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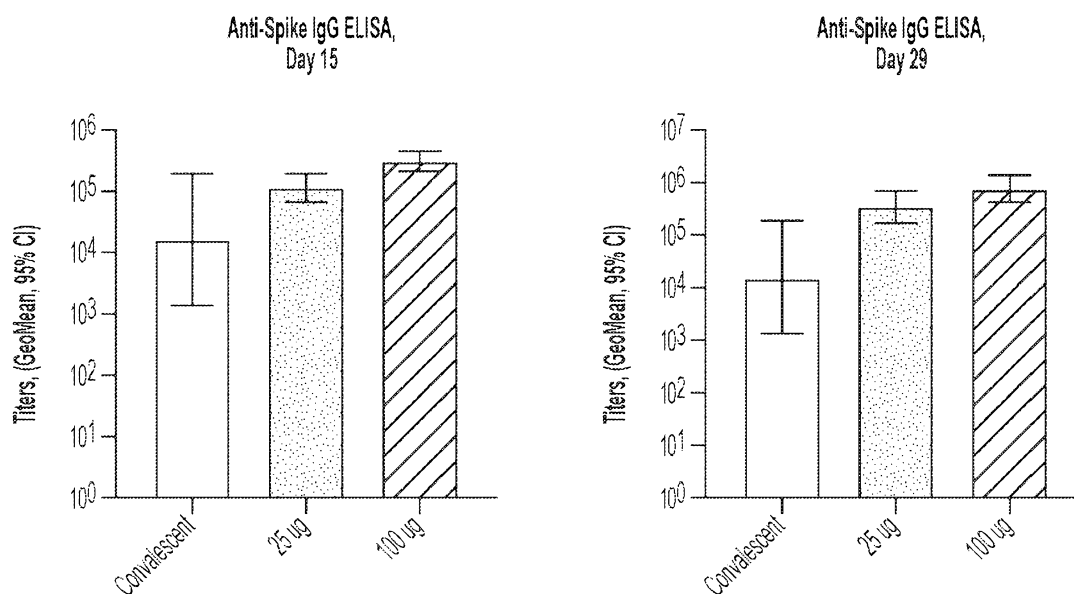


FIG. 1

(57) Abstract: The disclosure relates to SARS-CoV-2 messenger ribonucleic acid (mRNA) vaccine compositions as well as methods of using the vaccines.

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CORONAVIRUS RNA VACCINES AND METHODS OF USE

RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. § 119(e) of U.S. provisional application number 63/025918, filed May 15, 2020, and U.S. provisional application number 63/028,424, filed May 21, 2020.

BACKGROUND

Coronaviruses (CoV) are a large family of viruses that cause illness ranging from the common cold to more severe diseases, such as Middle East Respiratory Syndrome (MERS CoV) and Severe Acute Respiratory Syndrome (SARS-CoV). Coronaviruses are zoonotic, meaning they are transmitted between animals and people.

An outbreak of the coronavirus disease (COVID-19) caused by the 2019 novel coronavirus (SARS-nCoV-2) began in Wuhan, Hubei Province, China in December 2019, and has spread throughout China and to many other countries and territories, including the United States (WHO, 2020). A coronavirus ribonucleic acid (CoV-RNA) was quickly identified in some of these patients. As an RNA virus, SARS-CoV-2 still has the inherent feature of a high mutation rate, although like other coronaviruses, the mutation rate might be lower than other RNA viruses because of its genome-encoded exonuclease. This aspect could potentially enable SARS-CoV-2 to adapt and become more efficiently transmitted from person to person and possibly become more virulent.

There is currently no vaccine against SARS-CoV-2. Global efforts to evaluate novel antivirals and therapeutic strategies to treat severe SARS-CoV-2 infections have intensified, but no proven therapeutic currently exists. Therefore, there is an urgent public health need for rapid development of novel interventions to prevent the spread of this disease.

SUMMARY

The present disclosure provides a rapid-response vaccine platform based on a messenger RNA (mRNA) delivery system. The platform is based on the principle and data showing that cells *in vivo* can take up mRNA, translate it, and then express protein viral antigen(s) on the cell surface. The delivered mRNA does not enter the cellular nucleus or interact with the genome, is non-replicating, and is expressed transiently. The mRNA vaccines provided herein, in some embodiments, encode for the full-length spike protein (S protein) of SARS-CoV-2, modified to introduce two proline residues to stabilize the S protein into a prefusion conformation. The coronavirus S protein mediates attachment and entry of the virus into host cells (by fusion), making it a primary target for neutralizing antibodies that prevent infection. Preclinical studies

have demonstrated that coronavirus S proteins are immunogenic and S protein-based vaccines, including those based on mRNA delivery platforms, are protective in animals. The studies provided herein demonstrate that mRNA-based vaccines are safe and immunogenic.

mRNA vaccines have superior properties in that they produce much larger antibody
5 titers, better neutralizing immunity, produce more durable immune responses, and/or produce responses earlier than commercially-available vaccines.

Some aspects of the present disclosure provide a method comprising administering to a subject a composition comprising a messenger ribonucleic acid (mRNA) comprising an open reading frame (ORF) that encodes a SARS-CoV-2 prefusion stabilized Spike (S) protein,
10 wherein the mRNA is formulated in a lipid nanoparticle, and wherein the composition is administered in an effective amount to induce in the subject a neutralizing antibody response to SARS-CoV-2 S protein.

In some embodiments, the geometric mean titer (GMT) of anti-S protein IgG antibodies produced in the subject at Day 15 post-administration of a 25 µg or 100 µg dose of the
15 composition at Day 1 is at least 1 log higher than the GMT of antibodies produced in convalescent subjects who were infected with SARS-CoV-2. In some embodiments, the geometric mean titer (GMT) of anti-S protein IgG antibodies produced in the subject at Day 29 post-administration of a 25 µg or 100 µg dose of the composition at Day 1 is at least 1 log higher than the GMT of antibodies produced in convalescent subjects who were infected with SARS-
20 CoV-2.

In some embodiments, the method comprises administering to the subject at least two doses of the composition.

In some embodiments, a second dose of the composition is administered to the subject at least 28 days after a first dose of the composition is administered to the subject.

25 In some embodiments, the composition further comprises Tris buffer, sucrose, and sodium acetate.

In some embodiments, the composition comprises 10 mM – 30 mM Tris buffer, 75 mg/mL – 95 mg/mL sucrose, and 5 mM – 15 mM sodium acetate, optionally wherein the composition has a pH of 6-8. For example, the composition may comprise 20 mM Tris buffer, 87
30 mg/mL sucrose, and 10.7 mM sodium acetate, optionally wherein the composition has a pH of 7.5.

In some embodiments, the composition comprises 0.5 mg/mL of the mRNA.

In some embodiments, the composition is administered intramuscularly, optionally into a deltoid region of an arm.

In some embodiments, the SARS-CoV-2 prefusion stabilized S protein comprises an amino acid sequence having at least 90%, at least 95%, or at least 98% identity to the sequence of SEQ ID NO: 8. For example, the SARS-CoV-2 prefusion stabilized S protein may comprise the amino acid sequence of SEQ ID NO: 8.

5 In some embodiments, the ORF comprises a nucleotide sequence having at least 90%, at least 95%, or at least 98% identity to the sequence of SEQ ID NO: 7. For example, the ORF may comprise the nucleotide sequence of SEQ ID NO: 7.

In some embodiments, the mRNA comprises a nucleotide sequence having at least 90%, at least 95%, or at least 98% identity to the sequence of SEQ ID NO: 6. For example, the mRNA
10 may comprise the nucleotide sequence of SEQ ID NO: 6.

In some embodiments, the lipid nanoparticle comprises: ionizable cationic lipid; neutral lipid; sterol; and PEG-modified lipid. In some embodiments, the lipid nanoparticle comprises: 20-60 mol% ionizable cationic lipid; 5-25 mol% neutral lipid; 25-55 mol% sterol; and 0.5-15 mol% PEG-modified lipid. In some embodiments, the lipid nanoparticle comprises: 40-55 mol%
15 ionizable cationic lipid; 5-15 mol% neutral lipid; 35-45 mol% sterol; and 1-5 mol% PEG-modified lipid. For example, the lipid nanoparticle may comprise: (a) 47 mol% ionizable cationic lipid; 11.5 mol% neutral lipid; 38.5 mol% sterol; and 3.0 mol% PEG-modified lipid; (b) 48 mol% ionizable cationic lipid; 11 mol% neutral lipid; 38.5 mol% sterol; and 2.5 mol% PEG-modified lipid; (c) 49 mol% ionizable cationic lipid; 10.5 mol% neutral lipid; 38.5 mol% sterol;
20 and 2.0 mol% PEG-modified lipid; (d) 50 mol% ionizable cationic lipid; 10 mol% neutral lipid; 38.5 mol% sterol; and 1.5 mol% PEG-modified lipid; or (e) 51 mol% ionizable cationic lipid; 9.5 mol% neutral lipid; 38.5 mol% sterol; and 1.0 mol% PEG-modified lipid.

In some embodiments, the ionizable cationic lipid is heptadecan-9-yl 8 ((2 hydroxyethyl)(6 oxo 6-(undecyloxy)hexyl)amino)octanoate (Compound 1). In some
25 embodiments, the neutral lipid is 1,2 distearoyl sn glycerol-3 phosphocholine (DSPC). In some embodiments, the sterol is cholesterol. In some embodiments, the PEG-modified lipid is 1-monomethoxypolyethyleneglycol-2,3-dimyristylglycerol with polyethylene glycol of average molecular weight 2000 (PEG2000 DMG).

In some embodiments, the age of the subject is 18 to 54 years or 55 years or older.

30 In some embodiments, the subject is immunocompromised. In some embodiments, the subject has a chronic pulmonary disease, such as chronic obstructive pulmonary disease (COPD) or asthma. In some embodiments, the subject has an underlying comorbid condition, optionally selected from heart disease, diabetes, and lung disease.

Other aspects of the present disclosure provide a composition comprising a 25 µg dose of an mRNA of any one of the preceding claims formulated in a lipid nanoparticle, wherein the geometric mean titer (GMT) of anti-SARS-CoV-2 S protein IgG antibodies produced in a subject at Day 15 or Day 29 post-administration of the composition at Day 1 is at least 1 log higher than the GMT of antibodies produced in convalescent subjects who were infected with SARS-CoV-2.

Yet other aspects of the present disclosure provide a composition comprising a 100 µg dose of an mRNA of any one of the preceding claims formulated in a lipid nanoparticle, wherein the geometric mean titer (GMT) of anti-SARS-CoV-2 S protein IgG antibodies produced in a subject at Day 15 or Day 29 post-administration of the composition at Day 1 is at least 1 log higher than the GMT of antibodies produced in convalescent subjects who were infected with SARS-CoV-2.

A method of administering to a subject a composition comprising a messenger ribonucleic acid (mRNA) comprising an open reading frame (ORF) that encodes a SARS-CoV-2 antigen comprising the amino acid sequence of SEQ ID NO: 8, and a lipid nanoparticle in an effective amount to elicit IgG2a and IgG1 subclass S protein-binding antibodies is provided in other aspects. In some embodiments the titers of IgG2a to IgG1 are balanced compared to a protein vaccine control.

In some aspects a method comprising administering to a subject a composition comprising a messenger ribonucleic acid (mRNA) comprising an open reading frame (ORF) that encodes a SARS-CoV-2 antigen comprising the amino acid sequence of SEQ ID NO: 8 in an effective amount to elicit a robust CD8 T cell response is provided. In some embodiments a CD4-directed T cell response is not elicited in the subject.

In some aspects a method comprising administering to a subject a composition comprising a messenger ribonucleic acid (mRNA) comprising an open reading frame (ORF) that encodes a SARS-CoV-2 antigen comprising the amino acid sequence of SEQ ID NO: 8 in an effective amount to elicit a balanced Th1/Th2 response is provided.

In other aspects a method comprising administering to a subject a composition comprising a messenger ribonucleic acid (mRNA) comprising an open reading frame (ORF) that encodes a SARS-CoV-2 antigen comprising the amino acid sequence of SEQ ID NO: 8 in an effective amount to confer protection to SARS-CoV-2 challenge is provided. In some embodiments protection is conferred at least 7 weeks post administration of the composition.

In some aspects a method comprising administering to a subject a composition comprising a messenger ribonucleic acid (mRNA) comprising an open reading frame (ORF) that encodes a SARS-CoV-2 antigen comprising the amino acid sequence of SEQ ID NO: 8 in an

effective amount to elicit high levels of neutralizing antibodies that increase between week 2 and week 4 post administration of the composition is provided.

The entire contents of International Application No. PCT/US2016/058327 (Publication No. WO2017/07062) and International Application No. PCT/US2018/022777 (Publication No. WO2018/170347) are incorporated herein by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows anti-spike IgG titers 15 days (left graph) or 29 days (right graph) after a single injection of either 25 µg or 100 µg of the SARS-CoV-2 mRNA vaccine compared to anti-spike IgG titers measured in convalescent sera. The geometric means with 95% confidence intervals are shown.

FIG. 2 shows graphs of the geometric mean titer (GMT) of serum IgG antibody titers by time point and treatment group.

FIG. 3 shows graphs of the geometric mean area under the curve (AUC) values by time point and vaccination group.

FIG. 4 shows graphs of the Log2 dilution of plaque reduction neutralization test (PRNT) titer by time point and vaccination group.

FIG. 5 shows a graph showing data from immunogenicity studies in rats. Rats were immunized with SARS-CoV-2 mRNA vaccine (also referred to herein as mRNA-1273 and/or the Wuhan-Hu-1 Variant 9 mRNA vaccine) at day 1. Sera from day 0 (open diamonds) and day 34 post-immunization (solid diamonds) were analyzed by ELISA to assess binding to SARS-CoV-2 stabilized prefusion spike protein (SARS-CoV-2 S-2P). Each symbol represents an individual mouse, bars represent geometric mean titers (GMT), and error bars indicate geometric standard deviation (SD). Two-way ANOVA was used to compare pre-dose responses to post-immunization responses and mRNA doses (**** = p-value < 0.0001).

FIGS. 6A-6E show graphs showing data from a lethal challenge study in mice, demonstrating that MERS-CoV S-2P mRNA protects mice from lethal challenge. Humanized DPP4 288/330+/+ mice were immunized at weeks 0 and 3 weeks with 0.01, 0.1, or 1 µg of MERS-CoV S-2P mRNA. Mock-immunized mice were immunized with PBS (gray). Four weeks post-boost, mice were challenged with a lethal dose of mouse-adapted MERS-CoV (MA35c5). Following challenge, mice were monitored for weight loss (FIG. 6A). The mean of each group is represented by circles. Error bars represent SEMs. At days 3 and 5 post-challenge, lungs from 5 mice/group were harvested for analysis of viral titers (FIGS. 6B-6C) and hemorrhage (0 = no hemorrhage, 4 = severe hemorrhage in all lobes) (FIGS. 6D-6E). Bars represent the GMT (FIGS. 6B-6C) or mean (FIGS. 6D-6E) of each group, and error bars represent geometric SD or SEM,

respectively. Experimental groups were compared by one-way ANOVA with Kruskal-Wallis post-test. Weight loss in experimental groups was compared to the PBS-immunized group on each day post-challenge. * = p-value < 0.05, ** = p-value < 0.01, **** = p-value < 0.0001.

FIGs. 7A-7B show graphs of data demonstrating that one dose of mRNA-1273 elicits robust antibody responses. BALB/c mice were immunized with 0.1 µg, 1 µg, or 10 µg of mRNA-1273. Sera were collected 2 (open circles) and 4 (closed circles) weeks post-immunization and assessed for SARS-CoV-2 S-specific total IgG by ELISA (FIG. 7A) and neutralizing antibodies against homotypic SARS-CoV-2 pseudovirus (FIG. 7B). Two-way ANOVA with Dunnett's post-test was used to compare temporal and dose-dependent antibody responses post-immunization. Bars represent GMT, and error bars indicate geometric SD.

FIGs. 8A-8I show graphs of data demonstrating that mRNA-1273 elicits both IgG2A and IgG1 subclass spike-binding antibodies. BALB/c (FIGs. 8A-8C), C57BL/6 (FIGs. 8D-8F), or B6C3 (FIGs. 8G-8I) mice were immunized at weeks 0 and 3 weeks with 0.01, 0.1, or 1 µg of mRNA-1273 (FIGs. 8A, 8D, 8G) or SARS-CoV-2 S-2P protein adjuvanted with Sigma adjuvant system (SAS) (FIGs. 8B, 8E, 8H). Sera were collected 2 weeks post-boost and assessed by ELISA for SARS-CoV-2 S-specific IgG1 and IgG2a, IgG2c, or IgG2a/c, for BALB/c, C57BL/6, and B6C3 mice, respectively. Endpoint titers (FIGs. 8A-8B, 8D-8E, 8G-8H) and endpoint titer ratios of IgG2a to IgG1 (FIG. 8C), IgG2c to IgG1 (FIG. 8F), and IgG2a/c to IgG1 (FIG. 8I) were calculated. For endpoint titers for that did not reach the lower limit of detection (dotted line), ratios were not calculated. Each symbol represents an individual mouse. (FIGs. 8A-8B, 8D-8E, 8G-8H). Bars represent geometric mean titers (GMT), and error bars indicate geometric SD. Two-way ANOVA was used to compare IgG subclasses. (FIGs. 8C, 8F, 8I). Bars represent means, and error bars indicate SEM. Two-way ANOVA with Dunnett's post-test was used to compare mRNA-1273 to S-2P protein. * = p-value < 0.05, ** = p-value < 0.01, *** = p-value < 0.001, **** = p-value < 0.0001.

FIG. 9 shows a graph of data demonstrating a balanced response of IgG2a and IgG1 with mRNA-1273 and an unbalanced response for the adjuvanted protein vaccine. BALB/c mice were then immunized with 1 µg or 10 µg of mRNA-1273 or 10 µg CoV-2 S protein adjuvanted with 250 µg ALHYDROGEL® (Alum). Sera were collected 2 weeks post-immunization and assessed by ELISA for Spike-specific IgG2a (green) and IgG1 (orange), and IgG2a to IgG1 subclass ratios were calculated.

FIGs. 10A-10B show graphs of data demonstrating that mRNA-1273 induces a Spike-specific T cell response (expressing Th1 cytokines) and does not drive a Th2 response). BALB/c mice were immunized with 0.1 µg, 1 µg, or 10 µg of mRNA-1273, and CD8 and CD4 T cell

responses were assessed on Day 36 for cytokine expression. A CD8 T cell response was assessed by Th1 cytokine expression (IFN γ , IL-2, TNF α) (FIG. 10A), and a CD4 T cell response was assessed by Th2 cytokine expression (IL-4, IL-5, IL-9, IL-10, IL-13) (FIG. 10B).

DETAILED DESCRIPTION

The present disclosure provides vaccine compositions and vaccination methods that elicit potent neutralizing antibodies against coronavirus antigens. In some embodiments, a composition includes messenger RNA (mRNA) encoding a SARS-CoV-2 Spike (S) protein (*e.g.*, a prefusion stabilized form of the S protein) formulated in a lipid nanoparticle (LNP).

Vaccine Compositions

Provided herein are vaccine compositions for inducing a neutralizing antibody response to SARS-CoV-2 Spike (S) protein in a subject. The compositions provided herein can be used as therapeutically or prophylactically.

The compositions provided herein include messenger RNA (mRNA) encoding a SARS-CoV-2 S protein (*e.g.*, prefusion stabilized S protein).

In some embodiments, a composition containing a messenger RNA as described herein can be administered to a subject (*e.g.*, a mammalian subject, such as a human subject), and the mRNA is translated *in vivo* to produce an antigenic polypeptide (antigen), such as a SARS-CoV-2 S protein (*e.g.*, prefusion stabilized S protein) or S protein subunit.

In some embodiments, a vaccine composition comprises an approximately 25 μ g to 250 μ g dose of mRNA encoding a SARS-CoV-2 S protein (*e.g.*, prefusion stabilized S protein). In some embodiments, a vaccine composition comprises an approximately 25 μ g dose of mRNA encoding a SARS-CoV-2 S protein (*e.g.*, prefusion stabilized S protein). In some embodiments, a vaccine composition comprises an approximately 50 μ g dose of mRNA encoding a SARS-CoV-2 S protein (*e.g.*, prefusion stabilized S protein). In some embodiments, a vaccine composition comprises an approximately 100 μ g dose of mRNA encoding a SARS-CoV-2 S protein (*e.g.*, prefusion stabilized S protein). In some embodiments, a vaccine composition comprises an approximately 150 μ g dose of mRNA encoding a SARS-CoV-2 S protein (*e.g.*, prefusion stabilized S protein). In some embodiments, a vaccine composition comprises an approximately 200 μ g dose of mRNA encoding a SARS-CoV-2 S protein (*e.g.*, prefusion stabilized S protein). In some embodiments, a vaccine composition comprises an approximately 250 μ g dose of mRNA encoding a SARS-CoV-2 S protein (*e.g.*, prefusion stabilized S protein).

A composition may further comprise a buffer, for example a Tris buffer. For example, a composition may comprise 10 mM – 30 mM, 10 mM – 20 mM, or 20 mM – 30 mM Tris buffer.

In some embodiments, a composition comprises 10, 15, 20, 25, or 30 mM Tris buffer. In some embodiments, a composition comprises 20 mM Tris buffer.

In some embodiments, mRNA of a vaccine composition is formulated at a concentration of 0.1 – 1 mg/mL. In some embodiments, mRNA of a vaccine composition is formulated at a concentration of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, or 1 mg/mL. In some embodiments, mRNA of a vaccine composition is formulated at a concentration of 0.5 mg/mL.

In some embodiments, a composition comprises sucrose. For example, a composition may comprise 75 mg/mL – 95 mg/mL, 75 mg/mL – 85 mg/mL, or 85 mg/mL – 95 mg/mL sucrose. In some embodiments, a composition comprises 75, 80, 85, 86, 87, 88, 89, 90, or 95 mg/mL sucrose. In some embodiments, a composition comprises 87 mg/mL sucrose.

In some embodiments, a composition comprises sodium acetate. For example, a composition may comprise 5 mM – 15 mM, 5 mM – 10 mM, or 10 mM – 15 mM sodium acetate. In some embodiments, a composition comprises 5, 10, 11, 12, 13, 14, or 15 mM sodium acetate. In some embodiments, a composition comprises 10, 10.1, 10.2, 10.3, 10.4, 10.5, 10.6, 10.7, 10.8, 10.9, or 11 mM sodium acetate. In some embodiments, a composition comprises 10.7 mM sodium acetate.

A composition may have a pH value of 6-8. In some embodiments, a composition has a pH value of 6, 6.5, 7, 7.5, or 8. In some embodiments, a composition has a pH value of 7.5.

A composition, in some embodiments, is formulated to include mRNA at a concentration of 0.1 mg/mL – 1 mg/mL. In some embodiments, a composition comprises 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, or 1 mg/mL mRNA. In some embodiments, a composition comprises 0.5 mg/mL mRNA.

In some embodiments, the composition further comprises a mixture of lipids. The mixture of lipids typically forms a lipid nanoparticle. The mRNA described herein, in some embodiments, is formulated with a lipid nanoparticle (*e.g.*, for administration to a subject).

In some embodiments, the lipid mixture, and thus the lipid nanoparticle, comprises: an ionizable cationic lipid; a neutral lipid; a sterol; and a PEG-modified lipid. For example, the lipid mixture/lipid nanoparticle may comprise: 20-60 mol% ionizable cationic lipid; 5-25 mol% neutral lipid; 25-55 mol% sterol; and 0.5-15 mol% PEG-modified lipid. In some embodiments, the lipid nanoparticle comprises: 20-60 mol% ionizable cationic lipid; 5-25 mol% neutral lipid; 25-55 mol% sterol; and 0.5-15 mol% PEG-modified lipid. In some embodiments, the lipid nanoparticle comprises: 40-55 mol% ionizable cationic lipid; 5-15 mol% neutral lipid; 35-45 mol% sterol; and 1-5 mol% PEG-modified lipid. For example, the lipid nanoparticle may comprise: (a) 47 mol% ionizable cationic lipid; 11.5 mol% neutral lipid; 38.5 mol% sterol; and

3.0 mol% PEG-modified lipid; (b) 48 mol% ionizable cationic lipid; 11 mol% neutral lipid; 38.5 mol% sterol; and 2.5 mol% PEG-modified lipid; (c) 49 mol% ionizable cationic lipid; 10.5 mol% neutral lipid; 38.5 mol% sterol; and 2.0 mol% PEG-modified lipid; (d) 50 mol% ionizable cationic lipid; 10 mol% neutral lipid; 38.5 mol% sterol; and 1.5 mol% PEG-modified lipid; or (e) 51 mol% ionizable cationic lipid; 9.5 mol% neutral lipid; 38.5 mol% sterol; and 1.0 mol% PEG-modified lipid.

In some embodiments, the lipid mixture, and thus the lipid nanoparticle, comprises 20-55 mol%, 20-50 mol%, 20-45 mol%, 20-40 mol%, 25-60 mol%, 25-55 mol%, 25-50 mol%, 25-45 mol%, 25-40 mol%, 30-60 mol%, 30-55 mol%, 30-50 mol%, 30-45 mol%, 30-40 mol%, 35-60 mol%, 35-55 mol%, 35-50 mol%, 35-45 mol%, 35-40 mol%, 40-60 mol%, 40-55 mol%, 40-50 mol%, 40-45 mol%, 50-60 mol%, 50-55 mol%, or 55-60 mol% ionizable cationic lipid.

In some embodiments, the lipid mixture, and thus the lipid nanoparticle, comprises 5-20 mol%, 5-15 mol%, 5-10 mol%, 10-25 mol%, 10-20 mol%, 10-15 mol%, 15-25 mol%, 15-20 mol%, or 20-25 mol% neutral lipid.

In some embodiments, the lipid mixture, and thus the lipid nanoparticle, comprises 25-50 mol%, 25-45 mol%, 25-40 mol%, 25-35 mol%, 25-30 mol%, 30-55 mol%, 30-50 mol%, 30-45 mol%, 30-40 mol%, 30-35 mol%, 35-55 mol%, 35-50 mol%, 35-45 mol%, 35-40 mol%, 40-55 mol%, 40-50 mol%, 40-45 mol%, 45-55 mol%, 45-50 mol%, or 50-55 mol% sterol.

In some embodiments, the lipid mixture, and thus the lipid nanoparticle, comprises 0.5-10 mol%, 0.5-5 mol%, 0.5-1 mol%, 1-15%, 1-10 mol%, 1-5 mol%, 1.5-15%, 1.5-10 mol%, 1.5-5 mol%, 2-15%, 2-10 mol%, 2-5 mol%, 2.5-15%, 2.5-10 mol%, 2.5-5 mol%, 3-15%, 3-10 mol%, or 3-5 mol% PEG-modified lipid.

In some embodiments, the lipid mixture comprises: 50 mol% ionizable cationic lipid; 10 mol% neutral lipid; 38.5 mol% sterol; and 1.5 mol% PEG-modified lipid.

In some embodiments, the ionizable cationic lipid is heptadecan-9-yl 8 ((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate (Compound 1). In some embodiments, the neutral lipid is 1,2 distearoyl sn glycerophosphocholine (DSPC). In some embodiments, the sterol is cholesterol. In some embodiments, the PEG-modified lipid is 1-monomethoxypolyethyleneglycol-2,3-dimyristylglycerol with polyethylene glycol of average molecular weight 2000 (PEG2000 DMG).

A composition, may further include a pharmaceutically-acceptable excipient, inert or active. A pharmaceutically acceptable excipient, after administered to a subject, does not cause undesirable physiological effects. The excipient in the pharmaceutical composition must be “acceptable” also in the sense that it is compatible with mRNA and can be capable of stabilizing

it. One or more excipients (*e.g.*, solubilizing agents) can be utilized as pharmaceutical carriers for delivery of the mRNA. Examples of a pharmaceutically acceptable excipients include, but are not limited to, biocompatible vehicles (*e.g.*, LNPs), carriers, adjuvants, additives, and diluents to achieve a composition usable as a dosage form. Examples of other excipients include colloidal
5 silicon oxide, magnesium stearate, cellulose, and sodium lauryl sulfate. Additional suitable pharmaceutical excipients, as well as pharmaceutical necessities for their use, are described in Remington's Pharmaceutical Sciences.

In some embodiments, an mRNA is formulated using one or more excipients to: (1) increase stability; (2) increase cell transfection; (3) permit the sustained or delayed release (*e.g.*,
10 from a depot formulation); (4) alter the biodistribution (*e.g.*, target to specific tissues or cell types); (5) increase the translation of encoded protein *in vivo*; and/or (6) alter the release profile of encoded protein (antigen) *in vivo*. In addition to traditional excipients such as any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, excipients
15 can include, without limitation, lipidoids, liposomes, lipid nanoparticles, polymers, lipoplexes, core-shell nanoparticles, peptides, proteins, cells transfected with the RNA (*e.g.*, for transplantation into a subject), hyaluronidase, nanoparticle mimics and combinations thereof.

In some embodiments, a composition comprising mRNA does not include an adjuvant (they are adjuvant free).

20 Vaccine compositions may be sterile, pyrogen-free or both sterile and pyrogen-free. General considerations in the formulation and/or manufacture of pharmaceutical agents, such as vaccine compositions, may be found, for example, in Remington: The Science and Practice of Pharmacy 21st ed., Lippincott Williams & Wilkins, 2005 (incorporated herein by reference in its entirety).

25 Formulations of the vaccine compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the mRNA into association with an excipient (*e.g.*, a mixture of lipids and/or a lipid nanoparticle), and then, if necessary and/or desirable, dividing, shaping and/or packaging the product into a desired single- or multi-dose unit.

30 Relative amounts of the mRNA, the pharmaceutically-acceptable excipient, and/or any additional ingredients in a composition in accordance with the disclosure may vary, depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be administered.

Vaccination Methods

Provided herein are vaccine compositions and methods for inducing a neutralizing antibody response to SARS-CoV-2 Spike (S) protein in a subject. A subject may be any mammal, including anon-human primate and human subjects. Typically, a subject is a human subject.

Vaccine compositions herein (*e.g.*, mRNA encoding SARS-CoV-2 Spike (S) protein formulated in a lipid nanoparticle) can be used as therapeutic composition, prophylactic compositions, or both therapeutic and prophylactic compositions. In some embodiments, the compositions are used in the priming of immune effector cells, for example, to activate peripheral blood mononuclear cells (PBMCs) *ex vivo*, which are then infused (re-infused) into a subject. The mRNA encoding the SARS-CoV-2 S protein is expressed and translated *in vivo* to produce the antigen, which then stimulates an immune response in the subject.

The compositions provided herein are administered, in some embodiments, in “effective amounts,” for example, therapeutically-effective and/or prophylactically-effective amounts. In some embodiments, an effective amount of a composition induces a SARS-CoV-2 antigen-specific immune response. An effective amount of a composition (*e.g.*, comprising RNA) is based, at least in part, on the target tissue, target cell type, means of administration, physical characteristics of the RNA (*e.g.*, length, nucleotide composition, and/or extent of modified nucleosides), other components of the vaccine, and other determinants, such as age, body weight, height, sex and general health of the subject. Typically, an effective amount of a composition provides an induced or boosted immune response as a function of antigen production in the cells of the subject.

In some embodiments, an effective amount of the composition comprising mRNA having at least one chemical modifications are more efficient than a composition containing a corresponding unmodified polynucleotide encoding the same antigen or a peptide antigen. Increased antigen production may be demonstrated by increased cell transfection (the percentage of cells transfected with an RNA composition), increased protein translation and/or expression from the RNA, decreased nucleic acid degradation (as demonstrated, for example, by increased duration of protein translation from a modified polynucleotide), or altered antigen specific immune response of the host cell.

In some embodiments, the compositions (comprising polynucleotides and their encoded polypeptides) in accordance with the present disclosure may be administered prophylactically or therapeutically as part of an active immunization scheme to healthy individuals or early in infection during the incubation phase or during active infection after onset of symptoms. In some

embodiments, the amount of RNA provided to a cell, a tissue or a subject may be an amount effective for immune prophylaxis.

In some embodiments, an initial dose of a vaccine composition is administered followed by a booster dose. A booster dose is a dose that is given at a certain interval after completion of the primary dose or series of doses that is/are intended to boost immunity to, and therefore prolong protection against, the disease that is to be prevented. The time between administration of an initial dose of a composition and a booster dose may be, for example, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 2 years, 3 years, 4 years, or 5 years. In some embodiments, the time between administration of an initial dose of a composition and a booster dose is 20-30 days. For example, the time between administration of an initial dose of a composition and a booster dose may be 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 days. In some embodiments, the time between administration of an initial dose of a composition and a booster dose is 28 days.

A method of eliciting an immune response in a subject against a SARS-CoV-2 antigen (or multiple antigens) is provided in aspects of the present disclosure. In some embodiments, a method involves administering to the subject a composition comprising a mRNA having an open reading frame encoding a SARS-CoV-2 S protein (*e.g.*, prefusion stabilized SARS-CoV-2 S protein), thereby inducing in the subject an immune response specific to the SARS-CoV-2 S protein, wherein an anti-S protein antibody titer in the subject is increased following vaccination relative to an anti-S protein antibody titer in an unvaccinated subject who has not been infected with SARS-CoV-2 or who has been infected by has recovered. An “anti-S protein antibody” is a serum antibody the binds specifically to SARS-CoV-2 S protein.

A vaccine composition may be administered by any route that results in a therapeutically effective outcome. These include, but are not limited, to intradermal, intramuscular, intranasal, and/or subcutaneous administration. In some embodiments, a composition is administered intramuscularly (*e.g.*, into a deltoid muscle).

The present disclosure provides methods comprising administering vaccine compositions to a subject in need thereof. The exact amount required may vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the disease, the particular composition, its mode of administration, its mode of activity, and the like. The mRNA is typically formulated in dosage unit form for ease of administration and uniformity of dosage. It will be understood, however, that the total daily usage of the mRNA may be decided by the attending physician within the scope of sound medical judgment. The specific

therapeutically effective, prophylactically effective, or appropriate imaging dose level for any particular subject may depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts.

The effective amount of the RNA, as provided herein, may range from about 25 µg – 500 µg, administered as a single dose or as multiple (*e.g.*, booster) doses. In some embodiments, a single dose of a vaccine composition (*e.g.*, administered once, twice, three times, or more) comprises about 25 µg mRNA. In some embodiments, a single dose of a vaccine composition (*e.g.*, administered once, twice, three times, or more) comprises about 100 µg mRNA. In some embodiments, a single dose of a vaccine composition (*e.g.*, administered once, twice, three times, or more) comprises about 250 µg mRNA.

In some embodiments, a total amount of mRNA administered to a subject is about 25 µg, 50 µg, about 100 µg, about 200 µg, about 250 µg, or about 500 µg mRNA. In some embodiments, a total amount of mRNA administered to a subject is about 25 µg. In some embodiments, a total amount of mRNA administered to a subject is about 50 µg. In some embodiments, a total amount of mRNA administered to a subject is about 100 µg. In some embodiments, a total amount of mRNA administered to a subject is about 200 µg. In some embodiments, a total amount of mRNA administered to a subject is about 250 µg. In some embodiments, a total amount of mRNA administered to a subject is about 500 µg.

The mRNA described herein can be formulated into a dosage form described herein, such as an intranasal, intratracheal, or injectable (*e.g.*, intravenous, intraocular, intravitreal, intramuscular, intradermal, intracardiac, intraperitoneal, and subcutaneous).

Vaccine Efficacy

The vaccine compositions as provided herein are administered in effective amounts to induce an immune response to SARS-CoV-2.

As used herein, an immune response to a vaccine composition is the development in a subject of a humoral and/or a cellular immune response to a (one or more) coronavirus protein(s) present in the composition. For purposes of the present disclosure, a “humoral” immune response refers to an immune response mediated by antibody molecules, including, *e.g.*, secretory (IgA) or IgG molecules, while a “cellular” immune response is one mediated by T-lymphocytes (*e.g.*, CD4⁺ helper and/or CD8⁺ T cells (*e.g.*, CTLs) and/or other white blood cells. One important

aspect of cellular immunity involves an antigen-specific response by cytolytic T-cells (CTLs). CTLs have specificity for peptide antigens that are presented in association with proteins encoded by the major histocompatibility complex (MHC) and expressed on the surfaces of cells. CTLs help induce and promote the destruction of intracellular microbes or the lysis of cells infected with such microbes. Another aspect of cellular immunity involves an antigen-specific response by helper T-cells. Helper T-cells act to help stimulate the function, and focus the activity nonspecific effector cells against cells displaying peptide antigens in association with MHC molecules on their surface. A cellular immune response also leads to the production of cytokines, chemokines, and other such molecules produced by activated T-cells and/or other white blood cells including those derived from CD4+ and CD8+ T-cells.

In some embodiments, an immune response is assessed by determining [protein] antibody titer in the subject. In some embodiments, the ability of serum or antibody from an immunized subject is tested for its ability to neutralize viral uptake or reduce viral transformation of human B lymphocytes. In some embodiments, the ability to promote a robust T cell response(s) is measured.

In some embodiments, an antigen-specific immune response is characterized by measuring an anti-antigen antibody titer produced in a subject administered a composition as provided herein, wherein the antigen is a SARS-CoV-2 S protein (*e.g.*, prefusion stabilized S protein). An antibody titer is a measurement of the amount of antibodies within a subject, for example, antibodies that are specific to a particular antigen or epitope of an antigen. Antibody titer is typically expressed as the inverse of the greatest dilution that provides a positive result.

A variety of serological tests can be used to measure antibody against encoded antigen of interest, for example, SAR-CoV-2 virus or SAR-CoV-2 viral antigen, *e.g.*, SAR-CoV-2 spike or S protein, of domain thereof. These tests include the hemagglutination-inhibition test, complement fixation test, fluorescent antibody test, enzyme-linked immunosorbent assay (ELISA), and plaque reduction neutralization test (PRNT). Each of these tests measures different antibody activities. In exemplary embodiments, a plaque reduction neutralization test, or PRNT (*e.g.*, PRNT50 or PRNT80) is used as a serological correlate of protection. PRNT measures the biological parameter of *in vitro* virus neutralization and is the most serologically virus-specific test among certain classes of viruses, correlating well to serum levels of protection from virus infection.

The basic design of the PRNT allows for virus-antibody interaction to occur in a test tube or microtiter plate, and then measuring antibody effects on viral infectivity by plating the mixture on virus-susceptible cells, preferably cells of mammalian origin. The cells are overlaid

with a semi-solid media that restricts spread of progeny virus. Each virus that initiates a productive infection produces a localized area of infection (a plaque), that can be detected in a variety of ways. Plaques are counted and compared back to the starting concentration of virus to determine the percent reduction in total virus infectivity. In PRNT, the serum sample being
5 tested is usually subjected to serial dilutions prior to mixing with a standardized amount of virus. The concentration of virus is held constant such that, when added to susceptible cells and overlaid with semi-solid media, individual plaques can be discerned and counted. In this way, PRNT end-point titers can be calculated for each serum sample at any selected percent reduction of virus activity.

10 In functional assays intended to assess vaccinal immunogenicity, the serum sample dilution series for antibody titration should ideally start below the “seroprotective” threshold titer. Regarding SARS-CoV-2 neutralizing antibodies, the “seroprotective” threshold titer remains unknown; but a seropositivity threshold of 1:10 can be considered a seroprotection threshold in certain embodiments.

15 PRNT end-point titers are expressed as the reciprocal of the last serum dilution showing the desired percent reduction in plaque counts. The PRNT titer can be calculated based on a 50% or greater reduction in plaque counts (PRNT50). A PRNT50 titer is preferred over titers using higher cut-offs (*e.g.*, PRNT80) for vaccine sera, providing more accurate results from the linear portion of the titration curve.

20 There are several ways to calculate PRNT titers. The simplest and most widely used way to calculate titers is to count plaques and report the titer as the reciprocal of the last serum dilution to show >50% reduction of the input plaque count as based on the back-titration of input plaques. Use of curve fitting methods from several serum dilutions may permit calculation of a more precise result. There are a variety of computer analysis programs available for this (*e.g.*,
25 SPSS or GraphPad Prism).

In some embodiments, an antibody titer is used to assess whether a subject has had an infection or to determine whether immunizations are required. In some embodiments, an antibody titer is used to determine the strength of an autoimmune response, to determine whether a booster immunization is needed, to determine whether a previous vaccine was effective, and to
30 identify any recent or prior infections. In accordance with the present disclosure, an antibody titer may be used to determine the strength of an immune response induced in a subject by a vaccine composition.

In some embodiments, an anti-antigen antibody titer produced in a subject is increased by at least 1 log relative to a control. For example, anti-antigen antibody titer produced in a subject

may be increased by at least 1.5, at least 2, at least 2.5, or at least 3 log relative to a control. In some embodiments, the anti-antigen antibody titer produced in the subject is increased by 1, 1.5, 2, 2.5 or 3 log relative to a control. In some embodiments, the anti-antigen antibody titer produced in the subject is increased by 1-3 log relative to a control. For example, the anti-antigen antibody titer produced in a subject may be increased by 1-1.5, 1-2, 1-2.5, 1-3, 1.5-2, 1.5-2.5, 1.5-3, 2-2.5, 2-3, or 2.5-3 log relative to a control.

In some embodiments, the anti-antigen antibody titer produced in a subject is increased at least 2 times relative to a control. For example, the anti-antigen antibody titer produced in a subject may be increased at least 3 times, at least 4 times, at least 5 times, at least 6 times, at least 7 times, at least 8 times, at least 9 times, or at least 10 times relative to a control. In some embodiments, the anti-antigen antibody titer produced in the subject is increased 2, 3, 4, 5, 6, 7, 8, 9, or 10 times relative to a control. In some embodiments, the anti-antigen antibody titer produced in a subject is increased 2-10 times relative to a control. For example, the anti-antigen antibody titer produced in a subject may be increased 2-10, 2-9, 2-8, 2-7, 2-6, 2-5, 2-4, 2-3, 3-10, 3-9, 3-8, 3-7, 3-6, 3-5, 3-4, 4-10, 4-9, 4-8, 4-7, 4-6, 4-5, 5-10, 5-9, 5-8, 5-7, 5-6, 6-10, 6-9, 6-8, 6-7, 7-10, 7-9, 7-8, 8-10, 8-9, or 9-10 times relative to a control.

In some embodiments, antibody-mediated immunogenicity in a subject is assessed at one or more time points (*e.g.*, Day 1, Day 29, Day 57, Day 119, Day 209, and Day 394). Methods of assessing antibody-mediated immunogenicity are known and include geometric mean concentration (GMC) of antibody to antigen, geometric mean fold rise (GMFR) in serum antibody, geometric mean titer (GMT), median, minimum, maximum, 95% confidence interval (CI), geometric mean ratio (GMR) of post-baseline / baseline titers, and seroconversion rate.

The GMC is the average antibody concentration for a group of subjects calculated by multiplying all values and taking the n th root of this number, where n is the number of subjects with available data. GMT is the average antibody titer for a group of subjects calculated by multiplying all values and taking the n th root of this number, where n is the number of subjects with available data.

A control, in some embodiments, is an anti-antigen antibody titer produced in a subject who has not been administered a vaccine composition, or who has been administered a saline placebo (an unvaccinated subject). In some embodiments, a control is an anti-antigen antibody titer produced in a subject administered a recombinant or purified protein vaccine (*e.g.*, protein subunit vaccine). Recombinant protein vaccines typically include protein antigens that either have been produced in a heterologous expression system (*e.g.*, bacteria or yeast) or purified from

large amounts of the pathogenic organism. A control may be, for example, a subject administered a live attenuated viral vaccine or an inactivated viral vaccine.

In some embodiments, the ability of a vaccine composition to be effective is measured in a rodent (*e.g.*, murine or rabbit model). For example, a composition may be administered to a rodent model and the murine model assayed for induction of neutralizing antibody titers. Viral challenge studies may also be used to assess the efficacy of a composition of the present disclosure. For example, a composition may be administered to a rodent model, the rodent model challenged with virus, and the rodent model assayed for survival and/or immune response (*e.g.*, neutralizing antibody response, T cell response (*e.g.*, cytokine response)).

Vaccine efficacy may be assessed using standard analyses (see, *e.g.*, Weinberg et al., J Infect Dis. 2010 Jun 1;201(11):1607-10). For example, vaccine efficacy may be measured by double-blind, randomized, clinical controlled trials. Vaccine efficacy may be expressed as a proportionate reduction in disease attack rate (AR) between the unvaccinated (ARU) and vaccinated (ARV) study cohorts and can be calculated from the relative risk (RR) of disease among the vaccinated group with use of the following formulas:

$$\text{Efficacy} = (\text{ARU} - \text{ARV})/\text{ARU} \times 100; \text{ and}$$

$$\text{Efficacy} = (1 - \text{RR}) \times 100.$$

Likewise, vaccine effectiveness may be assessed using standard analyses (see, *e.g.*, Weinberg et al., J Infect Dis. 2010 Jun 1;201(11):1607-10). Vaccine effectiveness is an assessment of how a vaccine (which may have already proven to have high vaccine efficacy) reduces disease in a population. This measure can assess the net balance of benefits and adverse effects of a vaccination program, not just the vaccine itself, under natural field conditions rather than in a controlled clinical trial. Vaccine effectiveness is proportional to vaccine efficacy (potency) but is also affected by how well target groups in the population are immunized, as well as by other non-vaccine-related factors that influence the ‘real-world’ outcomes of hospitalizations, ambulatory visits, or costs. For example, a retrospective case control analysis may be used, in which the rates of vaccination among a set of infected cases and appropriate controls are compared. Vaccine effectiveness may be expressed as a rate difference, with use of the odds ratio (OR) for developing infection despite vaccination:

$$\text{Effectiveness} = (1 - \text{OR}) \times 100.$$

In some embodiments, efficacy of a vaccine composition is at least 60% relative to unvaccinated control subjects. For example, efficacy of a vaccine composition may be at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 95%, at least 98%, or 100% relative to unvaccinated control subjects.

Sterilizing Immunity. Sterilizing immunity refers to a unique immune status that prevents effective pathogen infection into the host. In some embodiments, the effective amount of a composition of the present disclosure is sufficient to provide sterilizing immunity in the subject for at least 1 year. For example, the effective amount of a composition of the present disclosure is sufficient to provide sterilizing immunity in the subject for at least 2 years, at least 3 years, at least 4 years, or at least 5 years. In some embodiments, the effective amount of a composition of the present disclosure is sufficient to provide sterilizing immunity in the subject at an at least 5-fold lower dose relative to control. For example, the effective amount may be sufficient to provide sterilizing immunity in the subject at an at least 10-fold lower, 15-fold, or 20-fold lower dose relative to a control.

Detectable Antigen. In some embodiments, the effective amount of a composition of the present disclosure is sufficient to produce detectable levels of SARS-CoV-2 antigen as measured in serum of the subject at 1-72 hours post administration.

Titer. An antibody titer is a measurement of the amount of antibodies within a subject, for example, antibodies that are specific to a particular antigen (*e.g.*, an anti-coronavirus antigen). Antibody titer is typically expressed as the inverse of the greatest dilution that provides a positive result. Enzyme-linked immunosorbent assay (ELISA) is a common assay for determining antibody titers, for example.

In some embodiments, the effective amount of a composition of the present disclosure is sufficient to produce a 1,000-10,000 neutralizing antibody titer produced by neutralizing antibody against the SARS-CoV-2 antigen as measured in serum of the subject at 1-72 hours post administration. In some embodiments, the effective amount is sufficient to produce a 1,000-5,000 neutralizing antibody titer produced by neutralizing antibody against the SARS-CoV-2 antigen as measured in serum of the subject at 1-72 hours post administration. In some embodiments, the effective amount is sufficient to produce a 5,000-10,000 neutralizing antibody titer produced by neutralizing antibody against the SARS-CoV-2 virus antigen as measured in serum of the subject at 1-72 hours post administration.

In some embodiments, the neutralizing antibody titer is at least 100 NT₅₀. For example, the neutralizing antibody titer may be at least 200, 300, 400, 500, 600, 700, 800, 900 or 1000 NT₅₀. In some embodiments, the neutralizing antibody titer is at least 10,000 NT₅₀.

In some embodiments, the neutralizing antibody titer is at least 100 neutralizing units per milliliter (NU/mL). For example, the neutralizing antibody titer may be at least 200, 300, 400, 500, 600, 700, 800, 900 or 1000 NU/mL. In some embodiments, the neutralizing antibody titer is at least 10,000 NU/mL.

In some embodiments, an anti-antigen antibody titer produced in the subject is increased by at least 1 log relative to a control. For example, an anti-antigen antibody titer produced in the subject may be increased by at least 2, 3, 4, 5, 6, 7, 8, 9 or 10 log relative to a control.

In some embodiments, an anti-antigen antibody titer produced in the subject is increased at least 2 times relative to a control. For example, an anti-coronavirus antigen antibody titer produced in the subject is increased by at least 3, 4, 5, 6, 7, 8, 9 or 10 times relative to a control.

In some embodiments, a geometric mean, which is the n th root of the product of n numbers, is generally used to describe proportional growth. Geometric mean, in some embodiments, is used to characterize antibody titer produced in a subject.

Antigens

Antigens, as used herein, are proteins capable of inducing an immune response (*e.g.*, causing an immune system to produce antibodies against the antigens). Herein, use of the term “antigen” encompasses immunogenic proteins and immunogenic fragments and subunits [an immunogenic fragment or subunit that induces (or is capable of inducing) an immune response to a (at least one) coronavirus], unless otherwise stated. It should be understood that the term “protein” encompasses peptides and the term “antigen” encompasses antigenic fragments, protein domains, and subunits. Other molecules may be antigenic such as bacterial polysaccharides or combinations of protein and polysaccharide structures, but for the viral vaccines included herein, viral proteins, fragments of viral proteins, and designed and/or mutated proteins derived from the betacoronavirus SARS-CoV-2 are the antigens provided herein.

Exemplary sequences of the SARS-CoV-2 antigens and the mRNA encoding the antigens of the compositions of the present disclosure are provided in Table 3.

In some embodiments, the coronavirus antigen is a prefusion stabilized spike (S) protein, which comprises an amino acid sequence that stabilizes the S protein in its prefusion conformation. A prefusion stabilized spike protein is more stable than the S protein in its postfusion conformation. In some embodiments, a prefusion stabilized S protein comprises a double proline stabilizing mutation. In some embodiments, a prefusion stabilized S protein comprises a double proline stabilizing mutation at position 986 (K986P) and 987 (V987P), relative to wild-type (native) S protein comprising the amino acid sequence of SEQ ID NO: 5.

In some embodiments, a composition comprises an RNA (*e.g.*, mRNA) that encodes an S protein that comprises a sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% identity to the sequence of SEQ ID NO: 8. In some embodiments, a composition comprises an RNA (*e.g.*, mRNA) that encodes an S protein that comprises a sequence having at least 80% identity to the sequence of SEQ ID NO: 8. In some embodiments,

a composition comprises an RNA (*e.g.*, mRNA) that encodes an S protein that comprises a sequence having at least 85% identity to the sequence of SEQ ID NO: 8. In some embodiments, a composition comprises an RNA (*e.g.*, mRNA) that encodes an S protein that comprises a sequence having at least 90% identity to the sequence of SEQ ID NO: 8. In some embodiments, a composition comprises an RNA (*e.g.*, mRNA) that encodes an S protein that comprises a sequence having at least 95% identity to the sequence of SEQ ID NO: 8. In some embodiments, a composition comprises an RNA (*e.g.*, mRNA) that encodes an S protein that comprises a sequence having at least 96% identity to the sequence of SEQ ID NO: 8. In some embodiments, a composition comprises an RNA (*e.g.*, mRNA) that encodes an S protein that comprises a sequence having at least 97% identity to the sequence of SEQ ID NO: 8. In some embodiments, a composition comprises an RNA (*e.g.*, mRNA) that encodes an S protein that comprises a sequence having at least 98% identity to the sequence of SEQ ID NO: 8. In some embodiments, a composition comprises an RNA (*e.g.*, mRNA) that encodes an S protein that comprises the amino acid sequence of SEQ ID NO: 8.

It should be understood that any one of the antigens encoded by the RNA described herein may or may not comprise a signal sequence.

20 Nucleic Acids

The compositions of the present disclosure comprise a (at least one) messenger RNA (mRNA) having an open reading frame (ORF) encoding a SARS-CoV-2 antigen. In some embodiments, the mRNA further comprises a 5' UTR, 3' UTR, a poly(A) tail, and/or a 5' cap analog.

It should also be understood that the vaccine composition of the present disclosure may include any 5' untranslated region (UTR) and/or any 3' UTR. UTRs may also be omitted from the mRNA provided herein.

Nucleic acids comprise a polymer of nucleotides (nucleotide monomers). Thus, nucleic acids are also referred to as polynucleotides. Nucleic acids may be or may include, for example, deoxyribonucleic acids (DNAs), ribonucleic acids (RNAs), threose nucleic acids (TNAs), glycol nucleic acids (GNAs), peptide nucleic acids (PNAs), locked nucleic acids (LNAs, including LNA having a β -D-ribo configuration, α -LNA having an α -L-ribo configuration (a diastereomer of LNA), 2'-amino-LNA having a 2'-amino functionalization, and 2'-amino- α -LNA having a 2'-amino functionalization), ethylene nucleic acids (ENA), cyclohexenyl nucleic acids (CeNA) and/or chimeras and/or combinations thereof.

Messenger RNA (mRNA) is any RNA that encodes a (at least one) protein (a naturally-occurring, non-naturally-occurring, or modified polymer of amino acids) and can be translated to produce the encoded protein *in vitro*, *in vivo*, *in situ*, or *ex vivo*. The skilled artisan will appreciate that, except where otherwise noted, nucleic acid sequences set forth in the instant application may recite “T”s in a representative DNA sequence but where the sequence represents RNA (*e.g.*, mRNA), the “T”s would be substituted for “U”s. Thus, any of the DNAs disclosed and identified by a particular sequence identification number herein also disclose the corresponding RNA (*e.g.*, mRNA) sequence complementary to the DNA, where each “T” of the DNA sequence is substituted with “U.”

An open reading frame (ORF) is a continuous stretch of DNA or RNA beginning with a start codon (*e.g.*, methionine (ATG or AUG)) and ending with a stop codon (*e.g.*, TAA, TAG or TGA, or UAA, UAG or UGA). An ORF typically encodes a protein. It will be understood that the sequences disclosed herein may further comprise additional elements, *e.g.*, 5′ and 3′ UTRs, but that those elements, unlike the ORF, need not necessarily be present in an mRNA of the present disclosure.

In some embodiments, the ORF comprises a nucleotide sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% identity to the sequence of SEQ ID NO: 7. In some embodiments, the ORF comprises a nucleotide sequence having at least 80% identity to the sequence of SEQ ID NO: 7. In some embodiments, the ORF comprises a nucleotide sequence having at least 85% identity to the sequence of SEQ ID NO: 7. In some embodiments, the ORF comprises a nucleotide sequence having at least 90% identity to the sequence of SEQ ID NO: 7. In some embodiments, the ORF comprises a nucleotide sequence having at least 95% identity to the sequence of SEQ ID NO: 7. In some embodiments, the ORF comprises the nucleotide sequence of SEQ ID NO: 7.

In some embodiments, the mRNA comprises a nucleotide sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% identity to the sequence of SEQ ID NO: 6. In some embodiments, the mRNA comprises a nucleotide sequence having at least 80% identity to the sequence of SEQ ID NO: 6. In some embodiments, the mRNA comprises a nucleotide sequence having at least 85% identity to the sequence of SEQ ID NO: 6. In some embodiments, the mRNA comprises a nucleotide sequence having at least 90% identity to the sequence of SEQ ID NO: 6. In some embodiments, the mRNA comprises a nucleotide sequence having at least 95% identity to the sequence of SEQ ID NO: 6. In some embodiments, the mRNA comprises the nucleotide sequence of SEQ ID NO: 6.

Variants

In some embodiments, the compositions of the present disclosure include mRNA that encodes a SARS-CoV-2 antigen variant. Antigen variants or other polypeptide variants refers to molecules that differ in their amino acid sequence from a wild-type, native, or reference sequence. The antigen/polypeptide variants may possess substitutions, deletions, and/or insertions at certain positions within the amino acid sequence, as compared to a native or reference sequence. Ordinarily, variants possess at least 50% identity to a wild-type, native or reference sequence. In some embodiments, variants share at least 80%, or at least 90% identity with a wild-type, native, or reference sequence.

Variant antigens/polypeptides encoded by nucleic acids of the disclosure may contain amino acid changes that confer any of a number of desirable properties, *e.g.*, that enhance their immunogenicity, enhance their expression, and/or improve their stability or PK/PD properties in a subject. Variant antigens/polypeptides can be made using routine mutagenesis techniques and assayed as appropriate to determine whether they possess the desired property. Assays to determine expression levels and immunogenicity are well known in the art and exemplary such assays are set forth in the Examples section. Similarly, PK/PD properties of a protein variant can be measured using art recognized techniques, *e.g.*, by determining expression of antigens in a vaccinated subject over time and/or by looking at the durability of the induced immune response. The stability of protein(s) encoded by a variant nucleic acid may be measured by assaying thermal stability or stability upon urea denaturation or may be measured using in silico prediction. Methods for such experiments and in silico determinations are known in the art.

In some embodiments, a composition comprises an mRNA or an mRNA ORF that comprises a nucleotide sequence of any one of the sequences provided herein (*see, e.g.*, Table 3), or comprises a nucleotide sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a nucleotide sequence of any one of the sequences provided herein.

The term “identity” refers to a relationship between the sequences of two or more polypeptides (*e.g.* antigens) or polynucleotides (nucleic acids), as determined by comparing the sequences. Identity also refers to the degree of sequence relatedness between or among sequences as determined by the number of matches between strings of two or more amino acid residues or nucleic acid residues. Identity measures the percent of identical matches between the smaller of two or more sequences with gap alignments (if any) addressed by a particular mathematical model or computer program (*e.g.*, “algorithms”). Identity of related antigens or nucleic acids can be readily calculated by known methods. “Percent (%) identity” as it applies to

polypeptide or polynucleotide sequences is defined as the percentage of residues (amino acid residues or nucleic acid residues) in the candidate amino acid or nucleic acid sequence that are identical with the residues in the amino acid sequence or nucleic acid sequence of a second sequence after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent identity. Methods and computer programs for the alignment are well known in the art. It is understood that identity depends on a calculation of percent identity but may differ in value due to gaps and penalties introduced in the calculation. Generally, variants of a particular polynucleotide or polypeptide (*e.g.*, antigen) have at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% but less than 100% sequence identity to that particular reference polynucleotide or polypeptide as determined by sequence alignment programs and parameters described herein and known to those skilled in the art. Such tools for alignment include those of the BLAST suite (Stephen F. Altschul, et al (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", *Nucleic Acids Res.* 25:3389-3402). Another popular local alignment technique is based on the Smith-Waterman algorithm (Smith, T.F. & Waterman, M.S. (1981) "Identification of common molecular subsequences." *J. Mol. Biol.* 147:195-197). A general global alignment technique based on dynamic programming is the Needleman-Wunsch algorithm (Needleman, S.B. & Wunsch, C.D. (1970) "A general method applicable to the search for similarities in the amino acid sequences of two proteins." *J. Mol. Biol.* 48:443-453). More recently a Fast Optimal Global Sequence Alignment Algorithm (FOGSAA) has been developed that purportedly produces global alignment of nucleotide and protein sequences faster than other optimal global alignment methods, including the Needleman-Wunsch algorithm.

As such, polynucleotides encoding peptides or polypeptides containing substitutions, insertions and/or additions, deletions and covalent modifications with respect to reference sequences, in particular the polypeptide (*e.g.*, antigen) sequences disclosed herein, are included within the scope of this disclosure. For example, sequence tags or amino acids, such as one or more lysines, can be added to peptide sequences (*e.g.*, at the N-terminal or C-terminal ends). Sequence tags can be used for peptide detection, purification or localization. Lysines can be used to increase peptide solubility or to allow for biotinylation. Alternatively, amino acid residues located at the carboxy and amino terminal regions of the amino acid sequence of a peptide or protein may optionally be deleted providing for truncated sequences. Certain amino acids (*e.g.*, C-terminal or N-terminal residues) may alternatively be deleted depending on the use of the sequence, as for example, expression of the sequence as part of a larger sequence which is soluble or linked to a solid support. In some embodiments, sequences for (or encoding) signal

sequences, termination sequences, transmembrane domains, linkers, multimerization domains (such as, *e.g.*, foldon regions) and the like may be substituted with alternative sequences that achieve the same or a similar function. In some embodiments, cavities in the core of proteins can be filled to improve stability, *e.g.*, by introducing larger amino acids. In other embodiments, buried hydrogen bond networks may be replaced with hydrophobic residues to improve stability. In yet other embodiments, glycosylation sites may be removed and replaced with appropriate residues. Such sequences are readily identifiable to one of skill in the art. It should also be understood that some of the sequences provided herein contain sequence tags or terminal peptide sequences (*e.g.*, at the N-terminal or C-terminal ends) that may be deleted, for example, prior to use in the preparation of an mRNA vaccine composition.

As recognized by those skilled in the art, protein fragments, functional protein domains, and homologous proteins are also considered to be within the scope of coronavirus antigens of interest. For example, provided herein is any protein fragment (meaning a polypeptide sequence at least one amino acid residue shorter than a reference antigen sequence but otherwise identical) of a reference protein, provided that the fragment is immunogenic and confers a protective immune response to the coronavirus. In addition to variants that are identical to the reference protein but are truncated or mutated, in some embodiments, an antigen includes 2, 3, 4, 5, 6, 7, 8, 9, 10, or more mutations, as shown in any of the sequences provided or referenced herein. Antigens/antigenic polypeptides can range in length from about 4, 6, or 8 amino acids to protein domains such as spike protein N-terminal domain (NTD) and receptor binding domain (RBD) and full length mature spike (S) proteins.

Stabilizing Elements

Naturally-occurring eukaryotic mRNA molecules can contain stabilizing elements, including, but not limited to untranslated regions (UTR) at their 5'-end (5' UTR) and/or at their 3'-end (3' UTR), in addition to other structural features, such as a 5'-cap structure or a 3'-poly(A) tail. Both the 5' UTR and the 3' UTR are typically transcribed from the genomic DNA and are elements of the premature mRNA. Characteristic structural features of mature mRNA, such as the 5'-cap and the 3'-poly(A) tail are usually added to the transcribed (premature) mRNA during mRNA processing.

In some embodiments, a composition includes an mRNA having an open reading frame encoding at least one antigenic polypeptide having at least one modification, at least one 5' terminal cap, and is formulated within a lipid nanoparticle. 5'-capping of polynucleotides may be completed concomitantly during the *in vitro*-transcription reaction using the following chemical RNA cap analogs to generate the 5'-guanosine cap structure according to manufacturer protocols:

3'-O-Me-m7G(5')ppp(5') G [the ARCA cap]; G(5')ppp(5')A; G(5')ppp(5')G; m7G(5')ppp(5')A; m7G(5')ppp(5')G (New England BioLabs, Ipswich, MA). 5'-capping of modified RNA may be completed post-transcriptionally using a Vaccinia Virus Capping Enzyme to generate the "Cap 0" structure: m7G(5')ppp(5')G (New England BioLabs, Ipswich, MA). Cap 1 structure may be generated using both Vaccinia Virus Capping Enzyme and a 2'-O methyl-transferase to generate: m7G(5')ppp(5')G-2'-O-methyl. Cap 2 structure may be generated from the Cap 1 structure followed by the 2'-O-methylation of the 5'-antepenultimate nucleotide using a 2'-O methyl-transferase. Cap 3 structure may be generated from the Cap 2 structure followed by the 2'-O-methylation of the 5'-preantepenultimate nucleotide using a 2'-O methyl-transferase. Enzymes may be derived from a recombinant source.

The 3'-poly(A) tail is typically a stretch of adenine nucleotides added to the 3'-end of the transcribed mRNA. It can, in some instances, comprise up to about 400 adenine nucleotides. In some embodiments, the length of the 3'-poly(A) tail may be an essential element with respect to the stability of the individual mRNA.

In some embodiments, an mRNA includes a stabilizing element. Stabilizing elements may include for instance a histone stem-loop. A stem-loop binding protein (SLBP), a 32 kDa protein has been identified. It is associated with the histone stem-loop at the 3'-end of the histone messages in both the nucleus and the cytoplasm. Its expression level is regulated by the cell cycle; it peaks during the S-phase, when histone mRNA levels are also elevated. The protein has been shown to be essential for efficient 3'-end processing of histone pre-mRNA by the U7 snRNP. SLBP continues to be associated with the stem-loop after processing, and then stimulates the translation of mature histone mRNAs into histone proteins in the cytoplasm. The RNA binding domain of SLBP is conserved through metazoa and protozoa; its binding to the histone stem-loop depends on the structure of the loop. The minimum binding site includes at least three nucleotides 5' and two nucleotides 3' relative to the stem-loop.

In some embodiments, an mRNA includes a coding region, at least one histone stem-loop, and optionally, a poly(A) sequence or polyadenylation signal. The poly(A) sequence or polyadenylation signal generally should enhance the expression level of the encoded protein. The encoded protein, in some embodiments, is not a histone protein, a reporter protein (*e.g.* Luciferase, GFP, EGFP, β -Galactosidase, EGFP), or a marker or selection protein (*e.g.* alpha-Globin, Galactokinase and Xanthine:guanine phosphoribosyl transferase (GPT)).

In some embodiments, an mRNA includes the combination of a poly(A) sequence or polyadenylation signal and at least one histone stem-loop, even though both represent alternative mechanisms in nature, acts synergistically to increase the protein expression beyond the level

observed with either of the individual elements. The synergistic effect of the combination of poly(A) and at least one histone stem-loop does not depend on the order of the elements or the length of the poly(A) sequence.

In some embodiments, an mRNA does not include a histone downstream element (HDE).

- 5 “Histone downstream element” (HDE) includes a purine-rich polynucleotide stretch of approximately 15 to 20 nucleotides 3' of naturally occurring stem-loops, representing the binding site for the U7 snRNA, which is involved in processing of histone pre-mRNA into mature histone mRNA. In some embodiments, the nucleic acid does not include an intron.

- 10 An mRNA may or may not contain an enhancer and/or promoter sequence, which may be modified or unmodified or which may be activated or inactivated. In some embodiments, the histone stem-loop is generally derived from histone genes and includes an intramolecular base pairing of two neighbored partially or entirely reverse complementary sequences separated by a spacer, consisting of a short sequence, which forms the loop of the structure. The unpaired loop region is typically unable to base pair with either of the stem loop elements. It occurs more often
15 in RNA, as is a key component of many RNA secondary structures but may be present in single-stranded DNA as well. Stability of the stem-loop structure generally depends on the length, number of mismatches or bulges, and base composition of the paired region. In some embodiments, wobble base pairing (non-Watson-Crick base pairing) may result. In some embodiments, the at least one histone stem-loop sequence comprises a length of 15 to 45
20 nucleotides.

In some embodiments, an mRNA has one or more AU-rich sequences removed. These sequences, sometimes referred to as AURES are destabilizing sequences found in the 3'UTR. The AURES may be removed from the RNA vaccines. Alternatively, the AURES may remain in an mRNA vaccine composition.

- 25 In some embodiments, an mRNA does not include a stabilizing element.

Signal Peptides

- 30 In some embodiments, a composition comprises an mRNA having an ORF that encodes a signal peptide fused to the coronavirus antigen. Signal peptides, comprising the N-terminal 15-60 amino acids of proteins, are typically needed for the translocation across the membrane on the secretory pathway and, thus, universally control the entry of most proteins both in eukaryotes and prokaryotes to the secretory pathway. In eukaryotes, the signal peptide of a nascent precursor protein (pre-protein) directs the ribosome to the rough endoplasmic reticulum (ER) membrane and initiates the transport of the growing peptide chain across it for processing. ER processing
35 produces mature proteins, wherein the signal peptide is cleaved from precursor proteins,

typically by a ER-resident signal peptidase of the host cell, or they remain uncleaved and function as a membrane anchor. A signal peptide may also facilitate the targeting of the protein to the cell membrane.

A signal peptide may have a length of 15-60 amino acids. For example, a signal peptide may have a length of 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60 amino acids. In some embodiments, a signal peptide has a length of 20-60, 25-60, 30-60, 35-60, 40-60, 45-60, 50-60, 55-60, 15-55, 20-55, 25-55, 30-55, 35-55, 40-55, 45-55, 50-55, 15-50, 20-50, 25-50, 30-50, 35-50, 40-50, 45-50, 15-45, 20-45, 25-45, 30-45, 35-45, 40-45, 15-40, 20-40, 25-40, 30-40, 35-40, 15-35, 20-35, 25-35, 30-35, 15-30, 20-30, 25-30, 15-25, 20-25, or 15-20 amino acids.

Signal peptides from heterologous genes (which regulate expression of genes other than coronavirus antigens in nature) are known in the art and can be tested for desired properties and then incorporated into a nucleic acid of the disclosure.

Fusion Proteins

In some embodiments, a composition of the present disclosure includes an mRNA encoding an antigenic fusion protein. Thus, the encoded antigen or antigens may include two or more proteins (*e.g.*, protein and/or protein fragment) joined together. Alternatively, the protein to which a protein antigen is fused does not promote a strong immune response to itself, but rather to the coronavirus antigen. Antigenic fusion proteins, in some embodiments, retain the functional property from each original protein.

Scaffold Moieties

The vaccine compositions as provided herein, in some embodiments, encode fusion proteins that comprise coronavirus antigens linked to scaffold moieties. In some embodiments, such scaffold moieties impart desired properties to an antigen encoded by a nucleic acid of the disclosure. For example scaffold proteins may improve the immunogenicity of an antigen, *e.g.*, by altering the structure of the antigen, altering the uptake and processing of the antigen, and/or causing the antigen to bind to a binding partner.

In some embodiments, the scaffold moiety is protein that can self-assemble into protein nanoparticles that are highly symmetric, stable, and structurally organized, with diameters of 10–150 nm, a highly suitable size range for optimal interactions with various cells of the immune system. In some embodiments, viral proteins or virus-like particles can be used to form stable nanoparticle structures. Examples of such viral proteins are known in the art. For example, in

some embodiments, the scaffold moiety is a hepatitis B surface antigen (HBsAg). HBsAg forms spherical particles with an average diameter of ~22 nm and which lacked nucleic acid and hence are non-infectious (Lopez-Sagasetta, J. et al. Computational and Structural Biotechnology Journal 14 (2016) 58–68). In some embodiments, the scaffold moiety is a hepatitis B core antigen (HBcAg) self-assembles into particles of 24–31 nm diameter, which resembled the viral cores obtained from HBV-infected human liver. HBcAg produced in self-assembles into two classes of differently sized nanoparticles of 300 Å and 360 Å diameter, corresponding to 180 or 240 protomers. In some embodiments, the coronavirus antigen is fused to HBsAg or HBcAg to facilitate self-assembly of nanoparticles displaying the coronavirus antigen.

In some embodiments, bacterial protein platforms may be used. Non-limiting examples of these self-assembling proteins include ferritin, lumazine and encapsulin.

Ferritin is a protein whose main function is intracellular iron storage. Ferritin is made of 24 subunits, each composed of a four-alpha-helix bundle, that self-assemble in a quaternary structure with octahedral symmetry (Cho K.J. et al. *J Mol Biol.* 2009;390:83–98). Several high-resolution structures of ferritin have been determined, confirming that *Helicobacter pylori* ferritin is made of 24 identical protomers, whereas in animals, there are ferritin light and heavy chains that can assemble alone or combine with different ratios into particles of 24 subunits (Granier T. et al. *J Biol Inorg Chem.* 2003;8:105–111; Lawson D.M. et al. *Nature.* 1991;349:541–544). Ferritin self-assembles into nanoparticles with robust thermal and chemical stability. Thus, the ferritin nanoparticle is well-suited to carry and expose antigens.

Lumazine synthase (LS) is also well-suited as a nanoparticle platform for antigen display. LS, which is responsible for the penultimate catalytic step in the biosynthesis of riboflavin, is an enzyme present in a broad variety of organisms, including archaea, bacteria, fungi, plants, and eubacteria (Weber S.E. *Flavins and Flavoproteins. Methods and Protocols, Series: Methods in Molecular Biology.* 2014). The LS monomer is 150 amino acids long, and consists of beta-sheets along with tandem alpha-helices flanking its sides. A number of different quaternary structures have been reported for LS, illustrating its morphological versatility: from homopentamers up to symmetrical assemblies of 12 pentamers forming capsids of 150 Å diameter. Even LS cages of more than 100 subunits have been described (Zhang X. et al. *J Mol Biol.* 2006;362:753–770).

Encapsulin, a novel protein cage nanoparticle isolated from thermophile *Thermotoga maritima*, may also be used as a platform to present antigens on the surface of self-assembling nanoparticles. Encapsulin is assembled from 60 copies of identical 31 kDa monomers having a thin and icosahedral T = 1 symmetric cage structure with interior and exterior diameters of 20 and 24 nm, respectively (Sutter M. et al. *Nat Struct Mol Biol.* 2008, 15: 939-947). Although the

exact function of encapsulin in *T. maritima* is not clearly understood yet, its crystal structure has been recently solved and its function was postulated as a cellular compartment that encapsulates proteins such as DyP (Dye decolorizing peroxidase) and Flp (Ferritin like protein), which are involved in oxidative stress responses (Rahmanpour R. et al. FEBS J. 2013, 280: 2097-2104).

5 In some embodiments, an RNA of the present disclosure encodes a coronavirus antigen (*e.g.*, SARS-CoV-2 S protein) fused to a foldon domain. The foldon domain may be, for example, obtained from bacteriophage T4 fibritin (*see, e.g.*, Tao Y, *et al. Structure*. 1997 Jun 15; 5(6):789-98).

10 **Linkers and Cleavable Peptides**

In some embodiments, the mRNAs of the disclosure encode more than one polypeptide, referred to herein as fusion proteins. In some embodiments, the mRNA further encodes a linker located between at least one or each domain of the fusion protein. The linker can be, for example, a cleavable linker or protease-sensitive linker. In some embodiments, the linker is
15 selected from the group consisting of F2A linker, P2A linker, T2A linker, E2A linker, and combinations thereof. This family of self-cleaving peptide linkers, referred to as 2A peptides, has been described in the art (see for example, Kim, J.H. et al. (2011) *PLoS ONE* 6:e18556). In some embodiments, the linker is an F2A linker. In some embodiments, the linker is a GGGS linker. In some embodiments, the fusion protein contains three domains with intervening linkers, having
20 the structure: domain-linker-domain-linker-domain.

Cleavable linkers known in the art may be used in connection with the disclosure. Exemplary such linkers include: F2A linkers, T2A linkers, P2A linkers, E2A linkers (See, *e.g.*, WO2017/127750). The skilled artisan will appreciate that other art-recognized linkers may be suitable for use in the constructs of the disclosure (*e.g.*, encoded by the nucleic acids of the
25 disclosure). The skilled artisan will likewise appreciate that other polycistronic constructs (mRNA encoding more than one antigen/polypeptide separately within the same molecule) may be suitable for use as provided herein.

Sequence Optimization

30 In some embodiments, an ORF encoding an antigen of the disclosure is codon optimized. Codon optimization methods are known in the art. For example, an ORF of any one or more of the sequences provided herein may be codon optimized. Codon optimization, in some embodiments, may be used to match codon frequencies in target and host organisms to ensure proper folding; bias GC content to increase mRNA stability or reduce secondary structures;
35 minimize tandem repeat codons or base runs that may impair gene construction or expression;

customize transcriptional and translational control regions; insert or remove protein trafficking sequences; remove/add post translation modification sites in encoded protein (*e.g.*, glycosylation sites); add, remove or shuffle protein domains; insert or delete restriction sites; modify ribosome binding sites and mRNA degradation sites; adjust translational rates to allow the various domains of the protein to fold properly; or reduce or eliminate problem secondary structures within the polynucleotide. Codon optimization tools, algorithms and services are known in the art – non-limiting examples include services from GeneArt (Life Technologies), DNA2.0 (Menlo Park CA) and/or proprietary methods. In some embodiments, the open reading frame (ORF) sequence is optimized using optimization algorithms.

In some embodiments, a codon optimized sequence shares less than 95% sequence identity to a naturally-occurring or wild-type sequence ORF (*e.g.*, a naturally-occurring or wild-type mRNA sequence encoding a coronavirus antigen). In some embodiments, a codon optimized sequence shares less than 90% sequence identity to a naturally-occurring or wild-type sequence (*e.g.*, a naturally-occurring or wild-type mRNA sequence encoding a coronavirus antigen). In some embodiments, a codon optimized sequence shares less than 85% sequence identity to a naturally-occurring or wild-type sequence (*e.g.*, a naturally-occurring or wild-type mRNA sequence encoding a coronavirus antigen). In some embodiments, a codon optimized sequence shares less than 80% sequence identity to a naturally-occurring or wild-type sequence (*e.g.*, a naturally-occurring or wild-type mRNA sequence encoding a coronavirus antigen). In some embodiments, a codon optimized sequence shares less than 75% sequence identity to a naturally-occurring or wild-type sequence (*e.g.*, a naturally-occurring or wild-type mRNA sequence encoding a coronavirus antigen).

In some embodiments, a codon optimized sequence shares between 65% and 85% (*e.g.*, between about 67% and about 85% or between about 67% and about 80%) sequence identity to a naturally-occurring or wild-type sequence (*e.g.*, a naturally-occurring or wild-type mRNA sequence encoding a coronavirus antigen). In some embodiments, a codon optimized sequence shares between 65% and 75% or about 80% sequence identity to a naturally-occurring or wild-type sequence (*e.g.*, a naturally-occurring or wild-type mRNA sequence encoding a coronavirus antigen).

In some embodiments, a codon-optimized sequence encodes an antigen that is as immunogenic as, or more immunogenic than (*e.g.*, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 100%, or at least 200% more), than a coronavirus antigen encoded by a non-codon-optimized sequence.

When transfected into mammalian host cells, the modified mRNAs have a stability of between 12-18 hours, or greater than 18 hours, *e.g.*, 24, 36, 48, 60, 72, or greater than 72 hours and are capable of being expressed by the mammalian host cells.

In some embodiments, a codon optimized mRNA may be one in which the levels of G/C are enhanced. The G/C-content of nucleic acid molecules (*e.g.*, mRNA) may influence the stability of the mRNA. mRNA having an increased amount of guanine (G) and/or cytosine (C) residues may be functionally more stable than mRNA containing a large amount of adenine (A) and thymine (T) or uracil (U) nucleotides. As an example, WO02/098443 discloses a pharmaceutical composition containing an mRNA stabilized by sequence modifications in the translated region. Due to the degeneracy of the genetic code, the modifications work by substituting existing codons for those that promote greater mRNA stability without changing the resulting amino acid. The approach is limited to coding regions of the RNA.

Chemically Unmodified Nucleotides

In some embodiments, an mRNA is not chemically modified and comprises the standard ribonucleotides consisting of adenosine, guanosine, cytosine and uridine. In some embodiments, nucleotides and nucleosides of the present disclosure comprise standard nucleoside residues such as those present in transcribed mRNA (*e.g.* A, G, C, or U). In some embodiments, nucleotides and nucleosides of the present disclosure comprise standard deoxyribonucleosides such as those present in DNA (*e.g.* dA, dG, dC, or dT).

Chemical Modifications

The compositions of the present disclosure comprise, in some embodiments, an mRNA having an open reading frame encoding a coronavirus antigen, wherein the nucleic acid comprises nucleotides and/or nucleosides that can be standard (unmodified) or modified as is known in the art. In some embodiments, nucleotides and nucleosides of the present disclosure comprise modified nucleotides or nucleosides. Such modified nucleotides and nucleosides can be naturally-occurring modified nucleotides and nucleosides or non-naturally occurring modified nucleotides and nucleosides. Such modifications can include those at the sugar, backbone, or nucleobase portion of the nucleotide and/or nucleoside as are recognized in the art.

In some embodiments, a naturally-occurring modified nucleotide or nucleoside of the disclosure is one as is generally known or recognized in the art. Non-limiting examples of such naturally occurring modified nucleotides and nucleosides can be found, *inter alia*, in the widely recognized MODOMICS database.

In some embodiments, a non-naturally occurring modified nucleotide or nucleoside of the disclosure is one as is generally known or recognized in the art. Non-limiting examples of such non-naturally occurring modified nucleotides and nucleosides can be found, inter alia, in published US application Nos. PCT/US2012/058519; PCT/US2013/075177;

5 PCT/US2014/058897; PCT/US2014/058891; PCT/US2014/070413; PCT/US2015/36773; PCT/US2015/36759; PCT/US2015/36771; or PCT/IB2017/051367 all of which are incorporated by reference herein.

Hence, nucleic acids of the disclosure (*e.g.*, DNA and RNA, such as mRNA) can comprise standard nucleotides and nucleosides, naturally-occurring nucleotides and nucleosides, 10 non-naturally-occurring nucleotides and nucleosides, or any combination thereof.

Nucleic acids of the disclosure, in some embodiments, comprise various (more than one) different types of standard and/or modified nucleotides and nucleosides. In some embodiments, a particular region of a nucleic acid contains one, two or more (optionally different) types of standard and/or modified nucleotides and nucleosides.

15 In some embodiments, a modified mRNA introduced to a cell or organism, exhibits reduced degradation in the cell or organism, respectively, relative to an unmodified nucleic acid comprising standard nucleotides and nucleosides.

In some embodiments, a modified mRNA nucleic acid introduced into a cell or organism, may exhibit reduced immunogenicity in the cell or organism, respectively (*e.g.*, a reduced innate 20 response) relative to an unmodified nucleic acid comprising standard nucleotides and nucleosides.

Nucleic acids, in some embodiments, comprise non-natural modified nucleotides that are introduced during synthesis or post-synthesis of the nucleic acids to achieve desired functions or properties. The modifications may be present on internucleotide linkages, purine or pyrimidine 25 bases, or sugars. The modification may be introduced with chemical synthesis or with a polymerase enzyme at the terminal of a chain or anywhere else in the chain. Any of the regions of a nucleic acid may be chemically modified.

The present disclosure provides for modified nucleosides and nucleotides of a nucleic acid. A “nucleoside” refers to a compound containing a sugar molecule (*e.g.*, a pentose or ribose) 30 or a derivative thereof in combination with an organic base (*e.g.*, a purine or pyrimidine) or a derivative thereof (also referred to herein as “nucleobase”). A “nucleotide” refers to a nucleoside, including a phosphate group. Modified nucleotides may be synthesized by any useful method, such as, for example, chemically, enzymatically, or recombinantly, to include one or more modified or non-natural nucleosides. Nucleic acids can comprise a region or regions of

linked nucleosides. Such regions may have variable backbone linkages. The linkages can be standard phosphodiester linkages, in which case the nucleic acids would comprise regions of nucleotides.

Modified nucleotide base pairing encompasses not only the standard adenosine-thymine, adenosine-uracil, or guanosine-cytosine base pairs, but also base pairs formed between nucleotides and/or modified nucleotides comprising non-standard or modified bases, wherein the arrangement of hydrogen bond donors and hydrogen bond acceptors permits hydrogen bonding between a non-standard base and a standard base or between two complementary non-standard base structures, such as, for example, in those nucleic acids having at least one chemical modification. One example of such non-standard base pairing is the base pairing between the modified nucleotide inosine and adenine, cytosine or uracil. Any combination of base/sugar or linker may be incorporated into nucleic acids of the present disclosure.

In some embodiments, modified nucleobases in nucleic acids (*e.g.*, mRNA) comprise 1-methyl-pseudouridine (m1 ψ), 1-ethyl-pseudouridine (e1 ψ), 5-methoxy-uridine (mo5U), 5-methyl-cytidine (m5C), and/or pseudouridine (ψ). In some embodiments, modified nucleobases in nucleic acids (*e.g.*, RNA nucleic acids, such as mRNA nucleic acids) comprise 5-methoxymethyl uridine, 5-methylthio uridine, 1-methoxymethyl pseudouridine, 5-methyl cytidine, and/or 5-methoxy cytidine. In some embodiments, the polyribonucleotide includes a combination of at least two (*e.g.*, 2, 3, 4 or more) of any of the aforementioned modified nucleobases, including but not limited to chemical modifications.

In some embodiments, an mRNA of the disclosure comprises 1-methyl-pseudouridine (m1 ψ) substitutions at one or more or all uridine positions of the nucleic acid.

In some embodiments, an mRNA of the disclosure comprises 1-methyl-pseudouridine (m1 ψ) substitutions at one or more or all uridine positions of the nucleic acid and 5-methyl cytidine substitutions at one or more or all cytidine positions of the nucleic acid.

In some embodiments, an mRNA of the disclosure comprises pseudouridine (ψ) substitutions at one or more or all uridine positions of the nucleic acid.

In some embodiments, an mRNA of the disclosure comprises pseudouridine (ψ) substitutions at one or more or all uridine positions of the nucleic acid and 5-methyl cytidine substitutions at one or more or all cytidine positions of the nucleic acid.

In some embodiments, an mRNA of the disclosure comprises uridine at one or more or all uridine positions of the nucleic acid.

In some embodiments, mRNAs are uniformly modified (*e.g.*, fully modified, modified throughout the entire sequence) for a particular modification. For example, a nucleic acid can be

uniformly modified with 1-methyl-pseudouridine, meaning that all uridine residues in the mRNA sequence are replaced with 1-methyl-pseudouridine. Similarly, a nucleic acid can be uniformly modified for any type of nucleoside residue present in the sequence by replacement with a modified residue such as those set forth above.

5 The nucleic acids of the present disclosure may be partially or fully modified along the entire length of the molecule. For example, one or more or all or a given type of nucleotide (*e.g.*, purine or pyrimidine, or any one or more or all of A, G, U, C) may be uniformly modified in a nucleic acid of the disclosure, or in a predetermined sequence region thereof (*e.g.*, in the mRNA including or excluding the poly(A) tail). In some embodiments, all nucleotides X in a nucleic
10 acid of the present disclosure (or in a sequence region thereof) are modified nucleotides, wherein X may be any one of nucleotides A, G, U, C, or any one of the combinations A+G, A+U, A+C, G+U, G+C, U+C, A+G+U, A+G+C, G+U+C or A+G+C.

 The nucleic acid may contain from about 1% to about 100% modified nucleotides (either in relation to overall nucleotide content, or in relation to one or more types of nucleotide, *i.e.*,
15 any one or more of A, G, U or C) or any intervening percentage (*e.g.*, from 1% to 20%, from 1% to 25%, from 1% to 50%, from 1% to 60%, from 1% to 70%, from 1% to 80%, from 1% to 90%, from 1% to 95%, from 10% to 20%, from 10% to 25%, from 10% to 50%, from 10% to 60%, from 10% to 70%, from 10% to 80%, from 10% to 90%, from 10% to 95%, from 10% to 100%, from 20% to 25%, from 20% to 50%, from 20% to 60%, from 20% to 70%, from 20% to 80%,
20 from 20% to 90%, from 20% to 95%, from 20% to 100%, from 50% to 60%, from 50% to 70%, from 50% to 80%, from 50% to 90%, from 50% to 95%, from 50% to 100%, from 70% to 80%, from 70% to 90%, from 70% to 95%, from 70% to 100%, from 80% to 90%, from 80% to 95%, from 80% to 100%, from 90% to 95%, from 90% to 100%, and from 95% to 100%). It will be understood that any remaining percentage is accounted for by the presence of unmodified A, G,
25 U, or C.

 The mRNAs may contain at a minimum 1% and at maximum 100% modified nucleotides, or any intervening percentage, such as at least 5% modified nucleotides, at least 10% modified nucleotides, at least 25% modified nucleotides, at least 50% modified nucleotides, at least 80% modified nucleotides, or at least 90% modified nucleotides. For example, the
30 nucleic acids may contain a modified pyrimidine such as a modified uracil or cytosine. In some embodiments, at least 5%, at least 10%, at least 25%, at least 50%, at least 80%, at least 90% or 100% of the uracil in the nucleic acid is replaced with a modified uracil (*e.g.*, a 5-substituted uracil). The modified uracil can be replaced by a compound having a single unique structure, or can be replaced by a plurality of compounds having different structures (*e.g.*, 2, 3, 4 or more

unique structures). In some embodiments, at least 5%, at least 10%, at least 25%, at least 50%, at least 80%, at least 90% or 100% of the cytosine in the nucleic acid is replaced with a modified cytosine (*e.g.*, a 5-substituted cytosine). The modified cytosine can be replaced by a compound having a single unique structure, or can be replaced by a plurality of compounds having different structures (*e.g.*, 2, 3, 4 or more unique structures).

Untranslated Regions (UTRs)

The mRNAs of the present disclosure may comprise one or more regions or parts which act or function as an untranslated region. Where mRNAs are designed to encode at least one antigen of interest, the nucleic may comprise one or more of these untranslated regions (UTRs). Wild-type untranslated regions of a nucleic acid are transcribed but not translated. In mRNA, the 5' UTR starts at the transcription start site and continues to the start codon but does not include the start codon; whereas, the 3' UTR starts immediately following the stop codon and continues until the transcriptional termination signal. There is growing body of evidence about the regulatory roles played by the UTRs in terms of stability of the nucleic acid molecule and translation. The regulatory features of a UTR can be incorporated into the polynucleotides of the present disclosure to, among other things, enhance the stability of the molecule. The specific features can also be incorporated to ensure controlled down-regulation of the transcript in case they are misdirected to undesired organs sites. A variety of 5'UTR and 3'UTR sequences are known and available in the art.

A 5' UTR is region of an mRNA that is directly upstream (5') from the start codon (the first codon of an mRNA transcript translated by a ribosome). A 5' UTR does not encode a protein (is non-coding). Natural 5'UTRs have features that play roles in translation initiation. They harbor signatures like Kozak sequences which are commonly known to be involved in the process by which the ribosome initiates translation of many genes. Kozak sequences have the consensus CCR(A/G)CCAUGG (SEQ ID NO: 9), where R is a purine (adenine or guanine) three bases upstream of the start codon (AUG), which is followed by another 'G'. 5'UTRs also have been known to form secondary structures which are involved in elongation factor binding.

In some embodiments of the disclosure, a 5' UTR is a heterologous UTR, *i.e.*, is a UTR found in nature associated with a different ORF. In another embodiment, a 5' UTR is a synthetic UTR, *i.e.*, does not occur in nature. Synthetic UTRs include UTRs that have been mutated to improve their properties, *e.g.*, which increase gene expression as well as those which are completely synthetic. Exemplary 5' UTRs include *Xenopus* or human derived α -globin or β -globin (US8278063; US9012219), human cytochrome b-245 a polypeptide, and hydroxysteroid (17b) dehydrogenase, and Tobacco etch virus (US8278063, US9012219). CMV immediate-early

1 (IE1) gene (US2014/0206753, WO2013/185069), the sequence GGGAUCCUACC (SEQ ID NO: 10) (WO2014/144196) may also be used. In another embodiment, 5' UTR of a TOP gene is a 5' UTR of a TOP gene lacking the 5' TOP motif (the oligopyrimidine tract) (*e.g.*, WO2015/101414, WO2015/101415, WO2015/062738, WO2015/024667, WO2015/024667; 5' UTR element derived from ribosomal protein Large 32 (L32) gene (WO2015/101414, WO2015/101415, WO2015/062738), 5' UTR element derived from the 5'UTR of an hydroxysteroid (17- β) dehydrogenase 4 gene (HSD17B4) (WO2015/024667), or a 5' UTR element derived from the 5' UTR of ATP5A1 (WO2015/024667) can be used. In some embodiments, an internal ribosome entry site (IRES) is used instead of a 5' UTR.

10 In some embodiments, a 5' UTR of the present disclosure comprises the sequence of SEQ ID NO: 2.

A 3' UTR is region of an mRNA that is directly downstream (3') from the stop codon (the codon of an mRNA transcript that signals a termination of translation). A 3' UTR does not encode a protein (is non-coding). Natural or wild type 3' UTRs are known to have stretches of adenosines and uridines embedded in them. These AU rich signatures are particularly prevalent in genes with high rates of turnover. Based on their sequence features and functional properties, the AU rich elements (AREs) can be separated into three classes (Chen et al, 1995): Class I AREs contain several dispersed copies of an AUUUA motif within U-rich regions. C-Myc and MyoD contain class I AREs. Class II AREs possess two or more overlapping UUAUUUA(U/A)(U/A) (SEQ ID NO: 11) nonamers. Molecules containing this type of AREs include GM-CSF and TNF- α . Class III AREs are less well defined. These U rich regions do not contain an AUUUA motif. c-Jun and Myogenin are two well-studied examples of this class. Most proteins binding to the AREs are known to destabilize the messenger, whereas members of the ELAV family, most notably HuR, have been documented to increase the stability of mRNA. HuR binds to AREs of all the three classes. Engineering the HuR specific binding sites into the 3' UTR of nucleic acid molecules will lead to HuR binding and thus, stabilization of the message *in vivo*.

Introduction, removal or modification of 3' UTR AU rich elements (AREs) can be used to modulate the stability of nucleic acids (*e.g.*, RNA) of the disclosure. When engineering specific nucleic acids, one or more copies of an ARE can be introduced to make nucleic acids of the disclosure less stable and thereby curtail translation and decrease production of the resultant protein. Likewise, AREs can be identified and removed or mutated to increase the intracellular stability and thus increase translation and production of the resultant protein. Transfection experiments can be conducted in relevant cell lines, using nucleic acids of the disclosure and

protein production can be assayed at various time points post-transfection. For example, cells can be transfected with different ARE-engineering molecules and by using an ELISA kit to the relevant protein and assaying protein produced at 6 hour, 12 hour, 24 hour, 48 hour, and 7 days post-transfection.

3' UTRs may be heterologous or synthetic. With respect to 3' UTRs, globin UTRs, including *Xenopus* β -globin UTRs and human β -globin UTRs are known in the art (US8278063, US9012219, US2011/0086907). A modified β -globin construct with enhanced stability in some cell types by cloning two sequential human β -globin 3' UTRs head to tail has been developed and is well known in the art (US2012/0195936, WO2014/071963). In addition α 2-globin, α 1-globin, UTRs and mutants thereof are also known in the art (WO2015/101415, WO2015/024667). Other 3' UTRs described in the mRNA constructs in the non-patent literature include CYBA (Ferizi et al., 2015) and albumin (Thess et al., 2015). Other exemplary 3' UTRs include that of bovine or human growth hormone (wild type or modified) (WO2013/185069, US2014/0206753, WO2014/152774), rabbit β globin and hepatitis B virus (HBV), α -globin 3' UTR and Viral VEEV 3' UTR sequences are also known in the art. In some embodiments, the sequence UUUGAAUU (WO2014/144196) is used. In some embodiments, 3' UTRs of human and mouse ribosomal protein are used. Other examples include rps9 3'UTR (WO2015/101414), FIG4 (WO2015/101415), and human albumin 7 (WO2015/101415).

In some embodiments, a 3' UTR of the present disclosure comprises the sequence of SEQ ID NO: 4.

Those of ordinary skill in the art will understand that 5' UTRs that are heterologous or synthetic may be used with any desired 3' UTR sequence. For example, a heterologous 5' UTR may be used with a synthetic 3' UTR with a heterologous 3' UTR.

Non-UTR sequences may also be used as regions or subregions within a nucleic acid. For example, introns or portions of introns sequences may be incorporated into regions of nucleic acid of the disclosure. Incorporation of intronic sequences may increase protein production as well as nucleic acid levels.

Combinations of features may be included in flanking regions and may be contained within other features. For example, the ORF may be flanked by a 5' UTR which may contain a strong Kozak translational initiation signal and/or a 3' UTR which may include an oligo(dT) sequence for templated addition of a poly-A tail. 5' UTR may comprise a first polynucleotide fragment and a second polynucleotide fragment from the same and/or different genes such as the 5' UTRs described in US Patent Application Publication No. 2010/0293625 and PCT/US2014/069155, herein incorporated by reference in its entirety.

It should be understood that any UTR from any gene may be incorporated into the regions of a nucleic acid. Furthermore, multiple wild-type UTRs of any known gene may be utilized. It is also within the scope of the present disclosure to provide artificial UTRs which are not variants of wild type regions. These UTRs or portions thereof may be placed in the same orientation as in the transcript from which they were selected or may be altered in orientation or location. Hence a 5' or 3' UTR may be inverted, shortened, lengthened, made with one or more other 5' UTRs or 3' UTRs. As used herein, the term "altered" as it relates to a UTR sequence, means that the UTR has been changed in some way in relation to a reference sequence. For example, a 3' UTR or 5' UTR may be altered relative to a wild-type or native UTR by the change in orientation or location as taught above or may be altered by the inclusion of additional nucleotides, deletion of nucleotides, swapping or transposition of nucleotides. Any of these changes producing an "altered" UTR (whether 3' or 5') comprise a variant UTR.

In some embodiments, a double, triple or quadruple UTR such as a 5' UTR or 3' UTR may be used. As used herein, a "double" UTR is one in which two copies of the same UTR are encoded either in series or substantially in series. For example, a double beta-globin 3' UTR may be used as described in US Patent Publication 2010/0129877, the contents of which are incorporated herein by reference in its entirety.

It is also within the scope of the present disclosure to have patterned UTRs. As used herein "patterned UTRs" are those UTRs which reflect a repeating or alternating pattern, such as ABABAB or AABBAABBAABB or ABCABCABC or variants thereof repeated once, twice, or more than 3 times. In these patterns, each letter, A, B, or C represent a different UTR at the nucleotide level.

In some embodiments, flanking regions are selected from a family of transcripts whose proteins share a common function, structure, feature or property. For example, polypeptides of interest may belong to a family of proteins which are expressed in a particular cell, tissue or at some time during development. The UTRs from any of these genes may be swapped for any other UTR of the same or different family of proteins to create a new polynucleotide. As used herein, a "family of proteins" is used in the broadest sense to refer to a group of two or more polypeptides of interest which share at least one function, structure, feature, localization, origin, or expression pattern.

The untranslated region may also include translation enhancer elements (TEE). As a non-limiting example, the TEE may include those described in US Publication No. 2009/0226470, herein incorporated by reference in its entirety, and those known in the art.

***In vitro* Transcription of RNA**

cDNA encoding the polynucleotides described herein may be transcribed using an *in vitro* transcription (IVT) system. *In vitro* transcription of mRNA is known in the art and is described in International Publication WO 2014/152027, which is incorporated by reference
5 herein in its entirety. In some embodiments, the RNA of the present disclosure is prepared in accordance with any one or more of the methods described in WO 2018/053209 and WO 2019/036682, each of which is incorporated by reference herein.

In some embodiments, the mRNA transcript is generated using a non-amplified, linearized DNA template in an *in vitro* transcription reaction to generate the mRNA transcript. In
10 some embodiments, the template DNA is isolated DNA. In some embodiments, the template DNA is cDNA. In some embodiments, the cDNA is formed by reverse transcription of an mRNA, for example, but not limited to coronavirus mRNA. In some embodiments, cells, *e.g.*, bacterial cells, *e.g.*, *E. coli*, *e.g.*, DH-1 cells are transfected with the plasmid DNA template. In some embodiments, the transfected cells are cultured to replicate the plasmid DNA which is then
15 isolated and purified. In some embodiments, the DNA template includes a RNA polymerase promoter, *e.g.*, a T7 promoter located 5' to and operably linked to the gene of interest.

In some embodiments, an *in vitro* transcription template encodes a 5' untranslated (UTR) region, contains an open reading frame, and encodes a 3' UTR and a poly(A) tail. The particular nucleic acid sequence composition and length of an *in vitro* transcription template will depend on
20 the mRNA encoded by the template.

A “5' untranslated region” (UTR) refers to a region of an mRNA that is directly upstream (*i.e.*, 5') from the start codon (*i.e.*, the first codon of an mRNA transcript translated by a ribosome) that does not encode a polypeptide. When RNA transcripts are being generated, the 5' UTR may comprise a promoter sequence. Such promoter sequences are known in the art. It
25 should be understood that such promoter sequences will not be present in a vaccine of the disclosure.

A “3' untranslated region” (UTR) refers to a region of an mRNA that is directly downstream (*i.e.*, 3') from the stop codon (*i.e.*, the codon of an mRNA transcript that signals a termination of translation) that does not encode a polypeptide.

An “open reading frame” is a continuous stretch of DNA beginning with a start codon (*e.g.*, methionine (ATG)), and ending with a stop codon (*e.g.*, TAA, TAG or TGA) and encodes a polypeptide.
30

A “poly(A) tail” is a region of mRNA that is downstream, *e.g.*, directly downstream (*i.e.*, 3'), from the 3' UTR that contains multiple, consecutive adenosine monophosphates. A poly(A)

tail may contain 10 to 300 adenosine monophosphates. For example, a poly(A) tail may contain 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290 or 300 adenosine monophosphates. In some embodiments, a poly(A) tail contains 50 to 250 adenosine monophosphates. In a relevant biological setting (*e.g.*, in cells, *in vivo*) the poly(A) tail functions to protect mRNA from enzymatic degradation, *e.g.*, in the cytoplasm, and aids in transcription termination, and/or export of the mRNA from the nucleus and translation.

In some embodiments, a nucleic acid includes 200 to 3,000 nucleotides. For example, a nucleic acid may include 200 to 500, 200 to 1000, 200 to 1500, 200 to 3000, 500 to 1000, 500 to 1500, 500 to 2000, 500 to 3000, 1000 to 1500, 1000 to 2000, 1000 to 3000, 1500 to 3000, or 2000 to 3000 nucleotides).

An *in vitro* transcription system typically comprises a transcription buffer, nucleotide triphosphates (NTPs), an RNase inhibitor and a polymerase.

The NTPs may be manufactured in house, may be selected from a supplier, or may be synthesized as described herein. The NTPs may be selected from, but are not limited to, those described herein including natural and unnatural (modified) NTPs.

Any number of RNA polymerases or variants may be used in the method of the present disclosure. The polymerase may be selected from, but is not limited to, a phage RNA polymerase, *e.g.*, a T7 RNA polymerase, a T3 RNA polymerase, a SP6 RNA polymerase, and/or mutant polymerases such as, but not limited to, polymerases able to incorporate modified nucleic acids and/or modified nucleotides, including chemically modified nucleic acids and/or nucleotides. Some embodiments exclude the use of DNase.

In some embodiments, the mRNA transcript is capped via enzymatic capping. In some embodiments, the RNA comprises 5' terminal cap, for example, 7mG(5')ppp(5')NlmpNp.

Chemical Synthesis

Solid-phase chemical synthesis. Nucleic acids the present disclosure may be manufactured in whole or in part using solid phase techniques. Solid-phase chemical synthesis of nucleic acids is an automated method wherein molecules are immobilized on a solid support and synthesized step by step in a reactant solution. Solid-phase synthesis is useful in site-specific introduction of chemical modifications in the nucleic acid sequences.

Liquid Phase Chemical Synthesis. The synthesis of nucleic acids of the present disclosure by the sequential addition of monomer building blocks may be carried out in a liquid phase.

Combination of Synthetic Methods. The synthetic methods discussed above each has its own advantages and limitations. Attempts have been conducted to combine these methods to overcome the limitations. Such combinations of methods are within the scope of the present disclosure. The use of solid-phase or liquid-phase chemical synthesis in combination with enzymatic ligation provides an efficient way to generate long chain nucleic acids that cannot be obtained by chemical synthesis alone.

Ligation of Nucleic Acid Regions or Subregions

Assembling nucleic acids by a ligase may also be used. DNA or RNA ligases promote intermolecular ligation of the 5' and 3' ends of polynucleotide chains through the formation of a phosphodiester bond. Nucleic acids such as chimeric polynucleotides and/or circular nucleic acids may be prepared by ligation of one or more regions or subregions. DNA fragments can be joined by a ligase catalyzed reaction to create recombinant DNA with different functions. Two oligodeoxynucleotides, one with a 5' phosphoryl group and another with a free 3' hydroxyl group, serve as substrates for a DNA ligase.

Purification

Purification of the nucleic acids described herein may include, but is not limited to, nucleic acid clean-up, quality assurance and quality control. Clean-up may be performed by methods known in the arts such as, but not limited to, AGENCOURT® beads (Beckman Coulter Genomics, Danvers, MA), poly-T beads, LNATM oligo-T capture probes (EXIQON® Inc, Vedbaek, Denmark) or HPLC based purification methods such as, but not limited to, strong anion exchange HPLC, weak anion exchange HPLC, reverse phase HPLC (RP-HPLC), and hydrophobic interaction HPLC (HIC-HPLC). The term "purified" when used in relation to a nucleic acid such as a "purified nucleic acid" refers to one that is separated from at least one contaminant. A "contaminant" is any substance that makes another unfit, impure or inferior. Thus, a purified nucleic acid (*e.g.*, DNA and RNA) is present in a form or setting different from that in which it is found in nature, or a form or setting different from that which existed prior to subjecting it to a treatment or purification method.

A quality assurance and/or quality control check may be conducted using methods such as, but not limited to, gel electrophoresis, UV absorbance, or analytical HPLC.

In some embodiments, the nucleic acids may be sequenced by methods including, but not limited to reverse-transcriptase-PCR.

Quantification

In some embodiments, the nucleic acids of the present disclosure may be quantified in exosomes or when derived from one or more bodily fluid. Bodily fluids include peripheral blood, serum, plasma, ascites, urine, cerebrospinal fluid (CSF), sputum, saliva, bone marrow, synovial fluid, aqueous humor, amniotic fluid, cerumen, breast milk, bronchoalveolar lavage fluid, semen, prostatic fluid, Cowper's fluid or pre-ejaculatory fluid, sweat, fecal matter, hair, tears, cyst fluid, pleural and peritoneal fluid, pericardial fluid, lymph, chyme, chyle, bile, interstitial fluid, menses, pus, sebum, vomit, vaginal secretions, mucosal secretion, stool water, pancreatic juice, lavage fluids from sinus cavities, bronchopulmonary aspirates, blastocyst cavity fluid, and umbilical cord blood. Alternatively, exosomes may be retrieved from an organ selected from the group consisting of lung, heart, pancreas, stomach, intestine, bladder, kidney, ovary, testis, skin, colon, breast, prostate, brain, esophagus, liver, and placenta.

Assays may be performed using construct specific probes, cytometry, qRT-PCR, real-time PCR, PCR, flow cytometry, electrophoresis, mass spectrometry, or combinations thereof while the exosomes may be isolated using immunohistochemical methods such as enzyme linked immunosorbent assay (ELISA) methods. Exosomes may also be isolated by size exclusion chromatography, density gradient centrifugation, differential centrifugation, nanomembrane ultrafiltration, immunoabsorbent capture, affinity purification, microfluidic separation, or combinations thereof.

These methods afford the investigator the ability to monitor, in real time, the level of nucleic acids remaining or delivered. This is possible because the nucleic acids of the present disclosure, in some embodiments, differ from the endogenous forms due to the structural or chemical modifications.

In some embodiments, the nucleic acid may be quantified using methods such as, but not limited to, ultraviolet visible spectroscopy (UV/Vis). A non-limiting example of a UV/Vis spectrometer is a NANODROP® spectrometer (ThermoFisher, Waltham, MA). The quantified nucleic acid may be analyzed in order to determine if the nucleic acid may be of proper size, check that no degradation of the nucleic acid has occurred. Degradation of the nucleic acid may be checked by methods such as, but not limited to, agarose gel electrophoresis, HPLC based purification methods such as, but not limited to, strong anion exchange HPLC, weak anion exchange HPLC, reverse phase HPLC (RP-HPLC), and hydrophobic interaction HPLC (HIC-HPLC), liquid chromatography-mass spectrometry (LCMS), capillary electrophoresis (CE) and capillary gel electrophoresis (CGE).

Lipid Nanoparticles (LNPs)

In some embodiments, the RNA (*e.g.*, mRNA) of the disclosure is formulated in a lipid nanoparticle (LNP). Lipid nanoparticles typically comprise ionizable cationic lipid, non-cationic lipid, sterol and PEG lipid components along with the nucleic acid cargo of interest. The lipid nanoparticles of the disclosure can be generated using components, compositions, and methods as are generally known in the art, see for example PCT/US2016/052352; PCT/US2016/068300; PCT/US2017/037551; PCT/US2015/027400; PCT/US2016/047406; PCT/US2016/000129; PCT/US2016/014280; PCT/US2016/014280; PCT/US2017/038426; PCT/US2014/027077; PCT/US2014/055394; PCT/US2016/52117; PCT/US2012/069610; PCT/US2017/027492; PCT/US2016/059575 and PCT/US2016/069491 all of which are incorporated by reference herein in their entirety.

Vaccine compositions of the present disclosure are typically formulated in lipid nanoparticle. In some embodiments, the lipid nanoparticle comprises at least one ionizable cationic lipid, at least one non-cationic lipid/neutral lipid, at least one sterol, and/or at least one polyethylene glycol (PEG)-modified lipid.

In some embodiments, the lipid nanoparticle comprises 20-60 mol% ionizable cationic lipid or 40-55 mol% ionizable cationic lipid. For example, the lipid nanoparticle may comprise 20-50 mol%, 20-40 mol%, 20-30 mol%, 30-60 mol%, 30-50 mol%, 30-40 mol%, 40-60 mol%, 40-50 mol%, or 50-60 mol% ionizable cationic lipid. In some embodiments, the lipid nanoparticle comprises 20 mol%, 30 mol%, 40 mol%, 50, or 60 mol% ionizable cationic lipid.

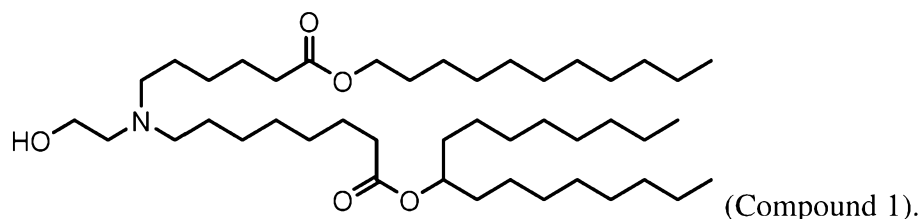
In some embodiments, the lipid nanoparticle comprises 5-25 mol% non-cationic lipid/neutral lipid or 5-15 mol% non-cationic lipid/neutral lipid. For example, the lipid nanoparticle may comprise 5-20 mol%, 5-15 mol%, 5-10 mol%, 10-25 mol%, 10-20 mol%, 10-25 mol%, 15-25 mol%, 15-20 mol%, or 20-25 mol% non-cationic lipid/neutral lipid. In some embodiments, the lipid nanoparticle comprises 5 mol%, 10 mol%, 15 mol%, 20 mol%, or 25 mol% non-cationic lipid/neutral lipid.

In some embodiments, the lipid nanoparticle comprises 25-55 mol% sterol or 35-45 mol% sterol. For example, the lipid nanoparticle may comprise 25-50 mol%, 25-45 mol%, 25-40 mol%, 25-35 mol%, 25-30 mol%, 30-55 mol%, 30-50 mol%, 30-45 mol%, 30-40 mol%, 30-35 mol%, 35-55 mol%, 35-50 mol%, 35-45 mol%, 35-40 mol%, 40-55 mol%, 40-50 mol%, 40-45 mol%, 45-55 mol%, 45-50 mol%, or 50-55 mol% sterol. In some embodiments, the lipid nanoparticle comprises 25 mol%, 30 mol%, 35 mol%, 40 mol%, 45 mol%, 50 mol%, or 55 mol% sterol.

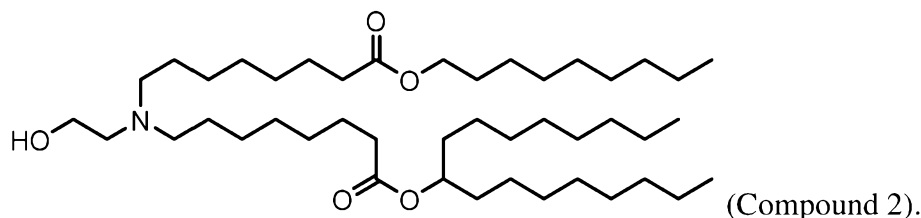
In some embodiments, the lipid nanoparticle comprises 0.5-15 mol% PEG-modified lipid or 1-5 mol% PEG-modified lipid. For example, the lipid nanoparticle may comprise 0.5-10 mol%, 0.5-5 mol%, 1-15 mol%, 1-10 mol%, 1-5 mol%, 2-15 mol%, 2-10 mol%, 2-5 mol%, 5-15 mol%, 5-10 mol%, or 10-15 mol%. In some embodiments, the lipid nanoparticle comprises 0.5 mol%, 1 mol%, 2 mol%, 3 mol%, 4 mol%, 5 mol%, 6 mol%, 7 mol%, 8 mol%, 9 mol%, 10 mol%, 11 mol%, 12 mol%, 13 mol%, 14 mol%, or 15 mol% PEG-modified lipid.

In some embodiments, the lipid nanoparticle comprises 20-60 mol% ionizable cationic lipid, 5-25 mol% non-cationic lipid, 25-55 mol% sterol, and 0.5-15 mol% PEG-modified lipid.

In some embodiments, an ionizable cationic lipid of the disclosure comprises heptadecan-9-yl 8 ((2 hydroxyethyl)(6 oxo 6-(undecyloxy)hexyl)amino)octanoate. Thus, in some embodiments, an ionizable cationic lipid of the disclosure comprises a compound having structure:



In some embodiments, an ionizable cationic lipid of the disclosure comprises a compound having structure:



In some embodiments, a non-cationic lipid of the disclosure comprises 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-diundecanoyl-sn-glycero-phosphocholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-sn-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine, 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinolenoyl-sn-glycero-3-

phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosaheptaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), sphingomyelin, and mixtures thereof.

5 In some embodiments, a PEG modified lipid of the disclosure comprises a PEG-modified phosphatidylethanolamine, a PEG-modified phosphatidic acid, a PEG-modified ceramide, a PEG-modified dialkylamine, a PEG-modified diacylglycerol, a PEG-modified dialkylglycerol, and mixtures thereof. In some embodiments, the PEG-modified lipid is DMG-PEG, PEG-c-DOMG (also referred to as PEG-DOMG), PEG-DSG and/or PEG-DPG.

10 In some embodiments, a sterol of the disclosure comprises cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, ursolic acid, alpha-tocopherol, and mixtures thereof.

In some embodiments, a LNP of the disclosure comprises an ionizable cationic lipid of Compound 1, wherein the non-cationic lipid is DSPC, the structural lipid that is cholesterol, and the PEG lipid is DMG-PEG.

15 In some embodiments, the lipid nanoparticle comprises 45 – 55 mole percent (mol%) ionizable cationic lipid. For example, lipid nanoparticle may comprise 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, or 55 mol% ionizable cationic lipid.

In some embodiments, the lipid nanoparticle comprises 5 – 15 mol% DSPC. For example, the lipid nanoparticle may comprise 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 mol% DSPC.

20 In some embodiments, the lipid nanoparticle comprises 35 – 40 mol% cholesterol. For example, the lipid nanoparticle may comprise 35, 36, 37, 38, 39, or 40 mol% cholesterol.

In some embodiments, the lipid nanoparticle comprises 1 – 2 mol% DMG-PEG. For example, the lipid nanoparticle may comprise 1, 1.5, or 2 mol% DMG-PEG.

25 In some embodiments, the lipid nanoparticle comprises 50 mol% ionizable cationic lipid, 10 mol% DSPC, 38.5 mol% cholesterol, and 1.5 mol% DMG-PEG.

In some embodiments, a LNP of the disclosure comprises an N:P ratio of from about 2:1 to about 30:1.

In some embodiments, a LNP of the disclosure comprises an N:P ratio of about 6:1.

In some embodiments, a LNP of the disclosure comprises an N:P ratio of about 3:1.

30 In some embodiments, a LNP of the disclosure comprises a wt/wt ratio of the ionizable cationic lipid component to the RNA of from about 10:1 to about 100:1.

In some embodiments, a LNP of the disclosure comprises a wt/wt ratio of the ionizable cationic lipid component to the RNA of about 20:1.

In some embodiments, a LNP of the disclosure comprises a wt/wt ratio of the ionizable cationic lipid component to the RNA of about 10:1.

In some embodiments, a LNP of the disclosure has a mean diameter from about 50 nm to about 150 nm.

5 In some embodiments, a LNP of the disclosure has a mean diameter from about 70 nm to about 120 nm.

Multivalent Vaccines

10 The compositions, as provided herein, may include mRNA or multiple mRNAs encoding two or more antigens of the same or different species. In some embodiments, composition includes an mRNA or multiple mRNAs encoding two or more coronavirus antigens. In some embodiments, the mRNA may encode 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or more coronavirus antigens.

15 In some embodiments, two or more different mRNAs encoding antigens may be formulated in the same lipid nanoparticle. In other embodiments, two or more different RNA encoding antigens may be formulated in separate lipid nanoparticles (each RNA formulated in a single lipid nanoparticle). The lipid nanoparticles may then be combined and administered as a single vaccine composition (*e.g.*, comprising multiple mRNAs encoding multiple antigens) or may be administered separately.

20

Combination Vaccines

The compositions, as provided herein, may include an mRNA or multiple mRNAs encoding two or more antigens of the same or different virus(es) or viral strain(s). In some embodiments, a composition includes RNA encoding at least one coronavirus antigen and at least one antigen of a different virus. In some embodiments, a composition includes mRNA encoding at a first coronavirus antigen and a second coronavirus antigen, wherein the first and second coronavirus antigens are different from each other. Thus, compositions (*e.g.*, mRNA vaccines) of the present disclosure may target one or more antigen(s) of the same strain/species, or one or more antigen(s) of different strains/species, *e.g.*, antigens which induce immunity to organisms which are found in the same geographic areas where the risk of coronavirus infection is high or organisms to which an individual is likely to be exposed to when exposed to a coronavirus.

35

EXAMPLES

Example 1: Phase 1 Clinical Trial

5 Study Design and Methodology

The study was an open-label, dose ranging clinical trial in males and non-pregnant females, at least 18 years of age, who were in good health and meet all eligibility criteria.

This study was designed to assess the safety, reactogenicity, and immunogenicity of a SARS-CoV-2 mRNA vaccine (SEQ ID NO: 6, ORF SEQ ID NO: 7, encoding SEQ ID NO: 8).

10 **Table 1: Treatment Arms**

Cohort	Sample Size	Stratum (Years of Age)	First and Second Dose
1	15	18-55	25 µg
2	15	18-55	100 µg
3	15	18-55	250 µg
4	10	56-70	25 µg
5	10	56-70	100 µg
6	10	56-70	250 µg
7	10	≥71	25 µg
8	10	≥71	100 µg
9	10	≥71	250 µg

One hundred and five (105) subjects were enrolled into one of nine cohorts (25 micrograms [µg], 100 µg, 250 µg). Subjects received an intramuscular (IM) injection (0.5 milliliter [mL]) of the SARS-CoV-2 mRNA vaccine on Days 1 and 29 in the deltoid muscle and were followed through 12 months post second vaccination (Day 394). The second dose of vaccine (0.5 mL) was administered preferably in the same arm used for the first dose.

Follow-up visits occurred 1, 2 and 4 weeks post each vaccination (Days 8, 15, 29, 36, 43, and 57), as well as 3, 6 and 12 months post second vaccination (Days 119, 209 and 394).

20 Reactogenicity was assessed at these visits and blood was drawn for immunogenicity assays. Additional safety and reactogenicity data were solicited via telephone calls to subjects 1 and 2 days post each vaccination (Days 2, 3, 30, and 31).

To determine early safety signals for this phase I study, vaccination proceeded in a staged fashion. Sentinel subject dosing began with 4 subjects in cohort 1 (25 µg). The 4 sentinel subjects for cohort 2 (100 µg) were enrolled no earlier than one day after enrollment of the last of the 4 sentinel subjects in cohort 1. If no halting rules were met after the 8 sentinel subjects had completed Day 5, then full enrollment proceeded first with the remaining subjects in cohort 1, followed by the remaining subjects in cohort 2 without interruption. If no halting rules were met after all subjects in cohort 2 had completed Day 8, then dosing of 4 sentinel subjects began in

cohort 3. If no halting rules were met after the 4 sentinel subjects in cohort 3 had completed Day 5, then full enrollment of cohort 3 proceeds.

If no halting rules were met after all subjects in cohorts 1 and 2 had completed Day 8, dosing began for cohorts 4 (25 µg; 56-70 years of age) and 5 (100 µg; 56-70 years of age). If no halting rules were met after all subjects in cohorts 4 and 5 had completed Day 8, dosing began for cohorts 7 (25 µg; ≥71 years of age) and 8 (100 µg; ≥71 years of age). If no halting rules were met after all subjects in cohorts 3, 7 and 8 had completed Day 8, dosing began for cohort 6 (250 µg; 56-70 years of age). If no halting rules were met after all subjects in cohort 6 had completed Day 8, dosing began for cohort 9 (250 µg; ≥71 years of age).

Reactogenicity was measured by the occurrence of solicited injection site and systemic reactions from the time of each vaccination through 7 days post each vaccination. Unsolicited non-serious adverse events (AEs) were collected from the time of each vaccination through 28 days post each vaccination. Serious adverse events (SAEs), new-onset chronic medical conditions (NOCMCs) and medically-attended adverse events (MAAEs) were collected through 12 months after the last vaccination (Day 394).

Clinical safety laboratory evaluations were performed at screening, as well as immediately prior to and 7 days post each vaccination (Days 1, 8, 29, and 36).

To support development of diagnostics, therapeutics and vaccines, a subset of subjects enrolled in cohorts 2, 3, 5, and 6 underwent leukapheresis to collect additional samples for secondary research.

The duration of the entire study was 18 months (from start of screening to last subject last visit). The duration for each individual subject was approximately 14 months (from first contact to last visit).

Objectives

The primary objective was to evaluate the safety and reactogenicity of a 2-dose vaccination schedule of the SARS-CoV-2 mRNA vaccine (SEQ ID NO: 6, ORF SEQ ID NO: 7, encoding SEQ ID NO: 8), given 28 days apart, across 3 dosages in healthy adults.

The secondary objective was to evaluate the immunogenicity as measured by IgG ELISA to the SARS-CoV-2 S (spike) protein following a 2-dose vaccination schedule of the SARS-CoV-2 mRNA vaccine at Day 57.

The exploratory objectives were (1) to evaluate the immunogenicity as measured by IgG ELISA to the SARS-CoV-2 S (spike) protein following a 2-dose vaccination schedule of the SARS-CoV-2 mRNA vaccine at all timepoints, other than Day 57; (2) to evaluate the immunogenicity as measured by IgM and IgA ELISA to the SARS-CoV-2 S (spike) protein

following a 2-dose vaccination schedule of the SARS-CoV-2 mRNA vaccine given 28 days apart; (3) to evaluate the immunogenicity as measured by pseudovirus neutralization following a 2-dose vaccination schedule of the SARS-CoV-2 mRNA vaccine given 28 days apart; (4) to evaluate the immunogenicity as measured by live wild-type SARS-CoV-2 neutralization following a 2-dose vaccination schedule of the SARS-CoV-2 mRNA vaccine given 28 days apart; (5) to assess, in at least a subset of samples, the SARS-CoV-2 S protein-specific T cell responses; and (6) to determine, in at least a subset of samples, the epitopes recognized by B cells and antibodies generated in response to the SARS-CoV-2 mRNA vaccine.

10 Study Population

Participants (males and females 18 years of age or older at time of consent) were included in the study if they were in good health according to the assessment of the investigator and could comply with study procedures.

15 Vaccine, Dosage, and Route of Administration

The SARS-CoV-2 mRNA vaccine (SEQ ID NO: 6, ORF SEQ ID NO: 7, encoding SEQ ID NO: 8) was a lipid nanoparticle (LNP) dispersion of an mRNA encoding the prefusion stabilized spike protein SARS-CoV-2 formulated in LNPs composed of four (4) lipids (50 mol% ionizable lipid heptadecan-9-yl 8 ((2 hydroxyethyl)(6 oxo 6-(undecyloxy)hexyl)amino)octanoate (Compound 1); 10 mol% 1,2 distearoyl sn glycerol-3 phosphocholine (DSPC); 38.5 mol% cholesterol; and 1.5 mol% 1-monomethoxypolyethyleneglycol-2,3-dimyristylglycerol with polyethylene glycol of average molecular weight 2000 (PEG2000 DMG)). The SARS-CoV-2 mRNA vaccine was provided as a sterile liquid for injection, white to off white dispersion in appearance, at a concentration of 0.5 mg/mL in 20 mM trometamol (Tris) buffer containing 87 mg/mL sucrose and 10.7 mM sodium acetate, at pH 7.5.

The diluent was 0.9% sodium chloride (normal saline) injection, United States Pharmacopeia (USP). The USP grade 0.9% NaCl or normal saline for injection was a sterile, nonpyrogenic, isotonic solution; each mL contains NaCl 9 mg. It contained no bacteriostatic agent, antimicrobial agent, preservatives, or added buffer and was supplied only in single-dose containers. The solution may have contained hydrochloric acid and/or sodium hydroxide for pH adjustment (pH 5.3, range 4.5-7.0). This product could be used to dilute the vaccine to the desired concentration.

The SARS-CoV-2 mRNA vaccine was administered as an intramuscular injection into the deltoid muscle on a 2-dose vaccination schedule on Day 1 and Day 29, with at least a 28-day interval between doses. Each vaccination contained 0.5 mL of the SARS-CoV-2 mRNA vaccine

diluted in 0.9% NaCl for injection, USP, to obtain 25 µg, 100 µg, or 250 µg of the SARS-CoV-2 mRNA vaccine in each 0.5 mL dose. Preferably, the vaccine was administered into the nondominant arm and the second dose of vaccine was administered in the same arm as used for the first dose.

5

Table 2: Dosing and Administration

Cohort	Vaccine Dose	Route	Frequency of Administration
1	25 mcg	IM	D1, D29
2	100 mcg	IM	D1, D29
3	250 mcg	IM	D1, D29
4	25 mcg	IM	D1, D29
5	100 mcg	IM	D1, D29
6	250 mcg	IM	D1, D29
7	25 mcg	IM	D1, D29
8	100 mcg	IM	D1, D29
9	250 mcg	IM	D1, D29

Primary Endpoints

The primary endpoints were the following:

- 10
- Frequency and grade of each solicited local and systemic reactogenicity AE during a 7-day follow-up period post each vaccination.
 - Frequency and grade of any unsolicited AEs during the 28-day follow-up period post each vaccination.
 - Frequency of any SAEs, NOCMCs and MAAEs from Day 1 to Day 394.

15 Analysis

Safety assessments included monitoring and recording of the following for each participant:

- 20
- Solicited local and systemic adverse events (AEs) occur during the 7 days following each injection (i.e., Days 1-8 for the first vaccination, and Days 29-36 for the second vaccination).
 - Unsolicited AEs from the time of study product administration through Day 57. After Day 57, only SAEs, MAAEs and NOCMCs will be reported through the end of the study (Day 394).

25 Safety and reactogenicity were assessed by clinical review of all relevant parameters including solicited AEs (local and systemic events), unsolicited AEs, SAEs, MAAEs, AEs leading to discontinuation, safety laboratory test results, vital signs, and physical examination findings.

Solicited ARs included injection site pain, injection site erythema, injection site edema/induration, headache, fatigue, myalgia, arthralgia, nausea, fever, and chills.

Secondary Endpoints

5 The secondary objectives were evaluated by the following endpoints:

- Geometric mean titer (GMT) of antibody at Day 57.
- Percentage of subjects who seroconverted, defined as a 4-fold change in antibody titer from baseline.
- The geometric mean fold rise (GMFR) in IgG titer from baseline.

10 *Analysis*

Seroconversion rates, GMFR and GMT for SARS-CoV-2 as measured by IgG ELISA were calculated at Days 1 (GMT only) and 57 by cohort and were summarized graphically.

Seroconversion rates, GMFR and GMT were presented with their corresponding 95% confidence interval (CI) estimates at each timepoint and overall peak GMT, and the pair-wise differences between seroconversion rates by cohort were summarized by study day along with 95% CIs.

Exploratory Endpoints

The exploratory endpoints were the following:

- 20 • GMT of antibody at each timepoint.
- Percentage of subjects who seroconverted at each timepoint.
- The GMFR in IgG titer from baseline for each post-vaccination timepoint.
- GMT at each timepoint.
- Percentage of subjects who seroconverted at each timepoint.
- 25 • The GMFR in IgM and IgA titer from baseline at each post-vaccination timepoint.
- GMT of neutralizing (Neut) antibody at each timepoint.
- Percentage of subjects who seroconverted, defined as a 4-fold change in Neut antibody titer from baseline at each timepoint.
- The GMFR Neut antibody titer from baseline at each post-vaccination timepoint.
- 30 • GMT of Neut antibody at each timepoint.
- Percentage of subjects who seroconverted, defined as a 4-fold change in Neut antibody titer from baseline at each timepoint.
- The GMFR in Neut antibody titer from baseline at each post-vaccination timepoint.

- Magnitude, phenotype, and percentage of cytokine producing S protein-specific T cells, as measured by flow cytometry at different timepoints post vaccination relative to baseline.
- Change in magnitude and phenotype of S protein-specific B cells as measured by flow cytometry at different timepoints post vaccination relative to baseline.
- Determination of major antigenic sites and amino acid residues on SARS-CoV-2 S protein recognized by representative B cell clones and corresponding sequences of B cell receptors and monoclonal antibodies generated by vaccination.

Analysis

Seroconversion rates, GMFR and GMT for SARS-CoV-2 as measured by IgG, IgA and IgM ELISA, neutralization assay using SARS-CoV-2 pseudovirus and neutralization assay using a live wild-type SARS-CoV-2 were calculated for specified timepoints by cohort and were summarized graphically. Seroconversion rates, GMFR and GMT were presented with their corresponding 95% CI estimates at each timepoint and overall peak GMT, and the pair-wise differences between seroconversion rates by cohort were summarized by study day along with 95% CI.

The magnitude, phenotype and percentage of cytokine producing S protein-specific T cells were summarized at each timepoint by vaccination group.

The magnitude and phenotype of S protein-specific B cells were summarized at each timepoint by vaccination group.

B-cell receptor sequence analysis to identify representative B cell clones and associated major antigenic sites and amino acid residues was described.

Immunogenicity Evaluations

Serological Immunogenicity Assays

The following serological immunogenicity assays were performed:

- IgG ELISA to the SARS-CoV-2 S (spike) protein.
- IgM and IgA ELISA to the SARS-CoV-2 S (spike) protein.
- Neutralization assay using a SARS-CoV-2 pseudovirus.
- Neutralization assay using a live wild-type SARS-CoV-2.

Cellular Immunology Assays

The trial also investigated T and B cell immune responses using multiparametric flow cytometry, as well as identification of major antigenic sites and amino acid residues on the SARS-CoV-2 S protein recognized by B cell clones.

DNA obtained from B-cells was sequenced to identify B cell receptors and monoclonal antibodies. The DNA data was used to synthesize antigen-specific antibodies to characterize antibody binding.

Additionally, stored PBMCs, including leukocyte samples obtained by leukapheresis, were used in secondary research for testing, including, but not limited to, other genomic analysis single nucleotide polymorphisms (SNP) arrays, human leukocyte antigen (HLA) typing, transcriptomic analysis, evaluation of the immune response to the vaccine, and/or evaluation of any AE from the vaccine.

10 Interim Immunogenicity Review

For public health reasons there were several immunogenicity reviews. The following reviews occurred once data is available:

- For sentinel subjects in cohorts 1 and 2, the ELISA IgG data through Day 29;
- For all subjects in cohorts 1 and 2, the ELISA IgG data through Day 29;
- 15 • For sentinel subjects in cohort 3, the ELISA IgG data through Day 29;
- For all subjects in cohort 3, the ELISA IgG data through Day 29;
- All subjects in cohorts 4, 5, 7 and 8, ELISA IgG data through Day 29;
- All subjects in cohorts 6 and 9, ELISA IgG data through Day 29;
- Sentinels in cohorts 1 and 2, ELISA IgG data through Day 57;
- 20 • All subjects in cohorts 1 and 2, ELISA IgG data through Day 57;
- Sentinels in cohort 3, ELISA IgG data through Day 57;
- All subjects in cohort 3, ELISA IgG data through Day 57;
- All subjects in cohorts 4, 5, 7 and 8, ELISA IgG data through Day 57;
- All subjects in cohorts 6 and 9, ELISA IgG data through Day 57.
- 25 • Additional data review of immunogenicity may be performed to inform public health decisions.

Interim Immunogenicity and Safety Review

An interim analysis of safety, reactogenicity, and immunologic response data was planned once all subjects in cohorts 1-3 have completed Day 57 and the data were entered in the study database, validated, and monitored according to the clinical monitoring plan (CMP). Additionally, an interim analysis was done once subjects in cohorts 4, 5, 7, and 8 had completed Day 57, and another interim analysis was done once subjects in cohorts 6 and 9 had completed Day 57.

Example 2 – GMT in Vaccinated Subjects v. Convalescent Subjects

ELISAs were performed to determine the anti-spike protein IgG titer levels in sera at 15 or 29 days post immunization with a single dose of 25 µg or 100 µg of the vaccine described in Example 1. As is shown in FIG. 1, surprisingly, both dose levels induce higher titers than the convalescent sera (*e.g.*, sera from a subject who has recovered from COVID-19).

Example 3 – Immunogenicity Data (IgG ELISA)

IgG ELISAs against the SARS-CoV-2 spike (S) protein were performed with the initial (baseline) samples, (Day 1), Day 15, Day 29, Day 36, and Day 43 in cohorts 1-5 (Table 1) (FIGs. 2-3). Antibody titers were increased in vaccination subjects, relative to baseline for all cohorts.

The plaque reduction neutralization test (PRNT) demonstrated the SARS-CoV-2 mRNA vaccine induces the production of neutralizing antibodies (FIG. 4).

Methods

Coating: His-Tagged SARS-CoV-2 S-2P (expressed from VRC7471_2019 nCoV S-2P-dFurin-F3CH2S_JSM). VRC-SARS-CoV-2 S-2P (15-1208)-3C-His8-Strep2x2, NCI lot: P200423.02 (2 µg/ml in PBS)

Detection: Goat anti-Human IgG (H+L) Cross-Adsorbed Secondary Antibody, HRP (1:32000 in 0.1% TPBS)

Pos ctrl: SARS-CoV-2S2-specific mAb S652-112 spiked in NHS (per plate); SARS-CoV-2 POS Pool 1: combination of 4 convalescent specimen with high and medium high binding responses, per batch

Neg ctrl: Assay Diluent (PBS with 0.1% Tween 20), per plate.

Example 4 – Immunogenicity in Rats

Rats were immunized with the SARS-CoV-2 mRNA vaccine (SEQ ID NO: 6, ORF SEQ ID NO: 7, encoding SEQ ID NO: 8) at day 1 (FIG. 5). Sera from day 0 (open diamonds) and day 34 post-immunization (solid diamonds) were analyzed by ELISA to assess binding to SARS-CoV-2 stabilized prefusion spike protein (SARS-CoV-2 S-2P). 30 µg, 60 µg, and 100 µg doses of the vaccine elicited significant spike-specific antibody in rats by 34 days post-immunization in a dose-independent manner.

Example 5 - MERS-CoV S-2P mRNA protects mice from lethal challenge - prototype pathogen approach to pandemic preparedness

Humanized DPP4 288/330+/+ mice were immunized at weeks 0 and 3 weeks with 0.01, 0.1, or 1 µg of MERS-CoV S-2P mRNA. Mock-immunized mice were immunized with PBS. Four weeks post-boost, mice were challenged with a lethal dose of mouse-adapted MERS-CoV

(MA35c5). Following challenge, mice were monitored for weight loss (FIG. 6A). At days 3 and 5 post-challenge, lungs from 5 mice/group were harvested for analysis of viral titers (FIGs. 6B-6C) and hemorrhage (0 = no hemorrhage, 4 = severe hemorrhage in all lobes) (FIGs. 6D-6E). MERS S-2P mRNA protects mice in a dose-dependent manner, and 0.01 µg appears to be the breakthrough dose. Subprotective level of responses, as shown by the 0.01 µg dose group, did not lead to enhanced weight loss, viral load, or lung hemorrhage.

Example 6 – One dose of SARS-CoV-2 mRNA vaccine elicits a robust antibody response

BALB/c mice were immunized with 0.1, 1 µg, or 10 µg of SARS-CoV-2 mRNA vaccine. Sera were collected 2 and 4 weeks post-immunization and assessed for SARS-CoV-2 S-specific total IgG by ELISA and neutralizing antibodies against homotypic SARS-CoV-2 pseudovirus (FIGs. 7A-7B). Results show that one dose of 1 µg or 10 µg of SARS-CoV-2 mRNA vaccine robustly elicits spike-binding antibodies, and is sufficient to confer protection to SARS-CoV-2 challenge at 7 weeks. 10 µg of SARS-CoV-2 mRNA vaccine elicits high levels of neutralizing antibodies that increase between week 2 and week 4.

Example 7 – SARS-CoV-2 mRNA vaccine elicits a balanced Th1/Th2 response

BALB/c, C57BL/6, or B6C3 mice were immunized at weeks 0 and 3 with 0.01, 0.1, or 1 µg of SARS-CoV-2 mRNA vaccine or SARS-CoV-2 S-2P protein adjuvanted with Sigma adjuvant system (SAS). Sera were collected 2 weeks post-boost and assessed by ELISA for SARS-CoV-2 S-specific IgG1 and IgG2a, IgG2c, or IgG2a/c, for BALB/c, C57BL/6, and B6C3 mice, respectively. SARS-CoV-2 mRNA vaccine and SARS-CoV-2 S-2P adjuvanted with the TLR4-agonist SAS elicit both IgG2a and IgG1 subclass spike-binding antibodies, indicating a balanced Th1/Th2 response (FIGs. 8A-8I).

BALB/c mice were then immunized with 1 µg or 10 µg of SARS-CoV-2 mRNA vaccine or 10 µg CoV-2 S protein adjuvanted with 250 µg ALHYDROGEL® (Alum). Sera were collected 2 weeks post-immunization and assessed by ELISA for Spike-specific IgG2a and IgG1 (FIG. 9). In contrast to the S protein plus adjuvant, a balanced response of IgG2a and IgG1 (Th2 associated) indicates that SARS-CoV-2 mRNA vaccine does not induce a Th2 response, which can be associated with disease enhancement. Results also show that the ratio of IgG2a to IgG1 indicate that disease enhancement is unlikely.

Example 8 – SARS-CoV-2 mRNA vaccine drives a robust CD8 response

BALB/c mice were immunized with 0.1 µg, 1 µg, or 10 µg of SARS-CoV-2 mRNA vaccine, and CD8 and CD4 T cell responses were assessed on Day 36 for cytokine expression. A CD8 T cell response was assessed by Th1 cytokine expression (IFNγ, IL-2, TNFα) (FIG. 10A),

and a CD4 T cell response was assessed by Th2 cytokine expression (IL-4, IL-5, IL-9, IL-10, IL-13) (FIG. 10B). Results show that 1 µg and 10 µg doses of SARS-CoV-2 mRNA vaccine drive a robust, Th1-directed CD8 T-cell response, and that the vaccine does not drive a CD4 T-cell response.

Methods

SARS-CoV-2 ELISA

To measure antibody binding, an ELISA was performed. SARS-CoV-2 pre-S was coated onto 96-well Nunc MaxiSorp™ flat-bottom plates (ThermoFisher, catalog #: 44-2401-21) in 100 µL of 1X PBS for 16 hours at 4 °C. Plates were washed 3 times with 250 µL PBS-Tween (PBST) (Medicago AB, catalog #: 09-9410-100). To prevent non-specific binding, plates were blocked with 200 µL PBST supplemented with 5% nonfat skim milk (BD Difco™, catalog #: 232100) (blocking buffer) for 1 hour at room temperature (RT). Plates were washed 3 times with 250 µL PBST. Sera were serially diluted (1:100, 4-fold, 8 times) in 100 µL blocking buffer and allowed to bind to antigen for 1 hour at RT, in duplicate. Plates were washed 3 times with 250 µL PBST. 100 µL goat anti-mouse IgG (H+L) cross-adsorbed secondary antibody conjugated to HRP (ThermoFisher, catalog #: G-21040) diluted in blocking buffer was added for 1 hour at RT. Plates were washed 3 times with 250 µL PBST. The enzyme-linked reaction was developed for 10 minutes with 100 µL KPL SureBlue 1-component TMB microwell peroxidase substrate (Sure Blue, catalog #: 5120-0077) and stopped with 100 µL 1N sulfuric acid (ThermoFisher, catalog #: SA 212-1). Spectramax Paradigm (Molecular Devices) was used to detect OD450. Sera endpoint titers were calculated as 4-fold above non-specific secondary antibody binding to antigen.

Table 3.

SARS-CoV-2 Spike (S) Protein		
SEQ ID NO: 1 consists of from 5' end to 3' end: 5' UTR SEQ ID NO: 2, mRNA ORF SEQ ID NO: 31, and 3' UTR SEQ ID NO: 4.		1
Chemistry	1-methylpseudouridine	
Cap	7mG(5')ppp(5')N1mpNp	
5' UTR	GGGAAAUAGAGAGAAAAAGAAGAGUAAGAAGAAUUAAGACCCCG GCGCCGCCACC	2
ORF of mRNA Construct (excluding the stop codon)	AUGUUCGUGUUCUGGUGCUGCUGCCCCUGGUGAGCAGCCAGUGCG UGAACCCUGACCACCCGGACCCAGCUGCCACCAGCCUACACCAACAG CUUACCCCGGGGCGUCUACUACCCCGACAAGGUGUUCGGAGCAGC GUCCUGCACAGCACCCAGGACCUUCCUGCCCUUCUUCAGCAACG UGACCUGGUUCCACGCCAUCCACGUGAGCGGCACCAACGGCACCAA GCGGUUCGACAACCCCGUGCUGCCCUUCAACGACGGCGUGUACUUC	3

	GCCAGCACCGAGAAGAGCAACAUCAUCCGGGGCUGGAUCUUCGGCA CCACCCUGGACAGCAAGACCCAGAGCCUGCUGAUCGUGAAUAACGC CACCAACGUGGUGAUCAAGGUGUGCGAGUUCAGUUCUGCAACGAC CCCUUCCUGGGCGUGUACUACCACAAGAACAACAAGAGCUGGAUGG AGAGCGAGUUCGGGUGUACAGCAGCGCCAACAACUGCACCUUCGA GUACGUGAGCCAGCCCUUCCUGAUGGACCUGGAGGGCAAGCAGGGC AACUUCAAGAACCUGCGGGAGUUCGUGUUAAGAACAUCGACGGCU ACUUCAAGAUCUACAGCAAGCACACCCCAUCAACCUGGUGCGGGA UCUGCCCCAGGGCUUCUCAGCCCUGGAGCCCCUGGUGGACCUGCCC AUCGGCAUCAACAUCACCCGGUUCAGACCCUGCUGGGCCUGCACC GGAGCUACCUGACCCCAGGCGACAGCAGCAGCGGGUGGACAGCAGG CGCGGCUGCUUACUACGUGGGCUACCUGCAGCCCCGGACCUUCCUG CUGAAGUACAACGAGAACGGCACCAUCACCGACGCCGUGGACUGCG CCCUGGACCCUCUGAGCGAGACCAAGUGCACCUCUGAAGAGCUUCAC CGUGGAGAAGGGCAUCUACCAGACCAGCAACUUCGGGUGCAGCCC ACCGAGAGCAUCGUGCGGUUCCCCAACAUACCAACCUGUGCCCCU UCGGCGAGGUGUUAACGCCACCCGGUUCGCCAGCGUGUACGCCUG GAACCGGAAGCGGAUCAGCAACUGCGUGGCCGACUACAGCGUGCUG UACAACAGCGCCAGCUUCAGCACCUUCAAGUGCUACGGCGUGAGCC CCACCAAGCUGAACGACCUGUGCUUCACCAACGUGUACGCCGACAG CUUCGUGAUCCGUGGCGACGAGGUGCGGCAGAUCCGACCCGGCCAG ACAGGCAAGAUCGCCGACUACAACUACAAGCUGCCCAGCAGCUUCA CCGGCUGCGUGAUCGCCUGGAACAGCAACAACCUCGACAGCAAGGU GGGCGGCAACUACAACUACCUGUACCGGCUGUCCGGAAGAGCAAC CUGAAGCCCUUUCGAGCGGGACAUACGACCCGAGAUCAACCAAGCCG GCUCCACCCCUUGCAACGGCGUGGAGGGCUUUAACUGCUACUCCCC UCUGCAGAGCUACGGCUUCCAGCCCACCAACGGCGUGGGCUACCAG CCCUACCGGGUGGUGGUGCUGAGCUUCGAGCUGCUGCAGCCCCAG CCACCGUGUGUGGGCCCCAAGAAGAGCACCAACCUGGUGAAGAACA GUGCGUGAACUUAACUUAACGGCCUUAACGGCACCGGCGUGCUG ACCGAGAGCAACAAGAAAUUCCUGCCCUUUCAGCAGUUCGGCCGGG ACAUCGCCGACACCACCGACGCUUGCGGGAUCCCCAGACCCUGGA GAUCCUGGACAUCACCCCUUGCAGCUUCGGCGGCUGAGCGUGAUC ACCCAGGCACCAACACCAGCAACCAGGUGGGCGUGCUGUACCAGG ACGUGAACUGCACCGAGGUGCCCGUGGCCAUCCACGCCGACCAGCU GACACCCACCUGGCGGGUCUACAGCACCGGCAGCAACGUGUCCAG ACCCGGGCGCGUUGCCUGAUCGGCGCCGAGCAGUGAACAAACAGCU ACGAGUGCGACAUCCCCAUCGGCGCCGGCAUCUGUGCCAGCUACCA GACCCAGACCAAUUCACCCCGGAGGGCAAGGAGCGUGGCCAGCCAG AGCAUCAUCGCCUACACCAUGAGCCUGGGCGCCGAGAACAGCGUGG CCUACAGCAACAACAGCAUCGCCAUCCCCACCAACUUCACCAUCAG CGUGACCACCGAGAUUCUGCCCGUGAGCAUGACCAAGACCAGCGUG GACUGCACCAUGUACAUCUGCGGCAGCAGCACCGAGUGCAGCAACC UGCUGCUGCAGUACGGCAGCUUCUGCACCCAGCUGAACCGGGCCCU GACCGGCAUCGCCGUGGAGCAGGACAAGAACACCCAGGAGGUGUUC GCCCAGGUGAAGCAGAUCAACAAGACCCUCCCAUCAAGGACUUCG GCGGCUUAACUUCAGCCAGAUCCUGCCCGACCCAGCAAGCCCAG CAAGCGGAGCUUCAUCGAGGACCUGCUGUUAACAAGGUGACCCUA GCCGACGCCGGCUUCAUCAAGCAGUACGGCGACUGCCUCGGCGACA UAGCCGCCCCGGGACCUGAUCUGCGCCCAGAAGUUAACGGCCUGAC CGUGCUGCCUCCCCUGCUGACCGACGAGAUGAUCGCCAGUACACC AGCGCCCUUGUAGCCGGAACCAUACACAGCGGCUGGACUUUCGGCG CUGGAGCCGCUUCGAGAUCCCCUUCGCCAUGCAGAUAGGCCUACCG GUUCAACGGCAUCGGCGUGACCCAGAACGUGCUGUACGAGAACCAG AAGCUGAUCGCCAACCAGUUAACAGCGCCAUCGGCAAGAUAACAGG ACAGCCUGAGCAGCACCGCUAGCGCCCUGGGCAAGCUGCAGGACGU GGUGAACCAAGACGCCAGGCCUGAACACCCUGGUGAAGCAGCUG AGCAGCAACUUCGGCGCCAUCAGCAGCGUGCUGAACGACAUCUGA	
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	GCGGCGUGGACAAGGUGGAGGCCGAGGUGCAGAU CGACCGGUGAU CACUGGCCGGCUGCAGAGCCUGCAGACCUACGUGACCCAGCAGCUG AUCCGGGCCCGCCGAGAUUCGGGCCAGCGCCAACUGGCCGCCACCA AGAUGAGCGAGUGCGUGCUGGGCCAGAGCAAGCGGGUGGACUUCUG CGGCAAGGGCUACCACCUGAUGAGCUUCCCCAGAGCGCACCCAC GGAGUGGUGUCCUGCACGUGACCUACGUGCCCCGCCAGGAGAAGA ACUUCACCACCGCCCCAGCCAUCUGCCACGACGGCAAGGCCACUU UCCCCGGGAGGGCGUGUUCGUGAGCAACGGCACCCACUGGUUCGUG ACCCAGCGGAACUUCUACGAGCCCCAGAUCAUACCACCGACAACA CCUUCGUGAGCGGCAACUGCGACGUGGUGAUCGGCAUCGUGAACAA CACCGUGUACGAUCCCCUGCAGCCCGAGCUGGACAGCUUCAAGGAG GAGCUGGACAAGUACUUAAGAAUCACACCAGCCCCGACGUGGACC UGGGCGACAUCAGCGGCAUCAACGCCAGCGUGGUGAACAUCCAGAA GGAGAUCGAUCGGCUGAACGAGGUGGCCAAGAACCUGAACGAGAGC CUGAUCGACCUGCAGGAGCUGGGCAAGUACGAGCAGUACAUAAGU GGCCUGGUACAUCUGGCUGGGCUUCAUCGCCGGCCUGAUCGCCAU CGUGAUGGUGACCAUCAUGCUGUGCUGCAUGACCAGCUGCUGCAGC UGCCUGAAGGGCUGUUGCAGCUGCGGCAGCUGCUGCAAGUUCGACG AGGACGACAGCGAGCCCGUGCUGAAGGGCGUGAAGCUGCACUACAC C	
3' UTR	UGAUAAUAGGCUAGGAGCCUCGGUGGCCUAGCUUCUUGCCCCUUGGG CCUCCCCCAGCCCCUCCUCCCCUCCUGCACCCGUACCCCGUGG UCUUUGAAUAAAAGUCUGAGUGGGCGGC	4
Corresponding amino acid sequence	MFVFLVLLPLVSSQCVNLTRTQLPPAYTNSFTRGVYYPDKVFRSS VLHSTQDLFLPFFSNVTWFHAIHVS GTNGTKRFDNPVLPFNDGVYF ASTEKSNIIRGWIFGTTLD SKTQSL LIVNNATNVVIKVCE FQFCND PFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQG NFKNLREFVFKNIDGYFKIYSKHTP INLVRDLPQGFSALEPLVDLP IGINITRFQTL LALHRSYLT PGDSSSGWTAGAAAYVGYLQPRFTL LKY NENGTITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQP TESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVL YNSASFSTFKCYGVSP TKLNDLCFTNVYADSFVIRGDEV RQIAPGQ TGKIADYNYKL PDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSN LKPFERDISTEIIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQ PYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVL TESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVI TPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQ TRAGCLIGA EHVNNSYEC DIPIGAGICASYQTQ TNSPRRARSVASQ SIIAYTMSLGAENSVAYSNN SIAIPTNFTISVTTEILPVSMTKTSV DCTMYICGDSTEC SNLL LQYGSFCTQLNRALTGI AVEQDKNTQEVF AQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTL ADAGFIKQYGDCLG DIAARDL ICAQKFNGLT VLPPLLTDEMIAQYT SALLAGTITSGWTFGAGAA LQIPFAMQMAYRFNGIGVTQNVLYENQ KLIANQFN SAIGKIQDSL SSTA S ALGKLQDVVNQNAQALNTLVKQL SSNFGAISSVLNDILSR LDKVEAEVQIDRLITGR LQSLQTYVTQQL IRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLSF PQSAPH GVVFLHVTYVPAQEKNFTTAPAICH DGKAHFPREGVFVSNGTHW FV TQRNFYEPQIITTDNTFVSGNCDV VIGIVNNTVYDPLQPELDSFKE ELDKYFKNHTSPD VDLGDISGINASVVNIQKEIDRLNEVAKNLNES LIDLQELGKYEQYIKWPWYIWLGF IAGLIAIVMVTIMLC CMTSCCS CLKGCCSCGSCCKFDEDDSEPV LKGVKLHYT	5
PolyA tail	100 nt	
SARS-CoV-2 S Protein Variant		
SEQ ID NO: 6 consists of from 5' end to 3' end: 5' UTR SEQ ID NO: 2, mRNA ORF SEQ ID		6

NO: 7, and 3' UTR SEQ ID NO: 4.		
Chemistry	1-methylpseudouridine	
Cap	7mG(5')ppp(5')NlmpNp	
5' UTR	GGGAAAUAAAGAGAGAAAAAGAAGAGUAAGAAGAAAUAAAGACCCCG GCGCCGCCACC	2
ORF of mRNA Construct (excluding the stop codon)	AUGUUCGUGUUCUGGUGCUGCUGCCCCUGGUGAGCAGCCAGUGCG UGAACCUGACCACCCGGACCCAGCUGCCACCAGCCUACACCAACAG CUUCACCCGGGGCGUCUACUACCCCGACAAGGUGUUCGGAGCAGC GUCCUGCACAGCACCCAGGACCUGUUCUGCCCUUCUUCAGCAACG UGACCUGGUUCCACGCCAUCCACGUGAGCGGCACCAACGGCACCAA GCGGUUCGACAACCCCGUGCUGCCCUUCAAACGACGGCGUGUACUUC GCCAGCACCGAGAAGAGCAACAUCAUCCGGGGCUGGAUCUUCGGCA CCACCCUGGACAGCAAGACCCAGAGCCUGCUGAUCGUGAAUAACGC CACCAACGUGGUGAUC AAGGUGUGCGAGUUCAGUUCUGCAACGAC CCCUUCCUGGGCGUGUACUACCACAAGAACAACAAGAGCUGGAUGG AGAGCGAGUUCGGGUGUACAGCAGCGCCAACAACUGCACCUUCGA GUACGUGAGCCAGCCUUCUGAUGGACCUGGAGGGCAAGCAGGGC AACUUCAAGAACCUGCGGGAGUUCGUGUUAAGAACAUCGACGGCU ACUUCAAGAUCUACAGCAAGCACACCCCAAUAACCUGGUGCGGGA UCUGCCCCAGGGCUUCUCAGCCUGGAGCCCCUGGUGGACCUGCCC AUCGGCAUCAACAUCACCCGGUUCAGACCCUGCUGGCCCUGCACC GGAGCUACCUGACCCAGGCGACAGCAGCAGCGGGUGGACAGCAGG CGCGGCUUCUACUACGUGGGCUACCUGCAGCCCCGGACCUUCCUG CUGAAGUACAACGAGAACGGCACCAUCACCGACGCCGUGGACUGCG CCCUGGACCCUCUGAGCGAGACCAAGUGCACCCUGAAGAGCUUCAC CGUGGAGAAGGGCAUCUACCAGACCAGCAACUCCGGGUGCAGCCC ACCGAGAGCAUCGUGCGGUUCCCCAACAUACCAACCUGUGCCCCU UCGGCGAGGUGUUAACGCCACCCGGUUCGCCAGCGUGUACGCCUG GAACCGGAAGCGGAUCAGCAACUGCGUGGCCGACUACAGCGUGCUG UACAACAGCGCCAGCUUCAGCACCUUCAAGUGCUACGGCGUGAGCC CCACCAAGCUGAACGACCUGUGCUUACCAACGUGUACGCCGACAG CUUCGUGAUCCUGGGCGACGAGGUGCGGCAGAUCCGACCCGGCCAG ACAGGCAAGAUCCGCCGACUACAACUACAAGCUGCCCGACGACUUA CCGGCUGCGUGAUCGCCUGGAACAGCAACAACCUAGACAGCAAGGU GGGCGGCAACUACAACUACCUGUACCGGCGUUCGGGAAGAGCAAC CUGAAGCCCCUUCGAGCGGGACAUACGACCCGAGAUCAACCAAGCCG GCUCCACCCCUUGCAACGGCGUGGAGGGCUUAACUGCUACUCCCC UCUGCAGAGCUACGGCUUCCAGCCCACCAACGGCGUGGGCUACCAG CCCUAACC GGUGGUGGUGCUGAGCUUCGAGCUGCUGCAGCCCCAG CCACCGUGUGUGGGCCCCAAGAAGAGCACCAACCUGGUGAAGAACA GUGCGUGAACUUAACUUAACGGCCUUAACGGCACCGGCGUGCUG ACCGAGAGCAACAAGAAAUUCCUGCCCUUUCAGCAGUUCGGCCGGG ACAUCGCCGACACCACCGACGCUUGCGGGAUCCCCAGACCCUGGA GAUCCUGGACAUCACCCCUUGCAGCUUCGGCGGCGUGAGCGUGAUC ACCCAGGCACCAACACCAGCAACCAGGUGGCCGUGCUGUACCAGG ACGUGAACUGCACCGAGGUGCCCGUGGCCAUCCACGCCGACCAGCU GACACCCACCUGGCGGGUCUACAGCACCGGCAGCAACGUGUUCAG ACCCGGGCCGGUUGCCUGAUCGGCGCCGAGCACGUGAACAACAGCU ACGAGUGCGACAUCCCCAUCGGCGCCGGCAUCUGUGCCAGCUACCA GACCCAGACCAAUUCACCCCGGAGGGCAAGGAGCGUGGCCAGCCAG AGCAUCAUCGCCUACACCAUGAGCCUGGGCGCCGAGAACAGCGUGG CCUACAGCAACAACAGCAUCGCCAUCCCCACCAACUUCACCAUCAG CGUGACCACCGAGAUUCUGCCCGUGAGCAUGACCAAGACCAGCGUG GACUGCACCAUGUACAUCUGCGGCGACAGCACCGAGUGCAGCAACC UGCUGCUGCAGUACGGCAGCUUCUGCACCCAGCUGAACCGGGCCCU	7

	GACCGGCAUCGCCGUGGAGCAGGACAAGAACACCCAGGAGGUGUUC GCCCAGGUGAAGCAGAUCAACAAGACCCUCCCAUCAAGGACUUCG GCGGCUUCAACUUCAGCCAGAUCCUGCCCGACCCAGCAAGCCCAG CAAGCGGAGCUUCAUCGAGGACCUGCUGUUAACAAGGUGACCCUA GCCGACGCCGGCUUCAUCAAGCAGUACGGCGACUGCCUCGGCGACA UAGCCGCCCCGGGACCUGAUCUGCGCCCAGAAGUUAACGGCCUGAC CGUGCUGCCUCCCCUGCUGACCGACGAGAUGAUCGCCAGUACACC AGCGCCUGUAGCCGGAACCAUCACCAGCGGCUGGACUUCGGCG CUGGAGCCGCUCUGCAGAUCCCCUUCGCCAUGCAGAUGGCCUACCG GUUCAACGGCAUCGGCGUGACCCAGAACGUGCUGUACGAGAACCAG AAGCUGAUCGCCAACCAGUUAACAGCGCCAUCGGCAAGAUAUCCAGG ACAGCCUGAGCAGCACCAGCUAGCGCCUGGGCAAGCUGCAGGACGU GGUGAACCAGAACGCCAGGCCUGAACACCCUGGUGAAGCAGCUG AGCAGCAACUUCGGCGCCAUCAGCAGCGUGCUGAACGACAUCCUGA GCCGGCUGGACCCUCCCGAGGCCGAGGUGCAGAUCCAGCGCUGAU CACUGGCCGGCUGCAGAGCCUGCAGACCUACGUGACCCAGCAGCUG AUCCGGGCCGCCGAGAUUCGGGCCAGCGCCAACCUGGCCGCCACCA AGAUGAGCGAGUGCGUGCUGGGCCAGAGCAAGCGGGUGGACUUCUG CGGCAAGGGCUACCACCUGAUGAGCUUCCCCAGAGCGCACCCCCAC GGAGUGGUGUUCUGCACGUGACCUACGUGCCCGCCAGGAGAAGA ACUUCACCACCGCCCCAGCCAUCUGCCACGACGGCAAGGCCACUU UCCCCGGGAGGGCGUGUUCGUGAGCAACGGCACCCACUGGUUCGUG ACCCAGCGGAACUUCUACGAGCCCCAGAUCAUACCACCGACAACA CCUUCGUGAGCGGCAACUGCGACGUGGUGAUCGGCAUCGUGAACAA CACCGUGUACGAUCCCCUGCAGCCCGAGCUGGACAGCUUCAAGGAG GAGCUGGACAAGUACUUAAGAAUCACACCAGCCCCGACGUGGACC UGGGCGACAUCAGCGGCAUCAACGCCAGCGUGGUGAACAUCAGAA GGAGAUCAUCGGCUGAACGAGGUGGCCAAGAACCUGAACGAGAGC CUGAUCGACCUGCAGGAGCUGGGCAAGUACGAGCAGUACAUAAGU GGCCUGGUACAUCUGGCUGGGCUUCAUCGCCGGCCUGAUCGCCAU CGUGAUGGUGACCAUCAUGCUGUGCUGCAUGACCAGCUGCUGCAGC UGCCUGAAGGGCUGUUGCAGCUGCGGCAGCUGCUGCAAGUUCGACG AGGACGACAGCGAGCCCGUGCUGAAGGGCGUGAAGCUGCACUACAC C	
3' UTR	UGAUAAUAGGCUGGAGCCUCGGUGGCCUAGCUUCUUGCCCCUUGGG CCUCCCCCAGCCCCUCCUCCCCUCCUGCACCCGUACCCCCGUGG UCUUUGAAUAAAGUCUGAGUGGGCGGC	4
Corresponding amino acid sequence	MFVFLVLLPLVSSQCVNLTRTQLPPAYTNSFTRGVYYPDKVFRSS VLHSTQDLFLPFFSNVTWFHAIHVS GTNGTKRFDNPVLPFNDGVYF ASTEKSNIIRGWIFGTTLDSKTQSLIVNNATNVVIKVECFQFCND PFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPF LMDLEGKQG NFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLP IGINITRFQTL LALHRSYLT PGDSSSGW TAGAAAYVGYLQPRFTL LKYNENGTITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQP TESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVL YNSASFSTFKCYGVSP TKLNDLCFTNVYADSFVIRGDEV RQIAPGQ TGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNLYRLFRKSN LKPFERDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVG YQ PYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVL TESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSF GGVS VI TPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQ TRAGCLIGA EHVNNSYECDIPIGAGICASYQTQTNSPRRARSVASQ SIIAYTMSLGAENSVAYSNN SIAIPTNFTISVTTEILPVSMTKTSV DCTMYICGDSTEC SNLLLQYGSFCTQLNRALTGI AVEQDKNTQE VF AQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTL ADAGFIKQYGDCLGDIAARDL ICAQKFNGLTVLPPLLTDEMIAQYT	8

	SALLAGTITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQ KLIANQFNSAIGKIQDSLSTASALGKLQDVVNQNAQALNTLVKQL SSNFGAISSVLNDILSRLDPPAEVQIDRLITGRQLQSLQTYVTQQL IRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPH GVVFLHVTYVPAQEKNFTTAPAICHGDKAHFPREGVFVSNGTHWV TQRNFYEPQIIITDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKE ELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNES LIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCS CLKGCCSCGSCCKFDEDDSEPVLKGVKLHYT	
PolyA tail	100 nt	

All references, patents and patent applications disclosed herein are incorporated by reference with respect to the subject matter for which each is cited, which in some cases may encompass the entirety of the document.

5 The indefinite articles “a” and “an,” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

10 In the claims, as well as in the specification above, all transitional phrases such as “comprising,” “including,” “carrying,” “having,” “containing,” “involving,” “holding,” “composed of,” and the like are to be understood to be open-ended, *i.e.*, to mean including but not limited to. Only the transitional phrases “consisting of” and “consisting essentially of” shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent
15 Office Manual of Patent Examining Procedures, Section 2111.03.

The terms “about” and “substantially” preceding a numerical value mean $\pm 10\%$ of the recited numerical value.

Where a range of values is provided, each value between the upper and lower ends of the range are specifically contemplated and described herein.

20 The entire contents of International Application Nos. PCT/US2015/02740, PCT/US2016/043348, PCT/US2016/043332, PCT/US2016/058327, PCT/US2016/058324, PCT/US2016/058314, PCT/US2016/058310, PCT/US2016/058321, PCT/US2016/058297, PCT/US2016/058319, and PCT/US2016/058314 are incorporated herein by reference.

CLAIMS

What is claimed is:

1. A method comprising administering to a human subject a composition comprising a messenger ribonucleic acid (mRNA) comprising an open reading frame (ORF) that encodes a SARS-CoV-2 prefusion stabilized Spike (S) protein, wherein the mRNA is formulated in a lipid nanoparticle, and wherein the composition is administered in an effective amount to induce in the subject a neutralizing antibody response to SARS-CoV-2 S protein, wherein the effective amount is a 25 µg or 100 µg dose.
2. The method of claim 1, wherein the geometric mean titer (GMT) of anti-S protein IgG antibodies produced in the subject at Day 15 post-administration of a 25 µg or 100 µg dose of the composition at Day 1 is at least 1 log higher than the GMT of antibodies produced in convalescent subjects who were infected with SARS-CoV-2.
3. The method of claim 1 or 2, wherein GMT of anti-S protein IgG antibodies produced in the subject at Day 29 post-administration of a 25 µg or 100 µg dose of the composition at Day 1 is at least 1 log higher than the GMT of antibodies produced in convalescent subjects who were infected with SARS-CoV-2.
4. The method of any one of claims 1-3, wherein the GMT of anti-S protein IgG antibodies produced in the subject at Day 36 post-administration of a 25 µg or 100 µg dose of the composition at Day 1 is at least 1 log higher than the GMT of antibodies produced in convalescent subjects who were infected with SARS-CoV-2.
5. The method of any one of claims 1-4, wherein the GMT of anti-S protein IgG antibodies produced in the subject at Day 43 post-administration of a 25 µg or 100 µg dose of the composition at Day 1 is at least 1 log higher than the GMT of antibodies produced in convalescent subjects who were infected with SARS-CoV-2.
6. The method of any one of claims 1-5, wherein the method comprises administering to the subject at least two doses of the composition.

7. The method of claim 6, wherein a second dose of the composition is administered to the subject at least 28 days after a first dose of the composition is administered to the subject.

8. The method of any one of claims 1-7, wherein the composition further comprises Tris buffer, sucrose, and sodium acetate.

9. The method of claim 8, wherein the composition comprises 10 mM – 30 mM Tris buffer, 75 mg/mL – 95 mg/mL sucrose, and 5 mM – 15 mM sodium acetate, optionally wherein the composition has a pH of 6-8.

10. The method of claim 9, wherein the composition comprises 20 mM Tris buffer, 87 mg/mL sucrose, and 10.7 mM sodium acetate, optionally wherein the composition has a pH of 7.5.

11. The method of any one of claims 1-10, wherein the composition comprises 0.5 mg/mL of the mRNA.

12. The method of any one of claims 1-11, wherein the composition is administered intramuscularly, optionally into a deltoid region of an arm.

13. The method of any one of claims 1-12, wherein the SARS-CoV-2 prefusion stabilized S protein comprises an amino acid sequence having at least 90%, at least 95%, or at least 98% identity to the sequence of SEQ ID NO: 8.

14. The method of claim 13, wherein the SARS-CoV-2 prefusion stabilized S protein comprises the amino acid sequence of SEQ ID NO: 8.

15. The method of any one of claims 1-14, wherein the ORF comprises a nucleotide sequence having at least 90%, at least 95%, or at least 98% identity to the sequence of SEQ ID NO: 7.

16. The method of claim 15, wherein the ORF comprises the nucleotide sequence of SEQ ID NO: 7.

17. The method of any one of claims 1-14, wherein the mRNA comprises a nucleotide sequence having at least 90%, at least 95%, or at least 98% identity to the sequence of SEQ ID NO: 6.

5 18. The method of claim 17, wherein the mRNA comprises the nucleotide sequence of SEQ ID NO: 6.

19. The method of any one of claims 1-18, wherein the lipid nanoparticle comprises: ionizable cationic lipid; neutral lipid; sterol; and PEG-modified lipid.

10

20. The method of claim 19, wherein the lipid nanoparticle comprises: 40-55 mol% ionizable cationic lipid; 5-15 mol% neutral lipid; 35-45 mol% sterol; and 1-5 mol% PEG-modified lipid.

21. The method of claim 20, wherein the lipid nanoparticle comprises:

15 47 mol% ionizable cationic lipid; 11.5 mol% neutral lipid; 38.5 mol% sterol; and 3.0 mol% PEG-modified lipid;

48 mol% ionizable cationic lipid; 11 mol% neutral lipid; 38.5 mol% sterol; and 2.5 mol% PEG-modified lipid;

20 49 mol% ionizable cationic lipid; 10.5 mol% neutral lipid; 38.5 mol% sterol; and 2.0 mol% PEG-modified lipid;

50 mol% ionizable cationic lipid; 10 mol% neutral lipid; 38.5 mol% sterol; and 1.5 mol% PEG-modified lipid; or

51 mol% ionizable cationic lipid; 9.5 mol% neutral lipid; 38.5 mol% sterol; and 1.0 mol% PEG-modified lipid.

25

22. The method of any one of claims 19-21, wherein the ionizable cationic lipid is heptadecan-9-yl 8 ((2 hydroxyethyl)(6 oxo 6-(undecyloxy)hexyl)amino)octanoate (Compound 1).

30 23. The method of any one of claims 19-21, wherein the neutral lipid is 1,2 distearoyl sn glycerol-3 phosphocholine (DSPC).

24. The method of any one of claims 19-21, wherein the sterol is cholesterol.

25. The method of any one of claims 19-24, wherein the PEG-modified lipid is 1-monomethoxypolyethyleneglycol-2,3-dimyristylglycerol with polyethylene glycol of average molecular weight 2000 (PEG2000 DMG).
- 5 26. The method of any one of claims 1-25, wherein the age of the subject is 18 to 54 years or 55 years or older.
27. The method of any one of claims 1-26, wherein the subject is immunocompromised.
- 10 28. The method of any one of claims 1-26, wherein the subject has a chronic pulmonary disease, such as chronic obstructive pulmonary disease (COPD) or asthma.
29. The method of any one of claims 1-27, wherein the subject has an underlying comorbid condition, optionally selected from heart disease, diabetes, and lung disease.
- 15 30. A composition comprising a 25 µg dose of an mRNA of any one of the preceding claims formulated in a lipid nanoparticle, wherein the geometric mean titer (GMT) of anti-SARS-CoV-2 S protein IgG antibodies produced in a human subject at Day 15 or Day 29 post-administration of the composition at Day 1 is at least 1 log higher than the GMT of antibodies produced in
20 convalescent subjects who were infected with SARS-CoV-2.
31. A composition comprising a 100 µg dose of an mRNA of any one of the preceding claims formulated in a lipid nanoparticle, wherein the geometric mean titer (GMT) of anti-SARS-CoV-2 S protein IgG antibodies produced in a human subject at Day 15 or Day 29 post-
25 administration of the composition at Day 1 is at least 1 log higher than the GMT of antibodies produced in convalescent subjects who were infected with SARS-CoV-2.
32. A method comprising administering to a subject a composition comprising a messenger ribonucleic acid (mRNA) comprising an open reading frame (ORF) that encodes a SARS-CoV-2
30 antigen comprising the amino acid sequence of SEQ ID NO: 8, and a lipid nanoparticle in an effective amount to elicit IgG2a and IgG1 subclass S protein-binding antibodies.
33. The method of claim 32, wherein the titers of IgG2a to IgG1 is balanced compared to a protein vaccine control.

34. A method comprising administering to a subject a composition comprising a messenger ribonucleic acid (mRNA) comprising an open reading frame (ORF) that encodes a SARS-CoV-2 antigen comprising the amino acid sequence of SEQ ID NO: 8 in an effective amount to elicit a robust CD8 T cell response.

35. A method comprising administering to a subject a composition comprising a messenger ribonucleic acid (mRNA) comprising an open reading frame (ORF) that encodes a SARS-CoV-2 antigen comprising the amino acid sequence of SEQ ID NO: 8 in an effective amount to elicit a balanced Th1/Th2 response.

36. A method comprising administering to a subject a composition comprising a messenger ribonucleic acid (mRNA) comprising an open reading frame (ORF) that encodes a SARS-CoV-2 antigen comprising the amino acid sequence of SEQ ID NO: 8 in an effective amount to confer protection to SARS-CoV-2 challenge.

37. The method of claim 36, wherein protection is conferred at least 7 weeks post administration of the composition.

38. A method comprising administering to a subject a composition comprising a messenger ribonucleic acid (mRNA) comprising an open reading frame (ORF) that encodes a SARS-CoV-2 antigen comprising the amino acid sequence of SEQ ID NO: 8 in an effective amount to elicit high levels of neutralizing antibodies that increase between week 2 and week 4 post administration of the composition.

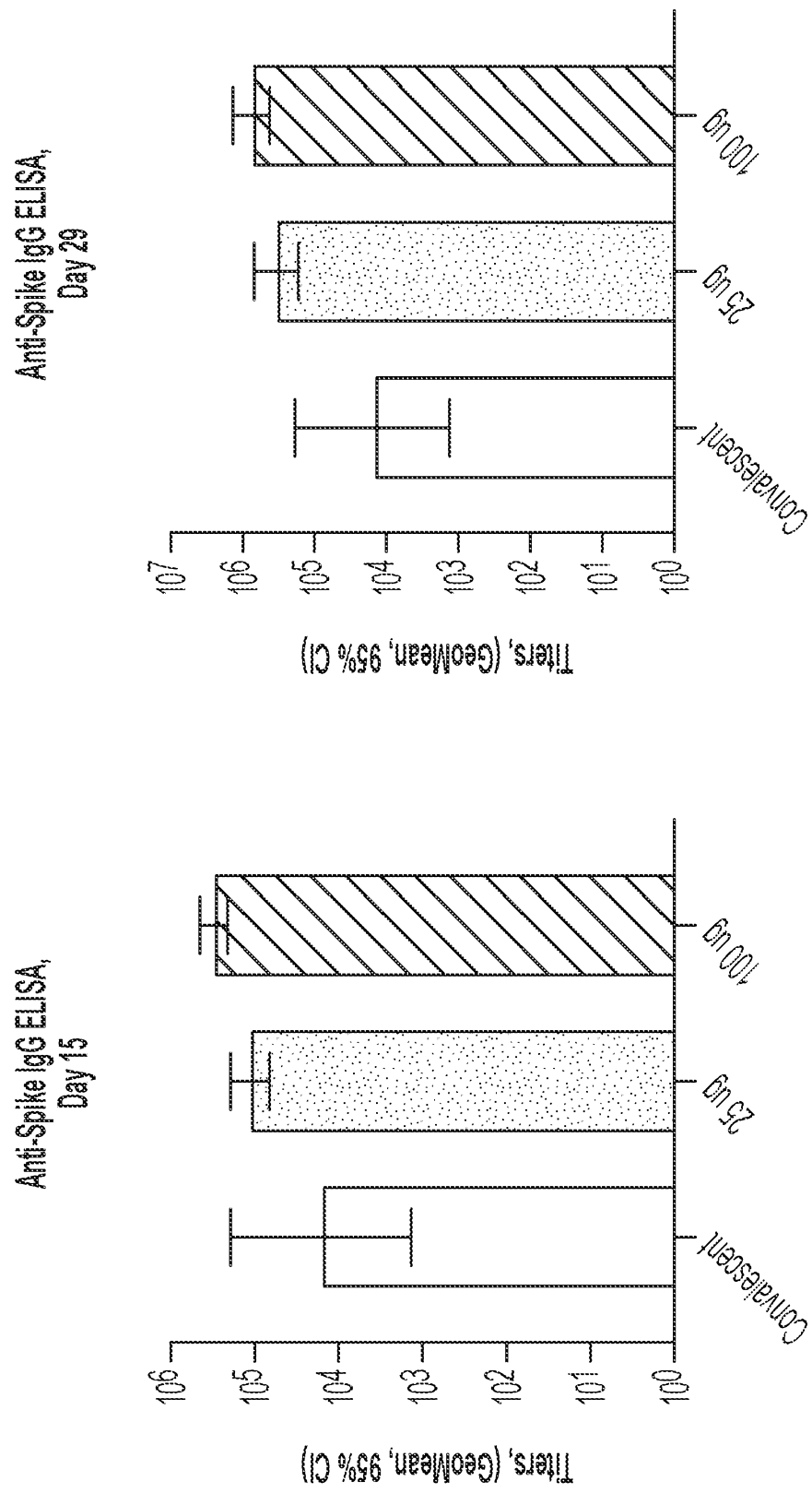


FIG. 1

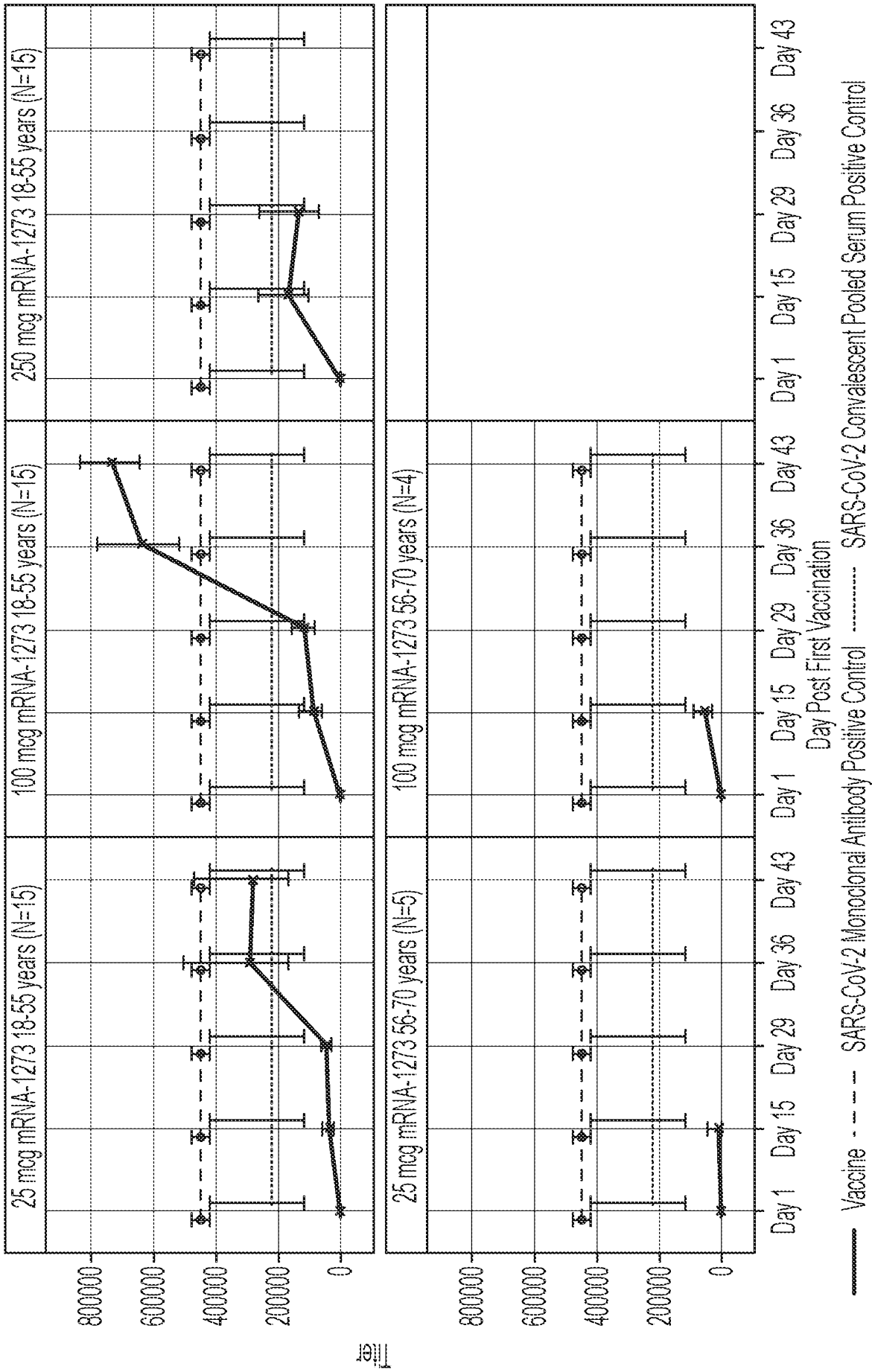


FIG. 2

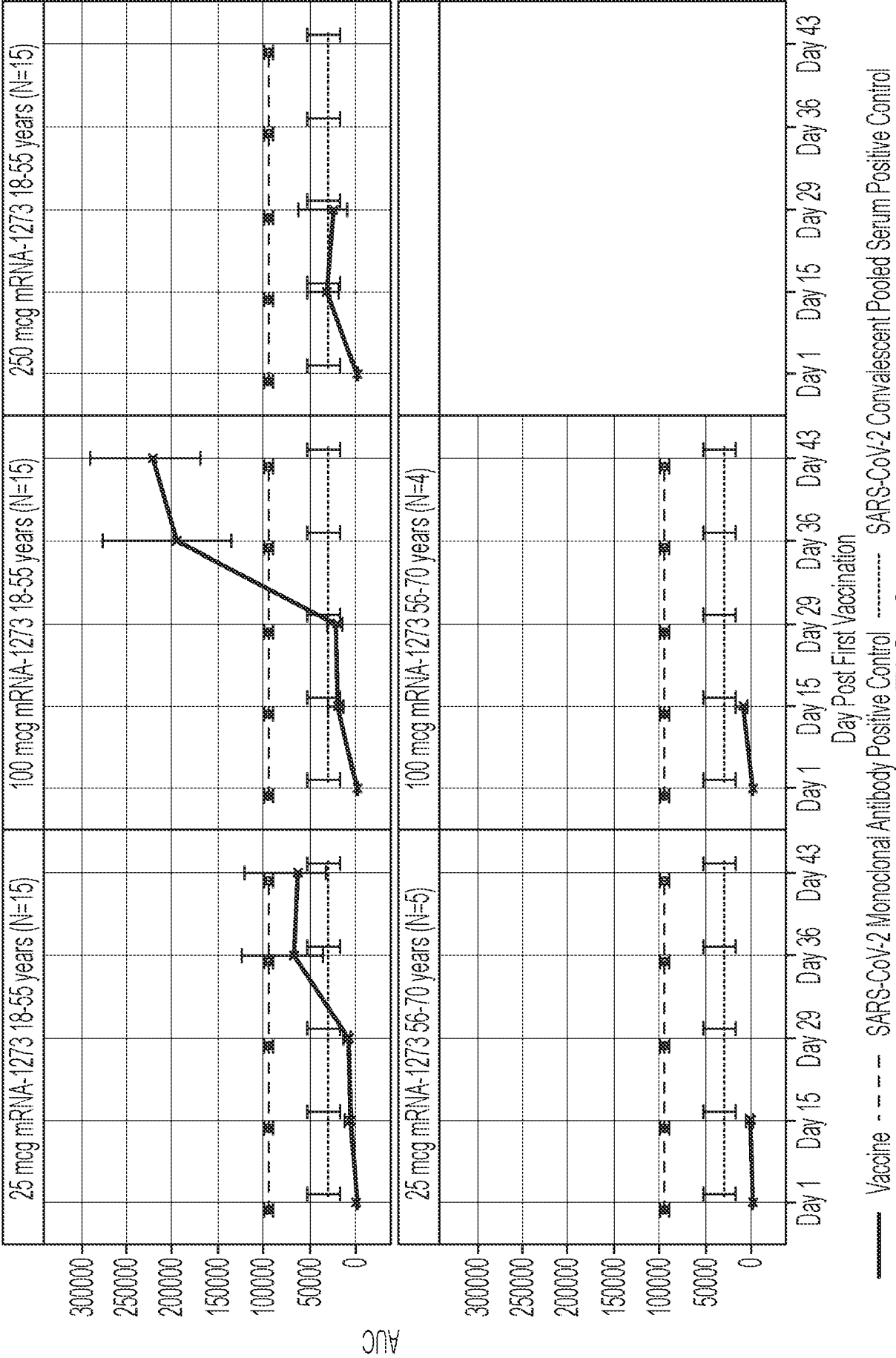


FIG. 3

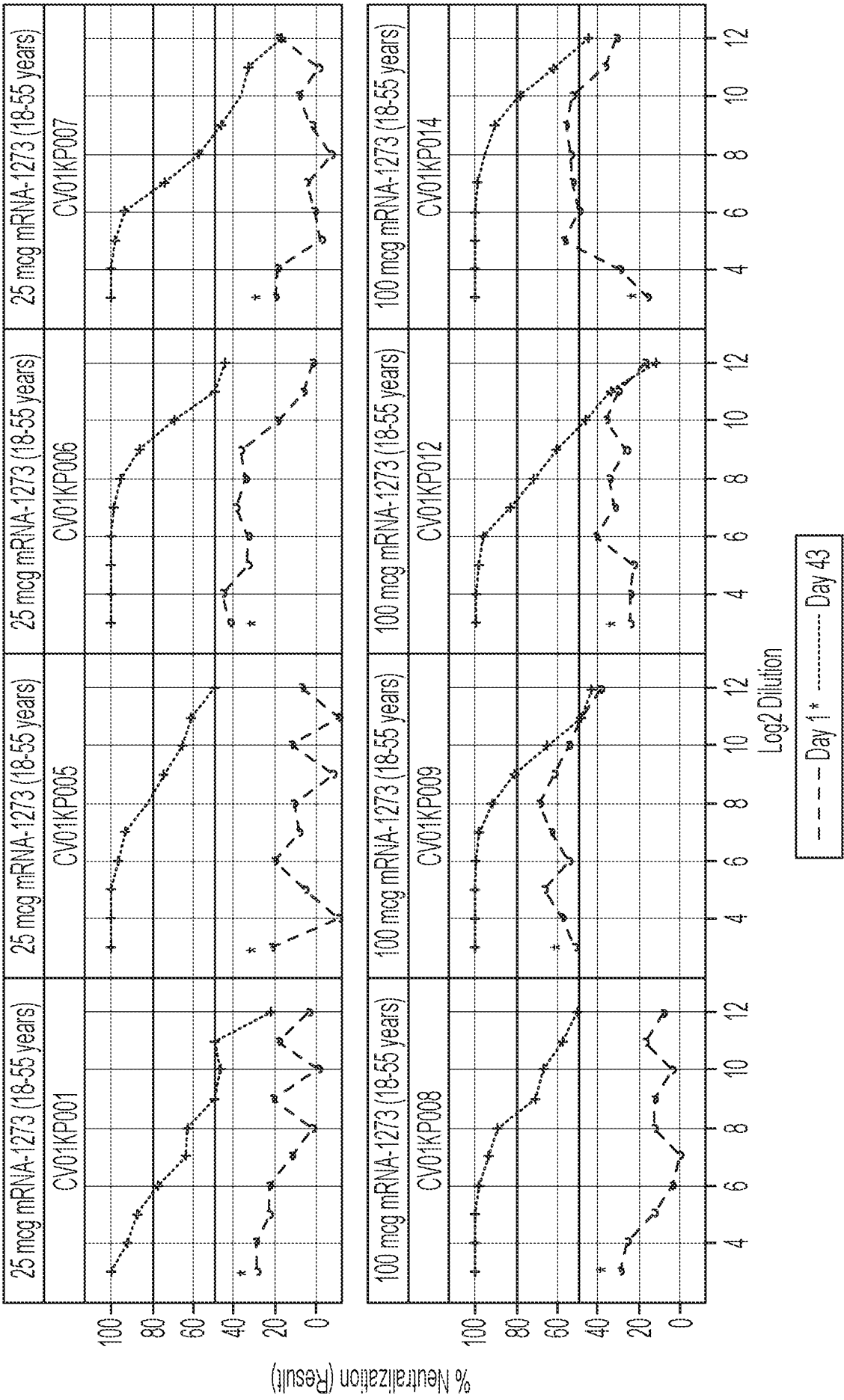


FIG. 4

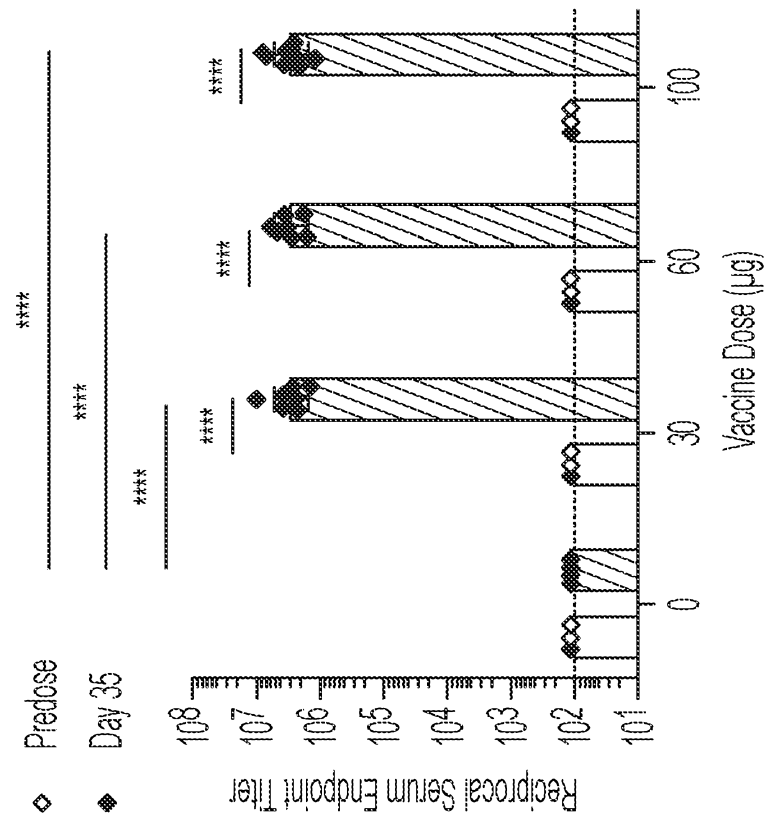


FIG. 5

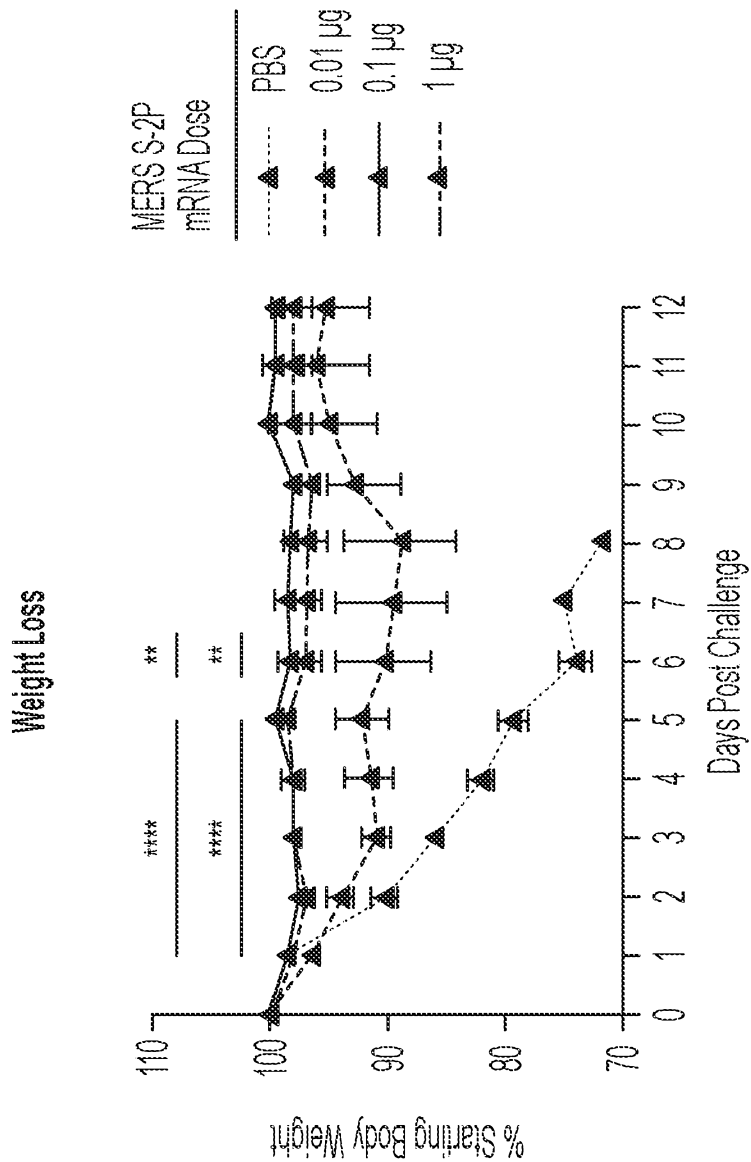


FIG. 6A

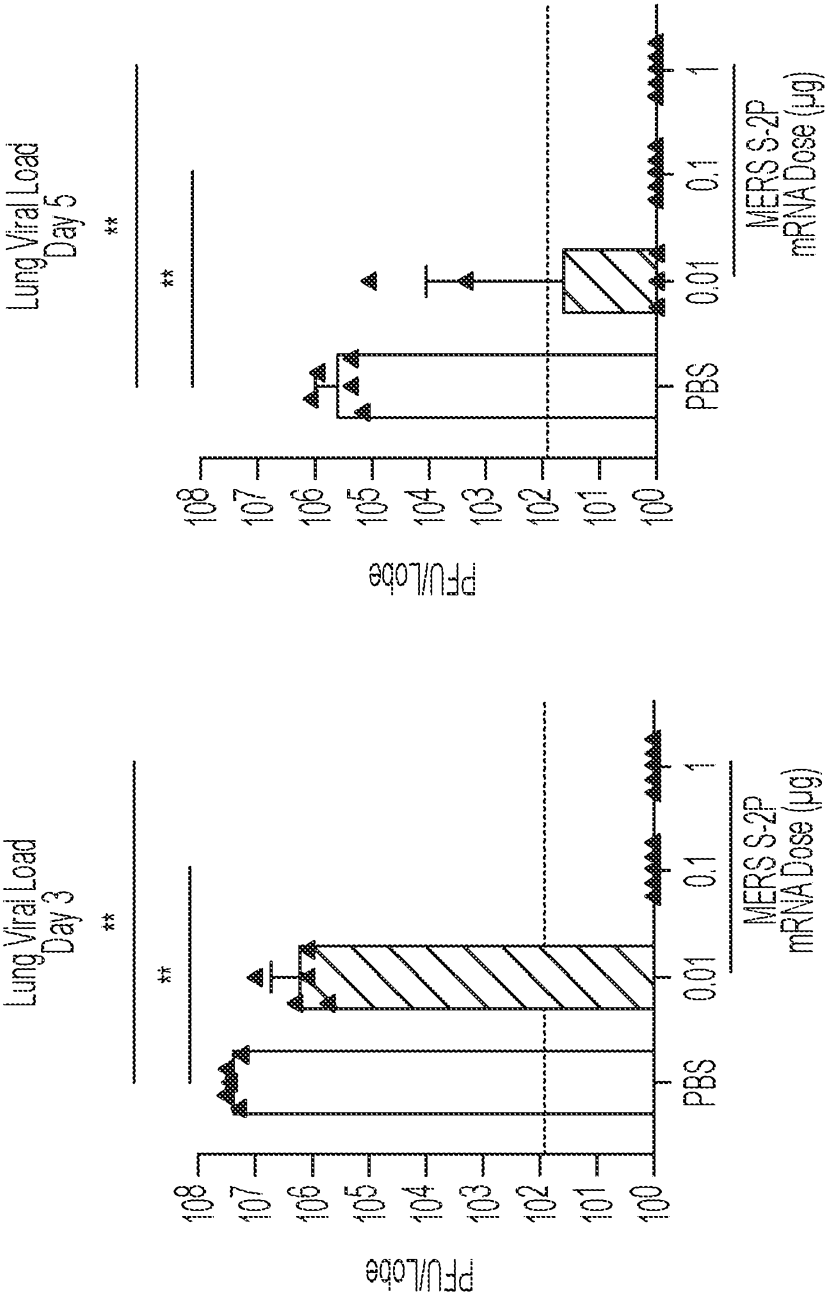


FIG. 6C

FIG. 6B

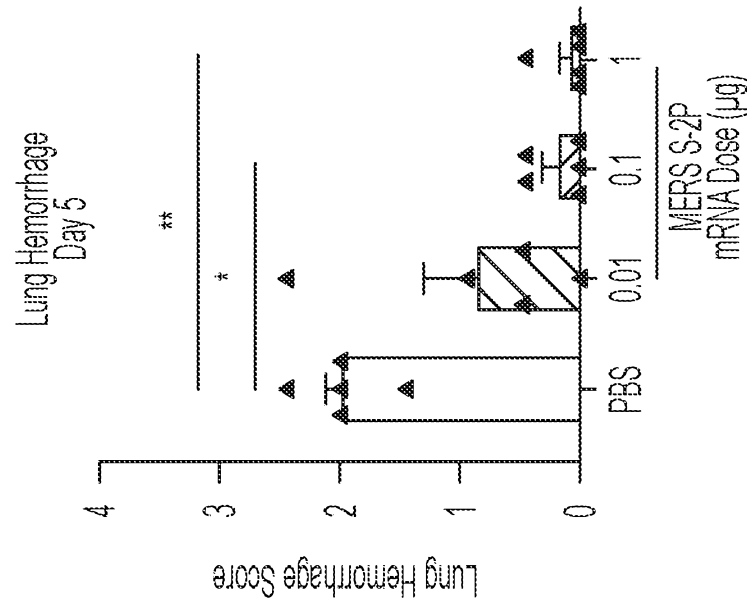


FIG. 6E

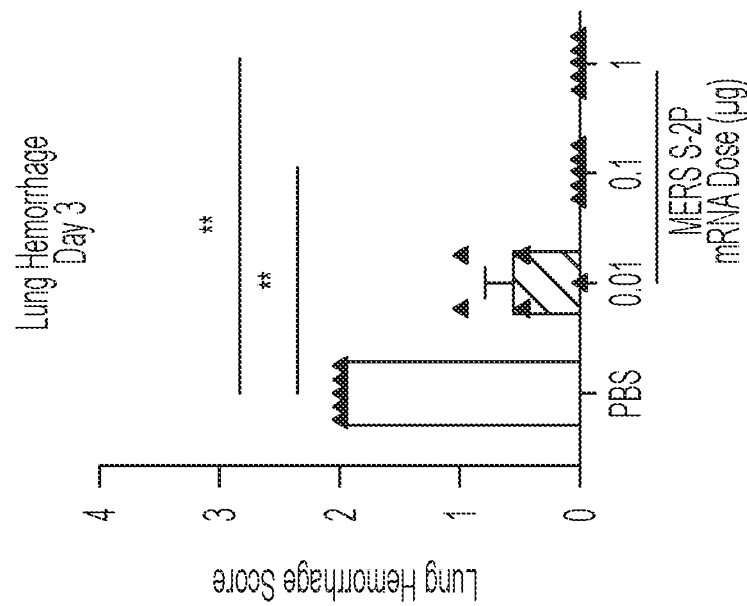


FIG. 6D

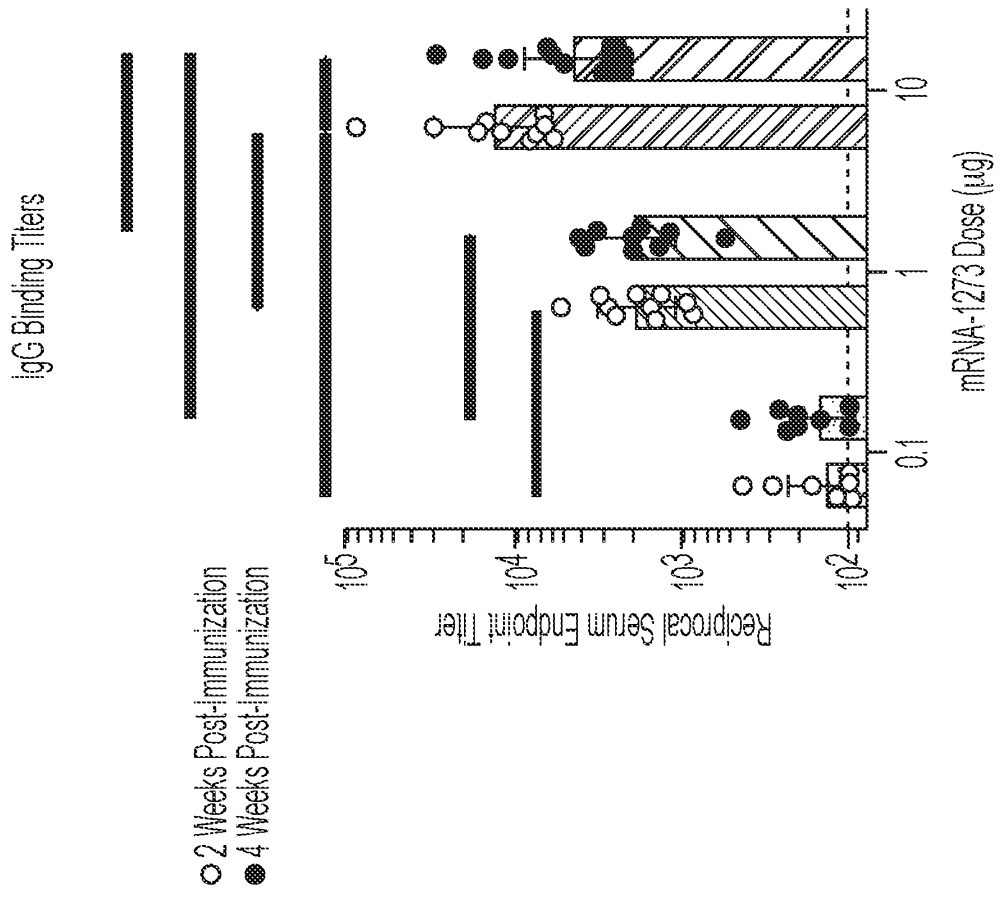


FIG. 7A

10/18

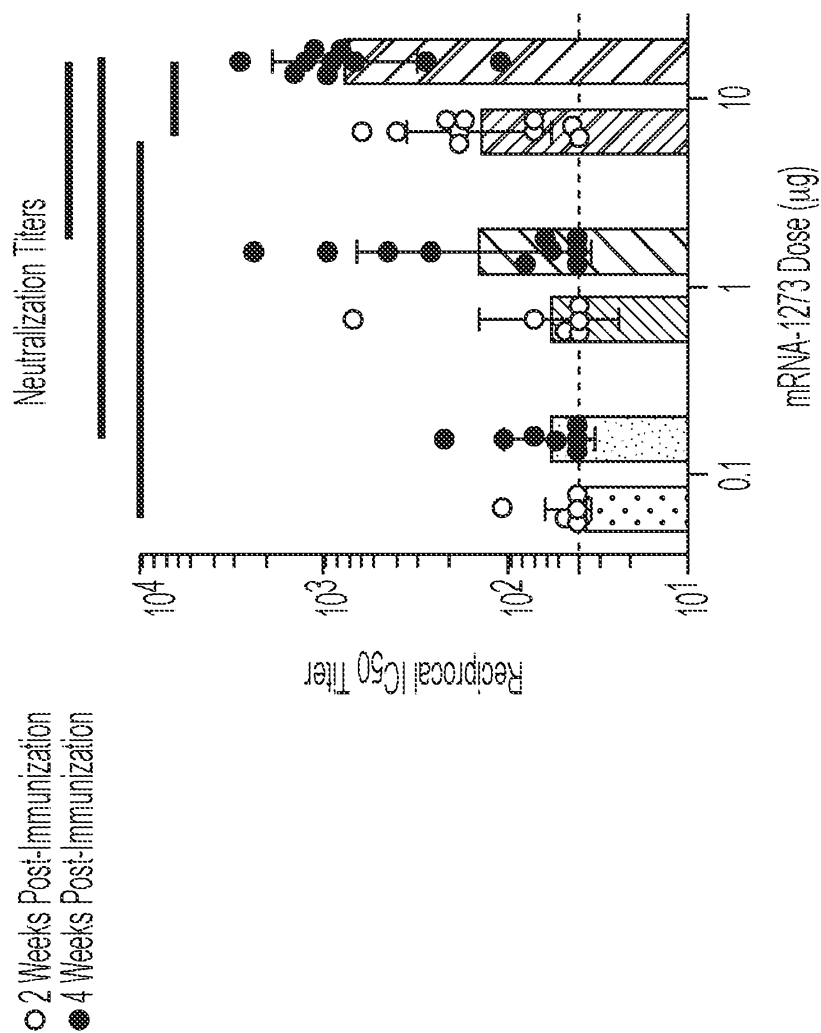


FIG. 7B

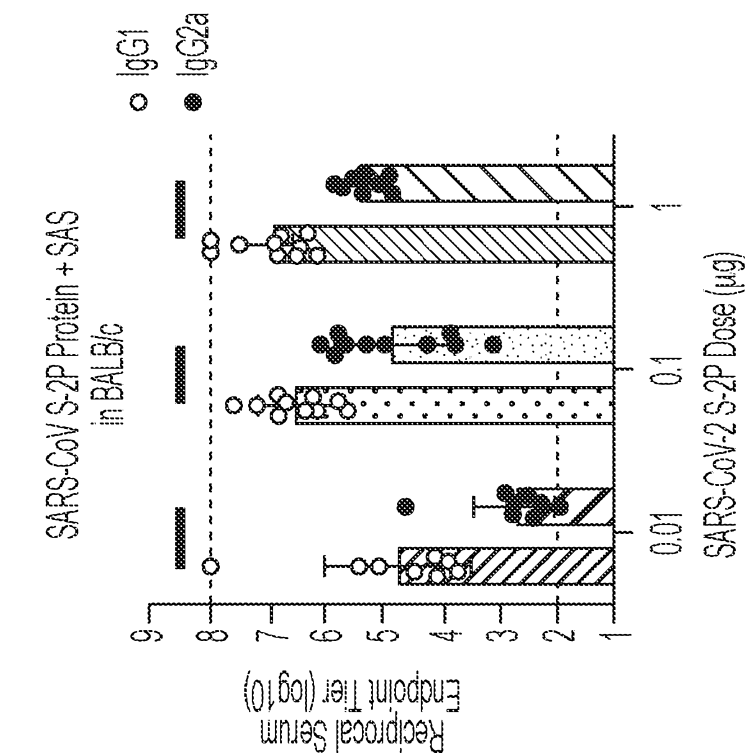


FIG. 8A

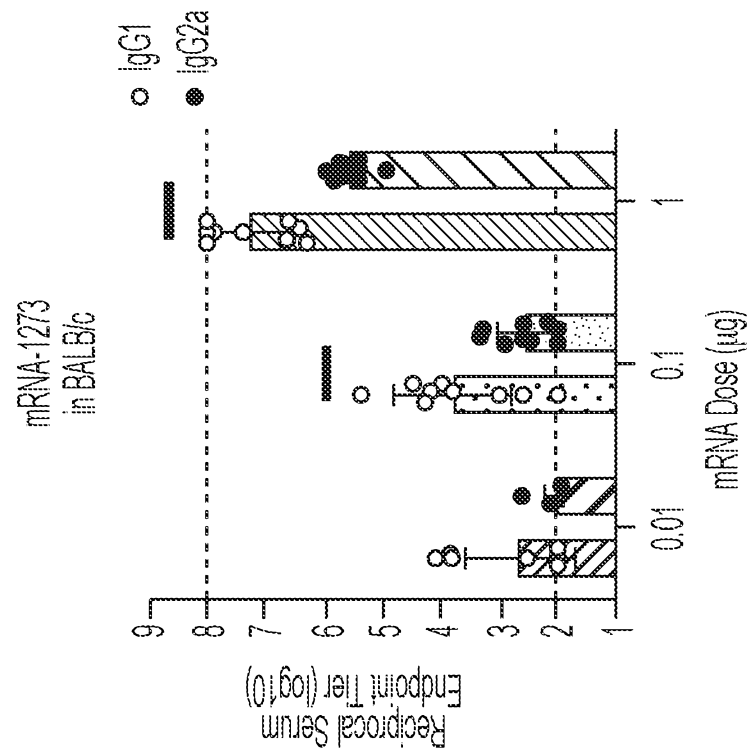


FIG. 8B

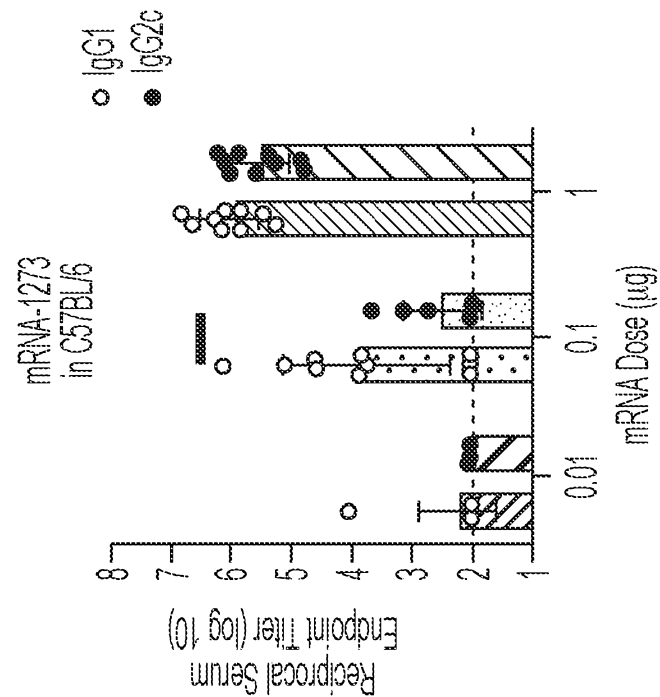


FIG. 8D

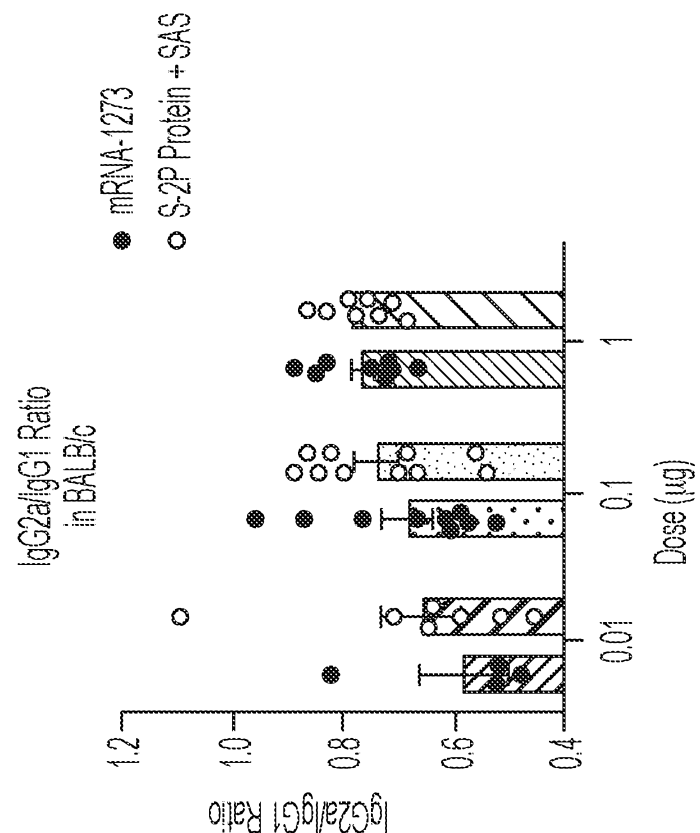
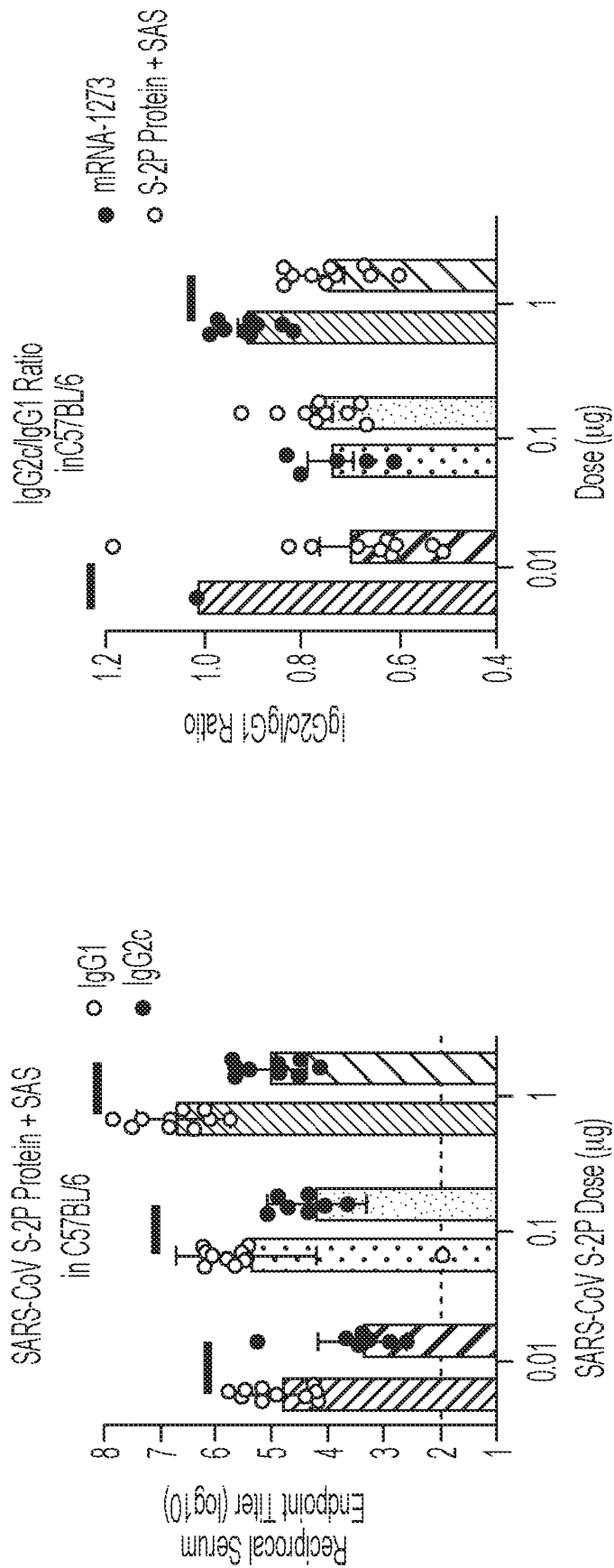


FIG. 8C



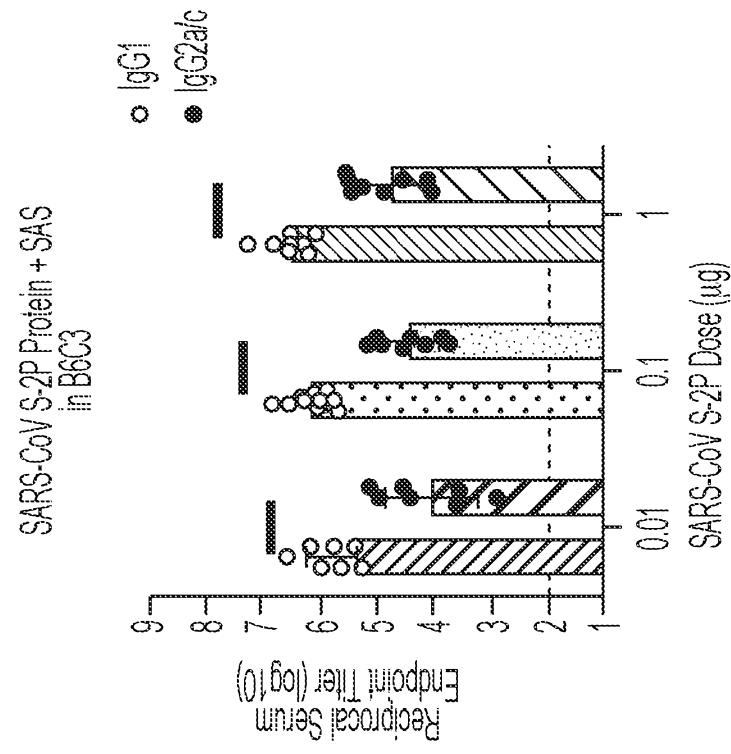


FIG. 8H

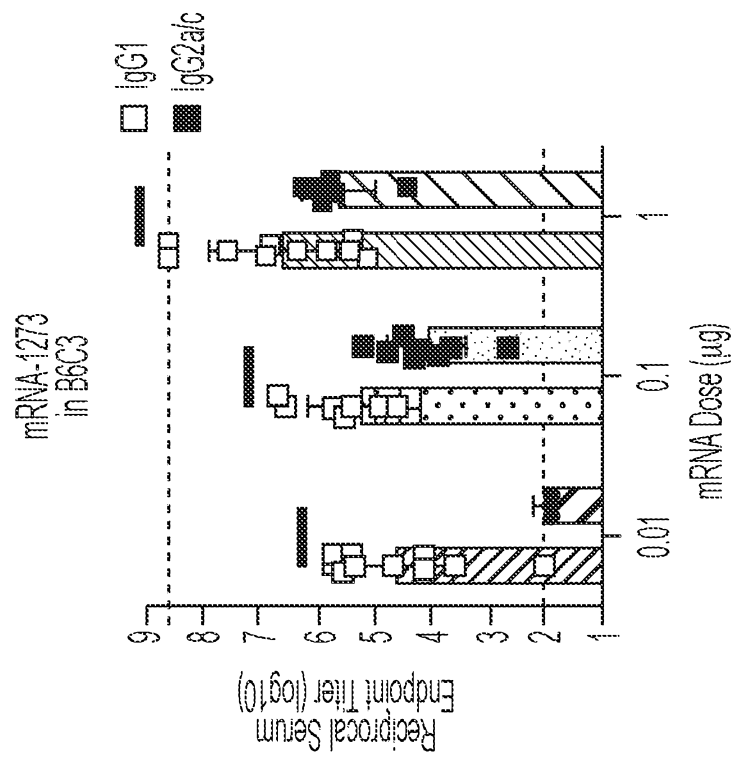


FIG. 8G

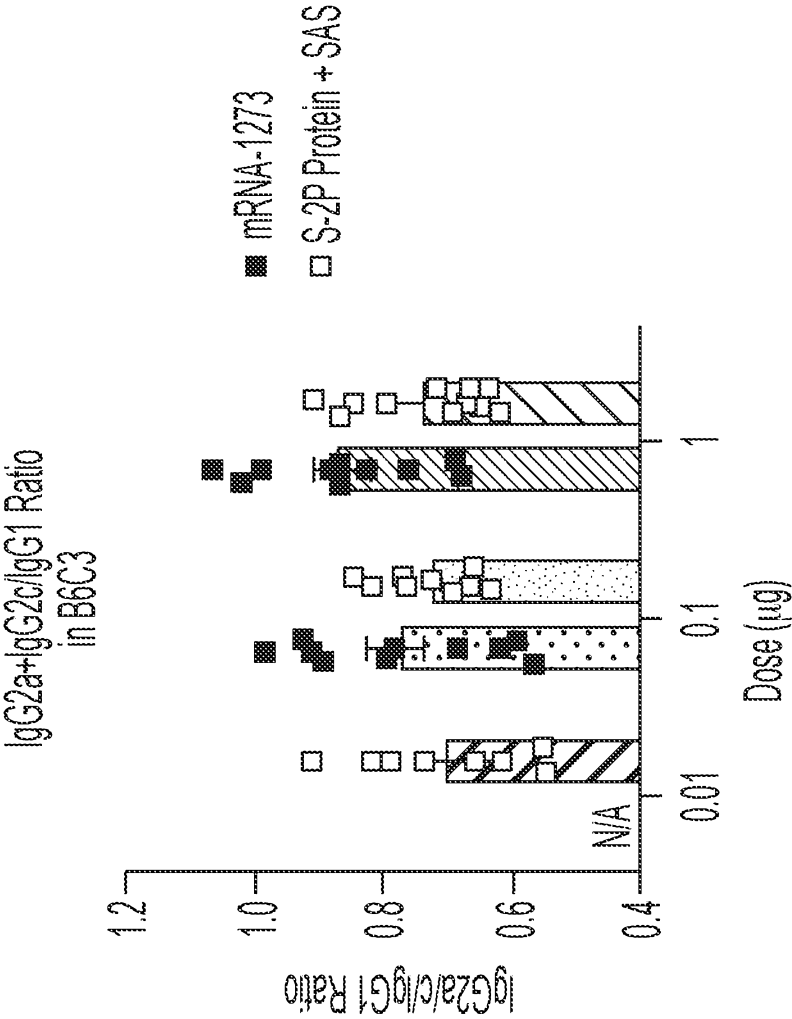


FIG. 8I

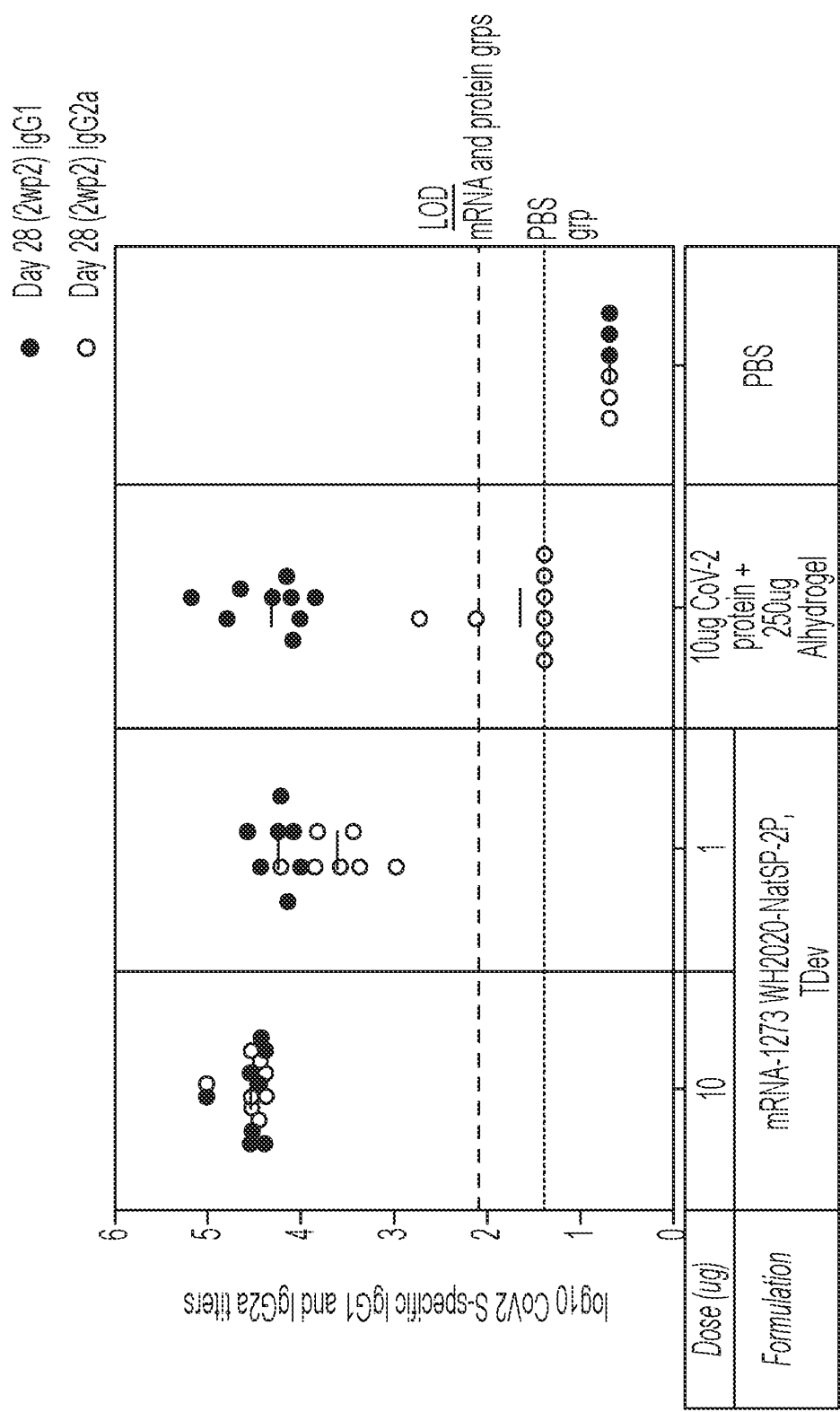


FIG. 9

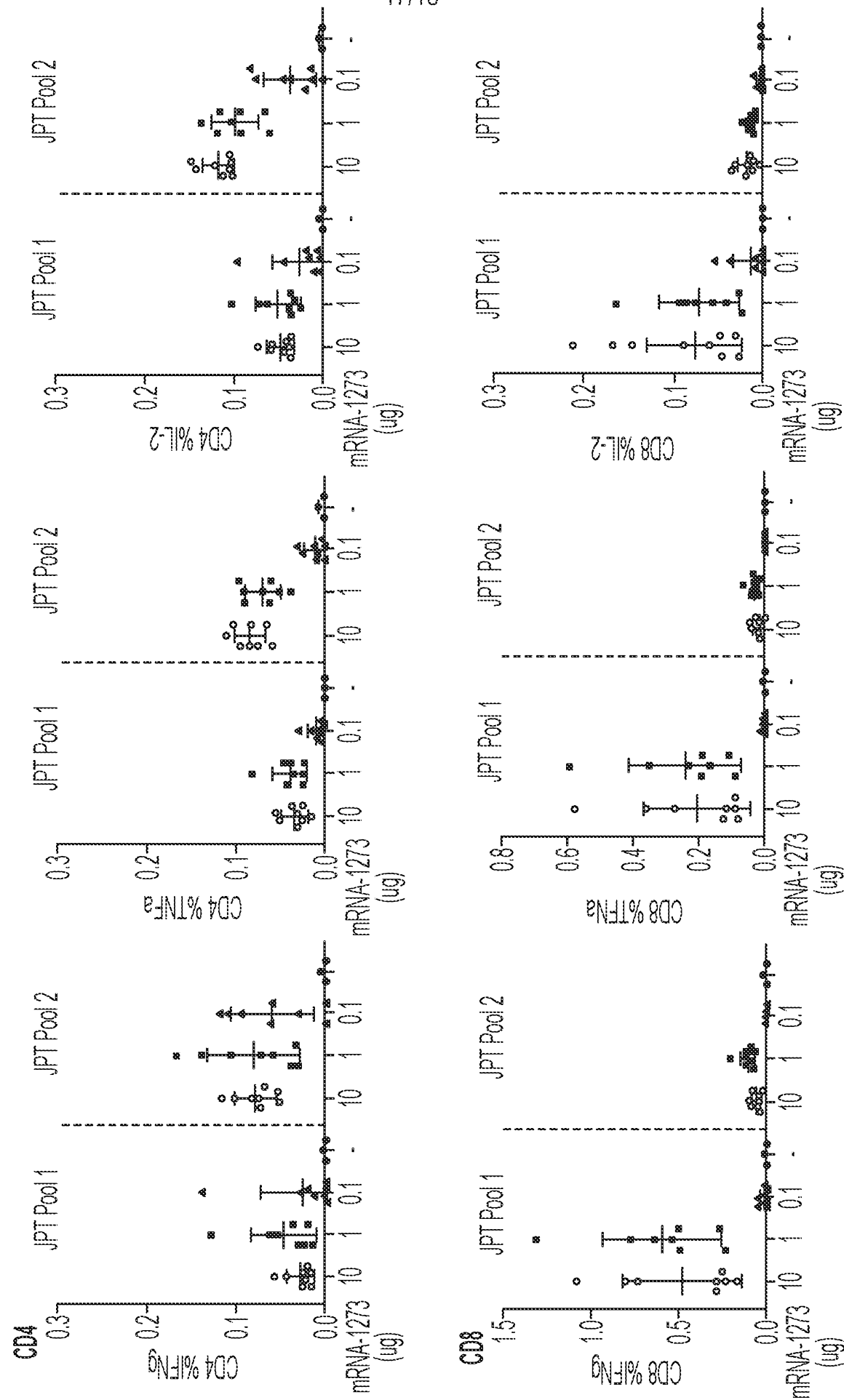


FIG. 10A

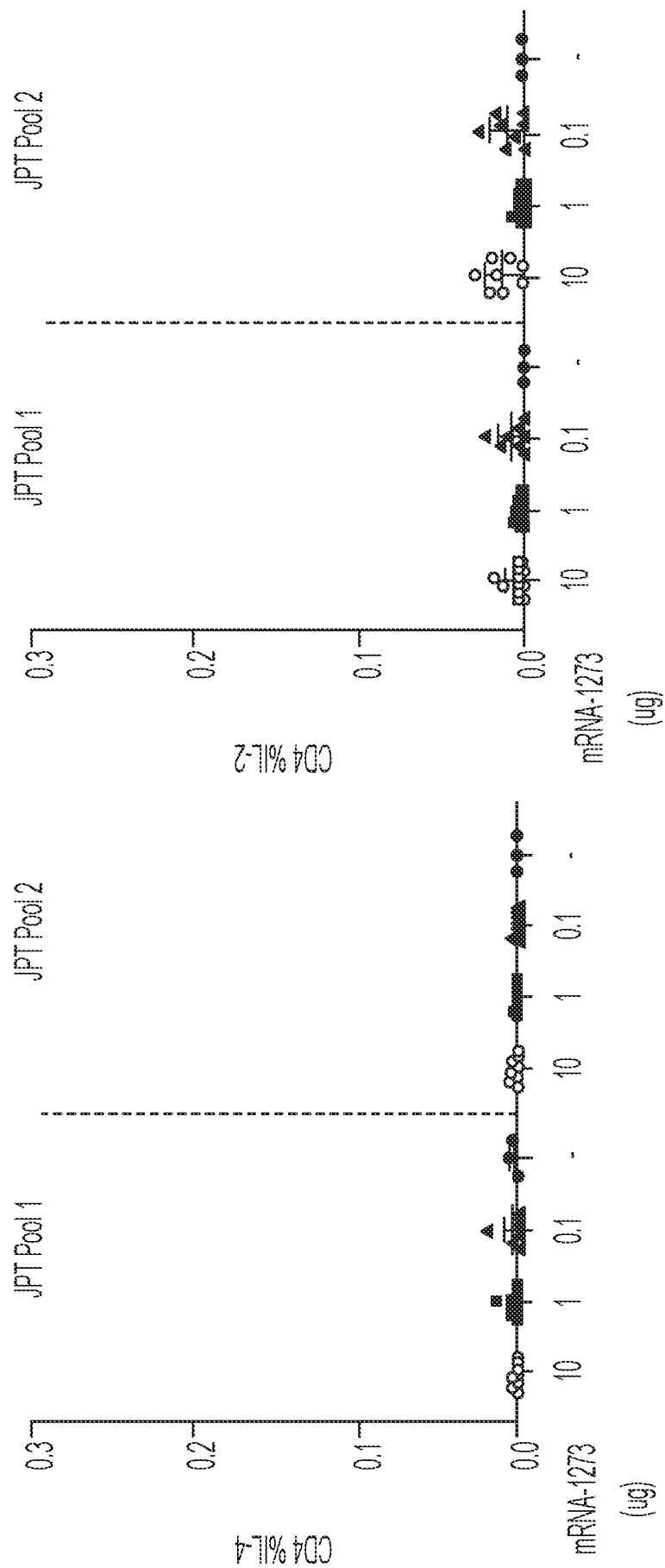


FIG. 10B