(72) Inventors: DOMINY, John; Novartis Institutes for Bio-Medical Research, Inc., 250 Massachusetts Avenue, Cambridge, MA 02139 (US). DUNN, Robert; Novartis Institute for Functional Genomics, Inc., DBA Genomics Institute of the Novartis Research Foundation (GNF), 10675 John Jay Hopkins Drive, San Diego, CA 92121 (US). GLASER, Scott; Novartis Institute for Functional Genomics, Inc., DBA Genomics Institute of the Novartis Research Foundation (GNF), 10675 John Jay Hopkins Drive, San Diego, CA 92 l2 1 (US). KEATING, Mark; 135 Orchard Avenue, Weston, MA 02493 (US). KLATTENHOFF, Carla; Novartis Institutes for BioMedical Research, Inc., 250 Massachusetts Avenue, Cambridge, MA 02139 (US). SPLAWSKI, Igor; Novartis Institutes for BioMedical Research, Inc., 250 Massachusetts Avenue, Cambridge, MA 02139 (US).

(54) Title: MRNA-MEDIATED IMMUNIZATION METHODS

Priming immunization		Boosting immunizations
1) mRNA	-- →	1) mRNA
2) mRNA	⎯→	2) Overexpressing cells
3) mRNA	·→	3) Virus like particles
4) Overexpressing cells	⎯→	4) mRNA
5) Virus like particles	·→	5) mRNA

FIG. 1A

(57) Abstract: The present disclosure is directed to methods of immunization and methods for generating antibodies using compositions comprising cationic lipids and polynucleotide molecules, such as polyribonucleotide molecules, e.g., mRNA, which code for immunogens (e.g., a target protein or a fragment thereof).

WO 2018/029586 A1

mRNA-MEDIATED IMMUNIZATION METHODS

[001]   This application claims the benefit of U.S. Provisional Application No. 62/371 ,834 filed on August 7, 2016 and U.S. Provisional Application No. 62/399,544 filed on September 26, 2016, each of which is hereby incorporated by reference in its entirety.

SEQUENCE LISTING

[002]   The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on August 3, 2017, is named PAT057169-WO-PCT_SL.txt and is 146,992 bytes in size.

FIELD

[003]   The present disclosure is in the field of immunology. In particular, this disclosure is directed to methods of immunization using compositions comprising cationic lipids and polynucleotide molecules, such as polyribonucleotide molecules, e.g., mRNA, which code for immunogens (e.g., a target protein or a fragment thereof). This disclosure is also directed to methods for producing antibodies (e.g., monoclonal antibodies) from immunized animals (e.g., non-human animals) for the purposes of making therapeutic antibodies, as well as to the antibodies themselves.

BACKGROUND

[004]   Therapeutic monoclonal antibody development in vivo is often limited by the ability to produce a high quality antigen that can be used for immunization. Ideally, the antigen should be a highly purified protein with an intact structural conformation and have enough sequence variation from the animal host strain as to break immunological tolerance and induce a robust humoral response. For many target proteins intended for use as antigens, however, meeting these requirements is not possible due to such issues as inherently poor biophysical properties of the protein that proscribe overexpression/purification, cytotoxicity in host production cells, and poor immunogenicity of the target protein's amino acid sequence.

[005]   Traditional methods of animal immunization have employed two general strategies for the generation of antibodies. The first involves repeated injections of full length protein antigen in purified format in the presence of an adjuvant to enhance the immune response. For small to medium-sized soluble proteins, this procedure can be a successful method for the generation of monoclonal antibodies against an antigen in its native conformation. For very large proteins,

transmembrane proteins, proteins with unusual post translational modifications, or proteins with poor solubility, this method is of very limited utility, as obtaining pure native, full length protein in the quantities needed for immunization is difficult. The second strategy entails immunization of animals with a DNA construct which encodes the antigen of interest. This strategy allows for the expression of difficult to purify proteins in their native state *in situ.* It suffers, however, from a relatively low antibody titer generation, which can ultimately correlate with a low yield of monoclonal hybridoma production (Howard et al. *Making and using antibodies: A Practical Handbook,* 2nd Edition CRC Press, 2013).

SUMMARY

[006]   The present disclosure is directed to a method for eliciting an immune response in an animal (e.g., non-human animal), comprising the steps of: (a) mixing at least one cationic lipid with a polynucleotide, such as polyribonucleotide (e.g., mRNA), coding for an antigenic determinant, thereby forming a cationic lipid-polynucleotide complex; and (b) administering the lipid-polynucleotide complex to the animal. The present disclosure is further directed to a genetic immunization method wherein the polynucleotide is a polyribonucleotide molecule such as an mRNA molecule which codes for an immunogen (e.g., a target protein or a fragment thereof). The present disclosure is further directed to a method for producing antibodies (e.g., polyclonal or monoclonal antibodies) comprising the use of genetic immunization method described herein, and further comprising the step of isolating the antibodies from the immunized animal.

[007]   The present disclosure is also directed to a method for producing monoclonal antibodies comprising the steps of: (a) mixing at least one cationic lipid with a polynucleotide, thereby forming a lipid-polynucleotide complex, wherein the polynucleotide comprises an mRNA sequence coding for an immunogen; (b) administering the lipid-polynucleotide complex to at least one mouse; (c) removing antibody-producing cells such as lymphocytes (e.g., B-lymphocytes) or splenocytes from the immunized mice; (d) fusing the B-lymphocytes from the immunized mice with myeloma cells, thereby producing hybridomas; (e) cloning the hybridomas; (f) selecting positive clones which produce anti-immunogen antibody; (g) culturing the anti-immunogen antibody-producing clones; and (h) isolating anti-immunogen antibodies from the cultures. In certain aspects, the methods provided herein for producing antibodies comprise further steps to determine the amino acid sequence of the heavy chain variable region and light chain variable region of such antibodies as well as the corresponding encoding nucleic acid sequences. In particular aspects, the methods provided herein for producing antibodies comprise further steps to generate a chimeric antibody or humanized antibody of the anti-immunogen antibody.

[008]   The present disclosure is also directed to a method in which immune tissues are collected from animals immunized with mRNA containing cationic lipid nanoparticles (LNPs) and B cells are selectively isolated. The B cells are directly screened for the production of an antibody with the desired properties and the antibody is directly cloned and expressed recombinantly, bypassing the need for generation of hybridomas.

[009]   The mRNA encapsulated LNPs of the present disclosure may also be used for the purpose of generating a recombinant antibody library from the immune tissues of an immunized host animal (e.g., rodents (e.g., mice and rats), rabbits, chickens, cows, camelids, pigs, sheep, goats, sharks, and non-human primates, etc.). This library can then be subsequently screened in a heterologous host system, such as phage or yeast display for the desired properties.

[0010]   The methods of polynucleotide-based, e.g., mRNA-based, immunization of the present disclosure have addressed many of the issues associated with the above-described difficulties inherent in antigen production and/or antibody generation. Among other things, said methods dispense with the need to directly express and purify the target protein antigen. An animal host's own cellular machinery is used to make the target protein and present it to the immune system. For eukaryotic target proteins, this has the added advantage of permitting the addition of eukaryotic-specific post-translational modifications and protein processing. In particular, unpurified mRNA that is used for immunization has a highly inflammatory character, due in part to the presence of double stranded RNA entities in the preparation. Double-stranded RNA can present pathogen-associated molecular patterns that are recognized by receptors comprising the innate immune system, most notably the toll-like receptors. Without being bound by any particular theory, it is believed that this serves as an adjuvant to boost the humoral response against the target protein and result in high titer antibody production.

[001 1]   Monoclonal antibody development for an immunogen, e.g., a target protein or a fragment thereof, can be expedited in particular through the immunization of animals with mRNAs which encode said target protein or a fragment thereof. This method offers considerable advantages for proteins against which it has historically been technically challenging to develop specific antibodies, such as transmembrane proteins (e.g., multi-pass transmembrane proteins), for example, G-protein coupled receptors (GPCRs), as there is no need to heterologously produce and purify the target protein. Without being bound by any particular theory, it is believed that host defense mechanisms elicited by the adjuvant-like properties of the mRNA result in a fast development of sera titers and make it a superior choice over DNA immunization or other conventional methods of immunization (e.g., recombinant protein immunization).

[0012]   Non-limiting embodiments of the present disclosure are described in the following aspects:

[0013]   Aspect 1. A method for producing antibodies (e.g., monoclonal antibodies) against a target protein, comprising the steps of: (a) mixing at least one cationic lipid with a polyribonucleotide such as an messenger RNA (mRNA) coding for the target protein or a fragment thereof, thereby forming a cationic lipid-polyribonucleotide complex (e.g., mRNA-LNP complex); (b) administering the lipid-polyribonucleotide complex to a non-human animal; and (c) obtaining antibodies that specifically bind to the target protein from the animal.

[0014]   Aspect 2. A method for producing antibodies (e.g., monoclonal antibodies) against a target protein, comprising the steps of: (a) administering a lipid-polyribonucleotide complex (e.g., mRNA-LNP complex) to a non-human animal, wherein the complex comprises at least one cationic lipid with a polyribonucleotide, such as mRNA, coding for the target protein or a fragment thereof, thereby inducing an immune response to the target protein; and (b) obtaining antibodies produced by the animal that specifically bind to the target protein.

[0015]   Aspect 3. The method of aspect 1 or 2, wherein the target protein is a transmembrane protein.

[0016]   Aspect 4. The method of aspect 3, wherein the transmembrane protein is selected from the following:

(i)   a G protein coupled receptor (GPCR);

(ii)   a single pass transmembrane protein receptor;

(iii)   a Tumor Necrosis Factor Receptor Superfamily (TNFRSF) member;

(iv)   an interleukin (IL) receptor;

(v)   an ion channel;

(vi)   a solute carrier;

(vii)   an immune receptor; and

(viii)   a multi-pass transmembrane protein.

[0017]   Aspect 5. The method of aspect 3 or 4, wherein the transmembrane protein is a multi-pass transmembrane protein such as a G protein coupled receptor (GPCR).

[0018]   Aspect 6. The method of aspect 5, wherein the GPCR is RXFP1, TSHR, APJ, GPR40, GPR64, GPR4, or GPR15.

[0019]   Aspect 7. The method of aspect 3 or 4, wherein the transmembrane protein is a single pass transmembrane protein receptor such as GP130 or a multi-pass transmembrane protein such as SLC52A2.

[0020]   Aspect 8. The method of aspect 3 or 4, wherein the transmembrane protein is an interleukin (IL) receptor, such as IL-1 receptor, IL-2 receptor, IL-3 receptor, IL-4 receptor, IL-5 receptor, IL-6 receptor, IL-7 receptor, IL-8 receptor, IL-9 receptor, IL-10 receptor, IL-1 1 receptor,

IL-12 receptor, IL-13 receptor, IL-14 receptor, IL-15 receptor, IL-16 receptor, IL-17 receptor, IL-18 receptor, IL-19 receptor, IL-20 receptor, IL-21 receptor, IL-22 receptor, IL-23 receptor, IL-24 receptor, IL-25 receptor, IL-26 receptor, IL-27 receptor, IL-28 receptor, IL-29 receptor, IL-30 receptor, IL-31 receptor, IL-32 receptor, IL-33 receptor, IL-35 receptor, or IL-36 receptor.

[0021]   Aspect 9. The method of aspect 3 or 4, wherein the transmembrane protein is a tumor necrosis factor receptor superfamily (TNFRSF) member selected from the group consisting of the following: TNFRSF1A, TNFRSF1 B, TNFRSF3, TNFRSF4, TNFRSF5, TNFRSF6, TNFRSF6B, TNFRSF7, TNFRSF8, TNFRSF9, TNFRSF10A, TNFRSF10B, TNFRSF10C, TNFRSF10D, TNFRSF1 1A, TNFRSF1 1B, TNFRSF12A, TNFRSF13B, TNFRSF13C, TNFRSF14, TNFRSF16, TNFRSF17, TNFRSF18, TNFRSF19, TNFRSF21 , TNFRSF25, and TNFRSF27.

[0022]   Aspect 10. The method of aspect 3 or 4, wherein the transmembrane protein is an ion channel such as TMEM16A.

[0023]   Aspect 11. The method of aspect 3 or 4, wherein the transmembrane protein is a solute carrier.

[0024]   Aspect 12.   The method of aspect 1 or 2, wherein the target protein is selected from the following:

ACKR1 , ACKR2, ACKR3, ACKR4, ADCYAP1 R 1, ADGRA1 , ADGRA2, ADGRA3, ADGRB1 , ADGRB2, ADGRB3, ADGRD1 , ADGRD2, ADGRE1 , ADGRE2, ADGRE3, ADGRE4P, ADGRE5, ADGRF1 , ADGRF2, ADGRF3, ADGRF4, ADGRF5, ADGRG1 , ADGRG2, ADGRG3, ADGRG4, ADGRG5, ADGRG6, ADGRG7, ADGRL1 , ADGRL2, ADGRL3, ADGRL4, ADGRV1 , ADORA1 , ADORA2A, ADORA2B, ADORA3, ADRA1A, ADRA1 B, ADRA1 D, ADRA2A, ADRA2B, ADRA2C, ADRB1 , ADRB2, ADRB3, AGTR1 , AGTR2, APLNR/APJ, ASGR1 , ASGR2, AVPR1 A, AVPR1 B, AVPR2, BDKRB1 , BDKRB2, BRS3, BRS3, C3AR1 , C5AR1 , C5AR2, CALCR, CALCRL, CASR, CCKAR, CCKBR, CCR1 , CCR10, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCRL2, CELSR1 , CELSR2, CELSR3, CHRM1 , CHRM2, CHRM3, CHRM4, CHRM5, CMKLR1 , CNR1 , CNR2, CRHR1 , CRHR2, CX3CR1 , CXCR1 , CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, CYSLTR1 , CYSLTR2, DRD1 , DRD2, DRD3, DRD4, DRD5, EDNRA, EDNRB, F2R, F2RL1 , F2RL2, F2RL3, FFAR1 , FFAR2, FFAR3, FFAR4, FPR1 , FPR2, FPR2, FPR3, FSHR, FZD1 , FZD10, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, GABBR1 , GABBR2, GALR1 , GALR2, GALR3, GCGR, GHRHR, GHSR, GIPR, GLP1 R, GLP2R, GNRHR, GNRHR2, GPBAR1 , GPER1 , GPR1 , GPR4, GPR12, GPR15, GPR17, GPR18, GPR19, GPR20, GPR21 , GPR22, GPR25, GPR26, GPR27, GPR3, GPR31 , GPR32, GPR33, GPR34, GPR35, GPR37, GPR37L1 , GPR39, GPR40, GPR42, GPR42, GPR45, GPR50, GPR52, GPR55, GPR6, GPR61 , GPR62, GPR63, GPR65, GPR68, GPR75, GPR78, GPR79, GPR82, GPR83, GPR84, GPR85, GPR87, GPR88, GPR101 , GPR107, GPR132, GPR135, GPR137, GPR139, GPR141 , GPR142, GPR143,

GPR146, GPR148, GPR149, GPR15, GPR150, GPR151 , GPR152, GPR153, GPR156, GPR157, GPR158, GPR160, GPR161 , GPR162, GPR171 , GPR173, GPR174, GPR176, GPR179, GPR182, GPR183, GPRC5A, GPRC5B, GPRC5C, GPRC5D, GPRC6A, GRM1 , GRM2, GRM3, GRM4, GRM5, GRM6, GRM7, GRM8, GRPR, HCAR1 , HCAR2, HCAR3, HCRTR1 , HCRTR2, HRH1 , HRH2, HRH3, HRH4, HTR1A, HTR1 B, HTR1 D, HTR1 E , HTR1 F, HTR2A, HTR2 B, HTR2C, HTR4, HTR5A, HTR5BP, HTR6, HTR7, KISS1 R , LGR3, LGR4, LGR5, LGR6, LHCGR, LPAR1 , LPAR2, LPAR3, LPAR4, LPAR5, LPAR6, LTB4R, LTB4R2 , MAS1 , MAS1 L, MC1 R , MC2 R , MC3R, MC4R, MC5R, MCHR1 , MCH R2, MLNR, MRG PRD, MRG PRE, MRGPRF, MRG PRG, MRG PRX1 , MRG PRX2, MRG PRX3, MRGPRX4,   MTNR1A, MTN R 1B , NMBR, NMUR1 , NMUR2 , NPBWR1 , NPBWR2 , NPFFR1 , NPFFR2 , NPSR1 , NPY1 R , NPY2 R , NPY4R, NPY5R, NPY6R, NTSR1 , NTSR2, OPN 3 , OPN 4 , OPN 5 , OPRD1 , OPRK1 , OPRL1 , OPRM 1, OR51 E 1 , OXER1 , OXGR1 , OXTR, P2 RY1 , P2 RY1 0 , P2 RY1 1, P2 RY1 2 , P2 RY1 3 , P2 RY1 4 , P2RY2, P2RY4, P2RY6, P2RY8, PRLHR, PROKR1 , PROKR2, PTAFR, PTGDR, PTGDR2, PTGER1 , PTGER2, PTGER3, PTGER4, PTGFR, PTGIR, PTH1 R , PTH2R, QRFPR, RXFP1 , RXFP2, RXFP3, RXFP4, S 1PR1 , S 1PR2 , S 1PR3 , S 1PR4 , S 1PR5 , SCTR, SMO, SSTR1 , SSTR2, SSTR3, SSTR4, SSTR5, SUCNR1 , TAAR1 , TAAR2, TAAR3, TAAR4P, TAAR5, TAAR6, TAAR8, TAAR9, TACR1 , TACR2, TACR3, TAS1 R 1 , TAS1 R2, TAS1 R3, TAS2R1 , TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R19, TAS2R20, TAS2R3, TAS2R30, TAS2R31 , TAS2R38, TAS2R39, TAS2R4, TAS2R40, TAS2R41 , TAS2R42, TAS2R43, TAS2R45, TAS2R46, TAS2R5, TAS2R50, TAS2R60, TAS2R7, TAS2R8, TAS2R9, TBXA2R, TPRA1 , TRHR, TSHR, UTS2R, VIPR1 , VIPR2, XCR1 , TCR-a, TCR-β, CD3, ζ-chain accessory, CD4, CD8, SIGIRR (Single Ig And TIR Domain Containing), mannose receptor (MR), asialoglycoprotein receptor family (e.g., asialoglycoprotein receptor macrophage galactose-type lectin (MGL)), DC-SIGN (CLEC4L), langerin (CLEC4K), myeloid DAP12-associating lectin (MDL)-1 (CLEC5A), dectin 1/CLEC7A, DNGR1/CLEC9A, Myeloid C-type lectin-like receptor (MICL) (CLEC12A), CLEC2 (also called CLEC1 B), CLEC12B, DCIR/CLEC4A, Dectin 2/CLEC6A, Blood DC antigen 2 (BDCA2) (CLEC4C), macrophage- inducible C-type lectin (CLEC4E), TLR1 , TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR1 1, TLR12, TLR13, FcyRI (CD64), FcyRIIA (CD32), FcyRIIBI (CD32), FCYRI IB2 (CD32), FcyRIIIA (CD16a), FcyRIIIB (CD16b), FceRI, FceRII (CD23), FcaR1 (CD89), Fca^R, FcRn, CD27, CD40, OX40, GITR, CD137, PD-1 , CTLA-4, PD-L1 , TIGIT, T-cell immunoglobulin domain and mucin domain 3 (TIM3), V-domain Ig suppressor of T cell activation (VISTA), CD28, CD122, ICOS, A2AR, B7-H3, B7-H4, B and T lymphocyte attenuator (BTLA), Indoleamine 2,3-dioxygenase (IDO), killer-cell immunoglobulin-like receptor (KIR), lymphocyte activation gene-3 (LAG3), FAM159B, HLA-A, HLA-B, HLA-C, HLA-DPA1 , HLA-DPB1 , HLA-DQA1 , HLA-DQB1 , HLA-DRA, HLA-DRB1 , gp130, IL-1 receptor, IL-2 receptor, IL-3 receptor, IL-4

receptor, IL-5 receptor, IL-6 receptor, IL-7 receptor, IL-8 receptor, IL-9 receptor, IL-10 receptor, IL-11 receptor, IL-12 receptor, IL-13 receptor, IL-14 receptor, IL-15 receptor, IL-16 receptor, IL-17 receptor, IL-18 receptor, IL-19 receptor, IL-20 receptor, IL-21 receptor, IL-22 receptor, IL-23 receptor, IL-24 receptor, IL-25 receptor, IL-26 receptor, IL-27 receptor, IL-28 receptor, IL-29 receptor, IL-30 receptor, IL-31 receptor, IL-32 receptor, IL-33 receptor, IL-35 receptor, IL-36 receptor, FGFR1 , FGFR2, FGFR3, FGFR4, TNFRSF1A, TNFRSF1 B, TNFRSF3, TNFRSF4, TNFRSF5, TNFRSF6, TNFRSF6B, TNFRSF7, TNFRSF8, TNFRSF9, TNFRSF10A, TNFRSF10B, TNFRSF10C, TNFRSF10D, TNFRSF1 1A, TNFRSF1 1B, TNFRSF12A, TNFRSF13B, TNFRSF13C, TNFRSF14, TNFRSF16, TNFRSF17, TNFRSF18, TNFRSF19, TNFRSF21 , TNFRSF25, TNFRSF27, SCN1A, SCN1 B, SCN2A, SCN2B, SCN3A, SCN3B, SCN4A, SCN5A, SCN7A, SCN8A, SCN9A, SCN10A, SCN1 1A, CACNA1A, CACNA1 B, CACNA1C, CACNA1 D, CACNA1 E, CACNA1 F, CACNA1G, CACNA1 H, CACNA1 I, CACNA1S, TRPA1 , TRPC1 , TRPC2, TRPC3, TRPC4, TRPC5, TRPC6, TRPC7, TRPM1 , TRPM2, TRPM3, TRPM4, TRPM5, TRPM6, TRPM7, TRPM8, MCOLN1 , MCOLN2, MCOLN3, PKD1 , PKD2, PKD2L1 , PKD2L2, TRPV1 , TRPV2, TRPV3, TRPV4, TRPV5, TRPV6, CATSPER1 , CATSPER2, CATSPER3, CATSPER4, TPCN1 , TPCN2, CNGA1 , CNGA2, CNGA3, CNGA4, CNGB1 , CNGB3, HCN1 , HCN2, HCN3, HCN4, KCNMA1 , KCNN1 , KCNN2, KCNN3, KCNN4, KCNT1 , KCNT2, KCNU1 , KCNA1 , KCNA2, KCNA3, KCNA4, KCNA5, KCNA6, KCNA7, KCNA10, KCNB1 , KCNB2, KCNC1 , KCNC2, KCNC3, KCNC4, KCND1 , KCND2, KCND3, KCNF1 , KCNG1 , KCNG2, KCNG3, KCNG4, KCNH1 , KCNH2, KCNH3, KCNH4, KCNH5, KCNH6, KCNH7, KCNH8, KCNQ1 , KCNQ2, KCNQ3, KCNQ4, KCNQ5, KCNS1 , KCNS2, KCNS3, KCNV1 , KCNV2, KCNJ1 , KCNJ2, KCNJ3, , KCNJ4, KCNJ5, KCNJ6, KCNJ8, KCNJ9, KCNJ10, KCNJ1 1, KCNJ12, KCNJ13, KCNJ14, KCNJ15, KCNJ16, KCNJ18, KCNK1 , KCNK2, KCNK3, KCNK4, KCNK5, KCNK6, KCNK7, KCNK9, KCNK10, KCNK12, KCNK13, KCNK15, KCNK16, KCNK17, KCNK18, HVCN1 , HTR3A, HTR3B, HTR3C, HTR3D, HTR3E, CHRNA1 , CHRNA2, CHRNA3, CHRNA4, CHRNA5, CHRNA6, CHRNA7, CHRNA9, CHRNA10, CHRNB1 , CHRNB2, CHRNB3, CHRNB4, CHRND, CHRNE, CHRNG, GABRA1 , GABRA2, GABRA3, GABRA4, GABRA5, GABRA6, GABRB1 , GABRB2, GABRB3, GABRD, GABRE, GABRG1 , GABRG2, GABRG3 ,GABRP, GABRQ, GABRR1 , GABRR2, GABRR3, GRIA1 , GRIA2, GRIA3, GRIA4, GRID1 , GRID2, GRIK1 , GRIK2, GRIK3, GRIK4, GRIK5, GRIN1 , GRIN2A, GRIN2B, GRIN2C, GRIN2D, GRIN3A, GRIN3B, GLRA1 , GLRA2, GLRA3, GLRA4, P2RX1 , P2RX2, P2RX3, P2RX4, P2RX5, P2RX6, P2RX7, ZACN, ASIC1 , ASIC2, ASIC3, ASIC4, AQP1 , AQP2, AQP3, AQP4, AQP5, AQP6, AQP7, AQP8, AQP9, AQP10, AQP1 1, AQP12A, AQP12B, MIP, CLCN1 , CLCN2, CLCN3, CLCN4, CLCN5, CLCN6, CLCN7, CLCNKA, CLCNKB, Cystic fibrosis transmembrane conductance regulator (CFTR), AN01 , AN02, AN03, AN04, AN05, AN06, AN07, AN08, AN09, ANO10, BEST1 , BEST2, BEST3, BEST4,

CLIC1 , CLIC2, CLIC3, CLIC4, CLIC5, CLIC6, GJA1 , GJA3, GJA4, GJA5, GJA6P, GJA8, GJA9,
GJA10, GJB1 , GJB2, GJB3, GJB4, GJB5, GJB6, GJB7, GJC1 , GJC2, GJC3, GJD2, GJD3,
GJD4, GJE1 , ITPR1 , ITPR2, ITPR3, PANX1 , PANX2, PANX3, RYR1 , RYR2, RYR3, NALCN,
SCNN1A, SCNN1 B, SCNN1 D, SCNN1G, TEM16A, ADAMTS7, ANGPTL3, ANGPTL4,
ANGPTL8, LPL, GDF15, galectin-1 , galectin-2, galectin-3, galectin-4, galectin-7, galectin-8,
galectin-9, galectin-1 0, galectin-12, galectin-1 3, matrix gla protein (MGP), PRNP, DGAT1 ,
GPAT3, DMC1 , BLM, BRCA2, members of the human endogenous retrovirus type K (HERV-K)
family, ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1), ectonucleoside
triphosphate diphosphohydrolase 2 (ENTPD2), SLC1A1 , SLC1A2, SLC1 A3, SLC1A4, SLC1A5,
SLC1A6, SLC1A7, SLC2A1 , SLC2A2, SLC2A3, SLC2A4, SLC2A5, SLC2A6, SLC2A7, SLC2A8,
SLC2A9, SLC2A10, SLC2A1 1, SLC2A12, SLC2A13, SLC2A14, SLC3A1 , SLC3A2, SLC4A1 ,
SLC4A2, SLC4A3, SLC4A4, SLC4A5, SLC4A6, SLC4A7, SLC4A8, SLC4A9, SLC4A10,
SLC4A1 1, SLC5A1 , SLC5A2, SLC5A3, SLC5A4, SLC5A5, SLC5A6, SLC5A7, SLC5A8, SLC5A9,
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SLC6A7, SLC6A8, SLC6A9, SLC6A10, SLC6A1 1, SLC6A12, SLC6A13, SLC6A14, SLC6A15,
SLC6A16, SLC6A17, SLC6A18, SLC6A19, SLC6A20, SLC7A5, SLC7A6, SLC7A7, SLC7A8,
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SLC9A2, SLC9A3, SLC9A4, SLC9A5, SLC9A6, SLC9A7, SLC9A8, SLC9A9, SLC9A10,
SLC9A1 1, SLC9B1 , SLC9B2, SLC10A1 , SLC10A2, SLC10A3, SLC10A4, SLC10A5, SLC10A6,
SLC10A7, SLC1 1A1 , SLC1 1A2, SLC12A1 , SLC12A2, SLC12A3, SLC12A4, SLC12A5, SLC12A6,
SLC12A7, SLC12A8, SLC12A9, SLC13A1 , SLC13A2, SLC13A3, SLC13A4, SLC13A5, SLC14A1 ,
SLC14A2, SLC15A1 , SLC15A2, SLC15A3, SLC15A4, SLC16A1 , SLC16A2, SLC16A3, SLC16A4,
SLC16A5, SLC16A6, SLC16A7, SLC16A8, SLC16A9, SLC16A10, SLC16A1 1, SLC16A12,
SLC16A13, SLC16A14, SLC17A1 , SLC17A2, SLC17A3, SLC17A4, SLC17A5, SLC17A6,
SLC17A7, SLC17A8, SLC17A9, SLC18A1 , SLC18A2, SLC18A3, SLC19A1 , SLC19A2, SLC19A3,
SLC20A1 , SLC20A2, SLC01A2, SLC01 B1 , SLC01 B3, SLC01C1 , SLC02A1 , SLC02B1 ,
SLC03A1 , SLC04A1 , SLC04C1 , SLC05A1 , SLC06A1 , SLC22A1 , SLC22A2, SLC22A3,
SLC22A4, SLC22A5, SLC22A6, SLC22A7, SLC22A8, SLC22A9, SLC22A10, SLC22A1 1,
SLC22A12, SLC22A13, SLC22A14, SLC22A15, SLC22A16, SLC22A17, SLC22A18,
SLC22A18AS,SLC22A19, SLC22A20, SLC22A23, SLC22A24, SLC22A25, SLC22A31 , SLC23A1 ,
SLC23A2, SLC23A3, SLC23A4, SLC24A1 , SLC24A2, SLC24A3, SLC24A4, SLC24A5, SLC24A6,
SLC25A1 , SLC25A2, SLC25A3, SLC25A4, SLC25A5, SLC25A6, SLC25A7, SLC25A8, SLC25A9,
SLC25A10, SLC25A1 1, SLC25A12, SLC25A13, SLC25A14, SLC25A15, SLC25A16, SLC25A17,
SLC25A18, SLC25A19.SLC25A20, SLC25A21 , SLC25A22, SLC25A23, SLC25A24, SLC25A25,
SLC25A26, SLC25A27, SLC25A28, SLC25A29, SLC25A30, SLC25A31 , SLC25A32, SLC25A33,

SLC25A34, SLC25A35, SLC25A36, SLC25A37,SLC25A38, SLC25A39, SLC25A40, SLC25A41 , SLC25A42, SLC25A43, SLC25A44, SLC25A45, SLC25A46, SLC26A1 , SLC26A2, SLC26A3, SLC26A4, SLC26A5, SLC26A6, SLC26A7, SLC26A8, SLC26A9, SLC26A10, SLC26A1 1, SLC27A1 , SLC27A2, SLC27A3, SLC27A4, SLC27A5, SLC27A6, SLC28A1 , SLC28A2, SLC28A3, SLC29A1 , SLC29A2, SLC29A3, SLC29A4, SLC30A1 , SLC30A2, SLC30A3, SLC30A4, SLC30A5, SLC30A6, SLC30A7, SLC30A8, SLC30A9, SLC30A10, SLC31A1 , SLC31A2, SLC32A1 , SLC33A1 , SLC34A1 , SLC34A2, SLC34A3, SLC35A1 , SLC35A2, SLC35A3, SLC35A4, SLC35A5, SLC35B1 , SLC35B2, SLC35B3, SLC35B4, SLC35C1 , SLC35C2, SLC35D1 , SLC35D2, SLC35D3, SLC35E1 , SLC35E2, SLC35E3, SLC35E4, SLC35F1 , SLC35F2, SLC35F3, SLC35F4, SLC35F5, SLC35G1 , SLC35G3, SLC35G4, SLC35G5, SLC35G6, SLC36A1 , SLC36A2, SLC36A3, SLC36A4, SLC37A1 , SLC37A2, SLC37A3, SLC37A4, SLC38A1 , SLC38A2, SLC38A3, SLC38A4, SLC38A5, SLC38A6, SLC38A7, SLC38A8, SLC38A9, SLC38A10, SLC38A1 1, SLC39A1 , SLC39A2, SLC39A3, SLC39A4, SLC39A5, SLC39A6, SLC39A7, SLC39A8, SLC39A9, SLC39A10, SLC39A1 1, SLC39A12, SLC39A13, SLC39A14, SLC40A1 , SLC41A1 , SLC41A2, SLC41A3, RhAG, RhBG, RhCG, SLC43A1 , SLC43A2, SLC43A3, SLC44A1 , SLC44A2, SLC44A3, SLC44A4, SLC44A5, SLC45A1 , SLC45A2, SLC45A3, SLC45A4, SLC46A1 , SLC46A2, SLC46A3, SLC47A1 , SLC47A2, HCP-1 , MFSD5, MFSD10, SLC50A1 , OSTa, ΟβΤβ, SLC52A1 , SLC52A2, and SLC52A3.

[0025]   Aspect 13. The method of aspect 1 or 2 wherein the target antigen is difficult to express or difficult to raise antibodies against.

[0026]   Aspect 14. The method of aspect 13, wherein expression of the target protein leads to cytotoxicity or increases in cytotoxicity in host production cells.

[0027]   Aspect 15. The method of aspect 13, wherein the target protein, when expressed recombinantly and/or purified, exhibits poor yield, stability, solubility, and/or functional activity.

[0028]   Aspect 16. The method of any one of the preceding aspects wherein said polyribonucleotide of the complex comprises one or more of the following: a consensus Kozak sequence; a 7-methylguanosine cap on the 5' end of the mRNA; a polyadenosine (polyA) tail found at the 3' terminus of the mRNA transcript; and 5'-and 3'-untranslated regions (UTRs).

[0029]   Aspect 17. The method of any one of the preceding aspects, wherein said administering is parenteral.

[0030]   Aspect 18. The method of any one of the preceding aspects, wherein said administering is intravenous.

[0031]   Aspect 19. The method of any one of the preceding aspects, wherein said administering is intramuscular.

[0032]   Aspect 20. The method of any one of the preceding aspects, wherein said administering is subcutaneous.

[0033]   Aspect 21. The method of any one of aspects 1-16, wherein said administering is intranasal.

[0034]   Aspect 22.   The method of any one of the preceding aspects, wherein said target protein is RXFP1 or a fragment thereof.

[0035]   Aspect 23.   The method of any one of the preceding aspects, wherein said complex comprises a polyribonucleotide comprising the nucleotide sequence of SEQ ID NO:4, or any one of SEQ ID NOs: 2, 4, and 37.

[0036]   Aspect 24.   The method of any one of the preceding aspects, wherein said target protein is SLC52A2 or a fragment thereof.

[0037]   Aspect 25.   The method of any one of the preceding aspects, wherein said complex comprises a polyribonucleotide comprising the nucleotide sequence of SEQ ID NO:7, or any one of SEQ ID NOs: 5, 7, and 40.

[0038]   Aspect 26.   The method of any one of the preceding aspects, wherein said target protein is ANGPTL8 or a fragment thereof.

[0039]   Aspect 27.   The method of any one of the preceding aspects, wherein said complex comprises a polyribonucleotide comprising the nucleotide sequence of SEQ ID NO:10, or any one of SEQ ID NOs: 8, 10, and 43.

[0040]   Aspect 28.   The method of any one of the preceding aspects, wherein said target protein is TSHR or a fragment thereof.

[0041]   Aspect 29.   The method of any one of the preceding aspects, wherein said complex comprises a polyribonucleotide comprising the nucleotide sequence of SEQ ID NO:16, or any one of SEQ ID NOs: 14, 16, and 46.

[0042]   Aspect 30.   The method of any one of the preceding aspects, wherein said target protein is APJ or a fragment thereof.

[0043]   Aspect 31.   The method of any one of the preceding aspects, wherein said complex comprises a polyribonucleotide comprising the nucleotide sequence of SEQ ID NO:19, or any one of SEQ ID NOs: 17, 19, and 49.

[0044]   Aspect 32.   The method of any one of the preceding aspects, wherein said target protein is gp130 or a fragment thereof.

[0045]   Aspect 33.   The method of any one of the preceding aspects, wherein said complex comprises a polyribonucleotide comprising the nucleotide sequence of SEQ ID NO:22, or any one of SEQ ID NOs: 20, 22, and 52.

[0046]   Aspect 34,   The method of any one of the preceding aspects, wherein said target protein is Galectin 3 or a fragment thereof.

[0047]   Aspect 35.   The method of any one of the preceding aspects, wherein said complex comprises a polyribonucleotide comprising the nucleotide sequence of SEQ ID NO:55, or any one of SEQ ID NOs: 26, 55, and 56.

[0048]   Aspect 36. The method of any one of aspects 1-35, wherein said complex has a diameter of approximately 30-150 nm.

[0049]   Aspect 37.   The method of any one of aspects 1-35, wherein the complex comprises helper lipids.

[0050]   Aspect 38. The method of any one of aspects 1-35, wherein the complex comprises any combination of (i) cationic lipid, (ii) a helper lipid, for example cholesterol, (iii) a neutral lipid, for example DSPC, and (iv) a stealth lipid, for example S010, S024, S027, S031, or S033.

[0051]   Aspect 39.   The method of any one of aspects 1-38, wherein the animal is administered with 5 μg, 10 μg, 12.5 μg, 20 μg, 25 μg, 30 μg, 40 μg, 50 μg, 60 μg, 70 μg, 80 μg, 90 μg, 100 μg, 110 μg, 120 μg, 130 μg, 140 μg or 150 μg polyribonucleotide.

[0052]   Aspect 40. The method of any one of aspects 1-39, wherein the cationic lipid is selected from the group consisting of: N,N-dioleyl-N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(1-(2,3-dioleoyloxy) propyl)-N,N,N-trimethylammonium chloride (DOTAP), 1,2-Dioleoyl-3-Dimethylammonium -propane (DODAP), N-(1-(2,3-dioleyloxy)propyl) -N,N,N-trimethylammonium chloride (DOTMA), 1,2-Dioleoylcarbamyl -3-Dimethylammonium-propane (DOCDAP), 1,2-Dilineoyl-3-Dimethylammonium-propane (DLINDAP), dilauryl($C_{12:0}$) trimethyl ammonium propane (DLTAP), Dioctadecylamidoglycyl spermine (DOGS), DC-Choi, Dioleoyloxy -N-[2-sperminecarboxamido)ethyl} -N,N-dimethyl-1-propanaminiumtrifluoroacetate (DOSPA), 1,2-Dimyristyloxypropyl-3-dimethyl -hydroxyethyl ammonium bromide (DMRIE), 3-Dimethylamino-2-(Cholest-5-en -3-beta-oxybutan-4-oxy) -1-(cis,cis-9,12 -octadecadienoxy)prop ane (CLinDMA), N,N-dimethyl-2,3-dioleyloxy)propylamine (DODMA), 2-[5'-(cholest-5-en-3[beta]-oxy)-3'-oxapentoxy) -3-dimethyl-1-(cis,cis-9',12'-octadecadienoxy) propane (CpLinDMA) and N,N-Dimethyl-3,4-dioleyloxybenzylamine (DMOBA), and 1,2-N,N'-Dioleylcarbamyl-3-dimethylaminopropane (DOcarbDAP).

[0053]   Aspect 41. The method of aspect 40, wherein the cationic lipid is DOTAP or DLTAP.

[0054]   Aspect 42. The method of any one of aspects 1-41 further comprising the step of generating hybridomas producing antibodies that specifically bind the target antigen.

[0055]   Aspect 43.   The method of any one of aspects 1-42 further comprising the step of purifying antibodies that specifically bind to the target protein.

[0056]   Aspect 44. The method of any one of aspects 1-43, further comprising the step of generating chimeric or humanized antibodies derived from the purified antibodies that specifically bind the target protein.

[0057]   Aspect 45. The method of any one of aspects 1-44, wherein said method produces higher antibody titer in sera from a first bleed or a second bleed relative to a method comprising immunization with cDNA, protein or peptide, a viral particle, or whole cell.

[0058]   Aspect 46. The method of any one of aspects 1-44, wherein said method produces a higher number of hybridomas producing target protein-specific antibodies than a method comprising immunization with cDNA, protein or peptide, a viral particle, or whole cell.

[0059]   Aspect 47. The method of any one of aspects 1-46, wherein the target protein is a human target protein, and the non-human animal is a mouse, rat, rabbit, sheep, cat, dog, camelid, shark, monkey, pig, or horse.

[0060]   Aspect 48. A hybridoma producing an antibody that specifically binds to the target protein, wherein the hybridoma is obtainable by the method of any one of aspects 1-47.

[0061]   Aspect 49. A mixture of polyclonal antibodies, which specifically bind to the target protein, wherein the mixture is obtainable from the method of any one of aspects 1-47.

[0062]   Aspect 50. An isolated monoclonal antibody which specifically binds to the target protein, wherein the monoclonal antibody is obtainable by the method of any one of aspects 1-47.

[0063]   Aspect 51. A method for eliciting an immune response to a target protein in a non-human animal, comprising the steps of: administering a lipid-polynucleotide complex to the animal, wherein the lipid-polynucleotide complex comprises a cationic lipid and an mRNA coding for a target protein, wherein the target protein is of a species different than the animal.

[0064]   Aspect 52. The method of aspect 51 wherein said complex comprises one or more of the following: a consensus Kozak sequence; a 7-methylguanosine cap on the 5' end of the mRNA ; a polyadenosine (polyA) tail found at the 3' terminus of the mRNA transcript; and 5'-and 3'-untranslated regions (UTRs).

[0065]   Aspect 53.   The method of aspect 51, wherein said administering is parenteral.

[0066]   Aspect 54.   The method of aspect 51, wherein said administering is intravenous.

[0067]   Aspect 55.   The method of aspect 51, wherein said administering is intramuscular.

[0068]   Aspect 56.   The method of aspect 51, wherein said administering is subcutaneous.

[0069]   Aspect 57.   The method of aspect 51, wherein said administering is intranasal.

[0070]   Aspect 58.   The method of any one of aspects 51-57, wherein said target protein is RXFP1 .

[0071]   Aspect 59.   The method of any one of aspects 51-57, wherein said complex comprises a polyribonucleotide comprising the nucleotide sequence of SEQ ID NO:4, or any one of SEQ ID NOs: 2, 4, and 37.

[0072]   Aspect 60.   The method of any one of aspects 51-57, wherein said target protein is SLC52A2.

[0073]   Aspect 6 1.   The method of any one of aspects 51-57, wherein said complex comprises a polyribonucleotide comprising the nucleotide sequence of SEQ ID NO:7, or any one of SEQ ID NOs: 5, 7, and 40.

[0074]   Aspect 62.   The method of any one of aspects 51-57, wherein said target protein is ANGPTL8.

[0075]   Aspect 63.   The method of any one of aspects 51-57, wherein said complex comprises a polyribonucleotide comprising the nucleotide sequence of SEQ ID NO:10, or any one of SEQ ID NOs: 8, 10, and 43.

[0076]   Aspect 64.   The method of any one of aspects 51-57, wherein said target protein is TSHR.

[0077]   Aspect 65.   The method of any one of aspects 51-57, wherein said complex comprises a polyribonucleotide comprising the nucleotide sequence of SEQ ID NO:16, or any one of SEQ ID NOs: 14, 16, and 46.

[0078]   Aspect 66.   The method of any one of aspects 51-57, wherein said target protein is APJ.

[0079]   Aspect 67.   The method of any one of aspects 51-57, wherein said complex comprises a polyribonucleotide comprising the nucleotide sequence of SEQ ID NO:19, or any one of SEQ ID NOs: 17, 19, and 49.

[0080]   Aspect 68.   The method of any one of aspects 51-57, wherein said target protein is GP130.

[0081]   Aspect 69.   The method of any one of aspects 51-57, wherein said complex comprises a polyribonucleotide comprising the nucleotide sequence of SEQ ID NO: 22, or any one of SEQ ID NOs: 20, 22, and 52.

[0082]   Aspect 70.   The method of aspect 5 1, wherein said target protein is Galectin 3.

[0083]   Aspect 7 1.   The method of aspect 5 1, wherein said complex comprises SEQ ID NO:55, or any one of SEQ ID NOs: 26, 55, and 56.

[0084]   Aspect 72.   The method of any one of aspects 51-71 , which further comprises the step of obtaining antibodies, which specifically binds the target protein, or an antibody-producing cell, from the animal.

[0085]   Aspect 73. The method of any one of aspects 51-72, wherein the target protein is a human target protein, and the non-human animal is a mouse, rat, rabbit, sheep, cat, dog, camelid, shark, monkey, pig, or horse.

[0086]   Aspect 74. The method of any one of aspects 51-73, wherein the complex comprises any combination of (i) cationic lipid, (ii) a helper lipid, for example cholesterol, (iii) a neutral lipid, for example DSPC, and (iv) a stealth lipid, for example S010, S024, S027, S031 , or S033.

[0087]   Aspect 75.   The method of any one of aspects 51-74, wherein the animal is administered with 5 μg, 10 μg, 12.5 μg, 20 μg, 25 μg, 30 μg, 40 μg, 50 μg, 60 μg, 70 μg, 80 μg, 90 μg, 100 μg, 110 μg, 120 μg, 130 μg, 140 μg or 150 μg polyribonucleotide (e.g., mRNA).

[0088]   Aspect 76. The method of any one of aspects 51-75, wherein the cationic lipid is selected from the group consisting of: N,N-dioleyl-N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(1-(2,3-dioleoyloxy) propyl)-N,N,N-trimethylammonium chloride (DOTAP), 1,2-Dioleoyl-3-Dimethylammonium -propane (DODAP), N-(1-(2,3-dioleyloxy)propyl) -N,N,N-trimethylammonium chloride (DOTMA), 1,2-Dioleoylcarbamyl -3-Dimethylammonium-propane (DOCDAP), 1,2-Dilineoyl-3-Dimethylammonium-propane (DLINDAP), dilauryl($C_{12:0}$) trimethyl ammonium propane (DLTAP), Dioctadecylamidoglycyl spermine (DOGS), DC-Choi, Dioleoyloxy -N-[2-sperminecarboxamido)ethyl} -N,N-dimethyl-1-propanaminiumtrifluoroacetate (DOSPA), 1,2-Dimyristyloxypropyl-3-dimethyl -hydroxyethyl ammonium bromide (DMRIE), 3-Dimethylamino-2-(Cholest-5-en -3-beta-oxybutan-4-oxy) -1-(cis,cis-9,12 -octadecadienoxy)prop ane (CLinDMA), N,N-dimethyl-2,3-dioleyloxy)propylamine (DODMA), 2-[5'-(cholest-5-en-3[beta]-oxy)-3'-oxapentoxy) -3-dimethyl-1-(cis,cis-9',12'-octadecadienoxy) propane (CpLinDMA) and N,N-Dimethyl-3,4-dioleyloxybenzylamine (DMOBA), and 1,2-N,N'-Dioleylcarbamyl-3-dimethylaminopropane (DOcarbDAP).

[0089]   Aspect 77. The method of aspect 76, wherein the cationic lipid is DOTAP or DLTAP.

[0090]   Aspect 78. The method of any one of aspects 51-77, wherein the target protein is selected from the following:

ACKR1 , ACKR2, ACKR3, ACKR4, ADCYAP1 R1, ADGRA1 , ADGRA2, ADGRA3, ADGRB1 , ADGRB2, ADGRB3, ADGRD1 , ADGRD2, ADGRE1 , ADGRE2, ADGRE3, ADGRE4P, ADGRE5, ADGRF1 , ADGRF2, ADGRF3, ADGRF4, ADGRF5, ADGRG1 , ADGRG2, ADGRG3, ADGRG4, ADGRG5, ADGRG6, ADGRG7, ADGRL1 , ADGRL2, ADGRL3, ADGRL4, ADGRV1 ,

14

ADORA1 , ADORA2A, ADORA2B, ADORA3, ADRA1A, ADRA1 B, ADRA1 D, ADRA2A, ADRA2B,
ADRA2C, ADRB1 , ADRB2, ADRB3, AGTR1 , AGTR2, APLNR/APJ, ASGR1 , ASGR2, AVPR1A,
AVPR1 B, AVPR2, BDKRB1 , BDKRB2, BRS3, BRS3, C3AR1 , C5AR1 , C5AR2, CALCR, CALCRL,
CASR, CCKAR, CCKBR, CCR1 , CCR10, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8,
CCR9, CCRL2, CELSR1 , CELSR2, CELSR3, CHRM1 , CHRM2, CHRM3, CHRM4, CHRM5,
CMKLR1 , CNR1 , CNR2, CRHR1 , CRHR2, CX3CR1 , CXCR1 , CXCR2, CXCR3, CXCR4, CXCR5,
CXCR6, CYSLTR1 , CYSLTR2, DRD1 , DRD2, DRD3, DRD4, DRD5, EDNRA, EDNRB, F2R,
F2RL1 , F2RL2, F2RL3, FFAR1 , FFAR2, FFAR3, FFAR4, FPR1 , FPR2, FPR2, FPR3, FSHR,
FZD1 , FZD10, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, GABBR1 , GABBR2,
GALR1 , GALR2, GALR3, GCGR, GHRHR, GHSR, GIPR, GLP1 R, GLP2R, GNRHR, GNRHR2,
GPBAR1 , GPER1 , GPR1 , GPR4, GPR12, GPR15, GPR17, GPR18, GPR19, GPR20, GPR21 ,
GPR22, GPR25, GPR26, GPR27, GPR3, GPR31 , GPR32, GPR33, GPR34, GPR35, GPR37,
GPR37L1 , GPR39, GPR40, GPR42, GPR42, GPR45, GPR50, GPR52, GPR55, GPR6, GPR61 ,
GPR62, GPR63, GPR65, GPR68, GPR75, GPR78, GPR79, GPR82, GPR83, GPR84, GPR85,
GPR87, GPR88, GPR101 , GPR107, GPR132, GPR135, GPR137, GPR139, GPR141 , GPR142,
GPR143, GPR146, GPR148, GPR149, GPR15, GPR150, GPR151 , GPR152, GPR153, GPR156,
GPR157, GPR158, GPR160, GPR161 , GPR162, GPR171 , GPR173, GPR174, GPR176,
GPR179, GPR182, GPR183, GPRC5A, GPRC5B, GPRC5C, GPRC5D, GPRC6A, GRM1 , GRM2,
GRM3, GRM4, GRM5, GRM6, GRM7, GRM8, GRPR, HCAR1 , HCAR2, HCAR3, HCRTR1 ,
HCRTR2, HRH1 , HRH2, HRH3, HRH4, HTR1A, HTR1 B, HTR1 D, HTR1 E, HTR1 F, HTR2A,
HTR2B, HTR2C, HTR4, HTR5A, HTR5BP, HTR6, HTR7, KISS1 R, LGR3, LGR4, LGR5, LGR6,
LHCGR, LPAR1 , LPAR2, LPAR3, LPAR4, LPAR5, LPAR6, LTB4R, LTB4R2, MAS1 , MAS1 L,
MC1 R, MC2R, MC3R, MC4R, MC5R, MCHR1 , MCHR2, MLNR, MRGPRD, MRGPRE, MRGPRF,
MRGPRG, MRGPRX1 , MRGPRX2, MRGPRX3, MRGPRX4, MTNR1A, MTNR1 B, NMBR,
NMUR1 , NMUR2, NPBWR1 , NPBWR2, NPFFR1 , NPFFR2, NPSR1 , NPY1 R, NPY2R, NPY4R,
NPY5R, NPY6R, NTSR1 , NTSR2, OPN 3, OPN 4, OPN 5, OPRD1 , OPRK1 , OPRL1 , OPRM1 ,
OR51 E1 , OXER1 , OXGR1 , OXTR, P2RY1 , P2RY10, P2RY1 1, P2RY12, P2RY13, P2RY14,
P2RY2, P2RY4, P2RY6, P2RY8, PRLHR, PROKR1 , PROKR2, PTAFR, PTGDR, PTGDR2,
PTGER1 , PTGER2, PTGER3, PTGER4, PTGFR, PTGIR, PTH1 R, PTH2R, QRFPR, RXFP1 ,
RXFP2, RXFP3, RXFP4, S 1PR1 , S 1PR2, S 1PR3, S 1PR4, S 1PR5, SCTR, SMO, SSTR1 , SSTR2,
SSTR3, SSTR4, SSTR5, SUCNR1 , TAAR1 , TAAR2, TAAR3, TAAR4P, TAAR5, TAAR6, TAAR8,
TAAR9, TACR1 , TACR2, TACR3, TAS1 R 1, TAS1 R2, TAS1 R3, TAS2R1 , TAS2R10, TAS2R13,
TAS2R14, TAS2R16, TAS2R19, TAS2R20, TAS2R3, TAS2R30, TAS2R31 , TAS2R38, TAS2R39,
TAS2R4, TAS2R40, TAS2R41 , TAS2R42, TAS2R43, TAS2R45, TAS2R46, TAS2R5, TAS2R50,
TAS2R60, TAS2R7, TAS2R8, TAS2R9, TBXA2R, TPRA1 , TRHR, TSHR, UTS2R, VIPR1 , VIPR2,

XCR 1, TCR-α, TCR-β, CD3, ζ-chain accessory, CD4, CD8, SIGIRR (Single Ig And TIR Domain Containing), mannose receptor (MR), asialoglycoprotein receptor family (e.g., asialoglycoprotein receptor macrophage galactose-type lectin (MGL)), DC-SIGN (CLEC4L), langerin (CLEC4K), myeloid DAP12-associating lectin (MDL)-1 (CLEC5A), dectin 1/CLEC7A, DNGR1/CLEC9A, Myeloid C-type lectin-like receptor (MICL) (CLEC12A), CLEC2 (also called CLEC1B), CLEC12B, DCIR/CLEC4A, Dectin 2/CLEC6A, Blood DC antigen 2 (BDCA2) (CLEC4C), macrophage-inducible C-type lectin (CLEC4E), TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11, TLR12, TLR13, FcγRI (CD64), FcγRIIA (CD32), FcγRIIBI (CD32), FCYRIIB2 (CD32), FcγRIIIA (CD16a), FcγRIIIB (CD16b), FceRI, FceRII (CD23), FcaR1 (CD89), Fca/pR, FcRn, CD27, CD40, OX40, GITR, CD137, PD-1, CTLA-4, PD-L1, TIGIT, T-cell immunoglobulin domain and mucin domain 3 (TIM3), V-domain Ig suppressor of T cell activation (VISTA), CD28, CD122, ICOS, A2AR, B7-H3, B7-H4, B and T lymphocyte attenuator (BTLA), Indoleamine 2,3-dioxygenase (IDO), killer-cell immunoglobulin-like receptor (KIR), lymphocyte activation gene-3 (LAG3), FAM159B, HLA-A, HLA-B, HLA-C, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, HLA-DRA, HLA-DRB1, gp130, IL-1 receptor, IL-2 receptor, IL-3 receptor, IL-4 receptor, IL-5 receptor, IL-6 receptor, IL-7 receptor, IL-8 receptor, IL-9 receptor, IL-10 receptor, IL-11 receptor, IL-12 receptor, IL-13 receptor, IL-14 receptor, IL-15 receptor, IL-16 receptor, IL-17 receptor, IL-18 receptor, IL-19 receptor, IL-20 receptor, IL-21 receptor, IL-22 receptor, IL-23 receptor, IL-24 receptor, IL-25 receptor, IL-26 receptor, IL-27 receptor, IL-28 receptor, IL-29 receptor, IL-30 receptor, IL-31 receptor, IL-32 receptor, IL-33 receptor, IL-35 receptor, IL-36 receptor, FGFR1, FGFR2, FGFR3, FGFR4, TNFRSF1A, TNFRSF1B, TNFRSF3, TNFRSF4, TNFRSF5, TNFRSF6, TNFRSF6B, TNFRSF7, TNFRSF8, TNFRSF9, TNFRSF10A, TNFRSF10B, TNFRSF10C, TNFRSF10D, TNFRSF11A, TNFRSF11B, TNFRSF12A, TNFRSF13B, TNFRSF13C, TNFRSF14, TNFRSF16, TNFRSF17, TNFRSF18, TNFRSF19, TNFRSF21, TNFRSF25, TNFRSF27, SCN1A, SCN1B, SCN2A, SCN2B, SCN3A, SCN3B, SCN4A, SCN5A, SCN7A, SCN8A, SCN9A, SCN10A, SCN11A, CACNA1A, CACNA1B, CACNA1C, CACNA1D, CACNA1E, CACNA1F, CACNA1G, CACNA1H, CACNA1I, CACNA1S, TRPA1, TRPC1, TRPC2, TRPC3, TRPC4, TRPC5, TRPC6, TRPC7, TRPM1, TRPM2, TRPM3, TRPM4, TRPM5, TRPM6, TRPM7, TRPM8, MCOLN1, MCOLN2, MCOLN3, PKD1, PKD2, PKD2L1, PKD2L2, TRPV1, TRPV2, TRPV3, TRPV4, TRPV5, TRPV6, CATSPER1, CATSPER2, CATSPER3, CATSPER4, TPCN1, TPCN2, CNGA1, CNGA2, CNGA3, CNGA4, CNGB1, CNGB3, HCN1, HCN2, HCN3, HCN4, KCNMA1, KCNN1, KCNN2, KCNN3, KCNN4, KCNT1, KCNT2, KCNU1, KCNA1, KCNA2, KCNA3, KCNA4, KCNA5, KCNA6, KCNA7, KCNA10, KCNB1, KCNB2, KCNC1, KCNC2, KCNC3, KCNC4, KCND1, KCND2, KCND3, KCNF1, KCNG1, KCNG2, KCNG3, KCNG4, KCNH1, KCNH2, KCNH3, KCNH4, KCNH5, KCNH6, KCNH7, KCNH8, KCNQ1, KCNQ2, KCNQ3, KCNQ4,

KCNQ5, KCNS1 , KCNS2, KCNS3, KCNV1 , KCNV2, KCNJ1 , KCNJ2, KCNJ3, , KCNJ4, KCNJ5, KCNJ6, KCNJ8, KCNJ9, KCNJ10, KCNJ1 1, KCNJ12, KCNJ13, KCNJ14, KCNJ15, KCNJ16, KCNJ18, KCNK1 , KCNK2, KCNK3, KCNK4, KCNK5, KCNK6, KCNK7, KCNK9, KCNK10, KCNK12, KCNK13, KCNK15, KCNK16, KCNK17, KCNK18, HVCN1 , HTR3A, HTR3B, HTR3C, HTR3D, HTR3E, CHRNA1 , CHRNA2, CHRNA3, CHRNA4, CHRNA5, CHRNA6, CHRNA7, CHRNA9, CHRNA10, CHRNB1 , CHRNB2, CHRNB3, CHRNB4, CHRND, CHRNE, CHRNG, GABRA1 , GABRA2, GABRA3, GABRA4, GABRA5, GABRA6, GABRB1 , GABRB2, GABRB3, GABRD, GABRE, GABRG1 , GABRG2, GABRG3 ,GABRP, GABRQ, GABRR1 , GABRR2, GABRR3, GRIA1 , GRIA2, GRIA3, GRIA4, GRID1 , GRID2, GRIK1 , GRIK2, GRIK3, GRIK4, GRIK5, GRIN1 , GRIN2A, GRIN2B, GRIN2C, GRIN2D, GRIN3A, GRIN3B, GLRA1 , GLRA2, GLRA3, GLRA4, P2RX1 , P2RX2, P2RX3, P2RX4, P2RX5, P2RX6, P2RX7, ZACN, ASIC1 , ASIC2, ASIC3, ASIC4, AQP1 , AQP2, AQP3, AQP4, AQP5, AQP6, AQP7, AQP8, AQP9, AQP10, AQP1 1, AQP12A, AQP12B, MIP, CLCN1 , CLCN2, CLCN3, CLCN4, CLCN5, CLCN6, CLCN7, CLCNKA, CLCNKB, Cystic fibrosis transmembrane conductance regulator (CFTR), AN01 , AN02, AN03, AN04, AN05, AN06, AN07, AN08, AN09, ANO10, BEST1 , BEST2, BEST3, BEST4, CLIC1 , CLIC2, CLIC3, CLIC4, CLIC5, CLIC6, GJA1 , GJA3, GJA4, GJA5, GJA6P, GJA8, GJA9, GJA10, GJB1 , GJB2, GJB3, GJB4, GJB5, GJB6, GJB7, GJC1 , GJC2, GJC3, GJD2, GJD3, GJD4, GJE1 , ITPR1 , ITPR2, ITPR3, PANX1 , PANX2, PANX3, RYR1 , RYR2, RYR3, NALCN, SCNN1A, SCNN1 B, SCNN1 D, SCNN1G, TEM16A, ADAMTS7, ANGPTL3, ANGPTL4, ANGPTL8, LPL, GDF15, galectin-1 , galectin-2, galectin-3, galectin-4, galectin-7, galectin-8, galectin-9, galectin-1 0, galectin-12, galectin-1 3, matrix gla protein (MGP), PRNP, DGAT1 , GPAT3, DMC1 , BLM, BRCA2, members of the human endogenous retrovirus type K (HERV-K) family, ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1), ectonucleoside triphosphate diphosphohydrolase 2 (ENTPD2), SLC1A1 , SLC1A2, SLC1 A3, SLC1A4, SLC1A5, SLC1A6, SLC1A7, SLC2A1 , SLC2A2, SLC2A3, SLC2A4, SLC2A5, SLC2A6, SLC2A7, SLC2A8, SLC2A9, SLC2A10, SLC2A1 1, SLC2A12, SLC2A13, SLC2A14, SLC3A1 , SLC3A2, SLC4A1 , SLC4A2, SLC4A3, SLC4A4, SLC4A5, SLC4A6, SLC4A7, SLC4A8, SLC4A9, SLC4A10, SLC4A1 1, SLC5A1 , SLC5A2, SLC5A3, SLC5A4, SLC5A5, SLC5A6, SLC5A7, SLC5A8, SLC5A9, SLC5A10, SLC5A1 1, SLC5A12, SLC6A1 , SLC6A2, SLC6A3, SLC6A4, SLC6A5, SLC6A6, SLC6A7, SLC6A8, SLC6A9, SLC6A10, SLC6A1 1, SLC6A12, SLC6A13, SLC6A14, SLC6A15, SLC6A16, SLC6A17, SLC6A18, SLC6A19, SLC6A20, SLC7A5, SLC7A6, SLC7A7, SLC7A8, SLC7A9, SLC7A10, SLC7A1 1, SLC7A13, SLC7A14, SLC8A1 , SLC8A2, SLC8A3, SLC9A1 , SLC9A2, SLC9A3, SLC9A4, SLC9A5, SLC9A6, SLC9A7, SLC9A8, SLC9A9, SLC9A10, SLC9A1 1, SLC9B1 , SLC9B2, SLC10A1 , SLC10A2, SLC10A3, SLC10A4, SLC10A5, SLC10A6, SLC10A7, SLC1 1A1 , SLC1 1A2, SLC12A1 , SLC12A2, SLC12A3, SLC12A4, SLC12A5, SLC12A6,

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[0091]   Aspect 79. The method of any one of the preceding aspects, wherein the step of obtaining antibodies that specifically bind to the target protein comprises obtaining antibody-

producing cells from the animal, generating hybridomas with the antibody-producing cells, selecting hybridomas that produce the antibodies that specifically bind to the target protein, and isolating the antibodies produced by the hybridoma.

[0092]   Aspect 80. The method of aspect 79, further comprising the step of determining the nucleic acid sequence encoding the antibody that specifically binds to the target protein.

[0093]   Aspect 8 1. The method of aspect 72, 79, or 80, wherein the antibody-producing cells are lymphocytes, splenocytes, or peripheral blood mononuclear cells (PBMCs).

[0094]   Aspect 82. The method of any one of the preceding aspects, further comprising the step of generating a chimeric antibody or humanized antibody based on the antibody that specifically binds to the target protein, wherein the chimeric antibody or humanized antibody is capable of binding to the target protein with comparable affinity.

[0095]   Aspect 83. The method of any one of the preceding aspects, wherein the animal has been genetically modified to produce human antibodies.

[0096]   Aspect 84. The method of any one of the preceding aspects, wherein the polyribonucleotide of the complex comprises pseudouridine.

[0097]   Aspect 85. The method of any one of the preceding aspects, wherein the polyribonucleotide of the complex comprises:

  (a) one or more of the following modified nucleotides for cytidine: 5-formylcytidine, 5-methylcytidine, 5-methoxycytidine, 5-hydroxycytidine, and 5-hydroxymethylcytidine;

  (b) one or more of the following modified nucleotides for uridine: 5-formyluridine, 5-methyluridine, 5-methoxyuridine, 5-carboxymethylesteruridine, pseudouridine, and N1-methylpseudouridine;

  (c) N6-methyladenosine as a modified nucleotide for adenosine: and/or

  (d) thienoguanosine as a modified nucleotide for guanosine.Aspect 86. The method of any one of the preceding aspects, wherein the complex comprises two or more different polyribonucleotides, such as mRNAs, encoding two or more different target proteins which are capable of binding to each other.

[0098]   Aspect 87. The method of any one of the preceding aspects, wherein said complex comprises a polyribonucleotide comprising a sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to any one of the sequences in Tables 1-7, e.g., SEQ ID NOs: 2, 4, 37, 5, 7, 40, 8, 10, 43, 14, 16, 46, 17, 19, 49, 20, 22, 52, 26, 55, or 56.

BRIEF DESCRIPTION OF THE DRAWINGS

[0099]   **Figures 1A-1D** depict an exemplary RXFP1 immunization strategy and resulting FACS-based sera response.

[00100]   **Figure 1A** is a schematic of an exemplary immunization strategy for human RXFP1 .

[00101]   **Figure 1B** depicts FACS-based sera responses from animals 10 days after the priming immunization, illustrating the rapid induction of target-specific titers by mRNA immunization compared to the more traditional immunization formats of whole cells overexpressing human RXFP1 (BaF/3) or virus like particles overexpressing human RXFP1 (VLP-300.19).

[00102]   **Figure 1C** depicts final sera responses of immunized mice prior to final boost and initiation of hybridoma fusion. Eight mice were selected for fusion and boosted with 100 ug of the indicated immunogen.

[00103]   **Figure 1D** depicts sample FACS profiles of three anti-human RXFP1 hybridoma clones obtained from the immunization campaign. 207 RXFP1 specific clones were obtained in total.

[00104]   **Figure 2** depicts an exemplary SLC52A2 immunization strategy and resulting FACS-based sera response. Illustration of the immunization strategy employed for the generation of anti-SLC52A antibodies and the corresponding sera titers. Traditional immunogens, such as overexpressing cells, virus like particles, and peptides encoding extracellular loops (EC2) failed to elicit significant target specific titers. A total of 228 hybridomas capable of yielding SLC52A2 specific antibodies were generated from 8 fused mice.

[00105]   **Figure 3** depicts an exemplary Galectin-3 immunization strategy and resulting ELISA sera response.

[00106]   **Figure** 4 shows bioanalyzer traces for purified human RXFP1 mRNA. Total amount of mRNA loaded per well is indicated. Samples synthesized using pseudouridine exhibit molecular weights that are closer to the predicted size (2687 bases) than transcripts synthesized with uridine.

[00107]   **Figure 5** depicts Western blotting of plasma membrane fractions prepared from HEK293 cells transiently transfected with increasing concentrations of human RXFP1 mRNA. As control comparators, non-transfected cells and cells transfected with a DNA plasmid encoding human RXFP1 were also loaded. Amount of nucleic acid used per transfected 6 well is indicated.

DETAILED DESCRIPTION

[00108]   The present disclosure is directed to methods of immunization using compositions comprising cationic lipids and polynucleotide molecules, such as polyribonucleotide molecules, e.g., mRNA, which code for immunogens (e.g., target proteins or fragments thereof). This disclosure is also directed to methods for producing polyclonal and monoclonal antibodies from

genetically immunized animals, as well as to the antibodies produced by the immunization methods provided herein, including chimeric and humanized variants of such antibodies. This disclosure is also directed hybridomas obtained by the immunization methods (e.g., mRNA-LNP immunization methods) provided herein.

[00109] The present disclosure is directed to a method for eliciting an immune response in an animal (e.g., non-human animal such as mouse, rat, or rabbit), comprising the steps of: (a) mixing at least one cationic lipid with a polynucleotide coding for an antigenic determinant, thereby forming a cationic lipid-polynucleotide complex (e.g., mRNA-LNP complex); and (b) administering the lipid-polynucleotide complex to the animal. The present disclosure is further directed to a genetic immunization method wherein the polynucleotide is an mRNA molecule which codes for an immunogen (e.g., transmembrane protein (e.g., multi-pass transmembrane protein) such as a GPCR. mRNA has been found to be a superior polynucleotide for quickly raising antibodies to challenging and complex protein targets (e.g., multi-pass transmembrane proteins such as GPCRs).

[001 10] The present disclosure is further directed to a method for producing polyclonal antibodies comprising the use of the genetic immunization method described above, and further comprising the step of isolating the polyclonal antibodies from the immunized animal.

[001 11] The present disclosure is also directed to a method for producing monoclonal antibodies comprising the steps of: (a) administering to a non-human animal (e.g., mouse) a composition comprising at least one cationic lipid and a polynucleotide (e.g., polyribonucleotide), wherein the polynucleotide comprises an mRNA sequence coding for an immunogen; and (b) obtaining antibodies which specifically bind to the immunogen. In specific aspects, the step of obtaining antibodies which specifically bind to the immunogen comprises one or more of the following steps: (a) obtaining antibody-producing cells such as lymphocytes (e.g., B-lymphocytes) or splenocytes, from the immunized animal; (b) fusing the antibody-producing cells from the immunized animal with myeloma cells, thereby producing hybridomas; (c) cloning the hybridomas; (d) selecting positive clones which produce anti-immunogen antibody (i.e., antibody which specifically binds to the immunogen); (e) culturing the anti-immunogen antibody-producing clones; and (f) isolating anti-immunogen antibodies from the cultures.

[001 12] The present disclosure is also directed to a method for producing monoclonal antibodies comprising the steps of: (a) mixing at least one cationic lipid with a polynucleotide (e.g., polyribonucleotide), thereby forming a lipid-polynucleotide complex, wherein the polynucleotide comprises an mRNA sequence coding for an immunogen; (b) administering the lipid-polynucleotide complex to at least one mouse; (c) removing antibody-producing cells such as lymphocytes (e.g., B-lymphocytes) or splenocytes, from the immunized mice; (d) using the B-

lymphocytes from the immunized mice with myeloma cells, thereby producing hybridomas; (e) cloning the hybridomas; (f) selecting positive clones which produce anti-immunogen antibody; (g) culturing the anti-immunogen antibody-producing clones; and (h) isolating anti-immunogen antibodies from the cultures.

[001 13] Various formulations of cationic lipids have been used to transfect cells in vitro (for example, WO 91/17424; WO 91/16024; U.S. Pat. No. 4,897,355; U.S. Pat. No. 4,946,787; U.S. Pat. No. 5,049,386; and U.S. Pat. No. 5,208,036). Cationic lipids have also been used to introduce foreign polynucleotides into frog and rat cells in vivo (see, e.g., Holt et al., *Neuron* 4:203-214 (1990); Hazinski et al., *Am. J. Respr. Cell. Mol. Biol.* 4: 206-209 (1991)). In specific embodiments provided herein, cationic lipids are used, generally, to deliver or to introduce biologically active substances (for example, see WO 91/17424; WO 91/16024; and WO 93/03709). In specific aspects described herein, cationic liposomes can provide an efficient carrier for the introduction of foreign polynucleotides such as polyribonucleotides (e.g., mRNA) into host cells for genetic immunization.

[001 14] Various cationic lipids well-known in the prior art can be used in the compositions and methods provided herein. One well-known cationic lipid is N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA). DOTMA, alone or in a 1:1 combination with dioleoylphosphatidylethanolamine (DOPE) can be formulated into liposomes using standard techniques. Feigner et al. *(Proc. Natl. Acad. Sci. U.S.A.* 84:7413-7417 (1987)), which is hereby incorporated by reference in its entirety, have shown that such liposomes provide efficient delivery of nucleic acids to cultured cells. A DOTMA:DOPE (1:1) formulation is sold under the name LIPOFECTIN™(GIBCO/BRL: Life Technologies, Inc., Gaithersburg, Md.). Another commercially available cationic lipid is 1,2-bis(oleoyloxy)-3-3-(trimethylammonia)propane (DOTAP), which differs from DOTMA in that the oleoyl moieties are linked via ester bonds, not ether bonds, to the propylamine. DOTAP is believed to be more readily degraded by target cells.

[001 15] Related groups of known compounds differ from DOTMA and DOTAP in that one of the methyl groups of the trimethylammonium group is replaced by a hydroxyethyl group. Compounds of this type are similar to the Rosenthal Inhibitor of phospholipase A (Rosenthal et al., *J. Biol. Chem.* 235:2202-2206 (1960), which has stearoyl esters linked to the propylamine core. The dioleoyl analogs of the Rosenthal Inhibitor (RI) are commonly abbreviated as DORI-ether and DORI-ester, depending upon the linkage of the fatty acid moieties to the propylamine core. The hydroxy group can be used as a site for further functionalization, for example, by esterification to carboxyspermine.

[001 16] Another class of known compounds has been described by Behr et al. *(Proc. Natl. Acad. Sci. USA* 86:6982-6986 (1989); EPO Publication 0 394 111), in which carboxyspermine has

been conjugated to two types of lipids, resulting in dipalmitoylphosphatidylethanolamine 5-carboxyspermylamide (DDPES).

[001 17]   Both DOGS and DPPES have been used to coat plasmids, forming a lipid aggregate complex that provides efficient transfection. The compounds are claimed to be more efficient and less toxic than DOTMA for transfection of certain cell lines. DOGS is available commercially as TRANSFECTAM™ (Promega, Madison, Wis.).

[001 18]   A cationic cholesterol derivative (DC-Choi) has been synthesized and formulated into liposomes in combination with DOPE (Gao et al., *Biochim. Biophys. Res. Comm.* 179:280-285 (1991)). Liposomes formulated with DC-Choi provide more efficient transfection and lower toxicity than DOTMA-containing liposomes for certain cell lines.

[001 19]   Lipopolylysine is formed by conjugating polylysine to DOPE. This compound has been reported to be especially effective for transfection in the presence of serum (Zhou et al., *Biochim. Biophys. Res. Comm.* 165:8-14 (1991)). Thus, lipopolylysine may be an effective carrier for immunization.

[00120]   Other non-limiting examples of lipids (e.g., cationic lipids, helper lipids, and stealth lipids) which can be used in the methods and compositions provided herein include those described in WO201 5/095346, WO201 5/095340, WO2016/037053, WO201 4/1 36086, and WO201 1/076807, each of which is hereby incorporated by reference in its entirety.

[00121]   In certain aspects, cationic lipids for the compositions and methods described herein include, but are not limited to, N,N-dioleyl-N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(1-(2,3-dioleoyloxy) propyl)-N,N,N-trimethylammonium chloride (DOTAP),
1,2-Dioleoyl-3-Dimethylammonium -propane (DODAP),
N-(1-(2,3-dioleyloxy)propyl) - N,N,N-trimethylammonium chloride (DOTMA),
1,2-Dioleoylcarbamyl -3-Dimethylammonium-propane (DOCDAP),
1,2-Dilineoyl-3-Dimethylammonium-propane (DLINDAP), dilauryl($C_{12:0}$) trimethyl ammonium propane (DLTAP), Dioctadecylamidoglycyl spermine (DOGS), DC-Choi,
Dioleoyloxy -N-[2-sperminecarboxamido)ethyl} -N,N-dimethyl-1-propanaminiumtrifluoroacetate (DOSPA), 1,2-Dimyristyloxypropyl-3-dimethyl -hydroxyethyl ammonium bromide (DMRIE), 3-Dimethylamino-2-(Cholest-5-en -3-beta-oxybutan-4-oxy) -1-(cis,cis-9,12 -octadecadienoxy)prop ane (CLinDMA), N,N-dimethyl-2,3-dioleyloxy)propylamine (DODMA),
2-[5'-(cholest-5-en-3[beta]-oxy)-3'-oxapentoxy) -3-dimethyl-1-(cis,cis-9',12'-octadecadienoxy) propane (CpLinDMA) and N,N-Dimethyl-3,4-dioleyloxybenzylamine (DMOBA), and
1,2-N,N'-Dioleylcarbamyl-3-dimethylaminopropane (DOcarbDAP). In one embodiment, the cationic lipid for the compositions and methods provided herein is DOTAP or DLTAP.

23

These compounds are useful either alone, or in combination with other lipid aggregate-forming components (such as DOPE or cholesterol) for formulation into liposomes or other lipid aggregates. Such aggregates are cationic and able to complex with anionic macromolecules such as DNA or RNA.

[00122]   The methods of mRNA-based immunization provided herein have addressed many of the issues associated with the above-described difficulties inherent in antigen production and/or antibody generation. Among other things, said methods dispense with the need to directly express and purify the target protein antigen. An animal host's own cellular machinery is used to make the target protein and present it to the immune system. For eukaryotic target proteins, this has the added advantage of permitting the addition of eukaryotic-specific post-translational modifications and protein processing. Without being bound to any particular theory, the unpurified mRNA that is used for immunization has a highly inflammatory character due in part to the presence of double stranded RNA entities in the preparation. In specific aspects, this serves as an adjuvant to boost the humoral response against the target protein.

[00123]   In particular aspects provided herein, monoclonal antibody development for a target protein can be expedited through the immunization of animals with mRNAs which encode said target protein. This method offers considerable advantages for proteins against which it has historically been technically challenging to develop specific antibodies, such as G-protein coupled receptors, as there is no need to heterologously produce and purify the target protein. Host defense mechanisms elicited by the adjuvant-like properties of the mRNA result in a fast development of sera titers.

[00124]   In certain aspects, hybridoma fusions using antibody-producing cells obtained from animals immunized with the encapsulated mRNA-based immunization methods provided herein have been highly productive. The encapsulated mRNAs are potent and highly immunogenic, which can shorten an immunization schedule and require fewer animals. All manner of proteins can be generated as antigens with the present methods, e.g., soluble, membrane bound, complexed/heteromeric, etc., regardless of complexity. Expression should result in the native confirmation of the antigen.

[00125]   The present methods provided herein also can result in the co-expression of multiple chains for complexed protein (e.g., IgGs, receptor complexes). In specific aspects of the mRNA-based immunization methods provided herein, expressed antigens are immunogenically clean, i.e, have no contaminants which would alter the specificity of the humoral response or infectious pathogens, since they are synthetic.

[00126]   In particular aspects, the present methods describe a stable method of antigen generation and reagent storage, as the lipid encapsulated mRNAs of the present invention are capable of remaining stable at 4°C for months. Furthermore, there is no IMPACT (Infectious Microbe PCR Amplification Test) pathogen testing required, as the mRNAs are all synthetic.

TERMINOLOGY

[00127]   "Cloning Vector" means plasmid or phage DNA or other DNA sequence which is able to replicate autonomously in a host cell, and which is characterized by one or a small number of restriction endonuclease recognition sites at which such DNA sequences may be cut in a determinable fashion without loss of an essential biological function of the vector, and into which a DNA fragment may be spliced in order to bring about its replication and cloning. The cloning vector may further contain a marker suitable for use in the identification of cells transformed with the cloning vector. Markers, for example, provide tetracycline resistance or ampicillin resistance.

[00128]   "Expression vector" is a vector similar to a cloning vector but which is capable of enhancing the expression of a gene which has been cloned into it, after transformation into a host. The cloned gene is usually placed under the control of (i.e., operably linked to) certain control sequences such as promoter sequences. Promoter sequences may be either constitutive or inducible.

[00129]   "Expression" is the cellular process by which a polypeptide is produced from a structural gene. The process involves transcription of a gene into messenger RNA (mRNA) and the translation of such mRNA into polypeptide(s). "Expression" can also include where applicable, but not limited to, for example, transcription, translation, folding, modification and processing. "Expression products" include RNA transcribed from a gene, and polypeptides obtained by translation of mRNA transcribed from a gene. In specific aspects, "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 processing); (3) translation of an RNA into a polypeptide or protein; and (4) post-translational modification of a polypeptide or protein.

[00130]   An "exogenous" nucleic acid is a nucleic acid (e.g., a modified synthetic mRNA described herein) that has been introduced by a process involving human intervention into a biological system such as a cell or organism in which it is not normally found, or in which it is found in lower amounts. A factor (e.g. a modified synthetic mRNA described herein) is exogenous if it is introduced into an immediate precursor cell or a progeny cell that inherits the substance. In contrast, an "endogenous" is a factor or expression product that is native to the biological system or cell (e.g., endogenous expression of a gene).

[00131]   "Isolated" means, in the case of a nucleic acid or polypeptide, a nucleic acid or polypeptide separated from at least one other component (e.g., nucleic acid or polypeptide) that is present with the nucleic acid or polypeptide as found in its natural source and/or that would be present with the nucleic acid or polypeptide when expressed by a cell, or secreted in the case of secreted polypeptides. A chemically synthesized nucleic acid or polypeptide or one synthesized using in vitro transcription/translation is considered "isolated".

[00132]   "Isolated cells" are cells that have been removed from an organism in which they originally found, or descendants of such cells. Optionally the cell has been cultured in vitro, e.g., in the presence of other cells. Optionally, the cell is later introduced into a second organism or re-introduced into the organism from which it (or the cell or population of cells from which it descended) was isolated.

[00133]   "Modified" means a changed state or structure of a molecule described herein. Molecules may be modified in many ways including chemically, structurally, and functionally. In one embodiment, the mRNA molecules described herein are modified by the introduction of natural and non-natural nucleosides and/or nucleotides. Modified may also mean any alteration which is different from the wild type. A "modified" RNA is an RNA molecule produced in vitro, which comprise at least one modified nucleoside.

[00134]   A "modified nucleoside" is a ribonucleoside that encompasses modification(s) relative to the standard guanine (G), adenine (A), cytidine (C), and uridine (U) nucleosides. Such modifications can include, for example, modifications normally introduced post-transcriptionally to mammalian cell mRNA, and artificial chemical modifications, as known to one of skill in the art. In one aspect, the following are non-limiting examples of modified nucleotides: 5-formylcytidine, 5-methylcytidine, 5-methoxycytidine, 5-hydroxycytidine, 5-hydroxymethylcytidine,5-formyluridine, 5-methyluridine, 5-methoxyuridine, 5-carboxymethylesteruridine, pseudouridine, N1-methylpseudouridine, N6-methyladenosine, and thienoguanosine.

[00135]   "Added co-transcriptionally" means the addition of a feature, e.g., a 5' methylguanosine cap or other modified nucleoside, to a modified synthetic mRNA of the invention during transcription of the RNA molecule (i.e., the modified RNA is not fully transcribed prior to the addition of the 5' cap).

[00136]   "Contacting" a cell means contacting with a factor (e.g., a modified synthetic mRNA described herein), optionally, including subjecting a cell to a transfection system. Where such a cell is in vivo, contacting the cell with a modified synthetic mRNA described herein includes administering a modified synthetic mRNA described herein in a formulation, such as a pharmaceutical composition, to a subject by an appropriate administration route, such that the compound contacts the cell in vivo.

[00137]　In general, a "recombinant host" may be any prokaryotic or eukaryotic microorganism or cell which contains the desired cloned genes in an expression vector or cloning vector. This term is also meant to include those microorganisms that have been genetically engineered to contain the desired gene(s) in the chromosome or genome of that organism.

[00138]　"Recombinant vector" is any cloning vector or expression vector which contains the desired cloned gene(s).

[00139]　A "host" means any prokaryotic or eukaryotic microorganism or cell that is the recipient of a replicable expression vector or cloning vector. A host also includes prokaryotic or eukaryotic microorganisms or cells that can be genetically engineered by well-known techniques to contain desired gene(s) on its chromosome or genome. For examples of such hosts, see Maniatis et al., *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1982).

[00140]　A "promoter" is a DNA sequence generally described as the 5' region of a gene, located proximal to the start codon. The transcription of an adjacent gene(s) is initiated at the promoter region. If a promoter is an inducible promoter, then the rate of transcription increases in response to an inducing agent. In contrast, the rate of transcription is not regulated by an inducing agent if the promoter is a constitutive promoter.

[00141]　"Gene" is a DNA sequence that contains information needed for expressing a polypeptide or protein.

[00142]　"Structural gene" is a nucleotide, e.g., DNA, sequence that is transcribed into messenger RNA (mRNA) that is then translated into a sequence of amino acids characteristic of a specific polypeptide.

[00143]　"Transfection" refers to the transformation of a host cell with polynucleotides, e.g., DNA or RNA. The recombinant host cell expresses protein which is encoded by the transfected polynucleotide, e.g., DNA or RNA. In specific aspects, "transfection" means the use of methods, such as chemical methods, to introduce exogenous nucleic acids, such as the modified synthetic mRNA described herein into a host cell, such as a eukaryotic cell. As used herein, the term "transfection" does not encompass viral-based methods of introducing exogenous nucleic acids into a cell. Non-limiting methods of transfection include physical treatments (e.g., electroporation, nanoparticles, magnetofection), and chemical-based transfection methods. Chemical-based transfection methods include, but are not limited to, cyclodextrin, polymers, liposomes, and nanoparticles.

[00144]　An "epitope" is the part of a non-immunoglobulin antigen to which the variable region of an antibody binds. An "antigenic determinant" is a protein or peptide which contains one or more epitopes. An "immunogen" is a protein or peptide which is capable of eliciting an immune

response due to the presence of one or more epitopes. The terms "antigen," "antigenic determinant," and "immunogen" are used synonymously herein. In specific aspects, epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and may have specific three dimensional structural characteristics, as well as specific charge characteristics. In particular aspects, conformational and nonconformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents. In certain aspects, an epitope may be a linear epitope comprising contiguous amino acid sequences of a fragment or portion of an antigen. In certain aspects, an epitope may be a conformational epitope comprising noncontiguous amino acid sequences of an antigen.

[00145]   A "transfection reagent" is an agent that induces uptake of polynucleotides such as DNA or RNA into a host cell. In specific aspects, also encompassed are agents that enhance uptake e.g., by at least 50-90%, compared to a modified synthetic mRNA described herein administered in the absence of such a reagent. In one embodiment, a cationic or non-cationic lipid molecule useful for preparing a pharmaceutical composition or for co-administration with a modified synthetic mRNA described herein is used as a transfection reagent. In other embodiments, the modified synthetic mRNA described herein comprises a chemical linkage to attach e.g., a ligand, a peptide group, a lipophilic group, a targeting moiety etc. In other embodiments, the transfection reagent comprises a charged lipid, an emulsion, a liposome, a cationic or non-cationic lipid, an anionic lipid, or a penetration enhancer as known in the art or described herein.

[00146]   "Innate immune response" or "interferon response" means a cellular defense response initiated by a cell in response to recognition of infection by a foreign organism, such as a virus or bacteria or a product of such an organism, e.g., an RNA lacking the modifications characteristic of RNAs produced in the subject cell. The innate immune response protects against viral and bacterial infection by inducing the death of cells that detect exogenous nucleic acids.

[00147]   A "therapeutically effective amount" or "effective amount" is the amount of the subject compound or combination that will elicit the biological or medical response of a tissue, system, animal or human that is being sought by the researcher, veterinarian, medical doctor or other clinician.

[00148]   "Primers" are also nucleic acid sequences. PCR primers are typically oligonucleotides of fairly short length (e.g., 8-30 nucleotides) that are used in polymerase chain reactions. PCR primers and hybridization probes can readily be developed and produced by those of skill in the art, using sequence information from the target sequence. See, Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Labs Press).

28

[00149] "Selectively binds to" or "specifically binds to" means the specific binding of one protein to another (e.g., an antibody, fragment thereof, or binding partner to a target protein), wherein the level of binding, as measured by any standard assay (e.g., an immunoassay), is statistically significantly higher than the background control for the assay.

[00150] A "conserved" nucleotide or amino acid is a residue of a polynucleotide sequence or polypeptide sequence, respectively, which occurs unaltered in the same position of two or more sequences being compared. Nucleotides or amino acids that are relatively conserved are those that are conserved amongst more related sequences than nucleotides or amino acids appearing elsewhere in the sequences. Two or more sequences are "completely conserved" if they are 100% identical to one another. In some embodiments, two or more sequences are "highly conserved" if they are at least 90% identical, to one another. In some embodiments, two or more bases are "conserved" if they are identical, to one another. Conservation of sequence may apply to the entire length of an oligonucleotide or polypeptide or may apply to a portion, region or feature thereof. In the context of polypeptides, the following eight groups contain amino acids that are conservative substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (see, e.g., Creighton, Proteins (1984)).

[00151] "Delivery" means the act or manner of delivering a compound, substance, entity, moiety, cargo or payload.

[00152] A "delivery agent" is any substance which facilitates, at least in part, the in vivo delivery of a nucleic acid molecule to targeted cells.

[00153] A "formulation" includes at least a modified nucleic acid molecule and a delivery agent.

[00154] "Homology" means the overall relatedness between polymeric molecules, e.g. between nucleic acid molecules (e.g. DNA molecules and/or RNA molecules) and/or between polypeptide molecules. In some embodiments, polymeric molecules are considered to be "homologous" to one another if their sequences are at least 25% identical. The term "homologous" necessarily refers to a comparison between at least two sequences (polynucleotide or polypeptide sequences). Two polynucleotide sequences are considered to be homologous if the polypeptides they encode are at least about 50% identical for at least one stretch of at least about 20 amino acids. In some embodiments, homologous polynucleotide sequences are characterized by the ability to encode a stretch of at least 4-5 uniquely specified amino acids. For polynucleotide sequences less than 60 nucleotides in length, homology is determined by the ability to encode a stretch of at least 4-5 uniquely specified amino acids. In accordance with the

invention, two protein sequences are considered to be homologous if the proteins are at least about 50% identical, at least about 60% identical for at least one stretch of at least about 20 amino acids.

[00155]   "Identity" means the overall relatedness between polymeric molecules, e.g., between oligonucleotide molecules (e.g., DNA molecules and/or RNA molecules) or between polypeptide molecules. Calculation of the percent identity of two polynucleotide sequences, for example, can be performed by aligning the two sequences for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second nucleic acid sequences for optimal alignment and non-identical sequences can be disregarded for comparison purposes). The nucleotides at corresponding nucleotide positions are then compared. When a position in the first sequence is occupied by the same nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which needs to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. Two non-limiting examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., Nuc. Acids Res. 25:3389-3402, 1977; and Altschul et al., J. Mol. Biol. 215:403-410, 1990, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information.

[00156]   The term "cationic liposome(s)" or "cationic lipid(s)" are structures that are made of positively charged lipids, which are capable of interacting with negatively charged DNA and cell membranes.

[00157]   The term "lipid nanoparticle" or "LNP" refers to a particle that comprises a plurality of (i.e. more than one) lipid molecules physically associated with each other by intermolecular forces. The lipid nanoparticles may be, e.g., microspheres (including unilamellar and multilamellar vesicles, e.g. liposomes), a dispersed phase in an emulsion, micelles or an internal phase in a suspension.

[00158]   The term "nucleic acid" is used herein interchangeably with the term "polynucleotide" and refers to deoxyribonucleotides (DNA) or ribonucleotides (RNA) or polyribonucleotides, including messenger RNA (mRNA), and polymers thereof in either single- or double-stranded form. The term encompasses nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar properties as the reference nucleic acid, and which are metabolized

in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs).

[00159]   Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. Specifically, as detailed below, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res. 19:5081, 1991; Ohtsuka et al., J. Biol. Chem. 260:2605-2608, 1985; and Rossolini et al., Mol. Cell. Probes 8:91-98, 1994).

[00160]   The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, 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.

[00161]   A "sample" is a subset of its tissues, cells or component parts (e.g., body fluids). A sample further may include a homogenate, lysate or extract prepared from a whole organism or a subset of its tissues, cells or component parts, or a fraction or portion thereof, including but not limited to, for example, plasma, serum, spinal fluid, lymph fluid, the external sections of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, milk, blood cells, tumors, organs. A sample further refers to a medium, such as a nutrient broth or gel, which may contain cellular components, such as proteins or nucleic acid molecule.

[00162]   "Synthetic" means produced, prepared, and/or manufactured by human intervention. Synthesis of polynucleotides or polypeptides or other molecules described herein may be chemical or enzymatic.

[00163]   As used herein, "pseudouridine" refers to the C-glycoside isomer of the nucleoside uridine.

[00164]   As used herein, "purify," "purified," "purification" means to make substantially pure or clear from unwanted components, material defilement, admixture or imperfection.

[00165]   The term "antibody" as used herein means a whole antibody and any antigen-binding fragment (*i.e.,* "antigen-binding portion") or single chain thereof. A whole antibody is a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable

region (abbreviated herein as VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The V H and V L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V H and V L is composed of three CDRs and four FRs arranged from amino-terminus to carboxy-terminus in the following order: FR1 , CDR1 , FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system *(e.g.,* effector cells) and the first component (Clq) of the classical complement system.

[00166]   The term "antigen-binding portion" or "antigen-binding fragment" of an antibody, as used herein, refers to one or more fragments of an intact antibody that retain the ability to specifically bind to a given antigen. Antigen-binding functions of an antibody can be performed by fragments of an intact antibody. Examples of binding fragments encompassed within the term antigen-binding portion or antigen-binding fragment of an antibody include a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; a F(ab)$_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; an Fd fragment consisting of the V H and CH1 domains; an Fv fragment consisting of the V L and V H domains of a single arm of an antibody; a single domain antibody (dAb) fragment (Ward *et al.,* 1989 Nature 341 :544-546), which consists of a V H domain or a V L domain; and an isolated complementarity determining region (CDR). In certain aspects, the CDRs of an antibody can be determined according to (i) the Kabat numbering system (Kabat et al. (1971) Ann. NY Acad. Sci. 190:382-391 and, Kabat et al. (1991) Sequences of Proteins of Immunological Interest Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242); or (ii) the Chothia numbering scheme (see, e.g., Chothia and Lesk, 1987, J. Mol. Biol., 196:901-917; Al- Lazikani et al, 1997, J. Mol. Biol., 273 :927-948; Chothia et al., 1992, J. Mol. Biol., 227:799-817; Tramontano A et al. , 1990, J. Mol. Biol. 215(1): 175-82; and U.S. Patent No. 7,709,226); or (iii) the ImMunoGeneTics (IMGT) numbering system, for example, as described in Lefranc, M.-P., 1999, The Immunologist, 7: 132-136 and Lefranc, M.-P. et al, 1999, Nucleic Acids Res., 27:209- 212 ("IMGT CDRs"); or (iv) MacCallum et al, 1996, J. Mol. Biol., 262:732-745. See also, e.g., Martin, A., "Protein Sequence and Structure Analysis of Antibody Variable Domains," in Antibody Engineering, Kontermann and Diibel, eds., Chapter 3 1, pp. 422-439, Springer- Verlag, Berlin (2001).

[00167]   Furthermore, although the two domains of the Fv fragment, V L and VH, are coded for by separate genes, they can be joined, using recombinant methods, by an artificial peptide linker

that enables them to be made as a single protein chain in which the V L and V H regions pair to form monovalent molecules (known as single chain Fv (scFv); see, *e.g.,* Bird *et al.,* 1988 Science 242:423-426; and Huston *et al.,* 1988 Proc. Natl. Acad. Sci. 85:5879-5883). Such single chain antibodies include one or more antigen-binding portions or fragments of an antibody. These antibody fragments are obtained using conventional techniques known to those of skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

[00168]   Antigen-binding fragments can also be incorporated into single domain antibodies, maxibodies, minibodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv (see, *e.g.,* Hollinger and Hudson, 2005, Nature Biotechnology, 23, 9, 1126-1 136). Antigen-binding portions of antibodies can be grafted into scaffolds based on polypeptides such as Fibronectin type III (Fn3) (see U.S. Pat. No. 6,703,199, which describes fibronectin polypeptide monobodies).

[00169]   Antigen-binding fragments can be incorporated into single chain molecules comprising a pair of tandem Fv segments (VH-CH1-VH-CH1) which, togetherwith complementary light chain polypeptides, form a pair of antigen-binding regions (Zapata et al. (1995) Protein Eng. 8(1 0): 1057-1062; and U.S. Pat. No. 5,641 ,870).

[00170]   As used herein, the term "affinity" refers to the strength of interaction between antibody and antigen at single antigenic sites. Within each antigenic site, the variable region of the antibody "arm" interacts through weak non-covalent forces with antigen at numerous sites; the more interactions, the stronger the affinity. As used herein, the term "high affinity" for an antibody or antigen-binding fragments thereof (e.g., a Fab fragment) generally refers to an antibody, or antigen-binding fragment, having a K_D of 10^{-9} M or less.

[00171]   The term "human antibody", as used herein, is intended to include antibodies having variable regions in which both the framework and CDR regions are derived from sequences of human origin. Furthermore, if the antibody contains a constant region, the constant region also is derived from such human sequences, *e.g.,* human germline sequences, or mutated versions of human germline sequences. In certain aspects, human antibodies, such as human monoclonal antibodies, may be produced by a hybridoma which includes an immortalized cell fused to a B cell obtained from a transgenic non-human animal, *e.g.,* a transgenic mouse, having a genome comprising a human heavy chain transgene and a human light chain transgene.

[00172]   A "humanized" antibody is an antibody that retains the reactivity of a non-human antibody while being less immunogenic in humans. This can be achieved, for instance, by retaining the non-human CDR regions and replacing the remaining parts of the antibody with their human counterparts *(i.e.,* the constant region as well as the framework portions of the variable region). See, *e.g.,* Morrison *et al.,* Proc. Natl. Acad. Sci. USA, 8 1:6851-6855, 1984; Morrison and Oi, Adv. Immunol., 44:65-92, 1988; Verhoeyen *et al.,* Science, 239:1534-1536, 1988; Padlan,

Molec. Immun., 28:489-498, 1991; and Padlan, Molec. Immun., 31:169-217, 1994. Other examples of human engineering technology include, but are not limited to XOMA technology disclosed in US 5,766,886.

[00173]   The term "hybridoma" refers to an immortalized cell derived from the fusion of an antibody-producing cell, such as B lymphoblasts, with a fusion partner, such as a myeloma cell. In specific aspects, the antibody-producing cell used to generate a hybridoma is obtained from an animal immunized with an antigen, for example, immunized according to the mRNA-based immunization methods described herein.

[00174]   The term "isolated antibody" refers to an antibody that is substantially free of other antibodies having different antigenic specificities and/or different amino acid sequence. In particular aspects, an isolated antibody that specifically binds an antigen may, however, have cross-reactivity to other antigens. In specific aspects, an isolated antibody may be substantially free of other cellular material and/or chemicals.

[00175]   The term "isotype" refers to the antibody class (*e.g.,* IgM, IgE, IgG such as IgG^ IgG$_2$, or IgG$_4$) that is provided by the heavy chain constant region genes. Isotype also includes modified versions of one of these classes, where modifications have been made to alter the Fc function, for example, to enhance or reduce effector functions or binding to Fc receptors. The term "monoclonal antibody" is a well known term of art that refers to an antibody obtained from a population of homogenous or substantially homogeneous antibodies displaying binding specificity and affinity for a particular epitope. The term "monoclonal" is not limited to any particular method for making the antibody. Generally, a population of monoclonal antibodies can be generated by cells, a population of cells, or a cell line. In particular aspects, a monoclonal antibody can be a chimeric antibody or a humanized antibody. In particular aspects, a monoclonal antibody is a monovalent antibody or multivalent (e.g., bivalent) antibody.

[00176]   As used herein, the term "polyclonal antibodies" refers to a heterologous antibody population comprising a variety of different antibodies that react against a specific antigen, but the different antibodies recognize different epitopes within the antigen.

[00177]   The term "recombinant human antibody", as used herein, includes all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies isolated from an animal (*e.g.,* a mouse) that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom, antibodies isolated from a host cell transformed to express the human antibody, *e.g.,* from a transfectoma, antibodies isolated from a recombinant, combinatorial human antibody library, and antibodies prepared, expressed, created or isolated by any other means that involve splicing of all or a portion of a human immunoglobulin gene, sequences to other DNA sequences. Such recombinant human antibodies

have variable regions in which the framework and CDR regions are derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies can be subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the V H and V L regions of the recombinant antibodies are sequences that, while derived from and related to human germline V H and V L sequences, may not naturally exist within the human antibody germline repertoire *in vivo.*

[00178]   As used herein, the term, "optimized" means that a nucleotide sequence has been altered to encode an amino acid sequence using codons that are preferred in the production cell or organism, generally a eukaryotic cell. In certain aspects, the optimized nucleotide sequence is engineered to retain completely or as much as possible the amino acid sequence originally encoded by the starting nucleotide sequence, which is also known as the "parental" sequence. In particular aspects, optimized sequences described herein have been engineered to have codons that are preferred in mammalian cells, such as murine cells. In other aspects, optimized expression of sequences in other eukaryotic cells or prokaryotic cells is also envisioned herein.

I. Target Proteins/Antigenic Determinants

[00179]   Provided herein are methods for inducing an immune response to a target protein (e.g. human protein, such as human transmembrane protein, e.g., human GPCR) or a fragment thereof in an animal (e.g., non-human animal), wherein the animal is administered a composition comprising a complex comprising a lipid and a polynucleotide, such as polyribonucleotide (e.g., mRNA), encoding the target protein or a fragment thereof, and related methods of producing antibodies against such target protein. The methods provided herein are useful to produce antibodies (e.g., monoclonal antibodies) against, or to induce an immune response to, any target protein of interest (e.g., transmembrane protein). In specific aspects, the target proteins are human target proteins and the immunized animals used in the methods described herein are non-human animals.

[00180]   In specific aspects, provided herein are methods for producing antibodies to a target protein (e.g. human protein, such as human transmembrane protein, e.g., human GPCR) or a fragment thereof in an animal (e.g., non-human animal), using a composition comprising a complex comprising a lipid and a polyribonucleotide, such as mRNA, encoding the target protein or a fragment thereof, wherein the target protein is (i) a G protein coupled receptor (GPCR); (ii) a single pass transmembrane protein receptor; (iii)   a Tumor Necrosis Factor Receptor Superfamily (TNFRSF) member; (iv) an interleukin (IL) receptor; (v) an ion channel; (vi)   a solute carrier; (vii)   an immune receptor; or (viii) a multi-pass transmembrane protein.

[00181]   In specific aspects, provided herein are methods for producing antibodies to a target protein (e.g. human protein, such as human transmembrane protein, e.g., human GPCR) or a fragment thereof in an animal (e.g., non-human animal), using a composition comprising a complex comprising a lipid and a polyribonucleotide, such as mRNA, encoding the target protein or a fragment thereof, wherein the target protein is selected from the following:

ACKR1 , ACKR2, ACKR3, ACKR4, ADCYAP1 R 1, ADGRA1 , ADGRA2, ADGRA3, ADGRB1 , ADGRB2, ADGRB3, ADGRD1 , ADGRD2, ADGRE1 , ADGRE2, ADGRE3, ADGRE4P, ADGRE5, ADGRF1 , ADGRF2, ADGRF3, ADGRF4, ADGRF5, ADGRG1 , ADGRG2, ADGRG3, ADGRG4, ADGRG5, ADGRG6, ADGRG7, ADGRL1 , ADGRL2, ADGRL3, ADGRL4, ADGRV1 , ADORA1 , ADORA2A, ADORA2B, ADORA3, ADRA1A, ADRA1 B, ADRA1 D, ADRA2A, ADRA2B, ADRA2C, ADRB1 , ADRB2, ADRB3, AGTR1 , AGTR2, APLNR/APJ, ASGR1 , ASGR2, AVPR1 A , AVPR1 B, AVPR2, BDKRB1 , BDKRB2, BRS3, BRS3, C3AR1 , C5AR1 , C5AR2, CALCR, CALCRL, CASR, CCKAR, CCKBR, CCR1 , CCR10, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCRL2, CELSR1 , CELSR2, CELSR3, CHRM1 , CHRM2, CHRM3, CHRM4, CHRM5, CMKLR1 , CNR1 , CNR2, CRHR1 , CRHR2, CX3CR1 , CXCR1 , CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, CYSLTR1 , CYSLTR2, DRD1 , DRD2, DRD3, DRD4, DRD5, EDNRA, EDNRB, F2R, F2RL1 , F2RL2, F2RL3, FFAR1 , FFAR2, FFAR3, FFAR4, FPR1 , FPR2, FPR2, FPR3, FSHR, FZD1 , FZD10, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, GABBR1 , GABBR2, GALR1 , GALR2, GALR3, GCGR, GHRHR, GHSR, GIPR, GLP1 R , GLP2R, GNRHR, GNRHR2, GPBAR1 , GPER1 , GPR1 , GPR4, GPR12, GPR15, GPR17, GPR18, GPR19, GPR20, GPR21 , GPR22, GPR25, GPR26, GPR27, GPR3, GPR31 , GPR32, GPR33, GPR34, GPR35, GPR37, GPR37L1 , GPR39, GPR40, GPR42, GPR42, GPR45, GPR50, GPR52, GPR55, GPR55, GPR6, GPR61 , GPR62, GPR63, GPR65, GPR68, GPR75, GPR78, GPR79, GPR82, GPR83, GPR84, GPR85, GPR87, GPR88, GPR101 , GPR107, GPR132, GPR135, GPR137, GPR139, GPR141 , GPR142, GPR143, GPR146, GPR148, GPR149, GPR15, GPR150, GPR151 , GPR152, GPR153, GPR156, GPR157, GPR158, GPR160, GPR161 , GPR162, GPR171 , GPR173, GPR174, GPR176, GPR179, GPR182, GPR183, GPRC5A, GPRC5B, GPRC5C, GPRC5D, GPRC6A, GRM1 , GRM2, GRM3, GRM4, GRM5, GRM6, GRM7, GRM8, GRPR, HCAR1 , HCAR2, HCAR3, HCRTR1 , HCRTR2, HRH1 , HRH2, HRH3, HRH4, HTR1A, HTR1 B, HTR1 D, HTR1 E, HTR1 F, HTR2A, HTR2B, HTR2C, HTR4, HTR5A, HTR5BP, HTR6, HTR7, KISS1 R , LGR3, LGR4, LGR5, LGR6, LHCGR, LPAR1 , LPAR2, LPAR3, LPAR4, LPAR5, LPAR6, LTB4R, LTB4R2, MAS1 , MAS1 L, MC1 R , MC2R, MC3R, MC4R, MC5R, MCHR1 , MCHR2, MLNR, MRGPRD, MRGPRE, MRGPRF, MRGPRG, MRGPRX1 , MRGPRX2, MRGPRX3, MRGPRX4, MTNR1A, MTNR1 B, NMBR, NMUR1 , NMUR2, NPBWR1 , NPBWR2, NPFFR1 , NPFFR2, NPSR1 , NPY1 R, NPY2R, NPY4R,

NPY5R, NPY6R, NTSR1 , NTSR2, OPN3, OPN4, OPN5, OPRD1 , OPRK1 , OPRL1 , OPRM1 ,

OR5 1E 1, OXER 1, OXG R 1, OXTR, P2RY1 , P2RY10, P2RY1 1, P2RY12, P2RY13, P2RY14,

P2 RY2 , P2 RY4, P2 RY6, P2 RY8, PRLH R , PROKR1 , PROKR2 , PTAFR, PTG DR, PTG DR2 ,

PTGER1 , PTGER2 , PTGER3, PTGER4, PTGFR, PTG IR, PTH1 R , PTH2 R , QRFPR, RXFP 1,

RXFP2 , RXFP3, RXFP4, S 1PR1 , S 1PR2 , S 1PR3, S 1PR4, S 1PR5, SCTR, SMO, SSTR1 , SSTR2,

SSTR3, SSTR4, SSTR5, SUCNR1 , TAAR1 , TAAR2 , TAAR3, TAAR4P, TAAR5, TAAR6, TAAR8,

TAAR9, TACR1 , TACR2 , TACR3, TAS1 R 1, TAS1 R2, TAS1 R3, TAS2R1 , TAS2R10, TAS2R13,

TAS2 R 14 , TAS2 R 16 , TAS2 R 19 , TAS2R20, TAS2 R3, TAS2 R30, TAS2R3 1, TAS2 R38, TAS2 R39,

TAS2R4, TAS2R40, TAS2R41 , TAS2R42, TAS2R43, TAS2R45, TAS2R46, TAS2R5, TAS2R50,

TAS2R60, TAS2R7, TAS2R8, TAS2R9, TBXA2R, TPRA1 , TRHR, TSHR, UTS2R, VIPR1 , VIPR2,

XCR1 , TCR-a, TCR- β, CD3, ζ-chain accessory, CD4, and CD8, mannose receptor (MR),

asialoglycoprotein receptor family (e.g., asialoglycoprotein receptor macrophage galactose-type

lectin (MGL)), DC-SIGN (CLEC4L), langerin (CLEC4K), myeloid DAP12-associating lectin

(MDL)-1 (CLEC5A), dectin 1/CLEC7A, DNGR1/CLEC9A, Myeloid C-type lectin-like receptor

(MICL) (CLEC12A), CLEC2 (also called CLEC1 B), CLEC12B, DCIR/CLEC4A, Dectin 2/CLEC6A,

Blood DC antigen 2 (BDCA2) (CLEC4C), macrophage- inducible C-type lectin (CLEC4E), TLR1 ,

TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR1 1, TLR12, TLR13, FcγRI

(CD64), FcγRIIA (CD32), FcγRIIBI (CD32), FCYRI IB2 (CD32), FcγRIIIA (CD16a), FcγRIIIB

(CD16b), FceRI, FceRII (CD23), FcaR1 (CD89), Fca^R, FcRn, CD27, CD40, OX40, GITR,

CD137, PD-1 , CTLA-4, PD-L1 , TIGIT, T-cell immunoglobulin domain and mucin domain 3 (TIM3),

V-domain Ig suppressor of T cell activation (VISTA), CD28, CD122, ICOS, A2AR, B7-H3, B7-H4,

B and T lymphocyte attenuator (BTLA), Indoleamine 2,3-dioxygenase (IDO), killer-cell

immunoglobulin-like receptor (KIR), lymphocyte activation gene-3 (LAG3), FAM159B, HLA-A,

HLA-B, HLA-C, HLA-DPA1 , HLA-DPB1 , HLA-DQA1 , HLA-DQB1 , HLA-DRA, HLA-DRB1 , gp130,

IL-1 receptor, IL-2 receptor, IL-3 receptor, IL-4 receptor, IL-5 receptor, IL-6 receptor, IL-7 receptor,

IL-8 receptor, IL-9 receptor, IL-10 receptor, IL-1 1 receptor, IL-12 receptor, IL-13 receptor, IL-14

receptor, IL-1 5 receptor, IL-1 6 receptor, IL-1 7 receptor, IL-1 8 receptor, IL-1 9 receptor, IL-20

receptor, IL-21 receptor, IL-22 receptor, IL-23 receptor, IL-24 receptor, IL-25 receptor, IL-26

receptor, IL-27 receptor, IL-28 receptor, IL-29 receptor, IL-30 receptor, IL-31 receptor, IL-32

receptor, IL-33 receptor, IL-35 receptor, IL-36 receptor, FGFR1 , FGFR2, FGFR3, FGFR4,

TNFRSF1A, TNFRSF1 B, TNFRSF3, TNFRSF4, TNFRSF5, TNFRSF6, TNFRSF6B, TNFRSF7,

TNFRSF8, TNFRSF9, TNFRSF10A, TNFRSF10B, TNFRSF10C, TNFRSF10D, TNFRSF1 1A,

TNFRSF1 1B, TNFRSF12A, TNFRSF13B, TNFRSF13C, TNFRSF14, TNFRSF16, TNFRSF17,

TNFRSF18, TNFRSF19, TNFRSF21 , TNFRSF25, TNFRSF27, SCN1A, SCN1 B, SCN2A, SCN2B,

SCN3A, SCN3B, SCN4A, SCN5A, SCN7A, SCN8A, SCN9A, SCN10A, SCN1 1A, CACNA1A,

CACNA1 B, CACNA1C, CACNA1 D, CACNA1 E, CACNA1 F, CACNA1G, CACNA1 H, CACNA1 I, CACNA1S, TRPA1 , TRPC1 , TRPC2, TRPC3, TRPC4, TRPC5, TRPC6, TRPC7, TRPM1 , TRPM2, TRPM3, TRPM4, TRPM5, TRPM6, TRPM7, TRPM8, MCOLN1 , MCOLN2, MCOLN3, PKD1 , PKD2, PKD2L1 , PKD2L2, TRPV1 , TRPV2, TRPV3, TRPV4, TRPV5, TRPV6, CATSPER1 , CATSPER2, CATSPER3, CATSPER4, TPCN1 , TPCN2, CNGA1 , CNGA2, CNGA3, CNGA4, CNGB1 , CNGB3, HCN1 , HCN2, HCN3, HCN4, KCNMA1 , KCNN1 , KCNN2, KCNN3, KCNN4, KCNT1 , KCNT2, KCNU1 , KCNA1 , KCNA2, KCNA3, KCNA4, KCNA5, KCNA6, KCNA7, KCNA10, KCNB1 , KCNB2, KCNC1 , KCNC2, KCNC3, KCNC4, KCND1 , KCND2, KCND3, KCNF1 , KCNG1 , KCNG2, KCNG3, KCNG4, KCNH1 , KCNH2, KCNH3, KCNH4, KCNH5, KCNH6, KCNH7, KCNH8, KCNQ1 , KCNQ2, KCNQ3, KCNQ4, KCNQ5, KCNS1 , KCNS2, KCNS3, KCNV1 , KCNV2, KCNJ1 , KCNJ2, KCNJ3, , KCNJ4, KCNJ5, KCNJ6, KCNJ8, KCNJ9, KCNJ10, KCNJ1 1, KCNJ12, KCNJ13, KCNJ14, KCNJ15, KCNJ16, KCNJ18, KCNK1 , KCNK2, KCNK3, KCNK4, KCNK5, KCNK6, KCNK7, KCNK9, KCNK10, KCNK12, KCNK13, KCNK15, KCNK16, KCNK17, KCNK18, HVCN1 , HTR3A, HTR3B, HTR3C, HTR3D, HTR3E, CHRNA1 , CHRNA2, CHRNA3, CHRNA4, CHRNA5, CHRNA6, CHRNA7, CHRNA9, CHRNA10, CHRNB1 , CHRNB2, CHRNB3, CHRNB4, CHRND, CHRNE, CHRNG, GABRA1 , GABRA2, GABRA3, GABRA4, GABRA5, GABRA6, GABRB1 , GABRB2, GABRB3, GABRD, GABRE, GABRG1 , GABRG2, GABRG3 .GABRP, GABRQ, GABRR1 , GABRR2, GABRR3, GRIA1 , GRIA2, GRIA3, GRIA4, GRID1 , GRID2, GRIK1 , GRIK2, GRIK3, GRIK4, GRIK5, GRIN1 , GRIN2A, GRIN2B, GRIN2C, GRIN2D, GRIN3A, GRIN3B, GLRA1 , GLRA2, GLRA3, GLRA4, P2RX1 , P2RX2, P2RX3, P2RX4, P2RX5, P2RX6, P2RX7, ZACN, ASIC1 , ASIC2, ASIC3, ASIC4, AQP1 , AQP2, AQP3, AQP4, AQP5, AQP6, AQP7, AQP8, AQP9, AQP10, AQP1 1, AQP12A, AQP12B, MIP, CLCN1 , CLCN2, CLCN3, CLCN4, CLCN5, CLCN6, CLCN7, CLCNKA, CLCNKB, Cystic fibrosis transmembrane conductance regulator (CFTR), AN01/TMEM16a, AN02, AN03, AN04, AN05, AN06, AN07, AN08, AN09, ANO10, BEST1 , BEST2, BEST3, BEST4, CLIC1 , CLIC2, CLIC3, CLIC4, CLIC5, CLIC6, GJA1 , GJA3, GJA4, GJA5, GJA6P, GJA8, GJA9, GJA10, GJB1 , GJB2, GJB3, GJB4, GJB5, GJB6, GJB7, GJC1 , GJC2, GJC3, GJD2, GJD3, GJD4, GJE1 , ITPR1 , ITPR2, ITPR3, PANX1 , PANX2, PANX3, RYR1 , RYR2, RYR3, NALCN, SCNN1A, SCNN1 B, SCNN1 D, SCNN1G, ADAMTS7, ANGPTL3, ANGPTL4, ANGPTL8, LPL, GDF15, galectin-1 , galectin-2, galectin-3, galectin-4, galectin-7, galectin-8, galectin-9, galectin-10, galectin-12, galectin-13, matrix gla protein (MGP), PRNP, DGAT1 , GPAT3, DMC1 , BLM, BRCA2, members of the human endogenous retrovirus type K (HERV-K) family, ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1), ectonucleoside triphosphate diphosphohydrolase 2 (ENTPD2), SLC1A1 , SLC1A2, SLC1 A3, SLC1A4, SLC1A5, SLC1A6, SLC1A7, SLC2A1 , SLC2A2, SLC2A3, SLC2A4, SLC2A5, SLC2A6, SLC2A7, SLC2A8, SLC2A9, SLC2A10, SLC2A1 1, SLC2A12, SLC2A13, SLC2A14, SLC3A1 , SLC3A2, SLC4A1 ,

SLC4A2, SLC4A3, SLC4A4, SLC4A5, SLC4A6, SLC4A7, SLC4A8, SLC4A9, SLC4A10,
SLC4A1 1, SLC5A1 , SLC5A2, SLC5A3, SLC5A4, SLC5A5, SLC5A6, SLC5A7, SLC5A8, SLC5A9,
SLC5A10, SLC5A1 1, SLC5A12, SLC6A1 , SLC6A2, SLC6A3, SLC6A4, SLC6A5, SLC6A6,
SLC6A7, SLC6A8, SLC6A9, SLC6A10, SLC6A1 1, SLC6A12, SLC6A13, SLC6A14, SLC6A15,
SLC6A16, SLC6A17, SLC6A18, SLC6A19, SLC6A20, SLC7A5, SLC7A6, SLC7A7, SLC7A8,
SLC7A9, SLC7A10, SLC7A1 1, SLC7A13, SLC7A14, SLC8A1 , SLC8A2, SLC8A3, SLC9A1 ,
SLC9A2, SLC9A3, SLC9A4, SLC9A5, SLC9A6, SLC9A7, SLC9A8, SLC9A9, SLC9A10,
SLC9A1 1, SLC9B1 , SLC9B2, SLC10A1 , SLC10A2, SLC10A3, SLC10A4, SLC10A5, SLC10A6,
SLC10A7, SLC1 1A1 , SLC1 1A2, SLC12A1 , SLC12A2, SLC12A3, SLC12A4, SLC12A5, SLC12A6,
SLC12A7, SLC12A8, SLC12A9, SLC13A1 , SLC13A2, SLC13A3, SLC13A4, SLC13A5, SLC14A1 ,
SLC14A2, SLC15A1 , SLC15A2, SLC15A3, SLC15A4, SLC16A1 , SLC16A2, SLC16A3, SLC16A4,
SLC16A5, SLC16A6, SLC16A7, SLC16A8, SLC16A9, SLC16A10, SLC16A1 1, SLC16A12,
SLC16A13, SLC16A14, SLC17A1 , SLC17A2, SLC17A3, SLC17A4, SLC17A5, SLC17A6,
SLC17A7, SLC17A8, SLC17A9, SLC18A1 , SLC18A2, SLC18A3, SLC19A1 , SLC19A2, SLC19A3,
SLC20A1 , SLC20A2, SLC01A2, SLC01 B1, SLC01 B3, SLC01C1 , SLC02A1 , SLC02B1 ,
SLC03A1 , SLC04A1 , SLC04C1 , SLC05A1 , SLC06A1 , SLC22A1 , SLC22A2, SLC22A3,
SLC22A4, SLC22A5, SLC22A6, SLC22A7, SLC22A8, SLC22A9, SLC22A10, SLC22A1 1,
SLC22A12, SLC22A13, SLC22A14, SLC22A15, SLC22A16, SLC22A17, SLC22A18,
SLC22A1 8AS, SLC22A1 9, SLC22A20, SLC22A23, SLC22A24, SLC22A25, SLC22A31 , SLC23A1 ,
SLC23A2, SLC23A3, SLC23A4, SLC24A1 , SLC24A2, SLC24A3, SLC24A4, SLC24A5, SLC24A6,
SLC25A1 , SLC25A2, SLC25A3, SLC25A4, SLC25A5, SLC25A6, SLC25A7, SLC25A8, SLC25A9,
SLC25A10, SLC25A1 1, SLC25A12, SLC25A13, SLC25A14, SLC25A15, SLC25A16, SLC25A17,
SLC25A18, SLC25A19.SLC25A20, SLC25A21 , SLC25A22, SLC25A23, SLC25A24, SLC25A25,
SLC25A26, SLC25A27, SLC25A28, SLC25A29, SLC25A30, SLC25A31 , SLC25A32, SLC25A33,
SLC25A34, SLC25A35, SLC25A36, SLC25A37, SLC25A38, SLC25A39, SLC25A40, SLC25A41 ,
SLC25A42, SLC25A43, SLC25A44, SLC25A45, SLC25A46, SLC26A1 , SLC26A2, SLC26A3,
SLC26A4, SLC26A5, SLC26A6, SLC26A7, SLC26A8, SLC26A9, SLC26A10, SLC26A1 1,
SLC27A1 , SLC27A2, SLC27A3, SLC27A4, SLC27A5, SLC27A6, SLC28A1 , SLC28A2, SLC28A3,
SLC29A1 , SLC29A2, SLC29A3, SLC29A4, SLC30A1 , SLC30A2, SLC30A3, SLC30A4, SLC30A5,
SLC30A6, SLC30A7, SLC30A8, SLC30A9, SLC30A10, SLC31A1 , SLC31A2, SLC32A1 ,
SLC33A1 , SLC34A1 , SLC34A2, SLC34A3, SLC35A1 , SLC35A2, SLC35A3, SLC35A4, SLC35A5,
SLC35B1 , SLC35B2, SLC35B3, SLC35B4, SLC35C1 , SLC35C2, SLC35D1 , SLC35D2,
SLC35D3, SLC35E1 , SLC35E2, SLC35E3, SLC35E4, SLC35F1 , SLC35F2, SLC35F3, SLC35F4,
SLC35F5, SLC35G1 , SLC35G3, SLC35G4, SLC35G5, SLC35G6, SLC36A1 , SLC36A2,
SLC36A3, SLC36A4, SLC37A1 , SLC37A2, SLC37A3, SLC37A4, SLC38A1 , SLC38A2, SLC38A3,

SLC38A4, SLC38A5, SLC38A6, SLC38A7, SLC38A8, SLC38A9, SLC38A10, SLC38A1 1,
SLC39A1 , SLC39A2, SLC39A3, SLC39A4, SLC39A5, SLC39A6, SLC39A7, SLC39A8, SLC39A9,
SLC39A10, SLC39A1 1, SLC39A12, SLC39A13, SLC39A14, SLC40A1 , SLC41A1 , SLC41A2,
SLC41A3, RhAG, RhBG, RhCG, SLC43A1 , SLC43A2, SLC43A3, SLC44A1 , SLC44A2,
SLC44A3, SLC44A4, SLC44A5, SLC45A1 , SLC45A2, SLC45A3, SLC45A4, SLC46A1 , SLC46A2,
SLC46A3, SLC47A1 , SLC47A2, HCP-1 , MFSD5, MFSD10, SLC50A1 , OSTa, OβTβ, SLC52A1 ,
SLC52A2, and SLC52A3.

[00182]   In specific aspects, the methods provided herein are effective at producing antibodies
against target proteins that are transmembrane proteins, such as transmembrane receptors, and
target proteins that are difficult to raise antibodies against or that are difficult to express, for
example, those that are difficult due to cytotoxicity, yield, solubility, aggregation and/or stability
issues.

[00183]   In certain aspects, target proteins (e.g., human target proteins) described herein
include integral membrane proteins (IMPs), which are classified as Type I, type II, single-anchor
type II, C-terminal anchor, and polytopic (e.g., see Ott and Lingappa, 2002, J. Cell Sci.,
115(Pt 10):2003-2009). In specific aspects, target proteins (e.g., human target proteins) described
herein are single pass transmembrane proteins. In specific aspects, target proteins (e.g., human
target proteins) described herein are multi-pass transmembrane proteins.

G protein coupled receptor (GPCR):

[00184]   In specific aspects, complexes for use in the methods provided herein comprise a lipid
and a polynucleotide (e.g., mRNA) encoding a target protein, wherein the target protein (e.g.,
human target protein) is a GPCR (e.g., human GPCR). GPCRs, also known as seven-
transmembrane (7TM) domain receptors and G protein-linked receptors (GPLR). The two main
signal transduction pathways involving GPCRs are the cAMP signal pathway and the
phosphatidylinositol signal pathway.

[00185]   Classes of GPCRs include class A (Rhodopsin-like), B (Secretin receptor family), C
(Metabotropic glutamate/pheromone), D (Fungal mating pheromone receptors), E (Cyclic AMP
receptors) and F (Frizzled/Smoothened).

[00186]   Non-limiting examples of target proteins which are GPCRs (Class A, B, C, Class
Frizzled, and other 7 transmembrane proteins) include:
ACKR1 , ACKR2, ACKR3, ACKR4, ADCYAP1 R 1, ADGRA1 , ADGRA2, ADGRA3, ADGRB1 ,
ADGRB2, ADGRB3, ADGRD1 , ADGRD2, ADGRE1 , ADGRE2, ADGRE3, ADGRE4P, ADGRE5,
ADGRF1 , ADGRF2, ADGRF3, ADGRF4, ADGRF5, ADGRG1 , ADGRG2, ADGRG3, ADGRG4,
ADGRG5, ADGRG6, ADGRG7, ADGRL1 , ADGRL2, ADGRL3, ADGRL4, ADGRV1 , ADORA1 ,

ADORA2A, ADORA2B, ADORA3, ADRA1A, ADRA1 B, ADRA1 D, ADRA2A, ADRA2B, ADRA2C, ADRB1 , ADRB2, ADRB3, AGTR1 , AGTR2, APLNR/APJ, AVPR1A, AVPR1 B, AVPR2, BDKRB1 , BDKRB2, BRS3, BRS3, C3AR1 , C5AR1 , C5AR2, CALCR, CALCRL, CASR, CCKAR, CCKBR, CCR1 , CCR10, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCRL2, CELSR1 , CELSR2, CELSR3, CHRM1 , CHRM2, CHRM3, CHRM4, CHRM5, CMKLR1 , CNR1 , CNR2, CRHR1 , CRHR2, CX3CR1 , CXCR1 , CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, CYSLTR1 , CYSLTR2, DRD1 , DRD2, DRD3, DRD4, DRD5, EDNRA, EDNRB, F2R, F2RL1 , F2RL2, F2RL3, FFAR1 , FFAR2, FFAR3, FFAR4, FPR1 , FPR2, FPR2, FPR3, FSHR, FZD1 , FZD10, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, GABBR1 , GABBR2, GALR1 , GALR2, GALR3, GCGR, GHRHR, GHSR, GIPR, GLP1 R , GLP2R, GNRHR, GNRHR2, GPBAR1 , GPER1 , GPR1 , GPR4, GPR12, GPR15, GPR17, GPR18, GPR19, GPR20, GPR21 , GPR22, GPR25, GPR26, GPR27, GPR3, GPR31 , GPR32, GPR33, GPR34, GPR35, GPR37, GPR37L1 , GPR39, GPR40, GPR42, GPR42, GPR45, GPR50, GPR52, GPR55, GPR6, GPR61 , GPR62, GPR63, GPR65, GPR68, GPR75, GPR78, GPR79, GPR82, GPR83, GPR84, GPR85, GPR87, GPR88, GPR101 , GPR107, GPR132, GPR135, GPR137, GPR139, GPR141 , GPR142, GPR143, GPR146, GPR148, GPR149, GPR15, GPR150, GPR151 , GPR152, GPR153, GPR156, GPR157, GPR158, GPR160, GPR161 , GPR162, GPR171 , GPR173, GPR174, GPR176, GPR179, GPR182, GPR183, GPRC5A, GPRC5B, GPRC5C, GPRC5D, GPRC6A, GRM1 , GRM2, GRM3, GRM4, GRM5, GRM6, GRM7, GRM8, GRPR, HCAR1 , HCAR2, HCAR3, HCRTR1 , HCRTR2, HRH1 , HRH2, HRH3, HRH4, HTR1A, HTR1 B, HTR1 D, HTR1 E, HTR1 F, HTR2A, HTR2B, HTR2C, HTR4, HTR5A, HTR5BP, HTR6, HTR7, KISS1 R , LGR4, LGR5, LGR6, LHCGR, LPAR1 , LPAR2, LPAR3, LPAR4, LPAR5, LPAR6, LTB4R, LTB4R2, MAS1 , MAS1 L, MC1 R , MC2R, MC3R, MC4R, MC5R, MCHR1 , MCHR2, MLNR, MRGPRD, MRGPRE, MRGPRF, MRGPRG, MRGPRX1 , MRGPRX2, MRGPRX3, MRGPRX4, MTNR1A, MTNR1 B, NMBR, NMUR1 , NMUR2, NPBWR1 , NPBWR2, NPFFR1 , NPFFR2, NPSR1 , NPY1 R , NPY2R, NPY4R, NPY5R, NPY6R, NTSR1 , NTSR2, 0PN3, 0PN4, 0PN5, 0PRD1 , 0PRK1 , 0PRL1 , 0PRM1 , 0R51 E 1, 0XER1 , 0XGR1 , OXTR, P2RY1 , P2RY10, P2RY1 1, P2RY12, P2RY13, P2RY14, P2RY2, P2RY4, P2RY6, P2RY8, PRLHR, PROKR1 , PROKR2, PTAFR, PTGDR, PTGDR2, PTGER1 , PTGER2, PTGER3, PTGER4, PTGFR, PTGIR, PTH1 R , PTH2R, QRFPR, RXFP1 , RXFP2, RXFP3, RXFP4, S 1PR1 , S 1PR2, S 1PR3, S 1PR4, S 1PR5, SCTR, SMO, SSTR1 , SSTR2, SSTR3, SSTR4, SSTR5, SUCNR1 , TAAR1 , TAAR2, TAAR3, TAAR4P, TAAR5, TAAR6, TAAR8, TAAR9, TACR1 , TACR2, TACR3, TAS1 R 1, TAS1 R2, TAS1 R3, TAS2R1 , TAS2R10, TAS2R13, TAS2R14, TAS2R16, TAS2R19, TAS2R20, TAS2R3, TAS2R30, TAS2R31 , TAS2R38, TAS2R39, TAS2R4, TAS2R40, TAS2R41 , TAS2R42, TAS2R43, TAS2R45, TAS2R46, TAS2R5, TAS2R50, TAS2R60, TAS2R7, TAS2R8, TAS2R9, TBXA2R, TPRA1 , TRHR, TSHR, UTS2R, VIPR1 , VIPR2, and XCR1 .

Transmembrane and membrane-associated immune receptors:

[00187] In specific aspects, complexes for use in the methods provided herein comprise a lipid and a polynucleotide (e.g., mRNA) encoding a target protein, wherein the target protein is an immune receptor, such as pattern recognition receptors (PRRs), Toll-like receptors (TLRs), killer activated and killer inhibitor receptors (KARs and KIRs), complement receptors, Fc receptors, B cell receptors and T cell receptors (e.g., TCR-a, TCR-β, CD3, ζ-chain accessory, CD4, and CD8), and major histocompatibility complexes.

[00188] Non-limiting examples of pattern recognition receptors (PRRs) include mannose receptor (MR), asialoglycoprotein receptor family (e.g., asialoglycoprotein receptor macrophage galactose-type lectin (MGL)), DC-SIGN (CLEC4L), langerin (CLEC4K), myeloid DAP12-associating lectin (MDL)-1 (CLEC5A), dectin 1/CLEC7A, DNGR1/CLEC9A, Myeloid C-type lectin-like receptor (MICL) (CLEC12A), CLEC2 (also called CLEC1B), CLEC12B, and DC immunoreceptor (DCIR) subfamily (e.g., DCIR/CLEC4A, Dectin 2/CLEC6A, Blood DC antigen 2 (BDCA2) (CLEC4C), and macrophage-inducible C-type lectin (CLEC4E)).

[00189] In specific aspects, complexes for use in the methods provided herein comprise a lipid and a polynucleotide (e.g., mRNA) encoding a target protein, wherein the target protein is a Toll-like receptor (TLR). TLRs are a class of proteins that play a key role in the innate immune system. They are single, membrane-spanning, non-catalytic receptors usually expressed in cells such as macrophages and dendritic cells that recognize structurally conserved molecules derived from microbes, and activate immune cell responses. Non-limiting examples of TLRs include TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11, TLR12, and TLR13.

[00190] Non-limiting examples of Fc receptors (e.g., Fc-gamma receptors, Fc-alpha receptors, and Fc-epsilon receptors) include polymeric immunoglobulin receptor (pIgR), FcγRI (CD64), FcγRIIA (CD32), FcγRIIBI (CD32), FCγRIIB2 (CD32), FcγRIIA (CD16a), FcγRIIB (CD16b), FceRI, FceRII (CD23), FcaR1 (CD89), Fca/pR, and FcRn.

[00191] In specific aspects, complexes for use in the methods provided herein comprise a lipid and a polynucleotide (e.g., mRNA) encoding a target protein, wherein the target protein is an immune receptor selected from the following: CD27, CD40, OX40, GITR, CD137, PD-1, CTLA-4, PD-L1, TIGIT, T-cell immunoglobulin domain and mucin domain 3 (TIM3), V-domain Ig suppressor of T cell activation (VISTA), CD28, CD122, ICOS, A2AR, B7-H3, B7-H4, B and T lymphocyte attenuator (BTLA), Indoleamine 2,3-dioxygenase (IDO), killer-cell immunoglobulin-like receptor (KIR), and lymphocyte activation gene-3 (LAG3).

[00192] In specific aspects, complexes for use in the methods provided herein comprise a lipid and a polynucleotide (e.g., mRNA) encoding a target protein, wherein the target protein is a part

of the major histocompatibility complex I or II (MHC I or II) or MHC in complex with a peptide fragment. MHC proteins are a part of the acquired immune system and play a role in the presentation of processed peptide fragments to T cells and eliciting humoral immune system activation. Non-limiting examples of MHC proteins include HLA-A, HLA-B, HLA-C, HLA-DPA1 , HLA-DPB1 , HLA-DQA1 , HLA-DQB1 , HLA-DRA, and HLA-DRB1 .

Cytokine receptors:

[00193]   In specific aspects, complexes for use in the methods provided herein comprise a lipid and a polynucleotide (e.g., mRNA) encoding a target protein, wherein the target protein is a cytokine receptor (e.g., interleukin (IL) receptor or fibroblast growth factor (FGF) receptors). Non-limiting examples of cytokine receptors include receptors for nerve growth factor (NGF), myostatin (GDF-8), growth differentiation factors (GDFs), granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), platelet derived growth factors (PDGF), erythropoietin (EPO), thrombopoietin (TPO), Epidermal growth factor (EGF), fibroblast growth factors (FGF), vascular endothelial growth factors (VEGF), tissue inhibitor or metalloproteinase (TIMP), matrix metalloproteinases (MMPs), macrophage stimulating factor (MSF), ciliary neurotrophic factor (CNTF), cardiotrophin, oncostatin M, leukemia inhibitory factor (LIF), transforming growth factor (TGF) -alpha and -beta, interferon (IFN) -beta and -gamma, and tumor necrosis factor (TNF) alpha. In a specific embodiment, complexes for use in the methods provided herein comprising a lipid and a polynucleotide (e.g., mRNA) encoding a target protein, wherein the target protein is gp130, which is a shared receptor utilized by several related cytokines, including IL-6, IL-1 1, IL-27, Leukemia Inhibitory Factor (LIF), Oncostatin M (OSM), Ciliary Neurotrophic Factor (CNTF), Cardiotrophin 1 (CT-1) and Cardiotrophin-like Cytokine (CLC).

[00194]   In specific aspects, complexes for use in the methods provided herein comprise a lipid and a polynucleotide (e.g., mRNA) encoding a target protein, wherein the target protein is an interleukin (IL) receptor. Non-limiting examples of IL receptors include IL-1 receptor, IL-2 receptor, IL-3 receptor, IL-4 receptor, IL-5 receptor, IL-6 receptor, IL-7 receptor, IL-8 receptor, IL-9 receptor, IL-10 receptor, IL-1 1 receptor, IL-1 2 receptor, IL-13 receptor, IL-14 receptor, IL-15 receptor, IL-16 receptor, IL-17 receptor, IL-18 receptor, IL-19 receptor, IL-20 receptor, IL-21 receptor, IL-22 receptor, IL-23 receptor, IL-24 receptor, IL-25 receptor, IL-26 receptor, IL-27 receptor, IL-28 receptor, IL-29 receptor, IL-30 receptor, IL-31 receptor, IL-32 receptor, IL-33 receptor, IL-35 receptor, and IL-36 receptor.

[00195]   In specific aspects, complexes for use in the methods provided herein comprise a lipid and a polynucleotide (e.g., mRNA) encoding a target protein, wherein the target protein is a fibroblast growth factor (FGF) receptor (FGFR). Non-limiting examples of FGF receptors include FGFR1 , FGFR2, FGFR3, and FGFR4.

[00196]   In specific aspects, complexes for use in the methods provided herein comprise a lipid and a polynucleotide (e.g., mRNA) encoding a target protein, wherein the target protein is a tumor necrosis factor receptor superfamily (TNFRSF) member. Non-limiting examples of TNFRSF members include TNFRSF1A, TNFRSF1 B, TNFRSF3, TNFRSF4, TNFRSF5, TNFRSF6, TNFRSF6B, TNFRSF7, TNFRSF8, TNFRSF9, TNFRSF10A, TNFRSF10B, TNFRSF10C, TNFRSF10D, TNFRSF1 1A, TNFRSF1 1B, TNFRSF12A, TNFRSF13B, TNFRSF13C, TNFRSF14, TNFRSF16, TNFRSF17, TNFRSF18, TNFRSF19, TNFRSF21 , TNFRSF25, and TNFRSF27.

Ion channels:

[00197]   In specific aspects, complexes for use in the methods provided herein comprise a lipid and a polynucleotide (e.g., mRNA) encoding a target protein, wherein the target protein is an ion channel.

[00198]   There are over 300 types of ion channels, and they can be classified by the nature of their gating, the species of ions passing through those gates, the number of gates (pores) and localization of proteins. For example, voltage-gated ion channels include, but are not limited to, voltage-gated sodium channels, voltage-gated calcium channels, voltage-gated potassium channels (K_v), hyperpolarization-activated cyclic nucleotide-gated channels, voltage-gated proton channels. Ligand-gated ion channels, include, but are not limited to, cation-permeable "nicotinic" Acetylcholine receptor, ionotropic glutamate-gated receptors and ATP-gated P2X receptors, and the anion-permeable γ-aminobutyric acid-gated GABA receptor. Classification of ion channels by type of ions include, but are not limited to, chloride channels, potassium channels (e.g., ATP-sensitive potassium ion channels), sodium channels (e.g., NaVs, ENaCs, CaVs), calcium channels, proton channels, and non-selective cation channels.

[00199]   Non-limiting examples of voltage-gated sodium channels include SCN1A, SCN1 B, SCN2A, SCN2B, SCN3A, SCN3B, SCN4A, SCN5A, SCN7A, SCN8A, SCN9A, SCN10A and SCN1 1A.

[00200]   Non-limiting examples of voltage-gated calcium channels include CACNA1A, CACNA1 B, CACNA1C, CACNA1 D, CACNA1 E, CACNA1 F, CACNA1G, CACNA1 H, CACNA1 I and CACNA1S.

[00201]   Non-limiting examples of transient receptor potential cation channels include TRPA1 , TRPC1 , TRPC2, TRPC3, TRPC4, TRPC5, TRPC6, TRPC7, TRPM1 , TRPM2, TRPM3, TRPM4,

TRPM5, TRPM6, TRPM7, TRPM8, MCOLN1, MCOLN2, MCOLN3, PKD1, PKD2, PKD2L1, PKD2L2, TRPV1, TRPV2, TRPV3, TRPV4, TRPV5 and TRPV6.

[00202]   Non-limiting examples of CatSper channels include CATSPER1, CATSPER2, CATSPER3 and CATSPER4.

[00203]   Non-limiting examples of two-pore channels include TPCN1 and TPCN2.

[00204]   Non-limiting examples of cyclic nucleotide-regulated channels include CNGA1, CNGA2, CNGA3, CNGA4, CNGB1, CNGB3, HCN1, HCN2, HCN3 and HCN4.

[00205]   Non-limiting examples of calcium-activated Potassium channels include KCNMA1, KCNN1, KCNN2, KCNN3, KCNN4, KCNT1, KCNT2, and KCNU1.

[00206]   Non-limiting examples of voltage-gated Potassium channels include KCNA1, KCNA2, KCNA3, KCNA4, KCNA5, KCNA6, KCNA7, KCNA10, KCNB1, KCNB2, KCNC1, KCNC2, KCNC3, KCNC4, KCND1, KCND2, KCND3, KCNF1, KCNG1, KCNG2, KCNG3, KCNG4, KCNH1, KCNH2, KCNH3, KCNH4, KCNH5, KCNH6, KCNH7, KCNH8, KCNQ1, KCNQ2, KCNQ3, KCNQ4, KCNQ5, KCNS1, KCNS2, KCNS3, KCNV1 and KCNV2.

[00207]   Non-limiting examples of inwardly rectifying Potassium channels include KCNJ1, KCNJ2, KCNJ3, KCNJ4, KCNJ5, KCNJ6, KCNJ8, KCNJ9, KCNJ10, KCNJ1 1, KCNJ12, KCNJ13, KCNJ14, KCNJ15, KCNJ16 and KCNJ18.

[00208]   Non-limiting examples of two-P Potassium channels include KCNK1, KCNK2, KCNK3, KCNK4, KCNK5, KCNK6, KCNK7, KCNK9, KCNK10, KCNK12, KCNK13, KCNK15, KCNK16, KCNK17 and KCNK18.

[00209]   Non-limiting examples of Hydrogen voltage-gated ion channels include HVCN1.

[00210]   Non-limiting examples of ionotropic 5-HT (serotonin) receptors include HTR3A, HTR3B, HTR3C, HTR3D and HTR3E.

[0021 1]   Non-limiting examples of nicotinic acetylcholine receptors include CHRNA1, CHRNA2, CHRNA3, CHRNA4, CHRNA5, CHRNA6, CHRNA7, CHRNA9, CHRNA10, CHRNB1, CHRNB2, CHRNB3, CHRNB4, CHRND, CHRNE and CHRNG.

[00212]   Non-limiting examples of GABA(A) receptors include GABRA1, GABRA2, GABRA3, GABRA4, GABRA5, GABRA6, GABRB1, GABRB2, GABRB3, GABRD, GABRE, GABRG1, GABRG2, GABRG3, GABRP, GABRQ, GABRR1, GABRR2 and GABRR3.

[0021 3]   Non-limiting examples of ionotropic Glutamate receptors include GRIA1, GRIA2, GRIA3, GRIA4, GRID1, GRID2, GRIK1, GRIK2, GRIK3, GRIK4, GRIK5, GRIN1, GRIN2A   , GRIN2B, GRIN2C, GRIN2D, GRIN3A and GRIN3B.

[00214]   Non-limiting examples of Glycine receptors include GLRA1, GLRA2, GLRA3 and GLRA4

[00215]   Non-limiting examples of ionotropic Purinergic receptors include P2RX1 , P2RX2, P2RX3, P2RX4, P2RX5, P2RX6 and P2RX7.

[00216]   Non-limiting examples of Zinc-activated channels include ZACN.

[00217]   Non-limiting examples of Acid-sensing (proton-gated) ion channels include ASIC1 , ASIC2, ASIC3, ASIC4.

[00218]   Non-limiting examples of Aquaporins include AQP1 , AQP2, AQP3, AQP4, AQP5, AQP6, AQP7, AQP8, AQP9, AQP10, AQP1 1, AQP12A, AQP12B and MIP.

[00219]   Non-limiting examples of voltage-sensitive Chloride channels include CLCN1 , CLCN2, CLCN3, CLCN4, CLCN5, CLCN6, CLCN7, CLCNKA and CLCNKB.

[00220]   Non-limiting examples of Cystic fibrosis transmembrane conductance regulators include CFTR.

[00221]   Non-limiting examples of Calcium activated chloride channels (CaCC) include AN01 , AN02, AN03, AN04, AN05, AN06, AN07, AN08, AN09, ANO10, BEST1 , BEST2, BEST3 and BEST4.

[00222]   Non-limiting examples of Chloride intracelluar channels include CLIC1 , CLIC2, CLIC3, CLIC4, CLIC5 and CLIC6.

[00223]   Non-limiting examples of Gap junction proteins (connexins) include GJA1 , GJA3, GJA4, GJA5, GJA6P, GJA8, GJA9, GJA10, GJB1 , GJB2, GJB3, GJB4, GJB5, GJB6, GJB7, GJC1 , GJC2, GJC3, GJD2, GJD3, GJD4 and GJE1 .

[00224]   Non-limiting examples of IP3 receptors include ITPR1 , ITPR2 and ITPR3.

[00225]   Non-limiting examples of Pannexins include PANX1, PANX2 and PANX3.

[00226]   Non-limiting examples of Ryanodine receptors include RYR1 , RYR2 and RYR3.

[00227]   A non-limiting example of non-selective Sodium leak channels includes NALCN.

[00228]   Non-limiting examples of nonvoltage-gated Sodium channels include SCNN1 A , SCNN1 B, SCNN1 D and SCNN1G.

Solute carrier proteins:

[00229]   In specific aspects, complexes for use in the methods provided herein comprise a lipid and a polynucleotide (e.g., mRNA) encoding a target protein, wherein the target protein is a solute carrier. Solute carrier proteins are integral membrane proteins that are characterized by their ability to transport a solute from one side of the lipid membrane to the other. This group of proteins includes secondary active transporters, which translocate solutes against an electrochemical gradient, and facilitative transporters, which translocate solutes in the direction of their electrochemical gradient. Solute carriers are organized into 52 families which encompass

over 300 proteins. The 52 families and non-limiting example members thereof are indicated below:

(1) The high-affinity glutamate and neutral amino acid transporter family, non-limiting examples include: SLC1A1 , SLC1A2, SLC1A3, SLC1A4, SLC1A5, SLC1A6, and SLC1A7;

(2) The facilitative glucose (GLUT) transporter family, non-limiting examples include: SLC2A1 , SLC2A2, SLC2A3, SLC2A4, SLC2A5, SLC2A6, SLC2A7, SLC2A8, SLC2A9, SLC2A10, SLC2A1 1, SLC2A12, SLC2A13, and SLC2A14;

(3) The heavy subunits of heterodimeric amino acid family, non-limiting examples include: SLC3A1 , and SLC3A2;

(4) The bicarbonate family. Examples include: SLC4A1 , SLC4A2, SLC4A3, SLC4A4, SLC4A5, SLC4A6, SLC4A7, SLC4A8, SLC4A9, SLC4A10, and SLC4A1 1;

(5) The sodium glucose cotransporter family. Examples include: SLC5A1 , SLC5A2, SLC5A3, SLC5A4, SLC5A5, SLC5A6, SLC5A7, SLC5A8, SLC5A9, SLC5A10, SLC5A1 1, and SLC5A12;

(6) The sodium- and chloride-dependent sodium:neurotransmitter symporter family. Examples include: SLC6A1 , SLC6A2, SLC6A3, SLC6A4, SLC6A5, SLC6A6, SLC6A7, SLC6A8, SLC6A9, SLC6A10, SLC6A1 1, SLC6A12, SLC6A13, SLC6A14, SLC6A15, SLC6A16, SLC6A17, SLC6A18, SLC6A19, and SLC6A20;

(7) The cationic amino acid transporter/glycoprotein-associated family, non-limiting examples include: (i) cationic amino acid transporters (SLC7A1 , SLC7A2, SLC7A3, SLC7A4) and (ii) glycoprotein-associated/light or catalytic subunits of heterodimeric amino acid transporters (SLC7A5, SLC7A6, SLC7A7, SLC7A8, SLC7A9, SLC7A10, SLC7A1 1, SLC7A13, SLC7A14);

(8) The Na+/Ca2+ exchanger family, non-limiting examples include: SLC8A1 , SLC8A2, and SLC8A3;

(9) The Na+/H+ exchanger family, non-limiting examples include: SLC9A1 , SLC9A2, SLC9A3, SLC9A4, SLC9A5, SLC9A6, SLC9A7, SLC9A8, SLC9A9, SLC9A10, SLC9A1 1, SLC9B1 , and SLC9B2;

(10) The sodium bile salt cotransport family, non-limiting examples include: SLC10A1 , SLC10A2, SLC10A3, SLC10A4, SLC10A5, SLC10A6, and SLC10A7;

(1 1) The proton coupled metal ion transporter family, non-limiting examples include: SLC1 1A1 and SLC1 1A2;

(12) The electroneutral cation-CI cotransporter family, non-limiting examples include: SLC12A1 , SLC12A1 , SLC12A2, SLC12A3, SLC12A4, SLC12A5, SLC12A6, SLC12A7, SLC12A8, and SLC12A9;

(13) The Na+-S042-/carboxylate cotransporter family; non-limiting examples include: SLC13A1 , SLC13A2, SLC13A3, SLC13A4, and SLC13A5;

(14) The urea transporter family, non-limiting examples include: SLC14A1 and SLC14A2;

(15) The proton oligopeptide cotransporter family, non-limiting examples include: SLC15A1 , SLC15A2, SLC15A3, and SLC15A4;

(16) The monocarboxylate transporter family, non-limiting examples include: SLC16A1 , SLC16A2, SLC16A3, SLC16A4, SLC16A5, SLC16A6, SLC16A7, SLC16A8, SLC16A9, SLC16A10, SLC16A1 1, SLC16A12, SLC16A13, and SLC16A14;

(17) The vesicular glutamate transporter family, non-limiting examples include: SLC17A1 , SLC17A2, SLC17A3, SLC17A4, SLC17A5, SLC1 7A6, SLC17A7, SLC17A8, and SLC17A9;

(18) The vesicular amine transporter family, non-limiting examples include: SLC1 8A1 , SLC18A2, and SLC18A3;

(19) The folate/thiamine transporter family, non-limiting examples include: SLC19A1 , SLC19A2, and SLC19A3;

(20) The type III Na+-phosphate cotransporter family, non-limiting examples include: SLC20A1 and SLC20A2;

(21) The organic anion transporter family; non-limiting examples include: (i) subfamily 1 SLC01A2, SLC01 B1, SLC01 B3, and SLC01C1 ; (ii) subfamily 2, SLC02A1 and SLC02B1 ; (iii) subfamily 3, SLC03A1 ; (iv) subfamily 4, SLC04A1 , SLC04C1 ; (v) subfamily 5, SLC05A1 ; and (vi) subfamily 6, SLC06A1 ;

(22) The organic cation/anion/zwitterion transporter family, non-limiting examples include: SLC22A1 , SLC22A2, SLC22A3, SLC22A4, SLC22A5, SLC22A6, SLC22A7, SLC22A8, SLC22A9, SLC22A10, SLC22A1 1, SLC22A12, SLC22A13, SLC22A14, SLC22A15, SLC22A16, SLC22A17, SLC22A18, SLC22A18AS,SLC22A19, SLC22A20, SLC22A23, SLC22A24, SLC22A25, and SLC22A31 .

(23) The Na+-dependent ascorbic acid transporter family, non-limiting examples include: SLC23A1 , SLC23A2, SLC23A3, and SLC23A4.

(24) The Na+/(Ca2+-K+) exchanger family, non-limiting examples include: SLC24A1 , SLC24A2, SLC24A3, SLC24A4, SLC24A5, and SLC24A6;

(25) The mitochondrial carrier family, non-limiting examples include: SLC25A1 , SLC25A2, SLC25A3, SLC25A4, SLC25A5, SLC25A6, SLC25A7, SLC25A8, SLC25A9, SLC25A10, SLC25A1 1, SLC25A12, SLC25A13, SLC25A14, SLC25A15, SLC25A16, SLC25A17, SLC25A18, SLC25A19,SLC25A20, SLC25A21 , SLC25A22, SLC25A23, SLC25A24, SLC25A25, SLC25A26, SLC25A27, SLC25A28, SLC25A29, SLC25A30, SLC25A31 , SLC25A32, SLC25A33, SLC25A34, SLC25A35, SLC25A36, SLC25A37,SLC25A38,

SLC25A39, SLC25A40, SLC25A41 , SLC25A42, SLC25A43, SLC25A44, SLC25A45, and SLC25A46;

(26) The multifunctional anion exchanger family, non-limiting examples include: SLC26A1 , SLC26A2, SLC26A3, SLC26A4, SLC26A5, SLC26A6, SLC26A7, SLC26A8, SLC26A9, SLC26A10, and SLC26A1 1;

(27) The fatty acid transport protein family, non-limiting examples include: SLC27A1 , SLC27A2, SLC27A3, SLC27A4, SLC27A5, and SLC27A6;

(28) The Na+-coupled nucleoside transport family, non-limiting examples include: SLC28A1 , SLC28A2, and SLC28A3;

(29) The facilitative nucleoside transporter family, non-limiting examples include: SLC29A1 , SLC29A2, SLC29A3, and SLC29A4;

(30) The zinc efflux family, non-limiting examples include: SLC30A1 , SLC30A2, SLC30A3, SLC30A4, SLC30A5, SLC30A6, SLC30A7, SLC30A8, SLC30A9, and SLC30A10;

(31) The copper transporter family, non-limiting examples include: SLC31A1 and SLC31A2;

(32) The vesicular inhibitory amino acid transporter family, a non-limiting example includes: SLC32A1 .

(33) The acetyl-CoA transporter family, a non-limiting example includes: SLC33A1 ;

(34) The type II Na+-phosphate cotransporter family, non-limiting examples include: SLC34A1 , SLC34A2, and SLC34A3;

(35) The nucleoside-sugar transporter family, non-limiting examples include: (i) subfamily A, SLC35A1 , SLC35A2, SLC35A3, SLC35A4, SLC35A5; (ii) subfamily B, SLC35B1 , SLC35B2, SLC35B3, SLC35B4; (iii) subfamily C, SLC35C1 , SLC35C2; (iv) subfamily D, SLC35D1 , SLC35D2, SLC35D3; (v) subfamily E, SLC35E1 , SLC35E2, SLC35E3, SLC35E4; (vi) subfamily F, SLC35F1 , SLC35F2, SLC35F3, SLC35F4, SLC35F5; (vii) subfamily G, SLC35G1 , SLC35G3, SLC35G4, SLC35G5, SLC35G6;

(36) The proton-coupled amino acid transporter family, non-limiting examples include: SLC36A1 , SLC36A2, SLC36A3, and SLC36A4;

(37) The sugar-phosphate/phosphate exchanger family, non-limiting examples include: SLC37A1 , SLC37A2, SLC37A3, and SLC37A4;

(38) The system A & N, sodium-coupled neutral amino acid transporter family, non-limiting examples include: SLC38A1 , SLC38A2, SLC38A3, SLC38A4, SLC38A5, SLC38A6, SLC38A7, SLC38A8, SLC38A9, SLC38A10, and SLC38A1 1;

(39) The metal ion transporter family, non-limiting examples include: SLC39A1 , SLC39A2, SLC39A3, SLC39A4, SLC39A5, SLC39A6, SLC39A7, SLC39A8, SLC39A9, SLC39A10, SLC39A1 1, SLC39A12, SLC39A13, and SLC39A14;

(40) The basolateral iron transporter family, a non-limiting example includes: SLC40A1;

(41) The MgtE-like magnesium transporter family, non-limiting examples include: SLC41A1, SLC41A2, and SLC41A3;

(42) The ammonia transporter family, non-limiting examples include: RhAG, RhBG, and RhCG;

(43) The Na+-independent, system-L like amino acid transporter family; non-limiting examples include: SLC43A1, SLC43A2, and SLC43A3;

(44) The choline-like transporter family; non-limiting examples include: SLC44A1, SLC44A2, SLC44A3, SLC44A4, and SLC44A5;

(45) The putative sugar transporter family, non-limiting examples include: SLC45A1, SLC45A2, SLC45A3, and SLC45A4;

(46) The folate transporter family; non-limiting examples include: SLC46A1, SLC46A2, and SLC46A3;

(47) The multidrug and toxin extrusion family; non-limiting examples include: SLC47A1 and SLC47A2;

(48) The heme transporter family, a non-limiting xample includes: HCP-1;

(49) Transporters of the major facilitator superfamily, non-limiting examples include MFSD5 and MFSD10;

(50) Sugar efflux transporters of the SWEET family, a non-limiting example includes SLC50A1;

(51) Transporters of steroid-derived molecules, non-limiting examples include OSTa and OSTβ;

(52) Riboflavin transporter family RFVT/SLC52, non-limiting examples include SLC52A1, SLC52A2, and SLC52A3.

Difficult to express target proteins:

[00230]   In specific aspects, conventional methods for producing antibodies, e.g., immunizing animals with a purified recombinant protein, may not be effective for difficult to express target proteins. Many factors can contribute to expression issues, such as cytotoxicity in host production cells, inherently poor biophysical properties (e.g., size, solubility, conformation, post-translational modifications (such as glycosylation) of the protein that proscribe overexpression/purification. Non-limiting examples of characteristics of difficult-to-express proteins include, but are not limited to, large proteins (e.g., proteins with a molecular weight $\geq$ 150 kDa), transmembrane proteins, proteins with unusual post translational modifications, or proteins with poor solubility, unstable

proteins, secreted proteins that do not contain a signal peptide, membrane associated proteins, intrinsically disordered proteins and proteins with a short half-life.

[00231]   Non-limiting examples of target proteins such as soluble target proteins, which may be difficult to express, for use in the methods provided herein include: ADAMTS7, ANGPTL3, ANGPTL4, ANGPTL8, LPL, GDF15, Galectin-1 , Galectin-2, Galectin-3, matrix gla protein (MGP), PRNP, DGAT1 , GPAT3, DMC1 , BLM, and BRCA2.

[00232]   Characteristics of difficult-to-express proteins can be assessed using methods described in the art, for example, solubility assays (e.g., dynamic light scattering, liquid chromatography mass spectrometry), stability assays (e.g., Differential scanning fluorimetry, Differential scanning calorimetry, circular dicroism), NMR, and chromatography. In certain aspects, a difficult-to-express protein may be more susceptible to aggregation (e.g., at least 5% aggregation, or at least 10% aggregation, or at least 20% aggregation, or at least 30% aggregation, or at least 40% aggregation, or at least 50% aggregation, or least 60% aggregation), for example, when kept in solution at room temperature or at 4°C for a period of more than a week, more than several weeks, more than a month, more than several months (e.g., 3 months, 4 months or 5 months), or more than 6 months or 1 year.

[00233]   In particular aspects, a difficult-to-express protein has a positive charge. In certain aspects, a difficult-to-express protein has a negative charge. In certain aspects, a difficult-to-express protein is hydrophobic.

[00234]   In certain aspects, a difficult-to-express protein has a short half-life, for example, a half-life of less than 24 hours, less than 20 hours, less than 15 hours, less than 12 hours, less than 10 hours, less than 8 hours, less than 6 hours, less than 4 hours, less than 2 hours, or less than 1 hour.

[00235]   In certain embodiments, target proteins described herein include positively charged proteins, negatively charged proteins, hydrophobic proteins, and glycoproteins.

[00236]   In certain embodiments, target proteins described herein include enzymes, such as secreted and membrane associated enzymes.

II. Cationic Liposomes

[00237]   Any of the cationic lipids known in the prior art may be employed in the practice of the claimed invention. See, for example, Feigner et al. *(Proc. Natl. Acad. Sci. U.S.A.* 84:7413-7417 (1987)); Feigner et al. *(Focus* 11:2 1-25 (1989)); Feigner ("Cationic Liposome-Mediated Transfection with Lipofectin™ Reagent," in *Gene Transfer and Expression Protocols Vol. 7,* Murray, E . J., Ed., Humana Press, New Jersey, pp. 81-89 (1991)); WO 91/17424; WO 91/16024; U.S. Pat. No. 4,897,355; U.S. Pat. No. 4,946,787; U.S. Pat. No. 5,049,386; U.S. Pat. No.

5,208,036; Behr et al. *{Proc. Natl. Acad. Sci. USA* 86:6982-6986 (1989); EPO Publication 0 394 111); Gao et al. *(Biochim. Biophys. Res. Comm.* 179:280-285 (1991)); Zhou et al., *(Biochim. Biophys. Res. Comm.* 165:8-14 (1991)); and Gebeychu et al. (co-owned U.S. application Ser. No. 07/937,508; filed Aug. 28, 1992), the contents of which are fully incorporated by reference.

[00238]   Other non-limiting examples of lipids (e.g., cationic lipids, neutral lipids, helper lipids, and stealth lipids) which can be used in the methods and compositions provided herein include those described in WO2016/037053, WO2016/010840, WO2015/095346, WO2015/095340, WO2016/037053, WO2014/136086, and WO2011/076807, each of which is hereby incorporated by reference in its entirety. In a specific aspect, cationic lipids suitable for the methods described herein include Lipid A, Lipid B, and Lipid C having the following chemical structure (which are described in more detail in WO2015/095346 and WO2015/095340):

Chemical structure of Lipid A;
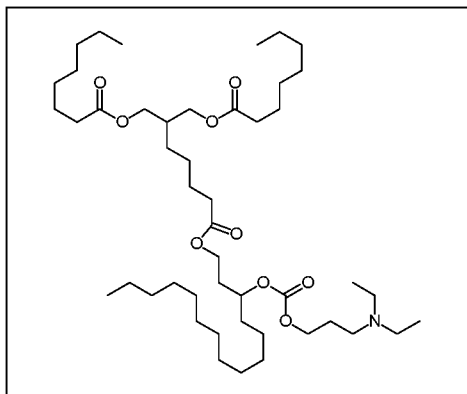
Chemical structure of Lipid B

Chemical structure of Lipid C

52

[00239]   In certain aspects, cationic lipids for the compositions and methods described herein include, but are not limited to, N,N-dioleyl-N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(1-(2,3-dioleoyloxy) propyl)-N,N,N-trimethylammonium chloride (DOTAP), 1,2-Dioleoyl-3-Dimethylammonium -propane (DODAP), N-(1-(2,3-dioleyloxy)propyl) -N,N,N-trimethylammonium chloride (DOTMA), 1,2-Dioleoylcarbamyl -3-Dimethylammonium-propane (DOCDAP), 1,2-Dilineoyl-3-Dimethylammonium-propane (DLINDAP), dilauryl($C_{12:0}$) trimethyl ammonium propane (DLTAP), Dioctadecylamidoglycyl spermine (DOGS), DC-Choi, Dioleoyloxy -N-[2-sperminecarboxamido)ethyl} -N,N-dimethyl-1-propanaminiumtrifluoroacetate (DOSPA), 1,2-Dimyristyloxypropyl-3-dimethyl -hydroxyethyl ammonium bromide (DMRIE), 3-Dimethylamino-2-(Cholest-5-en -3-beta-oxybutan-4-oxy) -1-(cis,cis-9,12 -octadecadienoxy)prop ane (CLinDMA), N,N-dimethyl-2,3-dioleyloxy)propylamine (DODMA), 2-[5'-(cholest-5-en-3[beta]-oxy)-3'-oxapentoxy) -3-dimethyl-1-(cis,cis-9',12'-octadecadienoxy) propane (CpLinDMA) and N,N-Dimethyl-3,4-dioleyloxybenzylamine (DMOBA), and 1,2-N,N'-Dioleylcarbamyl-3-dimethylaminopropane (DOcarbDAP). In one embodiment, the cationic lipid for the compositions and methods provided herein is DOTAP or DLTAP.

[00240]   In specific embodiments, cationic lipids for the compositions and methods described herein include N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium   chloride (DOTMA). DOTMA, alone or in a 1:1 combination with dioleoylphosphatidylethanolamine (DOPE) can be formulated into liposomes using standard techniques. A DOTMA:DOPE (1:1) formulation is sold under the name LIPOFECTIN™ (GIBCO/BRL: Life Technologies, Inc., Gaithersburg, Md.). In a particular embodiment, a commercially available cationic lipid is 1,2-bis(oleoyloxy)-3-3- (trimethylammonia)propane (DOTAP), which differs from DOTMA in that the oleoyl moieties are linked via ester bonds, not ether bonds, to the propylamine.

[00241]   In particular embodiments, a related group of cationic lipids for the compositions and methods described herein differ from DOTMA and DOTAP in that one of the methyl groups of the trimethylammonium group is replaced by a hydroxyethyl group. Compounds of this type are similar to the Rosenthal Inhibitor of phospholipase A (Rosenthal et al., supra), which has stearoyl esters linked to the propylamine core. The dioleoyl analogs of the Rosenthal Inhibitor (RI) are commonly abbreviated as DORI-ether and DORI-ester, depending upon the linkage of the fatty acid moieties to the propylamine core. The hydroxy group can be used as a site for further functionalization, for example, by esterification to carboxyspermine.

[00242]   In certain embodiments, another class of cationic lipids for the compositions and methods described herein include, carboxyspermine that has been conjugated to two types of lipids, resulting in 5-carboxylspermylglycine dioctadecylamide (DOGS). DOGS is available commercially as TRANSFECTAM™ (Promega, Madison, Wis.).
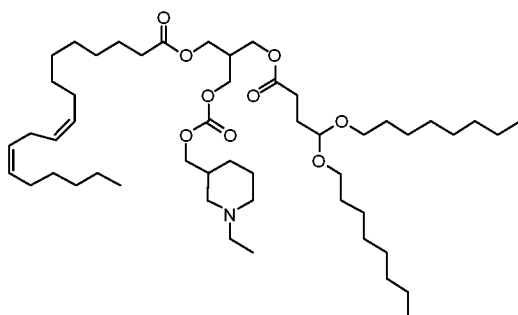
[00243]   Another class of known compounds has been described by Behr et al. *(Proc. Natl. Acad. Sci. USA* 86:6982-6986 (1989); EPO Publication 0 394 111), in which carboxyspermine has been conjugated to two types of lipids, resulting in dipalmitoylphosphatidylethanolamine 5-carboxyspermylamide (DDPES).

[00244]   In specific aspects, another cationic lipid for the compositions and methods described herein is a cholesterol derivative (DC-Choi) which has been synthesized and formulated into liposomes in combination with DOPE. In another specific embodiment, a cationic lipid for the compositions and methods described herein is lipopolylysine, which is formed by conjugating polylysine to DOPE.

[00245]   Further non-limiting examples of cationic lipids for the compositions and methods provided herein include the following, as well as those described in WO201 5/095346:

2-(10-dodecyl-3-ethyl-8,   14-dioxo-7,9, 13-trioxa-3-azaoctadecan-18-yl)propane-1   ,3-diyl dioctanoate;

2-(9-dodecyl-2-methyl-7,   13-dioxo-6,8, 12-trioxa-2-azaheptadecan-17-yl)propane-1   ,3-diyl dioctanoate;

2-(9-dodecyl-2-methyl-7,   13-dioxo-6,8, 12-trioxa-2-azapentadecan-15-yl)propane-1   ,3-diyl dioctanoate;

2-(10-dodecyl-3-ethyl-8,   14-dioxo-7,9, 13-trioxa-3-azahexadecan-16-yl)propane-1   ,3-diyl dioctanoate;

2-(8-dodecyl-2-methyl-6,   12-dioxo-5,7, 1 1 -trioxa-2-azaheptadecan-17-yl)propane-1   ,3-diyl dioctanoate;

2-(10-dodecyl-3-ethyl-8,   14-dioxo-7,9, 13-trioxa-3-azanonadecan-19-yl)propane-1   ,3-diyl dioctanoate;

2-(9-dodecyl-2-methyl-7,   13-dioxo-6,8, 12-trioxa-2-azaoctadecan-18-yl)propane-1   ,3-diyl dioctanoate;

2-(8-dodecyl-2-methyl-6,   12-dioxo-5,7, 1 1 -trioxa-2-azaoctadecan-18-yl)propane-1   ,3-diyl dioctanoate;

2-(10-dodecyl-3-ethyl-8,   14-dioxo-7,9,13-trioxa-3-azaicosan-20-yl)propane-1   ,3-diyl dioctanoate;

2- (9-dodecyl-2-methyl-7,   13-dioxo-6,8,12-trioxa-2-azanonadecan-19-yl)propane-1   ,3-diyl dioctanoate;

3- (((3-(diethylamino)propoxy)carbonyl)oxy)pentadecyl   4,4-bis(octyloxy)butanoate;

3-(((3-(diethylamino)propoxy)carbonyl)oxy)pentadecyl 4,4-bis((2-ethylhexyl)oxy)butanoate; 3-(((3-(diethylamino)propoxy)carbonyl)oxy)pentadecyl 4,4-bis((2-propylpentyl)oxy)butanoate; 3-(((3-(ethyl(methyl)amino)propoxy)carbonyl)oxy)pentadecyl 4,4-bis((2-propylpentyl)oxy)butanoate; 3-(((3-(dimethylamino)propoxy)carbonyl)oxy)pentadecyl 4,4-bis((2-propylpentyl)oxy)butanoate; 3-(((3-(diethylamino)propoxy)carbonyl)oxy)pentadecyl 6,6-bis(octyloxy)hexanoate; 3-(((3-(diethylamino)propoxy)carbonyl)oxy)pentadecyl 6,6-bis(hexyloxy)hexanoate; 3-(((3-(diethylamino)propoxy)carbonyl)oxy)pentadecyl 6,6-bis((2-ethylhexyl)oxy)hexanoate; 3-(((3-(diethylamino)propoxy)carbonyl)oxy)pentadecyl 8,8-bis(hexyloxy)octanoate; 3-(((3-(diethylamino)propoxy)carbonyl)oxy)pentadecyl 8,8-dibutoxyoctanoate; 3-(((3-(diethylamino)propoxy)carbonyl)oxy)pentadecyl 8,8-bis((2-propylpentyl)oxy)octanoate; 3-(((3-(ethyl(methyl)amino)propoxy)carbonyl)oxy)pentadecyl 8,8-bis((2-propylpentyl)oxy)octanoate; 3-(((3-(dimethylamino)propoxy)carbonyl)oxy)pentadecyl 8,8-bis((2-propylpentyl)oxy)octanoate; 3-(((3-(dimethylamino)propoxy)carbonyl)oxy)pentadecyl 3-octylundecanoate; 3-(((3-(dimethylamino)propoxy)carbonyl)oxy)pentadecyl 3-octylundec-2-enoate; 3-(((3-(dimethylamino)propoxy)carbonyl)oxy)pentadecyl 7-hexyltridec-6-enoate; 3-(((3-(dimethylamino)propoxy)carbonyl)oxy)pentadecyl 9-pentyltetradecanoate; 3-(((3-(dimethylamino)propoxy)carbonyl)oxy)pentadecyl 9-pentyltetradec-8-enoate; 3-(((3-(dimethylamino)propoxy)carbonyl)oxy)pentadecyl 5-heptyldodecanoate; 3-(((3-(dimethylamino)propoxy)carbonyl)oxy)tridecyl 5-heptyldodecanoate; 3-(((3-(dimethylamino)propoxy)carbonyl)oxy)undecyl 5-heptyldodecanoate; 1,3-bis(octanoyloxy)propan-2-yl (3-(((2-(dimethylamino)ethoxy)carbonyl)oxy)pentadecyl) succinate; 1,3-bis(octanoyloxy)propan-2-yl (3-(((3-(dimethylamino)propoxy)carbonyl)oxy)pentadecyl) succinate; 1-(3-(((3-(dimethylamino)propoxy)carbonyl)oxy)pentadecyl) 10-octyl decanedioate; 1-(3-(((3-(diethylamino)propoxy)carbonyl)oxy)pentadecyl) 10-octyl decanedioate; 1-(3-(((3-(ethyl(methyl)amino)propoxy)carbonyl)oxy)pentadecyl) 10-octyl decanedioate; 1-(3-(((3-(diethylamino)propoxy)carbonyl)oxy)pentadecyl) 10-(2-ethylhexyl) decanedioate; 1-(3-(((3-(ethyl(methyl)amino)propoxy)carbonyl)oxy)pentadecyl) 10-(2-ethylhexyl) decanedioate; 3-(((3-(dimethylamino)propoxy)carbonyl)oxy)pentadecyl 10-(octanoyloxy)decanoate; 8-dodecyl-2-methyl-6,12-dioxo-5,7,11-trioxa-2-azanonadecan-19-yl decanoate; 3-(((3-(diethylamino)propoxy)carbonyl)oxy)pentadecyl 10-(octanoyloxy)decanoate; 3-(((3-(ethyl(methyl)amino)propoxy)carbonyl)oxy)pentadecyl 10-(octanoyloxy)decanoate;

(9Z, 12Z)-3-(((3-(dimethylamino)propoxy)carbonyl)oxy)pentadecyl   octadeca-9, 12-dienoate; (9Z, 12Z)-3-(((3-(diethylamino)propoxy)carbonyl)oxy)pentadecyl   octadeca-9, 12-dienoate;

(9Z, 12Z)-3-(((3-(ethyl(methyl)amino)propoxy)carbonyl)oxy)pentadecyl   octadeca-9, 12-dienoate;

(9Z, 12Z)-3-(((2-(dimethylamino)ethoxy)carbonyl)oxy)pentadecyl   octadeca-9, 12-dienoate;

1 -((9Z, 12Z)-octadeca-9, 12-dienoyloxy)pentadecan-3-yl   1 ,4-dimethylpiperidine-4-carboxylate;

2- (((3-(diethylamino)propoxy)carbonyl)oxy)tetradecyl   4,4-bis((2-ethylhexyl)oxy)butanoate;   (9Z, 12Z)-(12Z, 15Z)-3-((3-(dimethylamino)propanoyl)oxy)henicosa-12,   15-dien-1 -yl octadeca-9, 12-dienoate;

(12Z, 15Z)-3-((4-(dimethylamino)butanoyl)oxy)henicosa-1   2 ,15-dien-1 -yl 3-octylundecanoate; (12Z, 15Z)-3-((4-(dimethylamino)butanoyl)oxy)henicosa-12,   15-dien-1 -yl 5-heptyldodecanoate;

(12Z, 15Z)-3-((4-(dimethylamino)butanoyl)oxy)henicosa-1   2 ,15-dien-1 -yl 7-hexyltridecanoate; (12Z, 15Z)-3-((4-(dimethylamino)butanoyl)oxy)henicosa-12,   15-dien-1 -yl 9-pentyltetradecanoate; (12Z, 15Z)-1 -((((9Z, 12Z)-octadeca-9, 12-dien-1 -yloxy)carbonyl)oxy)henicosa-12,   15-dien-3-yl 3-(dimethylamino)propanoate;

(13Z, 16Z)-4-(((2-(dimethylamino)ethoxy)carbonyl)oxy)docosa-13,   16-dien-1 -yl 2,2-bis(heptyloxy)acetate;

(13Z, 16Z)-4-(((3-(diethylamino)propoxy)carbonyl)oxy)docosa-13,   16-dien-1 -yl 2,2-bis(heptyloxy)acetate;

2,2-bis(heptyloxy)ethyl 3-((3-ethyl-10-((9Z, 12Z)-octadeca-9, 12-dien-1 -yl)-8, 15-dioxo-7,9, 14-trioxa-3-azaheptadecan-17-yl)disulfanyl)propanoate;

(13Z,16Z)-4-(((3-(dimethylamino)propoxy)carbonyl)oxy)docosa-13,16-dien-1-yl   heptadecan-9-yl succinate;

(9Z, 12Z)-2-(((1 1 Z, 14Z)-2-((3-(dimethylamino)propanoyl)oxy)icosa-1   1 , 14-dien-1 -yl)oxy)ethyl octadeca-9, 12-dienoate;

(9Z, 12Z)-3-(((3-(dimethylamino)propoxy)carbonyl)oxy)-13-(octanoyloxy)tridecyl   octadeca-9, 12-dienoate;

3- (((3-(dimethylamino)propoxy)carbonyl)oxy)-1   3-(octanoyloxy)tridecyl 3-octylundecanoate; 3-(((3-(dimethylamino)propoxy)carbonyl)oxy)-13-hydroxytridecyl   5-heptyldodecanoate;

3-(((3-(dimethylamino)propoxy)carbonyl)oxy)-1   3-(octanoyloxy)tridecyl 5-heptyldodecanoate; 3-(((3-(dimethylamino)propoxy)carbonyl)oxy)-13-(octanoyloxy)tridecyl   7-hexyltridecanoate; 3-(((3-(dimethylamino)propoxy)carbonyl)oxy)-13-hydroxytridecyl   9-pentyltetradecanoate;

3-(((3-(dimethylamino)propoxy)carbonyl)oxy)-13-(octanoyloxy)tridecyl   9-pentyltetradecanoate;

1- (3-(((3-(dimethylamino)propoxy)carbonyl)oxy)-13-(octanoyloxy)tridecyl)   10-octyl decanedioate;

3-(((3-(dimethylamino)propoxy)carbonyl)oxy)-13-(octanoyloxy)tridecyl   10-(octanoyloxy)decanoate;

(9Z, 12Z)-3-(((3-(dimethylamino)propoxy)carbonyl)oxy)-5-octyltridecyl   octadeca-9, 12-dienoate;

3-(((3-(dimethylamino)propoxy)carbonyl)oxy)-5-octyltridecyl   decanoate;

5-(((3-(dimethylamino)propoxy)carbonyl)oxy)-7-octylpentadecyl   octanoate;

(9Z, 12Z)-5-(((3-(dimethylamino)propoxy)carbonyl)oxy)-7-octylpentadecyl   octadeca-9, 12-
dienoate;

9-(((3-(dimethylamino)propoxy)carbonyl)oxy)-1   1 -octylnonadecyl octanoate;

9-(((3-(dimethylamino)propoxy)carbonyl)oxy)-1   1 -octylnonadecyl decanoate;

(9Z, 12Z)-9-(((3-(dimethylamino)propoxy)carbonyl)oxy)nonadecyl   octadeca-9, 12-dienoate; 9-(((3-
(dimethylamino)propoxy)carbonyl)oxy)nonadecyl   hexanoate;

9-(((3-(dimethylamino)propoxy)carbonyl)oxy)nonadecyl   3-octylundecanoate;

9-((4-(dimethylamino)butanoyl)oxy)nonadecyl   hexanoate;

9-((4-(dimethylamino)butanoyl)oxy)nonadecyl   3-octylundecanoate;

(9Z,9'Z, 12Z, 12'Z)-2-((4-(((3-(dimethylamino)propoxy)carbonyl)oxy)hexadecanoyl)oxy)propane-1
,3-diyl bis(octadeca-9, 12-dienoate);

(9Z,9'Z, 12Z, 12'Z)-2-((4-(((3-(diethylamino)propoxy)carbonyl)oxy)hexadecanoyl)oxy)propane-1
,3-diyl bis(octadeca-9, 12-dienoate);

(9Z,9'Z, 12Z, 12'Z, 15Z, 15'Z)-2-((4-(((3-(dimethylamino)propoxy)carbonyl)oxy)
hexadecanoyl)oxy)propane-1   ,3-diyl bis(octadeca-9, 12, 15-trienoate);

(Z)-2-((4-(((3-(dimethylamino)propoxy)carbonyl)oxy)hexadecanoyl)oxy)propane-1   ,3-diyl dioleate;

2- ((4-(((3-(diethylamino)propoxy)carbonyl)oxy)hexadecanoyl)oxy)propane-1   ,3-diyl
ditetradecanoate;

2-((4-(((3-(dimethylamino)propoxy)carbonyl)oxy)hexadecanoyl)oxy)propane-1   ,3-diyl
ditetradecanoate;

2-((4-(((3-(ethyl(methyl)amino)propoxy)carbonyl)oxy)hexadecanoyl)oxy)propane-1   ,3-diyl
ditetradecanoate;

2-((4-(((3-(dimethylamino)propoxy)carbonyl)oxy)hexadecanoyl)oxy)propane-1   ,3-diyl
didodecanoate;

2-((4-(((3-(diethylamino)propoxy)carbonyl)oxy)hexadecanoyl)oxy)propane-1   ,3-diyl
didodecanoate;

2-((4-(((3-(ethyl(methyl)amino)propoxy)carbonyl)oxy)hexadecanoyl)oxy)propane-1   ,3-diyl
didodecanoate;

2-((4-(((3-(diethylamino)propoxy)carbonyl)oxy)hexadecanoyl)oxy)propane-1   ,3-diyl
bis(decanoate);

2-((4-(((3-(ethyl(methyl)amino)propoxy)carbonyl)oxy)hexadecanoyl)oxy)propane-1   ,3-diyl
bis(decanoate);

2-((4-(((3-(diethylamino)propoxy)carbonyl)oxy)hexadecanoyl)oxy)propane-1   ,3-diyl dioctanoate;

2-((4-(((3-(ethyl(methyl)amino)propoxy)carbonyl)oxy)hexadecanoyl)oxy)propane-1   ,3-diyl dioctanoate;

2-(((1 3Z, 16Z)-4-(((3-(dimethylamino)propoxy)carbonyl)oxy)docosa-1   3,1 6-dienoyl)oxy)propane-1 ,3-diyl dioctanoate;

2-(((1 3Z, 16Z)-4-(((3-(diethylamino)propoxy)carbonyl)oxy)docosa-1   3, 16-dienoyl)oxy)propane-1 ,3-diyl dioctanoate;

(9Z,9'Z, 12Z, 12'Z)-2-((2-(((3-(diethylamino)propoxy)carbonyl)oxy)tetradecanoyl)oxy)propane-1   ,3-diyl bis(octadeca-9,12-dienoate);

(9Z,9'Z, 12Z, 12'Z)-2-((2-(((3-(dimethylamino)propoxy)carbonyl)oxy)dodecanoyl)oxy)propane-1   ,3-diyl bis(octadeca-9,12-dienoate);

(9Z,9'Z, 12Z, 12'Z)-2-((2-(((3-(dimethylamino)propoxy)carbonyl)oxy)tetradecanoyl)oxy)propane-1.3- diyl bis(octadeca-9,12-dienoate);

(9Z,9'Z, 12Z, 12'Z)-2-((2-(((3-(diethylamino)propoxy)carbonyl)oxy)dodecanoyl)oxy)propane-1   ,3-diyl bis(octadeca-9, 12-dienoate);

2-((2-(((3-(diethylamino)propoxy)carbonyl)oxy)tetradecanoyl)oxy)propane-1   ,3-diyl dioctanoate;

4.4- bis(octyloxy)butyl 4-(((3-(dimethylamino)propoxy)carbonyl)oxy)hexadecanoate;

4,4-bis(octyloxy)butyl 2-(((3-(diethylamino)propoxy)carbonyl)oxy)dodecanoate;

(9Z, 12Z)-10-dodecyl-3-ethyl-14-(2-((9Z,   12Z)-octadeca-9, 12-dienoyloxy)ethyl)-8, 13-dioxo-7,9-dioxa-3, 14-diazahexadecan-16-yl octadeca-9, 12-dienoate;

2- ((4-(((3-(diethylamino)propoxy)carbonyl)oxy)-1   1 -(octanoyloxy)undecanoyl)oxy)propane-1   ,3-diyl dioctanoate;

(9Z,9'Z, 12Z, 12'Z)-2-(9-dodecyl-2-methyl-7,   12-dioxo-6,8, 13-trioxa-2-azatetradecan-14-yl)propane-1 ,3-diyl bis(octadeca-9, 12-dienoate);

3- (((3-(dimethylamino)propoxy)carbonyl)oxy)pentadecyl   4,4-bis(octyloxy)butanoate;

3-(((3-(piperidin-1-yl)propoxy)carbonyl)oxy)pentadecyl   6,6-bis(octyloxy)hexanoate;

3-(((3-(piperazin-1-yl)propoxy)carbonyl)oxy)pentadecyl   6,6-bis(octyloxy)hexanoate;

3-(((4-(diethylamino)butoxy)carbonyl)oxy)pentadecyl   6,6-bis(octyloxy)hexanoate;

3-(((3-(4-methylpiperazin-1 -yl)propoxy)carbonyl)oxy)pentadecyl   6,6-bis(octyloxy)hexanoate; 3-((((1-methylpiperidin-4-yl)methoxy)carbonyl)oxy)pentadecyl   6,6-bis(octyloxy)hexanoate; 3-(((3-morpholinopropoxy)carbonyl)oxy)pentadecyl   6,6-bis(octyloxy)hexanoate;

3-(((2-(diethylamino)ethoxy)carbonyl)oxy)pentadecyl   6,6-bis(octyloxy)hexanoate;

3-(((3-(dimethylamino)propoxy)carbonyl)oxy)pentadecyl   6,6-bis(octyloxy)hexanoate;

3-(((3-(diethylamino)propoxy)carbonyl)oxy)pentadecyl   6,6-bis((2-propylpentyl)oxy)hexanoate; 3-(((3-(dimethylamino)propoxy)carbonyl)oxy)pentadecyl   6,6-bis((2-propylpentyl)oxy)hexanoate

58

LXR420: 3-(((3-(dimethylamino)propoxy)carbonyl)oxy)pentadecyl   6,6-bis((3-ethylpentyl)oxy)hexanoate;

(2R)-1 -((6,6-bis(octyloxy)hexanoyl)oxy)pentadecan-3-yl   1 -methylpyrrolidine-2-carboxylate;   (2S)-1-((6,6-bis(octyloxy)hexanoyl)oxy)pentadecan-3-yl   1-methylpyrrolidine-2-carboxylate;   (2R)-1-((6,6-bis(octyloxy)hexanoyl)oxy)pentadecan-3-yl   pyrrolidine-2-carboxylate;

1-((6,6-bis(octyloxy)hexanoyl)oxy)pentadecan-3-yl   1 ,3-dimethylpyrrolidine-3-carboxylate;   3-((3-(1-methylpiperidin-4-yl)propanoyl)oxy)pentadecyl   6,6-bis(octyloxy)hexanoate;

1- ((6,6-bis(octyloxy)hexanoyl)oxy)pentadecan-3-yl   1 ,4-dimethylpiperidine-4-carboxylate;   3-((5-(diethylamino)pentanoyl)oxy)pentadecyl   6,6-bis(octyloxy)hexanoate;

3-(((3-(diethylamino)propoxy)carbonyl)oxy)pentadecyl   5-(4,6-diheptyl-1 ,3-dioxan-2-yl)pentanoate;

3-(((3-(diethylamino)propoxy)carbonyl)oxy)undecyl   6,6-bis(octyloxy)hexanoate;

3-(((3-(diethylamino)propoxy)carbonyl)oxy)tridecyl   6,6-bis(octyloxy)hexanoate;

(12Z, 15Z)-3-(((3-(diethylamino)propoxy)carbonyl)oxy)henicosa-12,   15-dien-1 -yl 6,6-bis(octyloxy)hexanoate;

6-((6,6-bis(octyloxy)hexanoyl)oxy)-4-(((3-(diethylamino)propoxy)carbonyl)oxy)hexyl   octanoate;

4,4-bis(octyloxy)butyl 5-(((3- (diethylamino)propoxy)carbonyl)oxy)heptadecanoate;

4,4-bis(octyloxy)butyl (3-(diethylamino)propyl) pentadecane-1 ,3-diyl dicarbonate;

2- (5-((4-((1 ,4-dimethylpiperidine-4-carbonyl)oxy)hexadecyl)oxy)-5-oxopentyl)propane-1   ,3-diyl dioctanoate;

2-(5-((4-((1 ,3-dimethylpyrrolidine-3-carbonyl)oxy)hexadecyl)oxy)-5-oxopentyl)propane-1   ,3-diyl dioctanoate;

2-(5-oxo-5-((4-(((S)-pyrrolidine-2-carbonyl)oxy)hexadecyl)oxy)pentyl)propane-1   ,3-diyl dioctanoate;

2-(5-((4-(((((S)-1-methylpyrrolidin-3-yl)oxy)carbonyl)oxy)hexadecyl)oxy)-5-oxopentyl)propane-1   ,3-diyl dioctanoate;

2-(5-((4-(((((R)-1-methylpyrrolidin-3-yl)oxy)carbonyl)oxy)hexadecyl)oxy)-5-oxopentyl)propane-1   ,3-diyl dioctanoate;

2-(5-((4-(((((1-ethylpiperidin-3-yl)methoxy)carbonyl)oxy)hexadecyl)oxy)-5-oxopentyl)propane-1   ,3-diyl dioctanoate;

2-(5-((4-(((((1-methylpiperidin-4-yl)oxy)carbonyl)oxy)hexadecyl)oxy)-5-oxopentyl)propane-1   ,3-diyl dioctanoate;

2-(1 0-dodecyl-3-ethyl-8, 15-dioxo-7,9, 14-trioxa-3-azanonadecan-1 9-yl)propane-1 ,3-diyl dioctanoate;

2-(1 1 -dodecyl-3-ethyl-9, 15-dioxo-8, 10,14-trioxa-3-azanonadecan-19-yl)propane-1   ,3-diyl dioctanoate;

2-(5-((3-(((3-(1 H-imidazol-1-yl)propoxy)carbonyl)oxy)pentadecyl)oxy)-5-oxopentyl)propane-1 ,3-diyl dioctanoate;

2-(5-oxo-5-((3-(((3-(piperidin-1-yl)propoxy)carbonyl)oxy)pentadecyl)oxy)pentyl)propane-1 ,3-diyl dioctanoate; and

2-(12-dodecyl-3-ethyl-8, 14-dioxo-7,9, 13-trioxa-3-azaoctadecan-18-yl)propane-1 ,3-diyl dioctanoate.

[00246]   In specific aspects, these cationic lipid compounds are useful either alone, or in combination with other lipid aggregate-forming components (such as DOPE or cholesterol) for formulation into liposomes or other lipid aggregates. Such aggregates are cationic and able to complex with anionic macromolecules such as DNA or RNA.

[00247]   "Neutral lipids" suitable for use in a lipid composition and methods described herein include, for example, a variety of neutral, uncharged or zwitterionic lipids. Examples of neutral phospholipids suitable for use in the present invention include, but are not limited to: 5-heptadecylbenzene-1 ,3-diol (resorcinol), dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), phosphocholine (DOPC), dimyristoylphosphatidylcholine (DMPC), phosphatidylcholine (PLPC), l,2-distearoyl-sn-glycero-3-phosphocholine (DAPC), phosphatidylethanolamine (PE), egg phosphatidylcholine (EPC), dilauryloylphosphatidylcholine (DLPC), dimyristoylphosphatidylcholine (DMPC), l-myristoyl-2-palmitoyl phosphatidylcholine (MPPC), l-palmitoyl-2-myristoyl phosphatidylcholine (PMPC), l-palmitoyl-2-stearoyl phosphatidylcholine (PSPC), l,2-diarachidoyl-sn-glycero-3- phosphocholine (DBPC), l-stearoyl-2- palmitoyi phosphatidylcholine (SPPC), l,2-dieicosenoyl-sn-glycero -3-phosphocholine (DEPC), palmitoyloleoyi phosphatidylcholine (POPC), lysophosphatidyl choline, dioleoyl phosphatidylethanolamine (DOPE), dilinoleoylphosphatidylcholine distearoylphophatidylethanolamine (DSPE), dimyristoyl phosphatidylethanolamine (DMPE), dipalmitoyi phosphatidylethanolamine (DPPE), palmitoyloleoyi phosphatidylethanolamine (POPE), lysophosphatidylethanolamine and combinations thereof. In one embodiment, the neutral phospholipid is selected from the group consisting of distearoylphosphatidylcholine (DSPC) and dimyristoyl phosphatidyl ethanolamine (DMPE).

[00248]   "Helper lipids" are lipids that enhance transfection (e.g. transfection of the nanoparticle including the biologically active agent) to some extent. The mechanism by which the helper lipid enhances transfection may include, e.g., enhancing particle stability and/or enhancing membrane fusogenicity. Helper lipids include steroids and alkyl resorcinols. Helper lipids suitable for the compositions and methods described herein include, but are not limited to, cholesterol, 5-heptadecylresorcinol, and cholesterol hemisuccinate. Non-limiting examples of helper lipids for the compositions and methods described herein include those described in WO20 15/095346,

WO20 15/095340, WO2016/037053, WO201 4/1 36086, and WO201 1/076807, each of which is hereby incorporated by reference in its entirety.

[00249]   Stealth lipids are lipids that increase the length of time for which the nanoparticles can exist *in vivo* (e.g. in the blood). Stealth lipids suitable for the compositions and methods described herein include, but are not limited to, stealth lipids having a hydrophilic head group linked to a lipid moiety. Non-limiting examples of stealth lipids for the compositions and methods described herein include those described in WO201 5/095346, WO201 5/095340, WO201 6/037053, WO201 4/1 36086, and WO201 1/076807, each of which is hereby incorporated by reference in its entirety. In a certain aspect, examples of stealth lipids include compounds of formula (XI), as described in WO201 1/076807, and compounds listed in Table 1 of WO20 16/0 10840. In particular aspects, other stealth lipids suitable for use in a lipid composition described herein and information about the biochemistry of such lipids can be found in Romberg et al., Pharmaceutical Research, Vol. 25, No. 1, 2008, p.55-71 and Hoekstra et al., Biochimica et Biophysica Acta 1660 (2004) 41-52.

[00250]   In one aspect, a suitable stealth lipid comprises a group selected from PEG (sometimes referred to as poly(ethylene oxide) and polymers based on poly(oxazoline), polyvinyl alcohol), poly(glycerol), poly(N-vinylpyrrolidone), polyaminoacids and poly [N-(2-hydroxypropyl) methacrylamide], and additional suitable PEG lipids disclosed, e.g., in WO 2006/007712.

[00251]   In specific aspects, non-limiting examples of suitable stealth lipids include polyethyleneglycol-diacylglycerol or polyethyleneglycol-diacylglycamide (PEG-DAG) conjugates including those comprising a dialkylglycerol or dialkylglycamide group having alkyl chain length independently comprising from about C_4 to about C_{40} saturated or unsaturated carbon atoms. In further aspects, the dialkylglycerol or dialkylglycamide group can further comprise one or more substituted alkyl groups. In further aspects described herein, a PEG conjugate can be selected from PEG-dilaurylglycerol, PEG-dimyristylglycerol (PEG-DMG) (catalog # GM-020 from NOF, Tokyo, Japan), PEG-dipalmitoylglycerol, PEG-disterylglycerol, PEG-dilaurylglycamide, PEG-dimyristylglycamide, PEG-dipalmitoylglycamide, and PEG-disterylglycamide, PEG-cholesterol (I-[8'-(Cholest-5-en-3[beta]-oxy)carboxamido-3',6'-dioxaoctanyl]   carbamoyl- [omega] -methyl - poly(ethylene glycol), PEG-DMB (3,4-Ditetradecoxylbenzyl-[omega]-methyl-poly(ethylene glycol) ether), l,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-   [methoxy(polyethylene glycol)-2000] (catalog # 8801 50P from Avanti Polar Lipids, Alabaster, Alabama, USA). In one aspect, the stealth lipid is S010, S024, S027, S031 , or S033 (as described in WO201 6/037053, e.g., Table 1). In another aspect, the stealth lipid is S024 (as described in WO201 6/037053, e.g., Table 1).

III. Composition of the mRNA molecule

[00252]   In a specific embodiment, the polynucleotide to be used for the immunization methods of the invention is polyribonucleotide-based such as mRNA-based. This mRNA molecule should be able to directly encode and facilitate translation of the target protein(s) against which an antibody response is desired. As such, the molecule should contain several components. In specific aspects, the first component is the open reading frame corresponding to the amino acid sequence of a protein(s), for example, a human target protein or fragment thereof. The native codon sequence may be used or, alternatively, codon optimization may be performed for the host species (such as mouse or rabbit) to increase translational efficiency and ultimately protein expression levels of the target. Additional modifications may be made to the open reading frame to enhance protein expression/trafficking. For secreted or membrane proteins, this may include the use of heterologous signal peptides such as the secretion signal from interleukin 2 (IL-2). In a specific example for secreted proteins, an mRNA molecule may include a heterologous signal peptide, such as the signal peptide of human IL-2 or IgG kappa.

[00253]   In specific aspects, a second component is a consensus Kozak sequence. An exemplary Kozak DNA sequence is provided: GCCACCATG (SEQ ID NO: 1), wherein the nucleotides ATG represent the initiator methionine. An exemplary Kozak RNA sequence is provided: GCCACCAUG (SEQ ID NO: 11), wherein the nucleotides AUG represent the initiator methionine. Other non-limiting examples of a Kozak sequence include, as encoded by either RNA or DNA: (GCC)GCCRCCAUGG (SEQ ID NO: 12), AGNNAUGN (SEQ ID NO: 13), ANNAUGG (SEQ IDNO: 23), ACCAUGG (SEQ ID NO: 24), GACACCAUGG (SEQ ID NO: 25), GCCRCCATGG (SEQ ID NO: 57), CAAACATG (SEQ ID NO: 58), AAAAAATGTCT (SEQ ID NO: 28), AAAAAAATG RNA (SEQ ID NO: 29), NTAAAAATG RCT (SEQ ID NO: 30), TAAAAAATGAAN (SEQ ID NO: 31), GNCAAAATGG (SEQ ID NO: 32), NNNANNATGNC (SEQ ID NO: 33), and AACAATGGC (SEQ ID NO: 34), where "N" denotes any nucleotide (e.g., A, G, C or T in the context of DNA and A, G, C, or U in the context of RNA), and "R" denotes A or G. It is widely known that the inclusion of a Kozak sequence 5' of the open reading frame enhances translation in a eukaryotic host.

[00254]   In specific aspects, a third component is a 7-methylguanosine cap on the 5' end of an mRNA. This cap is essential for the recruitment of eukaryotic initiation factor eIF4E and assembly of a mature ribosome. The methylguanosine cap can be added enzymatically or chemically following generation of the mRNA transcript.

[00255]   In specific aspects, a fourth component is a polyadenosine (polyA) tail found at the 3' terminus of an mRNA transcript. A polyA tract is known to prolong the half-life of an mRNA in cells as well as to promote efficient ribosome assembly and protein translation. In a specific embodiment, an mRNA for the compositions and methods described herein comprises a polyA tail

of 120 nucleotides (SEQ ID NO: 59). In certain embodiments, an mRNA for the compositions and methods described herein comprises a polyA tail having 60-120 nucleotides. In particular embodiments, an mRNA for the compositions and methods described herein comprises a polyA tail of 60 nucleotides, 70 nucleotides, 80 nucleotides, 90 nucleotides, 100 nucleotides, 110 nucleotides, or 120 nucleotides. Inclusion of the polyA tract may be done through in vitro transcription or by enzymatic polyadenylation using poly(A) polymerase.

[00256]   In specific aspects, a fifth component of an mRNA molecule is the inclusion of 5'- and 3'-untranslated regions (UTRs). In specific embodiments, the 5' UTR is derived from tobacco etch virus and the 3' UTR is a tandem repeat of the 3' UTR found in human β-globin. It is widely accepted that the presence of UTRs can enhance the translation of a mRNA as well as increase its half-life within a cell (see, e.g., R.L. Tanguay and D.R. Gallie Molecular and Cellular Biology 1996 vol 16 no1 pp 146-156).

[00257]   Sufficient quantities of such RNA molecules may be obtained using in vitro transcription, followed by RNA purification. The technique of transcribing cloned DNA sequences in vitro using DNA-dependent RNA polymerases is well-known in the art (for example, see Sambrook et al., *Molecular Cloning: A Laboratory Manual, 2nd Edition,* Cold Spring Harbor Laboratory Press, 1989). Either naturally occurring ribonucleotides, such as uracil, guanine, cytosine, adenine, pseudouracil, or modified ribonucleotides may be used for mRNA synthesis so long as they still support appropriate codon recognition and protein translation. In this invention, guanine, cytosine, adenine, and pseudouracil were used for mRNA synthesis. In specific aspects, the use of pseudouracil instead of uracil permits more accurate estimates of mRNA size during quality control assessments on a BioAnalyzer (as described below).

IV. Use of the Lipid/Polynucleotide Complex

[00258]   According to the present disclosure, in specific aspects, the lipid/polynucleotide complex is used to carry out an *in vivo* transfection. Transfected cells express the protein encoded by the polynucleotide (e.g., polyribonucleotide such as mRNA), and may express or present the foreign protein, for example, on the cell surface. As a result, the host animal (e.g., non-human host animal) mounts an immune response to the foreign protein, or immunogen.

[00259]   Synthetic mRNA is transcribed *in vitro* using plasmid DNA template, rNTPs and T7 RNA polymerase. A 7-methylguanosine cap structure (Cap1) is enzymatically added to 5' end of mRNA to promote efficient translation. Capped mRNA is formulated into cationic lipid nanoparticles (LNPs) to protect mRNA from degradation and enhance cytoplasmic delivery. mRNA LNPs are stable at 4°C for 3-4 months and are ready to use for immunization.

In specific aspects, cationic lipid-polynucleotide complexes are formed by mixing a cationic lipid solution with an equal volume of polynucleotide solution. The cationic lipid and polynucleotides can be dissolved in any sterile physiologically-compatible aqueous carrier. In specific embodiments, cationic lipid and polynucleotides are dissolved in sterile saline (150 mM NaCl). The solutions are mixed at ambient temperatures. In certain embodiments, the solutions are mixed at 25°C. After mixing, the cationic lipid-polynucleotide complexes are incubated at room temperature, for example, for 15 to 45 minutes.

[00260]   Administration of lipid/polynucleotide complexes of the methods described herein may be by parenteral, intravenous, intramuscular, subcutaneous, intranasal, or any other suitable means. In mice, intravenous administration of mRNA LNPs has been found to be superior to subcutaneous delivery (see Figure 1B). The specific dosage administered may be dependent upon the age, weight, kind of current treatment, if any, and nature of the immunogen which will be expressed. The initial dose may be followed by booster dosages to enhance the immunogenic response. Immunization with mRNA LNPs can also be alternated with other immunogen formats (see Figure 1C).

[00261]   Because immunization generates the production of immunogen-specific antibodies in the host, the present disclosure is also directed to methods of producing immunogen-specific antibodies. Polyclonal antibodies may be isolated and purified from host animals using procedures well-known in the art (for example, see Harlow et al., *Antibodies: A Laboratory Manual,* Cold Spring Harbor Laboratory Press, 1988).

[00262]   This disclosure is also directed to the use of mRNA LNP-based immunization to produce monoclonal antibodies. According to this method, non-human animals (e.g., mice) are injected with a lipid/ mRNA complex, and antibody-producing cells (e.g., B-lymphocytes or splenocytes) are isolated from the immunized animal (e.g., mice). Monoclonal antibodies are produced by any method known in the art, for example, following the procedure of Kohler and Milstein *(Nature* 256:495-497 (1975) (for example, see Harlow et al., supra). Briefly, monoclonal antibodies can be produced by immunizing animals (e.g., mice) with a cationic lipid-mRNA complex, verifying the presence of antibody production by removing a serum sample, removing the spleen to obtain B-lymphocytes, fusing the B-lymphocytes with myeloma cells to produce hybridomas, cloning the hybridomas, selecting positive clones which produce anti-immunogen antibody, culturing the anti-immunogen antibody-producing clones, and isolating anti-immunogen antibodies from the hybridoma cultures.

V. RNA Modifications

[00263]   Polyribonucleotides such as mRNA for the compositions and methods described herein can include modifications to prevent rapid degradation by endo- and exo-nucleases and to avoid or reduce the cell's innate immune or interferon response to the RNA. Modifications include, but are not limited to, for example, (a) end modifications, e.g., 5' end modifications (phosphorylation, dephosphorylation, conjugation, inverted linkages, etc.), 3' end modifications (conjugation, DNA nucleotides, inverted linkages, etc.), (b) base modifications, e.g., replacement with modified bases, stabilizing bases, destabilizing bases, or bases that base pair with an expanded repertoire of partners, or conjugated bases, (c) sugar modifications (e.g., at the 2' position or 4' position) or replacement of the sugar, as well as (d) internucleoside linkage modifications, including modification or replacement of the phosphodiester linkages.

[00264]   In specific aspects, polyribonucleotides such as mRNA described herein can further comprise a 5' cap. In some embodiments of the aspects described herein, the modified synthetic mRNA comprises a 5' cap comprising a modified guanine nucleotide that is linked to the 5' end of an RNA molecule using a 5'-5' triphosphate linkage. The term "5' cap" is also intended to encompass other 5' cap analogs including, e.g., 5' diguanosine cap, tetraphosphate cap analogs having a methylene-bis(phosphonate)   moiety (see e.g., Rydzik, A M et al. (2009) Org Biomol Chem 7(22):4763-76), dinucleotide cap analogs having a phosphorothioate modification (see e.g., Kowalska, J. et al. (2008) RNA 14(6):1 119-1 131), cap analogs having a sulfur substitution for a non-bridging oxygen (see e.g., Grudzien-Nogalska, E. et al., (2007) RNA 13(10): 1745-1755), N7-benzylated dinucleoside tetraphosphate analogs (see e.g., Grudzien, E. et al. (2004) RNA 10(9):1479-1487), or anti-reverse cap analogs (see e.g., Jemielity, J. et al., (2003) RNA 9(9): 1108-1 122 and Stepinski, J. et al. (2001) RNA 7(10):1486-1495). In one such embodiment, the 5' cap analog is a 5' diguanosine cap. In some embodiments, the modified synthetic mRNA of the invention does not comprise a 5' triphosphate.

[00265]   The 5' cap is important for recognition and attachment of an mRNA to a ribosome to initiate translation. The 5' cap also protects modified synthetic mRNA described herein from 5' exonuclease mediated degradation.

[00266]   Polyribonucleotides such as mRNA described herein can further comprise a 5' and/or 3' untranslated region (UTR). Untranslated regions are regions of the RNA before the start codon (5') and after the stop codon (3'), and are therefore not translated by the translation machinery. Modification of an RNA molecule with one or more untranslated regions can improve the stability of an mRNA, since the untranslated regions can interfere with ribonucleases and other proteins involved in RNA degradation. In addition, modification of an RNA with a 5' and/or 3' untranslated region can enhance translational efficiency by binding proteins that alter ribosome binding to an mRNA. Modification of an RNA with a 3' UTR can be used to maintain a cytoplasmic localization

of the RNA, permitting translation to occur in the cytoplasm of the cell. In one embodiment, the modified synthetic mRNA of the invention does not comprise a 5' or 3' UTR. In another embodiment, the modified synthetic mRNA of the invention comprises either a 5' or 3' UTR. In another embodiment, the modified synthetic mRNA of the invention comprises both a 5' and a 3' UTR. In one embodiment, the 5' and/or 3' UTR is selected from an mRNA known to have high stability in the cell (e.g., a murine alpha-globin 3' UTR). In some embodiments, the 5' UTR, the 3' UTR, or both comprise one or more modified nucleosides.

[00267]   In some embodiments, polyribonucleotides such as mRNA described herein further comprise a Kozak sequence. The "Kozak sequence" refers to a sequence on eukaryotic mRNA having the consensus (gcc)gccRccAUGG (SEQ ID NO: 12), where R is a purine (adenine or guanine) three bases upstream of the start codon (AUG), which is followed by another 'G.' The Kozak consensus sequence is recognized by the ribosome to initiate translation of a polypeptide. Typically, initiation occurs at the first AUG codon encountered by the translation machinery that is proximal to the 5' end of the transcript. However, in some cases, this AUG codon can be bypassed in a process called leaky scanning. The presence of a Kozak sequence near the AUG codon will strengthen that codon as the initiating site of translation, such that translation of the correct polypeptide occurs. Furthermore, addition of a Kozak sequence to a modified synthetic mRNA described herein can promote more efficient translation, even if there is no ambiguity regarding the start codon. Thus, in some embodiments, the modified synthetic mRNA described herein further comprise a Kozak consensus sequence at the desired site for initiation of translation to produce the correct length polypeptide. In some such embodiments, the Kozak sequence comprises one or more modified nucleosides.

[00268]   In some embodiments, modified synthetic mRNA described herein further comprise a "poly (A) tail", which refers to a 3' homopolymeric tail of adenine nucleotides, which can vary in length (e.g., at least 5 adenine nucleotides) and can be up to several hundred adenine nucleotides). The inclusion of a 3' poly(A) tail can protect the modified synthetic mRNA of the invention from degradation in the cell, and also facilitates extra-nuclear localization to enhance translation efficiency. In some embodiments, the poly(A) tail comprises between 1 and 500 adenine nucleotides (SEQ ID NO: 60); in other embodiments the poly(A) tail comprises at least 5 adenine nucleotides or more. In one embodiment, the poly(A) tail comprises between 1 and 150 adenine nucleotides. In one embodiment, the poly(A) tail comprises between 60 and 120 adenine nucleotides. In another embodiment, the poly(A) tail comprises between 90 and 120 adenine nucleotides. In some such embodiments, the poly(A) tail comprises one or more modified nucleosides.

[00269]   The following are representative examples of target protein antigens that are amenable to production and expression according to the mRNA immunization methods provided herein. Generation of their mRNAs and immunization of host animals with the same demonstrate proof of concept for said methods, as described herein.

I.   RXFP1

[00270]   RXFP1 , or relaxin/insulin-like family peptide receptor 1, is a 757 amino acid class A G protein coupled receptor (GPCR) which contains a leucine-rich repeat N-terminal extracellular domain. Phylogenetically, it is a part of the same receptor subfamily which includes follicle stimulating hormone, luteinizing hormone, and thyroid stimulating hormone receptors. The endogenous ligand of RXFP1 is the protein hormone relaxin. RXFP1 and its ligand have been implicated in the control of menstruation and some of the physiological responses associated with pregnancy and parturition. In patients suffering from acute decompensated heart failure, a phase III clinical trial (RELAX-AHF) has shown that 48 h of recombinant relaxin infusion during hospitalization significantly reduced 6 month mortality.

[00271]   Establishing cell lines with high levels of RXFP1 expression is difficult due to cytotoxicity. Like many GPCRs, expression of purified, full length recombinant protein is also technically prohibitive.

[00272]   For the generation of human RXFP1 mRNA, the native human nucleotide sequence for the RXFP1 open reading frame (e.g., accession numbers NM_021634.3/NP_067647.2)   was subjected to codon optimization using GeneArt $^{®}$'s codon optimization algorithm for mice (see Table 1). In addition to changing codon sequences on the basis of mouse biases, sequences were altered to remove BamHI, RsrII, and BspQI restriction sites as these would be employed for subsequent subcloning and mRNA synthesis.

TABLE 1 Exemplary RXFP1 Polynucleotide and Polypeptide Sequences	
SEQ ID NO: and features	Sequence
SEQ ID NO: 1 Consensus Kozak sequence (DNA)	GCCACCATG
SEQ ID NO: 11 Consensus Kozak sequence (RNA)	GCCACCAUG U= Uridine and/or pseudouridine
SEQ ID NO: 35 RXFP1 native DNA sequence corresponding to	GTGCGTGTGTGTAAAGAAGGAGATTAGGACATTTAGAGAAGGAGGGCGGGGAGGAGA GATCCTGAGAATAGAAAGGAGGAAAGAAAAAAAGAGGAATGGAAAGAGACAGAGAAA GGAAATGGGAGTGGAAGGAGGGAGGACTGCTTTGTAACTGCTAAGATTGCAGACAGAA ATAGCACACAACCACTGTGAGCTGTATGCGATTCAGAAACCAAGACCAAATΓΓΓGCTCAC

SEQ ID NO: and features	Sequence
Protein Accession # NP_067647.2	TTTCATTAATCAGTTGCTCAGATAGAAGGAAATGACATCTGGTTCTGTCTTCTTCTACATC TTAATTTTTGGAAAATATTTTTTCTCATGGGGGTGGACAGGATGTCAAGTGCTCCCTTGGC TATTTCCCCTGTGGGAACATCACAAAGTGCTTGCCTCAGCTCCTGCACTGTAACGGTGTG GACGACTGCGGGAATCAGGCCGATGAGGACAACTGTGGAGACAACAATGGATGGTCTC TGCAATTTGACAAATATTTTGCCAGTTACTACAAAATGACTTCCCAATATCCTTTTGAGGC AGAAACACCTGAATGTTTGGTCGGTTCTGTGCCAGTGCAATGTCTTTGCCAAGGTCTGGA GCTTGACTGTGATGAAACCAATTTACGAGCTGTTCCATCGGTTTCTTCAAATGTGACTGCA ATGTCACTTCAGTGGAACTTAATAAGAAAGCTTCCTCCTGATTGCTTCAAGAATTATCATG ATCTTCAGAAGCTGTACCTGCAAAACAATAAGATTACATCCATCTCCATCTATGCTTTCAG AGGACTGAATAGCCTTACTAAACTGTATCTCAGTCATAACAGAATAACCTTCCTGAAGCC GGGTGTTTTTGAAGATCTTCACAGACTAGAATGGCTGATAATTGAAGATAATCACCTCAG TCGAATTTCCCCACCAACATTTTATGGACTAAATTCTCTTATTCTCTTAGTCCTGATGAATA ACGTCCTCACCCGTTACCTGATAAACCTCTCTGTCAACACATGCCAAGACTACATTGGCT GGACCTTGAAGGCAACCATATCCATAATTTAAGAAATTTGACTTTTATTTCCTGCAGTAAT TTAACTGTTTTTAGTGATGAGGAAAAACAAAATTAATCACTTAAATGAAAATACTTTTGCAC CTCTCCAGAAACTGGATGAATTGGATTTAGGAAGTAATAAGATTGAAAATCTTCCACCGC TTATATTCAAGGACCTGAAGGAGCTGTCACAATTGAATCTTTCCTATAATCCAATCCAGAA AATTCAAGCAAACCAATTTGATTATCTTGTCAAACTCAAGTCTCTCAGCCTAGAAGGGATT GAAATTTCAAATATCCAACAAAGGATGTTTAGACCTCTTATGAATCTCTCTCACATATATT TTAAGAAATTCCAGTACTGTGGGTATGCACCACATGTTCGCAGCTGTAAACCAAACACTG ATGGAATTTCATCTCTAGAGAATCTCTTGGCAAGCATTATTCAGAGAGTATTTGTCTGGG TTGTATCTGCAGTTACCTGCTTTGGAAACATTTTTGTCATTTGCATGCGACCTTATATCAG GTCTGAGAACAAGCTGTATGCCATGTCAATCATTTCTCTCTGCTGTGCCGACTGCTTAATG GGAATATATTTATTCGTGATCGGAGGCTTTGACCTAAAGTTTCGTGGAGAATACAATAAG CATGCGCAGCTGTGGATGGAGAGTACTCATTGTCAGCTTGTAGGATCTTTGGCCATTCTG TCCACAGAAGTATCAGTTTTTACTGTTAACATTTCTGACATTGGAAAAATACATCTGCATTG TCTATCCTTTTAGATGTGTGAGACCTGGAAAATGCAGAACAATTACAGTTCTGATTCTCAT TTGGATTACTGGTTTTTATAGTGGCTTTCATTCCATTGAGCAATAAGGAATTTTTTCAAAAAC TACTATGGCACCAATGGAGTATGCTTCCCTCTTCATTCAGAAGATACAGAAAGTATTGGA GCCCAGATTTATTCAGTGGCAATTTTTCTTGGTATTAATTTGGCCGCATTTATCATCATAG TTTTTTTCCTATGGAAGCATGTTTTTATAGTGTTCATCAAAGTGCCATAACAGCAACTGAAAT ACGGAATCAAGTTAAAAAAGAGATGATCCTTGCCAAACGTTTTTTTCTTTATAGTATTTACT GATGCATTATGCTGGATACCCATTTTTGTAGTGAAATTTCTTTCACTGCTTCAGGTAGAAA TACCAGGTACCATAACCTCTTGGGTAGTGATTTTTATTCTGCCCATTAACAGTGCTTTGAA CCCAATTCTCTATACTCTGACCACAAGACCATTTAAAGAAATGATTCATCGGTTTTGGTAT AACTACAGACAAAGAAAATCTATGGACAGCAAAGGTCAGAAAACATATGCTCCATCATT CATCTGGGTGGAAATGTGGCCACTGCAGGAGATGCCACCTGAGTTAATGAAGCCGGACC TTTTCACATACCCCTGTGAAATGTCACTGATTTCTCAATCAACGAGACTCAATTCCTATTCA TGACTGACTCTGAAATTCATTTCTTCGCAGAGAATACTGTGGGGGTGCTTCATGAGGGAT TTACTGGTATGAAATGAATACCACAAAATTAATTTATAATAATAGCTAAGATAAATATTTTT ACAAGGACATGAGGAAAAATAAAAATGACTAATGCTCTTACAAAGGGAAGTAATTATAT CAATAATGTATATATATTAGTAGACATTTTGCATAAGAAATTAAGAGAAATCTACTTCAGT AACATTCATTCATTTTTTCTAACATGCATTTATTGAGTACCCACTACTATGTGCATAGCATTG CAATATAGTCCTGGAAGTAGACAGTGCAGAACCTTTCAATCTGTAGATGGTGTTTAATGA CAAAAGACTATACAAAGTCCATCTGCAGTTCCTAGTTTAAAGTAGAGCTTTACCTGTCAT GTGCATCAGCAAGAATCATAGGCACTTTTAAATAAAGGTTTAAAGTTTTTGGAATACTCAG TGTATTTGCATCATAGAAAATGTCTGACTGTTTGCAAAATAATATTCTGTTTTTAAGAATCC ATCTTACCTCTCTTTAAGTTTCCATACACTTGAGAGCCAACACAACATATTTATTACTAAAA AGATGCTTTGCTAGAAACTCAAAAACAGCACTTCTTTTGGCACTTCCTGCCCAGTTTTCTC

SEQ_ID NO: and features	Sequence
	TTTGCTTTAAATGAACATCATCATATGGAATTGGAATAGGAGAGTATGAGTACGGCAGA GAAGTGGATCAGAAAAACTAGAATGAGGATAAACATTTACATTAGTGGAAACTCCTGAA ATAAATCCTTGTATTGTCAGTTAACTGATTTCAACAAGGATGCCAAGACAAAAAGGCTT TTCAACAAACCGTGCTGTTTTAAGAACAGACCTAAGTGGTTTAATTCACCCACTTTAGATG GGTGAATGTTATGGTGTGTGAAATATCTCAGTAAAGCAGTTAAAAGGAAAAAGAGCTGG AATGCACTGATTCAGGAACTTAATTTCAGGAAGGAAAGGTCTGTATGTACACATTTCACT TTAAGCAGAAAATCTTTCTTCAAGAAATGACTTTACTTTCTCTTTGCACTGCCAGCACGTG AGATACTAACTTTTTAACTAGTTGTTCTTCTCTAGTCTCTACGTTATTAGAATTTTTTGCTTT CATAATGTGAAACCTTTAAGCAGGAGAAGAAAATGTTTCAGATAGTTTCAAATACACCA AAAATGTTTGAAACACAAAAATACTGGAATCAAACCATAATGCACTTATTGAATATATAG TTGTATAGATTTGTTCTGAAAATAAATTATCTGAAATTTAACTATTAAAAAAAAAAAAAAA AAAAAAAAA
SEQ ID NO: 2 Native mRNA sequence corresponding to Protein Accession # NP_067647.2	GUGCGUGUGUGUAAAGAAGGAGAUUAGGACAUUUAGAGAAGGAGGG CGGGGAGGAGAGAUCCUGAGAAUAGAAAGGAGGAAAGAAAAAAAGA GGAAUGGAAAGAGACAGAGAAAGGAAAUGGGAGUGGAAGGAGGGAG GACUGCUUUGUAACUGCUAAGAUUGCAGACAGAAAUAGCACACAACC ACUGUGAGCUGUAUGCGAUUCAGAAACCAAGACCAAAUUUUGCUCA CUUUCAUUAAUCAGUUGCUCAGAUAGAAGGAAAUGACAUCUGGUUC UGUCUUCUUCUACAUCUUAAUUUUUGGAAAAUAUUUUUCUCAUGGG GGUGGACAGGAUGUCAAGUGCUCCCUUGGCUAUUUCCCCUGUGGG AACAUCACAAAGUGCUUGCCUCAGCUCCUGCACUGUAACGGUGUGG ACGACUGCGGGAAUCAGGCCGAUGAGGACAACUGUGGAGACAACAA UGGAUGGUCUCUGCAAUUUGACAAAUAUUUUGCCAGUUACUACAAAA UGACUUCCCAAUAUCCUUUUGAGGCAGAAACACCUGAAUGUUUGGU CGGUUCUGUGCCAGUGCAAUGUCUUUGCCAAGGUCUGGAGCUUGA CUGUGAUGAAACCAAUUUACGAGCUGUUCCAUCGGUUUCUUCAAAU GUGACUGCAAUGUCACUUCAGUGGAACUUAAUAAGAAAGCUUCCUC CUGAUUGCUUCAAGAAUUAUCAUGAUCUUCAGAAGCUGUACCUGCA AAACAAUAAGAUUACAUCCAUCUCCAUCUAUGCUUUCAGAGGACUGA AUAGCCUUACUAAACUGUAUCUCAGUCAUAACAGAAUAACCUUCCUG AAGCCGGGUGUUUUUGAAGAUCUUCACAGACUAGAAUGGCUGAUAA UUGAAGAUAAUCACCUCAGUCGAAUUUCCCCACCAACAUUUUAUGGA CUAAAUUCUCUUAUUCUCUUAGUCCUGAUGAAUAACGUCCUCACCC GUUUACCUGAUAAACCUCUCUGUCAACACAUGCCAAGACUACAUUGG CUGGACCUUGAAGGCAACCAUAUCCAUAAUUUAAGAAAUUUGACUUU UAUUUCCUGCAGUAAU UUAAC UGUUUUAGUGAU GAG GAAAAAC AAAA UUAAUCACUUAAAUGAAAAUACUUUUGCACCUCUCCAGAAACUGGAU GAAUUGGAUUUAGGAAGUAAUAAGAUUGAAAAUCUUCCACCGCUUAU AUUCAAGGACCUGAAGGAGCUGUCACAAUUGAAUCUUUCCUAUAAUC CAAUCCAGAAAAUUCAAGCAAACCAAUUUGAUUAUCUUGUCAAACUC AAGUCUCUCAGCCUAGAAGGGAUUGAAAUUUCAAAUAUCCAACAAAG GAUGUUUAGACCUCUUAUGAAUCUCUCUCACAUAUAUUUUAAGAAAU UCCAGUACUGUGGGUAUGCACCACAUGUUCGCAGCUGUAAACCAAA CACUGAUGGAAUUUCAUCUCUAGAGAAUCUCUUGGCAAGCAUUAUU CAGAGAGUAUUUGUCUGGGUUGUAUCUGCAGUUACCUGCUUUGGAA ACAUUUUUGUCAUUUGCAUGCGACCUUAUAUCAGGUCUGAGAACAA GCUGUAUGCCAUGUCAAUCAUUUCUCUCUGCUGUGCCGACUGCUUA AUGGGAAUAUAUUUAUUCGUGAUCGGAGGCUUUGACCUAAAGUUUC GUGGAGAAUACAAUAAGCAUGCGCAGCUGUGGAUGGAGAGUACUCA UUGUCAGCUUGUAGGAUCUUUGGCCAUUCUGUCCACAGAAGUAUCA GUUUUACUGUUAACAUUUCUGACAUUGGAAAAAUACAUCUGCAUUGU CUAUCCUUUUAGAUGUGUGAGACCUGGAAAAAUGCAGAACAAUUACA

	TABLE 1 Exemplary RXFP1 Polynucleotide and Polypeptide Sequences
SEQ ID NO: and features	Sequence
	GUUCUGAUUCUCAUUUGGAUUACUGGUUUUAUAGUGGCUUUCAUUC CAUUGAGCAAUAAGGAAUUUUUCAAAAACUACUAUGGCACCAAUGGA GUAUGCUUCCCUCUUCAUUCAGAAGAUACAGAAAGUAUUGGAGCCC AGAUUUAUUCAGUGGCAAUUUUUCUUGGUAUUAAUUUGGCCGCAUU UAUCAUCAUAGUUUUUUCCUAUGGAAGCAUGUUUUAUAGUGUUCAU CAAAGUGCCAUAACAGCAACUGAAAUACGGAAUCAAGUUAAAAAAGA GAUGAUCCUUGCCAAACGUUUUUUCUUUAUAGUAUUUACUGAUGCA UUAUGCUGGAUACCCAUUUUUGUAGUGAAAUUUCUUUCACUGCUUC AGGUAGAAAUACCAGGUACCAUAACCUCUUGGGUAGUGAUUUUUAU UCUGCCCAUUAACAGUGCUUUGAACCCAAUUCUCUAUACUCUGACCA CAAGACCAUUUAAAGAAAUGAUUCAUCGGUUUUGGUAUAACUACAGA CAAAGAAAUCUAUGGACAGCAAAGGUCAGAAACAUAUGCUCCAUC AUUCAUCUGGGUGGAAAUGUGGCCACUGCAGGAGAUGCCACCUGAG UUAAUGAAGCCGGACCUUUUCACAUACCCCUGUGAAAUGUCACUGA UUUCUCAAUCAACGAGACUCAAUUCCUAUUCAUGACUGACUCUGAAA UUCAUUUCUUCGCAGAGAAUACUGUGGGGGUGCUUCAUGAGGGAUU UACUGGUAUGAAAUGAAUACCACAAAAUUAAUUUAUAAUAAUAGCUA AGAUAAAUAUUUACAAGGACAUGAGGAAAAAUAAAAAUGACUAAUG CUCUUACAAAGGGAAGUAAUUAUAUCAAUAAUGUAUAUAUAUUAGUA GACAUUUUGCAUAAGAAAUUAAGAGAAAUCUACUUCAGUAACAUUCA UUCAUUUUUCUAACAUGCAUUUAUUGAGUACCCACUACUAUGUGCAU AGCAUUGCAAUAUAGUCCUGGAAGUAGACAGUGCAGAACCUUUCAA UCUGUAGAUGGUGUUUAAUGACAAAGACUAUACAAAGUCCAUCUGC AGUUCCUAGUUUAAAGUAGAGCUUUACCUGUCAUGUGCAUCAGCAA GAAUCAUAGGCACUUUUAAAUAAAGGUUUAAAGUUUUGGAAUACUCA GUGUAUUUGCAUCAUAGAAAAUGUCUGACUGUUUGCAAAAUAAUAUU CUGUUUUUAAGAAUCCAUCUUACCUCUCUUUAAGUUUCCAUACACUUG AGAGCCAACACAACAUAUUUAUUACUAAAAAGAUGCUUUGCUAGAAA CUCAAAAACAGCACUUCUUUUGGCACUUCCUGCCCAGUUUUCUCUU UGCUUUAAAUGAACAUCAUCAUAUGGAAUUGGAAUAGGAGAGUAUGA GUACGGCAGAGAAGUGGAUCAGAAAACUAGAAUGAGGAUAAACAUU UACAUUAGUGGAAACUCCUGAAAUAAAUCCUUGUAUUGUCAGUUAAC UGAUUUUCAACAAGGAUGCCAAGACAAAAAGGCUUUUCAACAAACCG UGCUGUUUUAAGAACAGACCUAAGUGGUUUAAUUCACCCACUUUAG AUGGGUGAAUGUUAUGGUGUGUGAAAUAUCUCAGUAAAGCAGUUAA AAGGAAAAAGAGCUGGAAUGCACUGAUUCAGGAACUUAAUUUCAGGA AGGAAAGGUCUGUAUGUACACAUUUCACUUUAAGCAGAAAAUCUUUC UUCAAGAAAUGACUUUACUUUCUCUUUGCACUGCCAGCACGUGAGA UACUAACUUUUUAACUAGUUGUUCUUCUCUAGUCUCUACGUUAUUA GAAUUUUUUGCUUUCAUAAUGUGAAACCUUUAAGCAGGAGAAGAAA UGUUUUCAGAUAGUUUCAAAUACACCAAAAAUGUUUGAAACACAAAA AUACUGGAAUCAAACCAUAAUGCACUUAUUGAAUAUAUAGUUGUAUA GAUUUGUUCUGAAAAUAAAUUAUCUGAAAUUUAACUAUUAAAAAAAAA AAAAAAAAAAAAAAAA U= Uridine and/or pseudouridine
SEQ ID NO: 3 Translated human RXFP1 from coding sequence (CDS) of the DNA construct of SEQ ID NO: 2	MTSGSVFFYILIFGKYFSHGGGQDVKCSLGYFPCGN ITKCLPQLLHCNGV DDCGNQADEDNCGDNNGWSLQFDKYFASYYKMTSQYPFEAETPECLVG SVPVQCLCQGLELDCDETNLRAVPSVSSNVTAMSLQWNLIRKLPPDCFK NYHDLQKLYLQNNKITSISIYAFRGLNSLTKLYLSHNRITFLKPGVFEDLHRL EWLIIEDNHLSRISPPTFYGLNSLILLVLMNNVLTRLPDKPLCQHMPRLHWL DLEGNH IHNLRNLTFISCSNLTVLVMRKNKINHLNENTFAPLQKLDELDLGS

SEQ ID NO: and features	Sequence
	NKIENLPPLIFKDLKELSQLNLSYNPIQKIQANQFDYLVKLKSLSLEGIEISNI QQRMFRPLMNLSHIYFKKFQYCGYAPHVRSCKPNTDGISSLENLLASIIQR VFVWVVSAVTCFGN IFVICMRPYIRSENKLYAMSI ISLCCADCLMGIYLFVIG GFDLKFRGEYNKHAQLWMESTHCQLVGSLAILSTEVSVLLLTFLTLEKYICI VYPFRCVRPGKCRTITVLILIWITGFIVAFIPLSNKEFFKNYYGTNGVCFPLH SEDTESIGAQIYSVAIFLGINLAAFI IIVFSYGSMFYSVHQSAITATEIRNQVK KEMILAKRFFFIVFTDALCWIPIFVVKFLSLLQVEIPGTITSWVVIFILPINSAL NPILYTLTTRPFKEMIHRFWYNYRQRKSMDSKGQKTYAPSFIWVEMWPL QEMPPELMKPDLFTYPCEMSLISQSTRLNSYS
SEQ ID NO: 36 (DNA) TEV-hRXFP1 - 2xhBG-1 20A Sequence features: Tobacco Etch Virus (TEV) 5' UTR: 37-1 90 Optimal Kozak sequence: 191-199 Human RXFP1 codon optimized, encoding amino acids Accession # NP_067647.2: 197-2467 2 stop codons: 2468-2473 2 copies of human beta-globin 3'UTR: 2492-2756 120 nucleotide polyA tail (SEQ ID NO: 59): 2764-2883	GGAGGCCGGAGAATTGTAATACGACTCACTATAGGGAGACGCGTGTTAAATAA CAAATCTCAACACAACATATACAAAACAAACGAATCTCAAGCAATCAAGCATTCT ACTTCTATTGCAGCAATTTAAATCATTTCTTTTAAAGCAAAAGCAATTTTCTGAAA ATTTTTCACCATTTACGAACGATAGCCGCCACCATGACAAGCGGCAGCGTGTTCTT CTACATCCTGATCTTCGGCAAGTACTTCAGCCACGGCGGAGGCCAGGACGTGAA GTGTAGCCTGGGCTACTTCCCCTGCGGCAACATCACCAAGTGCCTGCCCCAGCT GCTGCACTGCAACGGCGTGGACGATTGCGGCAACCAGGCCGACGAGGACAACT GCGGCGACAACAATGGCTGGTCCCTGCAGTTCGATAAGTACTTCGCCTCCTACT ACAAGATGACCAGCCAGTACCCCTTCGAGGCCGAGACACCTGAGTGCCTCGTGG GCTCTGTGCCTGTGCAGTGTCTGTGCCAGGGCCTGGAACTGGACTGCGACGAG ACAAACCTGAGAGCCGTGCCCAGCGTGTCCAGCAACGTGACAGCCATGAGCCT GCAGTGGAACCTGATCCGGAAGCTGCCCCCCGACTGCTTCAAGAACTACCACGA CCTGCAGAAGCTGTATCTGCAGAACAACAAGATCACCTCCATCAGCATCTACGCC TTCCGGGGCCTGAACAGCCTGACCAAGCTGTACCTGAGCCACAACCGGATCACC TTTCTGAAGCCCGGCGTGTTCGAGGACCTGCACAGACTGGAATGGCTGATCATC GAGGACAATCACCTGAGCCGGATCAGCCCCCCCACCTTCTACGGCCTGAACTCC CTGATCCTGCTGGTGCTGATGAACAACGTGCTGACCCGGCTGCCCGACAAGCCC CTGTGTCAGCACATGCCCAGACTGCACTGGCTGGACCTGGAAGGCAACCACATC CACAACCTGCGGAACCTGACCTTCATCAGCTGCAGCAACCTGACCGTGCTCGTG ATGCGGAAGAACAAGATTAACCACCTGAACGAGAACACCTTCGCCCCCCTGCAG AAACTGGACGAGCTGGATCTGGGCTCTAACAAGATCGAGAACCTGCCCCCTCTG ATCTTCAAGGACCTGAAAGAGCTGAGCCAGCTGAACCTGTCCTACAACCCCATC CAGAAGATCCAGGCCAACCAGTTCGACTACCTCGTGAAGCTGAAGTCCCTGTCC CTGGAAGGGATCGAGATCAGCAACATCCAGCAGCGGATGTTCCGGCCCCTGAT GAATCTGTCCCACATCTACTTCAAGAAGTTCCAGTACTGCGGCTACGCCCCCCAC GTGCGGAGCTGCAAGCCTAACACAGACGGCATCAGCAGCCTGGAAAACCTGCT GGCCTCCATCATCCAGCGGGTGTTCGTGTGGGTGGTGTCCGCCGTGACCTGCTT CGGCAATATCTTCGTGATCTGCATGCGGCCCTACATTCGGAGCGAGAACAAGCT GTATGCCATGAGCATCATCTCCCTGTGCTGCGCCGACTGCCTGATGGGCATCTAC CTGTTCGTGATCGGCGGCTTCGACCTGAAGTTCCGGGGCGAGTACAACAAGCAC GCCCAGCTGTGGATGGAAAGCACCCACTGCCAGCTCGTGGGCAGCCTGGCCAT CCTGAGCACTGAAGTGTCCGTGCTGCTGCTGACCTTCCTGACCCTGGAAAAGTA CATCTGCATCGTGTACCCTTTCAGATGCGTGCGGCCTGGCAAGTGCCGGACCAT CACAGTGCTGATCCTGATTTGGATCACCGGCTTCATCGTGGCCTTCATCCCCCTG AGCAACAAAGAGTTCTTCAAGAATTACTACGGCACCAATGGCGTGTGCTTCCCA

SEQ ID NO: and features	Sequence
	CTGCACTCCGAGGACACAGAGAGCATCGGCGCCCAGATCTACAGCGTGGCCAT CTTCCTGGGCATCAATCTGGCCGCCTTCATCATCATCGTGTTCAGCTACGGCTCC ATGTTCTACTCCGTGCACCAGAGCGCCATCACCGCCACCGAGATCCGGAACCAA GTGAAGAAAGAGATGATCCTGGCCAAGCGCTTCTTCTTCATTGTGTTCACCGAC GCCCTGTGTTGGATTCCAATCTTCGTCGTGAAGTTCCTGAGCCTGCTGCAGGTG GAAATCCCCGGCACAATCACCAGCTGGGTCGTGATCTTCATCCTGCCCATCAACA GCGCCCTGAACCCTATCCTGTACACCCTGACCACCCGGCCCTTCAAAGAAATGAT CCACCGGTTCTGGTACAACTACCGGCAGAGAAAGAGCATGGACAGCAAGGGCC AGAAAACCTACGCCCCTAGCTTCATCTGGGTGGAAATGTGGCCACTGCAGGAAA TGCCTCCCGAACTGATGAAGCCCGACCTGTTCACCTACCCCTGCGAGATGAGCCT GATCTCCCAGAGCACCCGGCTGAACAGCTACTCCTGATAACGGACCGGCGATAG ATGAAGCTCGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAG TCCAACTACTAAACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCTGC CTAATAAAAAACATTTATTTTCATTGCAGCTCGCTTTCTTGCTGTCCAATTTCTATT AAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAAACTGGGGGATATTATGAAGG GCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTATTTTCATTGCGGCCGC AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAA
SEQ ID NO: 4 (mRNA) TEV-hRXFP1 - 2xhBG-1 20A Sequence features: Tobacco Etch Virus (TEV) 5' UTR: 37-1 90 Optimal Kozak sequence: 191-199 Human RXFP1 codon optimized, encoding amino acids Accession # NP_067647.2: 197- 2467 2 stop codons: 2468-2473 2 copies of human beta-globin 3'UTR: 2492-2756 120 nucleotide polyA tail (SEQ ID NO: 59): 2764-2883	GGAGGCCGGAGAAU UGUAAUACGACUCACUAUAGGGAGACGCGUGU UAAA UAACAAAUCUCAACACAACAUAUACAAAACAAACGAAUCUCAAGCAAUCAAG CAU UCUACU UCUAUUGCAGCAAUU UAAAUCAU UUCU UU UAAAGCAAAAGCA AUU UUCUGAAAAUU UUCACCAUU UACGAACGAUAGCCGCCACCAUGACAAG CGGCAGCGUGUUCUUCUACAUCCUGAUCUUCGGCAAGUACUUCAGCCACGG CGGAGGCCAGGACGUGAAGUGUAGCCUGGGCUACUUCCCCUGCGGCAACAU CACCAAGUGCCUGCCCCAGCUGCUGCACUGCAACGGCGUGGACGAUUGCGG CAACCAGGCCGACGAGGACAACUGCGGCGACAACAAUGGCUGGUCCCUGCAG UUCGAUAAGUACU UCGCCUCCUACUACAAGAUGACCAGCCAGUACCCCUUCG AGGCCGAGACACCUGAGUGCCUCGUGGGCUCUGUGCCUGUGCAGUGUCUG UGCCAGGGCCUGGAACUGGACUGCGACGAGACAAACCUGAGAGCCGUGCCC AGCGUGUCCAGCAACGUGACAGCCAUGAGCCUGCAGUGGAACCUGAUCCGG AAGCUGCCCCCCGACUGCU UCAAGAACUACCACGACCUGCAGAAGCUGUAUC UGCAGAACAACAAGAUCACCUCCAUCAGCAUCUACGCCUUCCGGGGGCCUGAA CAGCCUGACCAAGCUGUACCUGAGCCACAACCGGAUCACCUUUCUGAAGCCC GGCGUGUUCGAGGACCUGCACAGACUGGAAUGGCUGAUCAUCGAGGACAA UCACCUGAGCCGGAUCAGCCCCCCCACCUUCUACGGCCUGAACUCCCUGAUC CUGCUGGUGCUGAUGAACAACGUGCUGACCCGGCUGCCCGACAAGCCCCUG UGUCAGCACAUGCCCAGACUGCACUGGCUGGACCUGGAAGGCAACCACAUCC ACAACCUGCGGAACCUGACCU UCAUCAGCUGCAGCAACCUGACCGUGCUCGU GAUGCGGAAGAACAAGAU UAACCACCUGAACGAGAACACCUUCGCCCCCCUG CAGAAACUGGACGAGCUGGAUCUGGGCUCUAACAAGAUCGAGAACCUGCCC CCUCUGAUCUUCAAGGACCUGAAAGAGCUGAGCCAGCUGAACCUG UCCUAC AACCCCAUCCAGAAGAUCCAGGCCAACCAGUUCGACUACCUCGUGAAGCUGA AGUCCCUGUCCCUGGAAGGGAUCGAGAUCAGCAACAUCCAGCAGCGGAUGU UCCGGCCCCUGAUGAAUCUGUCCCACAUCUACUUCAAGAAG UUCCAGUACU

SEQ ID NO: and features	Sequence
	GCGGCUACGCCCCCCACGUGCGGAGCUGCAAGCCUAACACAGACGGCAUCAG CAGCCUGGAAAACCUGCUGGCCUCCAUCAUCCAGCGGGUGUUCGUGUGGGU GGUGUCCGCCGUGACCUGCU UCGGCAAUAUCU UCGUGAUCUGCAUGCGGCC CUACAU UCGGAGCGAGAACAAGCUGUAUGCCAUGAGCAUCAUCUCCCUGUG CUGCGCCGACUGCCUGAUGGGCAUCUACCUGUUCGUGAUCGGCGGCUUCGA CCUGAAG UUCCGGGGGCGAG UACAACAAGCACGCCCAGCUGUGGAUGGAAAG CACCCACUGCCAGCUCGUGGGCAGCCUGGCCAUCCUGAGCACUGAAGUGUCC GUGCUGCUGCUGACCU UCCUGACCCUGGAAAAGUACAUCUGCAUCGUGUAC CCU UUCAGAUGCGUGCGGCCUGGCAAGUGCCGGACCAUCACAGUGCUGAUC CUGAU UUGGAUCACCGGCU UCAUCG UGGCCUUCAUCCCCCUGAGCAACAAA GAGUUCUUCAAGAAUUACUACGGCACCAAUGGCGUGUGCU UCCCACUGCAC UCCGAGGACACAGAGAGCAUCGGCGCCCAGAUCUACAGCGUGGCCAUCUUCC UGGGCAUCAAUCUGGCCGCCUUCAUCAUCAUCGUGUUCAGCUACGGCUCCA UGU UCUACUCCGUGCACCAGAGCGCCAUCACCGCCACCGAGAUCCGGAACCA AGUGAAGAAAGAGAUGAUCCUGGCCAAGCGCU UCU UCUUCAU UGUGU UCA CCGACGCCCUGUGUUGGAU UCCAAUCU UCGUCGUGAAGU UCCUGAGCCUGC UGCAGGUGGAAAUCCCCGGCACAAUCACCAGCUGGGUCGUGAUCUUCAUCC UGCCCAUCAACAGCGCCCUGAACCCUAUCCUGUACACCCUGACCACCCGGCCC UUCAAAGAAAUGAUCCACCGGU UCUGGUACAACUACCGGCAGAGAAAGAGC AUGGACAGCAAGGGCCAGAAAACCUACGCCCCUAGCU UCAUCUGGGUGGAA AUGUGGCCACUGCAGGAAAUGCCUCCCGAACUGAUGAAGCCCGACCUG UUC ACCUACCCCUGCGAGAUGAGCCUGAUCUCCCAGAGCACCCGGCUGAACAGCU ACUCCUGAUAACGGACCGGCGAUAGAUGAAGCUCGCUU UCU UGCUGUCCAA UU UCUAUUAAAGG UUCCU UUGUUCCCUAAGUCCAACUACUAAACUGGGGG AUAUUAUGAAGGGCCU UGAGCAUCUGGAUUCUGCCUAAUAAAAAACAUU U AUU UUCAUUGCAGCUCGCUU UCU UGCUGUCCAAUU UCUAUUAAAGGUUCC UU UGUUCCCUAAGUCCAACUACUAAACUGGGGGAUAU UAUGAAGGGCCU U GAGCAUCUGGAU UCUGCCUAAUAAAAAACAU UUAU UU UCAU UGCGGCCGCA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAA
SEQ ID NO: 37 RXFP1 RNA coding sequence of SEQ ID NO: 4 above	AUGACAAGCGGCAGCGUG UUCUUCUACAUCCUGAUCU UCGGCAAGUACU UC AGCCACGGCGGGAGGCCAGGACGUGAAGUGUAGCCUGGGCUACUUCCCCUGC GGCAACAUCACCAAGUGCCUGCCCCAGCUGCUGCACUGCAACGGCGUGGACG AUUGCGGCAACCAGGCCGACGAGGACAACUGCGGCGACAACAAUGGCUGGU CCCUGCAGU UCGAUAAGUACUUCGCCUCCUACUACAAGAUGACCAGCCAGU ACCCCUUCGAGGCCGAGACACCUGAGUGCCUCGUGGGCUCUGUGCCUGUGC AGUGUCUGUGCCAGGGCCUGGAACUGGACUGCGACGAGACAAACCUGAGAG CCGUGCCCAGCGUGUCCAGCAACGUGACAGCCAUGAGCCUGCAGUGGAACC UGAUCCGGAAGCUGCCCCCCGACUGCUUCAAGAACUACCACGACCUGCAGAA GCUGUAUCUGCAGAACAACAAGAUCACCUCCAUCAGCAUCUACGCCUUCCGG GGCCUGAACAGCCUGACCAAGCUGUACCUGAGCCACAACCGGAUCACCUUUC UGAAGCCCGGCGUGU UCGAGGACCUGCACAGACUGGAAUGGCUGAUCAUCG AGGACAAUCACCUGAGCCGGAUCAGCCCCCCCACCUUCUACGGCCUGAACUC CCUGAUCCUGCUGGUGCUGAUGAACAACGUGCUGACCCGGCUGCCCGACAA

73

<u>TABLE   1</u>
Exemplary   RXFP1 Polynucleotide   and Polypeptide   Sequences

SEQ ID NO: and features	Sequence
	GCCCCUG UGUCAGCACAUGCCCAGACUGCACUGGCUGGACCUGGAAGGCAA CCACAUCCACAACCUGCGGAACCUGACCUUCAUCAGCUGCAGCAACCUGACC GUGCUCGUGAUGCGGAAGAACAAGAU UAACCACCUGAACGAGAACACCU UC GCCCCCCUGCAGAAACUGGACGAGCUGGAUCUGGGCUCUAACAAGAUCGAG AACCUGCCCCCUCUGAUCUUCAAGGACCUGAAAGAGCUGAGCCAGCUGAACC UG UCCUACAACCCCAUCCAGAAGAUCCAGGCCAACCAGU UCGACUACCUCGU GAAGCUGAAGUCCCUGUCCCUGGAAGGGAUCGAGAUCAGCAACAUCCAGCA GCGGAUGU UCCGGCCCCUGAUGAAUCUGUCCCACAUCUACUUCAAGAAGU U CCAGUACUGCGGCUACGCCCCCCACGUGCGGAGCUGCAAGCCUAACACAGAC GGCAUCAGCAGCCUGGAAAACCUGCUGGCCUCCAUCAUCCAGCGGGUG UUC GUGUGGGUGGUGUCCGCCGUGACCUGCU UCGGCAAUAUCU UCGUGAUCUG CAUGCGGCCCUACAU UCGGAGCGAGAACAAGCUGUAUGCCAUGAGCAUCAU CUCCCUGUGCUGCGCCGACUGCCUGAUGGGCAUCUACCUGU UCGUGAUCGG CGGCU UCGACCUGAAGU UCCGGGGGCGAGUACAACAAGCACGCCCAGCUG UG GAUGGAAAGCACCCACUGCCAGCUCGUGGGCAGCCUGGCCAUCCUGAGCAC UGAAGUGUCCGUGCUGCUGCUGACCU UCCUGACCCUGGAAAAGUACAUCUG CAUCGUGUACCCU UUCAGAUGCGUGCGGCCUGGCAAGUGCCGGACCAUCAC AGUGCUGAUCCUGAU UUGGAUCACCGGCU UCAUCGUGGCCU UCAUCCCCCU GAGCAACAAAGAGU UCU UCAAGAAU UACUACGGCACCAAUGGCGUGUGCU U CCCACUGCACUCCGAGGACACAGAGAGCAUCGGCGCCCAGAUCUACAGCG UG GCCAUCU UCCUGGGCAUCAACUCGGCCGCCU UCAUCAUCAUCGUG UUCAGC UACGGCUCCAUG UUCUACUCCGUGCACCAGAGCGCCAUCACCGCCACCGAGA UCCGGAACCAAGUGAAGAAAGAGAUGAUCCUGGCCAAGCGCUUCUUCU UCA UUGUGUUCACCGACGCCCUGUGU UGGAU UCCAAUCUUCGUCGUGAAGUUC CUGAGCCUGCUGCAGGUGGAAAUCCCCGGCACAAUCACCAGCUGGGUCGUG AUCUUCAUCCUGCCCAUCAACAGCGCCCUGAACCCUAUCCGUACACCCUGA CCACCCGGCCCUUCAAAGAAAUGAUCCACCGGUUCUGGUACAACUACCGGCA GAGAAAGAGCAUGGACAGCAAGGGCCAGAAAACCUACGCCCCUAGCUUCAUC UGGGUGGAAAUG UGGCCACUGCAGGAAAUGCCUCCCGAACUGAUGAAGCCC GACCUGUUCACCUACCCCUGCGAGAUGAGCCUGAUCUCCCAGAGCACCCGGC UGAACAGCUACUCCUGAUAA

<u>1l.</u>   <u>SLC52A2</u>

[00273]   SLC52A2 (GPR172A) is a 445 amino acid multi-pass transmembrane protein predicted to have either 10 or 11 putative transmembrane helices. It has been shown to mediate the cellular uptake of riboflavin and has been reported to be a receptor for porcine endogenous retrovirus subgroup A . Certain genetic variants of SLC52A2 are associated with motor, sensory, and cranial neuronopathies.

[00274]   For the generation of a human SLC52A2 mRNA, a native human nucleotide sequence for this protein's open reading frame (e.g., accession numbers NM_001253816.1/ NP_001 240745) was subjected to codon optimization using GeneArt®'s codon optimization

algorithm for mice (see Table 2). In addition to changing codon sequences on the basis of mouse biases, sequences were altered to remove BamH I, Rsrl I, and BspQI restriction sites as these would be employed for subsequent subcloning and mRNA synthesis.

TABLE 2
Exemplary SLC52A2 Polynucleotide and Polypeptide Sequences

SEQ ID NO: and features	Sequence
SEQ ID NO: 38 Native DNA sequence of SLC52A2 corresponding to Protein Accession # NP_001240745	GGGCGGGACTTCCGGTCGTGGGCCATGCCGGGGGGCGGGCCCG GAACCGCCACGGCTAGAAGAAGTCTTCACTTCCCAGGAGAGCCA AAGCGTGTCTGGCCCTAGGTGGGAAAAGAACTGGCTGTGACCTT TGCCCTGACCTGGAAGGGCCCAGCCTTGGGCTGAATGGCAGCA CCCACGCCCGCCCGTCCGGTGCTGACCCACCTGCTGGTGGCTC TCTTCGGCATGGGCTCCTGGGCTGCGGTCAATGGGATCTGGGTG GAGCTACCTGTGGTGGTCAAAGAGCTTCCAGAGGGTTGGAGCCT CCCCTCTTACGTCTCTGTGCTTGTGGCTCTGGGGAACCTGGGTC TGCTGGTGGTGACCCTCTGGAGGAGGCTGGCCCCAGGAAAGGA CGAGCAGGTCCCCATCCGGGTGGTGCAGGTGCTGGGCATGGTG GGCACAGCCCTGCTGGCCTCTCTGTGGCACCATGTGGCCCCAGT GGCAGGACAGTTGCATTCTGTGGCCTTCTTAGCACTGGCCTTTGT GCTGGCACTGGCATGCTGTGCCTCGAATGTCACTTTCCTGCCCTT CTTGAGCCACCTGCCACCTCGCTTCTTACGGTCATTCTTCCTGGG TCAAGGCCTGAGTGCCCTGCTGCCCTGCGTGCTGGCCCTAGTGC AGGGTGTGGGCCGCCTCGAGTGCCCGCCAGCCCCCATCAACGG CACCCCTGGCCCCCCGCTCGACTTCCTTGAGCGTTTTCCCGCCA GCACCTTCTTCTGGGCACTGACTGCCCTTCTGGTCGCTTCAGCTG CTGCCTTCCAGGGTCTTCTGCTGCTGTTGCCGCCACCACCATCT GTACCCACAGGGGAGTTAGGATCAGGCCTCCAGGTGGGAGCCC CAGGAGCAGAGGAAGAGGTGGAAGAGTCCTCACCACTGCAAGA GCCACCAAGCCAGGCAGCAGGCACCACCCCTGGTCCAGACCCT AAGGCCTATCAGCTTCTATCAGCCCGCAGTGCCTGCCTGCTGGG CCTGTTGGCCGCCACCAACGCGCTGACCAATGGCGTGCTGCCTG CCGTGCAGAGCTTTTCCTGCTTACCCTACGGGCGTCTGGCCTAC CACCTGGCTGTGGTGCTGGGCAGTGCTGCCAATCCCCTGGCCTG CTTCCTGGCCATGGGTGTGCTGTGCAGGTCCTTGGCAGGGCTGG GCGGCCTCTCTGCTGGGCGTGTTCTGTGGGGGCTACCTGATG GCGCTGGCAGTCCTGAGCCCCTGCCCGCCCCTGGTGGGCACCT CGGCGGGGGTGGTCCTCGTGGTGCTGTCGTGGGTGCTGTGTCT TGGCGTGTTCTCCTACGTGAAGGTGGCAGCCAGCTCCCTGCTGC ATGGCGGGGGCCGGCCGGCATTGCTGGCAGCCGGCGTGGCCAT CCAGGTGGGCTCTCTGCTCGGCGCTGTTGCTATGTTCCCCCCGA CCAGCATCTATCACGTGTTCCACAGCAGAAAGGACTGTGCAGAC CCCTGTGACTCCTGAGCCTGGGCAGGTGGGGACCCCGCTCCCC AACACCTGTCTTTCCCTCAATGCTGCCACCATGCCTGAGTGCCTG CAGCCCAGGAGGCCCGCACACCGGTACACTCGTGGACACCTACA CACTCCATAGGAGATCCTGGCTTTCCAGGGTGGGCAAGGGCAAG GAGCAGGCTTGGAGCCAGGGACCAGTGGGGGCTGTAGGGTAAG CCCCTGAGCCTGGGACCTACATGTGGTTTGCGTAATAAAACATTT GTATTTAATGAGTTGGCATTAAAAAAAAAAAAAAAA
SEQ ID NO: 5 Native mRNA sequence of SLC52A2 corresponding to Protein Accession # NP_001240745	GGGCGGGACUUCCGGUCGUGGGCCAUGCCGGGGGGCGGGCCC GGAACCGCCACGGCUAGAAGAAGUCUUCACUUCCCAGGAGAGC CAAAGCGUGUCUGGCCCUAGGUGGGAAAAGAACUGGCUGUGAC CUUUGCCCUGACCUGGAAGGGCCCAGCCUUGGGCUGAAUGGC AGCACCCACGCCCGCCCGUCCGGUGCUGACCCACCUGCUGGU GGCUCUCUUCGGCAUGGGCUCCUGGGCUGCGGUCAAUGGGAU

TABLE 2
Exemplary SLC52A2 Polynucleotide and Polypeptide Sequences

SEQ ID NO: and features	Sequence
	CUGGGUGGAGCUACCUGUGGUGGUCAAAGAGCUUCCAGAGGG UUGGAGCCUCCCCUCUUACGUCUCUGUGCUUGUGGCUCUGGG GAACCUGGGUCUGCUGGUGGUGACCCUCUGGAGGAGGCUGGC CCCAGGAAAGGACGAGCAGGUCCCCAUCGGGUGGUGCAGGU GCUGGGCAUGGUGGGCACAGCCCUGCUGGCCUCUCUGUGGCA CCAUGUGGCCCCAGUGGCAGGACAGUUGCAUUCUGUGGCCUU CUUAGCACUGGCCUUUGUGCUGGCACUGGCAUGCUGUGCCUC GAAUGUCACUUUCCUGCCCUUCUUGAGCCACCUGCCACCUCGC UUCUUACGGUCAUUCUUCCUGGGUCAAGGCCUGAGUGCCCUG CUGCCCUGCGUGCUGGCCCUAGUGCAGGGUGUGGGCCGCCUC GAGUGCCCGCCAGCCCCCAUCAACGGCACCCCUGGCCCCCCGC UCGACUUCCUUGAGCGUUUUCCCGCCAGCACCUUCUUCGGGC ACUGACUGCCCUUCUGGUCGCUUCAGCUGCUGCCUUCCAGGG UCUUCUGCUGCUGUUGCCGCCACCACCAUCUGUACCCACAGGG GAGUUAGGAUCAGGCCUCCAGGUGGGAGCCCCAGGAGCAGAG GAAGAGGUGGAAGAGUCCUCACCACUGCAAGAGCCACCAAGCC AGGCAGCAGGCACCACCCCUGGUCCAGACCCUAAGGCCUAUCA GCUUCUAUCAGCCCGCAGUGCCUGCCUGCUGGGCCUGUUGGC CGCCACCAACGCGCUGACCAAUGGCGUGCUGCCUGCCGUGCA GAGCUUUUCCUGCUUACCCUACGGGCGUCUGGCCUACCACCUG GCUGUGGUGCUGGGCAGUGCUGCCAAUCCCCUGGCCUGCUUC CUGGCCAUGGGUGUGCUGUGCAGGUCCUUGGCAGGGCUGGGC GGCCUCUCUCUGCUGGGCGUGUUCUGUGGGGGGCUACCUGAUG GCGCUGGCAGUCCUGAGCCCCUGCCCGCCCCUGGUGGGCACC UCGGCGGGGGUGGUCCUCGUGGUGCUGUCGUGGGUGCUGUG UCUUGGCGUGUUCUCCUACGUGAAGGUGGCAGCCAGCUCCCU GCUGCAUGGCGGGGGCCGGCCGGCAUUGCUGGCAGCCGGCGU GGCCAUCCAGGUGGGCUCUCUGCUCGGCGCUGUUGCUAUGUU CCCCCCGACCAGCAUCUAUCACGUGUUCCACAGCAGAAAGGAC UGUGCAGACCCCUGUGACUCCUGAGCCUGGGCAGGUGGGGAC CCCGCUCCCCAACACCUGUCUUUCCCUCAAUGCUGCCACCAUG CCUGAGUGCCUGCAGCCCAGGAGGCCCGCACACCGGUACACUC GUGGACACCUACACACUCCAUAGGAGAUCCUGGCUUUCCAGGG UGGGCAAGGGCAAGGAGCAGGCUUGGAGCCAGGGACCAGUGG GGGCUGUAGGGUAAGCCCCUGAGCCUGGGACCUACAUGUGGU UUGCGUAAUAAACAUUUGUAUUUAAUGAGUUGGCAUUAAAAAA AAAAAAAAAA U= Uridine and/or pseudouridine
SEQ ID NO: 6 Translated human SLC52A2 from coding sequence (CDS) of the DNA construct of SEQ ID NO: 5	MAAPTPARPVLTHLLVALFGMGSWAAVNGIWVELPVVVKELPEGWS LPSYVSVLVALGNLGLLVVTLWRRLAPGKDEQVPIRVVQVLGMVGTA LLASLWHHVAPVAGQLHSVAFLALAFVLALACCASNVTFLPFLSHLP PRFLRSFFLGQGLSALLPCVLALVQGVGRLECPPAPINGTPGPPLDF LERFPASTFFWALTALLVASAAAFQGLLLLLPPPPSVPTGELGSGLQ VGAPGAEEEVEESSPLQEPPSQAAGTTPGPDPKAYQLLSARSACLL GLLAATNALTNGVLPAVQSFSCLPYGRLAYHLAVVLGSAANPLACFL AMGVLCRSLAGLGGLSLLGVFCGGYLMALAVLSPCPPLVGTSAGVV LVVLSWVLCLGVFSYVKVAASSLLHGGGRPALLAAGVAIQVGSLLGA VAMFPPTSIYHVFHSRKDCADPCDS
SEQ ID NO: 39 (DNA) TEV-hSLC52A2-2xhBG-120A	GGATCCGGAGGCCGGAGAATTGTAATACGACTCACTATAGGGAG ACGCGTGTTAAATAACAAATCTCAACACAACATATACAAAACAAAC GAATCTCAAGCAATCAAGCATTCTACTTCTATTGCAGCAATTTAAA

TABLE 2
Exemplary SLC52A2 Polynucleotide and Polypeptide Sequences

SEQ ID NO: and features	Sequence
Sequence features: Tobacco Etch Virus (TEV) 5' UTR: 37-190 Optimal Kozak sequence: 191-199 Human SLC52A2 codon optimized, Protein Accession # NP_001240745: 197-751 1 stop codon: 752-754 2 copies of human beta-globin 3'UTR: 773-1038 120 nucleotide polyA tail (SEQ ID NO: 59): 1045-1164	TCATTTCTTTTAAAGCAAAAGCAATTTTCTGAAAATTTTCACCATTT ACGAACGATAGCCGCCACCATGGCAGCACCCACGCCCGCCCGT CCGGTGCTGACCCACCTGCTGGTGGCTCTCTTCGGCATGGGCTC CTGGGCTGCGGTCAATGGGATCTGGGTGGAGCTACCTGTGGTG GTCAAAGAGCTTCCAGAGGGTTGGAGCCTCCCCTCTTACGTCTCT GTGCTTGTGGCTCTGGGGAACCTGGGTCTGCTGGTGGTGACCCT CTGGAGGAGGCTGGCCCCAGGAAAGGACGAGCAGGTCCCCATC CGGGTGGTGCAGGTGCTGGGCATGGTGGGCACAGCCCTGCTGG CCTCTCTGTGGCACCATGTGGCCCCAGTGGCAGGACAGTTGCAT TCTGTGGCCTTCTTAGCACTGGCCTTTGTGCTGGCACTGGCATGC TGTGCCTCGAATGTCACTTTCCTGCCCTTCTTGAGCCACCTGCCA CCTCGCTTCTTACGGTCATTCTTCCTGGGTCAAGGCCTGAGTGCC CTGCTGCCCTGCGTGCTGGCCCTAGTGCAGGGTGTGGGCCGCC TCGAGTGCCCGCCAGCCCCCATCAACGGCACCCCTGGCCCCCC GCTCGACTTCCTTGAGCGTTTTCCCGCCAGCACCTTCTTCTGGGC ACTGACTGCCCTTCTGGTCGCTTCAGCTGCTGCCTTCCAGGGTCT TCTGCTGCTGTTGCCGCCACCACCATCTGTACCCACAGGGGAGT TAGGATCAGGCCTCCAGGTGGGAGCCCCAGGAGCAGAGGAAGA GGTGGAAGAGTCCTCACCACTGCAAGAGCCACCAAGCCAGGCAG CAGGCACCACCCCTGGTCCAGACCCTAAGGCCTATCAGCTTCTA TCAGCCCGCAGTGCCTGCCTGCTGGGCCTGTTGGCCGCCACCAA CGCGCTGACCAATGGCGTGCTGCCTGCCGTGCAGAGCTTTTCCT GCTTACCCTACGGGCGTCTGGCCTACCACCTGGCTGTGGTGCTG GGCAGTGCTGCCAATCCCCTGGCCTGCTTCCTGGCAATGGGTGT GCTGTGCAGGTCCTTGGCAGGGCTGGGCGGCCTCTCTCTGCTG GGCGTGTTCTGTGGGGGCTACCTGATGGCGCTGGCAGTCCTGA GCCCCTGCCCGCCCCTGGTGGGCACCTCGGCGGGGGTGGTCCT CGTGGTGCTGTCGTGGGTGCTGTGTCTTGGCGTGTTCTCCTACG TGAAGGTGGCAGCCAGCTCCCTGCTGCATGGCGGGGGCCGGCC GGCATTGCTGGCAGCCGGCGTGGCCATCCAGGTGGGCTCTCTG CTCGGCGCTGTTGCTATGTTCCCCCCGACCAGCATCTATCACGT GTTCCACAGCAGAAAGGACTGTGCAGACCCCTGTGACTCCTGAC GGACCGGCGATAGATGAAGCTCGCTTTCTTGCTGTCCAATTTCTA TTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAAACTGGGGGA TATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAAC ATTTATTTTCATTGCAGCTCGCTTTCTTGCTGTCCAATTTCTATTAA AGGTTCCTTTGTTCCCTAAGTCCAACTACTAAACTGGGGGATATT ATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTT ATTTTCATTGCGGCCGCAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
SEQ ID NO: 7 (mRNA) TEV-hSLC52A2-2xhBG-120A Sequence features: Tobacco Etch Virus (TEV) 5' UTR: 37-190 Optimal Kozak sequence: 191-199 Human SLC52A2 codon optimized, Protein Accession #	GGGAGACGCGUGUUAAAUAACAAAUCUCAACACAACAUAUACAA AACAAACGAAUCUCAAGCAAUCAAGCAUUCUACUUCUAUUGCAG CAAUUUAAAUCAUUUCUUUUAAAGCAAAAGCAAUUUUCUGAAAA UUUUCACCAUUUACGAACGAUAGCCGCCACCAUGGCAGCACCC ACGCCCGCCCGUCCGGUGCUGACCCACCUGCUGGUGGCUCUC UUCGGCAUGGGCUCCUGGGCUGCGGUCAAUGGGAUCUGGGUG GAGCUACCUGUGGUGGUCAAAGAGCUUCCAGAGGGUUGGAGC CUCCCCUCUUACGUCUCUGUGCUUGUGGCUCUGGGGAACCUG GGUCUGCUGGUGGUGACCCUCUGGAGGAGGCUGGCCCCAGGA AAGGACGAGCAGGUCCCCAUCCGGGUGGUGCAGGUGCUGGGC AUGGUGGGCACAGCCCUGCUGGCCUCUCUGUGGCACCAUGUG GCCCCAGUGGCAGGACAGUUGCAUUCUGUGGCCUUCUUAGCAC

TABLE 2
Exemplary SLC52A2 Polynucleotide and Polypeptide Sequences

SEQ ID NO: and features	Sequence
NP_001240745: 197-751 1 stop codon: 752-754 2 copies of human beta-globin 3'UTR: 773-1038 120 nucleotide polyA tail (SEQ ID NO: 59): 1045-1164	UGGCCUUUGUGCUGGCACUGGCAUGCUGUGCCUCGAAUGUCA CUUUCCUGCCCUUCUUGAGCCACCUGCCACCUCGCUUCUUACG GUCAUUCUUCCUGGGUCAAGGCCUGAGUGCCCUGCUGCCCUG CGUGCUGGCCCUAGUGCAGGGUGUGGGCCGCCUCGAGUGCCC GCCAGCCCCCAUCAACGGCACCCCUGGCCCCCCGCUCGACUUC CUUGAGCGUUUUCCCGCCAGCACCUUCUUCUGGGCACUGACUG CCCUUCUGGUCGCUUCAGCUGCUGCCUUCCAGGGUCUUCUGC UGCUGUUGCCGCCACCACCAUCUGUACCCACAGGGGAGUUAGG AUCAGGCCUCCAGGUGGGAGCCCCAGGAGCAGAGGAAGAGGU GGAAGAGUCCUCACCACUGCAAGAGCCACCAAGCCAGGCAGCA GGCACCACCCCUGGUCCAGACCCUAAGGCCUAUCAGCUUCUAU CAGCCCGCAGUGCCUGCCUGCUGGGCCUGUUGGCCGCCACCA ACGCGCUGACCAAUGGCGUGCUGCCUGCCGUGCAGAGCUUUU CCUGCUUACCCUACGGGCGUCUGGCCUACCACCUGGCUGUGG UGCUGGGCAGUGCUGCCAAUCCCCUGGCCUGCUUCCUGGCAA UGGGUGUGCUGUGCAGGUCCUUGGCAGGGCUGGGCGGCCUCU CUCUGCUGGGCGUGUUCUGUGGGGGCUACCUGAUGGCGCUGG CAGUCCUGAGCCCCUGCCCGCCCCUGGUGGGCACCUCGGCGG GGGUGGUCCUCGUGGUGCUGUCGUGGGUGCUGUGUCUUGGC GUGUUCUCCUACGUGAAGGUGGCAGCCAGCUCCCUGCUGCAU GGCGGGGGCCGGCCGGCAUUGCUGGCAGCCGGCGUGGCCAUC CAGGUGGGCUCUCUGCUCGGCGCUGUUGCUAUGUUCCCCCCG ACCAGCAUCUAUCACGUGUUCCACAGCAGAAAGGACUGUGCAG ACCCCUGUGACUCCUGACGGACCGGCGAUAGAUGAAGCUCGCU UUCUUGCUGUCCAAUUUCUAUUAAAGGUUCCUUUGUUCCCUAA GUCCAACUACUAAACUGGGGGAUAUUAUGAAGGGCCUUGAGCA UCUGGAUUCUGCCUAAUAAAAAACAUUUAUUUUCAUUGCAGCUC GCUUUCUUGCUGUCCAAUUUCUAUUAAAGGUUCCUUUGUUCCC UAAGUCCAACUACUAAACUGGGGGAUAUUAUGAAGGGCCUUGA GCAUCUGGAUUCUGCCUAAUAAAAAACAUUUAUUUUCAUUGCG GCCGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA U= Uridine and/or pseudouridine
SEQ ID NO: 40 Human SLC52A2 RNA coding region sequence of SEQ ID NO: 7	AUGGCAGCACCCACGCCCGCCCGUCCGGUGCUGACCCACCUGC UGGUGGCUCUCUUCGGCAUGGGCUCCUGGGCUGCGGUCAAUG GGAUCGGGUGGAGCUACCUGUGGUGGUCAAAGAGCUUCCAG AGGGUUGGAGCCUCCCCUCUUACGUCUCUGUGCUUGUGGCUC UGGGGAACCUGGGUCUGCUGGUGGUGACCCUCGGAGGAGGC UGGCCCCAGGAAAGGACGAGCAGGUCCCCAUCCGGGUGGUGC AGGUGCUGGGCAUGGUGGGCACAGCCCUGCUGGCCUCUCUGU GGCACCAUGUGGCCCCAGUGGCAGGACAGUUGCAUUCUGUGG CCUUCUUAGCACUGGCCUUUGUGCUGGCACUGGCAUGCUGUG CCUCGAAUGUCACUUUCCUGCCCUUCUUGAGCCACCUGCCACC UCGCUUCUUACGGUCAUUCUUCCUGGGUCAAGGCCUGAGUGC CCUGCUGCCCUGCGUGCUGGCCCUAGUGCAGGGUGUGGGCCG CCUCGAGUGCCCGCCAGCCCCCAUCAACGGCACCCCUGGCCCC CCGCUCGACUUCCUUGAGCGUUUUCCCGCCAGCACCUUCUUCU GGGCACUGACUGCCCUUCUGGUCGCUUCAGCUGCUGCCUUCC AGGGUCUUCUGCUGCUGUUGCCGCCACCACCAUCUGUACCCAC AGGGGAGUUAGGAUCAGGCCUCCAGGUGGGAGCCCCAGGAGC AGAGGAAGAGGUGGAAGAGUCCUCACCACUGCAAGAGCCACCA

TABLE 2	
Exemplary SLC52A2 Polynucleotide and Polypeptide Sequences	
SEQ ID NO: and features	Sequence
	AGCCAGGCAGCAGGCACCACCCCUGGUCCAGACCCUAAGGCCU AUCAGCUUCUAUCAGCCCGCAGUGCCUGCCUGCUGGGCCUGU UGGCCGCCACCAACGCGCUGACCAAUGGCGUGCUGCCUGCCG UGCAGAGCUUUUCCUGCUUACCCUACGGGCGUCUGGCCUACCA CCUGGCUGUGGUGCUGGGCAGUGCUGCCAAUCCCCUGGCCUG CUUCCUGGCAAUGGGUGUGCUGUGCAGGUCCUUGGCAGGGCU GGGCGGCCUCUCUCUGCUGGGGCGUGUUCUGUGGGGGCUACCU GAUGGCGCUGGCAGUCCUGAGCCCCUGCCCGCCCCUGGUGGG CACCUCGGCGGGGGUGGUCCUCGUGGUGCUGUCGUGGGUGCU GUGUCUUGGCGUGUUCUCCUACGUGAAGGUGGCAGCCAGCUC CCUGCUGCAUGGCGGGGGCCGGCCGGCAUUGCUGGCAGCCGG CGUGGCCAUCCAGGUGGGCUCUCUGCUCGGCGCGCUGUUGCUAU GUUCCCCCCGACCAGCAUCUAUCACGUGUUCCACAGCAGAAAG GACUGUGCAGACCCCUGUGACUCCUGA

U= Uridine and/or pseudouridine

ML   ANGPTL8

[00275]   ANGPTL8 is a secreted protein, involved in lipid metabolism, which can be found in low ng/ml concentrations in human plasma. However, this protein is hard to express in heterologous systems in its native and soluble form. Furthermore, the biochemical function of this protein has not been described and thus there is no *in vitro* functional assay that could be used to measure the activity of recombinantly produced protein. Given the difficulty to generate and validate the quality of recombinant ANGPTL8 to use as an antigen, this protein was a good candidate for mRNA mediated immunization to generate monoclonal antibodies.

[00276]   The full length coding sequence of human ANGPTL8 (e.g., accession number NP_061 157) was codon optimized for expression in human cells and cloned into a vector that can sustain mRNA transcription by T7 polymerase and contains both 3 and 5' untranslated regions that help with mRNA stability and translatability (see Table 3 for sequence). mRNA was *in vitro* transcribed and encapsulated into lipid nanoparticles as described above.

TABLE 3	
Exemplary ANGPTL8 Polynucleotide and Polypeptide Sequences	
SEQ ID NO: and features	Sequence
SEQ ID NO: 4 1 ANGPTL8 native DNA sequence corresponding to Protein Accession # NP_061 157	ATACCTTAGACCCTCAGTCATGCCAGTGCCTGCTCTGTGCCTGCT CTGGGCCCTGGCAATGGTGACCCGGCCTGCCTCAGCGGCCCCC ATGGGCGGCCCAGAACTGGCACAGCATGAGGAGCTGACCCTGC TCTTCCATGGGACCCTGCAGCTGGGCCAGGCCCTCAACGGTGTG TACAGGACCACGGAGGGACGGCTGACAAAGGCCAGGAACAGCC TGGGTCTCTATGGCCGCACAATAGAACTCCTGGGGCAGGAGGTC

TABLE 3
Exemplary ANGPTL8 Polynucleotide and Polypeptide Sequences

SEQ ID NO: and features	Sequence
	AGCCGGGGCCGGGATGCAGCCCAGGAACTTCGGGCAAGCCTGT TGGAGACTCAGATGGAGGAGGATATTCTGCAGCTGCAGGCAGAG GCCACAGCTGAGGTGCTGGGGGAGGTGGCCCAGGCACAGAAGG TGCTACGGGACAGCGTGCAGCGGCTAGAAGTCCAGCTGAGGAG CGCCTGGCTGGGCCCTGCCTACCGAGAATTTGAGGTCTTAAAGG CTCACGCTGACAAGCAGAGCCACATCCTATGGGCCCTCACAGGC CACGTGCAGCGGCAGAGGCGGGAGATGGTGGCACAGCAGCATC GGCTGCGACAGATCCAGGAGAGACTCCACACAGCGGCGCTCCC AGCCTGAATCTGCCTGGATGGAACTGAGGACCAATCATGCTGCA AGGAACACTTCCACGCCCCGTGAGGCCCCTGTGCAGGGAGGAG CTGCCTGTTCACTGGGATCAGCCAGGGCGCCGGGCCCCACTTCT GAGCACAGAGCAGAGACAGACGCAGGCGGGGACAAAGGCAGAG GATGTAGCCCCATTGGGGAGGGGTGGAGGAAGGACATGTACCCT TTCATGCCTACACACCCCTCATTAAAGCAGAGTCGTGGCATCTCA AAAAAAAAAAAAAAAAAAA
SEQ ID NO: 8 ANGPTL8 native mRNA sequence of ANGPTL8 corresponding to Protein Accession # NP_061 157	AUACCUUAGACCCUCAGUCAUGCCAGUGCCUGCUCUGUGCCUG CUCUGGGCCCUGGCAAUGGUGACCCGGCCUGCCUCAGCGGCC CCCAUGGGCGGCCCAGAACUGGCACAGCAUGAGGAGCUGACCC UGCUCUUCCAUGGGACCCUGCAGCUGGGCCAGGCCCUCAACG GUGUGUACAGGACCACGGAGGGACGGCUGACAAAGGCCAGGAA CAGCCUGGGUCUCUAUGGCCGCACAAUAGAACUCCUGGGGCAG GAGGUCAGCCGGGGCCGGGAUGCAGCCCAGGAACUUCGGGCA AGCCUGUUGGAGACUCAGAUGGAGGAGGAUAUUCUGCAGCUGC AGGCAGAGGCCACAGCUGAGGUGCUGGGGGGAGGUGGCCCAGG CACAGAAGGUGCUACGGGACAGCGUGCAGCGGCUAGAAGUCCA GCUGAGGAGCGCCUGGCUGGGCCCUGCCUACCGAGAAUUUGA GGUCUUAAAGGCUCACGCUGACAAGCAGAGCCACAUCCUAUGG GCCCUCACAGGCCACGUGCAGCGGCAGAGGCGGGAGAUGGUG GCACAGCAGCAUCGGCUGCGACAGAUCCAGGAGAGACUCCACA CAGCGGCGCUCCCAGCCUGAAUCUGCCUGGAUGGAACUGAGGA CCAAUCAUGCUGCAAGGAACACUUCCACGCCCCGUGAGGCCCC UGUGCAGGGAGGAGCUGCCUGUUCACUGGGAUCAGCCAGGGC GCCGGGCCCCACUUCUGAGCACAGAGCAGAGACAGACGCAGGC GGGGACAAAGGCAGAGGAUGUAGCCCCAUUGGGGAGGGGUGG AGGAAGGACAUGUACCCUUUCAUGCCUACACACCCCUCAUUAAA GCAGAGUCGUGGCAUCU CAAAAAAAAAAAAAAAAAAA U= Uridine and/or pseudouridine
SEQ ID NO: 9 Translated human ANGPTL8 from coding sequence (CDS) of the DNA construct of SEQ ID NO: 8	MPVPALCLLWALAMVTRPASAAPMGGPELAQHEELTLLFHGTLQLG QALNGVYRTTEGRLTKARNSLGLYGRTIELLGQEVSRGRDAAQELR ASLLETQMEEDILQLQAEATAEVLGEVAQAQKVLRDSVQRLEVQLRS AWLGPAYREFEVLKAHADKQSHILWALTGHVQRQRREMVAQQHRL RQIQERLHTAALPA

TABLE 3
Exemplary ANGPTL8 Polynucleotide and Polypeptide Sequences

SEQ ID NO: and features	Sequence
SEQ ID NO: 42 (DNA) TEV-hANGPTL8-2xhBG-120A Sequence features: Tobacco Etch Virus (TEV) 5' UTR: 14-154 Optimal Kozak sequence: 155-163 Ikk signal peptide: 204-275 Human ANGPTL8 codon optimized , encoding amino acids 22-198 of Protein Accession # NP_061157, Flag-6His-Avi tag: 807-899 1 stop codon: 900-902 2 copies of human beta-globin 3'UTR: 921-1186 120 nucleotide polyA tail (SEQ ID NO: 59): 1187-1306	GGGATCCGGAGGCCGGAGAATTGTAATACGACTCACTATAGGGA GACGCGTGTTAAATAACAAATCTCAACACAACATATACAAAACAAA CGAATCTCAAGCAATCAAGCATTCTACTTCTATTGCAGCAATTTAA ATCATTTCTTTTAAAGCAAAAGCAATTTTCTGAAAATTTTCACCATT TACGAACGATAGCCGCCACCATGAAGACCTTCATCCTGCTGCTGT GGGTGCTGCTGCTGTGGGTCATCTTCCTGCTGCCTGGCGCCACA GCCGCTCCTATGGGAGGACCTGAACTGGCCCAGCACGAGGAACT GACCCTGCTGTTTCACGGCACCCTGCAGCTGGGACAGGCCCTGA ATGGCGTGTACAGAACCACCGAGGGCCGGCTGACCAAGGCCAG AAATAGCCTGGGCCTGTACGGCCGGACCATCGAACTGCTGGGGC AGGAAGTGTCCAGAGGCAGAGATGCCGCCCAGGAACTGAGAGC CAGCCTGCTGGAAACCCAGATGGAAGAGGACATCCTGCAGCTGC AGGCCGAGGCCACAGCTGAGGTGCTGGGAGAAGTGGCCCAGGC CCAGAAGGTGCTGAGAGACAGCGTGCAGCGGCTGGAAGTGCAG CTGAGATCTGCCTGGCTGGGCCCTGCCTACCGCGAGTTCGAAGT GCTGAAAGCCCACGCCGACAAGCAGAGCCACATCCTGTGGGCC CTGACAGGCCACGTGCAGAGACAGAGGCGGGAAATGGTGGCTC AGCAGCACAGACTGCGGCAGATCCAGGAACGGCTGCATACAGCT GCCCTGCCCGCCGACTACAAGGACGACGACGACAAGCACCACC ACCATCACCACGGCGGAGGCCTGAACGACATCTTCGAAGCCCAG AAAATCGAGTGGCACGAGTAACGGACCGGCGATAGATGAAGCTC GCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAA GTCCAACTACTAAACTGGGGGATATTATGAAGGGCCTTGAGCATC TGGATTCTGCCTAATAAAAAACATTTATTTTCATTGCAGCTCGCTT TCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCC AACTACTAAACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGA TTCTGCCTAATAAAAAACATTTATTTTCATTGCAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAA
SEQ ID NO: 10 (mRNA) TEV-hANGPTL8-2xhBG-120A Sequence features: Tobacco Etch Virus (TEV) 5' UTR: 14-154 Optimal Kozak sequence: 155-163 Ikk signal peptide: 204-275 Human ANGPTL8 codon optimized , encoding amino acids 22-198 of Protein Accession # NP_061157, Flag-6His-Avi tag:	GGGAGACGCGUGUUAAAUAACAAAUCUCAACACAACAUAUACAA AACAAACGAAUCUCAAGCAAUCAAGCAUUCUACUUCUAUUGCAG CAAUUUAAAUCAUUUCUUUUAAAGCAAAAGCAAUUUUCUGAAAA UUUUCACCAUUUACGAACGAUAGCCGCCACCAUGAAGACCUUC AUCCUGCUGCUGUGGGUGCUGCUGCUGUGGGUCAUCUUCCUG CUGCCUGGCGCCACAGCCGCUCCUAUGGGAGGACCUGAACUG GCCCAGCACGAGGAACUGACCCUGCUGUUUCACGGCACCCUGC AGCUGGGACAGGCCCUGAAUGGCGUGUACAGAACCACCGAGGG CCGGCUGACCAAGGCCAGAAAUAGCCUGGGCCUGUACGGCCG GACCAUCGAACUGCUGGGGCAGGAAGUGUCCAGAGGCAGAGAU GCCGCCCAGGAACUGAGAGCCAGCCUGCUGGAAACCCAGAUGG AAGAGGACAUCCUGCAGCUGCAGGCCGAGGCCACAGCUGAGGU GCUGGGAGAAGUGGCCCAGGCCCAGAAGGUGCUGAGAGACAG CGUGCAGCGGCUGGAAGUGCAGCUGAGAUCUGCCUGGCUGGG CCCUGCCUACCGCGAGUUCGAAGUGCUGAAAGCCCACGCCGAC AAGCAGAGCCACAUCCUGUGGGCCCUGACAGGCCACGUGCAGA GACAGAGGCGGGAAAUGGUGGCUCAGCAGCACAGACUGCGGCA GAUCCAGGAACGGCUGCAUACAGCUGCCCUGCCCGCCGACUAC AAGGACGACGACGACAAGCACCACCACCAUCACCACGGCGGAG GCCUGAACGACAUCUUCGAAGCCCAGAAAAUCGAGUGGCACGA

TABLE 3
Exemplary ANGPTL8 Polynucleotide and Polypeptide Sequences

SEQ ID NO: and features	Sequence
807-899 1 stop codon : 900-902 2 copies of human beta-globin 3'UTR: 921 - 1186 120 nucleotide polyA tail (SEQ ID NO: 59): 1187-1306	GUAACGGACCGGCGAUAGAUGAAGCUCGCUUUCUUGCUGUCCA AUUUCUAUUAAAGGUUCCUUUGUUCCCUAAGUCCAACUACUAAA CUGGGGGAUAUUAUGAAGGGCCUUGAGCAUCUGGAUUCUGCC UAAUAAAAAACAUUUAUUUUCAUUGCAGCUCGCUUUCUUGCUG UCCAAUUUCUAUUAAAGGUUCCUUUGUUCCCUAAGUCCAACUA CUAAACUGGGGGAUAUUAUGAAGGGCCUUGAGCAUCUGGAUUC UGCCUAAUAAAAAACAUUUAUUUUCAUUGCAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAA U= Uridine and/or pseudouridine
SEQ ID NO: 43 Human ANGPTL8 RNA coding sequence of SEQ ID NO: 10	AUGAAGACCUUCAUCCUGCUGCUGUGGGUGCUGCUGCUGUGG GUCAUCUUCCUGCUGCCUGGCGCCACAGCCGCUCCUAUGGGA GGACCUGAACUGGCCCAGCACGAGGAACUGACCCUGCUGUUUC ACGGCACCCUGCAGCUGGGACAGGCCCUGAAUGGCGUGUACA GAACCACCGAGGGCCGGCUGACCAAGGCCAGAAAUAGCCUGGG CCUGUACGGCCGGACCAUCGAACUGCUGGGGCAGGAAGUGUC CAGAGGCAGAGAUGCCGCCCAGGAACUGAGAGCCAGCCUGCUG GAAACCCAGAUGGAAGAGGACAUCCUGCAGCUGCAGGCCGAGG CCACAGCUGAGGUGCUGGGAGAAGUGGCCCAGGCCCAGAAGG UGCUGAGAGACAGCGUGCAGCGGCUGGAAGUGCAGCUGAGAU CUGCCUGGCUGGGCCCUGCCUACCGCGAGUUCGAAGUGCUGA AAGCCCACGCCGACAAGCAGAGCCACAUCCUGUGGGCCCUGAC AGGCCACGUGCAGAGACAGAGGCGGGAAAUGGUGGCUCAGCA GCACAGACUGCGGCAGAUCCAGGAACGGCUGCAUACAGCUGCC CUGCCCGCCGACUACAAGGACGACGACGACAAGCACCACCACC AUCACCACGGCGGAGGCCUGAACGACAUCUUCGAAGCCCAGAA AAUCGAGUGGCACGAGUAA U= Uridine and/or pseudouridine

JV.   TSHR

[00277]   The thyroid-stimulating hormone receptor (TSHR) is a G protein-coupled receptor, essential for thyroid growth and thyroid hormone production. It is also an autoantigen in Grave's disease. Prolonged activation of TSHR by TSHR-specific autoantibodies is one of the main cause underlying Graves' disease (Davies TF (2015) Expert Opin Ther Targets; 19:835-47).

[00278]   TSHR has a large extracellular domain (ECD) and a transmembrane domain (TMD). ECD has eleven leucine-rich repeat domains (LRD), which contains the binding sites for TSH and many autoantibodies. TSHR goes through extensive post-translational modifications and can form homodimers and polymers. TSHR has low baseline constitutive activities. Its signaling is promiscuous, mediated by Gs, Gi/o, Gq/1 1 or G12/13. TSHR is 51% identical to luteinizing

hormone, choriogonadotropin receptor (LHCGR) and 48% identical to follicle stimulating hormone receptor (FSHR).

[00279]   In normal thyroids, TSH activates TSHR, regulating thyrocyte proliferation and thyroid hormone release. In Graves' disease, agonistic autoantibodies are generated in patients. They displace TSH and over-activate the receptor in an unregulated manner: thyroid is enlarged and T3 and T4 levels are elevated.

[00280]   The full length coding sequence of human TSHR (e.g., Protein Accession No. NPJD00360.2) was codon optimized for expression in human cells and cloned into a vector that can sustain mRNA transcription by T7 polymerase and contains both 3 and 5' untranslated regions that help with mRNA stability and translatability (see Table 4 for sequence). mRNA was *in vitro* transcribed and encapsulated into lipid nanoparticles as described above.

TABLE 4 Exemplary TSHR Polynucleotide and Polypeptide Sequences	
SEQ ID NO: and features	Sequence

| SEQ ID NO: 44
TSHR native DNA
sequence
corresponding to
Protein Accession
NP_000360.2 | CCTCCTCCACAGTGGTGAGGTCACAGCCCCTTGGAGCCCTCCCTCTTCCCAC
CCCTCCCGCTCCCGGGTCTCCTTTGGCCTGGGGTAACCCGAGGTGCAGAGC
TGAGAATGAGGCGATTTCGGAGGATGGAGAAATAGCCCCGAGTCCCGTGGA
AAATGAGGCCGGCGGACTTGCTGCAGCTGGTGCTGCTGCTCGACCTGCCCA
GGGACCTGGGCGGAATGGGGTGTTCGTCTCCACCCTGCGAGTGCCATCAGG
AGGAGGACTTCAGAGTCACCTGCAAGGATATTCAACGCATCCCCAGCTTACC
GCCCAGTACGCAGACTCTGAAGCTTATTGAGACTCACCTGAGAACTATTCCAA
GTCATGCATTTTCTAATCTGCCCAATATTTCCAGAATCTACGTATCTATAGATG
TGACTCTGCAGCAGCTGGAATCACACTCCTTCTACAATTTGAGTAAAGTGACT
CACATAGAAATTCGGAATACCAGGAACTTAACTTACATAGACCCTGATGCCCT
CAAAGAGCTCCCCCTCCTAAAGTTCCTTGGCATTTTCAACACTGGACTTAAAA
TGTTCCCTGACCTGACCAAAGTTTATTCCACTGATATATTCTTTATACTTGAAA
TTACAGACAACCCTTACATGACGTCAATCCCTGTGAATGCTTTTCAGGGACTA
TGCAATGAAACCTTGACACTGAAGCTGTACAACAATGGCTTTACTTCAGTCCA
AGGATATGCTTTCAATGGGACAAAGCTGGATGCTGTTTACCTAAACAAGAATA
AATACCTGACAGTTATTGACAAAGATGCATTTGGAGGAGTATACAGTGGACCA
AGCTTGCTGGACGTGTCTCAAACCAGTGTCACTGCCCTTCCATCCAAAGGCC
TGGAGCACCTGAAGGAACTGATAGCAAGAAACACCTGGACTCTTAAGAAACT
TCCACTTTCCTTGAGTTTCCTTCACCTCACACGGGCTGACCTTTCTTACCCAA
GCCACTGCTGTGCTTTTAAGAATCAGAAGAAAATCAGAGGAATCCTTGAGTCC
TTGATGTGTAATGAGAGCAGTATGCAGAGCTTGCGCCAGAGAAAATCTGTGA
ATGCCTTGAATAGCCCCCTCCACCAGGAATATGAAGAGAATCTGGGTGACAG
CATTGTTGGGTACAAGGAAAAGTCCAAGTTCCAGGATACTCATAACAACGCTC
ATTATTACGTCTTCTTTGAAGAACAAGAGGATGAGATCATTGGTTTTGGCCAG
GAGCTCAAAAACCCCCAGGAAGAGACTCTACAAGCTTTTGACAGCCATTATGA
CTACACCATATGTGGGGACAGTGAAGACATGGTGTGTACCCCCAAGTCCGAT
GAGTTCAACCCGTGTGAAGACATAATGGGCTACAAGTTCCTGAGAATTGTGG
TGTGGTTCGTTAGTCTGCTGGCTCTCCTGGGCAATGTCTTTGTCCTGCTTATT
CTCCTCACCAGCCACTACAAACTGAACGTCCCCCGCTTTCTCATGTGCAACCT
GGCCTTTGCGGATTTCTGCATGGGGATGTACCTGCTCCTCATCGCCTCTGTA
GACCTCTACACTCACTCTGAGTACTACAACCATGCCATCGACTGGCAGACAG
GCCCTGGGTGCAACACGGCTGGTTTCTTCACTGTCTTTGCAAGCGAGTTATC
GGTGTATACGCTGACGGTCATCACCCTGGAGCGCTGGTATGCCATCACCTTC
GCCATGCGCCTGGACCGGAAGATCCGCCTCAGGCACGCATGTGCCATCATG
GTTGGGGGCTGGGTTTGCTGCTTCCTTCTCGCCCTGCTTCCTTTGGTGGGAA |

TAAGTAGCTATGCCAAAGTCAGTATCTGCCTGCCCATGGACACCGAGACCCC
TCTTGCTCTGGCATATATTGTTTTTGTTCTGACGCTCAACATAGTTGCCTTCGT
CATCGTCTGCTGCTGTTATGTGAAGATCTACATCACAGTCCGAAATCCGCAGT
ACAACCCAGGGGACAAAGATACCAAAATTGCCAAGAGGATGGCTGTGTTGAT
CTTCACCGACTTCATATGCATGGCCCCAATCTCATTCTATGCTCTGTCAGCAA
TTCTGAACAAGCCTCTCATCACTGTTAGCAACTCCAAAATCTTGCTGGTACTC
TTCTATCCACTTAACTCCTGTGCCAATCCATTCCTCTATGCTATTTTCACCAAG
GCCTTCCAGAGGGATGTGTTCATCCTACTCAGCAAGTTTGGCATCTGTAAACG
CCAGGCTCAGGCATACCGGGGGCAGAGGGTTCCTCCAAAGAACAGCACTGA
TATTCAGGTTCAAAAGGTTACCCACGAGATGAGGCAGGGTCTCCACAACATG
GAAGATGTCTATGAACTGATTGAAAACTCCCATCTAACCCCAAAGAAGCAAGG
CCAAATCTCAGAAGAGTATATGCAAACGGTTTTGTAAGTTAACACTACACTACT
CACAATGGTAGGGGAACTTACAAAATAATAGTTTCTTGAATATGCATTCCAATC
CCATGACACCCCCAACACATAGCTGCCCTCACTCTTGTGCAGGCGATGTTTC
AATGTTTCATGGGGCAAGAGTTTATCTCTGGAGAGTGATTAGTATTAACCTAA
TCATTGCCCCCAAGAAGGAAGTTAGGCTACCAGCATATTTGAATGCCAGGTG
AAATCAAAATAATCTACACTATCTAGAAGACTTTCTTGATGCCAAGTCCAGAGA
TGTCATTGTGTAGGATGTTCAGTAAATATTAACTGAGCTATGTCAATATAGAGC
TTCTCAGTTTTGTATAACATTTCATACTAAAGATTCAGCAAATGGAAAATGCTA
TTAATTTGGTTGGTGACCACAAGATAAAATCAGTCCCACGTTGGCTCAGTTCA
ACTAGATGTTCCCTGATACAAAGAGAACTTGATTTCCTTAAAACTGAAAAGCC
AAACACAGCTAGCTGTCATACAAGAAACAGCTATTATGAGACATGAAGGAGG
GTAAGAATTAGCTTTAAGTTTTGTTTTGCTTTGTTTTGTTTTTTAACTCAACCTA
TTAATCATCTCTTCACAAGAATCCACCTGATGTGACCAAGCTATTATGTGTTGC
CTGGAAAAACTGGCAAGATTTCAGCTTATGTGGCCTAGCAAACTAAGAATTGC
TCTTCTTGGCCAGCCTCATAGCATAAAGATGTGAACTCTAGGAAGTCTTTCT
GAGTAGCAATAAGTGGGAATTATGGGCAGAGCACACTCAATCCCCTGTTGAT
TAATAAAACAGGCTGGACACTAATTAACTATGGGACTTAAATCTGTAGAAATG
AAGGAGTCCAATAGCTTCTTCCAATTTTAAAACTCTAGTACATCCCTTTCCCTC
AAATATATATTTCTAAGATAAAGAGAAAGAAGAGCACTAAGTAAGTAGAATCTG
TTTTTCCTATTTTGTAGGGCTGCTGACTCCTAGTCCTTGAAGCCTAGACACAT
GACCCAGGAAATTTTTCCTTTGTTTCACTTTTGATTATGATGTCTGAGCCAAAA
ATTCAATTAAGTAAACATACTCGCCTGGATCTGAATCATTCATTTAATTACTAG
ATCTACCCAGCTGTTATATCAGGCCAAAACAGATTCGTGTTTATATAAAAGA
GTAAACGATGGTTGCAAATTTTGGCTATTTAGAGTTGCTACTTCACTATGAAGA
GTCACTTCAAAACACTTCGCTTGTCTTTAGGGATGATTTTTGCCATTTCCAGTC
CACGGTATGATACTAAAGCTGTCAAGAGAGGTTTCTTCTTTTCTGAAACTGCC
AGCTCTTTCCAGCCCTGTTGATCACTGGACATAAAGCTTCTTTTCCCCAATAAT
TCTTCTTTACTTAAAATAGTCAGGATCTTTATCTACAGATGTACTCTCCAGGTT
ACCTGTGATGATAGCCCCCTAATGTCCTGCTAGAAAAGTCTCCAAGCAGAGAT
GACATTACTTCTGAATGCTCATAAACCACACCATGAAATAAAAGCTCTTTGTTG
TTTTAAGATTGTGAAGTGTCGTTAATGGGTCCCCACAGATGGTCCCTGCTGGA
CTCACCTGGAATCTCTCCACAGCCATACCCACTCATCACTATCATTGAGACCT
GCACATCTTAATAGAAATATTATAAACATCGAAATCATGACTTACCTAGAAGT
TCGCTTGTAACTAATGAAATTAAACAAATGTGTTGCCTTTTGTCATGTGTTTCT
CTCCTGTGACATTTCAAAATATCACATCTTGATAAATAATGTGTTTCATCTTGA
ATAGCTGAACTAATTGCTTTGGAAACAGAGTCCTAGAAAAGTGACTTCAACAG
AATTGTTACTAAAATTTGCACTCACAACATGAAATAAATTTTCTTCCTATGGAAT
AATCGTGAAAAAAAAAA

| SEQ ID NO: 14
TSHR native
mRNA sequence
corresponding to
Protein Accession
NP_000360.2 | CCUCCUCCACAGUGGUGAGGUCACAGCCCCUUGGAGCCCUCCCUCUUCCC
ACCCUCCCGCUCCCGGGUCUCCUUUGGCCUGGGGUAACCCGAGGUGCAG
AGCUGAGAAUGAGGCGAUUUCGGAGGAUGGAGAAAUAGCCCCGAGUCCCG
UGGAAAAUGAGGCCGGCGGACUUGCUGCAGCUGGUGCUGCUGCUCGACCU
GCCCAGGGACCUGGGCGGAAUGGGGUGUUCGUCUCCACCCUGCGAGUGC
CAUCAGGAGGAGGACUUCAGAGUCACCUGCAAGGAUAUUCAACGCAUCCCC
AGCUUACCGCCCAGUACGCAGACUCUGAAGCUUAUUGAGACUCACCUGAGA
ACUAUUCCAAGUCAUGCAUUUUCUAAUCUGCCCAAUAUUUCCAGAAUCUAC
GUAUCUAUAGAUGUGACUCUGCAGCAGCUGGAAUCACACUCCUUCUACAAU
UUGAGUAAAGUGACUCACAUAGAAAUUCGGAAUACCAGGAACUUAACUUAC
AUAGACCCUGAUGCCCUCAAAGAGCUCCCCUCCUAAAGUUCCUUGGCAUU
UUCAACACUGGACUUAAAAUGUUCCCUGACCUGACCAAAGUUUAUUCCACU
GAUAUAUUCUUUAUACUUGAAAUUACAGACAACCCUUACAUGACGUCAAUC
CCUGUGAAUGCUUUUCAGGGACUAUGCAAUGAAACCUUGACACUGAAGCU
GUACAACAAUGGCUUUACUUCAGUCCAAGGAUAUGCUUUCAAUGGGACAAA
GCUGGAUGCUGUUUACCUAAACAAGAAUAAAUACCUGACAGUUAUUGACAA
AGAUGCAUUUGGAGGAGUAUACAGUGGACCAAGCUUGCUGGACGUGUCUC
AAACCAGUGUCACUGCCCUUCCAUCCAAAGGCCUGGAGCACCUGAAGGAAC
UGAUAGCAAGAAACACCUGGACUCUUAAGAAACUUCCACUUUCCUUGAGUU
UCCUUCACCUCACACGGGCUGACCUUUCUUACCCAAGCCACUGCUGUGCU
UUUAAGAAUCAGAAGAAAAUCAGAGGAAUCCUUGAGUCCUUGAUGUGUAAU
GAGAGCAGUAUGCAGAGCUUGCGCCAGAGAAAAUCUGUGAAUGCCUUGAA
UAGCCCCCUCCACCAGGAAUAUGAAGAGAAUCUGGGUGACAGCAUUGUUG
GGUACAAGGAAAAGUCCAAGUUCCAGGAUACUCAUAACAACGCUCAUUAUU
ACGUCUUCUUUGAAGAACAAGAGGAUGAGAUCAUUGGUUUUGGCCAGGAG
CUCAAAAACCCCCAGGAAGAGACUCUACAAGCUUUUGACAGCCAUUAUGAC
UACACCAUAUGUGGGGACAGUGAAGACAUGGUGUGUACCCCAAGUCCGA
UGAGUUCAACCCGUGUGAAGACAUAAUGGGCUACAAGUUCCUGAGAAUUG
UGGUGUGGUUCGUUAGUCUGCUGGCUCUCCUGGGCAAUGUCUUUGUCCU
GCUUAUUCUCCUCACCAGCCACUACAAACUGAACGUCCCCGCUUUCUCAU
GUGCAACCUGGCCUUUGCGGAUUUCUGCAUGGGGAUGUACCUGCUCCUCA
UCGCCUCUGUAGACCUCUACACUCACUCUGAGUACUACAACCAUGCCAUCG
ACUGGCAGACAGGCCCUGGGGUGCAACACGGCUGGUUUCUUCACUGUCUUU
GCAAGCGAGUUAUCGGUGUAUACGCUGACGGUCAUCACCCUGGAGCGCUG
GUAUGCCAUCACCUUCGCCAUGCGCCUGGACCGGAAGAUCCGCCUCAGGC
ACGCAUGUGCCAUCAUGGUUGGGGGGCUGGGUUUGCUGCUUCCUUCUCGC
CCUGCUUCCUUUGGUGGGAAUAAGUAGCUAUGCCAAAGUCAGUAUCUGCC
UGCCCAUGGACACCGAGACCCCUCUUGCUCUGGCAUAUAUUGUUUUUGUU
CUGACGCUCAACAUAGUUGCCUUCGUCAUCGUCUGCUGCUGUUAUGUGAA
GAUCUACAUCACAGUCCGAAAUCCGCAGUACAACCCAGGGGACAAAGAUAC
CAAAAUUGCCAAGAGGAUGGCUGUGUUGAUCUUCACCGACUUCAUAUGCAU
GGCCCCAAUCUCAUUCUAUGCUCUGUCAGCAAUUCUGAACAAGCCUCUCAU
CACUGUUAGCAACUCCAAAAUCUUGCUGGUACUCUUCUAUCCACUUAACUC
CUGUGCCAAUCCAUUCCUCUAUGCUAUUUUCACCAAGGCCUUCCAGAGGG
AUGUGUUCAUCCUACUCAGCAAGUUUGGCAUCUGUAAACGCCAGGCUCAG
GCAUACCGGGGGCAGAGGGUUCCUCCAAAGAACAGCACUGAUAUUCAGGU
UCAAAAGGUUACCCACGAGAUGAGGCAGGGUCUCCACAACAUGGAAGAUGU
CUAUGAACUGAUUGAAACUCCCAUCUAACCCCAAAGAAGCAAGGCCAAAU
CUCAGAAGAGUAUAUGCAAACGGUUUUGUAAGUUAACACUACACUACUCAC
AAUGGUAGGGGAACUUACAAAAUAAUAGUUUCUUGAAUAUGCAUUCCAAUC
CCAUGACACCCCCAACACAUAGCUGCCCUCACUCUUGUGCAGGCGAUGUU
UCAAUGUUUCAUGGGGCAAGAGUUUAUCUCUGGAGAGUGAUUAGUAUUAA
CCUAAUCAUUGCCCCCAAGAAGGAAGUUAGGCUACCAGCAUAUUUGAAUGC
CAGGUGAAAUCAAAAUAAUCUACACUAUCUAGAAGACUUUCUUGAUGCCAA |

	GUCCAGAGAUGUCAUUGUGUAGGAUGUUCAGUAAAUAUUAACUGAGCUAU GUCAAUAUAGAGCUUCUCAGUUUUGUAUAACAUUUCAUACUAAAGAUUCAG CAAAUGGAAAAUGCUAUUAAUUUGGUUGGUGACCACAAGAUAAAAUCAGUC CCACGUUGGCUCAGUUCAACUAGAUGUUCCCUGAUACAAAGAGAACUUGAU UUCCUAAAAACUGAAAAGCCAAACACAGCUAGCUGUCAUACAAGAAACAGC UAUUAUGAGACAUGAAGGAGGGUAAGAAUUAGCUUUAAGUUUUGUUUUGC UUUGUUUUGUUUUUUAACUCAACCUAUUAAUCAUCUCUUCACAAGAAUCCA CCUGAUGUGACCAAGCUAUUAUGUGUUGCCUGGAAAAACUGGCAAGAUUU CAGCUUAUGUGGCCUAGCAAACUAAGAAUUGCUCUUCUUGGCCAGCCUCA UAGCAUAAAAGAUGUGAACUCUAGGAAGUCUUUCUGAGUAGCAAUAAGUGG GAAUUAUGGGCAGAGCACACUCAAUCCCCUGUUGAUUAAUAAAACAGGCUG GACACUAAUUAACUAUGGGACUUAAAUCUGUAGAAAUGAAGGAGUCCAAUA GCUUCUUCCAAUUUUAAAACUCUAGUACAUCCCUUUCCCUCAAAUAUAUAU UUCUAAGAUAAAGAGAAAGAAGAGCACUAAGUAAGUAGAAUCUGUUUUUCC UAUUUUGUAGGGCUGCUGACUCCUAGUCCUUGAAGCCUAGACACAUGACC CAGGAAAUUUUUCCUUUGUUUCACUUUUGAUUAUGAUGUCUGAGCCAAAAA UUCAAUUAAGUAAACAUACUCGCCUGGAUCUGAAUCAUUCAUUUAAUUACU AGAUCUACCCAGCUGUUAUAUCAGGCCAAAAACAGAUUCGUGUUUAUAUAA AAGAGUAAACGAUGGUUGCAAAUUUUGGCUAUUUAGAGUUGCUACUUCACU AUGAAGAGUCACUUCAAAACACUUCGCUUGUCUUUAGGGAUGAUUUUUGCC AUUUCCAGUCCACGGUAUGAUACUAAAGCUGUCAAGAGAGGUUUCUUCUUU UCUGAAACUGCCAGCUCUUUCCAGCCCUGUUGAUCACUGGACAUAAAGCUU CUUUUCCCCAAUAAUUCUUCUUUACUUAAAAUAGUCAGGAUCUUUAUCUAC AGAUGUACUCUCCAGGUUACCUGUGAUGAUAGCCCCCUAAUGUCCUGCUA GAAAAGUCUCCAAGCAGAGAUGACAUUACUUCUGAAUGCUCAUAAACCACA CCAUGAAAUAAAAGCUCUUUGUUGUUUUAAGAUUGUGAAGUGUCGUUAAUG GGUCCCCACAGAUGGUCCCUGCUGGACUCACCUGGAAUCUCUCCACAGCC AUACCCACUCAUCACUAUCAUUGAGACCUGCACAUCUUAAUAGAAAUAUUA UAAACAUCGAAAUCAUGACUUACCUAGAAGUUCGCUUGUAACUAAUGAAA UUAAACAAAUGUGUUGCCUUUUGUCAUGUGUUUCUCUCCUGUGACAUUUC AAAAUAUCACAUCUUGAUAAAUAAUGUGUUUCAUCUUGAAUAGCUGAACUA AUUGCUUUGGAAACAGAGUCCUAGAAAAGUGACUUCAACAGAAUUGUUACU AAAAUUUGCACUCACAACAUGAAAUAAAUUUUCUUCCUAUGGAAUAAUCGU GAAAAAAAAAA U= Uridine and/or pseudouridine
SEQ ID NO: 15 Translated human TSHR from coding sequence (CDS) of the mRNA construct of SEQ ID NO: 14	MRPADLLQLVLLLDLPRDLGGMGCSSPPCECHQEEDFRVTCKDIQRIPSLPPSTQ TLKLIETHLRTIPSHAFSNLPNISRIYVSIDVTLQQLESHSFYNLSKVTH IEIRNTRNL TYIDPDALKELPLLKFLGIFNTGLKMFPDLTKVYSTDIFFILEITDNPYMTSIPVNAFQ GLCNETLTLKLYNNGFTSVQGYAFNGTKLDAVYLNKNKYLTVIDKDAFGGVYSGP SLLDVSQTSVTALPSKGLEHLKELIARNTWTLKKLPLSLSFLHLTRADLSYPSHCC AFKNQKKIRGILESLMCNESSMQSLRQRKSVNALNSPLHQEYEENLGDSIVGYKE KSKFQDTHNNAHYYVFFEEQEDEIIGFGQELKNPQEETLQAFDSHYDYTICGDSE DMVCTPKSDEFNPCEDIMGYKFLRIVVWFVSLLALLGNVFVLLILLTSHYKLNVPR FLMCNLAFADFCMGMYLLLIASVDLYTHSEYYNHAIDWQTGPGCNTAGFFTVFAS ELSVYTLTVITLERWYAITFAMRLDRKIRLRHACAIMVGGWVCCFLLALLPLVGISS YAKVSICLPMDTETPLALAYIVFVLTLN IVAFVIVCCCYVKIYITVRNPQYNPGDKDT KIAKRMAVLIFTDFICMAPISFYALSAILNKPLITVSNSKILLVLFYPLNSCANPFLYAI FTKAFQRDVFILLSKFGICKRQAQAYRGQRVPPKNSTDIQVQKVTHEMRQGLHN MEDVYELIENSHLTPKKQGQISEEYMQTVL

| SEQ ID NO: 45 (DNA)

TEV-hTSHR-2xhBG-120A

Sequence features:

Tobacco Etch Virus (TEV) 5' UTR: 37-190

Optimal Kozak sequence: 191-199

Human TSHR codon optimized, encoding amino acids Accession # NP_000360.2, 197-2488

2 stop codons: 2489-2495

2 copies of human beta-globin 3'UTR: 2513-2776

120 nucleotide polyA tail(SEQ ID NO: 59): 2785-2904 | GGAGGCCGGAGAATTGTAATACGACTCACTATAGGGAGACGCGTGTTAAATA
ACAAATCTCAACACAACATATACAAAACAAACGAATCTCAAGCAATCAAGCATT
CTACTTCTATTGCAGCAATTTAAATCATTTCTTTTAAAGCAAAAGCAATTTTCTG
AAAATTTTCACCATTTACGAACGATAGCCGCCACCATGAGGCCTGCCGACCT
GCTGCAGCTGGTGCTGCTGCTGGACCTGCCTAGAGATCTGGGCGGCATGGG
CTGTAGCAGCCCTCCATGCGAGTGCCACCAGGAAGAGGACTTCAGAGTGAC
CTGCAAGGACATCCAGAGAATCCCCAGCCTGCCCCCCAGCACCCAGACCCT
GAAGCTGATCGAGACACACCTGAGAACCATCCCTAGCCACGCCTTCAGCAAC
CTGCCCAACATCAGCAGAATCTACGTGTCCATCGACGTGACCCTGCAGCAGC
TGGAAAGCCACAGCTTCTACAACCTGAGCAAAGTGACCCACATCGAGATCAG
AAACACCCGGAACCTGACCTACATCGACCCCGACGCCCTGAAAGAGCTGCC
CCTGCTGAAGTTCCTGGGCATCTTCAACACCGGCCTGAAGATGTTCCCCGAC
CTGACCAAGGTGTACTCTACCGACATCTTCTTCATCCTGGAAATCACCGACAA
CCCCTACATGACCAGCATCCCCGTGAACGCCTTCCAGGGCCTGTGCAACGA
GACACTGACACTGAAGCTGTACAACAACGGCTTCACCAGCGTGCAGGGCTAC
GCCTTCAACGGCACAAAGCTGGACGCCGTGTACCTGAACAAGAACAAGTACC
TGACCGTGATCGACAAGGACGCCTTCGGCGGCGTGTACTCTGGACCTTCTCT
GCTGGACGTGTCCCAGACCAGCGTGACAGCCCTGCCTAGCAAGGGCCTGGA
ACACCTGAAAGAACTGATCGCCCGCAACACCTGGACTCTGAAGAAGCTGCCT
CTGAGCCTGAGCTTCCTGCACCTGACCAGAGCCGACCTGAGCTACCCAAGC
CACTGCTGCGCCTTCAAGAACCAGAAGAAGATCCGGGGAATCCTGGAATCCC
TGATGTGTAACGAGAGCAGCATGCAGAGCCTGAGACAGAGAAAGTCTGTGAA
CGCTCTGAACAGCCCCCTGCACCAGGAATACGAGGAAAACCTGGGCGACAG
CATCGTGGGCTACAAAGAGAAGTCCAAGTTCCAGGACACCCACAACAACGCC
CACTACTACGTGTTCTTCGAGGAACAGGAAGATGAGATCATCGGCTTCGGCC
AGGAACTGAAGAACCCTCAGGAAGAGACACTGCAGGCCTTCGACAGCCACTA
CGACTACACCATCTGCGGCGACAGCGAGGACATGGTGTGCACCCCTAAGAG
CGACGAGTTCAACCCCTGCGAGGATATTATGGGGTACAAGTTCCTGAGGATC
GTCGTGTGGTTCGTGTCCCTGCTGGCTCTGCTGGGCAACGTGTTCGTGCTGC
TGATCCTGCTGACCTCCCACTACAAGCTGAACGTGCCCAGATTCCTGATGTG
CAACCTGGCCTTCGCCGACTTCTGCATGGGCATGTACCTGCTGCTGATTGCC
AGCGTGGACCTGTACACCCACAGCGAGTACTACAACCACGCCATCGACTGGC
AGACCGGCCCTGGCTGTAACACCGCCGGCTTTTTCACCGTGTTCGCCAGCGA
GCTGAGCGTGTACACCCTGACAGTGATCACCCTGGAAAGGTGGTACGCCATC
ACCTTCGCCATGAGACTGGACAGAAAGATCAGACTGAGACACGCCTGCGCCA
TCATGGTGGGAGGCTGGGTGTGCTGTTTCCTGCTGGCCCTGCTGCCCCTCGT
GGGCATCAGCTCTTACGCCAAGGTGTCCATCTGCCTGCCCATGGACACCGAG
ACACCTCTGGCCCTGGCCTACATTGTGTTTGTGCTGACCCTGAACATCGTGG
CCTTCGTGATCGTGTGCTGCTGTTACGTGAAGATCTACATCACCGTGCGGAA
CCCCCAGTACAACCCCGGCGACAAGGATACCAAGATCGCCAAGAGAATGGC
CGTGCTGATCTTCACCGACTTCATCTGCATGGCCCCCATCAGCTTCTATGCCC
TGAGCGCCATTCTGAACAAGCCTCTGATCACCGTGTCCAACAGCAAAATCCT
GCTGGTGCTGTTCTACCCCCTGAACAGCTGCGCCAACCCCTTCCTGTACGCT
ATCTTCACCAAGGCCTTCCAGAGGGACGTGTTCATCCTGCTGTCTAAGTTCG
GCATCTGCAAGAGACAGGCCCAGGCCTACCGGGGGCCAGAGAGTGCCTCCTA
AGAACTCCACAGACATCCAGGTGCAGAAAGTGACACACGACATGAGACAGGG
CCTGCACAACATGGAAGATGTGTACGAGCTGATTGAGAACAGCCACCTGACC
CCCAAGAACAGGGACAGATCAGCGAAGAGTACATGCAGACCGTGCTGTGAT
AACGGACCGGCGATAGATGAAGCTCGCTTTCTTGCTGTCCAATTTCTATTAAA
GGTTCCTTTGTTCCCTAAGTCCAACTACTAAACTGGGGGATATTATGAAGGGC
CTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTATTTTCATTGCAGCTCGC
TTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTAC
TAAACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAA
AAACATTTATTTTCATTGCGGCCGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA |

| SEQ ID NO: 16 (mRNA) TEV-hTSHR-2xhBG-120A Sequence features: Tobacco Etch Virus (TEV) 5' UTR: 37-190 Optimal Kozak sequence: 191-199 Human TSHR codon optimized, encoding amino acids Accession # NP_000360.2, 197-2488 2 stop codons: 2489-2495 2 copies of human beta-globin 3'UTR: 2513-2776 120 nucleotide polyA tail (SEQ ID NO: 59): 2785-2904 | GGGAGACGCGUGUUAAAUAACAAAUCUCAACACAACAUAUACAAAACAAACG AAUCUCAAGCAAUCAAGCAUUCUACUUCUAUUGCAGCAAUUUAAAUCAUUU CUUUUAAAGCAAAAGCAAUUUUCUGAAAAUUUUCACCAUUUACGAACGAUA GCCGCCACCAUGAGGCCUGCCGACCUGCUGCAGCUGGUGCUGCUGCUGG ACCUGCCUAGAGAUCUGGGCGGCAUGGGCUGUAGCAGCCCUCCAUGCGAG UGCCACCAGGAAGAGGACUUCAGAGUGACCUGCAAGGACAUCCAGAGAAUC CCCAGCCUGCCCCCCAGCACCCAGACCCUGAAGCUGAUCGAGACACACCU GAGAACCAUCCCUAGCCACGCCUUCAGCAACCUGCCCAACAUCAGCAGAAU CUACGUGUCCAUCGACGUGACCCUGCAGCAGCUGGAAAGCCACAGCUUCU ACAACCUGAGCAAAGUGACCCACAUCGAGAUCAGAAACACCCGGAACCUGA CCUACAUCGACCCCGACGCCCUGAAGAGCUGCCCCUGCUGAAGUUCCUG GGCAUCUUCAACACCGGCCUGAAGAUGUUCCCCGACCUGACCAAGGUGUA CUCUACCGACAUCUUCUUCAUCCUGGAAAUCACCGACAACCCCUACAUGAC CAGCAUCCCCGUGAACGCCUUCCAGGGCCUGUGCAACGAGACACUGACAC UGAAGCUGUACAACAACGGCUUCACCAGCGUGCAGGGCUACGCCUUCAAC GGCACAAAGCUGGACGCCGUGUACCUGAACAAGAACAAGUACCUGACCGU GAUCGACAAGGACGCCUUCGGCGGCGUGUACUCUGGACCUUCUCUGCUGG ACGUGUCCCAGACCAGCGUGACAGCCCUGCCUAGCAAGGGCCUGGAACAC CUGAAAGAACUGAUCGCCCGCAACACCUGGACUCUGAAGAAGCUGCCUCU GAGCCUGAGCUUCCUGCACCUGACCAGAGCCGACCUGAGCUACCCAAGCC ACUGCUGCGCCUUCAAGAACCAGAAGAAGAUCCGGGGGAAUCCUGGAAUCC CUGAUGUGUAACGAGAGCAGCAUGCAGAGCCUGAGACAGAGAAAGUCUGU GAACGCUCUGAACAGCCCCCUGCACCAGGAAUACGAGGAAAACCUGGGCG ACAGCAUCGUGGGCUACAAAGAGAAGUCCAAGUUCCAGGACACCCACAACA ACGCCCACUACUACGUGUUCUUCGAGGAACAGGAAGAUGAGAUCAUCGGC UUCGGCCAGGAACUGAAGAACCCUCAGGAAGAGACACUGCAGGCCUUCGA CAGCCACUACGACUACACCAUCUGCGGCGACAGCGAGGACAUGGUGUGCA CCCCUAAGAGCGACGAGUUCAACCCCUGCGAGGAUAUUAUGGGGUACAAG UUCCUGAGGAUCGUCGUGUGGUUCGUGUCCCUGCUGGCUCUGCUGGGCA ACGUGUUCGUGCUGCUGAUCCUGCUGACCUCCCACUACAAGCUGAACGUG CCCAGAUUCCUGAUGUGCAACCUGGCCUUCGCCGACUUCUGCAUGGGCAU GUACCUGCUGCUGAUUGCCAGCGUGGACCUGUACACCCACAGCGAGUACU ACAACCACGCCAUCGACUGGCAGACCGGCCCUGGCUGUAACACCGCCGGC UUUUUCACCGUGUUCGCCAGCGAGCUGAGCGUGUACACCCUGACAGUGAU CACCCUGGAAAGGUGGUACGCCAUCACCUUCGCCAUGAGACUGGACAGAA AGAUCAGACUGAGACACGCCUGCGCCAUCAUGGUGGGAGGCUGGGUGUGC UGUUUCCUGCUGGCCCUGCUGCCCCUCGUGGGCAUCAGCUCUUACGCCAA GGUGUCCAUCUGCCUGCCCAUGGACACCGAGACACCUCUGGCCCUGGCCU ACAUUGUGUUUGUGCUGACCCUGAACAUCGUGGCCUUCGUGAUCGUGUGC UGCUGUUACGUGAAGAUCUACAUCACCGUGCGGAACCCCCAGUACAACCCC GGCGACAAGGAUACCAAGAUCGCCAAGAGAAUGGCCGUGCUGAUCUUCAC CGACUUCAUCUGCAUGGCCCCCAUCAGCUUCUAUGCCCUGAGCGCCAUUC UGAACAAGCCUCUGAUCACCGUGUCCAACAGCAAAAUCCUGCUGGUGCUG UUCUACCCCCUGAACAGCUGCGCCAACCCCUUCCUGUACGCUAUCUUCACC AAGGCCUUCCAGAGGGACGUGUUCAUCCUGCUGUCUAAGUUCGGCAUCUG CAAGAGACAGGCCCAGGCCUACCGGGGCCAGAGAGUGCCUCCUAAGAACU CCACAGACAUCCAGGUGCAGAAAGUGACACGACAUGAGACAGGGCCUGC ACAACAUGGAAGAUGUGUACGAGCUGAUUGAGAACAGCCACCUGACCCCCA AGAAACAGGGACAGAUCAGCGAAGAGUACAUGCAGACCGUGCUGUGAUAAC GGACCGGCGAUAGAUGAAGCUCGCUUUCUUGCUGUCCAAUUUCUAUUAAA GGUUCCUUUGUUCCCUAAGUCCAACUACUAAACUGGGGGAUAUUAUGAAG GGCCUUGAGCAUCUGGAUUCUGCCUAAUAAAAAACAUUUAUUUUCAUUGCA GCUCGCUUUCUUGCUGUCCAAUUUCUAUUAAAGGUUCCUUUGUU |

	CCCUAAGUCCAACUACUAAACUGGGGGAUAUUAUGAAGGGCCUUGAGCAU CUGGAUUCUGCCUAAUAAAAAACAUUUAUUUUCAUUGCGGCCGCAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAA U= Uridine and/or pseudouridine
SEQ ID NO: 46 TSHR RNA coding sequence of construct of SEQ ID NO: 16	AUGAGGCCUGCCGACCUGCUGCAGCUGGUGCUGCUGCUGGACCUGCCUA GAGAUCUGGGCGGCAUGGGCUGUAGCAGCCCUCCAUGCGAGUGCCACCA GGAAGAGGACUUCAGAGUGACCUGCAAGGACAUCCAGAGAAUCCCCAGCC UGCCCCCCAGCACCCAGACCCUGAAGCUGAUCGAGACACACCUGAGAACCA UCCCUAGCCACGCCUUCAGCAACCUGCCCAACAUCAGCAGAAUCUACGUGU CCAUCGACGUGACCCUGCAGCAGCUGGAAAGCCACAGCUUCUACAACCUG AGCAAAGUGACCCACAUCGAGAUCAGAAACACCCGGAACCUGACCUACAUC GACCCCGACGCCCUGAAGAGCUGCCCCUGCUGAAGUUCCUGGGCAUCUU CAACACCGGCCUGAAGAUGUUCCCCGACCUGACCAAGGUGUACUCUACCG ACAUCUUCUUCAUCCUGGAAAUCACCGACAACCCCUACAUGACCAGCAUCC CCGUGAACGCCUUCCAGGGCCUGUGCAACGAGACACUGACACUGAAGCUG UACAACAACGGCUUCACCAGCGUGCAGGGCUACGCCUUCAACGGCACAAA GCUGGACGCCGUGUACCUGAACAAGAACAAGUACCUGACCGUGAUCGACA AGGACGCCUUCGGCGGCGUGUACUCUGGACCUUCUCUGCUGGACGUGUC CCAGACCAGCGUGACAGCCCUGCCUAGCAAGGGCCUGGAACACCUGAAAG AACUGAUCGCCCGCAACACCUGGACUCUGAAGAAGCUGCCUCUGAGCCUG AGCUUCCUGCACCUGACCAGAGCCGACCUGAGCUACCCAAGCCACUGCUG CGCCUUCAAGAACCAGAAGAAGAUCCGGGGGAAUCCUGGAAUCCCUGAUGU GUAACGAGAGCAGCAUGCAGAGCCUGAGACAGAGAAAGUCUGUGAACGCU CUGAACAGCCCCCUGCACCAGGAAUACGAGGAAACCUGGGCGACAGCAU CGUGGGCUACAAAGAGAAGUCCAAGUUCCAGGACACCCACAACAACGCCCA CUACUACGUGUUCUUCGAGGAACAGGAAGAUGAGAUCAUCGGCUUCGGCC AGGAACUGAAGAACCCUCAGGAAGAGACACUGCAGGCCUUCGACAGCCAC UACGACUACACCAUCUGCGGCGACAGCGAGGACAUGGUGUGCACCCCUAA GAGCGACGAGUUCAACCCCUGCGAGGAUAUUAUGGGGUACAAGUUCCUGA GGAUCGUCGUGUGGUUCGUGUCCCUGCUGGCUCUGCUGGGCAACGUGUU CGUGCUGCUGAUCCUGCUGACCUCCCACUACAAGCUGAACGUGCCCAGAU UCCUGAUGUGCAACCUGGCCUUCGCCGACUUCUGCAUGGGCAUGUACCUG CUGCUGAUUGCCAGCGUGGACCUGUACACCCACAGCGAGUACUACAACCA CGCCAUCGACUGGCAGACCGGCCCUGGCUGUAACACCGCCGGCUUUUUCA CCGUGUUCGCCAGCGAGCUGAGCGUGUACACCCUGACAGUGAUCACCCUG GAAAGGUGGUACGCCAUCACCUUCGCCAUGAGACUGGACAGAAAGAUCAG ACUGAGACACGCCUGCGCCAUCAUGGUGGGAGGCUGGGUGUGCUGUUUC CUGCUGGCCCUGCUGCCCCUCGUGGGCAUCAGCUCUUACGCCAAGGUGU CCAUCUGCCUGCCCAUGGACACCGAGACACCUCUGGCCCUGGCCUACAUU GUGUUUGUGCUGACCCUGAACAUCGUGGCCUUCGUGAUCGUGUGCUGCU GUUACGUGAAGAUCUACAUCACCGUGCGGAACCCCCAGUACAACCCCGGC GACAAGGAUACCAAGAUCGCCAAGAGAAUGGCCGUGCUGAUCUUCACCGA CUUCAUCUGCAUGGCCCCCAUCAGCUUCUAUGCCCUGAGCGCCAUUCUGA ACAAGCCUCUGAUCACCGUGUCCAACAGCAAAAUCCUGCUGGUGCUGUUC UACCCCCUGAACAGCUGCGCCAACCCCUUCCUGUACGCUAUCUUCACCAA GGCCUUCCAGAGGGACGUGUUCAUCCUGCUGUCUAAGUUCGGCAUCUGCA AGAGACAGGCCCAGGCCUACCGGGGCCAGAGAGUGCCUCCUAAGAACUCC ACAGACAUCCAGGUGCAGAAAGUGACACGACAUGAGACAGGGCCUGCA CAACAUGGAAGAUGUGUACGAGCUGAUUGAGAACAGCCACCUGACCCCCAA GAAACAGGGACAGAUCAGCGAAGAGUACAUGCAGACCGUGCUGUGAUAA

<u>V.</u>   <u>APJ</u>

[00281]   Apelin receptor, also referred to as APJ, angiotension-like-1 receptor, angiotension II-like-1 receptor, and the like, is the previously orphan G-protein-coupled receptor (GPCR) that is cognate for the endogenous ligand Apelin. The apelin/APJ pathway is widely expressed in the cardiovascular system and apelin has shown major beneficial cardiovascular effects in preclinical models. Acute apelin administration in humans causes peripheral and coronary vasodilatation and increases cardiac output (Circulation. 2010; 121 :1818-1 827). As a result, APJ agonism is emerging as an important therapeutic target for patients with heart failure. Activation of the apelin receptor APJ is thought to increase cardiac contractility and provide cardioprotection, without the liabilities of current therapies.

[00282]   APJ is widely distributed not only in the heart but also in other organs and tissues including vessels, kidney, liver, adipose tissue and brain.

[00283]   The full length coding sequence of human APJ (e.g., Protein Accession No. NPJD05152.1) was codon optimized for expression in human cells and cloned into a vector that can sustain mRNA transcription by T7 polymerase and contains both 3 and 5' untranslated regions that help with mRNA stability and translatability (see Table 5 for sequence). mRNA was *in vitro* transcribed and encapsulated into lipid nanoparticles as described above.

TABLE 5
Exemplary APJ Polynucleotide and Polypeptide Sequences

SEQ ID NO: and features	Sequence

| SEQ ID NO: 47

APJ native DNA sequence corresponding to Protein Accession # NP_005152.1 | GGAAAGCCGACTTGCAAAACCACAGATAATGTTCAGCCCAGCACAGTAGG
GGTCAATTTGGTCCACTTGCTCAGTGACAAAAAGAAAAAAAAAGTGGGCT
GTCACTAAAGATTTTGACTCACAAGAGAGGGGCTGGTCTGGAGGTGGGA
GGAGGGAGTGACGAGTCAAGGAGGAGACAGGGACGCAGGAGGGTGCA
AGGAAGTGTCTTAACTGAGACGGGGGTAAGGCAAGAGAGGGTGGAGGA
AATTCTGCAGGAGACAGGCTTCCTCCAGGGTCTGGAGAACCCAGAGGCAG
CTCCTCCTGAGTGCTGGGAAGGACTCTGGGCATCTTCAGCCCTTCTTACTC
TCTGAGGCTCAAGCCAGAAATTCAGGCTGCTTGCAGAGTGGGTGACAGAG
CCACGGAGCTGGTGTCCCTGGGACCCTCTGCCCGTCTTCTCTCCACTCCCC
AGCATGGAGGAAGGTGGTGATTTTGACAACTACTATGGGGCAGACAACC
AGTCTGAGTGTGAGTACACAGACTGGAAATCCTCGGGGGCCCTCATCCCT
GCCATCTACATGTTGGTCTTCCTCCTGGGCACCACGGGCAACGGTCTGGTG
CTCTGGACCGTGTTTCGGAGCAGCCGGGAGAAGAGGCGCTCAGCTGATAT
CTTCATTGCTAGCCTGGCGGTGGCTGACCTGACCTTCGTGGTGACGCTGCC
CCTGTGGGCTACCTACACGTACCGGGACTATGACTGGCCCTTTGGGACCTT
CTTCTGCAAGCTCAGCAGCTACCTCATCTTCGTCAACATGTACGCCAGCGT
CTTCTGCCTCACCGGCCTCAGCTTCGACCGCTACCTGGCCATCGTGAGGCC
AGTGGCCAATGCTCGGCTGAGGCTGCGGGTCAGCGGGGCCGTGGCCACG
GCAGTTCTTTGGGTGCTGGCCGCCCTCCTGGCCATGCCTGTCATGGTGTTA
CGCACCACCGGGGACTTGGAGAACACCACTAAGGTGCAGTGCTACATGGA
CTACTCCATGGTGGCCACTGTGAGCTCAGAGTGGGCCTGGGAGGTGGGCC
TTGGGGTCTCGTCCACCACCGTGGGCTTTGTGGTGCCCTTCACCATCATGC
TGACCTGTTACTTCTTCATCGCCCAAACCATCGCTGGCCACTTCCGCAAGG
AACGCATCGAGGGCCTGCGGAAGCGGCGCCGGCTGCTCAGCATCATCGT
GGTGCTGGTGGTGACCTTTGCCCTGTGCTGGATGCCCTACCACCTGGTGA
AGACGCTGTACATGCTGGGCAGCCTGCTGCACTGGCCCTGTGACTTTGACC
TCTTCCTCATGAACATCTTCCCCTACTGCACCTGCATCAGCTACGTCAACAG
CTGCCTCAACCCCTTCCTCTATGCCTTTTTCGACCCCCGCTTCCGCCAGGCC
TGCACCTCCATGCTCTGCTGTGGCCAGAGCAGGTGCGCAGGCACCTCCCA
CAGCAGCAGTGGGGAGAAGTCAGCCAGCTACTCTTCGGGGCACAGCCAG
GGGCCCGGCCCCAACATGGGCAAGGGTGGAGAACAGATGCACGAGAAAT
CCATCCCCTACAGCCAGGAGACCCTTGTGGTTGACTAGGGCTGGGAGCAG
AGAGAAGCCTGGCGCCCTCGGCCCTCCCCGGCCTTTGCCCTTGCTTTCTGA
AAATCAGGTAGTGTGGCTACTCCTTGTCCTATGCACATCCTTTAACTGTCCC
CTGATTCTGCCCCGCCCTGTCCTCCTCTACTGCTTTATTCTTTCTCAGAGGTT
TGTGGTTTAGGGGAAAGAGACTGGGCTCTACAGACCTGACCCTGCACAAG
CCATTTAATCTCACTCAGCCTCAGTTTCTCCATTGGTATGAAATGGGGGAA
AGTCATATTGATCCTAAAATGTTGAAGCCTGAGTCTGGACGCAGTAAAAG
CTTGTTTCCCTCTGCTGCTTTCTTAGATCTGCAATCGTCTTTCCTCCCTTCTTT
CCTTGTAGTTTTTCCCCCACCACTCTCTGCAGCTGCCGCTCCTTATCCCTGCC
TTCTGGCACCAATCCCCTCCTACAGCTCGTCCCCCTCCCTCCATCCATCCTTC
TCCCCTGTCTACTTTCTTGTTCTGAAGGGCTACTAAGGGTTAAGGATCCCA
AAGCTTGCAGAGACTGACCCTGTTTAAGCTTTCTATCCTGTTTTCTGAGTGT
GAGGCAGGGAATGGGCTGGGGCCGGGGGTGGGCTGTGTGTCAGCAGAT
AATTAGTGCTCCAGCCCTTAGATCTGGGAGCTCCAGAGCTTGCCCTAAAAT
TGGATCACTTCCCTGTCATTTTG GGCATTG GGGCTAGTGTG ATTCCTGCAG
TTCCCCCATGGCACCATGACACTGACTAGATATGCTTTCTCCAAATTGTCCG
CAGACCCTTTCATCCTTCCTCTATTTTCTATGAGAATTGGAAGGCAGCAGG
GCTGATGAATGGATGTACTCCTTGGTTTCATTATGTGAGTGGGGGAGTTGG |

GAAGGGCAACTAGAGAGAGAGGATGGAGGGGTGTCTGCATTTAGTCCAG
ACACTGCTTGGCTCGCTCCCCGAGTCCTCCTGTTTCTGACTTCCTGCATAAC
TGTGAGCTGAAGGGTTTCCTCATCTCCCCATCTTACCCCATCATACTGATTT
CTTTCTTGGGCACTGGTGCTACTTGGTGCCAAGAATCATGTTGTTTGGGAT
GGAGATGCCTGCCTCTTGTCTGTGTGTGTTGTACTTATATGTCTATATGGAT
GAGCCTGGCATGAACAGCAGTGTGCCTGGGTCATTTGGACAAACCTCCTC
CCACCCCCCAATCCACTGCAACTCTGCTGTTCACACATTACCCTTGGCAGG
GGGTGGTGGGGGGCAGGGACACACTGAGGCAATGAAAAATGTAGAATA
AAAATGAGTCCACCCCCTACTGGATTTGGGGGCTCCAACGGCTGGTCCGT
GCTTTAGGAGCGAAGTTAATGTTTGCACCAGGCTTCCTGTAGGGAGATCC
CTCCCCAAAGCAGCTGGCGCCAAGGCTTGGGGGCGTCCTACTGAGCTGGG
TTCCTGCTCCTTCTTGGGCTCCATGAAGGAAGTAAGAGGCTAGTTGAGAG
CCTCCCTTGGCCCCTTTCCGGTGCCTCCCCGCCTGGCTTCAAATTTATGAGC
ATTGCCCTCATCGTCCTTTCTTGTTCCAGGGTCAGTGGCCCTCTTCCTAAGG
AGGCCTCCTGCTTGCCATGGGCCAAAAGGCACGGGGTGGGTTTTTTCTCTC
CCTACCCTCAGGATTGGACCTCTTGGCTTCTGCTGGATTGGGGATCTGGGA
ATAGGGACTGGAGCAAGTGTGCAGATAGCATGATGTCTACACTGCCAGAG
AGACCGTGAGGATGAAATTAATAGTGGGGCCTTTGTGAGCTAGAGGCTG
GGAGTGTCTATTCCGGGTTTTGTTCTTGGAGGACTATGAAAGTGAAGGAC
AAGACATGAGCGATGGAGATAAGAAAAGCCCAGCTTGATGTGAATGGAC
ATCTTGACCCTCCCTGGAATGACGCCAGCTCTGGGGGCAGAGGGAGGAG
GAGAGGGGAAGGGGCTCCTCACAGCCTAGTCTCCCCATCTTAAGATAGCA
TCTTTCACAGAGTCACCTCCTCTGCCCAGAGCTGTCCTCAAAGCATCCAGT
GAACACTGGAAGAGGCTTCTAGAAGGGAAGAAATTGTCCCTCTGAGGCC
GCCGTGGGTGACCTGCAGAGACTTCCTGCCTGGAACTCATCTGTGAACTG
GGACAGAAGCAGAGGAGGCTGCCTGCTGTGATACCCCCTTACCTCCCCCA
GTGCCTTCTTCAGAATATCTGCACTGTCTTCTGATCCTGTTAGTCACTGTGG
TTCATCAAATAAAACTGTTTGTGCAACTGTTGTGTCCAAA

| SEQ ID NO: 17 APJ Native mRNA sequence corresponding to Protein Accession # NP_0051 52.1 | GGAAAGCCGACUUGCAAAACCACAGAUAAUGU UCAGCCCAGCACAGUA GGGGUCAA UUUGGUCCACUUGCUCAG UGACAAAAAG AAAAAAAAG U GGGCUG UCACUAAAGAU UUUGACUCACAAGAGAGGGGCUGG UCUGGA GGUGGGAGGAGGGAGUGACGAGUCAAGGAGGAGACAGGGACGCAGGA GGGUGCAAGGAAGUGUCU UAACUGAGACGGGGG UAAGGCAAGAGAG GGUGGAGGAAAU UCUGCAGGAGACAGGCU UCCUCCAGGGUCUGGAGA ACCCAGAGGCAGCUCCUCCUGAGUGCUGGGAAGGACUCUGGGCAUCU UCAGCCCU UCU UACUCUCUGAGGCUCAAGCCAGAAAUUCAGGCUGCU U GCAGAG UGGGUGACAGAGCCACGGAGCUGGUGUCCCUGGGACCCUCU GCCCGUCUUCUCUCCACUCCCCAGCAUGGAGGAAGGUGGUGAUU UUG ACAACUACUAUGGGGCAGACAACCAGUCUGAGUGUGAGUACACAGACU GGAAAUCCUCGGGGGCCCUCAUCCCUGCCAUCUACAUGU UGGUCUUCC UCCUGGGCACCACGGGCAACGGUCUGGUGCUCUGGACCGUG UUUCGG AGCAGCCGGGAGAAGAGGCGCUCAGCUGAUAUCUUCAUUGCUAGCCU GGCGGUGGCUGACCUGACCUUCG UGG UGACGCUGCCCCUGUGGGCUA CCUACACGUACCGGGACUAUGACUGGCCCUU UGGGACCU UCUUCUGCA AGCUCAGCAGCUACCUCAUCU UCGUCAACAUGUACGCCAGCGUCU UCU GCCUCACCGGCCUCAGCUUCGACCGCUACCUGGCCAUCGUGAGGCCAG UGGCCAAUGCUCGGCUGAGGCUGCGGGUCAGCGGGGCCGUGGCCACG GCAGU UCUU UGGG UGCUGGCCGCCCUCCUGGCCAUGCCUGUCAUGGU G UUACGCACCACCGGGGACUUGGAGAACACCACUAAGGUGCAGUGCUA CAUGGACUACUCCAUGGUGGCCACUGUGAGCUCAGAGUGGGCCUGGG AGGUGGGCCU UGGGGUCUCG UCCACCACCGUGGGCU UUGUGGUGCCC UUCACCAUCAUGCUGACCUGU UACU UCU UCAUCGCCCAAACCAUCGCU GGCCACU UCCGCAAGGAACGCAUCGAGGGCCUGCGGAAGCGGCGCCGG CUGCUCAGCAUCAUCGUGG UGCUGG UGGUGACCU UUGCCCUGUGCUG GAUGCCCUACCACCUGG UGAAGACGCUGUACAUGCUGGGCAGCCUGCU GCACUGGCCCUGUGACU UUGACCUCUUCCUCAUGAACAUCU UCCCCUA CUGCACCUGCAUCAGCUACGUCAACAGCUGCCUCAACCCCU UCCUCUAU GCCU UU UUCGACCCCCGCUUCCGCCAGGCCUGCACCUCCAUGCUCUGC UG UGGCCAGAGCAGGUGCGCAGGCACCUCCCACAGCAGCAGUGGGGAG AAGUCAGCCAGCUACUCU UCGGGGCACAGCCAGGGGCCCGGCCCCAAC AUGGGCAAGGGUGGAGAACAGAUGCACGAGAAAUCCAUCCCCUACAGC CAGGAGACCCU UGUGGU UGACUAGGGCUGGGAGCAGAGAGAAGCCUG GCGCCCUCGGCCCUCCCCGGCCU UUGCCCU UGCUU UCUGAAAAUCAGG UAGUGUGGCUACUCCUUGUCCUAUGCACAUCCU U UAACUGUCCCCUG AUUCUGCCCCGCCCUGUCCUCCUCUACUGCUU UAU UCU UUCUCAGAGG UU UGUGGU UUAGGGGAAAGAGACUGGGCUCUACAGACCUGACCUGC ACAAGCCAU UUAAUCUCACUCAGCCUCAGUU UCUCCAU UGG UAUGAAA UGGGGGAAAGUCAUAUUGAUCCUAAAAUGU UGAAGCCUGAGUCUGGA CGCAGUAAAAGCU UGU UUCCCUCUGCUGCU UUCUUAGAUCUGCAAUC GUCUU UCCUCCCU UCU UUCCU UGUAGU UU UUCCCCCACCACUCUCUGC AGCUGCCGCUCCUUAUCCCUGCCUUCUGGCACCAAUCCCCUCCUACAGC UCGUCCCCCUCCCUCCAUCCAUCCU UCUCCCCUGUCUACUU UCUUGUU CUGAAGGGCUACUAAGGGU UAAGGAUCCCAAAGCU UGCAGAGACUGA CCCUGUU UAAGCUU UCUAUCCUG UU UUCUGAGUGUGAGGCAGGGAA UGGGCUGGGGCCGGGGGUGGGCUGUGUGUCAGCAGAUAAU UAGUGC UCCAGCCCU UAGAUCUGGGAGCUCCAGAGCU UGCCCUAAAAU UGGAUC ACU UCCCUG UCAUU UUGGGCAUUGGGGCUAGUGUGAUUCCUGCAGU |

UCCCCCAUGGCACCAUGACACUGACUAGAUAUGCUU UCUCCAAAUUGU
CCGCAGACCCUU UCAUCCU UCCUCUAUU UUCUAUGAGAAUUGGAAGG
CAGCAGGGCUGAUGAAUGGAUGUACUCCU UGGU UUCAUUAUGUGAG
UGGGGAGUUGGGAAGGGCAACUAGAGAGAGAGGAUGGAGGGGUGUC
UGCAU UUAGUCCAGACACUGCU UGGCUCGCUCCCCGAGUCCUCCUGU U
UCUGACU UCCUGCAUAACUGUGAGCUGAAGGGU UUCCUCAUCUCCCC
AUCU UACCCCAUCAUACUGAU UUCUU UCU UGGGCACUGGUGCUACU U
GGUGCCAAGAAUCAUGU UGU UUGGGAUGGAGAUGCCUGCCUCU UGU
CUGUGUGUGU UGUACUUAUAUGUCUAUAUGGAUGAGCCUGGCAUGA
ACAGCAGUGUGCCUGGGUCAUU UGGACAAACCUCCUCCCACCCCCCAA
UCCACUGCAACUCUGCUGU UCACACAU UACCCU UGGCAGGGGGUGG U
GGGGGGCAGGGACACACUGAGGCAAUGAAAAAUGUAGAAUAAAAAUG
AGUCCACCCCCUACUGGAU UUGGGGGGCUCCAACGGCUGGUCCG UGCU
UUAGGAGCGAAGUUAAUGU UUGCACCAGGCUUCCUGUAGGGAGAUCC
CUCCCCAAAGCAGCUGGCGCCAAGGCU UGGGGGGCG UCCUACUGAGCUG
GGUUCCUGCUCCU UCU UGGGCUCCAUGAAGGAAGUAAGAGGCUAGU U
GAGAGCCUCCCU UGGCCCCU UUCCGGUGCCUCCCCGCCUGGCU UCAAA
UU UAUGAGCAUUGCCCUCAUCGUCCUU UCU UG UUCCAGGGUCAG UGG
CCCUCU UCCUAAGGAGGCCUCCUGCU UGCCAUGGGCCAAAAGGCACGG
GGUGGGUU UUU UCUCUCCCUACCCUCAGGAU UGGACCUCUUGGCU UC
UGCUGGAUUGGGGAUCUGGGAAUAGGGACUGGAGCAAGUGUGCAGA
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UUGU UCU UGGAGGACUAUGAAAG UGAAGGACAAGACAUGAGCGAUG
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GGGCUCCUCACAGCCUAGUCUCCCCAUCUUAAGAUAGCAUCUU UCACA
GAGUCACCUCCUCUGCCCAGAGCUG UCCUCAAAGCAUCCAGUGAACAC
UGGAAGAGGCUUCUAGAAGGGAAGAAAU UG UCCCUCUGAGGCCGCCG
UGGGUGACCUGCAGAGACU UCCUGCCUGGAACUCAUCUGUGAACUGG
GACAGAAGCAGAGGAGGCUGCCUGCUGUGAUACCCCCUUACCUCCCCC
AGUGCCUUCUUCAGAAUAUCUGCACUG UCU UCUGAUCCUGU UAGUCA
CUGUGGUUCAUCAAAUAAACUGU UUGUGCAACUGU UGUGUCCAAA

U= Uridine and/or pseudouridine

SEQ ID NO: 18 Translated human APJ from coding sequence (CDS) of the DNA construct of SEQ ID NO: 47	M EEGGDFDNYYGADNQSECEYTDWKSSGALI PAIYM LVFLLGTTGNGLVLW TVFRSSREKRRSADI FIASLAVADLTFVVTLPLWATYTYRDYDWPFGTFFCKLSS YLI FVN MYASVFCLTGLSFDRYLAIVRPVANARLRLRVSGAVATAVLWVLAALL A M PVMVLRTTGDLENTTKVQCYM DYSMVATVSSEWAWEVGLGVSSTTVG FVVPFTI M LTCYFFIAQTIAGH FRKERI EGLRKRRRLLSI IVVLVVTFALCWM PYH LVKTLYMLGSLLHWPCDFDLFLM NIFPYCTCISYVNSCLNPFLYAFFDPRF RQACTSMLCCGQSRCAGTSHSSSGEKSASYSSGHSQGPGPNMGK GGEQMHEKSIPYSQETLVVD
SEQ ID NO: 48 (DNA) TEV-hAPJ-2xhBG-120A	GGAGGCCGGAGAATTGTAATACGACTCACTATAGGGAGACGCGTGTTAA ATAACAAATCTCAACACAACATATACAAAACAAACGAATCTCAAGCAATC AAGCATTCTACTTCTATTGCAGCAATTTAAATCATTTCTTTTAAAGC AAAAGCAATTTTCTGAAAATTTTCACCATTTACGAACGATAGCCGC CACCATGGAAGAGGGCGGCGACTTCGACAACTACTACGGCGCC GACAACCAGAGCGAGTGCGAGTACACCGACTGGAAGTCCTCTGG

Sequence features: Tobacco Etch Virus (TEV) 5' UTR: 37-1 90 Optimal Kozak sequence: 19 1- 199 Human APJ codon optimized, encoding amino acids 1-380 of Protein Accession #NP_0051 52.1 : 197-1336 1 stop codon : 1337-1349 2 copies of human beta-globin 3'UTR: 1358-1 623 120 nucleotide polyA tail (SEQ ID NO: 59): 1630-1 749	CGCCCTGATCCCCGCTATCTACATGCTGGTGTTTCTGCTGGGCA CCACCGGCAACGGACTGGTGCTGTGGACCGTGTTCAGAAGCAG CAGAGAGAAGCGGCGGAGCGCCGACATCTTTATCGCCAGCCTG GCCGTGGCCGACCTGACCTTTGTCGTGACACTGCCTCTGTGGGC CACCTACACCTACCGGGACTACGACTGGCCCTTCGGCACATTTT CTGCAAGCTGAGCAGCTACCTGATCTTCGTGAATATGTACGCCAG CGTGTTCTGCCTGACCGGCCTGAGCTTCGACAGATACCTGGCCA TCGTGCGGCCCGTGGCCAACGCTAGACTGCGGCTGAGAGTGTC TGGCGCCGTGGCTACAGCTGTGCTGTGGGTGCTGGCTGCCCTG CTGGCTATGCCTGTGATGGTGCTGAGAACCACCGGCGACCTGGA AAACACCACCAAGGTGCAGTGCTACATGGACTACAGCATGGTGG CCACAGTGTCCAGCGAGTGGGCCTGGGAAGTGGGACTGGGAGT GTCTAGCACCACCGTGGGCTTCGTGGTGCCCTTCACCATTATGC TGACCTGCTACTTCTTCATTGCCCAGACAATCGCCGGCCACTTCC GGAAAGAGCGGATCGAGGGCCTGCGGAAGAGAAGGCGGCTGCT GAGCATCATCGTGGTGCTGGTCGTGACCTTCGCCCTGTGCTGGA TGCCTTACCACCTCGTGAAAACCCTGTATATGCTGGGCAGCCTG CTGCACTGGCCCTGCGATTTCGACCTGTTCCTGATGAACATCTTC CCCTACTGCACCTGTATCAGCTACGTGAACAGCTGCCTGAACCC CTTCCTGTACGCCTTCTTCGACCCCCGGTTCAGACAGGCCTGCA CCTCCATGCTGTGCTGCGGCCAGTCTAGATGCGCCGGCACAAGC CACAGCAGCAGCGGCGAGAAGTCTGCCAGCTACAGCTCTGGCC ACAGCCAGGGCCCAGGCCCCAATATGGGAAAGGGCGGAGAGCA GATGCACGAGAAGTCCATCCCTTACAGCCAGGAAACCCTGGTGG TGGACTGACGGACCGGCGATAGATGAAGCTCGCTTTCTTGCTGT CCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAA ACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTA ATAAAAAACATTTATTTTCATTGCAGCTCGCTTTCTTGCTGTCCAA TTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAAACTG GGGGATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAA AAAACATTTATTTTCATTGCGGCCGCAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAA
SEQ ID NO: 19 (mRNA) TEV-hAPJ-2xhBG-120A Sequence features: Tobacco Etch Virus (TEV) 5' UTR: 37-1 90 Optimal Kozak sequence: 19 1- 199 Human APJ codon optimized, encoding amino acids 1-380 of Protein Accession #NP_0051 52.1 : 197-1336 1 stop codon : 1337-1349 2 copies of human beta-globin 3'UTR: 1358-1 623	GGGAGACGCGUGUUAAAUAACAAAUCUCAACACAACAUAUACAA AACAAACGAAUCUCAAGCAAUCAAGCAUUCUACUUCUAUUGCAG CAAUUUAAAUCAUUUCUUUUAAAGCAAAAGCAAUUUUCUGAAAA UUUUCACCAUUUACGAACGAUAGCCGCCACCAUGGAAGAGGGC GGCGACUUCGACAACUACUACGGCGCCGACAACCAGAGCGAGU GCGAGUACACCGACUGGAAGUCCUCUGGCGCCCUGAUCCCCG CUAUCUACAUGCUGGUGUUUCUGCUGGGCACCACCGGCAACG GACUGGUGCUGUGGACCGUGUUCAGAAGCAGCAGAGAGAAGC GGCGGAGCGCCGACAUCUUUAUCGCCAGCCUGGCCGUGGCCG ACCUGACCUUUGUCGUGACACUGCCUCUGUGGGCCACCUACAC CUACCGGGACUACGACUGGCCCUUCGGCACAUUUUCUGCAAG CUGAGCAGCUACCUGAUCUUCGUGAAUAUGUACGCCAGCGUGU UCUGCCUGACCGGCCUGAGCUUCGACAGAUACCUGGCCAUCG UGCGGCCCGUGGCCAACGCUAGACUGCGGCUGAGAGUGUCUG GCGCCGUGGCUACAGCUGUGCUGUGGGUGCUGGCUGCCCUGC UGGCUAUGCCUGUGAUGGUGCUGAGAACCACCGGCGACCUGG AAAACACCACCAAGGUGCAGUGCUACAUGGACUACAGCAUGGU GGCCACAGUGUCCAGCGAGUGGGCCUGGGAAGUGGGACUGGG AGUGUCUAGCACCACCGUGGGCUUCGUGGUGCCCUUCACCAU UAUGCUGACCUGCUACUUCUUCAUUGCCCAGACAAUCGCCGGC CACUUCCGGAAAGAGCGGAUCGAGGGCCUGCGGAAGAGAAGG CGGCUGCUGAGCAUCAUCGUGGUGCUGGUCGUGACCUUCGCC CUGUGCUGGAUGCCUUACCACCUCGUGAAAACCCUGUAUAUGC

120 nucleotide polyA tail (SEQ ID NO: 59): 1630-1 749	UGGGCAGCCUGCUGCACUGGCCCUGCGAUUUCGACCUGUUCC UGAUGAACAUCUUCCCCUACUGCACCUGUAUCAGCUACGUGAA CAGCUGCCUGAACCCCUUCCUGUACGCCUUCUUCGACCCCCG GUUCAGACAGGCCUGCACCUCCAUGCUGUGCUGCGGCCAGUC UAGAUGCGCCGGCACAAGCCACAGCAGCAGCGGCGAGAAGUCU GCCAGCUACAGCUCUGGCCACAGCCAGGGCCCAGGCCCCAAUA UGGGAAAGGGCGGAGAGCAGAUGCACGAGAAGUCCAUCCCUUA CAGCCAGGAAACCCUGGUGGUGGACUGACGGACCGGCGAUAG AUGAAGCUCGCUUUCUUGCUGUCCAAUUUCUAUUAAAGGUUCC UUUGUUCCCUAAGUCCAACUACUAAACUGGGGGAUAUUAUGAA GGGCCUUGAGCAUCUGGAUUCUGCCUAAUAAAAAACAUUUAUU UUCAUUGCAGCUCGCUUUCUUGCUGUCCAAUUUCUAUUAAAGG UUCCUUUGUUCCCUAAGUCCAACUACUAAACUGGGGGAUAUUA UGAAGGGCCUUGAGCAUCUGGAUUCUGCCUAAUAAAAAACAUU UAUUUUCAUUGCGGCCGC AAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA A U= Uridine and/or pseudouridine
SEQ ID NO: 49 APJ RNA Coding sequence of construct of SEQ ID NO: 19	AUGGAAGAGGGCGGCGACUUCGACAACUACUACGGCGCCGACA ACCAGAGCGAGUGCGAGUACACCGACUGGAAGUCCUCUGGCG CCCUGAUCCCCGCUAUCUACAUGCUGGUGUUUCUGCUGGGCA CCACCGGCAACGGACUGGUGCUGUGGACCGUGUUCAGAAGCA GCAGAGAGAAGCGGCGGAGCGCCGACAUCUUUAUCGCCAGCC UGGCCGUGGCCGACCUGACCUUUGUCGUGACACUGCCUCUGU GGGCCACCUACACCUACCGGGACUACGACUGGCCCUUCGGCA CAUUUUUCUGCAAGCUGAGCAGCUACCUGAUCUUCGUGAAUAU GUACGCCAGCGUGUUCUGCCUGACCGGCCUGAGCUUCGACAG AUACCUGGCCAUCGUGCGGCCCGUGGCCAACGCUAGACUGCG GCUGAGAGUGUCUGGCGCCGUGGCUACAGCUGUGCUGUGGGU GCUGGCUGCCCUGCUGGCUAUGCCUGUGAUGGUGCUGAGAAC CACCGGCGACCUGGAAAACACCACCAAGGUGCAGUGCUACAUG GACUACAGCAUGGUGGCCACAGUGUCCAGCGAGUGGGCCUGG GAAGUGGGACUGGGAGUGUCUAGCACCACCGUGGGCUUCGUG GUGCCCUUCACCAUUAUGCUGACCUGCUACUUCUUCAUUGCCC AGACAAUCGCCGGCCACUUCCGGAAAGAGCGGAUCGAGGGCC UGCGGAAGAGAAGGCGGCUGCUGAGCAUCAUCGUGGUGCUGG UCGUGACCUUCGCCCUGUGCUGGAUGCCUUACCACCUCGUGA AAACCCUGUAUAUGCUGGGCAGCCUGCUGCACUGGCCCUGCG AUUUCGACCUGUUCCUGAUGAACAUCUUCCCCUACUGCACCUG UAUCAGCUACGUGAACAGCUGCCUGAACCCCUUCCUGUACGCC UUCUUCGACCCCCGGUUCAGACAGGCCUGCACCUCCAUGCUG UGCUGCGGCCAGUCUAGAUGCGCCGGCACAAGCCACAGCAGC AGCGGCGAGAAGUCUGCCAGCUACAGCUCUGGCCACAGCCAG GGCCCAGGCCCCAAUAUGGGAAAGGGCGGAGAGCAGAUGCAC GAGAAGUCCAUCCCUUACAGCCAGGAAACCCUGGUGGUGGACU GA

VI.   GP130

[00284]   Glycoprotein 130 (GP130) is a 918 amino acid containing protein and is member of the type I single pass transmembrane protein receptor family. It is core component of the signal transduction complex used by many cytokines including interleukin 6, interleukin 11, ciliary neurotrophic factor, leukemia inhibitory factor, and oncostatin M. In the case of interleukin 6 (IL6),

GP130 binds to the IL6/IL6R (alpha chain) complex, resulting in the formation of high-affinity IL6 binding sites and initiation of signal transduction. GP130 contains five fibronectin type III domains and one Ig-like C2-type domain.

[00285] The full length coding sequence of human GP130 (e.g., Protein Accession No. NPJD02175.2 or AAI17405) was codon optimized for expression in human cells and cloned into a vector that can sustain mRNA transcription by T7 polymerase and contains both 3 and 5' untranslated regions that help with mRNA stability and translatability (see Table 6 for sequence). mRNA was *in vitro* transcribed and encapsulated into lipid nanoparticles as described above.

TABLE 6
Exemplary GP130 Polynucleotide and Polypeptide Sequences

SEQ ID NO: and features	Sequence
SEQ ID NO: 50 GP1 30 native DNA sequence corresponding to Protein Accession # NP_0021 75.2 or AAI1 7405	GAGCAGCCAAAAGGCCCGCGGAGTCGCGCTGGGCCGCCCCGGCGCA GCTGAACCGGGGGCCGCGCCTGCCAGGCCGACGGGTCTGGCCCAGC CTGGCGCCAAGGGGTTCGTGCGCTGTGGAGACGCGGAGGGTCGAGG CGGCGCGGCCTGAGTGAAACCCAATGGAAAAAGCATGACATTTAGAAG TAGAAGACTTAGCTTCAAATCCCTACTCCTTCACTTACTAATTTTGTGAT TTGGAAATATCCGCGCAAGATGTTGACGTTGCAGACTTGGGTAGTGCA AGCCTTGTTTATTTTCCTCACCACTGAATCTACAGGTGAACTTCTAGATC CATGTGGTTATATCAGTCCTGAATCTCCAGTTGTACAACTTCATTCTAAT TTCACTGCAGTTTGTGTGCTAAAGGAAAAATGTATGGATTATTTTCATGT AAATGCTAATTACATTGTCTGGAAAACAAACCATTTTACTATTCCTAAGG AGCAATATACTATCATAAACAGAACAGCATCCAGTGTCACCTTTACAGAT ATAGCTTCATTAAATATTCAGCTCACTTGCAACATTCTTACATTCGGACA GCTTGAACAGAATGTTTATGGAATCACAATAATTTCAGGCTTGCCTCCA GAAAAACCTAAAAATTTGAGTTGCATTGTGAACGAGGGGAAGAAAATGA GGTGTGAGTGGGATGGTGGAAGGGAAACACACTTGGAGACAAACTTCA CTTTAAAATCTGAATGGGCAACACACAAGTTTGCTGATTGCAAAGCAAA ACGTGACACCCCCACCTCATGCACTGTTGATTATTCTACTGTGTATTTTG TCAACATTGAAGTCTGGGTAGAAGCAGAGAATGCCCTTGGGAAGGTTA CATCAGATCATATCAATTTTGATCCTGTATATAAAGTGAAGCCCAATCCG CCACATAATTTATCAGTGATCAACTCAGAGGAACTGTCTAGTATCTTAAA ATTGACATGGACCAACCCAAGTATTAAGAGTGTTATAATACTAAAATATA ACATTCAATATAGGACCAAAGATGCCTCAACTTGGAGCCAGATTCCTCC TGAAGACACAGCATCCACCCGATCTTCATTCACTGTCCAAGACCTTAAA CCTTTTACAGAATATGTGTTTAGGATTCGCTGTATGAAGGAAGATGGTA AGGGATACTGGAGTGACTGGAGTGAAGAAGCAAGTGGGATCACCTATG AAGATAGACCATCTAAAGCACCAAGTTTCTGGTATAAAATAGATCCATC CCATACTCAAGGCTACAGAACTGTACAACTCGTGTGGAAGACATTGCCT CCTTTTGAAGCCAATGGAAAAATCTTGGATTATGAAGTGACTCTCACAA GATGGAAATCACATTTACAAAATTACACAGTTAATGCCACAAAACTGACA GTAAATCTCACAAATGATCGCTATCTAGCAACCCTAACAGTAAGAAATCT TGTTGGCAAATCAGATGCAGCTGTTTTAACTATCCCTGCCTGTGACTTT CAAGCTACTCACCCTGTAATGGATCTTAAAGCATTCCCCAAAGATAACA TGCTTTGGGTGGAATGGACTACTCCAAGGGAATCTGTAAAG AAATATAT ACTTGAGTGGTGTGTGTTATCAGATAAAGCACCCTGTATCACAGACTGG CAACAAGAAGATGGTACCGTGCATCGCACCTATTTAAGAGGGAACTTAG CAGAGAGCAAATGCTATTTGATAACAGTTACTCCAGTATATGCTGATGG ACCAGGAAGCCCTGAATCCATAAAGGCATACCTTAAACAAGCTCCACCT TCCAAAGGACCTACTGTTCGGACAAAAAAAGTAGGGAAAAACGAAGCT

TABLE 6
Exemplary GP130 Polynucleotide and Polypeptide Sequences

SEQ ID NO: and features	Sequence
	GTCTTAGAGTGGGACCAACTTCCTGTTGATGTTCAGAATGGATTTATCA GAAATTATACTATATTTTATAGAACCATCATTGGAAATGAAACTGCTGTG AATGTGGATTCTTCCCACACAGAATATACATTGTCCTCTTTGACTAGTGA CACATTGTACATGGTACGAATGGCAGCATACACAGATGAAGGTGGGAA GGATGGTCCAGAATTCACTTTTACTACCCCAAAGTTTGCTCAAGGAGAA ATTGAAGCCATAGTCGTGCCTGTTTGCTTAGCATTCCTATTGACAACTCT TCTGGGAGTGCTGTTCTGCTTTAATAAGCGAGACCTAATTAAAAAACAC ATCTGGCCTAATGTTCCAGATCCTTCAAAGAGTCATATTGCCCAGTGGT CACCTCACACTCCTCCAAGGCACAATTTTAATTCAAAAGATCAAATGTAT TCAGATGGCAATTTCACTGATGTAAGTGTTGTGGAAATAGAAGCAAATG ACAAAAAGCCTTTTCCAGAAGATCTGAAATCATTGGACCTGTTCAAAAA GGAAAAAATTAATACTGAAGGACACAGCAGTGGTATTGGGGGGTCTTC ATGCATGTCATCTTCTAGGCCAAGCATTTCTAGCAGTGATGAAAATGAA TCTTCACAAAACACTTCGAGCACTGTCCAGTATTCTACCGTGGTACACA GTGGCTACAGACACCAAGTTCCGTCAGTCCAAGTCTTCTCAAGATCCGA GTCTACCCAGCCCTTGTTAGATTCAGAGGAGCGGCCAGAAGATCTACA ATTAGTAGATCATGTAGATGGCGGTGATGGTATTTTGCCCAGGCAACAG TACTTCAAACAGAACTGCAGTCAGCATGAATCCAGTCCAGATATTTCAC ATTTTGAAAGGTCAAAGCAAGTTTCATCAGTCAATGAGGAAGATTTTGTT AGACTTAAACAGCAGATTTCAGATCATATTTCACAATCCTGTGGATCTG GGCAAATGAAAATGTTTCAGGAAGTTTCTGCAGCAGATGCTTTTGGTCC AGGTACTGAGGGACAAGTAGAAAGATTTGAAACAGTTGGCATGGAGGC TGCGACTGATGAAGGCATGCCTAAAAGTTACTTACCACAGACTGTACGG CAAGGCGGCTACATGCCTCAGTGAAGGACTAGTAGTTCCTGCTACAAC TTCAGCAGTACCTATAAAGTAAAGCTAAAATGATTTTATCTGTGAATTC
SEQ ID NO: 20 GP130 native mRNA sequence corresponding to Protein Accession # NP_0021 75.2 or AAI1 7405	GAGCAGCCAAAAGGCCCGCGGAGUCGCGCUGGGCCGCCCCGGCGCA GCUGAACCGGGGGCCGCGCCUGCCAGGCCGACGGGUCUGGCCCAGC CUGGCGCCAAGGGGUUCGUGCGCUGUGGAGACGCGGAGGGUCGAG GCGGCGCGGCCUGAGUGAAACCCAAUGGAAAAAGCAUGACAUUUAGA AGUAGAAGACUUAGCUUCAAAUCCCUACUCCUUCACUUACUAAUUUU GUGAUUUGGAAAUAUCCGCGCAAGAUGUUGACGUUGCAGACUUGGG UAGUGCAAGCCUUGUUUAUUUUCCUCACCACUGAAUCUACAGGUGAA CUUCUAGAUCCAUGUGGUUAUAUCAGUCCUGAAUCUCCAGUUGUACA ACUUCAUUCUAAUUUCACUGCAGUUUGUGUGCUAAAGGAAAAAUGUA UGGAUUAUUUUCAUGUAAAUGCUAAUUACAUUGUCUGGAAAACAAAC CAUUUUACUAUUCCUAAGGAGCAAUAUACUAUCAUAAACAGAACAGCA UCCAGUGUCACCUUUACAGAUAUAGCUUCAUUAAAUAUUCAGCUCAC UUGCAACAUUCUUACAUUCGGACAGCUUGAACAGAAUGUUUAUGGAA UCACAAUAAUUUCAGGCUUGCCUCCAGAAAAACCUAAAAAUUUGAGUU GCAUUGUGAACGAGGGGAAGAAAAUGAGGUGUGAGUGGGAUGGUGG AAGGGAAACACACUUGGAGACAAACUUCACUUUAAAAUCUGAAUGGG CAACACACAAGUUUGCUGAUUGCAAAGCAAAACGUGACACCCCCACC UCAUGCACUGUUGAUUAUUCUACUGUGUAUUUUGUCAACAUUGAAGU CUGGGUAGAAGCAGAGAAUGCCCUGGGGAAGGUUACAUCAGAUCAUA UCAAUUUUGAUCCUGUAUAUAAAGUGAAGCCCAAUCCGCCACAUAAU UUAUCAGUGAUCAACUCAGAGGAACUGUCUAGUAUCUUAAAAUUGAC AUGGACCAACCCAAGUAUUAAGAGUGUUAUAAUACUAAAAUAUAACAU UCAAUAUAGGACCAAAGAUGCCUCAACUUGGAGCCAGAUUCCUCCUG AAGACACAGCAUCCACCCGAUCUUCAUUCACUGUCCAAGACCUUAAA CCUUUUACAGAAUAUGUGUUUAGGAUUCGCUGUAUGAAGGAAGAUGG UAAGGGAUACUGGAGUGACUGGAGUGAAGAAGCAAGUGGGAUCACC UAUGAAGAUAGACCAUCUAAAGCACCAAGUUUCUGGUAUAAAAUAGAU

99

TABLE 6
Exemplary GP130 Polynucleotide and Polypeptide Sequences

SEQ ID NO: and features	Sequence
	CCAUCCCAUACUCAAGGCUACAGAACUGUACAACUCGUGUGGAAGAC AUUGCCUCCUUUUGAAGCCAAUGGAAAAAUCUUGGAUUAUGAAGUGA CUCUCACAAGAUGGAAAUCACAUUUACAAAAUUACACAGUUAAUGCCA CAAAACUGACAGUAAAUCUCACAAAUGAUCGCUAUCUAGCAACCCUAA CAGUAAGAAAUCUUGUUGGCAAAUCAGAUGCAGCUGUUUUAACUAUC CCUGCCUGUGACUUUCAAGCUACUCACCCUGUAAUGGAUCUUAAAGC AUUCCCCAAAGAUAACAUGCUUUGGGUGGAAUGGACUACUCCAAGGG AAUCUGUAAAGAAAUAUAUACUUGAGUGGUGUGUGUUAUCAGAUAAA GCACCCUGUAUCACAGACUGGCAACAAGAAGAUGGUACCGUGCAUCG CACCUAUUUUAAGAGGGAACUUAGCAGAGAGCAAAUGCUAUUUGAUAA CAGUUACUCCAGUAUAUGCUGAUGGACCAGGAAGCCCUGAAUCCAUA AAGGCAUACCUUAAACAAGCUCCACCUUCCAAAGGACCUACUGUUCG GACAAAAAAG UAGGGAAAAACG AAGC UGUCUUAGAGUGG GACC AAC UUCCUGUUGAUGUUCAGAAUGGAUUUAUCAGAAAUUAUACUAUAUUU UAUAGAACCAUCAUUGGAAAUGAAACUGCUGUGAAUGUGGAUUCUUC CCACACAGAAUAUACAUUGUCCUCUUUGACUAGUGACACAUUGUACA UGGUACGAAUGGCAGCAUACACAGAUGAAGGUGGGAAGGAUGGUCC AGAAUUCACUUUUACUACCCCAAAGUUUGCUCAAGGAGAAAUUGAAG CCAUAGUCGUGCCUGUUUGCUUAGCAUUCCUAUUGACAACUCUUCUG GGAGUGCUGUUCUGCUUUAAUAAGCGAGACCUAAUUAAAAAACACAU CUGGCCUAAUGUUCCAGAUCCUUCAAAGAGUCAUAUUGCCCAGUGGU CACCUCACACUCCUCCAAGGCACAAUUUUAAUUCAAAAGAUCAAAUGU AUUCAGAUGGCAAUUUCACUGAUGUAAGUGUUGUGGAAAUAGAAGCA AAUGACAAAAAGCCUUUUCCAGAAGAUCUGAAAUCAUUGGACCUGUU CAAAAAGGAAAAAAUUAAUACUGAAGGACACAGCAGUGGUAUUGGGG GGUCUUCAUGCAUGUCAUCUUCUAGGCCAAGCAUUUCUAGCAGUGAU GAAAAUGAAUCUUCACAAAACACUUCGAGCACUGUCCAGUAUUCUAC CGUGGUACACAGUGGCUACAGACACCAAGUUCCGUCAGUCCAAGUCU UCUCAAGAUCCGAGUCUACCCAGCCCUUGUUAGAUUCAGAGGAGCGG CCAGAAGAUCUACAAUUAGUAGAUCAUGUAGAUGGCGGUGAUGGUAU UUUGCCCAGGCAACAGUACUUCAAACAGAACUGCAGUCAGCAUGAAU CCAGUCCAGAUAUUUCACAUUUUGAAAGGUCAAAGCAAGUUUCAUCA GUCAAUGAGGAAGAUUUUGUUAGACUUAAAACAGCAGAUUUCAGAUCA UAUUUCACAAUCCUGUGGAUCUGGGCAAAUGAAAAUGUUUCAGGAAG UUUCUGCAGCAGAUGCUUUUGGUCCAGGUACUGAGGGACAAGUAGA AAGAUUUGAAACAGUUGGCAUGGAGGCUGCGACUGAUGAAGGCAUG CCUAAAAGUUACUUACCACAGACUGUACGGCAAGGCGGCUACAUGCC UCAGUGAAGGACUAGUAGUUCCUGCUACAACUUCAGCAGUACCUAUA AAGUAAAGCUAAAAUGAUUUUAUCUGUGAAUUC U= Uridine and/or pseudouridine
SEQ ID NO: 21 Translated human GP130 from coding sequence (CDS) of the DNA construct of SEQ ID NO: 20	MLTLQTWLVQALFIFLTTESTGELLDPCGYISPESPVVQLHSNFTAVCVLKEKCMDYF HVNANYIVWKTNHFTIPKEQYTIINRTASSVTFTDIASLNIQLTCNILTFGQLEQNVYGI TIISGLPPEKPKNLSCIVNEGKKMRCEWDRGRETHLETNFTLKSEWATHKFADC KAKRDTPTSCTVDYSTVYFVNIEVWVEAENALGKVTSDHINFDPVYKVKPN PPHNLSVINSEELSSILKLTWTNPSIKSVIILKYNIQYRTKDASTWSQIPPEDT ASTRSSFTVQDLKPFTEYVFRIRCMKEDGKGYWSDWSEEASGITYEDRPSKA PSFWYKIDPSHTQGYRTVQLVWKTLPPFEANGKILDYEVTLTRWKSHLQNYTV NATKLTVNLTNDRYVATLTVRNLVGKSDAAVLTIPACDFQATHPVMDLKAF PKDNMLWVEWTTPRESVKKYILEWCVLSDKAPCITDWQQEDGTVHRTYL RGNLAESKCYLITVTPVYADGPGSPESIKAYLKQAPPSKGPTVRTKKVGKN

100

TABLE 6
Exemplary GP130 Polynucleotide and Polypeptide Sequences

SEQ ID NO: and features	Sequence
	EAVLEWDQLPVDVQNGFIRNYTIFYRTI IGNETAVNVDSSHTEYTLSSLTSD TLYMVRMAAYTDEGGKDGPEFTFTTPKFAQGEIEAIVVPVCLAFLLTTLLGV LFCFNKRDLIKKH IWPNVPDPSKSHIAQWSPHTPPRHNFNSKDQMYSDGN FTDVSVVEIEANDKKPFPEDLKSLDLFKKEKINTEGHSSGIGGSSCMSSSRP SISSSDENESSQNTSSTVQYSTVVHSGYRHQVPSVQVFSRSESTQPLLDS EERPEDLQLVDHVDGGDGILPRQQYFKQNCSQHESSPDISHFERSKQVSS VNEEDFVRLKQQISDHISQSCGSGQMKMFQEVSAADAFGPGTEGQVERF ETVGMEAATDEGMPKSYLPQTVRQGGYMPQ
SEQ ID NO: 51 (DNA) TEV-hGP130-2xhBG-120A Sequence features: Tobacco Etch Virus (TEV) 5' UTR: 37-190 Optimal Kozak sequence: 191-199 Human GP130 codon optimized, encoding amino acids Accession # XM_011543376: 226-29791 stop codon: 2980-2982 2 copies of human beta-globin 3'UTR: 2983-3245 120 nucleotide polyA tail (SEQ ID NO: 59): 3249-3368	GATCCGGAGGCCGGAGAATTGTAATACGACTCACTATAGGGAGACGCGTGTTAA ATAACAAATCTCAACACAACATATACAAAACAAACGAATCTCAAGCAATCAAGCA TTCTACTTCTATTGCAGCAATTTAAATCATTTCTTTTAAAGCAAAAGCAATTTTCTG AAAATTTTCACCATTTACGAACGATAGCCGCCACCGCATCGTGAACGAGGGCAAG AAAATGCTGACCCTGCAGACCTGGCTGGTGCAGGCCCTGTTCATCTTCCTGACCA CCGAGAGCACCGGCGAGCTGCTGGACCCTTGTGGCTACATCAGCCCCGAGAGCC CTGTGGTGCAGCTGCATAGCAACTTCACCGCCGTGTGCGTGCTGAAAGAAAGT GCATGGACTACTTCCACGTGAACGCCAACTACATCGTGTGGAAAACAAACCACTT CACCATCCCCAAAGAGCAGTACACCATCATCAACAGAACCGCCAGCAGCGTGACC TTCACCGATATCGCCAGCCTGAACATCCAGCTGACCTGCAACATCCTGACCTTCGG CCAGCTGGAACAGAACGTGTACGGCATCACAATCATCAGCGGCCTGCCCCCCGA GAAGCCCAAGAACCTGAGCTGCATCGTGAACGAGGGCAAGAAAATGAGATGCG AGTGGGACGGCGGCAGAGAGACACACCTGGAAACAAACTTCACCCTGAAGTCCG AGTGGGCCACCCACAAGTTCGCCGACTGCAAGGCCAAGAGGGACACCCCCACCA GCTGTACCGTGGACTACAGCACCGTGTACTTCGTGAACATCGAAGTGTGGGTGG AAGCCGAGAACGCCCTGGGCAAAGTGACCAGCGACCACATCAACTTCGACCCTG TGTACAAAGTGAAGCCCAACCCCCCCCCACAACCTGAGCGTGATCAACAGCGAGG AACTGAGCAGCATCCTGAAGCTGACATGGACCAACCCCAGCATCAAGTCCGTGAT CATTCTGAAGTACAACATCCAGTACCGGACCAAGGACGCCAGCACCTGGTCCCAG ATCCCTCCAGAGGACACCGCCTCCACCAGATCCAGCTTCACAGTGCAGGACCTGA AGCCTTTCACCGAGTACGTGTTCAGGATTCGGTGCATGAAGGAAGATGGCAAGG GCTACTGGAGCGATTGGAGCGAGGAAGCCAGCGGCATCACCTACGAGGACAGA CCCTCTAAGGCCCCCAGCTTCTGGTACAAGATCGACCCCAGCCACACCCAGGGCT ACAGAACCGTGCAGCTCGTGTGGAAAACCCTGCCCCCATTCGAGGCCAACGGCA AGATCCTGGACTACGAAGTGACCCTGACCAGATGGAAGTCCCATCTGCAGAACTA CACCGTGAACGCTACCAAGCTGACCGTGAACCTGACAAACGACAGATACCTGGC CACCCTGACCGTGCGGAACCTCGTGGGCAAGTCTGATGCCGCCGTGCTGACCATC CCCGCATGCGATTTTCAAGCCACCCACCCCGTGATGGATCTGAAGGCTTTCCCCA AGGACAACATGCTGTGGGTGGAATGGACCACCCCCAGAGAAAGCGTGAAAAAG TACATCCTGGAATGGTGTGTGCTGAGCGACAAGGCCCCCTGCATCACCGATTGGC AGCAGGAAGATGGAACCGTGCACAGAACCTACCTGAGAGGCAACCTGGCCGAG AGCAAGTGCTACCTGATCACCGTGACCCCCGTGTACGCTGACGGCCCTGGAAGCC CTGAGAGCATCAAGGCCTACCTGAAGCAGGCCCCTCCCAGCAAGGGACCTACAG TGCGGACCAAGAAAGTGGGCAAGAACGAGGCCGTGCTGGAATGGGACCAGCTG CCTGTGGATGTGCAGAACGGCTTCATCAGAAACTACACCATCTTCTACAGGACCA TCATCGGCAACGAGACAGCCGTGAACGTGGACAGCAGCCACACAGAGTACACCC TGAGCAGCCTGACCTCCGACACCCTGTATATGGTGCGAATGGCCGCCTACACCGA CGAGGGCGGAAAGGATGGCCCCGAGTTCACCTTCACCACACCTAAGTTCGCTCA

<div align="center">TABLE 6</div>

Exemplary GP130 Polynucleotide and Polypeptide Sequences

SEQ ID NO: and features	Sequence
	GGGCGAGATCGAGGCCATCGTGGTGCCTGTGTGTCTGGCTTTCCTGCTGACCACC CTGCTGGGCGTGCTGTTCTGCTTCAACAAGCGGGACCTGATCAAGAAGCACATCT GGCCCAACGTGCCCGACCCTAGCAAGAGCCATATCGCCCAGTGGTCCCCCCACAC CCCCCCTAGACACAACTTCAACAGCAAGGACCAGATGTACAGCGACGGCAACTTT ACAGACGTGTCCGTGGTGGAAATCGAGGCTAACGATAAGAAGCCCTTCCCAGAA GATCTGAAGTCCCTGGATCTGTTCAAGAAAGAGAAGATCAACACAGAGGGCCAC AGCTCCGGCATCGGCGGCAGCTCTTGTATGAGCAGCAGCAGACCTAGCATCAGC AGCAGCGACGAGAACGAGAGCAGCCAGAACACCTCTAGCACCGTGCAGTACTCC ACCGTGGTGCACAGCGGCTACAGACACCAGGTGCCAAGCGTGCAGGTGTTCAGC AGAAGCGAGTCCACCCAGCCCCTGCTGGACAGCGAAGAGAGGCCTGAGGATCTG CAGCTGGTGGACCATGTGGACGGCGGAGATGGCATCCTGCCCAGACAGCAGTAC TTCAAGCAGAACTGCTCCCAGCACGAGTCCAGCCCCGACATCAGCCACTTCGAGA GAAGCAAACAGGTGTCCAGCGTGAACGAAGAGGACTTCGTGCGGCTGAAGCAG CAGATCAGCGATCACATCTCCCAGAGCTGCGGCAGCGGCCAGATGAAGATGTTC CAGGAAGTGTCCGCCGCTGACGCCTTCGGACCTGGAACTGAGGGCCAGGTGGAA AGATTCGAGACAGTGGGCATGGAAGCCGCCACAGACGAGGGCATGCCTAAGAG CTACCTGCCCCAGACTGTGCGGCAGGGCGGCTACATGCCTCAGTGAAGCTCGCTT TCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAAAC TGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATT TATTTTCATTGCAGCTCGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTC CCTAAGTCCAACTACTAAACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGAT TCTGCCTAATAAAAAACATTTATTTTCATTGCAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
SEQ ID NO: 22 (mRNA) TEV-hGP130-2xhBG-120A Sequence features: Tobacco Etch Virus (TEV) 5' UTR: 37-190 Optimal Kozak sequence: 191-199 Human GP130 codon optimized, encoding amino acids Accession # XM_011543376: 226-29791 stop codon: 2980-2982 2 copies of human beta-globin 3'UTR: 2983-3245 120 nucleotide polyA tail (SEQ ID NO: 59): 3249-3368	GGGAGACGCGUGUUAAAUAACAAAUCUCAACACAACAUAUACAAAACAAACG AAUCUCAAGCAAUCAAGCAU UCUACU UCUAUUGCAGCAAUU UAAAUCAU UUC UUUUAAAGCAAAAGCAAU UUUCUGAAAAU UUUCACCAU UUACG AACGAU AG CCGCCACCGCAUCGUGAACGAGGGCAAGAAAAUGCUGACCCUGCAGACCUGG CUGGUGCAGGCCCUGU UCAUCU UCCUGACCACCGAGAGCACCGGCGAGCUGC UGGACCCU UG UGGCUACAUCAGCCCCGAGAGCCCUGUGGUGCAGCUGCAUAG CAACU UCACCGCCGUGUGCGUGCUGAAAGAAAAG UGCAUGGACUACU UCCAC GUGAACGCCAACUACAUCGUG UGGAAAACAAACCACU UCACCAUCCCCAAAGA GCAGUACACCAUCAUCAACAGAACCGCCAGCAGCGUGACCUUCACCGAUAUCG CCAGCCUGAACAUCCAGCUGACCUGCAACAUCCUGACCUUCGGCCAGCUGGAA CAGAACGUGUACGGCAUCACAAUCAUCAGCGGCCUGCCCCCCGAGAAGCCCAA GAACCUGAGCUGCAUCGUGAACGAGGGCAAGAAAAUGAGAUGCGAGUGGGA CGGCGGCAGAGAGACACACCUGGAAACAAACUUCACCCUGAAGUCCGAGUGG GCCACCCACAAGU UCGCCGACUGCAAGGCCAAGAGGGACACCCCCACCAGCUG UACCGUGGACUACAGCACCGUGUACUUCGUGAACAUCGAAG UGUGGGUGGA AGCCGAGAACGCCCUGGGCAAAGUGACCAGCGACCACAUCAACUUCGACCCUG UGUACAAAGUGAAGCCCAACCCCCCCCACAACCUGAGCGUGAUCAACAGCGAG GAACUGAGCAGCAUCCUGAAGCUGACAUGGACCAACCCCAGCAUCAAGUCCG UGAUCAU UCUGAAGUACAACAUCCAGUACCGGACCAAGGACGCCAGCACCUG GUCCCAGAUCCCUCCAGAGGACACCGCCUCCACCAGAUCCAGCUUCACAGUGC AGGACCUGAAGCCUU UCACCGAG UACGUGU UCAGGAUUCGGUGCAUGAAGG

TABLE 6
Exemplary GP130 Polynucleotide and Polypeptide Sequences

SEQ ID NO: and features	Sequence
	AAGAUGGCAAGGGCUACUGGAGCGAUUGGAGCGAGGAAGCCAGCGGCAUCA CCUACGAGGACAGACCCUCUAAGGCCCCCAGCU UCUGGUACAAGAUCGACCCC AGCCACACCCAGGGCUACAGAACCGUGCAGCUCGUGUGGAAAACCCUGCCCCC AUUCGAGGCCAACGGCAAGAUCCUGGACUACGAAG UGACCCUGACCAGAUGG AAGUCCCAUCUGCAGAACUACACCGUGAACGCUACCAAGCUGACCGUGAACC UGACAAACGACAGAUACCUGGCCACCCUGACCGUGCGGAACCUCGUGGGCAA GUCUGAUGCCGCCGUGCUGACCAUCCCCGCAUGCGAUU UUCAAGCCACCCAC CCCGUGAUGGAUCUGAAGGCU UUCCCCAAGGACAACAUGCUGUGGG UGGAA UGGACCACCCCCAGAGAAAGCGUGAAAAAGUACAUCCUGGAAUGGUGUGUGC UGAGCGACAAGGCCCCCUGCAUCACCGAUUGGCAGCAGGAAGAUGGAACCGU GCACAGAACCUACCUGAGAGGCAACCUGGCCGAGAGCAAGUGCUACCUGAUC ACCGUGACCCCCGUG UACGCUGACGGCCCUGGAAGCCCUGAGAGCAUCAAGG CCUACCUGAAGCAGGCCCCUCCCAGCAAGGGACCUACAGUGCGGACCAAGAAA GUGGGCAAGAACGAGGCCGUGCUGGAAUGGGACCAGCUGCCUGUGGAUGUG CAGAACGGCU UCAUCAGAAACUACACCAUCUUCUACAGGACCAUCAUCGGCA ACGAGACAGCCGUGAACGUGGACAGCAGCCACACAGAGUACACCCUGAGCAG CCUGACCUCCGACACCCUGUAUAUGGGUGCGAAUGGCCGCCUACACCGACGAG GGCGGAAAGGAUGGCCCCGAGUUCACCU UCACCACACCUAAGU UCGCUCAGG GCGAGAUCGAGGCCAUCGUGGUGCCUGUGUGUCUGGCU UUCCUGCUGACCA CCCUGCUGGGCGUGCUGUUCUGCU UCAACAAGCGGGACCUGAUCAAGAAGCA CAUCUGGCCCAACGUGCCCGACCCUAGCAAGAGCCAUAUCGCCCAGUGGUCCC CCCACACCCCCCCUAGACACAACU UCAACAGCAAGGACCAGAUGUACAGCGAC GGCAACUU UACAGACGUGUCCGUGGUGGAAAUCGAGGCUAACGAUAAGAAG CCCUUCCCAGAAGAUCUGAAGUCCCUGGAUCUGUUCAAGAAAGAGAAGAUCA ACACAGAGGGCCACAGCUCCGGCAUCGGCGGCAGCUCU UGUAUGAGCAGCAG CAGACCUAGCAUCAGCAGCAGCGACGAGAACGAGAGCAGCCAGAACACCUCUA GCACCGUGCAGUACUCCACCGUGGUGCACAGCGGCUACAGACACCAGGUGCC AAGCGUGCAGGUGUUCAGCAGAAGCGAGUCCACCCAGCCCCUGCUGGACAGC GAAGAGAGGCCUGAGGAUCUGCAGCUGGUGGACCAUGUGGACGGCGGAGAU GGCAUCCUGCCCAGACAGCAGUACU UCAAGCAGAACUGCUCCCAGCACGAGU CCAGCCCCGACAUCAGCCACU UCGAGAGAAGCAAACAGGUGUCCAGCGUGAAC GAAGAGGACUUCGUGCGGCUGAAGCAGCAGAUCAGCGAUCACAUCUCCCAGA GCUGCGGCAGCGGCCAGAUGAAGAUG UUCCAGGAAGUG UCCGCCGCUGACGC CU UCGGACCUGGAACUGAGGGCCAGGUGGAAAGAUUCGAGACAGUGGGCAU GGAAGCCGCCACAGACGAGGGCAUGCCUAAGAGCUACCUGCCCCAGACUGUG CGGCAGGGCGGCUACAUGCCUCAGUGAAGCUCGCUU UCU UGCUGUCCAAUU UCUAU UAAAGGU UCCUU UGU UCCCUAAGUCCAACUACUAAACUGGGGGAUA UUAUGAAGGGCCU UGAGCAUCUGGAU UCUGCCUAAUAAAAACAU UUAU UU UCAU UGCAGCUCGCU UUCU UGCUGUCCAAU UUCUAU UAAAGGU UCCUU UGU UCCCUAAGUCCAACUACUAAACUGGGGGAUAUUAUGAAGGGCCU UGAGCAU CUGGAUUCUGCCUAAUAAAAACAUU UAU UUUCAU UGCAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

U= URIDINE AND/OR PSEUDOURIDINE

TABLE 6
Exemplary GP130 Polynucleotide and Polypeptide Sequences

SEQ ID NO: and features	Sequence
SEQ ID NO: 52 GP130 RNA coding sequence of construct of SEQ ID NO: 22	AUGCUGACCCUGCAGACCUGGCUGGUGCAGGCCCUGUUCAUCUUCCUGACCA CCGAGAGCACCGGCGAGCUGCUGGACCCUUGUGGCUACAUCAGCCCCGAGAG CCCUGUGGUGCAGCUGCAUAGCAACUUCACCGCCGUGUGCGUGCUGAAAGAA AAGUGCAUGGACUACU UCCACGUGAACGCCAACUACAUCGUGUGGAAAACAA ACCACU UCACCAUCCCCAAAGAGCAGUACACCAUCAUCAACAGAACCGCCAGC AGCGUGACCU UCACCGAUAUCGCCAGCCUGAACAUCCAGCUGACCUGCAACA UCCUGACCUUCGGCCAGCUGGAACAGAACGUGUACGGCAUCACAAUCAUCAG CGGCCUGCCCCCCGAGAAGCCCAAGAACCUGAGCUGCAUCGUGAACGAGGGC AAGAAAAUGAGAUGCGAGUGGGACGGCGGCAGAGAGACACACCUGGAAACAA ACUUCACCCUGAAGUCCGAGUGGGCCACCCACAAGU UCGCCGACUGCAAGGC CAAGAGGGACACCCCCACCAGCUGUACCGUGGACUACAGCACCGUGUACUUC GUGAACAUCGAAGUGUGGGUGGAAGCCGAGAACGCCCUGGGCAAAGUGACC AGCGACCACAUCAACUUCGACCCUGUGUACAAAGUGAAGCCCAACCCCCCCCA CAACCUGAGCGUGAUCAACAGCGAGGAACUGAGCAGCAUCCUGAAGCUGACA UGGACCAACCCCAGCAUCAAGUCCGUGAUCAU UCUGAAGUACAACAUCCAGU ACCGGACCAAGGACGCCAGCACCUGGUCCCAGAUCCCUCCAGAGGACACCGCC UCCACCAGAUCCAGCUUCACAG UGCAGGACCUGAAGCCU UUCACCGAGUACG UGU UCAGGAUUCGGUGCAUGAAGGAAGAUGGCAAGGGCUACUGGAGCGAU UGGAGCGAGGAAGCCAGCGGCAUCACCUACGAGGACAGACCCUCUAAGGCCC CCAGCU UCUGGUACAAGAUCGACCCCAGCCACACCCAGGGCUACAGAACCGUG CAGCUCGUGUGGAAAACCCUGCCCCCAU UCGAGGCCAACGGCAAGAUCCUGG ACUACGAAGUGACCCUGACCAGAUGGAAGUCCCAUCUGCAGAACUACACCGU GAACGCUACCAAGCUGACCGUGAACCUGACAAACGACAGAUACCUGGCCACCC UGACCGUGCGGAACCUCGUGGGCAAGUCUGAUGCCGCCGUGCUGACCAUCCC CGCAUGCGAU UU UCAAGCCACCCACCCCCGUGAUGGAUCUGAAGGCU UUCCCC AAGGACAACAUGCUGUGGGUGGAAUGGACCACCCCCAGAGAAAGCGUGAAAA AGUACAUCCUGGAAUGGUGUG UGCUGAGCGACAAGGCCCCCUGCAUCACCGA UUGGCAGCAGGAAGAUGGAACCGUGCACAGAACCUACCUGAGAGGCAACCUG GCCGAGAGCAAGUGCUACCUGAUCACCGUGACCCCCGUGUACGCUGACGGCC CUGGAAGCCCUGAGAGCAUCAAGGCCUACCUGAAGCAGGCCCCUCCCAGCAAG GGACCUACAGUGCGGACCAAGAAAGUGGGCAAGAACGAGGCCG UGCUGGAA UGGGACCAGCUGCCUGUGGAUGUGCAGAACGGCUUCAUCAGAAACUACACCA UCUUCUACAGGACCAUCAUCGGCAACGAGACAGCCGUGAACGUGGACAGCAG CCACACAGAGUACACCCUGAGCAGCCUGACCUCCGACACCCUGUAUAUGGUGC GAAUGGCCGCCUACACCGACGAGGGCGGAAAGGAUGGCCCCGAGU UCACCUU CACCACACCUAAGUUCGCUCAGGGCGAGAUCGAGGCCAUCGUGGUGCCUGUG UGUCUGGCU UUCCUGCUGACCACCCUGCUGGGCGUGCUGUUCUGCUUCAAC AAGCGGGACCUGAUCAAGAAGCACAUCUGGCCCAACGUGCCCGACCCUAGCAA GAGCCAUAUCGCCCAGUGG UCCCCCCACACCCCCCCUAGACACAACUUCAACA GCAAGGACCAGAUG UACAGCGACGGCAACUU UACAGACGUGUCCG UGGUGG AAAUCGAGGCUAACGAUAAGAAGCCCU UCCCAGAAGAUCUGAAGUCCCUGGA UCUGU UCAAGAAAGAGAAGAUCAACACAGAGGGCCACAGCUCCGGCAUCGGC GGCAGCUCU UG UAUGAGCAGCAGCAGACCUAGCAUCAGCAGCAGCGACGAGA ACGAGAGCAGCCAGAACACCUCUAGCACCG UGCAGUACUCCACCGUGGUGCAC AGCGGCUACAGACACCAGGUGCCAAGCGUGCAGGUGUUCAGCAGAAGCGAGU CCACCCAGCCCCUGCUGGACAGCGAAGAGAGGCCUGAGGAUCUGCAGCUGGU

TABLE 6
Exemplary GP130 Polynucleotide and Polypeptide Sequences

SEQ ID NO: and features	Sequence
	GGACCAUGUGGACGGCGGAGAUGGCAUCCUGCCCAGACAGCAGUACU UCAAG CAGAACUGCUCCCAGCACGAGUCCAGCCCCGACAUCAGCCACU UCGAGAGAAG CAAACAGGUGUCCAGCGUGAACGAAGAGGACU UCGUGCGGCUGAAGCAGCAG AUCAGCGAUCACAUCUCCCAGAGCUGCGGCAGCGGCCAGAUGAAGAUGUUCC AGGAAGUGUCCGCCGCUGACGCCUUCGGACCUGGAACUGAGGGCCAGGUGG AAAGAUUCGAGACAGUGGGCAUGGAAGCCGCCACAGACGAGGGCAUGCCUAA GAGCUACCUGCCCCAGACUGUGCGGCAGGGCGGCUACAUGCCUCAGUGA

VII.   Galectin-3

[00286]   Galectin-3 is a 26 kDa protein and is a member of the β-galactoside-binding lectin family. It contains a collagen-like N-terminal domain and a C-terminal carbohydrate recognition domain which confers the ability of galectin-3 to bind carbohydrates. Via its N-terminal domain, galectin-3 is able to form higher order oligomers. Galectin-3 has been suggested to play a role in cell attachment, differentiation, metastasis, embryogenesis, inflammation, and fibrosis.

[00287]   The full length coding sequence of human galectin-3 (e.g., Protein Accession No. NPJD02297) was codon optimized for expression in human cells and cloned into a vector that can sustain mRNA transcription by T7 polymerase and contains both 3 and 5' untranslated regions that help with mRNA stability and translatability (see Table 7 for sequence). mRNA was *in vitro* transcribed and encapsulated into lipid nanoparticles as described above.

TABLE 7
Exemplary Galectin 3 Polynucleotide and Polypeptide Sequences

SEQ ID NO: and features	Sequence
SEQ ID NO: 53 Galectin-3 native DNA sequence corresponding to Protein Accession # NP_002297	GAGTATTTGAGGCTCGGAGCCACCGCCCCGCCGGCGCCCGCAGCACCTCCTCGCCAGCAG CCGTCCGGAGCCAGCCAACGAGCGGAAAATGGCAGACAATTTTTCGCTCCATGATGCGTT ATCTGGGTCTGGAAACCCAAACCCTCAAGGATGGCCTGGCGCATGGGGGAACCAGCCTG CTGGGGCAGGGGGCTACCCAGGGGCTTCCTATCCTGGGGCCTACCCCGGGCAGGCACCC CCAGGGGCTTATCCTGGACAGGCACCTCCAGGCGCCTACCCTGGAGCACCTGGAGCTTAT CCCGGAGCACCTGCACCTGGAGTCTACCCAGGGCCACCCAGCGGCCCTGGGGCCTACCCA TCTTCTGGACAGCCAAGTGCCACCGGAGCCTACCCTGCCACTGGCCCCTATGGCGCCCCTG CTGGGCCACTGATTGTGCCTTATAACCTGCCTTTGCCTGGGGGAGTGGTGCCTCGCATGCT GATAACAATTCTGGGCACGGTGAAGCCCAATGCAAACAGAATTGCTTTAGATTTCCAAAG AGGGAATGATGTTGCCTTCCACTTTAACCCACGCTTCAATGAGAACAACAGGAGAGTCATT GTTTGCAATACAAAGCTGGATAATAACTGGGGAAGGGAAGAAAGACAGTCGGTTTTCCCA TTTGAAAGTGGGAAACCATTCAAAATACAAGTACTGGTTGAACCTGACCACTTCAAGGTTG CAGTG AATG ATG CTCACTTGTTG CAGTACAATCATCG GGTTAAAAAACTCAATG AAATCAG CAAACTGGGAATTTCTGGTGACATAGACCTCACCAGTGCTTCATATACCATGATATAATCT GAAAGGGGCAGATTAAAAAAAAAAAAAAAGAATCTAAACCTTACATGTGTAAAGGTTTCATG

TTCACTGTGAGTGAAAATTTTTACATTCATCAATATCCCTCTTGTAAGTCATCTACTTAATAA
ATATTACAGTGAATTACCTGTCTCAATATGTCAAAAAAAAAAAAAAAAAA

SEQ ID NO: 26 Native mRNA sequence corresponding to Protein Accession # NP_002297	GAGUAUUUGAGGCUCGGAGCCACCGCCCCGCCGGCGCCCGCAGCAC CUCCUCGCCAGCAGCCGUCCGGAGCCAGCCAACGAGCGGAAAAUGG CAGACAAUUUUUCGCUCCAUGAUGCGUUAUCUGGGUCUGGAAACCCA AACCCUCAAGGAUGGCCUGGCGCAUGGGGGAACCAGCCUGCUGGGG CAGGGGGCUACCCAGGGGCUUCCUAUCCUGGGGGCCUACCCCGGGCA GGCACCCCCAGGGGCUUAUCCUGGACAGGCACCUCCAGGCGCCUAC CCUGGAGCACCUGGAGCUUAUCCCGGAGCACCUGCACCUGGAGUCU ACCCAGGGCCACCCAGCGGCCCUGGGGCCUACCCAUCUUCUGGACA GCCAAGUGCCACCGGAGCCUACCCUGCCACUGGCCCCUAUGGCGCC CCUGCUGGGCCACUGAUUGUGCCUUAUAACCUGCCUUUGCCUGGGG GAGUGGUGCCUCGCAUGCUGAUAACAAUUCUGGGCACGGUGAAGCC CAAUGCAAACAGAAUUGCUUUAGAUUUCCAAAGAGGGAAUGAUGUUG CCUUCCACUUUAACCCACGCUUCAAUGAGAACAACAGGAGAGUCAUU GUUUGCAAUACAAAGCUGGAUAAUAACUGGGGGAAGGGAAGAAAGACA GUCGGUUUUCCCAUUUGAAAGUGGGAAACCAUUCAAAAUACAAGUAC UGGUUGAACCUGACCACUUCAAGGUUGCAGUGAAUGAUGCUCACUUG UUGCAGUACAAUCAUCGGGUUAAAAAACUCAAUGAAUCAGCAAACUG GGAAUUUCUGGUGACAUAGACCUCACCAGUGCUUCAUAUACCAUGAU AUAAUCUGAAAGGGGCAGAUUAAAAAAAAAAAAAAGAAUCUAAACCUUA CAUGUGUAAAGGUUUCAUGUUCACUGUGAGUGAAAAUUUUUACAUUC AUCAAUAUCCCUCUUGUAAGUCAUCUACUUAAUAAAUAUUACAGUGAA UUACCUGUCU CAA UA UG UCAAAAAAAAAAAAAAAAAAAA U= Uridine and/or pseudouridine
SEQ ID NO: 27 Translated human galectin-3 from coding sequence (CDS) of the DNA construct of SEQ ID NO: 26	MADNFSLHDALSGSGNPNPQGWPGAWGNQPAGAGGYPGASYPGAYPG QAPPGAYPGQAPPGAYPGAPGAYPGAPAPGVYPGPPSGPGAYPSSGQP SATGAYPATGPYGAPAGPLIVPYNLPLPGGVVPRMLITILGTVKPNANRIAL DFQRGNDVAFHFNPRFNENNRRVIVCNTKLDNNWGREERQSVFPFESGK PFKIQVLVEPDHFKVAVNDAHLLQYNHRVKKLNEISKLGISGDIDLTSASYT MI
SEQ ID NO: 54 (DNA) TEV-hGalectin-3-2xhBG-1 20A Sequence features: Tobacco Etch Virus (TEV) 5' UTR: 37-1 90 Optimal Kozak sequence: 191-199 Human Galectin-3 codon optimized , encoding amino acids Accession #	GATCCGGAGGCCGGAGAATTGTAATACGACTCACTATAGGGAGACGCGTGTTAA ATAACAAATCTCAACACAACATATACAAAACAAACGAATCTCAAGCAATCAAGCA TTCTACTTCTATTGCAGCAATTTAAATCATTTCTTTTAAAGCAAAAGCAATTTTCTG AAAATTTTCACCATTTACGAACGATAGCCGCCACCATGGCCGACAACTTCAGCCT GCACGATGCCCTGAGCGGCAGCGGCAACCCTAATCCTCAGGGATGGCCTGGCGC TTGGGGCAATCAGCCTGCTGGCGCTGGCGGATATCCTGGCGCATCTTACCCAGGC GCTTACCCCGGACAGGCTCCTCCAGGCGCATATCCAGGCCAGGCACCTCCTGGG GCTTATCCTGGGGCACCTGGCGCCTACCCTGGCGCTCCTGCTCCTGGCGTGTAC CCTGGACCTCCTTCTGGACCCGGCGCATACCCTAGCTCTGGCCAGCCA TCTGCTACCGGCGCCTATCCAGCCACAGGACCTTATGGCGCTCCAGCC GGACCTCTGATCGTGCCCTACAACCTGCCTCTGCCTGGCGGCGTGGTG CCCAGAATGCTGATCACAATCCTGGGCACCGTGAAGCCCAACGCCAAC AGAATCGCCCTGGACTTCCAGAGGGGCAACGACGTGGCCTTCCACTTC

NP_002297: 202-951 stop codon: 952-954. 2 copies of human beta-globin 3'UTR: 973-1 238. 120 nucleotide polyA tail (SEQ ID NO: 59): 1245-1 364.	AACCCCAGATTCAACGAGAACAATCGGCGCGTGATCGTGTGCAACACC AAGCTGGACAACAACTGGGGCAGAGAAGAAAGACAGAGCGTGTTCCCA TTCGAGAGCGGCAAGCCATTCAAGATCCAGGTGCTGGTGGAACCCGAC CACTTCAAGGTGGCCGTGAACGACGCCCATCTGCTGCAGTACAACCAC AGAGTGAAGAAGCTGAACGAGATCAGCAAGCTGGGCATCAGCGGCGA CATCGACCTGACCAGCGCCTCCTACACCATGATCTGACGGACCGGCGA TAGATGAAGCTCGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTG TTCCCTAAGTCCAACTACTAAACTGGGGGATATTATGAAGGGCCTTGAG CATCTGGATTCTGCCTAATAAAAAACATTTATTTTCATTGCAGCTCGCTT TCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACT ACTAAACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCT AATAAAAAACATTTATTTTCATTGCGGCCGCAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
SEQ ID NO: 55 (mRNA) TEV-hGalectin3-2xhBG-1 20A Sequence features: Tobacco Etch Virus (TEV) 5' UTR: 37-1 90 Optimal Kozak sequence: 19 1-1 99 Human Galectin-3 codon optimized , encoding amino acids Accession # NP_002297: 202-951 stop codon: 952-954. 2 copies of human beta-globin 3'UTR: 973-1 238. 120 nucleotide polyA tail (SEQ ID NO: 59): 1245-1 364.	GGGAGACGCGUGUUAAAUAACAAAUCUCAACACAACAUAUACAAAACA AACGAAUCUCAAGCAAUCAAGCAUUCUACUUCUAUUGCAGCAAUUUAA AUCAUUUCUUUUAAAGCAAAAGCAAUUUUCUGAAAAUUUUCACCAUUU ACGAACGAUAGCCGCCACCAUGGCCGACAACUUCAGCCUGCACGAUG CCCUGAGCGGCAGCGGCAACCCUAAUCCUCAGGGAUGGCCUGGCGC UUGGGGCAAUCAGCCUGCUGGCGCUGGCGGAUAUCCUGGCGCAUCU UACCCAGGCGCUUACCCCGGACAGGCUCCUCCAGGCGCAUAUCCAG GCCAGGCACCUCCUGGGGCUUAUCCUGGGGCACCUGGCGCCUACCC UGGCGCUCCUGCUCCUGGCGUGUACCCUGGACCUCCUUCUGGACCC GGCGCAUACCCUAGCUCUGGCCAGCCAUCUGCUACCGGCGCCUAUC CAGCCACAGGACCUUAUGGCGCUCCAGCCGGACCUCUGAUCGUGCC CUACAACCUGCCUCUGCCUGGCGGCGUGGUGCCCAGAAUGCUGAUC ACAAUCCUGGGCACCGUGAAGCCCAACGCCAACAGAAUCGCCCUGGA CUUCCAGAGGGGCAACGACGUGGCCUUCCACUUCAACCCCAGAUUCA ACGAGAACAAUCGGCGCGUGAUCGUGUGCAACACCAAGCUGGACAAC AACUGGGGCAGAGAAGAAAGACAGAGCGUGUUCCCAUUCGAGAGCG GCAAGCCAUUCAAGAUCCAGGUGCUGGUGGAACCCGACCACUUCAAG GUGGCCGUGAACGACGCCCAUCUGCUGCAGUACAACCACAGAGUGAA GAAGCUGAACGAGAUCAGCAAGCUGGGCAUCAGCGGCGACAUCGACC UGACCAGCGCCUCCUACACCAUGAUCUGACGGACCGGCGAUAGAUGA AGCUCGCUUUCUUGCUGUCCAAUUUCUAUUAAAGGUUCCUUUGUUCC CUAAGUCCAACUACUAAACUGGGGGAUAUUAUGAAGGGCCUUGAGCA UCUGGAUUCUGCCUAAUAAAAAACAUUUAUUUUCAUUGCAGCUCGCU UUCUUGCUGUCCAAUUUCUAUUAAAGGUUCCUUUGUUCCCUAAGUCC AACUACUAAACUGGGGGAUAUUAUGAAGGGCCUUGAGCAUCUGGAUU CUGCCUAAUAAAAAACAUUUAUUUUCAUUGCGGCCGCAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAA U= URIDINE AND/OR PSEUDOURIDINE
SEQ ID NO: 56 Galectin 3 RNA coding sequence of construct of SEQ ID NO: 55	AUGGCCGACAACUUCAGCCUGCACGAUGCCCUGAGCGGCAGCGGCA ACCCUAAUCCUCAGGGAUGGCCUGGCGCUUGGGGCAAUCAGCCUGC UGGCGCUGGCGGAUAUCCUGGCGCAUCUUACCCAGGCGCUUACCCC GGACAGGCUCCUCCAGGCGCAUAUCCAGGCCAGGCACCUCCUGGGG CUUAUCCUGGGGCACCUGGCGCCUACCCUGGCGCUCCUGCUCCUGG CGUGUACCCUGGACCUCCUUCUGGACCCGGCGCAUACCCUAGCUCU GGCCAGCCAUCUGCUACCGGCGCCUAUCCAGCCACAGGACCUUAUG GCGCUCCAGCCGGACCUCUGAUCGUGCCCUACAACCUGCCUCUGCC UGGCGGCGUGGUGCCCAGAAUGCUGAUCACAAUCCUGGGCACCGUG AAGCCCAACGCCAACAGAAUCGCCCUGGACUUCCAGAGGGGCAACGA

CGUGGCCUUCCACUUCAACCCCAGAUUCAACGAGAACAAUCGGCGCG
UGAUCGUGUGCAACACCAAGCUGGACAACAACUGGGGCAGAGAAGAA
AGACAGAGCGUGUUCCCAUUCGAGAGCGGCAAGCCAUUCAAGAUCCA
GGUGCUGGUGGAACCCGACCACUUCAAGGUGGCCGUGAACGACGCC
CAUCUGCUGCAGUACAACCACAGAGUGAAGAAGCUGAACGAGAUCAG
CAAGCUGGGCAUCAGCGGCGACAUCGACCUGACCAGCGCCUCCUACA
CCAUGAUCUGA

U= URIDINE AND/OR PSEUDOURIDINE

V. Encapsulated nucleic acid nanoparticles

[00288]   The term "lipid nanoparticle" or "LNP" or "LNPs" refers to a particle that comprises a plurality of (i.e. more than one) lipid molecules physically associated (e.g., covalently or non-covalently) with each other by intermolecular forces. The lipid nanoparticles may be, e.g., microspheres (including unilamellar and multilamellar vesicles, e.g. liposomes), a dispersed phase in an emulsion, micelles or an internal phase in a suspension.

[00289]   The term "lipid nanoparticle host" refers to a plurality of lipid molecules physically associated with each other by intermolecular forces/ electrostatic interactions to encapsulate one or more nucleic acid molecules, such as an mRNA.

[00290]   Certain embodiments provide an encapsulated nucleic acid nanoparticle composition comprising a pharmaceutically acceptable carrier and an encapsulated nucleic acid nanoparticle. The encapsulated nucleic acid nanoparticle includes a lipid nanoparticle host and a nucleic acid, e.g., polyribonucleotide such as mRNA that is encapsulated in the lipid nanoparticle host.

[00291]   The term "pharmaceutically acceptable carrier" as used herein, means a non-toxic, inert diluent. Materials which can serve as pharmaceutically acceptable carriers include, but are not limited to, pyrogen-free water, deionized water, isotonic saline, Ringer's solution, and phosphate buffer solutions. In preferred embodiments, the encapsulated nucleic acid nanoparticle has an average size of about 40 to about 70 nm and a polydispersity index of less than about 0.1 as determined by dynamic light scattering, e.g., using a Malvern Zetasizer Nano ZS. The lipid nanoparticle host comprises a degradable cationic lipid, a lipidated polyethylene glycol, cholesterol, and 1,2-distearoyl-sn-glycero-3-phosphocholine components as described elsewhere herein.

[00292]   Provided herein are methods of preparing an encapsulated nucleic acid nanoparticle composition comprising a cationic lipid and another lipid component. Another embodiment provides a method using a cationic lipid and a helper lipid, for example cholesterol. Another embodiment provides for a method using a cationic lipid, a helper lipid, for example cholesterol, and a neutral lipid, for example DSPC. Another embodiment of the present invention provides a method using a cationic lipid, a helper lipid, for example cholesterol, a neutral lipid, for example DSPC, and a stealth lipid, for example S010, S024, S027, S031 , or S033. Another embodiment

of the present invention provides for a method of encapsulating a nucleic acid in a lipid nanoparticle host where the nanoparticle comprises a cationic lipid, a helper lipid, for example cholesterol, a neutral lipid, for example DSPC, a stealth lipid, for example S010, S024, S027, S031 , or S033, and the nucleic acid is, for example an RNA or DNA. Another embodiment of the present invention provides a method of using a cationic lipid, a helper lipid, for example cholesterol, a neutral lipid, for example DSPC, and a stealth lipid, for example S010, S024, S027, S031 , or S033, where the nucleic acid is, for example, mRNA, mRNA or DNA.

[00293]   In some embodiments, the lipid solution/stream(s) contain a cationic lipid compound, a helper lipid (cholesterol), an optional neutral lipid (DSPC) and a stealth lipid (e.g., S010, S024, S027, or S031). Where a formulation contains four lipid components, the molar ratios of the lipids may range from 20 to 70 mole percent for the cationic lipid with a target of 40 to 60, the mole percent of helper lipid ranges from 20 to 70 with a target of 30 to 50, the mole percent of neutral lipid ranges from 10 to 30, the mole percent of PEG lipid has a range from 1 to 6 with a target of 2 to 5.

[00294]   In some embodiments, the lipid solution/stream(s) contain 30-60% of a compound of formula (III), 30-60% cholesterol / 5-10% DSPC, and 1-5% PEG-DMG, S010, S01 1 or S024.

[00295]   Another embodiment of the present disclosure provides a method of encapsulating a nucleic acid in a lipid nanoparticle host using a cationic lipid and a helper lipid, for example cholesterol, in a lipid molar ratio of about 40-55 cationic lipid /about 40-55 helper lipid. Another embodiment provides a method using a cationic lipid, a helper lipid, for example cholesterol, and a neutral lipid, for example DSPC in a lipid molar ratio of about 40-55 a cationic lipid / about 40-55 helper lipid /about 5-1 5 neutral lipid. Another embodiment provides a method using a cationic lipid, a helper lipid, for example cholesterol, a neutral lipid, for example DSPC, and a stealth lipid, for example S01 0, S024, S027, S031 , or S033 in a lipid molar ratio of about 40-55 cationic lipid /about 40-55 helper lipid / about 5-15 neutral lipid/ about 1-10 stealth lipid.

[00296]   Another embodiment of the present disclosure provides a method of encapsulating a nucleic acid in a lipid nanoparticle host using a cationic lipid and a helper lipid, for example cholesterol, in a lipid molar ratio of about 40-50 cationic lipid / about 40-50 helper lipid. Another embodiment provides a method using a cationic lipid, a helper lipid, for example cholesterol, and a neutral lipid, for example DSPC in a lipid molar ratio of about 40-50 cationic lipid / about 40-50 helper lipid /about 5-1 5 neutral lipid. Another embodiment provides a method using a cationic lipid, a helper lipid, for example cholesterol, a neutral lipid, for example DSPC, and a stealth lipid, for example S01 0, S024, S027, S031 , or S033 in a lipid molar ratio of about 40-50 cationic lipid / about 40-50 helper lipid / about 5-15 neutral lipid/ about 1-5 stealth lipid.

[00297]   Another embodiment of the present disclosure provides a method of encapsulating a nucleic acid in a lipid nanoparticle host using a cationic lipid and a helper lipid, for example cholesterol, in a lipid molar ratio of about 43-47 cationic lipid / about 43-47 helper lipid. Another embodiment provides a method using a cationic lipid, a helper lipid, for example cholesterol, and a neutral lipid, for example DSPC in a lipid molar ratio of about 43-47 cationic lipid / about 43-47 helper lipid /about 7-12 neutral lipid. Another embodiment provides a method using a cationic lipid, a helper lipid, for example cholesterol, a neutral lipid, for example DSPC, and a stealth lipid, for example S010, S024, S027, S031, or S033 in a lipid molar ratio of about 43-47 cationic lipid / about 43-47 helper lipid / about 7-12 neutral lipid/ about 1-4 stealth lipid.

[00298]   Another embodiment of the present disclosure provides a method of encapsulating a nucleic acid in a lipid nanoparticle host using a cationic lipid and a helper lipid, for example cholesterol, in a lipid molar ratio of about 45% cationic lipid and about 44% helper lipid. Another embodiment provides a method using a cationic lipid, a helper lipid, for example cholesterol, and a neutral lipid, for example DSPC in a lipid molar ratio of about 45% cationic lipid, about 44% helper lipid, and about 9% neutral lipid. Another embodiment provides a method using a cationic lipid, a helper lipid, for example cholesterol, a neutral lipid, for example DSPC, and a stealth lipid, for example S010, S024, S027, S031, or S033 in a lipid molar ratio of about 45% cationic lipid, about 44% helper lipid, about 9% neutral lipid, and about 2% stealth lipid.

[00299]   One embodiment of the present disclosure provides a method of preparing an encapsulated nucleic acid nanoparticle composition comprising a cationic lipid and another lipid component. Another embodiment provides a method using a compound of formula (I) and a helper lipid, for example cholesterol. Another embodiment provides for a method using a cationic lipid, a helper lipid, for example cholesterol, and a neutral lipid, for example DSPC. Another embodiment of the present disclosure provides a method using a cationic lipid, a helper lipid, for example cholesterol, a neutral lipid, for example DSPC, and a stealth lipid, for example S010, S024, S027, S031, or S033. Another embodiment of the present disclosure provides for a method of encapsulating a nucleic acid in a lipid nanoparticle host where the nanoparticle comprises a cationic lipid, a helper lipid, for example cholesterol, a neutral lipid, for example DSPC, a stealth lipid, for example S010, S024, S027, S031, or S033, and the nucleic acid is, for example an RNA or DNA. Another embodiment of the present disclosure provides a method of using cationic lipid, a helper lipid, for example cholesterol, a neutral lipid, for example DSPC, and a stealth lipid, for example S010, S024, S027, S031, or S033, where the nucleic acid is, for example, mRNA, mRNA or DNA.

[00300]   Another embodiment of the present disclosure provides a method of encapsulating a nucleic acid in a lipid nanoparticle host using a cationic lipid and a helper lipid, for example

cholesterol, in a lipid molar ratio of about 40-55 compound of formula (I) /about 40-55 helper lipid. Another embodiment provides a method using a cationic lipid, a helper lipid, for example cholesterol, and a neutral lipid, for example DSPC in a lipid molar ratio of about 40-55 cationic lipid / about 40-55 helper lipid /about 5-15 neutral lipid. Another embodiment provides a method using a cationic lipid, a helper lipid, for example cholesterol, a neutral lipid, for example DSPC, and a stealth lipid, for example S01 0, S024, S027, S031 , or S033 in a lipid molar ratio of about 40-55 compound of formula (I) /about 40-55 helper lipid / about 5-1 5 neutral lipid/ about 1-10 stealth lipid.

[00301]   Another embodiment of the present disclosure provides a method of encapsulating a nucleic acid in a lipid nanoparticle host using a cationic lipid and a helper lipid, for example cholesterol, in a lipid molar ratio of about 40-50 cationic lipid / about 40-50 helper lipid. Another embodiment provides a method using a cationic lipid, a helper lipid, for example cholesterol, and a neutral lipid, for example DSPC in a lipid molar ratio of about 40-50 cationic lipid / about 40-50 helper lipid /about 5-1 5 neutral lipid. Another embodiment provides a method using a cationic lipid, a helper lipid, for example cholesterol, a neutral lipid, for example DSPC, and a stealth lipid, for example S01 0, S024, S027, S031 , or S033 in a lipid molar ratio of about 40-50 cationic lipid / about 40-50 helper lipid / about 5-15 neutral lipid/ about 1-5 stealth lipid.

[00302]   Another embodiment of the present disclosure provides a method of encapsulating a nucleic acid in a lipid nanoparticle host using a cationic lipid and a helper lipid, for example cholesterol, in a lipid molar ratio of about 43-47 cationic lipid / about 43-47 helper lipid. Another embodiment provides a method using a cationic lipid, a helper lipid, for example cholesterol, and a neutral lipid, for example DSPC in a lipid molar ratio of about 43-47 cationic lipid / about 43-47 helper lipid /about 7-12 neutral lipid. Another embodiment provides a method using a cationic lipid, a helper lipid, for example cholesterol, a neutral lipid, for example DSPC, and a stealth lipid, for example S01 0, S024, S027, S031 , or S033 in a lipid molar ratio of about 43-47 cationic lipid / about 43-47 helper lipid / about 7-12 neutral lipid/ about 1-4 stealth lipid.

[00303]   Another embodiment of the present disclosure provides a method of encapsulating a nucleic acid in a lipid nanoparticle host using a cationic lipid and a helper lipid, for example cholesterol, in a lipid molar ratio of about 45% cationic lipid and about 44% helper lipid. Another embodiment provides a method using a cationic lipid, a helper lipid, for example cholesterol, and a neutral lipid, for example DSPC in a lipid molar ratio of about 45% cationic lipid, about 44% helper lipid, and about 9% neutral lipid. Another embodiment provides a method using a cationic lipid, a helper lipid, for example cholesterol, a neutral lipid, for example DSPC, and a stealth lipid, for example S010, S024, S027, S031 , or S033 in a lipid molar ratio of about 45% cationic lipid, about 44% helper lipid, about 9% neutral lipid, and about 2% stealth lipid.

[00304]   The ratio of lipids:nucleic acid (e.g. polyribonucleotide such as mRNA) in the processes of the disclosure may be approximately 15-20:1 (wt/wt). In certain embodiments, the ratio of lipids:nucleic acid is about 17-19:1 . In other embodiments, the ratio of lipids:nucleic acid is about 18.5:1 . In other embodiments, the ratio of lipids:nucleic acid is at least about 30:1 , 25:1 , 24:1 , 23:1 , 22:1 , 21:1 , 20:1 , 19:1 , 18:1 , 17:1 , 16:1 , 15:1 , 14:1 , 13:1 , 12:1 , 11:1, or 10:1 (wt/wt).

[00305]   In certain aspects, the nanoparticles produced by the processes of the disclosure have an average/mean diameter and a distribution of sizes around the average value. A narrower range of particle sizes corresponds to a more uniform distribution of particle sizes. Particle size may be determined at the time of collection of the nanoparticles, after an incubation time, or after fully processing (e.g., dilution, filtration, dialysis, etc.) a nanoparticle formulation. For example, particle size determination is typically done after a 60 min incubation period and/or after full sample processing. Average particle sizes are reported as either a Z-Average or a number average. Z-Averages are measured by dynamic light scattering on a Malvern Zetasizer. The nanoparticle sample is diluted in phosphate buffered saline (PBS) so that the count rate is approximately 200 - 400 kcts. The data is presented as a weighted average of the intensity measure. Dynamic light scattering also provides a polydispersity index (PDI) that quantifies the width of the particle size distribution. A larger PDI correlates with a larger particle size distribution and vice versa. Number averages, on the other hand, can be determined by measurement under a microscope.

[00306]   In some embodiments, the encapsulated nucleic acid nanoparticles produced by the processes of the disclosure have an average diameter of about 30 to about 150 nm. In other embodiments, the particles have an average diameter of about 30 to about 40 nm. In other embodiments, the particles have an average diameter of about 40 to about 70 nm. In other embodiments, the particles have an average diameter of about 65 to about 80 nm. In other embodiments, the particles have a Z-average of about 50 to about 80 nm and/or a number average of about 40 to about 80 nm. In still other embodiments, the particles have a Z-average of about 50 to about 70 nm and/or a number average of about 40 to about 65 nm. In yet other embodiments, the particles have a Z-average of about 70 to about 80 nm and/or a number average of about 60 to about 80 nm. The particular size of the particles obtained may depend on the linear velocity of the nucleic acid and lipid streams, the use of an optional dilution step, and the particular nucleic acid or lipids used. Greater linear velocities and maintaining the organic solvent concentration in the first outlet solution < 33% tend to produce smaller particle sizes.

[00307]   In some embodiments, the encapsulated mRNA nanoparticles produced by the processes of the disclosure have an average diameter of about 30 to about 150 nm. In other

embodiments, the particles have an average diameter of about 30 to about 40 nm. In other embodiments, the particles have an average diameter of about 40 to about 70 nm. In other embodiments, the particles have an average diameter of about 65 to about 80 nm. In other embodiments, the particles have a Z-average of about 50 to about 80 nm and/or a number average of about 40 to about 80 nm. In still other embodiments, the particles have a Z-average of about 50 to about 70 nm and/or a number average of about 40 to about 65 nm. In yet other embodiments, the particles have a Z-average of about 70 to about 80 nm and/or a number average of about 60 to about 80 nm. In still other embodiments, encapsulated mRNA nanoparticles produced by the processes of the disclosure may have average diameters of about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 65, about 70, about 75, or about 80 nm. . In still other embodiments, encapsulated mRNA nanoparticles produced by the processes of the disclosure may have average diameters of at least about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 65, about 70, about 75, or about 80 nm. In still other embodiments, encapsulated mRNA nanoparticles produced by the processes of the disclosure may have average diameters of less than about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 65, about 70, about 75, or about 80 nm.

[00308]   Using dynamic light scattering (e.g., Malvern Zetasizer NanoZS), the polydispersity index (PDI) may range from 0 to 1.0. In certain embodiments, the PDI is less than about 0.2. In other embodiments, the PDI is less than about 0.1. In some embodiments., the PDI is less than 1.5, less than 1.4, less than 1.3, less than 1.2, less than 1.1, less than 1.0, less than 0.9, less than 0.8, less than 0.7, less than 0.6, less than 0.5, less than 0.4, less than 0.3, less than 0.2 or less than 0.1.

[00309]   The processes of the present disclosure may be further optimized by one skilled in the art by combining cationic lipids with the desired pKa range, stealth lipids, helper lipids, and neutral lipids into formulations, including, e.g., liposome formulations, lipid nanoparticles (LNP) formulations, and the like for delivery to specific cells and tissues in vivo. In one embodiment, further optimization is obtained by adjusting the lipid molar ratio between these various types of lipids. In one embodiment, further optimization is obtained by adjusting one or more of: the desired particle size, N/P ratio, and/or process parameters. The various optimization techniques known to those of skill in the art pertaining to the above listed embodiments are considered as part of this invention.

Processes for Encapsulating a Nucleic Acid in a Lipid Nanoparticle Host

[00310]   The following methods can be used to make lipid nanoparticles provided herein. Non-limiting methods of making lipid nanoparticles have been described, for example, see PCT International Patent Application Publication Nos. WO 2016/010840, WO2016/037053, WO20 15/095346, WO201 5/095340, WO201 4/1 36086, and WO201 1/076807, each of which is incorporated by reference herein in its entirety. To achieve size reduction and/or to increase the homogeneity of size in the particles, the skilled person may use the method steps set out below, experimenting with different combinations. Additionally, the skilled person could employ sonication, filtration or other sizing techniques which are used in liposomal formulations.

[0031 1]   The process for making a composition provided herein typically comprises providing an aqueous solution, such as citrate buffer, comprising a nucleic acid in a first reservoir, providing a second reservoir comprising an organic solution, such as an organic alcohol, for example ethanol, of the lipid(s) and then mixing the aqueous solution with the organic lipid solution. The first reservoir is optionally in fluid communication with the second reservoir. The mixing step is optionally followed by an incubation step, a filtration or dialysis step, and a dilution and/or concentration step. The incubation step comprises allowing the solution from the mixing step to stand in a vessel for about 0 to about 24 hours (preferably about 1 hour) at about room temperature and optionally protected from light. In one embodiment, a dilution step follows the incubation step. The dilution step may involve dilution with aqueous buffer (e.g. citrate buffer or pure water) e.g., using a pumping apparatus (e.g. a peristaltic pump). The filtration step may be ultrafiltration or dialysis. Ultrafiltration comprises concentration of the diluted solution followed by diafiltration, e.g., using a suitable pumping system (e.g. pumping apparatus such as a peristaltic pump or equivalent thereof) in conjunction with a suitable ultrafiltration membrane (e.g. GE Hollow fiber cartridges or equivalent). Dialysis comprises solvent (buffer) exchange through a suitable membrane (e.g. 10,000 mwc snakeskin membrane).

[00312]   In one embodiment, the mixing step provides a clear single phase. In one embodiment, after the mixing step, the organic solvent is removed to provide a suspension of particles, wherein the nucleic acid is encapsulated by the lipid(s).

[0031 3]   The selection of an organic solvent will typically involve consideration of solvent polarity and the ease with which the solvent can be removed at the later stages of particle formation.   The organic solvent, which is also used as a solubilizing agent, is preferably in an amount sufficient to provide a clear single phase mixture of nucleic acid and lipids. Suitable organic solvents include those described by Strickley, Pharmaceutical Res. (2004), 2 1, 201-230 for use as co-solvents for injectable formulations. For example, the organic solvent may be selected from one or more (e.g. two) of ethanol, propylene glycol, polyethylene glycol 300,

polyethylene glycol 400, glycerin, dimethylacetamide (DMA), N-methyl-2-pyrrolidone (NMP), and dimethylsulfoxide (DMSO). In one embodiment, the organic solvent is ethanol.

[00314]   There is herein disclosed an apparatus for making a composition of the present disclosure. The apparatus typically includes at least one reservoir for holding an aqueous solution comprising a nucleic acid and another one or more reservoirs for holding an organic lipid solution. The apparatus also typically includes a pump mechanism configured to pump the aqueous and the organic lipid solutions into a mixing region or mixing chamber. In some embodiments, the mixing region or mixing chamber comprises a cross coupling, or equivalent thereof, which allows the aqueous and organic fluid streams to combine as input into the cross connector and the resulting combined aqueous and organic solutions to exit out of the cross connector into a collection reservoir or equivalent thereof. In other embodiments, the mixing region or mixing chamber comprises a T coupling or equivalent thereof, which allows the aqueous and organic fluid streams to combine as input into the T connector and the resulting combined aqueous and organic solutions to exit out of the T connector into a collection reservoir or equivalent thereof.

[00315]   In certain embodiments, the concentration of nucleic acid in the one or more nucleic acid streams is about 0.1 to about 1.5 mg/mL and the concentration of lipids in the one or more lipid streams is about 10 to about 25 mg/mL. In other embodiments, the concentration of nucleic acid in the one or more nucleic acid streams is about 0.2 to about 0.9 mg/mL and the concentration of lipids in the one or more lipid streams is about 15 to about 20 mg/mL. In other embodiments, the concentration of nucleic acid in the one or more nucleic acid streams is from about 0.225, 0.3, 0.33, or 0.45 to about 0.675 mg/mL, and the concentration of lipids in the one or more lipid streams is about 16-18 mg/mL. In other embodiments, the concentration of nucleic acid in the one or more nucleic acid streams is about 0.225, 0.3, 0.33, 0.45, or 0.675 mg/mL and the concentration of lipids in the one or more lipid streams is about 16.7 mg/mL.

[00316]   The lipid streams comprise a mixture of one or more lipids in an organic solvent. The one or more lipids may be a mixture of a cationic lipid, a neutral lipid, a helper lipid, and a stealth lipid, each of which may be present in about the same relative amounts as described elsewhere hereinabove for the final encapsulated nucleic acid nanoparticle. The organic solvent used in the lipid stream is one capable of solubilizing the lipids and that is also miscible with aqueous media. Suitable organic solvents include ethanol, propylene glycol, polyethylene glycol 300, polyethylene glycol 400, glycerin, dimethylacetamide (DMA), N-methyl-2-pyrrolidone (NMP), and dimethylsulfoxide (DMSO). In one aspect, the organic solvent comprises about 80% or more ethanol. In a particular aspect, the organic solvent comprises about 90% or more ethanol. In a specific aspect, the organic solvent is ethanol. In certain embodiments, the lipid stream comprises an optional buffer solution, such as a buffer solution of sodium citrate (e.g., 25 mM).

[0031 7]   The nucleic acid stream comprises a mixture of a suitable nucleic acid in a first aqueous solution. The first aqueous solution may include no salts or at least one salt. For example, the first aqueous solution may include a suitable nucleic acid in deionized or distilled water without an added salt. In certain embodiments, the first aqueous solution is a first buffer solution that includes at least one salt such as, for example sodium chloride and/or sodium citrate. In the first aqueous solution, sodium chloride may be present in concentrations ranging from about 0 to about 300 mM. In certain embodiments, the concentration of sodium chloride is about 50, 66, 75, 100, or 150 mM. The first aqueous solution may include sodium citrate in a concentration of about 0 mM to about 100 mM. The first buffer solution preferably has a pH of about 4 to about 6.5, more preferably about 4.5 -5.5. In some embodiments, the pH of the first buffer solution is about 5 and the sodium citrate concentration is about 25 mM. In other embodiments, the pH of the first buffer solution is about 6 and the concentration of sodium citrate is about 100 mM. In specific embodiments, the first buffer solution has a pH that is less than the pKa of the cationic lipid. For the embodiments of the disclosure that include no salt in the aqueous solution, the lipid stream includes the optional buffer solution. In the absence of a salt (e.g., sodium citrate) in either the nucleic acid stream or lipid stream, no encapsulation occurs.

[00318]   Other possible buffers include, but are not limited to, sodium acetate/acetic acid, Na_2HPO_4/citric acid, potassium hydrogen phthalate/sodium hydroxide, disodium hydrogen phthalate/sodium dihydrogen orthophosphate, dipotassium hydrogen phthalate/potassium dihydrogen orthophosphate, potassium dihydrogen orthophosphate/sodium hydroxide.

[00319]   In certain embodiments, the organic solvent comprises ethanol and the first outlet solution comprises about 20-25% ethanol, about 0.15-0.25 mg/mL nucleic acid, and about 3-4.5 mg/mL lipids. In other embodiments, the organic solvent comprises ethanol and the first outlet solution comprises about 20% ethanol, about 0.15-0.2 mg/mL nucleic acid, and about 3-3.5 mg/mL lipids. In yet other embodiments, the organic solvent comprises ethanol and the first outlet solution comprises about 20% ethanol, about 0.18 mg/mL nucleic acid, and about 3.3 mg/mL lipids. In other embodiments, the organic solvent comprises ethanol and the first outlet solution comprises about 25% ethanol, about 0.2-0.25 mg/mL nucleic acid, and about 4-4.5 mg/mL lipids. In still other embodiments, the organic solvent comprises ethanol and the first outlet solution comprises about 25% ethanol, about 0.23 mg/mL nucleic acid, and about 4.2 mg/mL lipids.

[00320]   In some embodiments of the present disclosure, the concentrations of the nucleic acid and the lipids may both be lowered or raised together. For example, although it is generally desirable to keep concentrations as high as possible for a more efficient process, it is possible to

lower the concentrations of the nucleic acid to about 0.045 mg/mL and the lipids to about 1.67 mg/mL. At still lower concentrations, however, particle aggregation tends to increase.

[00321] In certain embodiments, the concentration of nucleic acid in the one or more nucleic acid streams is about 0.1 to about 1.5 mg/mL and the concentration of lipids in the one or more lipid streams is about 10 to about 25 mg/mL. In other embodiments, the concentration of nucleic acid in the one or more nucleic acid streams is about 0.2 to about 0.9 mg/mL and the concentration of lipids in the one or more lipid streams is about 15 to about 20 mg/mL. In other embodiments, the concentration of nucleic acid in the one or more nucleic acid streams is from about 0.225, 0.3, 0.33, or 0.45 to about 0.675 mg/mL, and the concentration of lipids in the one or more lipid streams is about 16-18 mg/mL. In other embodiments, the concentration of nucleic acid in the one or more nucleic acid streams is about 0.225, 0.3, 0.33, 0.45, or 0.675 mg/mL and the concentration of lipids in the one or more lipid streams is about 16.7 mg/mL. Generally, higher nucleic acid concentrations require a correspondingly increased level of dilution from the dilution stream 50 to maintain the nucleic acid concentration in the first outlet stream 60 in a preferred range (e.g., about 0.15-0.25 mg/mL).

[00322] In particular embodiments, the mass ratio of lipids:nucleic acid is about 15-20:1 or about 17-19:1 and the concentration of the organic solvent in the outlet solution is about 20-25%. In other particular embodiments, the mass ratio of lipids:nucleic acid is about 18.5:1 and the concentration of the organic solvent in the outlet solution is about 25%.

[00323] In particular embodiments, the mass ratio of lipids:nucleic acid is about 17-19:1. In other particular embodiments, the mass ratio of lipids:nucleic acid is about 18.5:1.

[00324] In particular embodiments, the mass ratio of lipids:nucleic acid is about 15-20:1 or about 17-19:1 and the concentration of the organic solvent in the outlet solution is about 20-25%. In other particular embodiments, the mass ratio of lipids:nucleic acid is about 18.5:1 and the concentration of the organic solvent in the outlet solution is about 20% or 25%.

[00325] In particular embodiments, the mass ratio of lipids:nucleic acid is about 17-19:1. In other particular embodiments, the mass ratio of lipids:nucleic acid is about 18.5:1. In particular embodiments, the mass ratio of lipids:nucleic acid is about 15-20:1 or about 17-19:1 and the concentration of the organic solvent in the outlet solution is about 20-25%. In other particular embodiments, the mass ratio of lipids:nucleic acid is about 18.5:1 and the concentration of the organic solvent in the outlet solution is about 20%.

[00326] In particular embodiments, the mass ratio of lipids:nucleic acid is about 17-19:1. In other particular embodiments, the mass ratio of lipids:nucleic acid is about 18.5:1.

[00327] In certain aspects, the encapsulation rate is >60%. In certain aspects, the encapsulation rate is >65%. In certain aspects, the encapsulation rate is >70%. In some

117

embodiments of the present disclosure, 75% or more of the nucleic acid is encapsulated. In other embodiments, 80% or 85% of the nucleic acid is encapsulated. In still other embodiments, 90% or more of the nucleic acid is encapsulated. In other embodiments about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% of the nucleic acid is encapsulated.

[00328]   In certain aspects, following formation of the encapsulated nucleic acid nanoparticles as described herein, the first outlet solution may be incubated for about 60 minutes at room temperature. After incubation, the solution may be mixed with a second dilution solvent to dilute the first outlet solution by about 2-fold to provide a second outlet solution. The second dilution solvent may be a third buffer solution or water. The dilution step may be carried out by mixing the incubated first outlet solution with the second dilution solvent (water), for example, in a T connector. The incubated first outlet solution and the second dilution solvent may be supplied to the T connector at any suitable flow rate or velocity, such as, for example, about 0.5 to 1 meter/second. Following the dilution step, the concentration of organic solvent in the second outlet solution is reduced by one-half relative to the first outlet solution. Thus, in some embodiments, the concentration of organic solvent (e.g., ethanol) in the second outlet solution is less than 16.5%. In other embodiments, the concentration of organic solvent (e.g., ethanol) in the second outlet solution is about 10-15%, about 10-12.5%, about 12.5%, or about 10%. The second outlet solution may be concentrated by tangential flow filtration and subjected to a 15X diafiltration with phosphate buffered saline (PBS) to remove the starting buffer and ethanol, which are replaced with PBS. After tangential flow filtration, the pool of concentrated encapsulated nucleic acid nanoparticles in PBS may be collected and sterile filtered. Encapsulated nucleic acid nanoparticles present in formulations produced by the foregoing additional process steps may be storage stable at 4 °C for greater than 6 months.

[00329]   According to each of the embodiments disclosed herein, are further embodiments where the nucleic acid is a polyribonucleotide such as an mRNA. For example, according to the embodiments described herein are further embodiments where the nucleic stream is an mRNA stream comprising a mixture of one or more mRNA molecules in a buffer solution and having the linear velocities disclosed herein.

VI. Immunization of animals

[00330]   Host animals used for immunization encompass any species which can generate a humoral (antibody)-mediated immune response. Non-limiting examples of host animals, such as non-human animals, used for immunization include mouse, rat, rabbit, goat, sheep, camelid, horse, chicken, dog, cat, pig, donkey, cow, monkey and shark.

[00331]   In a specific aspect of the present disclosure for generating human antibodies against a target protein, transgenic or transchromosomic mice carrying parts of the human immune system rather than the mouse immune system may be used as host animals immunized with the mRNA-LNP complexes described herein. Non-limiting examples of these transgenic and/or transchromosomic mice include mice referred to herein as HuMAb mice and KM mice, respectively, and are collectively referred to herein as "human Ig mice."

[00332]   The HuMAb mouse® (Medarex, Inc.) contains human immunoglobulin gene miniloci that encode un-rearranged human heavy (μ and γ) and κ light chain immunoglobulin sequences, together with targeted mutations that inactivate the endogenous μ and κ chain loci (see e.g., Lonberg, et al., 1994 Nature 368(6474): 856-859). Accordingly, the mice exhibit reduced expression of mouse IgM or κ, and in response to immunization, the introduced human heavy and light chain transgenes undergo class switching and somatic mutation to generate high affinity human IgGK monoclonal (Lonberg, N. et al., 1994 supra; reviewed in Lonberg, N., 1994 Handbook of Experimental Pharmacology 113:49-101 ; Lonberg, N. and Huszar, D., 1995 Intern. Rev. Immunol. 13: 65-93, and Harding, F. and Lonberg, N., 1995 Ann. N.Y. Acad. Sci. 764:536-546). The preparation and use of HuMAb mice, and the genomic modifications carried by such mice, is further described in Taylor, L. et al., 1992 Nucleic Acids Research 20:6287-6295; Chen, J. et at., 1993 International Immunology 5: 647-656; Tuaillon et al., 1993 Proc. Natl. Acad. Sci. USA 94:3720-3724; Choi et al., 1993 Nature Genetics 4:1 17-123; Chen, J. et al., 1993 EMBO J. 12: 821-830; Tuaillon et al., 1994 J. Immunol. 152:2912-2920; Taylor, L. et al., 1994 International Immunology 579-591 ; and Fishwild, D. et al., 1996 Nature Biotechnology 14: 845-851 , the contents of all of which are hereby specifically incorporated by reference in their entirety. See further, U.S. Patent Nos. 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,789,650; 5,877,397; 5,661 ,016; 5,814,318; 5,874,299; and 5,770,429; all to Lonberg and Kay; U.S. Patent No. 5,545,807 to Surani et al.; PCT Publication Nos. WO 92103918, WO 93/12227, WO 94/25585, WO 971 13852, WO 98/24884 and WO 99/45962, all to Lonberg and Kay; and PCT Publication No. WO 01/14424 to Korman et al.

[00333]   In another embodiment, human antibodies can be raised by the mRNA-immunization methods provided herein using a mouse that carries human immunoglobulin sequences on transgenes and transchomosomes such as a mouse that carries a human heavy chain transgene and a human light chain transchromosome. Such mice, referred to herein as "KM mice", are described in detail in PCT Publication WO 02/43478 to Ishida et al.

119

Still further, alternative transgenic animal systems expressing human immunoglobulin genes are available in the art and can be used as host animals for the mRNA-immunization methods provided herein. For example, an alternative transgenic system referred to as the Xenomouse (Abgenix, Inc.) can be used. Such mice are described in, e.g., U.S. Patent Nos. 5,939,598; 6,075,181 ; 6,1 14,598; 6, 150,584 and 6,162,963 to Kucherlapati et al.

[00334]   Moreover, alternative transchromosomic animal systems expressing human immunoglobulin genes are available in the art and can be used as host animals for the mRNA-immunization methods provided herein. For example, mice carrying both a human heavy chain transchromosome and a human light chain tranchromosome, referred to as "TC mice" can be used; such mice are described in Tomizuka et al., 2000 Proc. Natl. Acad. Sci. USA 97:722-727. Furthermore, cows carrying human heavy and light chain transchromosomes have been described in the art (Kuroiwa et al., 2002 Nature Biotechnology 20:889-894) and can be used as host animals for the mRNA-immunization methods provided herein.

[00335]   Following encapsulated mRNA-mediated immunization, antibody secreting cells (e.g., such as lymphocytes, bone marrow cells, plasma cells, or splenocytes) from host animals may be harvested and screened for antibodies generated against the protein target which contain the desired properties. This may be done through the use of hybridoma-based technology, direct screening of antibody producing B cells followed by cloning and recombinant antibody production, or the generation of a recombinant antibody library from B cells followed by expression and screening in a heterologous expression system such as phage or yeast display.

[00336]   In a particular aspects, antibody secreting cells (e.g., such as lymphocytes, bone marrow cells, plasma cells, or splenocytes) from host animals are fused with fusion partner cells (e.g., immortal B cell cancer cells, for example, an immortalized myeloma cells), such as F0 cells (ATCC®, CRL-1646) and SP2/0 myeloma cells (ATCC®, CRL-1581). Cell fusion can be carried out by various methods, such as, electrofusion or chemical protocols, for example, using polyethylene glycol.

[00337]   In the case of immunizations wherein mice are the host animal, hybridoma-based antibody generation followed by FACS or ELISA-based screening offers an effective antibody expression and screening platform. Circulating levels of target-specific antibodies (i.e. sera titers) can be monitored over the course of a hybridoma-based immunization campaign to evaluate the effectiveness of the humoral response. Given the high degree of target specificity that is associated with mRNA-based immunization, sera titers for integral or membrane proteins can be efficiently monitored by FACS using cells which overexpress the target protein. Titers for soluble proteins can also be assayed by ELISA. Depending upon sera titers, dosing can be adjusted to achieve levels that are deemed suitable for initiation of B cell isolation and myeloma fusion.

Route of mRNA administration (e.g. intravenous, subcutaneous, intramuscular, etc.) can also be altered to vary the degree and perhaps diversity of the immune response. Intravenous administration of encapsulated mRNA has been found to be a particularly efficacious route for generating rapid target-specific titers.

[00338]   One generalizable immunization schedule is outlined below, as an example:

Day 0: draw blood to establish baseline titers in immunologically naive mice

Day 1 (1st immunization): Inject 4 mice subcutaneously and 4 mice intravenously with 5-100 μg, e.g., 25-50 μg, of encapsulated mRNA.

Day 10: Withdraw blood to monitor sera titers.

Day 21 (2nd immunization): Inject 4 mice subcutaneously and 4 mice intravenously with 5-100 μg, e.g., 25-50 μg, of encapsulated mRNA.

Day 31: Withdraw blood to monitor sera titers.

Day 42 (Final immunization): Inject mice intravenously with 5-100 μg, e.g., 25-50 μg, of encapsulated mRNA.

Day 45: Harvest spleens for isolation of splenocytes and hybridoma fusion.

[00339]   In certain aspects, a generalizable immunization schedule may include combinations of immunization with encapsulated mRNA and other conventional immunization methods, such as recombinant protein immunization or whole cell/whole cell extract immunization. For example, the 1st immunization comprises immunization with encapsulated mRNA followed by a 2nd immunization by conventional immunization methods, such as recombinant protein immunization or whole cell/whole cell extract immunization. In specific aspects, the number of days in between immunization, blood withdrawal to monitor sera titers, and subsequence rounds of immunizations may vary by 1, 2, 3, 4, 5, 6, or 7 days.

VIII.   Antibody Production

Generation of monoclonal antibodies

[00340]   Monoclonal antibodies (mAbs) can be produced by a variety of techniques, including conventional monoclonal antibody methodology *e.g.,* the standard somatic cell hybridization technique of Kohler and Milstein, 1975 Nature 256: 495. Many techniques for producing monoclonal antibody can be employed *e.g.,* viral or oncogenic transformation of B lymphocytes.

[00341]   Animal systems for preparing hybridomas include the murine, rat and rabbit systems. Hybridoma production in the mouse is a well established procedure. Immunization protocols are described herein and techniques for isolation of immunized splenocytes for fusion are known in

the art. Fusion partners *(e.g.,* murine myeloma cells) and fusion procedures are also known and have been described.

[00342]   Chimeric or humanized antibodies of the present disclosure can be prepared based on the sequence of a non-human, e.g., murine, monoclonal antibody prepared as described herein. DNA encoding the heavy and light chain immunoglobulins can be obtained from the hybridoma of interest and engineered to contain non-murine *(e.g.,.* human) immunoglobulin sequences using standard molecular biology techniques.   For example, to create a chimeric antibody, the murine variable regions can be linked to human constant regions using methods known in the art (see *e.g.,* U.S. Patent No. 4,816,567 to Cabilly *etai.).* To create a humanized antibody, the murine CDR regions can be inserted into a human framework using methods known in the art. See *e.g.,* U.S. Patent No. 5225539 to Winter, and U.S. Patent Nos. 5530101 ; 5585089; 5693762 and 6180370 to Queen *et al.*

[00343]   A chimeric antibody is a molecule in which different portions of the antibody are derived from different immunoglobulin molecules. For example, a chimeric antibody can contain a variable region of a mouse or rat monoclonal antibody fused to a constant region of a human antibody. Methods for producing chimeric antibodies are known in the art. See, e.g., Morrison, 1985, Science 229: 1202; Oi et al., 1986, BioTechniques 4:214; Gillies et al., 1989, J. Immunol.

[00344]   In a certain aspects, the antibodies of the present disclosure are human monoclonal antibodies. Such human monoclonal antibodies can be generated using transgenic or transchromosomic mice carrying parts of the human immune system rather than the mouse system. These transgenic and/or transchromosomic mice include mice referred to herein as HuMAb mice and KM mice, respectively, and are collectively referred to herein as "human Ig mice."

[00345]   Immunology 579-591 ; and Fishwild, D. *et al.,* 1996 Nature Biotechnology 14: 845-851 , the contents of all of which are hereby specifically incorporated by reference in their entirety. See further, U.S. Patent Nos. 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,789,650; 5,877,397; 5,661 ,016; 5,814,318; 5,874,299; and 5,770,429; all to Lonberg and Kay; U.S. Patent No. 5,545,807 to Surani *et al.;* PCT Publication Nos. WO 92103918, WO 93/12227, WO 94/25585, WO 971 13852, WO 98/24884 and WO 99/45962, all to Lonberg and Kay; and PCT Publication No. WO 01/14424 to Korman *et al.*

[00346]   Antibodies or antigen-binding fragments produced using techniques such as those described herein can be isolated using standard, well known techniques. For example, antibodies or antigen-binding fragments can be suitably separated from, e.g., culture medium, ascites fluid, serum, cell lysate, synthesis reaction material or the like by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose,_hydroxylapatite

chromatography, gel electrophoresis, dialysis, or affinity chromatography.

EXAMPLES

[00347]   The following examples are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

EXAMPLE 1

Encapsulated mRNA Production Workflow

Step One: Design of cDNA and cloning into in-vitro transcription vector

Design of the cDNA construct

[00348]   Native cDNA sequences may be used for the purposes of subcloning if it does not contain any consensus sites for the restriction enzymes used in the subcloning strategy or in the linearization of the final construct prior to transcription. In this particular example, the restriction enzymes used for subcloning are BamHI and RsrII, the restriction enzyme used for linearization is BspQI. However, any suitable restriction enzyme and corresponding restriction site can be used. For example, certain restriction sites that are not present in a particular cDNA encoding a target protein of interest can be selected for subcloning strategy and linearization.

[00349]   The native cDNA sequence can also be codon optimized for expression in a non-human animal, such as mouse or rabbit, using conventional methods, for example, using the GeneOptimizer® software (ThermoFisher Scientific, Inc.). The process of codon optimization involves one or more of the following: (i) elimination of cryptic splice sites and RNA destabilizing sequence elements for increased RNA stability; (ii) addition of RNA stabilizing sequence elements; (iii) codon optimization and G/C content adaptation for a particular expression system; (iv) intron removal; and (v) avoidance of stable RNA secondary structures. In specific aspects using the the GeneOptimizer® software, codon optimization settings were adjusted to protect the 573' restriction and exclude them from the rest of the molecule. BspQI consensus sequences (both forward [GCTCTTC] and reverse [GAAGAGC], as BspQI is not a palindromic sequence restriction enzyme) should also be excluded.

<u>TABLE 8</u>
Materials and Reagents for cloning of cDNA into transcription vector

<u>Reagent</u>	<u>Vendor</u>	<u>Catalog #</u>
BspQ1	New England Biolabs	R0712S
BamH1	New England Biolabs	R0136S
Rsrll	New England Biolabs	R0501S
Stbl3 competent cells	Life technologies	C7373-03
Quick ligase	NEB	M220S

Description of the vector

[00350]   cDNA encoding a target protein (e.g., see Tables 1-7) was cloned into a vector designed to drive RNA polymerase-mediated transcription from a T7 RNA polymerase promoter. Immediately downstream of the T7 promoter is a sequence which encodes the 5' untranslated region (UTR) of the tobacco etch virus (TEV). This UTR has been shown to improve translational efficiency in eukaryotic cells. Downstream of the TEV UTR, the cDNA of the target protein is placed. A Kozak consensus sequence (ccgccacc) was inserted upstream of the initiator methionine/start codon to enhance translation. Two stop codons were placed at the end of the cDNA followed by a Rsrll restriction site. Two tandem human beta-globin 3' UTRs follow the cDNA sequence. This element has been shown to enhance mRNA stability in cells. A C-terminal element of the transcriptionally-relevant components of the vector is a polyA tail. In specific embodiments, a polyA tail of an mRNA encoding a target protein or a fragment thereof is approximately 50 bps to 120 bps or 60 bps to 120 bps. In particular embodiments, a polyA tail of an mRNA encoding a target protein or a fragment thereof is approximately 60 bps or 120 bps. In certain embodiments a polyA tail of an mRNA encoding a target protein or a fragment thereof is approximately 70 bps, 80 bps, 90 bps, 100 bps, or 110 bps.

Cloning of cDNA into the in vitro transcription vector

[00351]   Digestion of the cDNA construct along with the vector with the restriction enzymes BamHI/Rsrll generated compatible fragments that were purified by agarose gel electrophoresis, and the purified cDNA subsequently were ligated to the digested vector to yield the desired transcription vector construct.

[00352]   The ligation mixture was transformed into stbl3 competent bacterial cells and plated onto ampicillin plates. The plates were incubated overnight at 37°C.

Prior to sequencing, colonies were triaged by digesting with the subcloning restriction enzymes to verify the appropriately sized insert and backbone. Colonies were also digested in parallel with

Rsrll and SapI (an isoschizimer of BspQ1 that cuts efficiently at 37°C) to establish the integrity of the polyA tail. Plasmids from a sequence-verified clone were expanded and used for mRNA generation.

Step Two: transcript linearization, in vitro transcription and capping

[00353]   Circular plasmid DNA was prepared according to conventional methods. Purified plasmid DNA was digested with BspQI restriction endonuclease. Plasmid DNA was combined with the appropriate reaction buffer (Buffer 4, New England Biolabs, 10X stock) and BspQI enzyme (1,250 U per mg of DNA). The reaction was incubated at 50°C for 2 hours and then placed on ice or at 4°C. A small sample of the reaction was run on a standard agarose electrophoresis gel to confirm complete linearization of the circular plasmid DNA. The linearized DNA template was purified by ethanol precipitation. DNA pellet from the ethanol precipitation step was dissolved using nuclease-free water to a concentration of >0.5 mg/ml.

In vitro transcription and capping of modified synthetic mRNA

[00354]   The modified synthetic mRNA of this EXAMPLE was generated by *in vitro* transcription (IVT), purified by lithium chloride (LiCl) purification, and then capped using a commercially available kit from New England Biolabs® (Beverly, MA USA). Materials and reagents are shown in TABLE 9.

TABLE 9
Materials and Reagents for *In vitro* Transcription and Capping

Reagent	Vendor	Catalog #
Nuclease-free water		
Tris-HCl pH 8.0	Life Technologies/ThermoFisher	AM9855G
MgCl$_2$	Life Technologies/ThermoFisher	AM9530G
ATP, CTP, GTP, UTP	New England Biolabs	N0450L
Pseudouridine (Ψ)	TriLink Biotech	N-1019
DTT	Sigma-Aldrich	43816
Spermidine	Sigma-Aldrich	85558
Linearized plasmid DNA		
Pyrophosphatase	New England Biolabs	M2403L
RNase inhibitor	New England Biolabs	M0307L
T7 RNA polymerase	New England Biolabs	M0251 L
DNase	New England Biolabs	M0303
LiCl	Life Technologies/ThermoFisher	AM9480
Vaccinia capping system	New England Biolabs	M2080S
mRNA cap 2'-0-methyltransferase	New England Biolabs	M0366S

[00355]   Transcription reactions are assembled, for example, as listed in TABLE 10, with care towards the use of RNase-free tubes, tips and practices.

TABLE 10
In vitro Transcription Reaction

Reagent	Concentration	Notes
Nuclease-free water	Remaining volume	
Tris-HCl pH 8.0 (mM)	40	
$MgCl_2$ (mM)	20	
ATP, CTP, GTP, UTP (mM)	4	
Pseudouridine (mM)	4	To make 100% pseudouridine mRNA, do not include UTP in reaction. To make 100% unmodified mRNA, do not include pseudouridine in reaction
DTT (mM)	10	
Spermidine (mM)	2	Dilute 1M stock 1:10 in water
Linearized plasmid DNA (µg/µL)	0.05	
Pyrophosphatase (U/ML)	0.004	
RNase inhibitor (U/ML)	1	
T7 RNA polymerase (U/µl)	5	

[00356] The procedure in this EXAMPLE for making modified synthetic mRNA was carried out as follows:

1. The materials above were incubated for 2 hours at 30°C, while monitoring the temperature. The DNA template was digested by adding 0.04 ʊ/µL DNase, and this reaction mixture was incubated for 30 minutes at 37°C.

2. LiCl was added to a final concentration of 2.81 M, and the reaction mixture was mixed well and incubated for over an hour at -20°C. The mixture then was centrifuged at 4°C for 15 minutes at a maximum speed of approximately 20,000xg (max speed). The supernatant was removed and the pellet was washed with 1 mL 70% ethanol. The preparation was centrifuged as described immediately above for 10 minutes. Then the supernatant was removed, and the remaining pellet was centrifuged again as described above for less than one minute.

3. The remaining ethanol was removed, and the pellet was resuspended in nuclease-free water. The concentration was measured and adjusted to approximately 1µg/µL.

4. To the preparation, 10% volume of 3M sodium acetate pH 5.5 was added, and the preparation was mixed well. Then, 1 volume of room temperature isopropanol was added to the preparation, and mixed well. The preparation was incubated overnight at -20°C. Subsequently, the preparation was centrifuged at 4°C for 15 minutes at a maximum speed of approximately 20,000xg (max speed), the supernatant was removed, and the remaining pellet was washed with 1 mL 70% ethanol. Again, the preparation was centrifuged as described immediately above for 10 minutes, followed by removal of the supernatant, and the centrifuge step was carried out again as described above for less than one minute.

5. The remaining ethanol was removed, and the pellet was resuspended in nuclease-free water. The concentration of the preparation was measured and adjusted to approximately 4 μg/μL.

[00357]   The modified synthetic mRNA can then be stored at -80°C until capping, and the concentration measured again upon thawing.

[00358]   For capping, the procedure used was that of New England BioLabs. The synthetic mRNA and water mixture was heat denatured at 65°C for 10 minutes, and then transferred to cold block to quench for 5 minutes. The stock solution of S-adenosyl methionine (SAM) (32 mM) was diluted 1:8 in water to 4 mM immediately before use, then the remaining reaction components were added in the order specified in TABLE 11.

TABLE 11: Capping Reaction		
Reagent	Stock concentration	Final concentration
mRNA (Mg/μl)		0.5
Water		Remaining volume
10x capping buffer (x)	10x	1x
GTP (mM)	10	0.5
SAM (mM)	4	0.2
RNase Inhibitor (U/μL)	40	1
Vaccinia capping enzyme (U/μL)	10	0.5
mRNA Cap 2'-0-Methyltransferase (U/μL)	50	2.5

[00359]   Then, the mixture was incubated for one hour at 37°C. The sample was purified by LiCl precipitation as described above, and then stored at -80°C.

Step Three: Determine mRNA Quality and Functionality

[00360]   The synthetic mRNA were analyzed for quality and integrity using an Agilent 2100

Bioanalyzer after the initial *in vitro* transcription reaction and/or after the capping reaction. The Agilent 2100 BioAnalyzer is a nanofluidics device that preforms size fractionation and quantification of small samples of DNA, RNA, or Protein. The analysis was performed using an Agilent RNA 6000 Nano Kit (Cat. # 5067-151 1).

[00361]   All of the kit reagents must be equilibrated to room temperature for 30 minutes prior to use. The synthetic mRNA sample and ladder from the kit were stored on ice.

Prepare gel, gel/dye mix, and samples

[00362]   A gel matrix (550 µL) was pipetted into a spin filter, and centrifuged at 1,500g for 10 minutes at room temperature, then stored at 4°C (for use within 1 month). Dye stock (1 µl) was added to a 65 µl aliquot of filtered gel matrix. The dye and gel matrix mix was vortexed and then centrifuged at 13,000g for 10 minutes at room temperature (for use within 1 day). The mRNA samples and ladder and kit standard were heat denatured at 70°C for 3-5 minutes to break apart any higher order structures, then quenched on ice prior to analysis.

Decontaminate Bioanalyzer electrodes

[00363]   The Bioanalyzer electrodes were decontaminated with 350 µl of RNaseZap electrode cleaner and nuclease-free water.

Load gel/dye mix onto chip

[00364]   A new chip was placed on the priming station of the Bioanalyzer (platform at position C, clip at top position), and 9 µl of gel/dye mixture was added into a well.

Load samples onto chip

[00365]   A volume of 5 µl of marker was added to ladder well and sample wells, and a volume of 1 µl of mRNA sample (< 1 µg) was added to sample wells. An IKA Vortexer was used to vortex for 1 minute at 2400 rpm. Then the samples were run on the Agilent 2100 Bioanalyzer using the mRNA assay method. See Figure 4 for a sample bioanalyzer trace of mRNA prepared for human RXFP1 (both codon and non-codon optimized) synthesized using adenine, guanine, cytidine, and either uridine or pseudouridine.

Transfection of mRNA into cultured mammalian cells and western blot to confirm expression of encoded protein

[00366]   Translatability was assessed by *in vitro* transfection of the mRNA into cultured mammalian cells using Lipofectamine ® 2000 reagent from Thermo Fisher Scientific. The

transfected cells were then lysed 24 to 48 hours later, and the proteins in the lysates were resolved using polyacrylamide gel electrophoresis followed by immunoblot with antibodies specific to the protein encoded by the mRNA (see Figure 5 for a sample Western blot illustrating confirmation of human RXFP1 expression from mRNA).

Step 4. Packaging of Modified Synthetic mRNA in a Delivery Vehicle

[00367]   The materials in this EXAMPLE used were as follows:

TABLE 12
Materials for Packaging of Modified Synthetic mRNA

Item	Vendor	Catalog #
Cationic lipid	Novartis	Selected from Cationic Lipid A, Cationic Lipid B or Cationic Lipid C
1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC)	Corden	LP-R4-076
Cholesterol	Sigma	C8667
Lipidated Polyethylene Glycol (PEG lipid)	Novartis	S024
Ethanol	Sigma	459844
Nuclease-free water	Life Technologies	10977
100 mM citrate buffer, pH 6.0	Teknova	Q2446
Amicon Ultra-15 Centrifugal Filter unit, 30K MWCO	Millipore	UFC903024
RNaseZap	Life Technologies	AM9780
Syringe Pump	KD Scientific	KDS220
10X PBS	Lonza	S1226
SnakeSkin dialysis tubing 10,000 MWCO	Thermo Scientific	68100
Minimate TFF system, 110 V	PALL Corporation	OAPMP1 10
Minimate tangential flow filtration capsule, Omega 500K membrane	PALL Corporation	OA500C12
Quant-iT Ribogreen RNA Assay Kit	Life Technologies	R 1 1490
TE buffer	Promega	V6231
Triton X-100	Sigma	T8787
Zetasizer Nano ZS	Malvern	ZEN3600

130

Chemical structure of Lipid A

Molecular Formula:
$C_{47}H_{89}NO_9$

Monoisotopic Mass:
811.6537

Chemical structure of Lipid B

Molecular Formula:	$C_{47}H_{81}NO_6$
Monoisotopic Mass:	755.6064
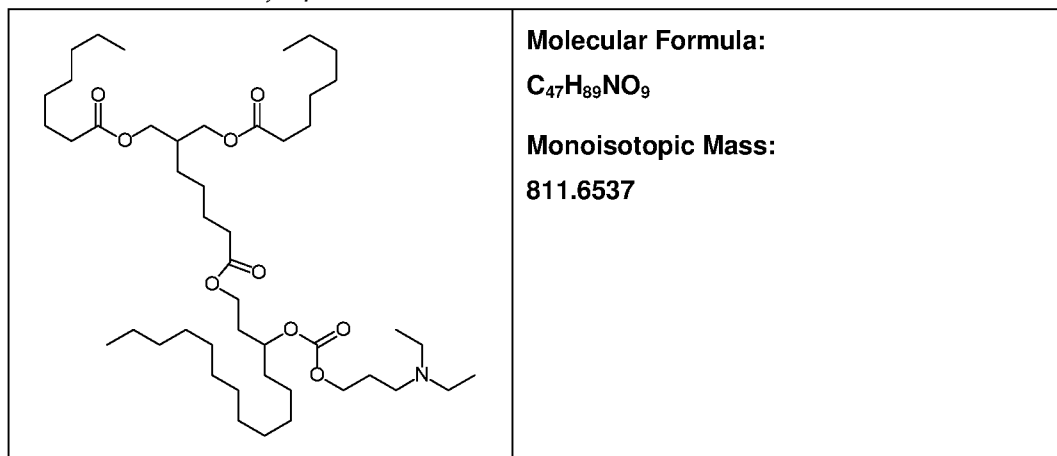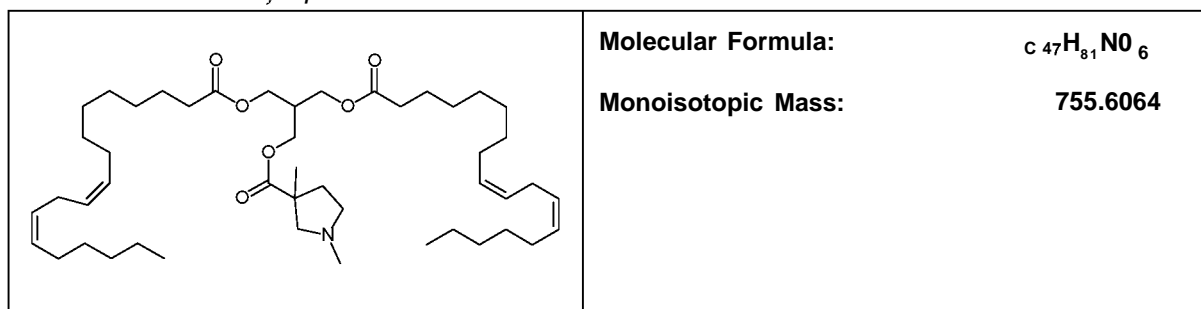
Chemical structure of Lipid C
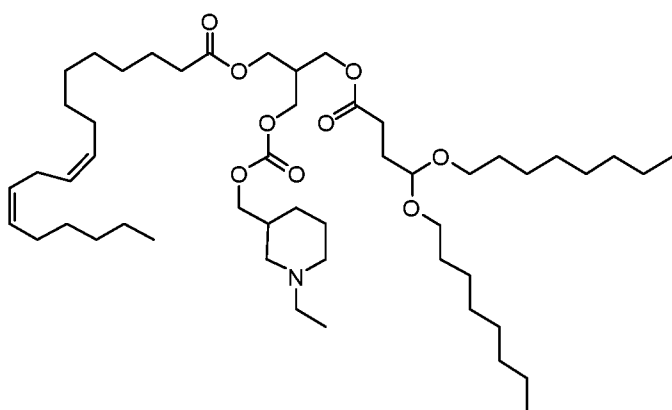
1.   Modified synthetic mRNAs encoding target protein (e.g., see Tables 1-7) were packaged into lipid nanoparticles at a cationic lipid amine group to mRNA phosphate group (N:P) molar ratio = 4:1 , dialyzed, and concentrated. As an example, amounts are shown for the protocol resulting in ~2 mg packaged modified synthetic mRNA in a concentration of >0.4 mg/mL mRNA.

2.   Using RNase-free reagents, tubes, tips, and practices, the lipid nanoparticle mixture reagents were weighed and mixed in a vial as described in TABLE 13.

TABLE 13

Lipid Nanoparticle Mixture

Reagent	Final concentration (mM)
Cationic lipid	6
DSPC	1.5
Cholesterol	7.2
PEG lipid	0.3

3.   Ethanol was added to the lipids, representing a 1.1x ratio of the volume needed, for ease of processing. The mixture was briefly sonicated and gently agitated for 5 minutes at 37°C. Subsequently, the mixture was incubated without agitation at 37°C until ready for use.

4.   The modified synthetic mRNA was exchanged from water into pH 6.0 buffer by loading mRNA solution onto Amicon Ultra-15 centrifugal device, and centrifuging for 15 minutes at 4,000 rpm at 4°C. The concentrated mRNA was resuspended in pH 6.0 citrate buffer and the mRNA concentration was measured.

5.   The final modified synthetic mRNA concentration of 0.5 mg/mL in pH 6.0 citrate buffer was prepared in a rinsed scintillation vial (4 mg mRNA in 8 mL), and the final concentration of the mRNA solution was measured. The mRNA dilution was incubated at 37°C until ready for use.

6.   Three 10 ml syringes were prepared, with 8 mL of each: (a) lipid mixture; (b) mRNA solution; (c) citrate buffer. Syringes (a) and (b) were attached to the Luer fittings of the T-shaped junction. Briefly, a P727 T-mixerwith 0.5 mm inner diameter attached to P652 adaptors (IDEX, Oak Harbor WA USA). Syringes (a) and (b) were attached to P658 Luer fittings (IDEX). The syringes (a) and (b) were connected to the T-mixer by PTFE 0.8 mm inner diameter tubing (#3200068, Dolomite, Royston, UK) with P938x nuts and ferrules (IDEX). Syringe (c) was attached to a Luer fitting connected to a final single tubing by P938x a nut and ferrule. The ends of the tubing were secured together over pre-rinsed beaker with stir bar and gently stirred.

7.   The syringe pump settings were set to appropriate syringe manufacturer and size, and a volume (8 mL) and flow rate of 1.0 mL/min were entered. The pump was started, and the resulting material collected into RNase-free 50 mL plastic beaker with a stir bar. The suspension of lipid nanoparticles containing mRNA was transferred to dialysis tubing, 2-3 mL per bag and dialyzed into phosphate-buffered saline (PBS) at 4°C overnight.

8.   The divided material was pooled into one 15 mL conical tube. The lipid nanoparticle (LNP) suspension was concentrated using tangential flow filtration (TFF). Using fresh tubing to connect fresh 500K molecular weight cut-off capsule to the Minimate system, the TFF system was prepared by rinsing with 500 mL RNA-free water at a flow rate of 150 rpm.

9.   The lipid nanoparticle/modified synthetic mRNA suspension was loaded into TFF unit reservoir and concentrated at a flow rate of 75 mL/min to 2-3 ml final volume.

10.   The percent encapsulation of modified synthetic mRNA was determined using Quant-iT Ribogreen RNA Assay kit from Life Technologies (Grand Island NY USA). The lipid nanoparticle / modified synthetic mRNA suspension was assayed by fluorescence measurement in buffer (mRNA outside the particle) and in buffer plus detergent (total mRNA). A 1000 ng/mL stock from the provided ribosomal RNA was prepared and used to generate a standard curve for the Ribogreen assay. For the assay, samples are prepared in TE buffer or TE plus Triton and the fluorescent reagent is added to each. The difference calculated is the mRNA inside the particle.

TABLE 14
Standard Curve (Preparation for Duplicate Samples)

RNA concentration (ng/mL)	Volume 1000 ng/mL stock (uL)	Volume buffer (uL)
0	0	250
20	5	245
100	25	225
500	125	125
1000	250	0

11.   Samples were prepared in TE buffer and TE buffer + 0.75% Triton X-100 with appropriate dilution so that reading is in the standard curve (400-600 fold). 100 μL standard/sample were added per well in a 96-well plate. The Ribogreen reagent was diluted 1:200 in TE buffer and 100 μL was added to each well.

12.   The sample fluorescence was measured using a fluorescence microplate reader, excitation at 480 nm, emission at 520 nm. The fluorescence value of the reagent blank was subtracted from the fluorescence value for each RNA sample to generate a standard curve of fluorescence versus RNA concentration. The fluorescence value of the reagent blank was subtracted from that of each of the samples and the RNA concentration of the sample from the standard curve was determined. The percent encapsulation of the sample was determined by dividing the difference in concentrations between sample plus Triton and just sample by the sample plus Triton concentration. A 6-fold dilution of the lipid nanoparticle / modified synthetic

suspension was made, and the diameter and polydispersity index determined using a Zetasizer Nano ZS instrument (Malvern Instruments, Ltd, Worcestershire, UK).

Table 15. Example of encapsulation properties for RXFP1 formulation.

Sample	Diameter Average (nm)	Poly dispersity Index	Encapsulation (%)	RNA concentration (ug/mL)	Total volume (mL)	Total amount (mg)	Yield (%)
RXFP1	112.6	0.053	93.5	1362.07	5.5	7 5	59.9

EXAMPLE 2- Immunization Strategy for RXFP1

[00368]   An overview of immunization strategies for a GPCR target protein, such as human RXFP1 , is shown in Table 16.

Table 16

Priming Immunization	Boosting Immunization	Final Boost
mRNA	mRNA	mRNA
mRNA	mRNA	Virus-like Particles
mRNA	Overexpressing Cells	mRNA
mRNA	Virus-like Particles	Virus-like Particles
Virus-like Particles	mRNA	Virus-like Particles
Overexpressing Cells	mRNA	
mRNA	Overexpressing Cells	

[00369]   Female BALB/c mice were immunized, via subcutaneous (s.c.) or intravenous (i.v.) route, with either 100 μg packaged human RXFP1 mRNA (e.g., see Table 1), 10^6 cells/ml of Ba/F3 cells overexpressing hRXFPI , or 50 μg virus-like particles (VLPs) overexpressing human RXFP1 derived from either 300.19 or HEK293 cells. Titers were checked 10 days after the priming immunization (as seen in Fig 1A). Boosting immunizations were delivered 2 1 days after the previous immunization.

[00370]   A total of 207 hybridomas producing RXFP1-specific antibodies were obtained and further screened to identify 10 anti-RXFP1 monoclonal antibodies with high affinities that were in the pM and nM range.

[00371]   Fig. 1B shows that at 10 days post-immunization, immunization with virus-like particles or cells overexpressing RXFP1 (exemplary target protein immunogens) failed to elicit any significant antibody titer. Immunization with mRNA-LNPs, by contrast, produced titers that were between 2- and 12-fold above background. This data suggest that mRNA-LNP immunization

confers a more robust immune response (e.g., higher antibody titer in sera) to the target protein immunogen than conventional methods, such as with virus-like particles or with cells overexpressing the target gene immunogen.

[00372]   Fig. 1C shows antibody titers of animals (Fig. 1B) following the boosting immunization step (e.g., 2nd administration of immunogen). Mice immunized (s.c.) with mRNA for both the 1st and 2nd immunizations exhibited higher antibody titers than mice immunized (s.c.) with mRNA for only one of the two immunizations (priming immunization and boosting immunization) and (s.c.) with VLPs or with cells overexpressing RXFP1 for one of the other immunizations. For immunization via i.v. administration, the difference in antibody titer for an immunization strategy with mRNA for both the priming and boosting immunizations and an immunization strategy with mRNA for only one of the two immunizations was less. On average the titer of all i.v. immunizations was higher than s.c. immunizations.

[00373]   Figure 1D shows FACS plots of three sample RXFP1-specific monoclonal hybridoma cultures obtained from mRNA-based immunization. Clones show minimal cross-reactivity to non-RXFP1 expressing cells (300.19 parental) and significant binding to cells overexpressing human RXFP1 (300.19 hRXFPI and Ba/F3 hRXFPI).

[00374]   Figure 2 depicts immunization strategies for a multipass transmembrane protein, SLC52A2, and the resulting FACS-based sera response. Immunization with mRNA encoding SLC52A2 (e.g., see Table 2) for only two rounds was the only antigen able to elicit target-specific antibody titers; immunization with traditional antigens, such as overexpressing cells, VLPs, and peptides encoding extracellular loops (EC2), for two to four rounds, and in various combinations, failed to elicit target-specific titers. Because there were no detectable target-specific IgGs in the sera from mice immunized with these immunogen formats, hybridoma fusion was not initiated. Failure to detect target-specific IgGs in plasma from animals immunized with these traditional antigens suggest that SLC52A2 is a poorly immunogenic protein. In mice immunized with mRNA-LNPs, however, hybridoma fusion was initiated. A total of 228 hybridomas capable of yielding SLC52A2-specific antibodies were identified from a pool of 12,880 hybridoma wells, generally about one third (1/3) of these wells contain hybridomas (approximately over 4,290 hybridomas). Thus, the data presented in Figure 2 suggest that the mRNA immunization methods described herein are surprisingly superior to traditional antigen formats for transmembrane proteins, e.g., multi-pass transmembrane proteins, for example SLC52A2, as it was the only means by which target specific sera titers could be produced.

EXAMPLE 3- <u>Immunization Strategy for ANGPTL8</u>

[00375]   An overview of immunization strategies for difficult-to-express target proteins, such as human ANGPTL8, is shown in Table 17. Examples of issues associated with recombinant expression of ANGPTL8 for raising specific antibodies include low yield, poor secretion, and aggregation. For instance, using standard expression protocols the resulting protein appeared to be more than 90% aggregated.

Table 17

Priming Immunization	Boost	Final Boost
mRNA (iv)	mRNA (sc)	Fusion protein (iv)
mRNA (sc)	Fusion protein (sc)	mRNA (iv)
Fusion protein (sc)	Fusion protein (sc)	mRNA (iv)

[00376]   Female BALB/c mice were immunized with either 50 μg packaged human ANGPTL8 mRNA (e.g., see Table 3), or 50 μg of HSA-ANGPTL8 fusion protein. Titers were checked 10 days after the priming immunization and after the first boost. The first boosting immunizations were delivered 21 days after the priming immunization and the final boost 25 days after the first boost. Dosing mice with mRNA encoding human ANGPTL8 or with purified recombinant human ANGPTL8 protein resulted in roughly equally potent immune responses against human ANGPTL8 in mice. Hybridomas producing ANGPTL8-specific antibodies were generated, and high affinity ANGPTL8 antibodies were obtained from further screens. These results confirm that mRNA-LNP immunization methods provided herein are effective strategies for producing antibodies to difficult-to-express target proteins, such as human ANGPTL8.

EXAMPLE 4 - <u>Immunization strategy for Galectin-3</u>

[00377]   An overview of the immunization strategies for lectin-binding proteins, such as galectin-3, is shown in Table 18.

[00378]   BALB/c mice were immunized with 2 mg/kg mRNA, complexed with LNPs, or 20 μg recombinant protein as indicated in Table 18. Plasma anti-galectin-3 IgG titers were assayed 7 days after the final boost, which was delivered at day 55.

[00379]   Figure 3 shows that the use of galectin-3 mRNA as a final boosting agent resulted in a significantly higher target-specific IgG titer than when purified recombinant protein (a traditional immunogen) was used. This effect was observed regardless of whether the antigens were delivered subcutaneously or intravenously.

[00380]   Hybridomas producing galectin-3-specific antibodies were generated, and high affinity monoclonal anti-galectin-3 antibodies were obtained from further screens.

Table 18

Priming Immunization (Day 0)	Boost (Day 7)	Final Boost (Day 55)
mRNA (I.V.)	mRNA (I.V.)	mRNA (I.V.)
mRNA (I.V.)	mRNA (I.V.)	Recombinant protein (I.V.)
mRNA (S.C.)	mRNA (S.C.)	mRNA (S.C.)
mRNA (S.C.)	mRNA (S.C.)	Recombinant protein (S.C.)

Summary of the hit rates attainable by mRNA-mediated immunization

[00381]   Table 19 provides a target protein-specific summary of the total number of hybridoma wells (generally about one third (1/3) of these wells contain hybridomas) screened and the number of confirmed target-specific antibodies obtained from those hybridomas wells following the use of lipid-encapsulated mRNA as an immunogen.

[00382]   Table 20 provides a comparison of mRNA-LNP immunization methods with other conventional methods of immunization by number of hybridomas producing target-specific antibodies. In general, these data suggest that mRNA-LNP immunization is an effective method for inducing an immune response to a target protein antigen and for obtaining a higher number/rate of target protein-specific antibodies. In particular, these results confirm that mRNA-LNP immunization is surprisingly more effective than conventional immunization methods for obtaining antibodies specific for transmembrane proteins, e.g., multi-pass transmembrane proteins, such as GPCRs, which are difficult to raise antibodies against, and for poorly immunogenic proteins (e.g., proteins which produce low or no detectable target-specific IgGs in plasma of animals immunized with traditional antigen).

Table 19

Protein target	Type of protein	Number of hybridoma wells screened	Number of hybridomas producing target-specific antibodies
RXFP1	Multi-pass Transmembrane protein/GPCR	20240	207
SLC52A2	Multi-pass Transmembrane protein	12880	228
ANGPTL8	Soluble protein	22816	542

TSHR	Transmembrane protein/GPCR	TBD	130
APJ	Transmembrane protein/GPCR	22080	230
GP130	Single-pass Transmembrane protein	23920	614

Table 20: Method of immunization and number of hybridomas producing target-specific antibodies

Protein target	Type of protein	mRNA-LNP[1]	Whole cells only	Virus-like particles only	cDNA only	Protein/ peptide only
RXFP1	GPCR/multi-pass	207	66	ND	ND	ND
SLC52A2	multi-pass	228	NST	NST	ND	NST
TSHR	GPCR/multi-pass	130	ND	ND	4[2]	41[3]
APJ	GPCR/multi-pass	230	9	46	21	ND

[1]- Immunization with mRNA-LNP alone or in combination with another antigen format (e.g., protein/peptide).

[2]-Sanders et al. 2002 Thyroid stimulating monoclonal antibodies *Thyroid* 12(1 2): 1043-1050.

[3]-Oda et al. 2000. Epitope analysis of the human thyrotropin (TSH) receptor using monoclonal antibodies. Thyroid 10(12): 1051-1059.

ND-Not determined; antigen format not tested

NST-No specific titers detected. Because no target-specific IgG titers were detectable in plasma, hybridoma generation was not initiated on these groups.

[00383]   In general, successful generation of hybridomas producing antigen-specific antibodies have been achieved for at least 15 different targets utilizing mRNA-LNP immunization methods as exemplified herein. These results show that the mRNA immunization methods described herein are capable of eliciting an immune response against a wide range of antigens (e.g., transmembrane proteins, for example multi-pass transmembrane proteins, such as GPCRs) in host animals, and are effective methods for producing high affinity monoclonal antibodies, which can serve as parentals for generation of chimeric variants, humanized variants, and affinity matured variants.

[00384] **Incorporation By Reference**

All references cited herein, including patents, patent applications, papers, text books, and the like, and the references cited therein, to the extent that they are not already, are hereby incorporated herein by reference in their entirety.

[00385] **Equivalents**

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The foregoing description and examples detail certain embodiments of the invention. It will be appreciated, however, that no matter how detailed the foregoing may appear in text, the invention may be practiced in many ways and the invention should be construed in accordance with the appended claims and any equivalents thereof.

Claims:

1.   A method for producing monoclonal antibodies against a target protein, comprising the steps of: (a) mixing at least one cationic lipid with a polyribonucleotide such as an messenger RNA (mRNA) coding for the target protein or a fragment thereof, thereby forming a cationic lipid-polyribonucleotide complex; (b) administering the lipid-polyribonucleotide complex to a non-human animal; and (c) obtaining antibodies that specifically bind to the target protein from the animal.

2.   A method for producing monoclonal antibodies against a target protein, comprising the steps of: (a) administering a lipid-polyribonucleotide complex to a non-human animal, wherein the complex comprises at least one cationic lipid with a polyribonucleotide, such as mRNA, coding for the target protein or a fragment thereof, thereby inducing an immune response to the target protein; and (b) obtaining from the animal antibodies that specifically bind to the target protein.

3.   A method for eliciting an immune response to a target protein in a non-human animal, comprising the steps of: administering a lipid-polynucleotide complex to the animal, wherein the lipid-polynucleotide complex comprises a cationic lipid and an mRNA coding for a target protein, wherein the target protein is of a species different than the animal.

4.   The method of claim 1, 2, or 3, wherein the target protein is a transmembrane protein.

5.   The method of claim 4, wherein the transmembrane protein is selected from the following:

(i)   a G protein coupled receptor (GPCR);

(ii)   a single pass transmembrane protein receptor;

(iii)   a Tumor Necrosis Factor Receptor Superfamily (TNFRSF) member;

(iv)   an interleukin (IL) receptor;

(v)   an ion channel;

(vi)   a solute carrier;

(vii)   an immune receptor; and

(viii)   multi-pass transmembrane protein.

6.   The method of claim 4 or 5, wherein the transmembrane protein is a G protein coupled receptor (GPCR).

7.   The method of claim 6, wherein the GPCR is RXFP1 , TSHR, APJ, GPR40, GPR64, GPR4, or GPR15.

8.   The method of claim 4 or 5, wherein the transmembrane protein is a single pass transmembrane protein receptor such as GP130.

9.   The method of claim 4 or 5, wherein the transmembrane protein is a multi-pass transmembrane protein such as SLC52A2.

10.   The method of claim 4 or 5, wherein the transmembrane protein is an interleukin (IL) receptor, such as IL-1 receptor, IL-2 receptor, IL-3 receptor, IL-4 receptor, IL-5 receptor, IL-6 receptor, IL-7 receptor, IL-8 receptor, IL-9 receptor, IL-10 receptor, IL-1 1 receptor, IL-12 receptor, IL-13 receptor, IL-14 receptor, IL-15 receptor, IL-16 receptor, IL-17 receptor, IL-18 receptor, IL-19 receptor, IL-20 receptor, IL-21 receptor, IL-22 receptor, IL-23 receptor, IL-24 receptor, IL-25 receptor, IL-26 receptor, IL-27 receptor, IL-28 receptor, IL-29 receptor, IL-30 receptor, IL-31 receptor, IL-32 receptor, IL-33 receptor, IL-35 receptor, or IL-36 receptor.

11.   The method of claim 4 or 5, wherein the transmembrane protein is a tumor necrosis factor receptor superfamily (TNFRSF) member selected from the group consisting of the following: TNFRSF1A, TNFRSF1 B, TNFRSF3, TNFRSF4, TNFRSF5, TNFRSF6, TNFRSF6B, TNFRSF7, TNFRSF8, TNFRSF9, TNFRSF10A, TNFRSF10B, TNFRSF10C, TNFRSF10D, TNFRSF1 1A, TNFRSF1 1B, TNFRSF12A, TNFRSF13B, TNFRSF13C, TNFRSF14, TNFRSF16, TNFRSF17, TNFRSF18, TNFRSF19, TNFRSF21 , TNFRSF25, and TNFRSF27.

12.   The method of claim 4 or 5, wherein the transmembrane protein is an ion channel such as TMEM16A.

13.   The method of claim 4 or 5, wherein the transmembrane protein is a solute carrier.

14.   The method of claim 1, 2, or 3, wherein the target protein is selected from the following:

ACKR1 , ACKR2, ACKR3, ACKR4, ADCYAP1 R 1, ADGRA1 , ADGRA2, ADGRA3, ADGRB1 , ADGRB2, ADGRB3, ADGRD1 , ADGRD2, ADGRE1 , ADGRE2, ADGRE3, ADGRE4P, ADGRE5, ADGRF1 , ADGRF2, ADGRF3, ADGRF4, ADGRF5, ADGRG1 , ADGRG2, ADGRG3, ADGRG4, ADGRG5, ADGRG6, ADGRG7, ADGRL1 , ADGRL2, ADGRL3, ADGRL4, ADGRV1 , ADORA1 , ADORA2A, ADORA2B, ADORA3, ADRA1A, ADRA1 B, ADRA1 D, ADRA2A, ADRA2B, ADRA2C, ADRB1 , ADRB2, ADRB3, AGTR1 , AGTR2, APLNR/APJ, ASGR1 , ASGR2, AVPR1 A , AVPR1 B, AVPR2, BDKRB1 , BDKRB2, BRS3, BRS3, C3AR1 , C5AR1 , C5AR2, CALCR, CALCRL, CASR, CCKAR, CCKBR, CCR1 , CCR10, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCRL2, CELSR1 , CELSR2, CELSR3, CHRM1 , CHRM2, CHRM3, CHRM4, CHRM5, CMKLR1 , CNR1 , CNR2, CRHR1 , CRHR2, CX3CR1 , CXCR1 , CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, CYSLTR1 , CYSLTR2, DRD1 , DRD2, DRD3, DRD4, DRD5, EDNRA, EDNRB, F2R, F2RL1 ,

F2RL2, F2RL3, FFAR1 , FFAR2, FFAR3, FFAR4, FPR1 , FPR2, FPR2, FPR3, FSHR, FZD1 ,
FZD10, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, GABBR1 , GABBR2, GALR1 ,
GALR2, GALR3, GCGR, GHRHR, GHSR, GIPR, GLP1 R , GLP2R, GNRHR, GNRHR2, GPBAR1 ,
GPER1 , GPR1 , GPR4, GPR12, GPR15, GPR17, GPR18, GPR19, GPR20, GPR21 , GPR22,
GPR25, GPR26, GPR27, GPR3, GPR31 , GPR32, GPR33, GPR34, GPR35, GPR37, GPR37L1 ,
GPR39, GPR40, GPR42, GPR42, GPR45, GPR50, GPR52, GPR55, GPR6, GPR61 , GPR62,
GPR63, GPR65, GPR68, GPR75, GPR78, GPR79, GPR82, GPR83, GPR84, GPR85, GPR87,
GPR88, GPR101 , GPR107, GPR132, GPR135, GPR137, GPR139, GPR141 , GPR142, GPR143,
GPR146, GPR148, GPR149, GPR15, GPR150, GPR151 , GPR152, GPR153, GPR156, GPR157,
GPR158, GPR160, GPR161 , GPR162, GPR171 , GPR173, GPR174, GPR176, GPR179,
GPR182, GPR183, GPRC5A, GPRC5B, GPRC5C, GPRC5D, GPRC6A, GRM1 , GRM2, GRM3,
GRM4, GRM5, GRM6, GRM7, GRM8, GRPR, HCAR1 , HCAR2, HCAR3, HCRTR1 , HCRTR2,
HRH1 , HRH2, HRH3, HRH4, HTR1A, HTR1 B, HTR1 D, HTR1 E , HTR1 F, HTR2A, HTR2B,
HTR2C, HTR4, HTR5A, HTR5BP, HTR6, HTR7, KISS1 R , LGR3, LGR4, LGR5, LGR6, LHCGR,
LPAR1 , LPAR2, LPAR3, LPAR4, LPAR5, LPAR6, LTB4R, LTB4R2, MAS1 , MAS1 L , MC1 R ,
MC2R, MC3R, MC4R, MC5R, MCHR1 , MCHR2, MLNR, MRGPRD, MRGPRE, MRGPRF,
MRGPRG, MRGPRX1 , MRGPRX2, MRGPRX3, MRGPRX4, MTNR1A, MTNR1 B, NMBR,
NMUR1 , NMUR2, NPBWR1 , NPBWR2, NPFFR1 , NPFFR2, NPSR1 , NPY1 R , NPY2R, NPY4R,
NPY5R, NPY6R, NTSR1 , NTSR2, 0PN3, 0PN4, 0PN5, 0PRD1 , 0PRK1 , 0PRL1 , 0PRM1 ,
0R51 E 1 , 0XER1 , 0XGR1 , OXTR, P2RY1 , P2RY10, P2RY1 1, P2RY12, P2RY13, P2RY14,
P2RY2, P2RY4, P2RY6, P2RY8, PRLHR, PROKR1 , PROKR2, PTAFR, PTGDR, PTGDR2,
PTGER1 , PTGER2, PTGER3, PTGER4, PTGFR, PTGIR, PTH1 R , PTH2R, QRFPR, RXFP1 ,
RXFP2, RXFP3, RXFP4, S 1 PR1 , S 1 PR2, S 1 PR3, S 1 PR4, S 1 PR5, SCTR, SMO, SSTR1 , SSTR2,
SSTR3, SSTR4, SSTR5, SUCNR1 , TAAR1 , TAAR2, TAAR3, TAAR4P, TAAR5, TAAR6, TAAR8,
TAAR9, TACR1 , TACR2, TACR3, TAS1 R 1 , TAS1 R2, TAS1 R3, TAS2R1 , TAS2R10, TAS2R13,
TAS2R14, TAS2R16, TAS2R19, TAS2R20, TAS2R3, TAS2R30, TAS2R31 , TAS2R38, TAS2R39,
TAS2R4, TAS2R40, TAS2R41 , TAS2R42, TAS2R43, TAS2R45, TAS2R46, TAS2R5, TAS2R50,
TAS2R60, TAS2R7, TAS2R8, TAS2R9, TBXA2R, TPRA1 , TRHR, TSHR, UTS2R, VIPR1 , VIPR2,
XCR1 , TCR-a, TCR- β, CD3, ζ-chain accessory, CD4, CD8, SIGIRR, mannose receptor (MR),
asialoglycoprotein receptor family (e.g., asialoglycoprotein receptor macrophage galactose-type
lectin (MGL)), DC-SIGN (CLEC4L), langerin (CLEC4K), myeloid DAP12-associating lectin
(MDL)-1 (CLEC5A), dectin 1/CLEC7A, DNGR1/CLEC9A, Myeloid C-type lectin-like receptor
(MICL) (CLEC12A), CLEC2 (also called CLEC1 B), CLEC12B, DCIR/CLEC4A, Dectin 2/CLEC6A,
Blood DC antigen 2 (BDCA2) (CLEC4C), macrophage- inducible C-type lectin (CLEC4E), TLR1 ,
TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR1 1, TLR12, TLR13, FcγRI

(CD64), FCYRIIA (CD32), FCYRIIB1 (CD32), FCYRIIB2 (CD32), FCYRIIIA (CD16a), FCYRIIIB (CD16b), FceRI, FceRII (CD23), FcaR1 (CD89), Fca/pR, FcRn, CD27, CD40, OX40, GITR, CD137, PD-1, CTLA-4, PD-L1, TIGIT, T-cell immunoglobulin domain and mucin domain 3 (TIM3), V-domain Ig suppressor of T cell activation (VISTA), CD28, CD122, ICOS, A2AR, B7-H3, B7-H4, B and T lymphocyte attenuator (BTLA), Indoleamine 2,3-dioxygenase (IDO), killer-cell immunoglobulin-like receptor (KIR), lymphocyte activation gene-3 (LAG3), FAM159B, HLA-A, HLA-B, HLA-C, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, HLA-DRA, HLA-DRB1, gp130, IL-1 receptor, IL-2 receptor, IL-3 receptor, IL-4 receptor, IL-5 receptor, IL-6 receptor, IL-7 receptor, IL-8 receptor, IL-9 receptor, IL-10 receptor, IL-11 receptor, IL-12 receptor, IL-13 receptor, IL-14 receptor, IL-15 receptor, IL-16 receptor, IL-17 receptor, IL-18 receptor, IL-19 receptor, IL-20 receptor, IL-21 receptor, IL-22 receptor, IL-23 receptor, IL-24 receptor, IL-25 receptor, IL-26 receptor, IL-27 receptor, IL-28 receptor, IL-29 receptor, IL-30 receptor, IL-31 receptor, IL-32 receptor, IL-33 receptor, IL-35 receptor, IL-36 receptor, FGFR1, FGFR2, FGFR3, FGFR4, TNFRSF1A, TNFRSF1B, TNFRSF3, TNFRSF4, TNFRSF5, TNFRSF6, TNFRSF6B, TNFRSF7, TNFRSF8, TNFRSF9, TNFRSF10A, TNFRSF10B, TNFRSF10C, TNFRSF10D, TNFRSF11A, TNFRSF11B, TNFRSF12A, TNFRSF13B, TNFRSF13C, TNFRSF14, TNFRSF16, TNFRSF17, TNFRSF18, TNFRSF19, TNFRSF21, TNFRSF25, TNFRSF27, SCN1A, SCN1B, SCN2A, SCN2B, SCN3A, SCN3B, SCN4A, SCN5A, SCN7A, SCN8A, SCN9A, SCN10A, SCN11A, CACNA1A, CACNA1B, CACNA1C, CACNA1D, CACNA1E, CACNA1F, CACNA1G, CACNA1H, CACNA1I, CACNA1S, TRPA1, TRPC1, TRPC2, TRPC3, TRPC4, TRPC5, TRPC6, TRPC7, TRPM1, TRPM2, TRPM3, TRPM4, TRPM5, TRPM6, TRPM7, TRPM8, MCOLN1, MCOLN2, MCOLN3, PKD1, PKD2, PKD2L1, PKD2L2, TRPV1, TRPV2, TRPV3, TRPV4, TRPV5, TRPV6, CATSPER1, CATSPER2, CATSPER3, CATSPER4, TPCN1, TPCN2, CNGA1, CNGA2, CNGA3, CNGA4, CNGB1, CNGB3, HCN1, HCN2, HCN3, HCN4, KCNMA1, KCNN1, KCNN2, KCNN3, KCNN4, KCNT1, KCNT2, KCNU1, KCNA1, KCNA2, KCNA3, KCNA4, KCNA5, KCNA6, KCNA7, KCNA10, KCNB1, KCNB2, KCNC1, KCNC2, KCNC3, KCNC4, KCND1, KCND2, KCND3, KCNF1, KCNG1, KCNG2, KCNG3, KCNG4, KCNH1, KCNH2, KCNH3, KCNH4, KCNH5, KCNH6, KCNH7, KCNH8, KCNQ1, KCNQ2, KCNQ3, KCNQ4, KCNQ5, KCNS1, KCNS2, KCNS3, KCNV1, KCNV2, KCNJ1, KCNJ2, KCNJ3, , KCNJ4, KCNJ5, KCNJ6, KCNJ8, KCNJ9, KCNJ10, KCNJ11, KCNJ12, KCNJ13, KCNJ14, KCNJ15, KCNJ16, KCNJ18, KCNK1, KCNK2, KCNK3, KCNK4, KCNK5, KCNK6, KCNK7, KCNK9, KCNK10, KCNK12, KCNK13, KCNK15, KCNK16, KCNK17, KCNK18, HVCN1, HTR3A, HTR3B, HTR3C, HTR3D, HTR3E, CHRNA1, CHRNA2, CHRNA3, CHRNA4, CHRNA5, CHRNA6, CHRNA7, CHRNA9, CHRNA10, CHRNB1, CHRNB2, CHRNB3, CHRNB4, CHRND, CHRNE, CHRNG, GABRA1, GABRA2, GABRA3, GABRA4, GABRA5, GABRA6, GABRB1, GABRB2, GABRB3, GABRD, GABRE, GABRG1, GABRG2, GABRG3, GABRP,

GABRQ, GABRR1 , GABRR2, GABRR3, GRIA1 , GRIA2, GRIA3, GRIA4, GRID1 , GRID2, GRIK1 , GRIK2, GRIK3, GRIK4, GRIK5, GRIN1 , GRIN2A, GRIN2B, GRIN2C, GRIN2D, GRIN3A, GRIN3B, GLRA1 , GLRA2, GLRA3, GLRA4, P2RX1 , P2RX2, P2RX3, P2RX4, P2RX5, P2RX6, P2RX7, ZACN, ASIC1 , ASIC2, ASIC3, ASIC4, AQP1 , AQP2, AQP3, AQP4, AQP5, AQP6, AQP7, AQP8, AQP9, AQP10, AQP1 1, AQP12A, AQP12B, MIP, CLCN1 , CLCN2, CLCN3, CLCN4, CLCN5, CLCN6, CLCN7, CLCNKA, CLCNKB, Cystic fibrosis transmembrane conductance regulator (CFTR), AN01 , AN02, AN03, AN04, AN05, AN06, AN07, AN08, AN09, AN01 0, BEST1 , BEST2, BEST3, BEST4, CLIC1 , CLIC2, CLIC3, CLIC4, CLIC5, CLIC6, GJA1 , GJA3, GJA4, GJA5, GJA6P, GJA8, GJA9, GJA10, GJB1 , GJB2, GJB3, GJB4, GJB5, GJB6, GJB7, GJC1 , GJC2, GJC3, GJD2, GJD3, GJD4, GJE1 , ITPR1 , ITPR2, ITPR3, PANX1 , PANX2, PANX3, RYR1 , RYR2, RYR3, NALCN, SCNN1A, SCNN1 B, SCNN1 D, SCNN1G, TEM16A, ADAMTS7, ANGPTL3, ANGPTL4, ANGPTL8, LPL, GDF15, galectin-1 , galectin-2, galectin-3, galectin-4, galectin-7, galectin-8, galectin-9, galectin-10, galectin-12, galectin-13, matrix gla protein (MGP), PRNP, DGAT1 , GPAT3, DMC1 , BLM, BRCA2, members of the human endogenous retrovirus type K (HERV-K) family, , ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1), ectonucleoside triphosphate diphosphohydrolase 2 (ENTPD2), SLC1A1 , SLC1A2, SLC1A3, SLC1A4, SLC1A5, SLC1A6, SLC1A7, SLC2A1 , SLC2A2, SLC2A3, SLC2A4, SLC2A5, SLC2A6, SLC2A7, SLC2A8, SLC2A9, SLC2A10, SLC2A1 1, SLC2A12, SLC2A13, SLC2A14, SLC3A1 , SLC3A2, SLC4A1 , SLC4A2, SLC4A3, SLC4A4, SLC4A5, SLC4A6, SLC4A7, SLC4A8, SLC4A9, SLC4A10, SLC4A1 1, SLC5A1 , SLC5A2, SLC5A3, SLC5A4, SLC5A5, SLC5A6, SLC5A7, SLC5A8, SLC5A9, SLC5A10, SLC5A1 1, SLC5A12, SLC6A1 , SLC6A2, SLC6A3, SLC6A4, SLC6A5, SLC6A6, SLC6A7, SLC6A8, SLC6A9, SLC6A10, SLC6A1 1, SLC6A12, SLC6A13, SLC6A14, SLC6A15, SLC6A16, SLC6A17, SLC6A18, SLC6A19, SLC6A20, SLC7A5, SLC7A6, SLC7A7, SLC7A8, SLC7A9, SLC7A10, SLC7A1 1, SLC7A13, SLC7A14, SLC8A1 , SLC8A2, SLC8A3, SLC9A1 , SLC9A2, SLC9A3, SLC9A4, SLC9A5, SLC9A6, SLC9A7, SLC9A8, SLC9A9, SLC9A10, SLC9A1 1, SLC9B1 , SLC9B2, SLC10A1 , SLC10A2, SLC10A3, SLC10A4, SLC10A5, SLC10A6, SLC10A7, SLC1 1A1 , SLC1 1A2, SLC12A1 , SLC12A2, SLC12A3, SLC12A4, SLC12A5, SLC12A6, SLC12A7, SLC12A8, SLC12A9, SLC13A1 , SLC13A2, SLC13A3, SLC13A4, SLC13A5, SLC14A1 , SLC14A2, SLC15A1 , SLC15A2, SLC15A3, SLC15A4, SLC16A1 , SLC16A2, SLC16A3, SLC16A4, SLC16A5, SLC16A6, SLC16A7, SLC16A8, SLC16A9, SLC16A10, SLC16A1 1, SLC16A12, SLC16A13, SLC16A14, SLC17A1 , SLC17A2, SLC17A3, SLC17A4, SLC17A5, SLC17A6, SLC17A7, SLC17A8, SLC17A9, SLC18A1 , SLC18A2, SLC18A3, SLC19A1 , SLC19A2, SLC19A3, SLC20A1 , SLC20A2, SLC01A2, SLC01 B1, SLC01 B3, SLC01C1 , SLC02A1 , SLC02B1 , SLC03A1 , SLC04A1 , SLC04C1 , SLC05A1 , SLC06A1 , SLC22A1 , SLC22A2, SLC22A3, SLC22A4, SLC22A5, SLC22A6, SLC22A7, SLC22A8, SLC22A9, SLC22A10,

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15.   The method of any one of the preceding claims wherein the target antigen is difficult to express or difficult to raise antibodies against.

16. The method of any one of claims 1-15, wherein expression of the target protein leads to cytotoxicity in host production cells.

17. The method of any one of claims 1-16, wherein the target protein, when expressed recombinantly and/or purified, exhibits poor yield, stability, solubility, and/or functional activity.

18.   The method of any one of the preceding claims wherein said polyribonucleotide or mRNA of the complex comprises one or more of the following: a consensus Kozak sequence; a 7-

methylguanosine cap on the 5' end of the mRNA; a polyadenosine (polyA) tail found at the 3' terminus of the mRNA transcript; and 5'-and 3'-untranslated regions (UTRs).

19.   The method of any one of the preceding claims, wherein said administering is parenteral, intravenous, intramuscular, intranasal, or subcutaneous.

20.   The method of any one of the preceding claims, wherein said target protein is RXFP1 .

21.   The method of any one of the preceding claims, wherein said complex comprises a polyribonucleotide or mRNA comprising the nucleic acid sequence of SEQ ID NO:4, or any one of SEQ ID NOs: 2, 4, and 37.

22.   The method of any one of the preceding claims, wherein said target protein is SLC52A2.

23.   The method of any one of the preceding claims, wherein said complex comprises a polyribonucleotide or mRNA comprising the nucleic acid sequence of SEQ ID NO:7, or any one of SEQ ID NOs: 5, 7, and 40.

24.   The method of any one of the preceding claims, wherein said target protein is ANGPTL8.

25.   The method of any one of the preceding claims, wherein said complex comprises a polyribonucleotide or mRNA comprising the nucleic acid sequence of SEQ ID NO: 10, or any one of SEQ ID NOs: 8, 10, and 43.

26.   The method of any one of the preceding claims, wherein said target protein is TSHR.

27.   The method of any one of the preceding claims, wherein said complex comprises a polyribonucleotide or mRNA comprising the nucleic acid sequence of SEQ ID NO: 16, or any one of SEQ ID NOs: 14, 16, and 46.

28.   The method of any one of the preceding claims, wherein said target protein is APJ.

29.   The method of any one of the preceding claims, wherein said complex comprises a polyribonucleotide or mRNA comprising the nucleic acid sequence of SEQ ID NO: 19, or any one of SEQ ID NOs: 17, 19, and 49.

30.   The method of any one of the preceding claims, wherein said target protein is gp130.

31.   The method of any one of the preceding claims, wherein said complex comprises a polyribonucleotide or mRNA comprising the nucleic acid sequence of SEQ ID NO:22, or any one of SEQ ID NOs: 20, 22, and 52.

32.   The method of any one of the preceding claims, wherein said target protein is Galectin 3.

33.   The method of any one of the preceding claims, wherein said complex comprises a polyribonucleotide or mRNA comprising the nucleic acid sequence of SEQ ID NO:55, or any one of SEQ ID NOs: 26, 55, and 56.

34. The method of any one of claims 1-33, wherein said complex has a diameter of approximately 30-150 nm.

35.   The method of any one of claims 1-34, wherein the complex comprises helper lipids.

36.   The method of any one of claims 1-35, wherein the complex comprises any combination of (i) cationic lipid, (ii) a helper lipid, for example cholesterol, (iii) a neutral lipid, for example DSPC, and (iv) a stealth lipid, for example S010, S024, S027, S031, or S033.

37.   The method of any one of claims 1-36, wherein the animal is administered with 10 μg, 12.5 μg, 20 μg, 25 μg, 30 μg, 40 μg, 50 μg, 60 μg, 70 μg, 80 μg, 90 μg, 100 μg, 110 μg, 120 μg, 130 μg, 140 μg or 150 μg polyribonucleotide.

38.   The method of any one of claims 1-37, wherein the cationic lipid is selected from the group consisting of: N,N-dioleyl-N,N-dimethylammonium   chloride (DODAC), N,N-distearyl-N,N-dimethylammonium   bromide (DDAB), N-(1-(2,3-dioleoyloxy) propyl)-N,N,N-trimethylammonium chloride (DOTAP), 1,2-Dioleoyl-3-Dimethylammonium   -propane (DODAP), N-(1-(2,3-dioleyloxy)propyl)   -N,N,N-thmethylammonium chloride (DOTMA), 1,2-Dioleoylcarbamyl   -3-Dimethylammonium-propane   (DOCDAP), 1,2-Dilineoyl-3-Dimethylammonium-propane   (DLINDAP), **dilauryl(Ci$_{2:0}$)** trimethyl ammonium propane (DLTAP), Dioctadecylamidoglycyl spermine (DOGS), DC-Choi, Dioleoyloxy -N-[2-sperminecarboxamido)ethyl}   -N,N-dimethyl-1-propanaminiumtrifluoroac etate (DOSPA), 1,2-Dimyristyloxypropyl-3-dimethyl   -hydroxyethyl ammonium bromide (DMRIE), 3-Dimethylamino-2-(Cholest-5-en   -3-beta-oxybutan-4-oxy)   -1-(cis,cis-9,12   -octadecadieno

xy)propane (CLinDMA), N,N-dimethyl-2,3-dioleyloxy)propylamine (DODMA), 2-[5'-(cholest-5-en-3[beta]-oxy)-3'-oxapentoxy) -3-dimethyl-1-(cis,cis-9',12'-octadecadieno xy) propane (CpLinDMA) and N,N-Dimethyl-3,4-dioleyloxybenzylamine (DMOBA), and 1,2-N,N'-Dioleylcarbamyl-3-dimethylaminopropane (DOcarbDAP).

39. The method of claim 38, wherein the cationic lipid is DOTAP or DLTAP.

40. The method of any one of claims 1-39 further comprising the step of generating hybridomas producing antibodies that specifically bind the target antigen.

41. The method of any one of claims 1-40 further comprising the step of purifying antibodies that specifically bind to the target protein.

42. The method of any one of claims 1-41, further comprising the step of generating chimeric or humanized antibodies derived from the purified antibodies that specifically bind the target protein.

43. The method of any one of claims 1-42, wherein said method produces higher antibody titer in sera from a first bleed or a second bleed relative to a method comprising immunization with cDNA, protein or peptide, a viral particle, or whole cell.
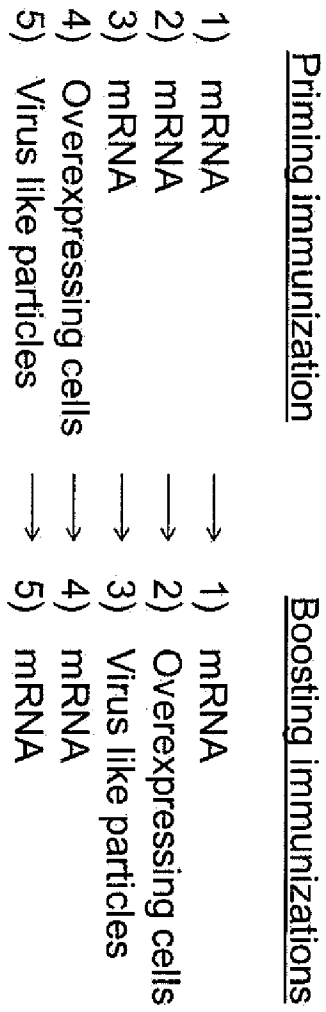
44. The method of any one of claims 1-43, wherein said method produces a higher number of hybridomas producing target protein-specific antibodies than a method comprising immunization with cDNA, protein or peptide, a viral particle, or whole cell.

45. The method of any one of claims 1-44, wherein the target protein is a human target protein, and the non-human animal is a mouse, rat, rabbit, sheep, cat, dog, camelid, shark, monkey, pig, or horse.

46. A hybridoma producing an antibody that specifically binds to the target protein, wherein the hybridoma was obtained by the method of any one of claims 1-45.

47. A mixture of polyclonal antibodies, which specifically bind to the target protein, wherein the mixture was obtained from the method of any one of claims 1-45.

48. An isolated monoclonal antibody which specifically binds to the target protein, wherein the monoclonal antibody was obtained by the method of any one of claims 1-45.
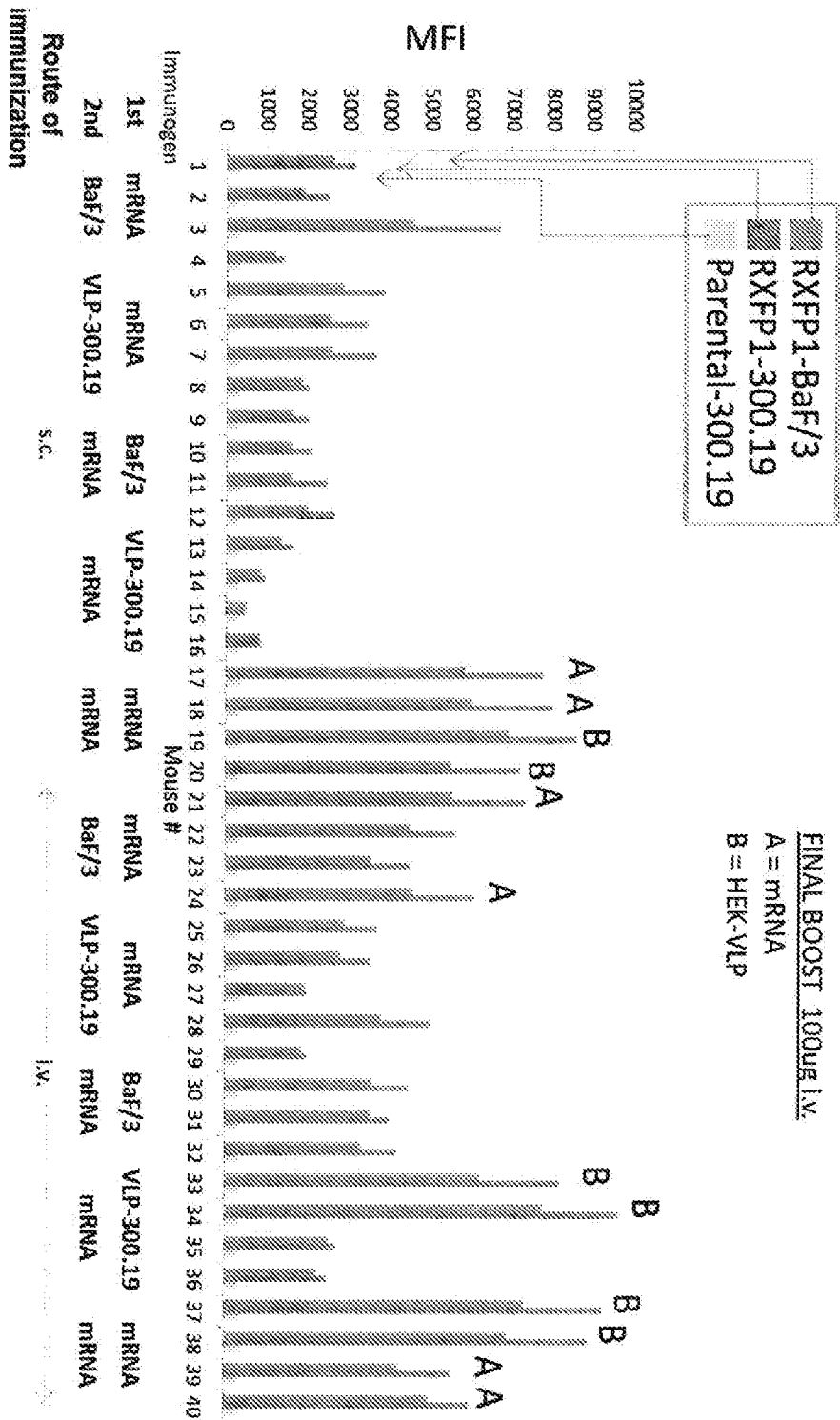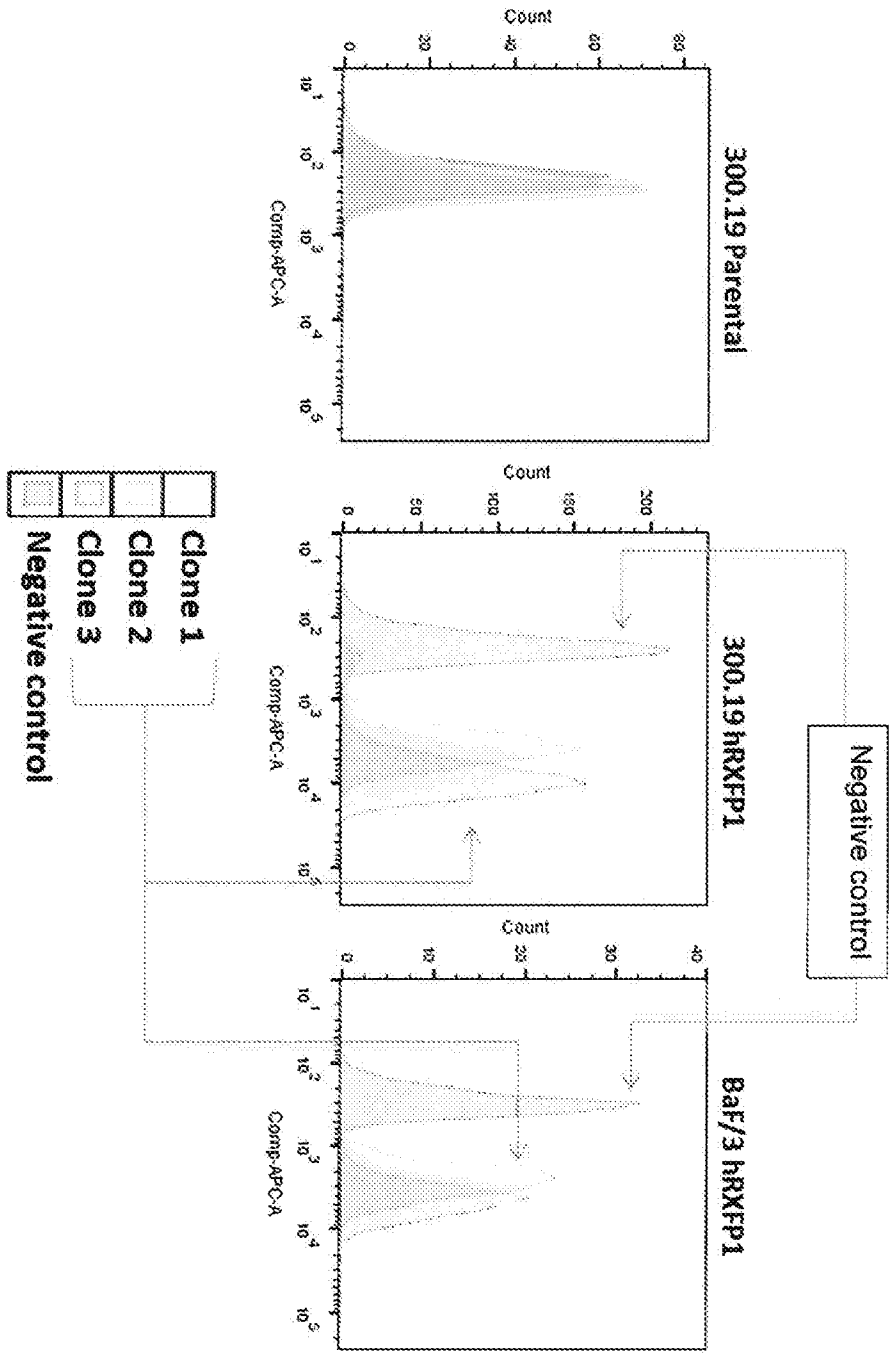
Priming immunization

1) mRNA
2) mRNA
3) mRNA
4) Overexpressing cells
5) Virus like particles

→
→
→
→
→

Boosting immunizations

1) mRNA
2) Overexpressing cells
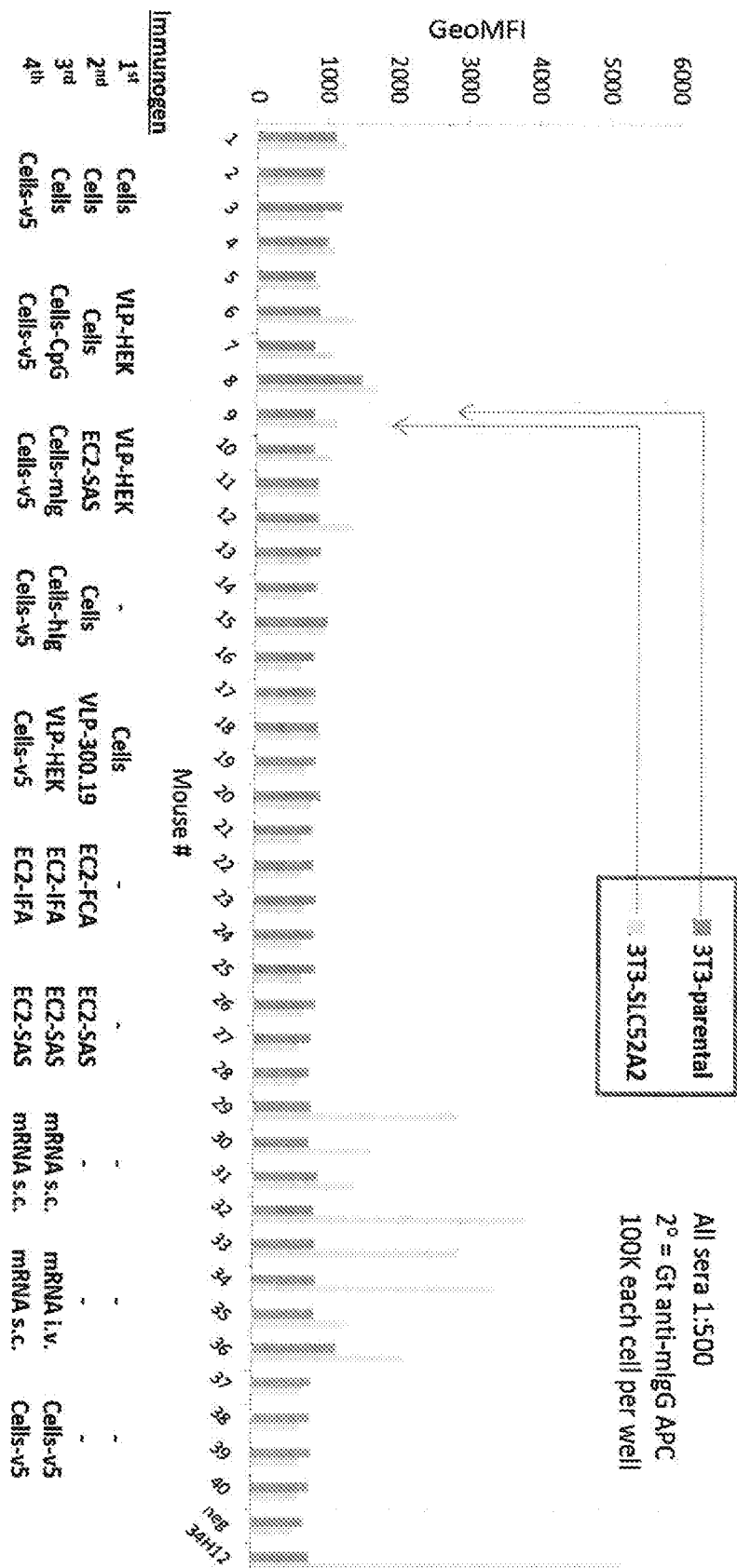3) Virus like particles
4) mRNA
5) mRNA
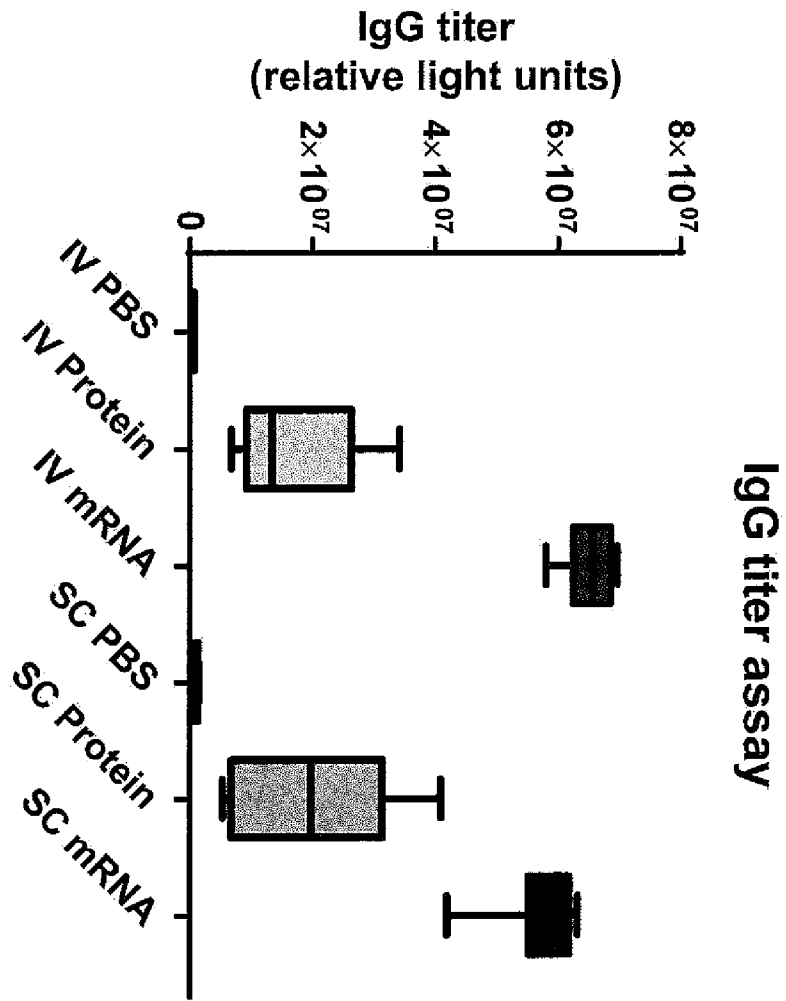
FIG. 1A

FIG. 1B

FIG. 1C

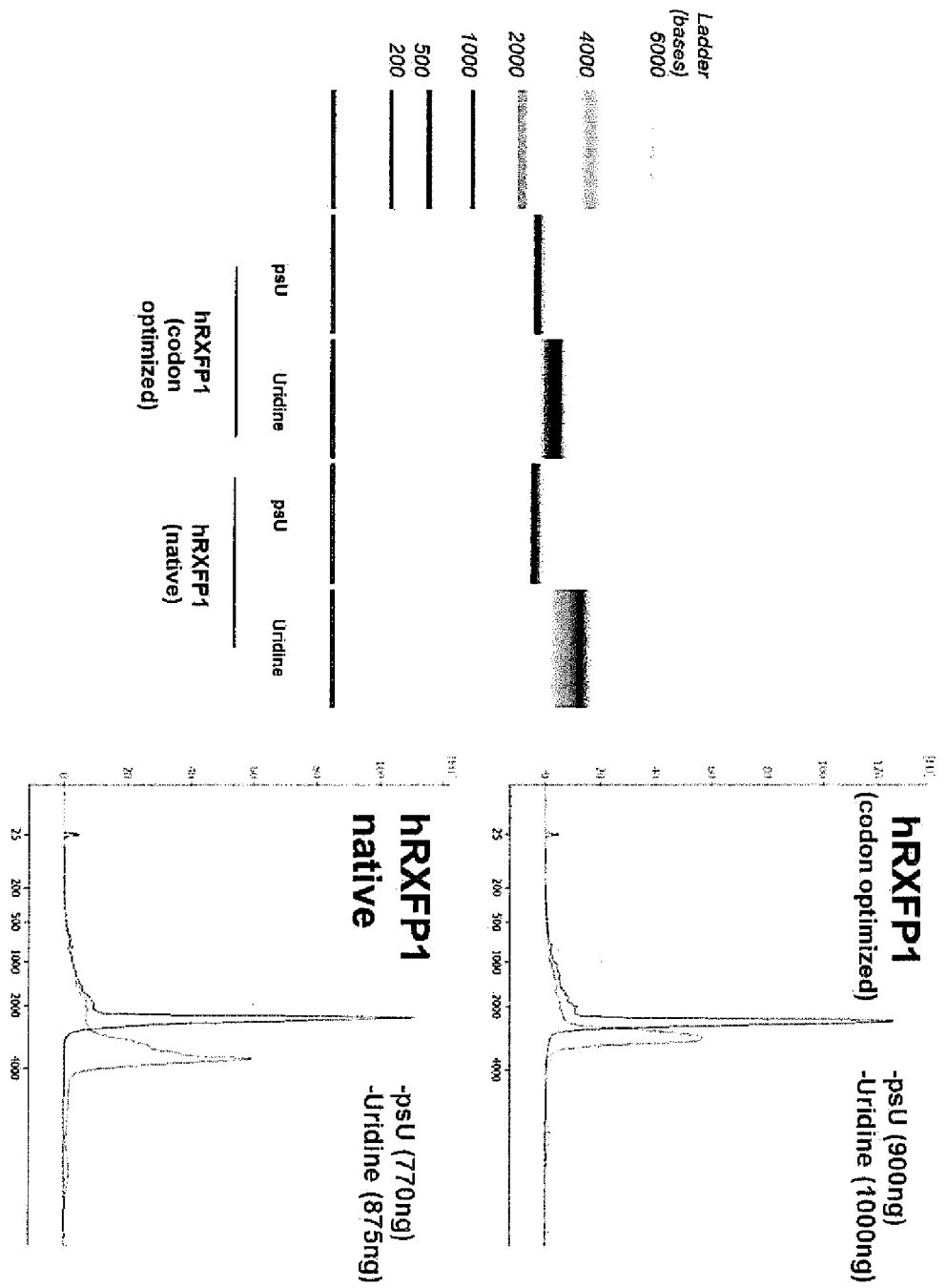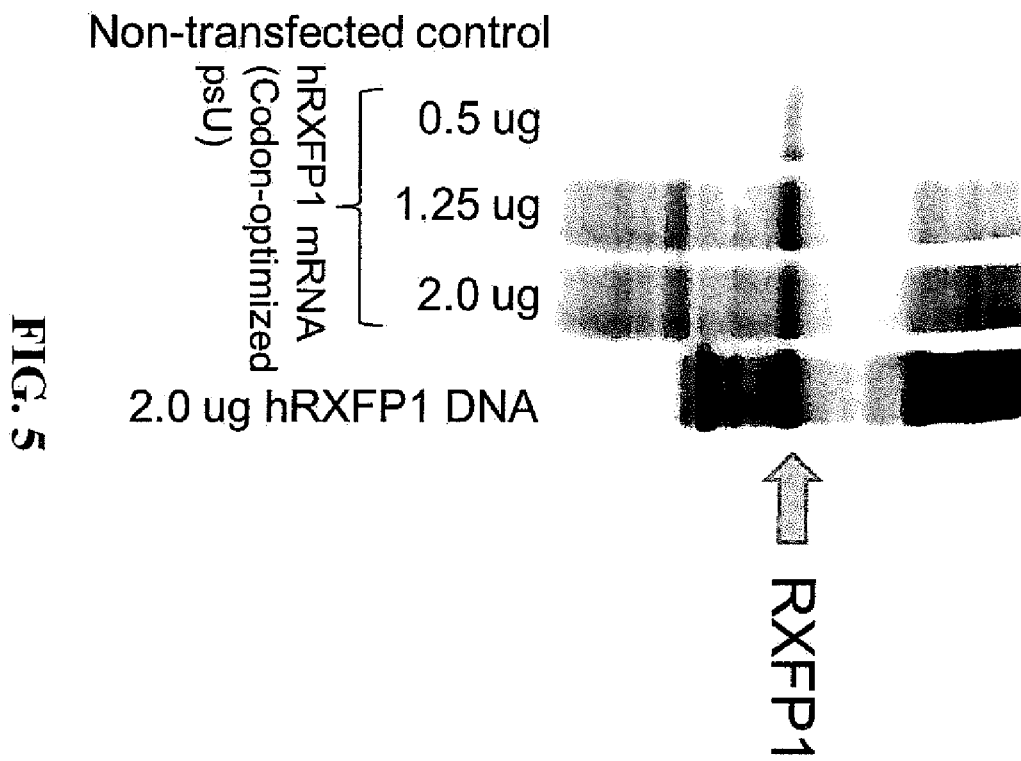FIG. 1D

FIG. 2

FIG. 3

FIG. 4

FIG. 5

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2017/054801

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K39/00 C07K16/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal , BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94/27435 AI (LIFE TECHNOLOGIES INC [US]) 8 December 1994 (1994-12-08) claim 15 page 15, line 1 - page 19, line 8 page 14, line 3 - line 24 page 22, line 5 - line 10 page 23, line 28 - page 31, line 9 page 5, line 22 - page 9, line 21 page 23, line 15 - line 21 -----	1-48
A	WO 2015/010840 AI (NOVARTIS AG [CH] ; BOWMAN KEITH A [US] ; GARDNER NOAH [US] ; JEANNOTTE TR) 21 January 2016 (2015-01-21) cited in the application ----- -/--	1-48

☒ Further documents are listed in the continuation of Box C.   ☒ See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 October 2017	13/11/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Marinoni J-C

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation).   DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2016/037053 A1 (NOVARTIS AG [CH] ; BECKWITH ROHAN ERIC JOHN [US] ; BRITO LUIS [US] ; DECH) 10 March 2016 (2016-03-10) cited in the application -----	1-48
A	WO 2015/095346 A1 (NOVARTIS AG [CH] ; BRITO LUIS [US] ; CHEN DELAI [US] ; GAMBER GABRIEL GRA) 25 June 2015 (2015-06-25) cited in the application -----	1-48
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