.0 kb, 1.25 kb, 1.5 kb, 1.75 kb, 2.0 kb, 2.5 kb, 3.0 kb, 3.5 kb, 4.0 kb in length.

[0098] As used herein, the term “light chain” encompasses all types of naturally-occurring light chains of different classes of immunoglobulins, including but not limited to κ or λ isotypes, and biologically active variants thereof. Typically, a light chain according to the present invention comprises an N-terminal variable domain (V_L). In some embodiments, a light chain according to the present invention contains a C-terminal constant domain (C_L). In some embodiments, an mRNA encoding a light chain of an antibody is of or greater than 0.1 kb, 0.2 kb, 0.3 kb, 0.4 kb, 0.5 kb, 0.6 kb, 0.7 kb, 0.8 kb, 0.9 kb, 1.0 kb, 1.25 kb, 1.5 kb, 1.75 kb, 2.0 kb, 2.5 kb, or 3.0 kb in length.

[0099] According to the present invention, a heavy chain and light chain of an antibody may be encoded and delivered by a single mRNA or separate mRNAs. It is contemplated that it may be advantageous to deliver heavy chain encoding mRNA and light chain encoding mRNA at varying ratios in order to optimize production of fully assembled functional antibodies.

[0100] In some embodiments, the mRNA may additionally encode one or more secretory leader sequences which are operably linked to and direct secretion of an antibody, antibody fragment(s), or other protein(s). Suitable secretory leader sequences are described, for example, in US 2008/0286834 A1. While one embodiment of the present invention relates to methods and compositions useful for conferring immunity to a subject (e.g., via the translation of mRNA encoding functional antibodies), the inventions disclosed herein and

contemplated hereby are broadly applicable. In an alternative embodiment the compositions of the present invention encode antibodies that may be used to transiently or chronically effect a functional response in subjects. For example, the mRNA of the present invention may encode a functional monoclonal or polyclonal antibody, which upon translation and secretion from target cell may be useful for targeting and/or inactivating a biological target (e.g., a stimulatory cytokine such as tumor necrosis factor).

Lipid Carrier Vehicles

[0101] The use of lipid carrier vehicles to facilitate the delivery of nucleic acids to target cells is contemplated by the present invention. Lipid carrier vehicles (e.g., liposomes and lipid-derived nanoparticles) are generally useful in a variety of applications in research, industry, and medicine, particularly for their use as transfer vehicles of diagnostic or therapeutic compounds *in vivo* (Lasic, Trends Biotechnol., 16: 307-321, 1998; Drummond *et al.*, Pharmacol. Rev., 51: 691-743, 1999) and are usually characterized as microscopic vesicles having an interior aqua space sequestered from an outer medium by a membrane of one or more bilayers. Bilayer membranes of liposomes are typically formed by amphiphilic molecules, such as lipids of synthetic or natural origin that comprise spatially separated hydrophilic and hydrophobic domains (Lasic, Trends Biotechnol., 16: 307-321, 1998). Bilayer membranes of the liposomes can also be formed by amphiphilic polymers and surfactants (e.g., polymerosomes, niosomes, etc.).

[0102] In the context of the present invention, a lipid carrier vehicle typically serves to transport mRNA to a target cell. One unexpected and advantageous feature of the current invention, was the observation that pulmonary administration of mRNA, which is encapsulated within a lipid carrier vehicle, results in delivery of mRNA and/or the protein to non-lung tissue and cells. For the purposes of the present invention, the liposomal transfer vehicles are prepared to contain the desired nucleic acids. The process of incorporation of a desired entity (e.g., a nucleic acid) into a liposome is often referred to as “loading” (Lasic, *et al.*, FEBS Lett., 312: 255-258, 1992). The liposome-incorporated nucleic acids may be completely or be 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 a 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 of the present invention, the selected transfer vehicle is capable of enhancing the stability of the mRNA contained therein. The liposome can allow the encapsulated mRNA to reach the target cell and/or may preferentially allow the encapsulated mRNA to reach non-lung tissue and cells, following pulmonary delivery.

[0103] In some embodiments, a suitable lipid carrier vehicle is formulated as a lipid nanoparticle. As used herein, the phrase “lipid nanoparticle” and “lipid carrier vehicle” and “lipid-derived nanoparticle” are all used interchangeably, and refer to a delivery vehicle comprising one or more lipids (e.g., cationic lipids, non-cationic lipids, cholesterol-based lipids, and PEG-modified lipids). The contemplated lipid nanoparticles may be prepared by including multi-component lipid mixtures of varying ratios employing one or more cationic lipids, non-cationic lipids, cholesterol-based lipids, and PEG-modified lipids. Examples of suitable lipids include, for example, the phosphatidyl compounds (e.g., phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebroside, and gangliosides).

[0104] Cationic liposome/mRNA complexes can help to protect mRNA from enzymatic degradation and facilitate intracellular delivery by interacting with the negatively charged cell membrane. However, the cationic surface of these lipoplexes also mediates strong interactions with negatively charged proteins that serve to reduce the half-life of the lipoplexes in vivo. This effect may be reduced by employing one or more of a mechanism to reduce the interaction between the cationic liposome/mRNA complex and negatively charged proteins. In most embodiments, the delivery vehicles used in the compositions and methods of the invention comprise nanoparticles constructed from a combination of one or more cationic lipids, non-cationic lipids, such as neutral or helper lipids, and PEG-modified lipids.

[0105] Lipid Nanoparticles

[0106] In some embodiments, a suitable delivery vehicle is formulated as a lipid nanoparticle. Lipid nanoparticles of the current invention comprise one or more lipids (e.g., cationic lipids, non-cationic lipids, cholesterol-based lipids, and PEG-modified lipids). Also contemplated is the use of polymers as transfer vehicles, whether alone or in combination with other delivery vehicles. In some embodiments, the delivery vehicle is selected based upon its ability to facilitate pulmonary delivery and translocation to non-lung tissue.

[0107] As used herein, liposomal delivery vehicles, e.g. lipid nanoparticles, are usually characterized as microscopic vesicles having an interior aqueous space sequestered from an outer medium by a membrane of one or more bilayers. Bilayer membranes of liposomes are typically formed by amphiphilic molecules, such as lipids of synthetic or natural origin that comprise spatially separated hydrophilic and hydrophobic domains (Lasic, Trends Biotechnol., 16: 307-321, 1998). Bilayer membranes of the liposomes can also be formed by amphiphilic polymers and surfactants (e.g., polymersomes, niosomes, etc.). In the context of the present invention, a liposomal delivery vehicle typically serves to transport a desired mRNA to a target tissue. 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 a 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.

[0108] In certain embodiments of the invention, the carrier is formulated using a polymer as a carrier, alone or in combination with other carriers. Suitable polymers may include, for example, polyacrylates, polyalkylcyanoacrylates, polylactide, polylactide-polyglycolide copolymers, polycaprolactones, dextran, albumin, gelatin, alginate, collagen, chitosan, cyclodextrins, protamine, PEGylated protamine, PLL, PEGylated PLL and polyethylenimine (PEI). When PEI is present, it may be branched PEI of a molecular weight ranging from 10 to 40 kDa, e.g., 25 kDa branched PEI (Sigma #408727).

[0109] In some embodiments, a suitable delivery vehicle contains a cationic lipid. As used herein, the phrase "cationic lipid" refers to any of a number of lipid species that have a net positive charge at a selected pH, such as physiological pH. Several cationic lipids have

been described in the literature, many of which are commercially available. Particularly suitable cationic lipids for use in the compositions and methods of the invention include those described in international patent publications WO 2010/053572 (and particularly, CI 2-200 described at paragraph [00225]) and WO 2012/170930.

In certain embodiments, the compositions and methods of the invention employ a lipid nanoparticles comprising an ionizable cationic lipid described in U.S. provisional patent application 61/617,468, filed March 29, 2012 ,

such as, e.g, (15Z, 18Z)-N,N-dimethyl-6-(9Z, 12Z)-octadeca-9, 12-dien-1-yl)tetracos-15,18-dien-1-amine (HGT5000), (15Z, 18Z)-N,N-dimethyl-6-(9Z, 12Z)-octadeca-9, 12-dien-1-yl)tetracos-4,15,18-trien-1-amine (HGT5001), and (15Z,18Z)-N,N-dimethyl-6-(9Z, 12Z)-octadeca-9, 12-dien-1-yl)tetracos-5, 15, 18-trien-1-amine (HGT5002).

[0110] In some embodiments, the cationic lipid N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride or "DOTMA" is used. (Feigner et al. (Proc. Nat'l Acad. Sci. 84, 7413 (1987); U.S. Pat. No. 4,897,355). DOTMA can be formulated alone or can be combined with the neutral lipid, dioleoylphosphatidyl-ethanolamine or "DOPE" or other cationic or non-cationic lipids into a liposomal transfer vehicle or a lipid nanoparticle, and such liposomes can be used to enhance the delivery of nucleic acids into target cells. Other suitable cationic lipids include, for example, 5-carboxyspermylglycinedioctadecylamide or "DOGS," 2,3-dioleoyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminium or "DOSPA" (Behr et al. Proc. Nat'l Acad. Sci. 86, 6982 (1989); U.S. Pat. No. 5,171,678; U.S. Pat. No. 5,334,761), 1,2-Dioleoyl-3-Dimethylammonium-Propane or "DODAP", 1,2-Dioleoyl-3-Trimethylammonium-Propane or "DOTAP". Contemplated cationic lipids also include 1,2-distearoyloxy-N,N-dimethyl-3-aminopropane or "DSDMA", 1,2-dioleoyloxy-N,N-dimethyl-3-aminopropane or "DODMA", 1,2-dilinoleoyloxy-N,N-dimethyl-3-aminopropane or "DLinDMA", 1,2-dilinolenyloxy-N,N-dimethyl-3-aminopropane or "DLenDMA", N-dioleoyl-N,N-dimethylammonium chloride or "DODAC", N,N-distearyl-N,N-dimethylammonium bromide or "DDAB", N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide or "DMRIE", 3-dimethylamino-2-(cholest-5-en-3-beta-oxybutan-4-oxy)-1-(cis,cis-9,12-octadecadienoxy)propane or "CLinDMA", 2-[5'-(cholest-5-en-3-beta-oxy)-3'-oxapentoxo]-3-dimethyl-1-(cis,cis-9',12'-octadecadienoxy)propane or "CpLinDMA", N,N-dimethyl-3,4-dioleoyloxybenzylamine or "RE-1" (di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate) or "RE-2" ((6Z,25Z)-diethyl 16-((4-

(dimethylamino)butanoyl)oxy)hentriaconta-6,25-dienedioate) or "RE-3" ((9Z,28Z)-dimethyl 19-((4-(dimethylamino)butanoyl)oxy)heptatriaconta-9,28-dienedioate) (See, US2012/0027803) or "GL-67" (Andries et al, Molecular Pharmaceutics, 9: 2136-2145 (2012); Zhao et al., "Cationic Liposomes in Different Structural Levels for Gene Delivery", Non-Viral Gene Therapy, InTech publishing, 13: 293-318 (2011)) or "DMOBA", 1,2-N,N'-dioleylecarbonyl-3-dimethylaminopropane or "DOcarbDAP", 2,3-Dilinoleoyloxy-N,N'-dimethylpropylamine or "DLinDAP", 1,2-N,N'-Dilinoleylecarbonyl-3-dimethylaminopropane or "DLincarbDAP", 1,2-Dilinoleylecarbonyl-3-dimethylaminopropane or "DLinCDAP", 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane or "DLin-DMA", 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane or "DLin-K-XTC2-DMA", and 2-(2,2-di((9Z,12Z)-octadeca-9,12-dien-1-yl)-1,3-dioxolan-4-yl)-N,N-dimethylethanamine (DLin-KC2-DMA)) (See, WO 2010/042877; Semple et al., Nature Biotech. 28: 172-176 (2010)), or mixtures thereof. (Heyes, J., et al., J Controlled Release 107: 276-287 (2005); Morrissey, DV., et al., Nat. Biotechnol. 23(8): 1003-1007 (2005); PCT Publication WO2005/121348A1). In some embodiments, the cationic lipid is not "GL-67".

[0111] In some embodiments, one or more of the cationic lipids present in such a composition comprise at least one of an imidazole, dialkylamino, or guanidinium moiety.

[0112] In some embodiments, one or more of the cationic lipids present in such a composition are chosen from XTC (2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane), MC3 (((6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate), ALNY-100 ((3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-amine)), NC98-5 (4,7,13-tris(3-oxo-3-(undecylamino)propyl)-N1,N16-diundecyl-4,7,10,13-tetraazahexadecane-1,16-diamide), DODAP (1,2-dioleyle-3-dimethylammonium propane), HGT4003 (WO 2012/170889), ICE (WO 2011/068810), HGT5000 (U.S. Provisional Patent Application No. 61/617,468) or HGT5001 (cis or trans) (Provisional Patent Application No. 61/617,468), aminoalcohol lipidoids such as those disclosed in WO2010/053572, DOTAP (1,2-dioleyle-3-trimethylammonium propane), DOTMA (1,2-di-o-octadecenyl-3-trimethylammonium propane), DLinDMA (Heyes, J.;

Palmer, L.; Bremner, K.; MacLachlan, I. "Cationic lipid saturation influences intracellular delivery of encapsulated nucleic acids" *J. Contr. Rel.* 2005, 107, 276-287), DLin-KC2-DMA (Semple, S.C. et al. "Rational Design of Cationic Lipids for siRNA Delivery" *Nature Biotech.* 2010, 28, 172-176), C12-200 (Love, K.T. et al. "Lipid-like materials for low-dose in vivo gene silencing" *PNAS* 2010, 107, 1864-1869).

[0113] In some embodiments, a suitable delivery vehicle contains one or more non-cationic lipids. In some embodiments, a non-cationic lipid is a neutral lipid, i.e., a lipid that does not carry a net charge in the conditions under which the composition is formulated and/or administered. Such exemplary non-cationic or neutral lipids can be chosen from DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine), DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine), DOPE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine), DPPE (1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine), DMPE (1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine), DOPG (1,2-dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol)), and cholesterol.

[0114] The use of cholesterol-based cationic lipids is also contemplated by the present invention. Such cholesterol-based cationic lipids can be used, either alone or in combination with other cationic or non-cationic lipids. Suitable cholesterol-based cationic lipids include, for example, DC-Choi (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), or ICE.

[0115] In other embodiments, suitable lipid nanoparticles comprising one or more cleavable lipids, such as, for example, one or more cationic lipids or compounds that comprise a cleavable disulfide (S-S) functional group (e.g., HGT4001, HGT4002, HGT4003, HGT4004 and HGT4005), as further described in U.S. Provisional Application No: 61/494,745.

[0116] In addition, several reagents are commercially available to enhance transfection efficacy. Suitable examples include LIPOFECTIN (DOTMA:DOPE) (Invitrogen, Carlsbad, Calif.), LIPOFECTA INE (DOSPA:DOPE) (Invitrogen), LIPOFECTAMINE2000. (Invitrogen), FUGENE, TRANSFECTAM (DOGS), and EFFECTENE.

[0117] In some embodiments, the cationic lipid may comprise a molar ratio of about 1% to about 90%, about 2% to about 70%, about 5% to about 50%, about 10% to about 40% of the total lipid present in the transfer vehicle, or preferably about 20% to about 70% of the total lipid present in the transfer vehicle.

[0118] 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 lipids together which comprise the transfer vehicle (e.g., a lipid nanoparticle). Contemplated PEG-modified lipids include, but is 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 cell, (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).

[0119] Particularly useful exchangeable lipids are PEG-ceramides having shorter acyl chains (e.g., C14 or C18). 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 15%, about 4% to about 10%, or about 2% of the total lipid present in the liposomal transfer vehicle.

[0120] 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 H, 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), palmitoyl-oleoylphosphatidylcholine (POPC), palmitoyl-oleoyl-phosphatidylethanolamine (POPE), dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl phosphatidyl 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. When used in combination with a cationic lipid, the non-cationic lipid may comprise a molar ratio of 5% to about 90%, or preferably about 10 % to about 70% of the total lipid present in the transfer vehicle.

[0121] In particular embodiments, a suitable transfer vehicle (e.g., a lipid nanoparticle) is prepared by combining multiple lipid and/or polymer components. For example, a transfer vehicle may be prepared using C12-200, DOPE, chol, DMG-PEG2K at a molar ratio of 40:30:25:5, or DODAP, DOPE, cholesterol, DMG-PEG2K at a molar ratio of 18:56:20:6, or HGT5000, DOPE, chol, DMG-PEG2K at a molar ratio of 40:20:35:5, or HGT5001, DOPE, chol, DMG-PEG2K at a molar ratio of 40:20:35:5. The selection of cationic lipids, non-cationic lipids and/or PEG-modified lipids which comprise the lipid nanoparticle, as well as the relative molar ratio of such lipids to each other, is based upon the characteristics of the selected lipid(s), the nature of the intended target cells, the characteristics of the mRNA to be delivered. 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. For example, in embodiments, the percentage of cationic lipid in the lipid nanoparticle may be greater than 10%, greater than 20%, greater than 30%, greater than 40%, greater than 50%, greater than 60%, or greater than 70%. The percentage of non-cationic lipid in the lipid nanoparticle may be greater than 5%, greater than 10%, greater than 20%, greater than 30%, or greater than 40%. The percentage of cholesterol in the lipid nanoparticle may be greater than 10%, greater than 20%, greater than 30%, or greater than 40%. The percentage of PEG-modified lipid in the lipid nanoparticle may be greater than 1%, greater than 2%, greater than 5%, greater than 10%, or greater than 20%.

[0122] In certain embodiments, suitable lipid nanoparticles of the invention comprise at least one of the following cationic lipids: C12-200, HGT4003, HGT5000, HGT5001, RE-1, RE-2, RE3, GL-67 and ICE. In some specific embodiments, a suitable lipid nanoparticle is formulated without using the cationic lipid GL-67. In some embodiments, suitable transfer vehicle comprises cholesterol and/or a PEG-modified lipid. In some embodiments, suitable transfer vehicles comprises DMG-PEG2K. In some embodiments, suitable transfer vehicle

comprises one of the following lipid combinations: C12-200, DOPE, cholesterol, DMG-PEG2K; DODAP, DOPE, cholesterol, DMG-PEG2K; HGT5000, DOPE, cholesterol, DMG-PEG2K; HGT5001, DOPE, cholesterol, DMG-PEG2K; XTC, DSPC, cholesterol, PEG-DMG; MC3, DSPC, cholesterol, PEG-DMG; and ALNY-100, DSPC, cholesterol, DLinKC2-DMA, , DODMA, DLinDMA, CLinDMA PEG-DSG.

[0123] The lipid carrier vehicles for use in the compositions of the invention can be prepared by various techniques which are presently known in the art. Multilamellar 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 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.

[0124] In certain embodiments of this invention, the compositions of the present invention comprise a transfer vehicle wherein the mRNA is associated on both the surface of the transfer vehicle 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 mRNA through electrostatic interactions. For example, during preparation of the compositions of the present invention, cationic liposomal transfer vehicles may associate with the mRNA through electrostatic interactions.

[0125] In some embodiments, the compositions and methods of the invention comprise mRNA encapsulated in a lipid carrier vehicle. In some embodiments, the one or more mRNA species may be encapsulated in the same lipid carrier vehicle. In some embodiments, the one or more mRNA species may be encapsulated in different lipid carrier vehicles. In some embodiments, the mRNA is encapsulated in one or more lipid carrier vehicles, 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 lipid carrier vehicles may have a different composition of cationic lipids, neutral lipid, PEG-modified lipid and/or combinations thereof. In some embodiments the one or more lipid carrier vehicles may have a different molar ratio of cationic lipid, neutral lipid, cholesterol and PEG-modified lipid used to create the lipid carrier vehicle.

Delivery Methods

[0126] The route of delivery used in the methods of the invention allows for non-invasive, self-administration of the therapeutic compositions of the invention. The methods of the invention involve intratracheal or pulmonary administration by aerosolization, nebulization, or instillation of compositions comprising mRNA encoding a therapeutic protein in a suitable transfection or lipid carrier vehicles as described above.

[0127] Although the local cells and tissues of the lung represent a potential target capable of functioning as a biological depot or reservoir for production and secretion of the protein encoded by the mRNA, applicants have discovered that administration of the compositions of the invention to the lung via aerosolization, nebulization, or instillation results in the distribution of even non-secreted proteins outside the lung cells. Without wishing to be bound by any particular theory, it is contemplated that nanoparticle compositions of the invention pass, through the lung airway-blood barrier, resulting in translocation of the intact nanoparticle to non-lung cells and tissues, such as, e.g., the heart, the liver, the spleen, where it results in the production of the encoded protein in these non-lung tissues. Thus, the utility of the compositions and methods of the invention extend beyond production of therapeutic protein in lung cells and tissues of the lung and can be used to delivery to non-lung target cells and/or tissues. They are useful in the management and treatment of a large number of diseases, and in particular peripheral diseases which result from both secreted and non-secreted protein and/or enzyme deficiencies (e.g., one or more lysosomal storage disorders). In certain embodiments, the compositions of the invention, used in the methods of the invention result in the distribution of the mRNA encapsulated nanoparticles and production of the encoded protein in the liver, spleen, heart, and/or other non-lung cells. For example, administration of the compositions of the present inventions, such as, e.g., a nanoparticle comprising mRNA encoding beta galactosidase (a non-secreted protein), by aerosolization, nebulization, or instillation to the lung will result in the composition itself and its protein product (e.g., functional beta galactosidase protein) will be detectable in both the local cells and tissues of the lung, as well as in peripheral target cells, tissues and organs as a result of translocation of the mRNA and delivery vehicle to non-lung cells.

[0128] In certain embodiments, the compositions of the invention may be employed in the methods of the invention to specifically target peripheral cells or tissues. Following the pulmonary delivery, it is contemplated that the compositions of the invention cross the lung airway-blood barrier and distribute into cells other than the local lung cells. Accordingly, the compositions disclosed herein may be administered to a subject by way of the pulmonary route of administration, using a variety of approach known by those skilled in the art (e.g., by inhalation), and distribute to both the local target cells and tissues of the lung, as well as in peripheral non-lung cells and tissues (e.g., cells of the liver, spleen, kidneys, heart, skeletal muscle, lymph nodes, brain, cerebrospinal fluid, and plasma). As a result, both the local cells of the lung and the peripheral non-lung cells can serve as biological reservoirs or depots capable of producing and/or secreting a translation product encoded by one or more polynucleotides. Accordingly, the present inventions are not limited to the treatment of lung diseases or conditions, but rather can be used as a non-invasive means of facilitating the delivery of polynucleotides, or the production of enzymes and proteins encoded thereby, in peripheral organs, tissues and cells (e.g., hepatocytes) which would otherwise be achieved only by systemic administration. Exemplary peripheral non-lung cells include, but are not limited to, hepatocytes, epithelial cells, hematopoietic cells, epithelial cells, endothelial cells, bone cells, stem cells, mesenchymal cells, neural cells, cardiac cells, adipocytes, vascular smooth muscle cells, cardiomyocytes, skeletal muscle cells, beta cells, pituitary cells, synovial lining cells, ovarian cells, testicular cells, fibroblasts, B cells, T cells, reticulocytes, leukocytes, granulocytes and tumor cells.

[0129] Following administration of the composition to the subject, the protein product encoded by the mRNA (e.g., a functional protein or enzyme) is detectable in the peripheral target tissues for at least about one to seven days or longer following administration of the composition to the subject. The amount of protein product necessary to achieve a therapeutic effect will vary depending on the condition being treated, the protein encoded, and the condition of the patient. For example, the protein product may be detectable in the peripheral target tissues at a concentration (e.g., a therapeutic concentration) of at least 0.025-1.5 µg/ml (e.g., at least 0.050 µg/ml, at least 0.075 µg/ml, at least 0.1 µg/ml, at least 0.2 µg/ml, at least 0.3 µg/ml, at least 0.4 µg/ml, at least 0.5 µg/ml, at least 0.6 µg/ml, at least 0.7 µg/ml, at least 0.8 µg/ml, at least 0.9 µg/ml, at least 1.0 µg/ml, at least 1.1 µg/ml, at least 1.2 µg/ml, at least 1.3 µg/ml, at least 1.4 µg/ml, or at least 1.5 µg/ml), for at least about 1, 2, 3, 4, 5, 6, 7, 8, 9,

10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45 days or longer following administration of the composition to the subject.

[0130] It has been demonstrated that nucleic acids can be delivered to the lungs by intratracheal administration of a liquid suspension of the nucleic acid composition and inhalation of an aerosol mist produced by a liquid nebulizer or the use of a dry powder apparatus such as that described in U.S. patent 5,780,014.

[0131] In certain embodiments, the compositions of the invention may be formulated such that they may be aerosolized or otherwise delivered as a particulate liquid or solid prior to or upon administration to the subject. Such compositions may be administered with the assistance of one or more suitable devices for administering such solid or liquid particulate compositions (such as, e.g., an aerosolized aqueous solution or suspension) to generate particles that are easily respirable or inhalable by the subject. In some embodiments, such devices (e.g., a metered dose inhaler, jet-nebulizer, ultrasonic nebulizer, dry-powder-inhalers, propellant-based inhaler or an insufflator) facilitate the administration of a predetermined mass, volume or dose of the compositions (e.g., about 0.5 mg/kg of mRNA per dose) to the subject. For example, in certain embodiments, the compositions of the invention are administered to a subject using a metered dose inhaler containing a suspension or solution comprising the composition and a suitable propellant. In certain embodiments, the compositions of the invention may be formulated as a particulate powder (e.g., respirable dry particles) intended for inhalation. In certain embodiments, compositions of the invention formulated as respirable particles are appropriately sized such that they may be respirable by the subject or delivered using a suitable device (e.g., a mean D50 or D90 particle size less than about 500 μ m, 400 μ m, 300 μ m, 250 μ m, 200 μ m, 150 μ m, 100 μ m, 75 μ m, 50 μ m, 25 μ m, 20 μ m, 15 μ m, 12.5 μ m, 10 μ m, 5 μ m, 2.5 μ m or smaller). In yet other embodiments, the compositions of the invention are formulated to include one or more pulmonary surfactants (e.g., lamellar bodies). In some embodiments, the compositions of the invention are administered to a subject such that a concentration of at least 0.05 mg/kg, at least 0.1 mg/kg, at least 0.5 mg/kg, at least 1.0 mg/kg, at least 2.0 mg/kg, at least 3.0 mg/kg, at least 4.0 mg/kg, at least 5.0 mg/kg, at least 6.0 mg/kg, at least 7.0 mg/kg, at least 8.0 mg/kg, at least 9.0 mg/kg, at least 10 mg/kg, at least 15 mg/kg, at least 20 mg/kg, at least 25 mg/kg, at least 30 mg/kg, at least 35 mg/kg, at least 40 mg/kg, at least 45 mg/kg, at least 50 mg/kg, at least 55 mg/kg, at least 60 mg/kg, at least 65 mg/kg, at least 70 mg/kg, at least 75 mg/kg, at least

80 mg/kg, at least 85 mg/kg, at least 90 mg/kg, at least 95 mg/kg, or at least 100 mg/kg body weight is administered in a single dose. In some embodiments, the compositions of the invention are administered to a subject such that a total amount of at least 0.1 mg, at least 0.5 mg, at least 1.0 mg, at least 2.0 mg, at least 3.0 mg, at least 4.0 mg, at least 5.0 mg, at least 6.0 mg, at least 7.0 mg, at least 8.0 mg, at least 9.0 mg, at least 10 mg, at least 15 mg, at least 20 mg, at least 25 mg, at least 30 mg, at least 35 mg, at least 40 mg, at least 45 mg, at least 50 mg, at least 55 mg, at least 60 mg, at least 65 mg, at least 70 mg, at least 75 mg, at least 80 mg, at least 85 mg, at least 90 mg, at least 95 mg or at least 100 mg mRNA is administered in one or more doses.

EXAMPLES

Example 1: Comparison of unmodified and modified mRNA in naked form or in nanoparticles by intratracheal administration

[0132] Overview: Mice were intratracheally (IT) sprayed either with unmodified or modified mRNA (25% of both cytidine-5'-triphosphate and uridine-5'-triphosphate were replaced by 5-methylcytidine-5'-triphosphate and 2-thiouridine-5'-triphosphate, respectively) coding for firefly luciferase (FFL) either in naked form or encapsulated in lipid based nanoparticles (NPs) for single dose administration. Luciferase production was measured by in vivo Bioluminescence Imaging (BLI) at different time points post IT spray. Organs from mice treated with C12-200 based NPs at a dose amounting to 20 µg/mouse were prepared for histopathological analysis. To assess biodistribution of complexes post IT spray, in vitro luciferase production was measured in the organs prepared from the euthanized mice treated with doses corresponding to 5 and 10 µg C12-200 based NPs per mouse.

A. IT spray of naked mRNA and mRNA in C12-200 based nanoparticles - 20 ug per mouse

[0133] Lipid Nanoparticle Formulation: Aliquots of 50 mg/mL ethanolic solutions of C12-200, DOPE, Cholesterol, and DMG-PEG2000 were mixed in a molar ratio of 40:30:25:5, respectively, and diluted with ethanol to 3 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of FFL or modified FFL mRNA was prepared from a 1 mg/mL stock. 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 with 1x PBS (pH 7.4), concentrated and stored at 2-8°C.

[0134] Messenger RNA was synthesized via in vitro transcription process using a cDNA template encoding FFL protein with predetermined untranslated regions. The resulting RNA construct was processed further incorporating a Cap 1 structure on the 5' end and a poly-A tail length of ~200 adenosine bases.

[0135] Modified messenger RNA was synthesized in a similar fashion as stated above with 25% of the uridine bases substituted with 2-thiouridine triphosphate and 25% of the cytidine bases substituted with 5-methylcytidine triphosphate.

[0136] Female Balb/c mice were purchased from Elevage-Janvier, France. The mice were 10 weeks old at the start of the experiment. Mice were weighed prior to the start of the experiment and assigned to one of the following four group (n=6 mice per group): group I – IT spray with FFL mRNA; group II – IT spray with modified FFL mRNA; group III – IT spray with FFL-mRNA in C12-200 based lipid nanoparticle; group IV – IT spray with modified FFL-mRNA in C12-200 based lipid nanoparticle. Each mouse was sprayed with 20µg of the respective mRNA/NPs. The required amount of mRNA/NPs per group were suspended just before application in DEPC treated (0.1%) RNase free water (Serva, Catalog number: 39798, Lot P060383), to a total volume of 50 µl/mouse. NPs were also characterized by size and zeta potential measurements. These measurements were performed in water and are tabulated as Table 5.

Table 5. Particle size and zeta potential measurements made in PBS

	Size (nm) ± polydispersity	Zeta Potential
FFL-NP	81.64 ± 0.177	+50 ± 6.36
FFL-mod-NP	84.14 ± 0.197	+52.5 ± 16.2

[0137] Luciferase production was measured by in vivo BLI at 6 hours post application. Whereas almost negligible amount of exogenous mRNA-derived protein could be detected with naked mRNA, the nanoparticle formulations, independent of modifications, showed significant levels of luciferase production in the entire thoracic region and upper

abdomen (**Figure 1**). Compared to modified FFL, the unmodified FFL mRNA resulted in approximately 2-3 fold higher luminescence at 6 hours (**Figure 2**).

[0138] (**Figures 1A and B**), very low (close to background) level luminescence could be detected with naked mRNA. In mice treated with lipid nanoparticles, independent of modifications, increased luciferase production (~2-3 fold), compared to 6 hour time point, was observed 24 hours after treatment (**Figure 3A and B**). No significant difference could be observed between luciferase production from FFL or modified FFL mRNA (**Figure 3C**).

[0139] The mice treated with naked mRNA, were followed further in the experiment and two additional doses at weekly intervals were applied. BLI was performed at different time points post application. The BLI images at 24 hours post application, the time point of maximum luminescence (**Figure 3**), are shown in **Figure 4** (naked FFL mRNA) and **Figure 5** (naked modified FFL mRNA). With a few exceptions, no noticeable luciferase production was observed for any of the measured mice (compare the scales in **Figures 4 and 5** with **Figure 3**).

B. IT spray of FFL and modified FFL mRNA in C12-200 based nanoparticles -5 µg per mouse and 10 µg per mouse

[0140] IT spray experiments were performed with reduced doses of 5 and 10 µg/mouse. The C12-200 based nanoparticle formulation was as described in Example 1.

[0141] Experimental Design: Female Balb/c mice were purchased from Elevage-Janvier, France. The mice were 19 weeks old at the start of the experiment. Mice were weighed prior to the start of the experiment. The C12-200 based lipid nanoparticles were suspended just before application in DEPC treated (0.1%) RNase free water (Serva, Catalog number: 39798, Lot P060383), to a total volume of 50 µl/mouse. The following four groups were tested (n=5 mice per group): group I – IT spray with FFL mRNA in C12-200 based nanoparticles (5 µg/mouse); group II – IT spray with FFL mRNA in C12-200 based nanoparticles (10 µg/mouse); group III – IT spray with modified FFL mRNA in C12-200 based nanoparticles (5 µg/mouse); and group IV – IT spray with modified FFL mRNA in C12-200 based nanoparticles (10 µg/mouse). One mouse from group III and IV died during IT spray. Thus, the remaining number of animals for these groups was four. At 6 hours post application, all animals showed piloerection and reduced motility. Moreover, one mouse

from each of the higher dose groups (groups II and IV) was dead at this time point. BLI imaging was performed for the mice at 6 hours post application.

[0142] Using FFL mRNA at the doses of 5 µg/mouse resulted in extremely low levels of luciferase production. With the 10 µg/mouse dose, greater production was observed which was concentrated in the liver (**Figure 6A**). The difference between the applied doses was not very evident in the modified FFL mRNA groups (**Figure 6B**). Comparing the mice from group II (FFL mRNA) with group IV (modified FFL mRNA) revealed higher luminescence in the former (compare **Figure 6A** panel 1, 2 with panel 1 in **Figure 6B**).

[0143] Luciferase production at 24 hours was significantly enhanced compared to 6 hours post IT spray (**Figure 7A and 7B**). Moreover, higher production was observed using FFL mRNA as compared to modified FFL mRNA (**Figure 7C**). Similar results were obtained in Example 1 with the dose of 20 µg/mouse. Internal organs (heart, liver, lungs, liver, spleen and kidney) were frozen in liquid nitrogen for in-vitro luciferase measurements.

[0144] Biodistribution after IT spray: The isolated organs were homogenized in the frozen state using a mortar and pestel, weighed and lysed in a solution containing Lysis-buffer (25 mM TRIS-Cl 0,1% Triton x-100; pH 7.4) and Complete-Protease-Inhibitor (Roche). Spleen, heart and kidneys were lysed in 250 µl, whereas lungs and liver were lysed in 400 µl. After incubation on ice for 20 min, samples were centrifuged at 10.000 rpm, 4 °C for 10 min. Luciferase activity was measured using 100 µl of the supernatant. Each sample was measured in duplicates and mean values from duplicates were used in analysis. All organs except the kidneys were positive for luciferase activity (**Figure 8**). In accordance with our BLI data, maximum luminescence was observed in liver and lungs. FFL-mRNA resulted in higher protein production compared to modified -FFL-mRNA and a dose dependency was evident.

C. IT spray of modified FFL mRNA in HGT5001 based nanoparticles– 20µg per mouse

[0145] An IT spray experiment was performed with an HGT5001 based nanoparticle formulation.

[0146] Lipid nanoparticle formulation: Aliquots of 50 mg/mL ethanolic solutions of HGT5001:DOPE:Cholesterol:DMG-PEG2K were mixed in a molar ratio of 40:20:35:5,

respectively, and diluted with ethanol to 3 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of FFL or modified FFL mRNA was prepared from a 1 mg/mL stock. 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 with 1x PBS (pH 7.4) followed by distilled RNase-free water, concentrated and stored at 2-8°C.

[0147] Experimental Design: Female Balb/c mice were purchased from Elevage-Janvier, France. The mice were 13 weeks old at the start of the experiment. Mice were weighed prior to the start of the experiment. The lipid nanoparticles were suspended just before application in DEPC treated (0.1%) RNase free water (Serva, Catalog number: 39798, Lot P060383), to a total volume of 50 µl/mouse.

[0148] IT Spray and BLI: Each mouse was IT sprayed with 20 µg of the HGT5001 based nanoparticle formulation in a total volume of 50 µl/mouse. BLI imaging was performed for the mice at 6 hrs post application.

[0149] Significantly lower luminescence values were observed with the HGT5001 based nanoparticles when compared to the corresponding time point with the C12-200 based nanoparticles and no increase in protein production from 6 to 24 hours was observed (**Figure 9 and Figure 10**).

[0150] In an independent experiment testing C12-200 and HGT5001 based nanoparticle formulations, following BLI imaging (**Figure 11**), mice were euthanized and organs (heart, lungs, liver, spleen and kidney) were evaluated by histology (**Figure 12**). FFL production was confirmed in the lung and liver for both C12-200 based NPs and HGT5011 based NPs.

D. IT spray of Modified FFL mRNA -- non- nanoparticle delivery

[0151] Naked mRNA resulted in low efficiency without perfluorocarbon treatment. IT aerosolization of encapsulated mRNA lead to protein production in lungs, liver, spleen, and heart. FFL and modified FFL were equally efficient with respect to protein production and with a dose-response.

[0152] Various delivery vehicles were tested, including polyethylenimines (L-PEI 22kDa, br-PEI 25 kDa), copolymers of oligo(ethylene glycol) methyl ether methacrylate (OEGMA) and N,N-dimethylaminoethyl methacrylate (DMAEMA), MLRI:DOPE, DOTAP, DMRIE-C, and Lipofectamine, and did not show luminescence in non-lung cells. In contrast, C12-200 and HGT5001 based lipid nanoparticle formulations resulted in significant protein production in non-lung cells following pulmonary delivery.

[0153] These observations indicate that only the nanoparticle formulations were able to translocate intact, by either active or passive means, from the lung to the systemic blood supply and subsequently to be deposited in different tissues, such as the liver. This translocation of an intact mRNA encoding a cytosolic protein, firefly luciferase, constitutes non-invasive systemic delivery of an active pharmaceutical ingredient beyond the lung to result in the production of a functional protein to systemically accessible tissues.

E. Nebulization of modified FFL mRNA with PEI based lipid nanoparticles

[0154] Mice that received modified FFL mRNA in PEI based nanoparticles showed luminescence in the lung (**Figure 13**). Luciferase production was greater with modified FFL mRNA (panels 1, 2) compared to unmodified FFL mRNA (panels 3, 4).

Example 2: Evaluation of nanoparticle migration by measuring lipid in non-lung target cells

[0155] To identify the passage of intact nanoparticles in non-lung tissues, aliquots of 50 mg/mL ethanolic solutions of C12-200, DOPE, Chol, DMG-PEG2000 and 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (ammonium salt) are mixed in a molar ratio of 40:29:25:5:1, respectively, and diluted with ethanol to 3 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of non-secreted protein, such as beta-galactosidase or FFL (modified or unmodified) mRNA is prepared from a 1 mg/mL stock. The lipid solution is injected rapidly into the aqueous mRNA solution and shaken to yield a final suspension in 20% ethanol. The resulting nanoparticle suspension is filtered, diafiltrated with 1x PBS (pH 7.4), concentrated and stored at 2-8°C.

[0156] Messenger RNA is synthesized via in vitro transcription process using a cDNA template encoding beta-galactosidase or FFL protein with predetermined untranslated regions. The resulting mRNA construct is processed further incorporating a Cap 1 structure on the 5' end and a poly-A tail length of ~200 adenosine bases.

[0157] Modified messenger RNA is synthesized in a similar fashion as stated above with 25% of the uridine bases substituted with 2-thiouridine triphosphate and 25% of the cytidine bases substituted with 5-methylcytidine triphosphate.

[0158] Female Balb/c mice are purchased from Elevage-Janvier, France. The mice are 10 weeks old at the start of the experiment. Mice are weighed prior to the start of the experiment. Each mouse is sprayed with 20µg of the respective mRNA/NPs comprising unmodified and modified mRNA in fluorescently labeled C12-200 based lipid nanoparticles. The mRNA encapsulated nanoparticles are suspended just before application in DEPC treated (0.1%) RNase free water (Serva, Catalog number: 39798, Lot P060383), to a total volume of 50 µl/mouse. Six hours after treatment mice are killed and organs are excised for histological examination of NP distribution by fluorescence microscopy on 6 µm cryosections.

[0159] Alternatively, mRNA is radioactively labeled with, e.g. I¹²³ according to the method of Commerford as described in detail by Terebesi et al (Terebesi J, Kwok KY, Rice KG. Anal Biochem. 1998 Oct 1;263(1):120-3). The labeling mixture is separated using a PD-10 gel filtration column (Amersham Biosciences, Freiburg, Germany) with water as eluent. The iodinated mRNA is mixed with unlabeled mRNA resulting in the desired amounts of mRNA which is formulated with lipids as described above and IT aerosolized to the mice lungs. At a desired time point, mice are killed and radioactivity of the organs is measured using a gamma counter.

[0160] The above examples demonstrate mRNA can be effectively delivered to non-lung cells or tissues through pulmonary administration using the methods and compositions described herein. In the representative examples above, mRNA delivery was evaluated using the fluorescent firefly luciferase reporter protein encoded by a codon optimized sequence of modified mRNA. However, it will be appreciated by those skilled in the art, that such examples are merely representative of a wide range of mRNAs and proteins that can be delivered according to the present invention. In particular, it will be readily apparent to one skilled in the art that the compositions and methods of the current invention may be used to

delivery mRNA encoding various therapeutic proteins to non-lung cells or tissues within a subject for the treatment of associated diseases, disorders or conditions.

[0161] From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

CLAIMS:

1. Use of a composition comprising mRNA encoding a protein and a lipid carrier vehicle comprising one or more PEG-modified lipids formulated for pulmonary delivery of mRNA, wherein administering to the lung delivers mRNA to a non-lung cell or tissue, and wherein the protein encoded by the mRNA is detectable in the non-lung cell or tissue for at least 6 hours following delivery to the lung.
2. The method of claim 1, wherein the composition is formulated for an administration to the lung by aerosolization, inhalation or nebulization.
3. The use of claim 1, wherein the non-lung cell is selected from the group consisting of hepatocytes, epithelial cells, hematopoietic cells, endothelial cells, bone cells, stem cells, mesenchymal cells, neural cells, cardiac cells, adipocytes, vascular smooth muscle cells, cardiomyocytes, skeletal muscle cells, beta cells, pituitary cells, synovial lining cells, ovarian cells, testicular cells, fibroblasts, B cells, T cells, reticulocytes, leukocytes, granulocytes, macrophages, neutrophils, antigen presenting cells (dendritic cells), fibroblasts, tumor cells and combination thereof.
4. The use of claim 1, wherein the non-lung tissue is selected from the group consisting of heart, liver, spleen, kidneys, skeletal muscle, lymph nodes, skin, brain, cerebrospinal fluid, plasma and combination thereof.
5. The use of claim 1, wherein the mRNA encodes a protein selected from the group consisting of a cytosolic protein, a secreted protein and a therapeutic protein.
6. The use of claim 5, wherein the cytosolic protein is selected from the group consisting of enzymes, transcription factors, chaperones, and combination thereof.
7. The use of claim 5, wherein the therapeutic protein is selected from the group consisting of alpha galactosidase, erythropoietin, al-antitrypsin, carboxypeptidase N,

alpha- L-iduronidase, iduronate-2-sulfatase, N-acetylglucosamine-1-phosphate transferase, N- acetylglucosaminidase, lysosomal acid lipase, arylsulfatase-A alpha-glucosaminide acetyltransferase, N-acetylglucosamine 6-sulfatase, N-acetylgalactosamine-4-sulfatase, beta- glucosidase, galactose-6-sulfate sulfatase, beta-galactosidase, beta-glucuronidase, glucocerebrosidase, heparan sulfamidase, hyaluronidase, galactocerebrosidase, human growth hormone, ornithine transcarbamylase (OTC), carbamyl phosphate synthetase-1 (CPSI), argininosuccinate synthetase-1 (ASS1), argininosuccinate lyase (ASL), arginase-1 (ARG 1), cystic fibrosis transmembrane conductance regulator (CFTR), Factor VII, Factor VIII, Factor IX, heparan-N-sulfatase, and combination thereof.

8. The use of any one of claims 1-7, wherein the protein is detectable in the non-lung cell or tissue for at least 1 day following delivery to the lung.
9. The use of any one of claims 1-8, wherein the protein is detectable in the non-lung cell or tissue for at least 2 days following delivery to the lung.
10. The use of any one of claims 1-9, wherein the composition comprises an amount greater than 0.5 mg/kg of mRNA per dose.
11. The use of any one of claims 1-10, wherein the lipid carrier vehicle is a liposome.
12. The use of claim 11, wherein the liposome comprises one or more cationic lipids, one or more non-cationic lipids, and one or more cholesterol-based lipids.
13. The use of claim 12, wherein the one or more cationic lipids are selected from the group consisting of C12-200, HGT4003, HGT5000, HGT5001, ICE, DLinKC2-DMA, DODAP, DODMA, DLinDMA, CLinDMA, and combination thereof.
14. The use of claim 1, wherein the composition further comprises one or more pulmonary surfactants.

15. The use of claim 1, wherein the composition is formulated as respirable particles.
16. The use of claim 15, wherein the respirable particles have a size less than 500 μm .
17. The use of any one of claims 1-16, wherein the composition is formulated as nebulizable lipid or as dry powder.
18. The use of claim 1, wherein the non-lung cell or tissue is affected by a disease or disorder.
19. The composition of claim 1, wherein the lipid carrier vehicle is a liposome further comprising one or more cationic lipids, one or more non-cationic lipids, and one or more cholesterol-based lipids.
20. Use of a composition comprising mRNA encoding a protein and a lipid carrier vehicle comprising one or more PEG-modified lipids for delivering mRNA to a non-lung cell or tissue by nebulization delivers mRNA, and wherein the protein encoded by the mRNA is detectable in the non-lung cell or tissue for at least 6 hours following delivery to the lung.
21. A composition for pulmonary delivery of messenger RNA (mRNA) to a non-lung cell or tissue comprising mRNA encoding a protein and a lipid carrier vehicle comprising one or more PEG-modified lipids, wherein the composition is formulated for pulmonary delivery and administration to the lung, wherein the protein encoded by the mRNA is detectable in the non-lung cell or tissue for at least 6 hours following administration to the lung.

1 / 13

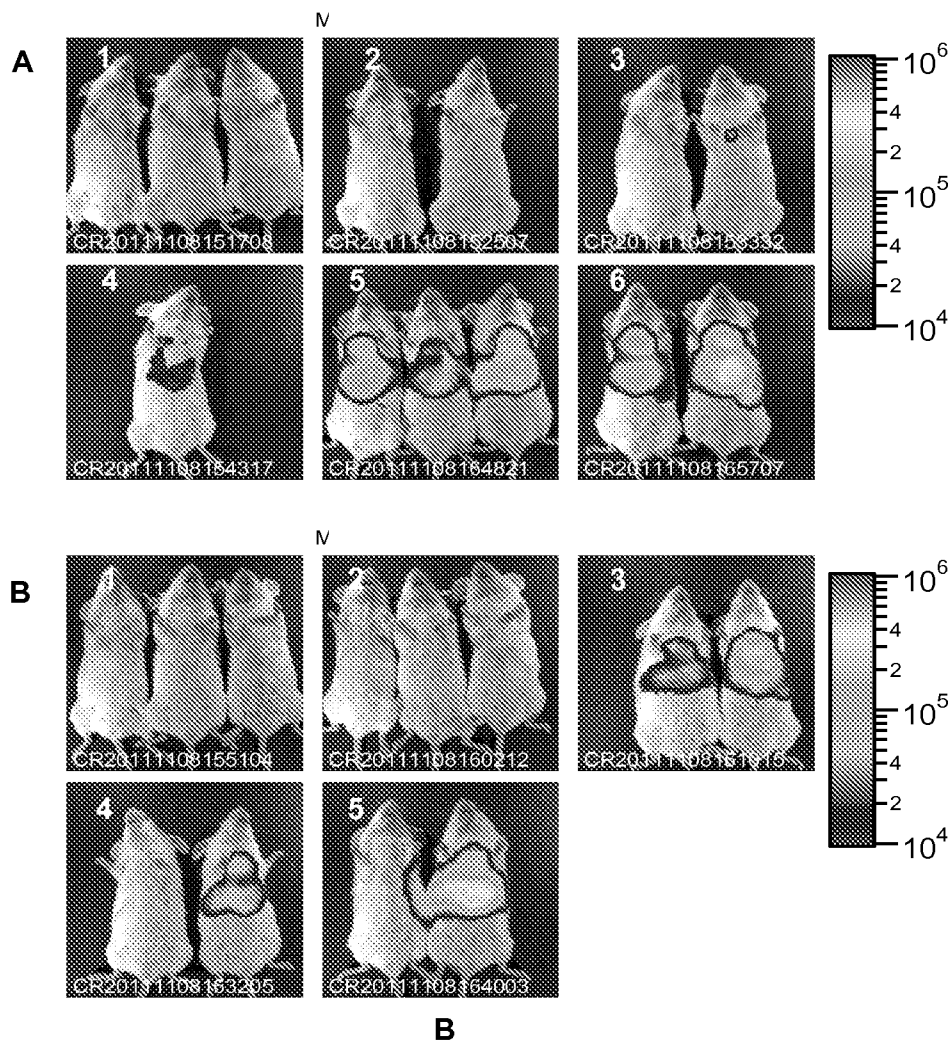


Figure 1

2 / 13

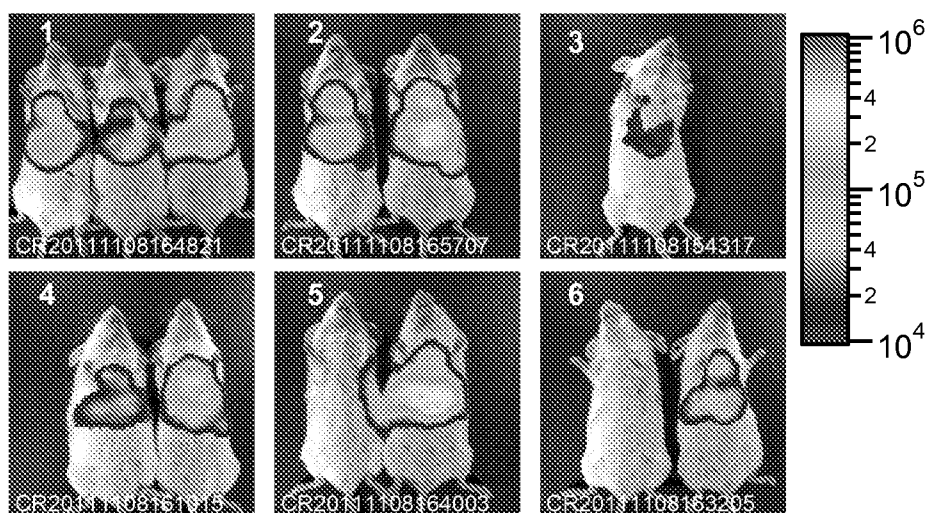


Figure 2

3 / 13

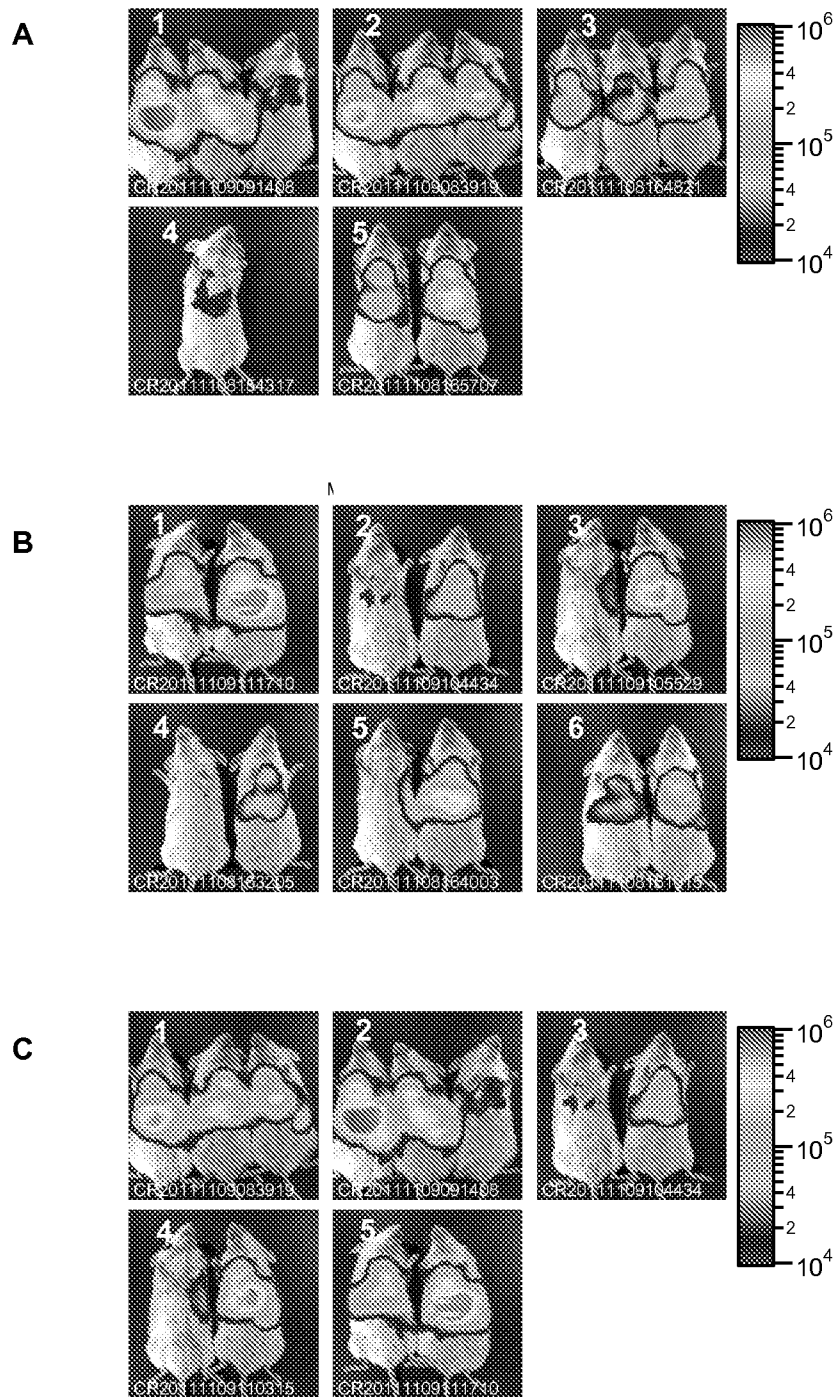


Figure 3

4 / 13

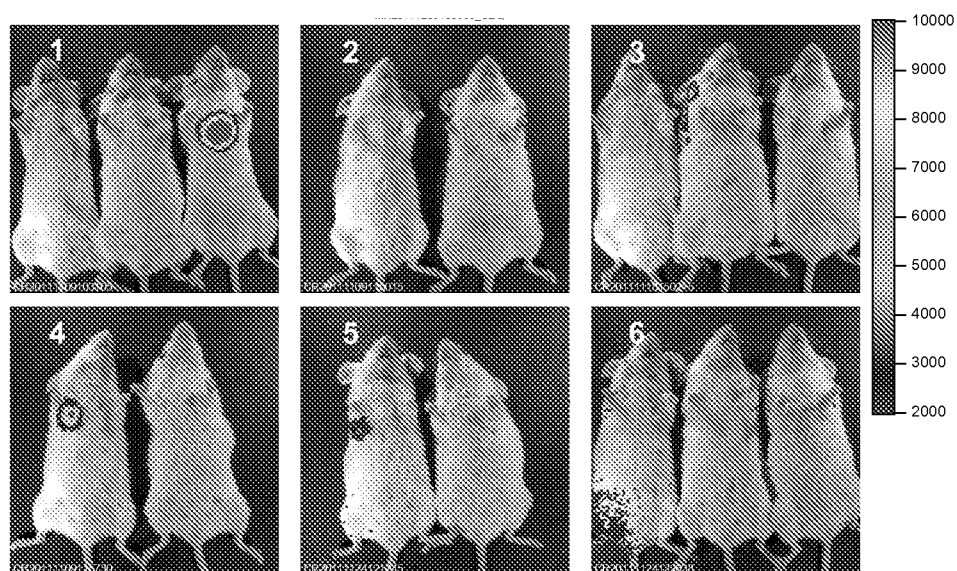


Figure 4

5 / 13

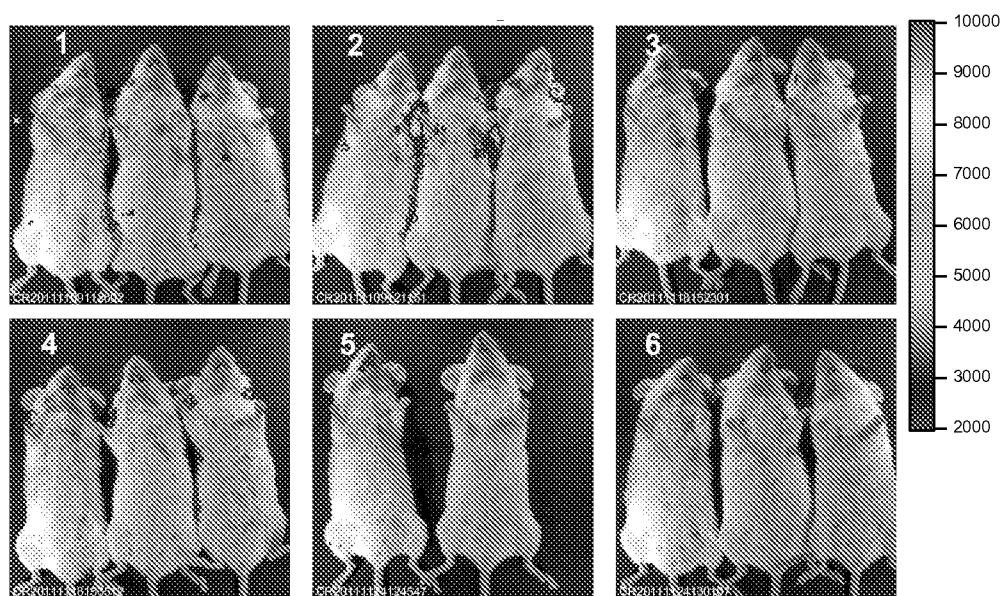


Figure 5

6 / 13

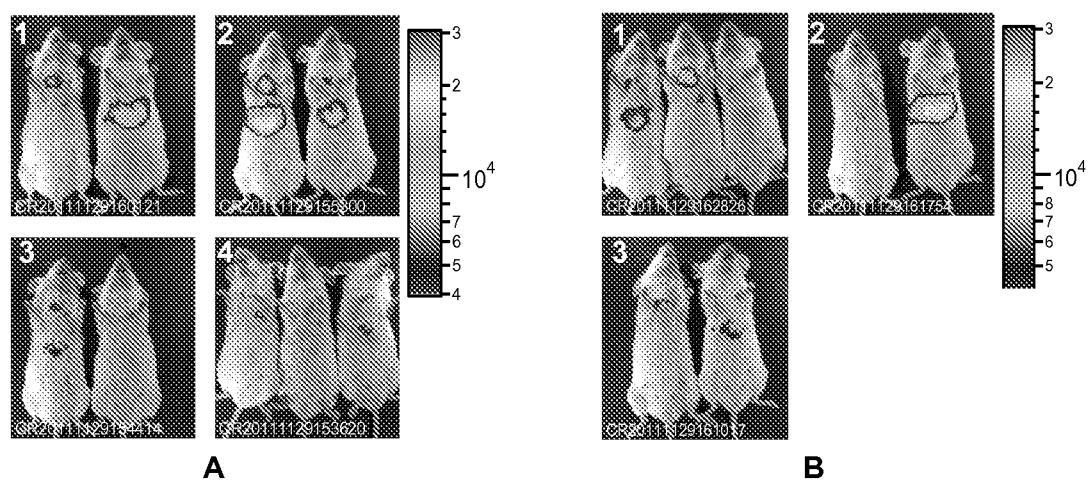


Figure 6

7 / 13

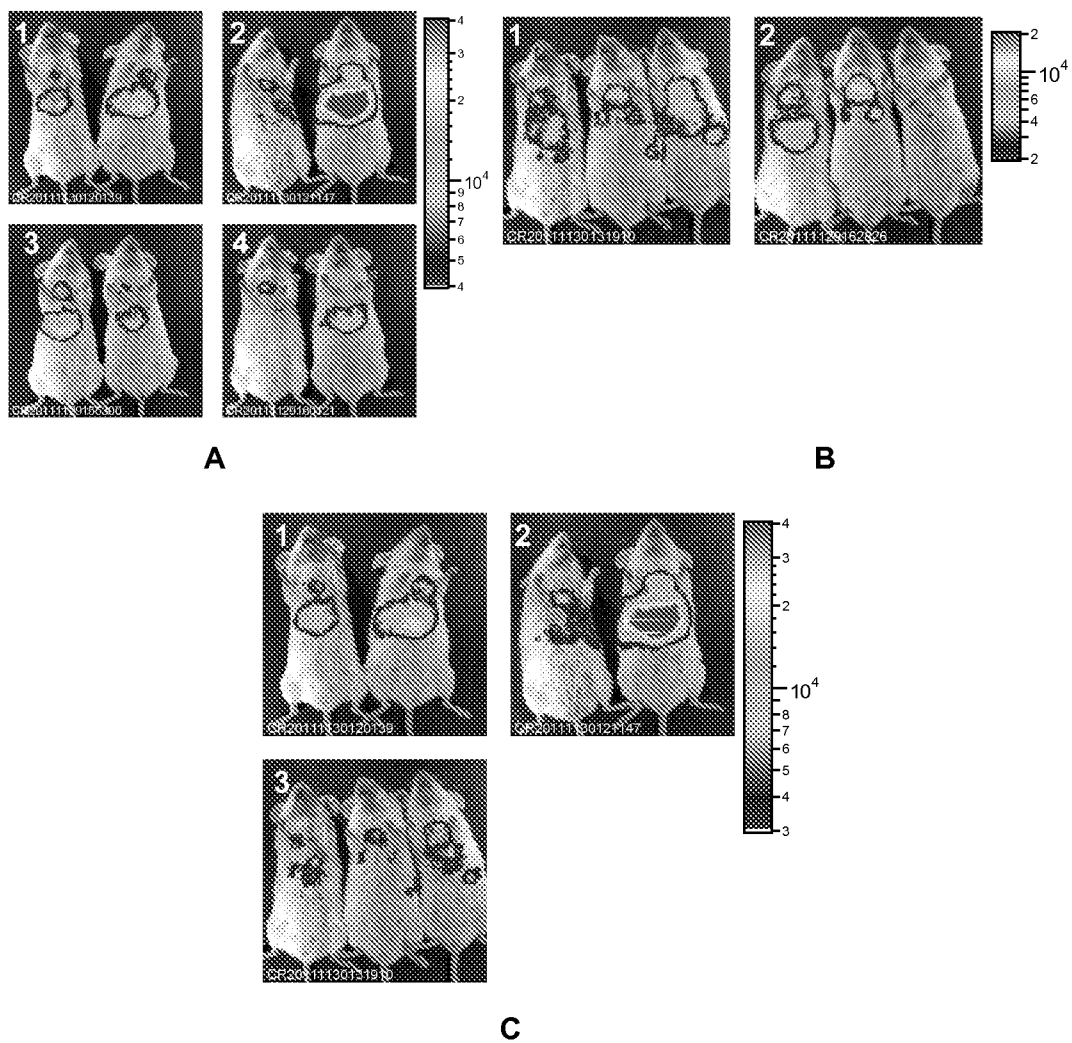


Figure 7

8 / 13

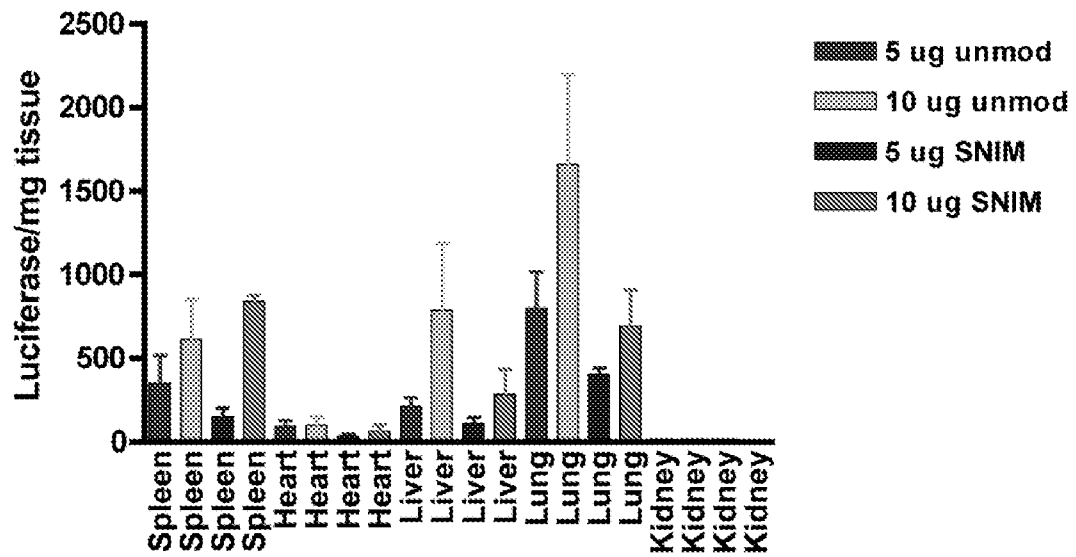


Figure 8

9 / 13

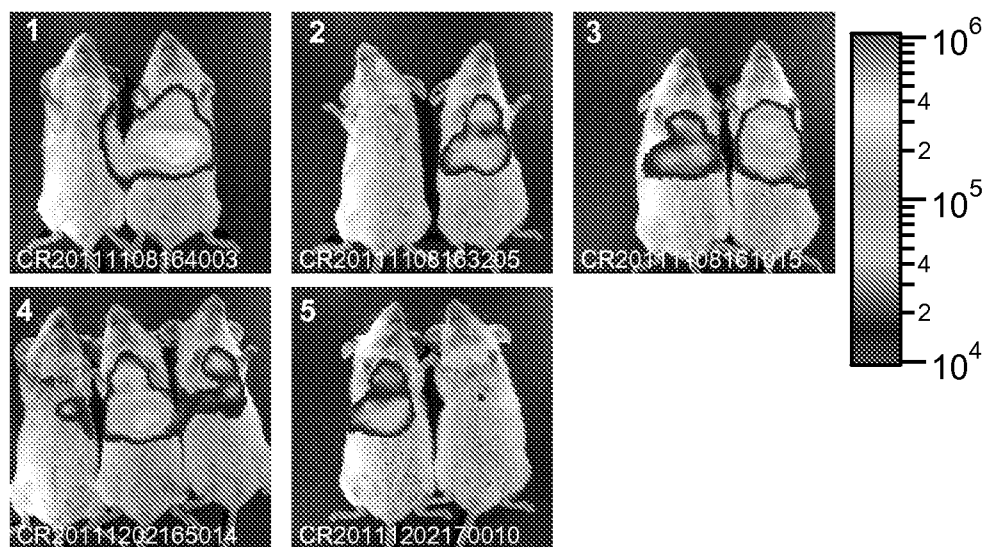


Figure 9

10 / 13

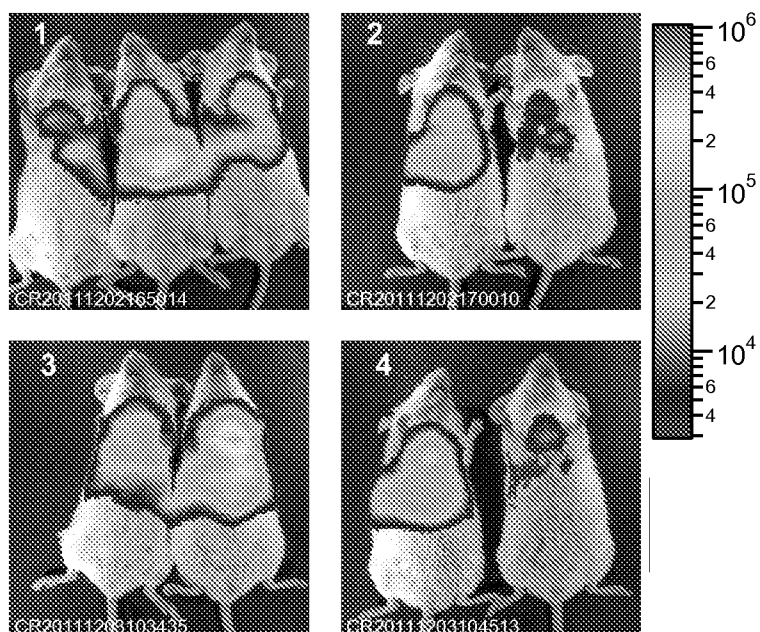


Figure 10

11 / 13

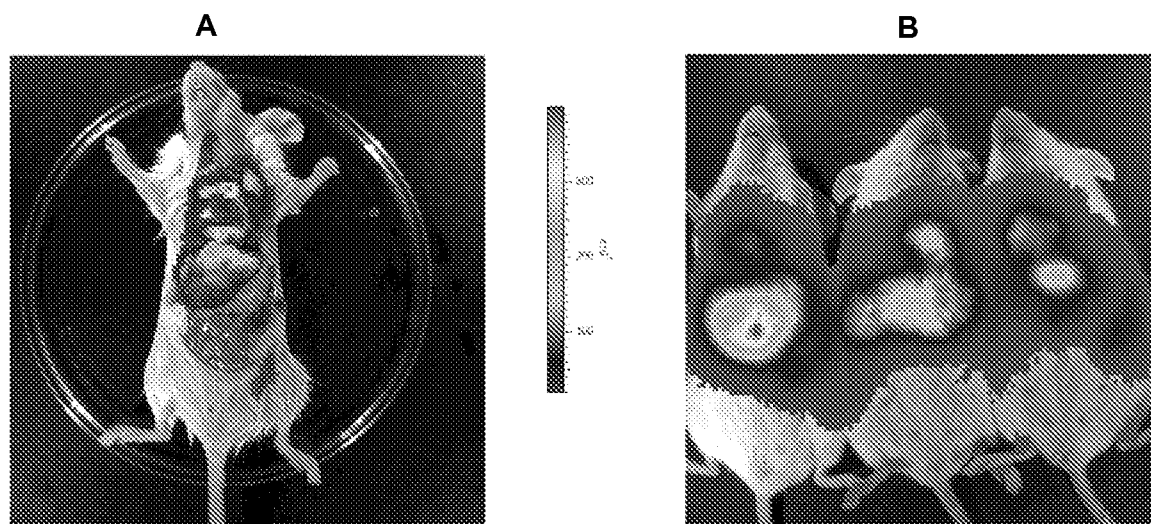


Figure 11

12 / 13

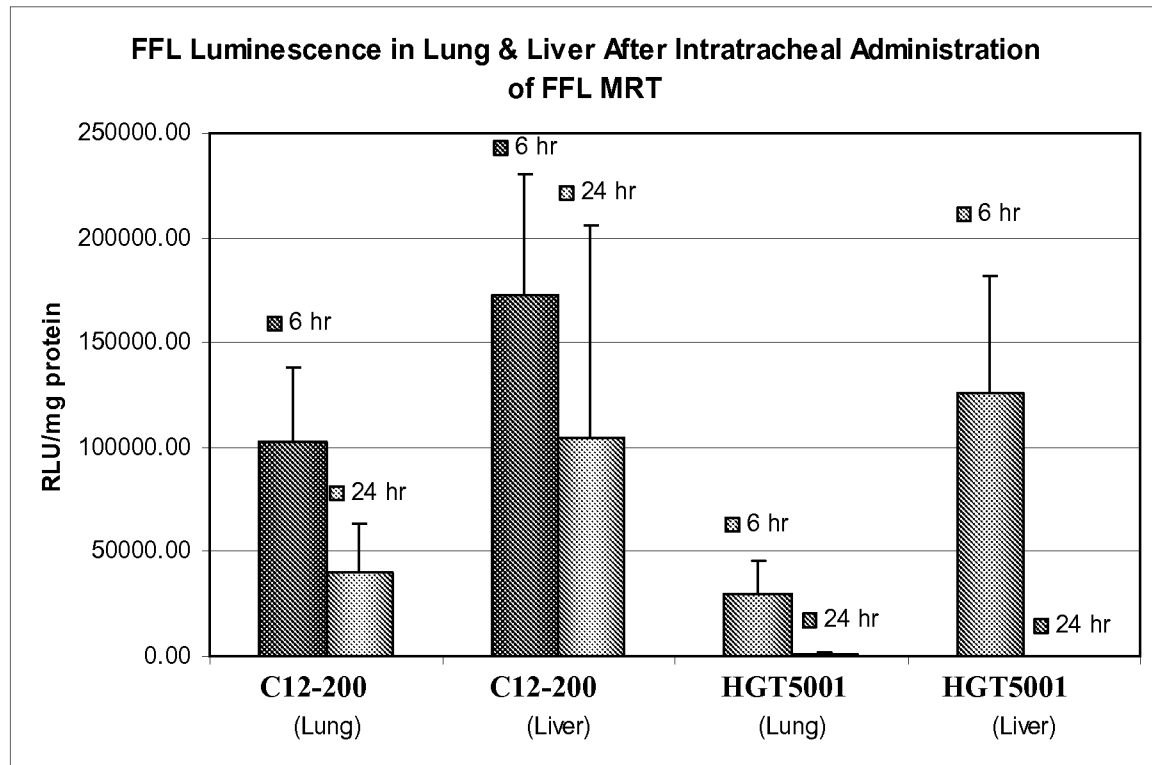
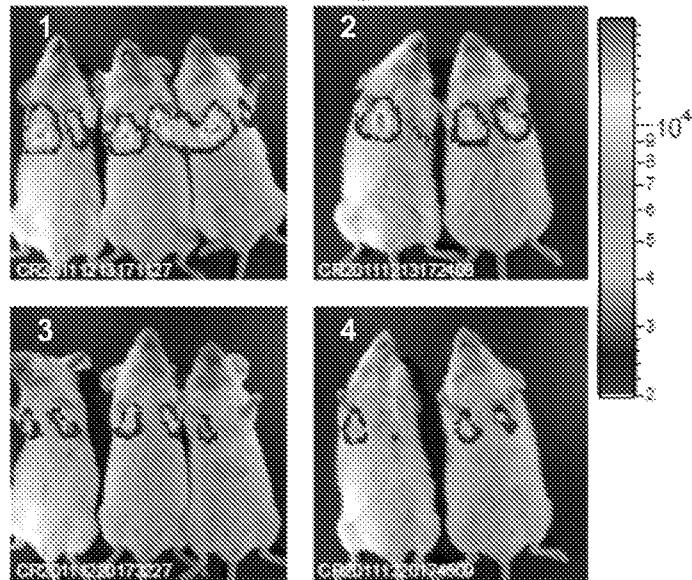


Figure 12

13 / 13



FFL Luminescence in Lung & Liver After Intratracheal Administration of FFL MRT

