

RSV F VACCINE FORMULATIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 63/483,119, which was filed on February 3, 2023. This application is incorporated herein in its entirety for all purposes.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

[0002] The contents of the electronic sequence listing (NOVV_104_01WO_SeqList_ST26.xml; Size: 852,414 bytes; and Date of Creation: February 3, 2024) are herein incorporated by reference in its entirety.

FIELD

[0003] The present disclosure is generally related to RSV F glycoproteins and nanoparticles useful for stimulating immune responses. The nanoparticles provide antigens, for example, glycoprotein antigens, associated with a detergent core and are typically produced using recombinant approaches. The nanoparticles have improved stability and enhanced epitope presentation. The disclosure also provides compositions containing the nanoparticles, methods for producing them, and methods of stimulating immune responses.

BACKGROUND OF THE INVENTION

[0004] Infectious diseases remain a problem throughout the world. While progress has been made on developing vaccines against some pathogens, many remain a threat to human health. Each year respiratory syncytial virus (RSV) causes 6000-10,000 deaths among adults 65 and older and 100-300 deaths in children younger than 5 years old. The development of vaccines to prevent or reduce the severity of life-threatening infectious diseases like RSV is desirable.

SUMMARY OF THE INVENTION

[0005] The present disclosure provides non-naturally occurring respiratory syncytial virus (RSV) fusion (F) glycoproteins suitable for inducing immune responses against respiratory syncytial virus (RSV). The disclosure also provides nanoparticles

containing the glycoproteins as well as methods of stimulating immune responses against RSV.

[0006] Provided herein are RSV F glycoproteins comprising an F1 domain, an F2 domain, and p27; wherein the glycoprotein comprises one or more modifications selected from the group consisting of: (a) deletion of one or more of amino acids 137-146; (b) an inactivated primary furin cleavage site; (c) an inactivated secondary furin cleavage site; (d) S155C; (e) S290C; (f) S190F; (g) V207L; and (h) N116Q; wherein the modifications are numbered according to the RSV F glycoprotein of SEQ ID NO: 330. In embodiments, the RSV F glycoproteins comprise S155C and S290C mutations. In embodiments, the RSV F glycoprotein comprise S190F and V207L mutations. In embodiments, the RSV F glycoproteins comprise S155C, S290C, S190F, and V207L mutations. In embodiments, the RSV F glycoproteins comprise a N116Q mutation. In embodiments, the RSV F glycoproteins comprise an inactivated primary furin cleavage site having the amino acid sequence of KKQKQQ (SEQ ID NO: 342), an S190F mutation, a V207 mutation, and deletion of amino acids 137-146. In embodiments, the RSV F glycoproteins comprise an inactivated primary furin cleavage site having the amino acid sequence of KKQKQQ (SEQ ID NO: 342), an S190F mutation, a V207 mutation, and deletion of amino acids 137-146, wherein the RSV F glycoprotein has at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identity to an RSV F glycoprotein of SEQ ID NO: 337. In embodiments, the RSV F glycoproteins comprise: (i) an F1 domain having an amino acid sequence with at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identity to the F1 domain of SEQ ID NO: 352; and (ii) an F2 domain having an amino acid sequence with at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identity to the F2 domain of SEQ ID NO: 357. In embodiments, the RSV F glycoprotein is at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identical to any one of SEQ ID NOS: 333-340 and 364-366. In embodiments, the RSV F glycoprotein is at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at

least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identical to the RSV F glycoprotein of SEQ ID NO: 337.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007]Fig. 1 shows that binding of the BV2279 glycoprotein to antibodies recognizing the site II (palivizumab), site IV (RSV.42, referred to as “RSV.42.2”), site ϕ (D25), and site VIII (hRSV90) epitopes.

[0008]Fig. 2 shows anti-RSV F IgG titers in cotton rat sera 42 days after immunization with either the BV2279 (labeled “prefusion RSV F Cav1”) or BV1184 (labeled “prefusogenic RSV F”) vaccine compositions of Example 2.

[0009]Fig. 3 shows the RSV neutralization titers against the RSV A (Long) and RSV B (18537) strains after immunization with either the BV2279 (labeled “prefusion RSV F Cav1”) or BV1184 (labeled “prefusogenic RSV F”) vaccine compositions of Example 2.

[0010]Fig. 4 shows competing antibody equivalent titers induced by immunization with either the BV2279 (labeled “prefusion RSV F Cav1”) or BV1184 (labeled “prefusogenic RSV F”) vaccine compositions of Example 2.

[0011]Fig. 5 shows the RSV viral titers in cotton rat tissue four days post viral challenge. The rats were immunized with either the BV2279 (labeled “prefusion RSV F Cav1”) or BV1184 (labeled “prefusogenic RSV F”) vaccine compositions of Example 2.

[0012]Fig. 6 shows transmission electron microscopy (TEM) images of influenza HA detergent core nanoparticles alone (left), saponin adjuvant (i.e., a first iscom particle containing Fraction A of *Quillaja Saponaria* Molina and not Fraction C of *Quillaja Saponaria* Molina, and a second iscom particle containing Fraction C of *Quillaja Saponaria* Molina and not Fraction A of *Quillaja Saponaria* Molina, wherein Fraction A accounts for 85 % of the sum of the weights of fraction A of *Quillaja Saponaria* Molina and fraction C of *Quillaja Saponaria* Molina in the adjuvant, and fraction C of *Quillaja Saponaria* Molina accounts for the remainder) (middle), and Hemagglutinin Saponin Matrix Nanoparticles (HaSMaNs) (right).

[0013]Fig. 7A shows the primary structure of a wild-type SARS-CoV-2 S polypeptide, containing a signal peptide, numbered with respect to SEQ ID NO: 1. **Fig. 7B** shows the primary structure of a wild-type SARS-CoV-2 S polypeptide, without a signal peptide, numbered with respect to SEQ ID NO: 2.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

[0014] As used herein, and in the appended claims, the singular forms “a”, “an”, and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “a protein” can refer to one protein or to mixtures of such protein, and reference to “the method” includes reference to equivalent steps and/or methods known to those skilled in the art, and so forth.

[0015] As used herein, the term “adjuvant” refers to a compound that, when used in combination with an immunogen, augments or otherwise alters or modifies the immune response induced against the immunogen. Modification of the immune response may include intensification or broadening the specificity of either or both antibody and cellular immune responses.

[0016] As used herein, the term “about” or “approximately” when preceding a numerical value indicates the value plus or minus a range of 10%. For example, “about 100” encompasses 90 and 110.

[0017] As used herein, the terms “immunogen,” “antigen,” and “epitope” refer to substances such as proteins, including glycoproteins, and peptides that are capable of eliciting an immune response.

[0018] As used herein, an “immunogenic composition” is a composition that comprises an antigen where administration of the composition to a subject results in the development in the subject of a humoral and/or a cellular immune response to the antigen.

[0019] As used herein, “substantially” refers to isolation of a substance (e.g. a compound, polynucleotide, or polypeptide) such that the substance forms the majority percent of the sample in which it is contained. For example, in a sample, a substantially purified component comprises 85%, preferably 85%-90%, more preferably at least 95%-99.5%, and most preferably at least 99% of the sample. If a component is substantially replaced the amount remaining in a sample is less than or equal to about 0.5% to about 10%, preferably less than about 0.5% to about 1.0%.

[0020] The terms “treat,” “treatment,” and “treating,” as used herein, refer to an approach for obtaining beneficial or desired results, for example, clinical results. For the purposes of this disclosure, beneficial or desired results may include inhibiting or suppressing the initiation or progression of an infection or a disease; ameliorating, or

reducing the development of, symptoms of an infection or disease; or a combination thereof.

[0021] “Prevention,” as used herein, is used interchangeably with “prophylaxis” and can mean complete prevention of an infection or disease, or prevention of the development of symptoms of that infection or disease; a delay in the onset of an infection or disease or its symptoms; or a decrease in the severity of a subsequently developed infection or disease or its symptoms.

[0022] As used herein an “effective dose” or “effective amount” refers to an amount of an immunogen sufficient to induce an immune response that reduces at least one symptom of pathogen infection. An effective dose or effective amount may be determined e.g., by measuring amounts of neutralizing secretory and/or serum antibodies, e.g., by plaque neutralization, complement fixation, enzyme-linked immunosorbent (ELISA), or microneutralization assay.

[0023] As used herein, the term “vaccine” refers to an immunogenic composition, such as an immunogen derived from a pathogen, which is used to induce an immune response against the pathogen that provides protective immunity (e.g., immunity that protects a subject against infection with the pathogen and/or reduces the severity of the disease or condition caused by infection with the pathogen). The protective immune response may include formation of antibodies and/or a cell-mediated response. Depending on context, the term “vaccine” may also refer to a suspension or solution of an immunogen that is administered to a subject to produce protective immunity.

[0024] As used herein, the term “subject” includes humans and other animals. Typically, the subject is a human. For example, the subject may be an adult, a teenager, a child (2 years to 14 years of age), an infant (birth to 2 year), or a neonate (up to 2 months). In particular aspects, the subject is up to 4 months old, or up to 6 months old. In aspects, the adults are seniors about 65 years or older, or about 60 years or older. In embodiments, the subject is five years old or younger. In embodiments, the subject is 65 years old or older. In aspects, the subject is a pregnant woman or a woman intending to become pregnant. In other aspects, subject is not a human; for example a non-human primate; for example, a baboon, a chimpanzee, a gorilla, or a macaque. In certain aspects, the subject may be a pet, such as a dog or cat.

[0025] The term “percent identity” in the context of two or more nucleic acid or polypeptide sequences, refers to two or more sequences or subsequences that have a specified percentage of nucleotides or amino acid residues that are the same, when compared. Unless otherwise indicated, percent identity is calculated over the entire length of the compared sequences using the tool EMBOSS Needle, which is available online at ebi.ac.uk/jdispatcher/psa/emboss_needle. When the percent identity of two polypeptides is compared, the following default parameters are used: Output Format = pair; Matrix = BLOSUM62; Gap Open = 10; Gap Extend 0.5, End Gap = false, End Gap Open = 10, End Gap Extend = 0.5. When the percent identity of two nucleic acids is compared, the following default parameters are used: Output Format = pair; Matrix = DNAfull; Gap Open = 10; Gap Extend 0.5, End Gap = false, End Gap Open = 10, End Gap Extend = 0.5.

[0026] As used herein, the term “vaccine” refers to an immunogenic composition, such as an immunogen derived from a pathogen, which is used to induce an immune response against the pathogen that provides protective immunity (e.g., immunity that protects a subject against infection with the pathogen and/or reduces the severity of the disease or condition caused by infection with the pathogen). The protective immune response may include formation of antibodies and/or a cell-mediated response. Depending on context, the term “vaccine” may also refer to a suspension or solution of an immunogen that is administered to a subject to produce protective immunity.

[0027] In aspects, the subject is immunocompromised. In embodiments, the immunocompromised subject is administered a medication that causes immunosuppression. Non-limiting examples of medications that cause immunosuppression include corticosteroids (e.g., prednisone), alkylating agents (e.g., cyclophosphamide), antimetabolites (e.g., azathioprine or 6-mercaptopurine), transplant-related immunosuppressive drugs (e.g., cyclosporine, tacrolimus, sirolimus, or mycophenolate mofetil), mitoxantrone, chemotherapeutic agents, methotrexate, tumor necrosis factor (TNF)-blocking agents (e.g., etanercept, adalimumab, infliximab). In embodiments, the immunocompromised subject is infected with a virus (e.g., human immunodeficiency virus or Epstein-Barr virus). In embodiments, the virus is a respiratory virus, such as respiratory syncytial virus, influenza, parainfluenza, adenovirus, or a picornavirus. In embodiments, the immunocompromised subject has acquired immunodeficiency syndrome (AIDS). In embodiments, the

immunocompromised subject is a person living with human immunodeficiency virus (HIV). In embodiments, the immunocompromised subject is immunocompromised due to a treatment regimen designed to prevent inflammation or prevent rejection of a transplant. In embodiments, the immunocompromised subject is a subject who has received a transplant. In embodiments, the immunocompromised subject has undergone radiation therapy or a splenectomy. In embodiments, the immunocompromised subject has been diagnosed with cancer, an autoimmune disease, tuberculosis, a substance use disorder (e.g., an alcohol, opioid, or cocaine use disorder), stroke or cerebrovascular disease, a solid organ or blood stem cell transplant, sickle cell disease, thalassemia, autoimmune lymphoproliferative syndrome (ALPS), autoimmune polyglandular syndrome type 1 (APS-1), B-cell expansion with NF- κ B and T-cell anergy (BENTA) disease, caspase eight deficiency state (CEDS), chronic granulomatous disease (CGD), common variable immunodeficiency (CVID), congenital neutropenia syndromes, a deficiency in the cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), a DOCK8 deficiency, a GATA2 deficiency, a glycosylation disorder with immunodeficiency, a hyper-immunoglobulin E syndrome (HIES), hyper-immunoglobulin M syndrome, diabetes, type 1 diabetes, type 2 diabetes, interferon gamma deficiency, interleukin 12 deficiency, interleukin 23 deficiency, leukocyte adhesion deficiency, lipopolysaccharide-responsive beige-like anchor (LRBA) deficiency, PI3 kinase disease, PLCG2-associated antibody deficiency and immune dysregulation (PLAID), severe combined immunodeficiency (SCID), STAT3 dominant-negative disease, STAT3 gain-of-function disease, warts, hypogammaglobulinemia, infections, and myelokathexis (WHIM) syndrome, Wiskott-Aldrich syndrome (WAS), X-linked agammaglobulinemia (XLA), X-linked lymphoproliferative disease (XLP), uremia, malnutrition, or XMEN disease. In embodiments, the immunocompromised subject is a current or former cigarette smoker. In embodiments, the immunocompromised subject has a B-cell defect, T-cell defect, macrophage defect, cytokine defect, phagocyte deficiency, phagocyte dysfunction, complement deficiency or a combination thereof.

[0028] In embodiments, the subject is overweight or obese. In embodiments, an overweight subject has a body mass index (BMI) ≥ 25 kg/m² and < 30 kg/m². In embodiments, an obese subject has a BMI that is ≥ 30 kg/m². In embodiments, the

subject has a mental health condition. In embodiments, the mental health condition is depression, schizophrenia, or anxiety.

[0029] As used herein, the term "pharmaceutically acceptable" means being approved by a regulatory agency of a U.S. Federal or a state government or listed in the U.S. Pharmacopeia, European Pharmacopeia or other generally recognized pharmacopeia for use in mammals, and more particularly in humans. These compositions can be useful as a vaccine and/or antigenic compositions for inducing a protective immune response in a vertebrate.

[0030] As used herein, the term "modification" as it refers to an RSV F glycoprotein refers to mutation, deletion, or addition of one or more amino acids of the RSV F glycoprotein. The location of a modification within an RSV F glycoprotein can be determined based by aligning the sequence of the RSV F glycoprotein to a native RSV F glycoprotein (e.g., SEQ ID NO: 330 (an RSV F glycoprotein containing a signal peptide) or SEQ ID NO: 329 (a mature RSV F glycoprotein lacking a signal peptide). The location of a modification within a SARS-CoV-2 Spike (S) glycoprotein (alternatively referred to herein as a "CoV S glycoprotein") can be determined by aligning the sequence of the polypeptide to SEQ ID NO: 1 (CoV S glycoprotein containing signal peptide) or SEQ ID NO: 2 (mature CoV S glycoprotein lacking a signal peptide). The sequences of the native RSV F glycoproteins having the amino acid sequences of SEQ ID NO: 329 and SEQ ID NO: 330 and the native CoV S glycoproteins having the amino acid sequences of SEQ ID NO: 1 and SEQ ID NO: 2 are found in the table below.

Sequence	SEQ ID NO:
QNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKENKCNG TDAKVKLIKQELDKYKNAVTELQLLMQSTPPTNNRARRELPRFMN YTLNNAKKTNTVLSKKRKRRFLGFLGVSASGVAVSKVLHLEG EVNKIKSALLSTNKAVVSLNNGVSVLTSKVLDLKNYIDKQLLPVNVK QSCSISNIETVIEFQQKNNRLEITREFSVNAGVTTPVSTYMLTNSE LLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMSIIKEEVLAYVVQL PLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDGRGWYCDNAGS VSFFPQAETCKVQSNRVFCDTMNSLTLPSEINLCNVDFNPKYDC KIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIIKTFSNGCD YVSNKGMDTVSVGNTLYYVNVKQEGKSLYVKGEPIINFYDPLVFPS DEFDASISQVNEKINQSLAFIRKSDELLHNVNAGKSTTNIMITIIIVII VILLSLIAVGLLLYCKARSTPVTLSKDQLSGINNIAFSN	329
MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSAL RTGWYTSVITIELSNIKENKCNGTDAKVKLIKQELDKYKNAVTELQ LMQSTPPTNNRARRELPRFMNYTLNNAKKTNTVLSKKRKRRFLG	330

Sequence	SEQ ID NO:
FLLGVGSAIASGVAVSKVLHLEGEVNIKISALLSTNKAVVSLSNGV SVLTSKVLDLKNIYIDKQLLPVNVKQSCSISNIETVIEFQQKNNRLEI TREFSVNAGVTPPVSTYMLTNSSELLSLINDMPITNDQKKLMSNNV QIVRQQSYSIMSIIKEEVLAYVVQLPLYGVIDTPCWKLHTSPLCTTN TKEGSNICLTRTDGRGWYCDNAGSVSFFPQAETCKVQSNRVFCDT MNSLTLPLSEINLCNVDIFNPKYDCKIMTSKTDVSSSVITSLGAIVSC YGKTKCTASNKNRGIKTFNSNGCDYVSNKGMDTVSVGNTLYYVVK QEGKSlyVKGEPIINFYDPLVFPSPDEFDASISQVNEKINQSLAFIRK SDELLHNVNAGKSTTNIMITTHIIVIVILLSLIAVGLLLYCKARSTPVTL SKDQLSGINNIAFSN	
MFVFLVLLPLVSSQCVNLTTTRTQLPPAYTNSFTRGVYYPDKVFRS SVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGV YFASTEKSNIIRGWIFGTTLDSKTQSLNIVNATNVVIKVCEFQFCN DPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEG KQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLV DLPIGINITRFQTLALHRSYLTTPGDSSSGWTAGAAAYYVGYLQPR TFLLKYNNGTITDAVDCALDPLSETKCTLKSTVEKGIYQTSNFR VQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRRKRISNCVADY SVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQA PGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNLYRLF RKSNNLKPFERDISTEIYQAGSTPCNGVEGFNCFYPLQSYGFQPTN GVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNG LTGTGVLTESNKKFLPFQFGRDIADTTDAVRDPQTLEILDITPCSF GGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYS TGSNVFQTRAGCLIGAHEVNNSECDIPIGAGICASYQTQTNSPR RARSVASQSIIAYTMSLGAENSVAYSNNNSIAIPTNFTISVTTEILPVS MTKTSVDCTMYICGDSTECNLLLQYGSFCTQLNRALTGIAVEQD KNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLL FNKVTLADAGFIKQYGDCLGDIAARDLCAQKFNGTLVLPPLLTDE MIAQYTSALLAGTITSGWTFGAGAAALQIPFAMQMAYRFNGIGVTQ NVLYENQKLIANQFNSAIGKIQDSLSTASALGKLQDVVNQNAQAL NTLVKQLSSNFGAISSVLNDILSRDLKVEAEVQIDRLITGRLQSLQT YVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMS FPQSAPHGVVFLHVTVYVPAQEKNFTTAPAICHGKAHFPREGVVFV SNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQ PELDSFKEELDKYFKNHTSPDVLGDIGINASVVNIQKEIDRLNEV AKNLNESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCC MTSCCCLKGCCSCGSCCKFDEDDSEPVLKGVKLHYT	1
QCVNLTTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPF FSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRG WIFGTTLDSKTQSLNIVNATNVVIKVCEFQFCNDPFLGVYYHKNN KSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFV FKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTL LALHRSYLTTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNNGTIT DAVDCALDPLSETKCTLKSTVEKGIYQTSNFRVQPTESIVRFPNIT	2

Sequence	SEQ ID NO:
NLCPFGEVFNATRFASVYAWNRRKRISNCVADYSVLYNSASFSTFK CYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNY KLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDI STEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVV LSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNK KFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNT SNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAG CLIGAETHVNNSECDIPIGAGICASYQTQTNPRRARSVASQSIIAY TMSLGAENSVAYSNNIAIPTNFTISVTTEILPVSMTKTSVDCTMYI CGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVK QIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLDAGFI KQYGDCLGDIAARDLICAQKFNGLTVLPPLTDEMIAQYTSALLAG TITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIAN QFNSAIGKIQDSLSTASALGKLQDVVNQNAQALNTLVKQLSSNF GAISSVLNDILSRDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEI RASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVV FLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGTHWFVT QRNFYEPQIITDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEE LDKYFKNHTSPDVLGDISGINASVVNIQKEIDRLNEVAKNLNESLI DLQELGKYEQYIKWPWYIWLGFIAGLIAIVMTIMLCCMTSCCSCL KGCCSCGSCCKFDEDDSEPVKGVKLHYT	

[0031] The term “efficacy” of an immunogenic composition or vaccine composition described herein refers to the percentage reduction of infection (e.g., with an RSV virus) in a group administered an immunogenic composition as compared to a group that is not administered the immunogenic composition. In embodiments, efficacy (E) is calculated using the following equation: $E\% = (1 - RR) \times 100$, where RR = relative risk of incidence rates between the group administered the immunogenic composition and the group that is not administered the immunogenic composition. In embodiments, immunogenic compositions described herein have an efficacy against an RSV virus that is at least about 50 %, at least about 55 %, at least about 60 %, at least about 65 %, at least about 70 %, at least about 75 %, at least about 80 %, at least about 85 %, at least about 90 %, at least about 91 %, at least about 92 %, at least about 93 %, at least about 94 %, at least about 95 %, at least about 96 %, at least about 97 %, at least about 98 %, at least about 99 %, between about 50 % and about 99 %, between about 50 % and about 98 %, between about 60 % and about 99 %, between about 60 % and about 98 %, between about 70 % and about 98 %, between about 70 % and about 95 %, between about 70 % and about 99 %, between about 80 % and about 99

%, between about 80 % and about 98 %, between about 80 % and about 95 %, between about 85 % and about 99 %, between about 85 % and about 98 %, between about 85 % and about 95 %, between about 90 % and about 95 %, between about 90 % and 98 %, or between about 90 % and about 99 %.

[0032] The term SARS-CoV-2 “variant”, used interchangeably herein with a “heterogeneous SARS-CoV-2 strain,” refers to a SARS-CoV-2 virus comprising a CoV S polypeptide having one or more modifications as compared to a SARS-CoV S polypeptide having the amino acid sequence of SEQ ID NO: 2. For example, a SARS-CoV-2 variant may have at least about 2, at least about 3, at least about 4, at least about 5, at least about 6, at least about 7, at least about 8, at least about 9, at least about 10, at least about 11, at least about 12, at least about 13, at least about 14, at least about 15, at least about 16, at least about 17, at least about 18, at least about 19, at least about 20, at least about 21, at least about 22, at least about 23, at least about 24, at least about 25, at least about 26, at least about 27, at least about 28, at least about 29, at least about 30, at least about 31, at least about 32, at least about 33, at least about 34, or at least about 35 modifications, as compared to a CoV S polypeptide having the amino acid sequence of SEQ ID NO: 2. For example, a SARS-CoV-2 variant may have at least one and up to 2, up to 3, up to 4, up to 5, up to 6, up to 7, up to 8, up to 9, up to 10, up to 11, up to 12, up to 13, up to 14, up to 15, up to 16, up to 17, up to 18, up to 19, up to 20, up to 21, up to 22, up to 23, up to 24, up to 25, up to 26, up to 27, up to 28, up to 29, up to 30, up to 31, up to 32, up to 33, up to 34, up to 35 modifications, up to 40 modifications, up to 45 modifications, up to 50 modifications, up to 55 modifications, up to 60 modifications, up to 65 modifications, up to 70 modifications, up to 75 modifications, up to 80 modifications, up to 85 modifications, up to 90 modifications, up to 95 modifications, or up to 100 modifications as compared to a CoV S polypeptide having the amino acid sequence of SEQ ID NO: 2. In aspects, a SARS-CoV-2 variant may have between about 2 and about 35 modifications, between about 5 and about 10 modifications, between about 5 and about 20 modifications, between about 10 and about 20 modifications, between about 15 and about 25 modifications, between about 20 and 30 modifications, between about 20 and about 40 modifications, between about 25 and about 45 modifications, between about 25 and about 100 modifications, between about 25 and about 45 modifications, or between about 35 and about 100 modifications, as compared to a CoV S polypeptide having the amino acid sequence of SEQ ID NO: 2.

[0033] As used herein, the terms "intranasal administration" and "nasal administration" refer to administration of an immunogenic composition described herein to the nasal cavity of a subject. In embodiments, intranasal administration is achieved using a liquid preparation (e.g., an aqueous preparation), an aerosolized preparation, or a dry powder preparation. In embodiments, the liquid preparation, aerosolized preparation, or dry powder preparation is administered via an externally propelled nasal delivery device. In embodiments, the liquid preparation, aerosolized preparation, or dry powder preparation is administered via a self-propelled (i.e., via inhalation) nasal delivery device. In embodiments, the liquid preparation, aerosolized preparation, or dry powder preparation is administered via nasal insufflation (when an immunogenic composition is blown into the nose) or nasal instillation (when an immunogenic composition is dropped into the nose). In embodiments, the liquid preparation, aerosolized preparation, or dry powder preparation is administered via a gel, cream, ointment, lotion, or paste applied to one or more nasal epithelium (e.g., olfactory epithelium or nasal respiratory epithelium). In embodiments, the immunogenic composition is applied to mucus in the nasal cavity of a subject.

[0034] As used herein, the term "non-invasive nasal delivery device" refers an instrument that is capable of delivering an immunogenic composition to the nasal cavity without piercing the epithelium of the subject. Non-limiting examples of non-invasive nasal delivery devices include propellant (e.g., a pressurized inhaler) and non-propellant (e.g., a pump-type inhaler) types of aerosol or atomizer devices, particle dispersion devices, nebulizers, and pressurized olfactory delivery devices for delivery of liquid or powder formulations.

[0035] As used herein, the term "dry powder composition" refers to a lyophilized or spray dried form of an immunogenic composition described herein. In embodiments, a dry powder composition contains less than 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less residual water content.

[0036] As used herein, the term "permeability enhancer" refers to a component of an immunogenic composition formulated for intranasal administration which promotes the passage of a viral glycoprotein through the nasal epithelium.

[0037] As used herein, the terms "bedside mix," "bedside formulations," "bedside vaccine compositions," "bedside vials," "bedside vial formulations" refer to vaccine formulations that are prepared immediately prior to administration. Such vaccine formulations contain viral antigens and adjuvants that are separately stored in different

containers and are administered to a subject (e.g., either administering two consecutive injections, or combining the antigens and the adjuvants into one injection prior to administration).

[0038] As used herein, the terms “co-formulation mix,” “co-formulation,” “co-formulation immunogenic compositions,” “prefilled syringes,” “pre-mix,” refer to vaccine formulations that are prepared for short to long-term storage prior to the time of administration to a subject. Such vaccine formulations contain a combination of antigens and adjuvant in the same container and prepared in advance of administration. In embodiments, formulations contain hemagglutinin and adjuvant (e.g., saponin adjuvant) and form HaSMaNs (Hemagglutinin Saponin Matrix Nanoparticles).

[0039] As used herein, the term “split-virion” refers to a virus (e.g., an influenza virus or a SARS-CoV-2 virus), which has a viral membrane that has been disrupted with a surfactant. Examples of surfactants are described throughout this disclosure. Split-virions do not undergo further purification, so they typically contain multiple viral proteins.

[0040] As used herein, the term “recombinant” as it refers to a protein (e.g. hemagglutinin) that is produced in a cell by transcription and translation of a nucleic acid that is introduced into a cell. The nucleic acid may be introduced via a vector or a virus encoding the nucleic acid.

[0041] As used herein, the term “whole influenza virus” refers to a virus that comprises all of its envelope, viral membrane, nucleocapsid, and genetic material. In embodiments, the whole influenza virus is inactivated.

[0042] As used herein, the term “inactivated virus” refers to a virus that has undergone treatment to substantially reduce or eliminate its virulence compared to the wild-type virus.

[0043] The term “bronchoalveolar lavage,” also referred to as “BAL” refers to a fluid sample retrieved from a patient’s lungs. In embodiments, the BAL is retrieved during a bronchoscopy. During a bronchoscopy, a bronchoscope containing a solution (e.g., saline) is passed through the mouth or nose into the lungs. The solution is subsequently collected from the lungs.

[0044] The term “mucosal immunity” refers to the cellular and humoral immune response that occurs in mucosal membranes. In embodiments, the methods of the disclosure provided herein result in mucosal immunity in the respiratory system.

[0045] The term “atomization” refers to the generation of fine, inhalable droplets of a liquid. The typical dimensions of atomized droplets are in the range of several microns.

[0046] The term “aerosol” refers to a dispersion of solid or liquid particles in a gas phase. In embodiments, the gas phase is air.

Immunogenic Compositions and Vaccine Compositions Containing RSV F glycoproteins

[0047] The disclosure provides non-naturally occurring RSV F glycoproteins, nanoparticles containing RSV F glycoproteins, and immunogenic compositions and vaccine compositions containing either non-naturally occurring RSV F glycoproteins or nanoparticles containing RSV F glycoproteins. In embodiments, provided herein are methods of using RSV F glycoproteins, nanoparticles comprising the same, immunogenic compositions comprising RSV F glycoproteins and/or nanoparticles, and vaccine compositions to stimulate an immune response against an RSV virus.

[0048] Also provided herein are methods of manufacturing the nanoparticles and vaccine compositions. Advantageously, the methods provide nanoparticles that are substantially free from contamination by other proteins, such as proteins associated with recombinant expression of proteins in insect cells. In embodiments, expression occurs in baculovirus/Sf9 systems.

Non-naturally occurring RSV F Glycoprotein Antigens

[0049] In embodiments, provided herein are RSV F glycoproteins that have one or more modifications compared to a native (also referred to as “wild-type”) RSV F glycoprotein. The wild-type RSV F glycoprotein is synthesized as a single-chain inactive precursor called F0 that contains three subunits: F1, F2, and a 27-amino acid glycopeptide called pep27 (“p27”). The F1 subunit is located at amino acids 137-574 of the RSV F glycoprotein of SEQ ID NO: 330. The F2 subunit is located at amino acids 26-110 of the RSV F glycoprotein of SEQ ID NO: 330. p27 is located at amino acids 111-136 of the RSV F glycoprotein of SEQ ID NO: 330. The C-terminal F1 subunit contains a transmembrane domain, two heptad repeats, and an N-terminal fusion peptide. The F0 precursor is cleaved by a furin-like protease to form the mature, fusion competent protein. The mature RSV F protein contains an F1 subunit and an F2 subunit connected by a disulfide bond. Additional sources that describe the RSV F protein structure are found at Swanson et al. A Monomeric Uncleaved Respiratory Syncytial Virus F Antigen Retains Prefusion-Specific Neutralizing Epitopes. Journal of

Virology, 2014, 88, 11802–11810. Jason S. McLellan et al. Structure of RSV Fusion Glycoprotein Trimer Bound to a Prefusion-Specific Neutralizing Antibody. Science, 2013, 340, 1113–1117.

[0050] In embodiments, an RSV F glycoprotein from the wild type RSV F A2 strain is the native RSV F glycoprotein. The RSV F glycoprotein from the wild type RSV F A2 strain comprises the amino acid sequence of SEQ ID NO: 330. The wild type RSV F A2 strain has sequencing errors (A102P, V379I, V447M). These sequencing errors are corrected in the non-naturally occurring RSV F glycoproteins described herein, i.e., amino acid 102 is alanine, amino acid 379 is valine, and amino acid 447 is valine.

[0051] In embodiments, the RSV F glycoproteins have one or more modifications selected from the group consisting of:

- (a) deletion of from 1 to 10 amino acids of the fusion peptide;
- (b) inactivation of the primary furin cleavage site (also referred to as Site II) through mutation of one or more of amino acids 131-136;
- (c) inactivation of the secondary furin cleavage site (also referred to as Site I) through mutation of one or more of amino acids 106-109;
- (d) S155C;
- (e) S290C;
- (f) S190F;
- (g) V207L; and
- (h) N116Q;

wherein the modifications are numbered according to the RSV F glycoprotein of SEQ ID NO: 330.

[0052] In embodiments, the RSV F glycoproteins have one or more modifications selected from the group consisting of:

- (a) deletion of from 1 to 10 amino acids of the fusion peptide;
- (b) inactivation of the primary furin cleavage site (also referred to as Site II) through mutation of one or more of amino acids 131-136;
- (c) inactivation of the secondary furin cleavage site (also referred to as Site I) through mutation of one or more of amino acids 106-109;
- (d) S155C;
- (e) S290C;

(f) S190F; and

(g) V207L;

wherein the modifications are numbered according to the RSV F glycoprotein of SEQ ID NO: 330.

[0053] In embodiments, the RSV F glycoproteins described herein exhibit one or more of the antigenic sites Ø, II, IV, and V. In embodiments, the RSV F proteins described herein exhibit mutations, which result in the presence of one or more of the RSV F antigenic sites Ø, II, IV, and V. The RSV F antigenic site Ø, which is located at the membrane-distal apex of the prefusion RSV F trimer, is targeted by potent RSV F neutralizing antibodies, including 5C4, AM22, and D25. The RSV F antigenic site II, also referred to herein as the palivizumab site, is the target of the antibodies palivizumab and motavizumab. Antigenic site II is composed of a helix-turn-helix motif that spans residues 253-278 of the RSV F glycoprotein of SEQ ID NO: 330. The RSV F antigenic site IV is located on the F1 subunit of the RSV F glycoprotein and encompasses residues 422-471 of the RSV F glycoprotein of SEQ ID NO: 330. The RSV F antigenic site V is composed of α 2- α 3 and β 3- β 4.

[0054] In embodiments, a particular antigenic site contains epitopes associated with pre-fusion or post-fusion structure. For example, the RSV F antigenic sites Ø and V are exhibited in pre-fusion structures of the RSV F glycoprotein. In contrast, the RSV F antigenic sites II and IV are exhibited in both pre-fusion and post-fusion structures of the RSV F glycoprotein. In embodiments, RSV F proteins contain antigenic sites that induce the formation of neutralizing antibodies against the pre-fusion conformation of the RSV F glycoprotein. In embodiments, the RSV F glycoproteins contain antigenic sites that induce the formation of neutralizing antibodies against the post-fusion conformation of the RSV F glycoprotein. In embodiments, the RSV F glycoproteins described herein contain antigenic sites that induce the production of neutralizing antibodies against both pre-fusion and post-fusion conformations of the RSV F glycoprotein.

[0055] In embodiments, the RSV F glycoproteins comprise a deletion of amino acids 137-146; an inactivated primary furin cleavage site; a S155C mutation; and a S290C mutation, wherein the modifications are numbered according to the RSV F glycoprotein of SEQ ID NO: 330. In embodiments, the RSV F glycoproteins comprise a deletion of amino acids 137-146; an inactivated primary furin cleavage site; a S155C

mutation; a S290C mutation; a S190F mutation; and a V207L mutation, wherein the modifications are numbered according to the RSV F glycoprotein of SEQ ID NO: 330. In embodiments, the RSV F glycoproteins comprise a deletion of amino acids 137-146; an inactivated primary furin cleavage site; a S190F mutation; and a V207L mutation, wherein the modifications are numbered according to the RSV F glycoprotein of SEQ ID NO: 330.

[0056] In embodiments, the RSV F glycoproteins comprise a deletion of amino acids 137-146; an inactivated primary furin cleavage site; and a N116Q mutation, wherein the modifications are numbered according to the RSV F glycoprotein of SEQ ID NO: 330. In embodiments, the RSV F glycoproteins comprise a deletion of amino acids 137-146; an inactivated primary furin cleavage site; a S155C mutation; a S290C mutation; and a N116Q mutation, wherein the modifications are numbered according to the RSV F glycoprotein of SEQ ID NO: 330. In embodiments, the RSV F glycoproteins comprise a deletion of amino acids 137-146; an inactivated primary furin cleavage site; a S190F mutation; a V207L mutation; and a N116Q mutation, wherein the modifications are numbered according to the RSV F glycoprotein of SEQ ID NO: 330. In embodiments, the RSV F glycoproteins comprise a deletion of amino acids 137-146; an inactivated primary furin cleavage site; a S155C mutation; a S290C mutation; a S190F mutation; a V207L mutation; and a N116Q mutation, wherein the modifications are numbered according to the RSV F glycoprotein of SEQ ID NO: 330.

Non-naturally occurring RSV F Glycoprotein Antigens—Mutations in Furin Cleavage Sites

[0057] In embodiments, particular mutations cause the mature RSV F proteins of the disclosure to retain p27 at the N-terminus of the F1 subunit. RSV F proteins which retain p27 contain a p27-F1 subunit and an F2 subunit that are connected by a disulfide bond. In embodiments, a mutation in the primary furin cleavage site leads to retention of p27.

[0058] The primary furin cleavage site, also referred to as Site II herein, is located at residues 131 to 136 of SEQ ID NO: 330. The wild-type primary furin cleavage site has the amino acid sequence of KKRKRR (SEQ ID NO: 341). Inactivation of the primary fusion cleavage site may be achieved by mutating residues in the site, with the result that furin can no longer recognize the consensus site. For example, inactivation of the primary furin cleavage site may be accomplished by introducing at least one, at least two, or three amino acid substitutions at positions corresponding to arginine 133,

arginine 135, and arginine 136 of the wild-type RSV F protein (SEQ ID NO: 330). In particular aspects, one, two, or all three of the arginines are mutated to glutamine. In other aspects, inactivation is accomplished by mutating the wild-type primary furin cleavage site to one of the following sequences: KKQKQQ (SEQ ID NO: 342), QKQKQQ (SEQ ID NO: 343), KKQKRQ (SEQ ID NO: 344), and GRRQQR (SEQ ID NO: 345).

[0059] In embodiments, RSV F glycoproteins described herein comprise a wild-type secondary furin cleavage site. The secondary furin cleavage site, also referred to as Site I herein, is located at residues 106 to 109 of SEQ ID NO: 330. The wild-type secondary furin cleavage site has the amino acid sequence RARR (SEQ ID NO: 6). In embodiments, RSV F glycoproteins described herein comprise an inactive secondary furin cleavage site. In embodiments, inactivation of the secondary furin cleavage site is accomplished by mutating the wild-type site to one of the following sequences: QQAQ (SEQ ID NO: 7), RAQQ (SEQ ID NO: 348), or RANN (SEQ ID NO: 349). In embodiments, the inactivated secondary furin cleavage site comprises the amino acid sequence of any one of SEQ ID NOS: 7-34, 97, 111, 348, and 349. In embodiments the inactivated secondary furin cleavage site comprises the amino acid sequence of QQAQ (SEQ ID NO: 7).

Non-naturally occurring RSV F Glycoprotein Antigens—Modifications to the F1 Domain

[0060] In embodiments, the non-naturally occurring RSV F glycoproteins described herein may have a deletion, an insertion, or mutation of up to about 1, up to about 2, up to about 3, up to about 4, up to about 5, up to about 10, up to about 15, up to about 20, up to about 25, or up to about 30 amino acids compared to the amino acid sequence of the F1 domain of SEQ ID NO: 330. The wild-type F1 domain has the amino acid sequence of SEQ ID NO: 350. In embodiments, the RSV F glycoproteins described herein comprise an F1 domain with at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, at least 99.5 %, identity, or 100 % identity to the F1 domain of SEQ ID NO: 350. The F1 domain of an RSV F glycoprotein herein may have a deletion, an insertion, or mutation of from 1 to about 30 amino acids, from 1 to about 25 amino acids, from 1 to about 20 amino acids, from 1 to about 15 amino acids, from 1 to about 10 amino acids, from about 1 to about 5 amino acids, from about 3 to about 10 amino acids, from about

5 to 10 amino acids, from about 8 to 12 amino acids, from about 10 to 15 amino acids, from about 12 to 17 amino acids, from about 15 to 20 amino acids, from about 18 to 23 amino acids, from about 20 to 25 amino acids, from about 22 to about 27 amino acids, or from about 25 to 30 amino acids as compared to the F1 domain of SEQ ID NO: 350.

[0061] In embodiments, the F1 domain of the RSV F glycoproteins comprise one or more mutations selected from the group consisting of S190F, V207L, S155C, S290C, I379A, and M447V, wherein the modifications are numbered according to the RSV F glycoprotein of SEQ ID NO: 330. In embodiments, the F1 domain of the RSV F glycoproteins comprise S190F and V207 L mutations, wherein the modifications are numbered according to the RSV F glycoprotein of SEQ ID NO: 330. In embodiments, the F1 domain of the RSV F glycoproteins comprise S155C and S290C mutations, wherein the mutations are numbered according to the RSV F glycoprotein of SEQ ID NO: 2. In embodiments, the F1 domain of the RSV F glycoproteins comprise S190F, V207L, S155C, and S290C mutations, wherein the mutations are numbered according to the RSV F glycoprotein of SEQ ID NO: 330.

[0062] In embodiments, the F1 domain of the RSV F glycoproteins comprises an amino acid sequence with at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95%, at least 96 %, at least 97%, at least 98 %, at least 99%, at least 99.5 % identity, or 100 % identity any one of SEQ ID NOS: 350-355.

Non-naturally occurring RSV F Glycoprotein Antigens—Deletions in Fusion Peptide of the F1 Domain

[0063] In embodiments, the RSV F glycoproteins of the disclosure lack one or more amino acids of the RSV F fusion peptide (amino acids 137-146 of SEQ ID NO: 330). The fusion peptide of the RSV F glycoprotein of SEQ ID NO: 330 has the amino acid sequence of FLGFLGVGS (SEQ ID NO: 363). In embodiments, provided herein are RSV F glycoproteins that lack the fusion peptide. In embodiments, provided herein are RSV F glycoproteins lacking from 1 to 10, from 1 to 9, from 1 to 8, from 1 to 7, from 1 to 6 from 1 to 5, from 1 to 4, from 1 to 3, or from 1 to 2 contiguous amino acids of the fusion peptide. In embodiments, provided herein are RSV F glycoproteins lacking from 1 to 10, from 1 to 9, from 1 to 8, from 1 to 7, from 1 to 6 from 1 to 5, from 1 to 4, from 1 to 3, or from 1 to 2 non-contiguous amino acids of the fusion peptide. In embodiments, up to 1, up to 2, up to 3, up to 4, up to 5, up to 6, up to 7, up to 8, up to

9, or up to 10 amino acids within the fusion peptide may be deleted. In embodiments, at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 amino acids, but no more than 10 amino acids of the fusion peptide are deleted.

Non-naturally occurring RSV F Glycoprotein Antigens—Modifications to the F2 Domain

[0064] In embodiments, the non-naturally occurring RSV F glycoproteins described herein may have a deletion, an insertion, or mutation of up to about 1, up to about 2, up to about 3, up to about 4, up to about 5, up to about 10, up to about 15, up to about 20, up to about 25, or up to about 30 amino acids compared to the amino acid sequence of the F2 domain of SEQ ID NO: 330. The wild-type F2 domain has the amino acid sequence of SEQ ID NO: 356. In embodiments, the RSV F glycoproteins described herein comprise an F1 domain with at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, at least 99.5 %, identity, or 100 % identity to the F2 domain of SEQ ID NO: 356. The F2 domain of an RSV F glycoprotein herein may have a deletion, an insertion, or mutation of between about 1 and about 5 amino acids, between about 3 and about 10 amino acids, between about 5 and 10 amino acids, between about 8 and 12 amino acids, between about 10 and 15 amino acids, between about 12 and 17 amino acids, between about 15 and 20 amino acids, between about 18 and 23 amino acids, between about 20 and 25 amino acids, between about 22 and about 27 amino acids, or between about 25 and 30 amino acids as compared to the F2 domain of SEQ ID NO: 356.

[0065] In embodiments, the F2 domain of the RSV F glycoproteins may comprise a mutation of P102A, wherein the mutation is numbered according to the RSV F glycoprotein of SEQ ID NO: 330.

[0066] In embodiments, the F2 domain of the RSV F glycoproteins comprises an amino acid sequence with at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, at least 99.5 % identity, or 100 % identity any one of SEQ ID NOS: 356-357.

Non-naturally occurring RSV F Glycoprotein Antigens—Modifications to p27

[0067] In embodiments, the non-naturally occurring RSV F glycoproteins described herein may have a deletion, an insertion, or mutation of up to about 1, up to about 2, up to about 3, up to about 4, up to about 5, up to about 10, up to about 15, up to about 20, up to about 25, or up to about 30 amino acids compared to the amino acid sequence of p27 of SEQ ID NO: 330. The wild-type p27 has the amino acid sequence of SEQ ID NO: 359. In embodiments, the RSV F glycoproteins described herein comprise a p27 with at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, at least 99.5 %, identity, or 100 % identity to the p27 of SEQ ID NO: 330. The p27 of an RSV F glycoprotein herein may have a deletion, an insertion, or mutation of from about 1 to about 5 amino acids, from about 3 to about 10 amino acids, from about 5 to 10 amino acids, from about 8 to 12 amino acids, from about 10 to 15 amino acids, from about 12 to 17 amino acids, from about 15 to 20 amino acids, from about 18 to 23 amino acids, from about 20 to 25 amino acids, from about 22 to about 27 amino acids, or from about 25 and 30 amino acids as compared to the p27 of SEQ ID NO: 330.

[0068] In embodiments, the p27 domain of the RSV F glycoproteins may comprise a mutation of N116Q, wherein the mutation is numbered according to the RSV F glycoprotein of SEQ ID NO: 330.

[0069] In embodiments, p27 of the RSV F glycoproteins comprises an amino acid sequence with at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, at least 99.5 % identity, or 100 % identity to any one of SEQ ID NOS: 358-359.

Non-naturally occurring RSV F Glycoprotein Antigens—Transmembrane domain

[0070] In embodiments, the RSV F glycoproteins described herein comprise a transmembrane domain. In embodiments, the transmembrane domain has the sequence of ITTIIIVIIILLSLIAVGLLLYCKARSTPVTLSKDQLSGINNIAFSN (SEQ ID NO: 362). In embodiments, the RSV F glycoproteins described herein may have a deletion, an insertion, or mutation of up to about 1, up to about 2, up to about 3, up to about 4, up to about 5, up to about 10, up to about 15, up to about 20, up to about 25, or up to about 30 amino acids compared to the amino acid sequence of the

transmembrane domain. In embodiments, the RSV F glycoprotein lacks the transmembrane domain.

Non-naturally occurring RSV F Glycoprotein Antigens—Signal Peptide

[0071] In embodiments, the RSV F glycoproteins described herein are expressed with an N-terminal signal peptide. In embodiments, the N-terminal signal peptide has an amino acid sequence of SEQ ID NO: 360 (MELLILKANAITTILTAVTFCFASG). In embodiments, the signal peptide may be replaced with any signal peptide that enables expression of the RSV F glycoprotein. In embodiments, one or more of the RSV F glycoprotein signal peptide amino acids may be deleted or mutated. An initiating methionine residue is maintained to initiate expression. In embodiments, the RSV F glycoprotein is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 361.

[0072] Following expression of the RSV F glycoprotein in a host cell, the N-terminal signal peptide is cleaved to provide the mature RSV F glycoprotein sequence (SEQ ID NOS: 331 and 337-340). In embodiments, the signal peptide is cleaved by host cell proteases. In aspects, the full-length protein may be isolated from the host cell and the signal peptide cleaved subsequently.

[0073] Following cleavage of the signal peptide from the RSV F glycoprotein with an amino acid sequence corresponding to SEQ ID NOS: 332-336 during expression and purification, a mature polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOS: 331 and 337-340 is obtained and used to produce an RSV F nanoparticle vaccine or RSV F nanoparticles.

[0074] Advantageously, the disclosed RSV F glycoproteins may have enhanced protein expression, stability, and immunogenicity relative to the native RSV F glycoproteins.

[0075] In embodiments, the RSV F glycoproteins described herein contain further modifications from the native RSV F glycoprotein (SEQ ID NO: 330). In embodiments, the RSV F glycoproteins described herein exhibit at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identity to the native RSV F glycoprotein.

[0076] In embodiments, the RSV F glycoproteins described herein are at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95%, at least 96 %, at least 97%, at least 98 %, at least 99%, at least 99.5 % identical to an RSV F glycoprotein having an amino acid selected from the group consisting of SEQ ID NOS: 329-340. In embodiments, provided herein is an RSV F glycoprotein with at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95%, at least 96 %, at least 97%, at least 98 %, at least 99%, at least 99.5 %, or 100 % identity to an RSV F glycoprotein of SEQ ID NO: 337. A RSV F glycoprotein may have a deletion, an insertion, or mutation of up to about 1, up to about 2, up to about 3, up to about 4, up to about 5, up to about 10, up to about 15, up to about 20, up to about 25, up to about 30, up to about 35, up to about 40, up to about 45, or up to about 50 amino acids compared to the amino acid sequence of the RSV F glycoprotein having an amino acid sequence of any one of SEQ ID NO: 329-340. A RSV F glycoprotein may have a deletion, an insertion, or mutation of from about 1 to about 5 amino acids, from about 3 to about 10 amino acids, from about 5 to 10 amino acids, from about 8 to 12 amino acids, from about 10 to 15 amino acids, from about 12 to 17 amino acids, from about 15 to 20 amino acids, from about 18 to 23 amino acids, from about 20 to 25 amino acids, from about 22 to about 27 amino acids, from about 25 to 30 amino acids, from about 30 to 35 amino acids, from about 35 to 40 amino acids, from about 40 to 45 amino acids, or from about 45 to 50 amino acids, as compared from the RSV F glycoprotein having an amino acid sequence of any one of SEQ ID NOS: 329-340. In embodiments, RSV F glycoproteins described herein comprise about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, or about 25 modifications compared to the RSV F glycoprotein having an amino acid sequence of any one of SEQ ID NOS: 329-340.

[0077] In embodiments, the RSV F glycoprotein is extended at the N-terminus, the C-terminus, or both the N-terminus and the C-terminus. In aspects, the extension is a tag useful for a function, such as purification or detection. In aspects the tag contains an epitope. For example, the tag may be a polyglutamate tag, a FLAG-tag, a HA-tag, a polyHis-tag (having about 5-10 histidines) (SEQ ID NO: 34), a hexahistidine tag (SEQ ID NO: 35), an 8X-His-tag (having eight histidines) (SEQ ID NO: 36), a Myc-tag, a Glutathione-S-transferase-tag, a Green fluorescent protein-tag, Maltose binding

protein-tag, a Thioredoxin-tag, or an Fc-tag. In other aspects, the extension may be an N-terminal signal peptide fused to the protein to enhance expression. While such signal peptides are often cleaved during expression in the cell, some nanoparticles may contain the antigen with an intact signal peptide. Thus, when a nanoparticle comprises an antigen, the antigen may contain an extension and thus may be a fusion protein when incorporated into nanoparticles. For the purposes of calculating identity to the sequence, extensions are not included. In embodiments, the tag is a protease cleavage site. Non-limiting examples of protease cleavage sites include the HRV3C protease cleavage site, chymotrypsin, trypsin, elastase, endopeptidase, caspase-1, caspase-2, caspase-3, caspase-4, caspase-5, caspase-6, caspase-7, caspase-8, caspase-9, caspase-10, enterokinase, factor Xa, Granzyme B, TEV protease, and thrombin. In embodiments, the protease cleavage site is an HRV3C protease cleavage site.

[0078] In embodiments, the RSV F glycoprotein comprises a fusion protein. In embodiments, the RSV F glycoprotein comprises an N-terminal fusion protein. In embodiments, the RSV F glycoprotein comprises a C-terminal fusion protein. In embodiments, the fusion protein encompasses a tag useful for protein expression, purification, or detection. In embodiments, the tag is a polyHis-tag (having about 5-10 histidines) (SEQ ID NO: 34), a Myc-tag, a Glutathione-S-transferase-tag, a Green fluorescent protein-tag, Maltose binding protein-tag, a Thioredoxin-tag, a Strep-tag, a Twin-Strep-tag, or an Fc-tag. In embodiments, the tag is an Fc-tag. In embodiments, the Fc-tag is monomeric, dimeric, or trimeric. In embodiments, the tag is a hexahistidine tag, e.g. a polyHis-tag which contains six histidines (SEQ ID NO: 35).

Immunogenic Compositions and/or Vaccine Compositions containing RSV F glycoproteins and Additional Viral Glycoproteins

[0079] In embodiments, provided herein are immunogenic compositions comprising RSV F glycoproteins. In embodiments, the immunogenic compositions are vaccine compositions. In embodiments, provided herein are immunogenic compositions and vaccine compositions comprising nanoparticles comprising the RSV F glycoproteins. In embodiments, the immunogenic compositions comprise from about 1 to about 50, from about 1 to about 45, from about 1 to about 40, from 1 to about 35, from about 1 to about 30, from 1 to about 25, from 1 to about 20, from 1 to about 15, from 1 to about 10, or from 1 to about 5 different RSV F glycoproteins.

[0080] In another embodiment, the disclosures provide for a pharmaceutical pack or kit comprising one or more containers filled with one or more of the components of the vaccine compositions.

[0081] Compositions disclosed herein may be used either prophylactically or therapeutically, but will typically be prophylactic. Accordingly, the disclosure includes methods for treating or preventing infection. The methods involve administering to the subject a therapeutic or prophylactic amount of the immunogenic compositions of the disclosure. Preferably, the pharmaceutical composition is a vaccine composition that provides a protective effect. In other aspects, the protective effect may include amelioration of a symptom associated with infection in a percentage of the exposed population. For example, the composition may prevent or reduce one or more virus disease symptoms selected from: fever fatigue, muscle pain, headache, sore throat, vomiting, diarrhea, rash, symptoms of impaired kidney and liver function, internal bleeding and external bleeding, compared to an untreated subject.

[0082] The nanoparticles may be formulated for administration as vaccines in the presence of various excipients, buffers, and the like. For example, the vaccine compositions may contain sodium phosphate, sodium chloride, and/or histidine. Sodium phosphate may be present at about 10 mM to about 50 mM, about 15 mM to about 25 mM, or about 25 mM; in particular cases, about 22 mM sodium phosphate is present. Histidine may be present about 0.1% (w/v), about 0.5% (w/v), about 0.7% (w/v), about 1% (w/v), about 1.5% (w/v), about 2% (w/v), or about 2.5% (w/v). Sodium chloride, when present, may be about 150 mM. In certain compositions, the sodium chloride may be present in higher concentrations, for example from about 200 mM to about 500 mM. In embodiments, the sodium chloride is present in a high concentration, including but not limited to about 200 mM, about 250 mM, about 300 mM, about 350 mM, about 400 mM, about 450 mM, or about 500 mM.

[0083] In embodiments, the nanoparticles described herein have improved stability at certain pH levels. In embodiments, the nanoparticles are stable at slightly acidic pH levels. For example, the nanoparticles that are stable at a slightly acidic pH, for example from pH 5.8 to pH 7.0. In embodiments, the nanoparticles and compositions containing nanoparticles may be stable at pHs ranging from about pH 5.8 to about pH 7.0, including about pH 5.9 to about pH 6.8, about pH 6.0 to about pH 6.5, about pH 6.1 to about pH 6.4, about pH 6.1 to about pH 6.3, or about pH 6.2. In embodiments, the nanoparticles and compositions described herein are stable at neutral pHs,

including from about pH 7.0 to about pH 7.4. In embodiments, the nanoparticles and compositions described herein are stable at slightly alkaline pHs, for example from about pH 7.0 to about pH 8.5, from about pH 7.0 to about pH 8.0, or from about pH 7.0 to about pH 7.5, including all values and ranges in between.

[0084] In embodiments, in addition to RSV F glycoproteins, the compositions described herein comprise one or more additional viral glycoproteins. In embodiments, the additional viral glycoproteins are selected from the group consisting of: a SARS-CoV-2 S glycoprotein, an influenza hemagglutinin glycoprotein, and an influenza neuraminidase. In embodiments, the immunogenic compositions comprise an RSV F glycoprotein described herein, an influenza glycoprotein, and a SARS-CoV-2 S glycoprotein. In embodiments, the immunogenic compositions comprise an RSV F glycoprotein described herein and an influenza glycoprotein. In embodiments, the immunogenic compositions comprise an RSV F glycoprotein described herein, and a SARS-CoV-2 S glycoprotein.

[0085] In embodiments, the immunogenic compositions comprise from about 1 to about 50, from about 1 to about 45, from about 1 to about 40, from 1 to about 35, from about 1 to about 30, from 1 to about 25, from 1 to about 20, from 1 to about 15, from 1 to about 10, or from 1 to about 5 different viral glycoproteins. Viral glycoproteins that are "different" have different amino acid sequences. In embodiments, different viral glycoproteins may be from different viruses, different strains, or sub-types of the same virus, or may differ from one another by mutation.

[0086] In embodiments, in addition to an RSV F glycoprotein, the immunogenic composition comprises from 1 to about 50 different influenza glycoproteins (e.g., hemagglutinin and/or neuraminidase). In embodiments, the influenza glycoprotein (e.g., hemagglutinin or neuraminidase) may be from any influenza virus strain.

[0087] In embodiments, the immunogenic compositions comprise 1, 2, 3, 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45, about 46, about 47, about 48, about 49, or about 50 influenza glycoproteins, including all values and ranges therebetween.

[0088] In embodiments, the influenza glycoprotein is a hemagglutinin. In embodiments, the influenza HA glycoprotein is selected from any one of the following subtypes: H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, H17, and H18. Phylogenetically, the influenza is split into groups. In embodiments, the HA glycoprotein is a Group 1 HA glycoprotein (i.e., a glycoprotein from the H1, H2, H5, H6, H8, H9, H11, H12, H13, H16, H17, or H18 subtypes). In embodiments, the HA glycoprotein is a Group 2 HA glycoprotein (i.e., a glycoprotein from the H3, H4, H7, H10, H14, or H15 subtypes).

[0089] Multiple examples of influenza HA glycoproteins are described in the following patent documents, which are each incorporated by reference herein in their entirety for all purposes: International Publication No. 2017/041100 and International Publication No. 2019/022930.

[0090] In embodiments, an immunogenic composition of the disclosure comprises at least three different HA glycoproteins or at least four different HA glycoproteins. In embodiments, an immunogenic composition of the disclosure comprises three or four different HA glycoproteins. In embodiments, each of the HA glycoproteins are from a different influenza strain. In embodiments, three HA glycoproteins are from a Type A influenza strain, and one HA glycoprotein is from a Type B influenza strain. In embodiments, two HA glycoproteins are from a Type A influenza strain, and two HA glycoproteins are from a Type B influenza strain. In embodiments, two HA glycoproteins are from a Type A influenza strain, and one HA glycoprotein is from a Type B influenza strain.

[0091] In embodiments, each of the at least three different HA glycoproteins is isolated separately using an egg-based manufacturing process. In embodiments, an egg-based manufacturing process comprises (a) propagating an influenza virus in an egg and (b) harvesting the influenza virus.

[0092] In embodiments, the influenza virus is a live virus. In embodiments, the influenza virus is a weakened or “attenuated” virus. In embodiments, the influenza virus is optimized to grow in an egg. In embodiments, the optimized influenza virus lacks the polybasic cleavage site of hemagglutinin. The following article describes the development of an optimized influenza virus (referred to as a “candidate vaccine virus”) in detail and is incorporated by reference herein in its entirety: Belser et al. *Virology*. 2017 Nov; 511: 135–141.

[0093] In embodiments, the egg is a chicken egg. In embodiments, the chicken egg is an embryonated chicken egg. In embodiments, the egg is a pathogen-free egg. In embodiments, an influenza virus is propagated by inoculating the virus in the allantoic cavity of an embryonated chicken egg. The following article describes an exemplary inoculation method and is incorporated by reference herein in its entirety: Brauer et al. J Vis Exp. 2015; (97): 52421.

[0094] In embodiments, the egg-based manufacturing process comprises purifying the influenza virus. In embodiments, an influenza virus is purified using any of the following techniques: centrifugation, chromatography, precipitation, or nanofiltration. In embodiments, the centrifugation technique is ultracentrifugation. In embodiments, the virus is purified using zonal centrifugation. In embodiments, the zonal centrifugation is continuous flow zonal centrifugation.

[0095] In embodiments, the egg-based manufacturing process comprises inactivating (also referred to herein as “killing”) an influenza virus. In embodiments, an influenza virus is inactivated using low pH (e.g., a pH from about 3.5-5.5), heat, ethanol, ultraviolet light, exposure to a detergent (e.g, octylphenol), or exposure to a chemical (e.g., 2-propanol, ethanol, iodopovidone). In embodiments, the purified virus is inactivated with ultraviolet light, betapropiolactone, sodium deoxycholate, formaldehyde, or any combination thereof.

[0096] In embodiments, the egg-based manufacturing process comprises exposing the influenza virus to a surfactant. The surfactant may be sodium taurodeoxycholate, octylphenol ethoxylate (Triton®-X 100), or cetyl trimethyl ammonium bromide. Exposing an influenza virus to a surfactant results in the formation of an influenza split-virion. In embodiments, the at least three HA glycoproteins are in the form of an influenza split-virion.

[0097] In embodiments, the egg-based manufacturing process comprises purifying an influenza antigen (e.g., hemagglutinin) from the virus.

[0098] The following Food and Drug Administration (FDA) approved influenza vaccines are produced using an egg-based manufacturing process: AFLURIA® QUADRIVALENT, FLUARIX® QUADRIVALENT, FLULAVAL QUADRIVALENT, FLUZONE® QUADRIVALENT, FLUZONE® HIGH-DOSE QUADRIVALENT, FLUMIST® QUADRIVALENT, FLUAD®, and FLUAD® QUADRIVALENT.

[0099] In embodiments, each of the at least three HA glycoproteins is isolated using a cell-culture based process. In embodiments, a cell-culture based process comprises

(i) growing an influenza virus in a cell and (ii) harvesting the virus from the cell. In embodiments, a cell-culture based process comprises (i) transfecting a cell with a vector comprising a hemagglutinin and (ii) harvesting the hemagglutinin from the cell. In embodiments, a cell-culture based process comprises (i) transducing a cell with a virus encoding a hemagglutinin and (ii) harvesting the hemagglutinin from the cell. In embodiments, the harvested hemagglutinin is recombinant hemagglutinin.

[00100] In embodiments, the cell is an animal cell, a bacterial cell, an insect cell, or a fungal cell. In embodiments, the animal is a human, a bird (e.g., a chicken), a dog, a reptile, a goat, a pig, a mouse, a rabbit, or a rat.

[00101] In embodiments, the virus encoding a hemagglutinin is a baculovirus, a lentivirus, or an adeno-associated virus.

[00102] In embodiments, an influenza virus produced using a cell-culture based process is purified. Any of the purification techniques described to purify influenza virus produced using an egg-based manufacturing process can be used to purify influenza virus produced using a cell-cultured based process.

[00103] In embodiments, a hemagglutinin produced using a cell-culture based process is purified. Purification techniques include chromatography, centrifugation, precipitation, and nanofiltration.

[00104] The Food and Drug Administration (FDA) approved influenza vaccine FLUCELVAX® QUADRIVALENT is produced using a cell-culture based process.

[00105] In embodiments, each of the at least three HA glycoproteins is a recombinant hemagglutinin. The Food and Drug Administration (FDA) approved influenza vaccine FLUBLOK® QUADRIVALENT is a recombinant hemagglutinin.

[00106] In embodiments, the at least three hemagglutinins are in the form of recombinant hemagglutinin. Recombinant hemagglutinin is isolated from a cell that produces hemagglutinin. In embodiments, a cell that produces hemagglutinin has been transfected with a vector encoding hemagglutinin. In embodiments, a cell that produces hemagglutinin has been transduced with a virus encoding hemagglutinin.

[00107] In embodiments, the compositions disclosed herein comprise detergent-core nanoparticles comprising hemagglutinin from an influenza virus. The aforementioned detergent-core nanoparticles are described in detail in U.S. Patent No. 10,426,829, which is incorporated herein by reference in its entirety for all purposes.

[00108] Detergent-core nanoparticles comprise a hemagglutinin from an influenza virus, which is associated with a detergent core. In embodiments, the hemagglutinin is a trimer. Each nanoparticle may contain 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 trimers. In embodiments, the nanoparticle contains between about 2 to about 9, about 2 to about 6, or about 5 hemagglutinin trimers. The hemagglutinin is associated with the non-ionic detergent containing core of the nanoparticle. In embodiments, the detergent is selected from polysorbate-20 (PS20), polysorbate-40 (PS40), polysorbate-60 (PS60), polysorbate-65 (PS65) and polysorbate-80 (PS80). The presence of the detergent facilitates formation of the nanoparticles by forming a core that organizes and presents the antigens. In embodiments, the nanoparticles may contain the antigens assembled into multi-oligomeric glycoprotein-PS80 protein-detergent nanoparticles with the head regions projecting outward and hydrophobic regions and PS80 detergent forming a central core surrounded by the antigens.

[00109] In embodiments, the detergent-core nanoparticles or HaSMaNs are trypsin-resistant nanoparticles produced using neutral pH purification. Trypsin resistance is achieved by neutral pH range of above 6.9 to 8.5 during purification and formulation of the HA nanoparticles. Trypsin resistant influenza glycoproteins and trypsin resistant influenza nanoparticles; and methods of making thereof are described in detail in U.S. Patent No. 10,426,829.

[00110] In embodiments, the hemagglutinin of the detergent-core nanoparticles or HaSMaNs described herein comprises the full-length wild type hemagglutinin amino acid sequence. In embodiments, the hemagglutinin is a hemagglutinin variant. In embodiments, the hemagglutinin exhibits at least 80%, at least 85 %, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99 % identity to the wild-type hemagglutinin protein.

[00111] HA is a homotrimer with each monomer consisting of ~550 amino acid residues. Each monomer of HA has been conceptually divided into three domains: the ectodomain of ~515 residues constitutes the extraviral part of the molecule; a single stretch of 27 residues defines the transmembrane (TM) domain; and ~10 residues constitute the cytoplasmic tail (CT). While some changes may be made to hemagglutinin, formation of both detergent core nanoparticles and HaSMaNs requires an intact transmembrane domain (TM). Thus, in particular examples, a modified HA protein sequence comprises 100% identity to the wild-type TM and CT domains with

some flexibility in the remaining ectodomain portion, where identity may be at least 90% or at least 95%. The domains may be identified by homology to the amino acid sequences of the TM domains and CT of Japan/305/57 HA shown in Figure 1 of Melikyan et al. (Mol Biol Cell. 1999 Jun; 10(6): 1821–1836) though it should be noted that the boundaries between ectodomain, TM, and CT domains may vary from HA protein to HA protein by up to three amino acids.

[00112] In embodiments, the immunogenic compositions and vaccine compositions described herein comprise a HaSMaN (Hemagglutinin Saponin Matrix Nanoparticle.) **Fig. 6** (right panel) illustrates HaSMaN structures as observed under an electron microscope. The HA glycoproteins decorate the Matrix cage-like structures. The HaSMaN structures are formed by preparing detergent-core nanoparticles comprising hemagglutinin from an influenza virus and then incubating them with ISCOM matrix adjuvant particles for a period of time. ISCOM matrix particles are shown in the center panel of Fig. 6. Notably, the HaSMaNs form readily with Type A influenza HA proteins, but not Type B influenza HA proteins.

[00113] HaSMaNs disclosed herein are produced by incubating the detergent-core nanoparticles with an ISCOM Matrix adjuvant comprising a saponin fraction, cholesterol and a phospholipid. In embodiments, a HaSMaN is formed by incubating a detergent-core nanoparticle with an ISCOM matrix adjuvant for between about 24 hours and about 48 hours. For example, the detergent-core nanoparticle may be incubated with an ISCOM matrix adjuvant for about 24 hours, about 25 hours, about 26 hours, about 27 hours, about 28 hours, about 29 hours, about 30 hours, about 31 hours, about 32 hours, about 33 hours, about 34 hours, about 35 hours, about 36 hours, about 37 hours, about 38 hours, about 39 hours, about 40 hours, about 41 hours, about 42 hours, about 43 hours, about 44 hours, about 45 hours, about 46 hours, about 47 hours, about 48 hours, or more. In some embodiments, a HaSMaN is formed by incubating a detergent-core nanoparticle with an ISCOM matrix adjuvant for at least about 24 hours, at least about 25 hours, at least about 26 hours, at least about 27 hours, at least about 28 hours, at least about 29 hours, at least about 30 hours, at least about 31 hours, at least about 32 hours, at least about 33 hours, at least about 34 hours, at least about 35 hours, at least about 36 hours, at least about 37 hours, at least about 38 hours, at least about 39 hours, at least about 40 hours, at least about 41 hours, at least about 42 hours, at least about 43 hours, at least about 44 hours, at least about 45 hours, at least about 46 hours, at least about 47 hours, or

at least about 48 hours. In embodiments, a HaSMaN is formed by incubating a detergent-core nanoparticle with an ISCOM matrix adjuvant at a temperature from about 4°C to about 25°C. For example, the HaSMaN may be formed by incubating a detergent-core nanoparticle with an ISCOM matrix adjuvant at a temperature of about 4°C, about 5 °C, about 6 °C, about 7 °C, about 8 °C, about 9 °C, about 10 °C, about 11 °C, about 12 °C, about 13 °C, about 14 °C, about 15 °C, about 16 °C, about 17 °C, about 18 °C, about 19 °C, about 20 °C, about 21 °C, about 22 °C, about 23 °C, about 24 °C, about 25°C, or higher. In embodiments, a HaSMaN is formed by incubating a detergent-core nanoparticle with an ISCOM matrix adjuvant at a temperature of at least about 4 °C, at least about 5 °C, at least about 6 °C, at least about 7 °C, at least about 8 °C, at least about 9 °C, at least about 10 °C, at least about 11 °C, at least about 12 °C, at least about 13 °C, at least about 14 °C, at least about 15 °C, at least about 16 °C, at least about 17 °C, at least about 18 °C, at least about 19 °C, at least about 20 °C, at least about 21 °C, at least about 22 °C, at least about 23 °C, at least about 24 °C, at least about 25°C. Typically, about 24 to 48 hours at 4°C or 25°C incubation is required for formation. Formation of HaSMaNs is promoted by higher temperatures. In embodiments, formation of HaSMaN occurs by incubation of detergent-core nanoparticles with an ISCOM Matrix adjuvant for at least 24 hours at about 25°C. Mixing detergent core nanoparticles with ISCOM Matrix adjuvant shortly prior to administering to a subject—i.e. bedside mix, does not produce HaSMaNs. Longer incubation periods do not negatively impact HaSMaNs formation.

[00114] In embodiments, in addition to an RSV F glycoprotein, the immunogenic composition comprises from 1 to about 50 different SARS-CoV-2 S glycoproteins (“CoV S glycoproteins”). In embodiments, in addition to an RSV F glycoprotein, the immunogenic composition comprises about 5 different SARS-CoV-2 CoV S glycoproteins. The CoV S glycoprotein is synthesized as an inactive precursor (S0) that is proteolytically cleaved at the furin cleavage site into S1 and S2 subunits which remain non-covalently linked to form prefusion trimers. The S2 domain of the CoV S glycoprotein comprises a fusion peptide (FP), two heptad repeats (HR1 and HR2), a transmembrane (TM) domain, and a cytoplasmic tail (CT). The S1 domain of the SARS-CoV-2 S protein folds into four distinct domains: the N-terminal domain (NTD) and the C-terminal domain, which contains the receptor binding domain (RBD) and two subdomains SD1 and SD2. The prefusion SARS-CoV-2 S protein trimers undergo a structural rearrangement from a prefusion to a postfusion conformation upon S-

protein receptor binding and cleavage. In embodiments, the CoV S polypeptides are glycoproteins, due to post-translational glycosylation. The glycoproteins comprise one or more domains, including a signal peptide, an S1 subunit, an S2 subunit, a NTD, a RBD, two subdomains (SD1 and SD2, labeled SD1/2 in Figs. 1A-B and referred to as "SD1/2" herein), an intact or modified fusion peptide, an HR1 domain, an HR2 domain, a TM, and a CD. In embodiments, the amino acids for each domain are given in **Fig. 7A** (shown according to SEQ ID NO: 1), **Fig. 7B** (shown according to SEQ ID NO: 2). SARS-CoV-2 S glycoproteins also contain a furin cleavage site.

[00115] In embodiments, each of the CoV S glycoproteins described herein contains proline at amino acid positions 973 and 974 and comprises an inactive furin cleavage site, wherein the CoV S glycoprotein is numbered according to the CoV S glycoprotein of SEQ ID NO: 2.

[00116] In embodiments, the CoV S glycoproteins described herein are expressed with an N-terminal signal peptide. In embodiments, the N-terminal signal peptide has an amino acid sequence of SEQ ID NO: 5 (MFVFLVLLPLVSS). In embodiments, the N-terminal signal peptide has an amino acid sequence of SEQ ID NO: 117 (MFVFLVLLPLVSI). In embodiments, the N-terminal signal peptide has an amino acid sequence of SEQ ID NO: 154 (MFVFFVLLPLVSS). In embodiments, the N-terminal signal peptide has an amino acid sequence of SEQ ID NO: 193 (MFGFLVLLPLVSS). In embodiments, the signal peptide may be replaced with any signal peptide that enables expression of the CoV S glycoprotein. In embodiments, one or more of the CoV S glycoprotein signal peptide amino acids may be deleted or mutated. An initiating methionine residue is maintained to initiate expression. In embodiments, the CoV S glycoproteins are encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 47, SEQ ID NO: 50, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 96, SEQ ID NO: 60, SEQ ID NO: 131, SEQ ID NO: 135, SEQ ID NO: 142, SEQ ID NO: 145, SEQ ID NO: 148, SEQ ID NO: 150, SEQ ID NO: 196, SEQ ID NO: 197; SEQ ID NO: 198; SEQ ID NO: 199; SEQ ID NO: 201; SEQ ID NO: 202; SEQ ID NO: 204; SEQ ID NO: 206; SEQ ID NO: 208; SEQ ID NO: 210; SEQ ID NO: 212; SEQ ID NO: 214; and SEQ ID NO: 216. In embodiments, the N-terminal signal peptide of the CoV S glycoprotein contains a mutation at Ser-13 relative to the native CoV Spike (S) signal polypeptide (SEQ ID NO: 5). In embodiments, Ser-

13 is mutated to any natural amino acid. In embodiments, Ser-13 is mutated to alanine, methionine, isoleucine, leucine, threonine, or valine. In embodiments, Ser-13 is mutated to isoleucine.

[00117] Following expression of the CoV S glycoprotein in a host cell, the N-terminal signal peptide is cleaved to provide the mature CoV glycoprotein sequence (SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 106, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 132, SEQ ID NO: 144, SEQ ID NO: 151, SEQ ID NO: 153, SEQ ID NO: 156, SEQ ID NO: 158, SEQ ID NO: 174, SEQ ID NO: 175, SEQ ID NO: 176, SEQ ID NO: 181, SEQ ID NO: 182, SEQ ID NO: 183, SEQ ID NO: 184, SEQ ID NO: 186, SEQ ID NO: 188, SEQ ID NO: 190, SEQ ID NO: 192, SEQ ID NO: 195, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224, SEQ ID NO: 225, SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235, SEQ ID NO: 236, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 274, SEQ ID NO: 276, SEQ ID NO: 278, SEQ ID NO: 280, SEQ ID NO: 284, SEQ ID NO: 288, SEQ ID NO: 292, SEQ ID NO: 296, SEQ ID NO: 300, SEQ ID NO: 304, SEQ ID NO: 308, SEQ ID NO: 312, SEQ ID NO: 316, SEQ ID NO: 320, SEQ ID NO: 324, SEQ ID NO: 328). In embodiments, the signal peptide is cleaved by host cell proteases. In aspects, the full-length protein may be isolated from the host cell and the signal peptide cleaved subsequently.

[00118] Following cleavage of the signal peptide from a CoV Spike (S) polypeptide with an amino acid sequence corresponding to any one of SEQ ID NO: 88, SEQ ID NO: 105, SEQ ID NO: 130, SEQ ID NO: 136, SEQ ID NO: 143, SEQ ID NO: 149, SEQ ID NO: 152, SEQ ID NO: 155, SEQ ID NO: 157, SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 167, SEQ ID NO: 170, SEQ ID NO: 173, SEQ ID NO: 177, SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180, SEQ ID NO: 185, SEQ ID NO: 187, SEQ ID NO: 189, SEQ ID NO: 191, SEQ ID NO: 194, SEQ ID NO: 200, SEQ ID NO: 203, SEQ ID NO: 205, SEQ ID NO: 207, SEQ ID NO: 209, SEQ ID NO: 211, SEQ ID NO: 213, SEQ ID NO: 215, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 242, SEQ ID NO: 243, SEQ ID NO: 244, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252, SEQ ID NO: 253, SEQ ID NO: 254, SEQ ID NO: 266, SEQ ID NO: 268, SEQ ID NO: 270, SEQ ID NO: 272, SEQ ID NO: 282, SEQ ID NO: 286, SEQ ID NO: 290, SEQ ID NO: 295, SEQ ID NO: 299, SEQ ID NO: 303,

SEQ ID NO: 307, SEQ ID NO: 311, SEQ ID NO: 315, SEQ ID NO: 319, SEQ ID NO: 323, and SEQ ID NO: 327 during expression and purification, a mature polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 106, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 132, SEQ ID NO: 144, SEQ ID NO: 151, SEQ ID NO: 153, SEQ ID NO: 156, SEQ ID NO: 158, SEQ ID NO: 174, SEQ ID NO: 175, SEQ ID NO: 176, SEQ ID NO: 181, SEQ ID NO: 182, SEQ ID NO: 183, SEQ ID NO: 184, SEQ ID NO: 186, SEQ ID NO: 188, SEQ ID NO: 190, SEQ ID NO: 192, SEQ ID NO: 195, SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224, SEQ ID NO: 225, SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235, SEQ ID NO: 236, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 274, SEQ ID NO: 276, SEQ ID NO: 278, SEQ ID NO: 280, SEQ ID NO: 284, SEQ ID NO: 288, SEQ ID NO: 292, SEQ ID NO: 296, SEQ ID NO: 300, SEQ ID NO: 304, SEQ ID NO: 308, SEQ ID NO: 312, SEQ ID NO: 316, SEQ ID NO: 320, SEQ ID NO: 324, and SEQ ID NO: 328 is obtained and used to produce a CoV S nanoparticle vaccine or CoV S nanoparticles.

[00119] Advantageously, the disclosed CoV S polypeptides may have enhanced protein expression and stability relative to a native CoV Spike (S) protein.

[00120] In embodiments, the CoV S polypeptides described herein contain further modifications from a native coronavirus S protein (e.g., SEQ ID NO: 2). In embodiments, the coronavirus S proteins described herein exhibit at least 80 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 % identity to a native CoV S glycoprotein. A person of skill in the art would use known techniques to calculate the percent identity of the recombinant coronavirus S protein to the native protein or to any of the CoV S glycoproteins described herein.

[00121] In embodiments, the CoV S polypeptides described herein are at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or at least 99.5 % identical to the CoV S polypeptide having an amino acid sequence of any one of SEQ ID NO: 87, SEQ ID NO: 174, SEQ ID NO: 175, SEQ ID NO: 176, SEQ ID NOS: 181-184, SEQ ID NO: 186, SEQ ID NO: 188, SEQ ID NO: 190, SEQ ID NO: 195; SEQ ID NOS: 217-228,

SEQ ID NOS: 233-236, and SEQ ID NO: 243, SEQ ID NOS 255-328. A CoV S polypeptide may have a deletion, an insertion, or mutation of up to about 1, up to about 2, up to about 3, up to about 4, up to about 5, up to about 10, up to about 15, up to about 20, up to about 25, up to about 30, up to about 35, up to about 40, up to about 45, or up to about 50 amino acids compared to the amino acid sequence of the CoV S polypeptide having an amino acid sequence of any one of SEQ ID NO: 87, SEQ ID NO: 174, SEQ ID NO: 175, SEQ ID NO: 176, SEQ ID NOS: 181-184, SEQ ID NO: 186, SEQ ID NO: 188, SEQ ID NO: 190, SEQ ID NO: 195; SEQ ID NOS: 217-228, SEQ ID NOS: 233-236, SEQ ID NO: 243, and SEQ ID NOS: 255-328. A CoV S polypeptide may have may have a deletion, an insertion, or mutation of from 1 to about 70 amino acids, from 1 to about 5 amino acids, from about 3 to about 10 amino acids, from about 5 to about 10 amino acids, from about 8 to about 12 amino acids, from about 10 to about 15 amino acids, from about 12 to about 17 amino acids, from about 15 to about 20 amino acids, from about 18 to about 23 amino acids, from about 20 to about 25 amino acids, from about 22 to about 27 amino acids, from about 25 to about 30 amino acids, from about 30 to about 35 amino acids, from about 35 to about 40 amino acids, from about 40 to about 45 amino acids, or from about 45 to about 50 amino acids, as compared to the CoV S polypeptide having an amino acid sequence of any one of SEQ ID NO: 87, SEQ ID NO: 174, SEQ ID NO: 175, SEQ ID NO: 176, SEQ ID NOS: 181-184, SEQ ID NO: 186, SEQ ID NO: 188, SEQ ID NO: 190, SEQ ID NO: 195; SEQ ID NOS: 217-228, SEQ ID NOS: 233-236, SEQ ID NO: 243, and SEQ ID NOS: 255-328. In embodiments, the CoV S polypeptides described herein comprise about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45, about 46, about 47, about 48, about 49, about 50, about 51, about 52, about 53, about 54, about 55, about 56, about 57, about 58, about 59, about 60, about 61, about 62, about 63, about 64, about 65, about 66, about 67, about 68, about 69, about 70, about 71, about 72, about 73, about 74, or about 75 deletions, insertions, or mutations compared to the coronavirus S protein having an amino acid sequence of any one of SEQ ID NO: 87, SEQ ID NO: 174, SEQ ID NO: 175, SEQ ID NO: 176, SEQ ID NOS: 181-184, SEQ ID NO: 186, SEQ ID NO: 188, SEQ ID NO: 190, SEQ ID NO:

195; SEQ ID NOS: 217-228, SEQ ID NOS: 233-236, SEQ ID NO: 243, and SEQ ID NOS: 255-328.

[00122] In embodiments, the coronavirus S polypeptide is extended at the N-terminus, the C-terminus, or both the N-terminus and the C-terminus. In aspects, the extension is a tag useful for a function, such as purification or detection. In aspects the tag contains an epitope. For example, the tag may be a polyglutamate tag, a FLAG-tag, a HA-tag, a polyHis-tag (having about 5-10 histidines) (SEQ ID NO: 101), a hexahistidine tag (SEQ ID NO: 100), an 8X-His-tag (having eight histidines) (SEQ ID NO: 102), a Myc-tag, a Glutathione-S-transferase-tag, a Green fluorescent protein-tag, Maltose binding protein-tag, a Thioredoxin-tag, or an Fc-tag. In other aspects, the extension may be an N-terminal signal peptide fused to the protein to enhance expression. While such signal peptides are often cleaved during expression in the cell, some nanoparticles may contain the antigen with an intact signal peptide. Thus, when a nanoparticle comprises an antigen, the antigen may contain an extension and thus may be a fusion protein when incorporated into nanoparticles. For the purposes of calculating identity to the sequence, extensions are not included. In embodiments, the tag is a protease cleavage site. Non-limiting examples of protease cleavage sites include the HRV3C protease cleavage site, chymotrypsin, trypsin, elastase, endopeptidase, caspase-1, caspase-2, caspase-3, caspase-4, caspase-5, caspase-6, caspase-7, caspase-8, caspase-9, caspase-10, enterokinase, factor Xa, Granzyme B, TEV protease, and thrombin. In embodiments, the protease cleavage site is an HRV3C protease cleavage site. In embodiments, the protease cleavage site comprises an amino acid sequence of SEQ ID NO: 98.

[00123] In embodiments, the CoV S glycoprotein comprises a fusion protein. In embodiments, the CoV S glycoprotein comprises an N-terminal fusion protein. In embodiments, the CoV S glycoprotein comprises a C-terminal fusion protein. In embodiments, the fusion protein encompasses a tag useful for protein expression, purification, or detection. In embodiments, the tag is a polyHis-tag (having about 5-10 histidines), a Myc-tag, a Glutathione-S-transferase-tag, a Green fluorescent protein-tag, Maltose binding protein-tag, a Thioredoxin-tag, a Strep-tag, a Twin-Strep-tag, or an Fc-tag. In embodiments, the tag is an Fc-tag. In embodiments, the Fc-tag is monomeric, dimeric, or trimeric. In embodiments, the tag is a hexahistidine tag, e.g. a polyHis-tag which contains six histidines (SEQ ID NO: 100). In embodiments, the tag is a Twin-Strep-tag with an amino acid sequence of SEQ ID NO: 99.

[00124] In embodiments, the CoV S polypeptide is a fusion protein comprising another coronavirus protein. In embodiments, the other coronavirus protein is from the same coronavirus. In embodiments, the other coronavirus protein is from a different coronavirus.

[00125] In aspects, the CoV S glycoprotein may be truncated. For example, the N-terminus may be truncated by about 10 amino acids, about 30 amino acids, about 50 amino acids, about 75 amino acids, about 100 amino acids, or about 200 amino acids. The C-terminus may be truncated instead of or in addition to the N-terminus. For example, the C-terminus may be truncated by about 10 amino acids, about 30 amino acids, about 50 amino acids, about 75 amino acids, about 100 amino acids, or about 200 amino acids. For purposes of calculating identity to the protein having truncations, identity is measured over the remaining portion of the protein.

[00126] In embodiments, the CoV S glycoproteins contain one or more modifications to the S1 subunit having an amino acid sequence of SEQ ID NO: 121.

[00127] The amino acid sequence of the S1 subunit (SEQ ID NO: 121) is shown below.

[00128] QCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFS
NVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSTQSL
LIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQ
PFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPI
GINITRFQTLALHRSYLT PGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDA
VDCALDPLSETKCTLSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATR
FASVYAWNRRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVI
RGDEVQRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRK
SNLKPFERDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLS
FELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIA
DTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHAD
QLTPTWRVYSTGSNVFQTRAGCLIGAHEVNNSECDIPIGAGICASYQTQTNSPRR
AR

[00129] In embodiments, the CoV S polypeptides described herein comprise an S1 subunit with at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, at least 99.5 %, or 100 % identity to the S1 subunit of SEQ ID NO: 1 or SEQ ID NO: 2. The S1 subunit may have a deletion, an insertion, or mutation of up to about 1, up to about 2,

up to about 3, up to about 4, up to about 5, up to about 10, up to about 15, up to about 20, up to about 25, up to about 30 amino acids, up to about 35 amino acids, up to about 40 amino acids, up to about 45 amino acids, or up to about 50 amino acids compared to the amino acid sequence of the S1 subunit of SEQ ID NO: 1 or SEQ ID NO: 2. The S1 subunit may have a deletion, an insertion, or mutation of between about 1 and about 5 amino acids, between about 3 and about 10 amino acids, between about 5 and 10 amino acids, between about 8 and 12 amino acids, between about 10 and 15 amino acids, between about 12 and 17 amino acids, between about 15 and 20 amino acids, between about 18 and 23 amino acids, between about 20 and 25 amino acids, between about 22 and about 27 amino acids, or between about 25 and 30 amino acids as compared to the S1 subunit of SEQ ID NO: 1 or SEQ ID NO: 2.

[00130] In embodiments, the S1 subunit may contain any combination of modifications shown in Table 1A.

Table 1A

Modifications to S1 (SEQ ID NO: 121)			
* amino acids 14-685 of SEQ ID NO: 1 and amino acids 1-672 of SEQ ID NO: 2			
Position within SEQ ID NO: 1	Position within SEQ ID NO: 2	Position within SEQ ID NO: 121	Potential Modifications
14-305	1-292	1-292	deletion of up to about 1, 2, 3, 4, 5, 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 292 amino acids
18	5	5	- mutation to phenylalanine - mutation to tyrosine - mutation to tryptophan
19	6	6	- mutation to arginine - mutation to lysine - mutation to histidine - mutation to isoleucine
20	7	7	- mutation to asparagine - mutation to glutamine - mutation to isoleucine - mutation to valine
24	11	11	- mutation to serine - mutation to threonine

Modifications to S1 (SEQ ID NO: 121)			
* amino acids 14-685 of SEQ ID NO: 1 and amino acids 1-672 of SEQ ID NO: 2			
Position within SEQ ID NO: 1	Position within SEQ ID NO: 2	Position within SEQ ID NO: 121	Potential Modifications
			• deletion
25	12	12	• insertion of amino acids proline-proline-alanine (PPA) after amino acid 25 • deletion
26	13	13	• mutation to serine • mutation to threonine • deletion
27	14	14	• mutation to serine • mutation to threonine
52	39	39	• mutation to arginine • mutation to lysine • mutation to histidine
64	51	51	• mutation to histidine • mutation to lysine • mutation to arginine
66	53	53	• mutation to tryptophan • mutation to tyrosine • mutation to phenylalanine
67	54	54	• mutation to valine • mutation to isoleucine • mutation to leucine
69	56	56	• Deletion of amino acid
70	57	57	• Deletion of amino acid • Mutation to phenylalanine • Mutation to tyrosine • Mutation to tryptophan
75	62	62	• Mutation to valine • Mutation to leucine • Mutation to isoleucine
76	63	63	• Mutation to isoleucine • Mutation to valine

Modifications to S1 (SEQ ID NO: 121)			
* amino acids 14-685 of SEQ ID NO: 1 and amino acids 1-672 of SEQ ID NO: 2			
Position within SEQ ID NO: 1	Position within SEQ ID NO: 2	Position within SEQ ID NO: 121	Potential Modifications
			• Mutation to leucine
80	67	67	• mutation to alanine • mutation to glycine
83	70	70	• mutation to alanine
95	82	82	• mutation to beta branched amino acid • mutation to isoleucine • mutation to valine
138	125	125	• mutation to tyrosine • mutation to phenylalanine • mutation to tryptophan
142	129	129	• mutation to aspartic acid • mutation to glutamic acid
143	130	130	• deletion of amino acid
144	131	131	• Deletion of amino acid • Mutation to serine
145	132	132	• Deletion of amino acid • Mutation to histidine • Mutation to asparagine • Mutation to glutamine
146	133	133	• mutation to aromatic amino acid • mutation to tyrosine • mutation to phenylalanine • mutation to tryptophan • mutation to glutamine
147	134	134	• mutation to glutamic acid • mutation to aspartic acid
152	139	139	• mutation to cysteine • mutation to methionine • mutation to serine • mutation to threonine • mutation to arginine

Modifications to S1 (SEQ ID NO: 121)			
* amino acids 14-685 of SEQ ID NO: 1 and amino acids 1-672 of SEQ ID NO: 2			
Position within SEQ ID NO: 1	Position within SEQ ID NO: 2	Position within SEQ ID NO: 121	Potential Modifications
			• mutation to lysine
156	143	143	• mutation to glycine • mutation to alanine
157	144	144	• deletion of amino acid • mutation to leucine • mutation to isoleucine • mutation to valine • mutation to beta-branched amino acid
158	145	145	• deletion of amino acid
180	167	167	• mutation to beta-branched amino acid • mutation to valine • mutation to isoleucine • mutation to leucine
183	170	170	• mutation to glutamic acid • mutation to aspartic acid
190	177	177	• mutation to serine • mutation to threonine • mutation to cysteine
210	197	197	• mutation to valine • mutation to isoleucine • mutation to leucine • mutation to beta branched amino acid
211	198	198	• mutation to isoleucine • mutation to valine • mutation to leucine • mutation to beta branched amino acid • deletion of amino acid
212	199	199	• mutation to isoleucine • mutation to valine • mutation to leucine • mutation to beta branched amino acid • deletion of amino acid
213	200	200	• mutation to valine

Modifications to S1 (SEQ ID NO: 121)			
* amino acids 14-685 of SEQ ID NO: 1 and amino acids 1-672 of SEQ ID NO: 2			
Position within SEQ ID NO: 1	Position within SEQ ID NO: 2	Position within SEQ ID NO: 121	Potential Modifications
			• mutation to leucine • mutation to isoleucine • mutation to beta branched amino acid • mutation to proline • mutation to glycine • mutation to glutamic acid • mutation to aspartic acid
214	201	201	• mutation to arginine • mutation to lysine • mutation to histidine • mutation to aspartic acid • mutation to glutamic acid • insertion of amino acids glutamic acid-proline-glutamic acid (EPE) after 214
215	202	202	• mutation to glycine • mutation to alanine • insertion of amino acids glutamic acid-proline-glutamic acid (EPE) after 215
222	209	209	• mutation to valine • mutation to leucine • mutation to isoleucine
241-244	228-231	228-231	• deletion of 1, 2, 3, or 4 amino acids •
242	229	229	• mutation to histidine • mutation to lysine • mutation to arginine
246	233	233	• mutation to beta-branched amino acid • mutation to isoleucine • mutation to valine • mutation to threonine • mutation to asparagine
247	234	234	• deletion of amino acid
248	235	235	• deletion of amino acid

Modifications to S1 (SEQ ID NO: 121)			
* amino acids 14-685 of SEQ ID NO: 1 and amino acids 1-672 of SEQ ID NO: 2			
Position within SEQ ID NO: 1	Position within SEQ ID NO: 2	Position within SEQ ID NO: 121	Potential Modifications
249	236	236	• deletion of amino acid
250	237	237	• deletion of amino acid
251	238	238	• deletion of amino acid
252	239	239	• deletion of amino acid • mutation to valine • mutation to leucine • mutation to isoleucine • mutation to beta branched amino acid • mutation to glycine
253	240	240	• mutation to glycine • deletion of amino acid
257	244	244	• mutation to serine • mutation to threonine • mutation to asparagine • mutation to glutamine
258	245	245	• mutation to isoleucine • mutation to valine • mutation to leucine • mutation to beta branched amino acid
339	326	326	• mutation to aspartic acid • mutation to glutamic acid • mutation to histidine
346	333	333	• mutation to lysine • mutation to arginine • mutation to histidine • mutation to threonine • mutation to serine
368	355	355	• mutation to isoleucine • mutation to leucine • mutation to valine • mutation to beta-branched amino acid
371	358	358	• mutation to leucine

Modifications to S1 (SEQ ID NO: 121)			
* amino acids 14-685 of SEQ ID NO: 1 and amino acids 1-672 of SEQ ID NO: 2			
Position within SEQ ID NO: 1	Position within SEQ ID NO: 2	Position within SEQ ID NO: 121	Potential Modifications
			• mutation to isoleucine • mutation to valine • mutation to phenylalanine
373	360	360	• mutation to proline
375	362	362	• mutation to phenylalanine • mutation to tyrosine • mutation to tryptophan
376	363	363	• mutation to alanine • mutation to glycine
405	392	392	• mutation to asparagine • mutation to glutamine
408	395	395	• mutation to serine • mutation to threonine
417	404	404	• mutation to asparagine • mutation to threonine • mutation to isoleucine • mutation to valine • mutation to serine • mutation to glutamine • mutation to beta-branched amino acid
432	419	419	• mutation to lysine • mutation to arginine • mutation to histidine
439	426	426	• mutation to lysine • mutation to arginine • mutation to histidine
440	427	427	• Mutation to lysine • Mutation to arginine • Mutation to histidine
444	431	431	• Mutation to threonine • Mutation to serine

Modifications to S1 (SEQ ID NO: 121)			
* amino acids 14-685 of SEQ ID NO: 1 and amino acids 1-672 of SEQ ID NO: 2			
Position within SEQ ID NO: 1	Position within SEQ ID NO: 2	Position within SEQ ID NO: 121	Potential Modifications
445	432	432	• Mutation to proline
446	433	433	• Mutation to serine • Mutation to threonine • Mutation to asparagine • Mutation to glutamine
452	439	439	• mutation to arginine • mutation to lysine • mutation to histidine • mutation to glutamine • mutation to asparagine
453	440	440	• mutation to phenylalanine • mutation to tryptophan
456	443	443	• mutation to leucine • mutation to valine • mutation to isoleucine
460	447	447	• mutation to lysine • mutation to arginine
477	464	464	• mutation to asparagine • mutation to glutamine
478	465	465	• mutation to lysine • mutation to arginine • mutation to histidine
484	471	471	• mutation to alanine • mutation to lysine • mutation to arginine • mutation to histidine • mutation to glutamine • mutation to asparagine
486	473	473	• mutation to valine • mutation to leucine • mutation to isoleucine • mutation to serine • mutation to threonine

Modifications to S1 (SEQ ID NO: 121)			
* amino acids 14-685 of SEQ ID NO: 1 and amino acids 1-672 of SEQ ID NO: 2			
Position within SEQ ID NO: 1	Position within SEQ ID NO: 2	Position within SEQ ID NO: 121	Potential Modifications
			• mutation to proline
490	477	477	• mutation to serine • mutation to threonine
493	480	480	• mutation to lysine • mutation to arginine • mutation to histidine
494	481	481	• mutation to proline
496	483	483	• mutation to serine • mutation to threonine
498	485	485	• mutation to lysine • mutation to arginine • mutation to histidine
501	488	488	• mutation to tyrosine • mutation to phenylalanine • mutation to tryptophan
505	492	492	• mutation to histidine
521	508	508	• mutation to serine • mutation to threonine
547	534	534	• mutation to lysine • mutation to arginine • mutation to histidine
570	557	557	• Mutation to aspartic acid • Mutation to glutamic acid
604	591	591	• Mutation to isoleucine • Mutation to leucine • Mutation to valine • Mutation to beta branched amino acid
613	600	600	• Mutation to histidine • Mutation to lysine

Modifications to S1 (SEQ ID NO: 121)			
* amino acids 14-685 of SEQ ID NO: 1 and amino acids 1-672 of SEQ ID NO: 2			
Position within SEQ ID NO: 1	Position within SEQ ID NO: 2	Position within SEQ ID NO: 121	Potential Modifications
			• Mutation to arginine
614	601	601	• Mutation to glycine • Mutation to alanine
639	626	626	• Mutation to valine • Mutation to leucine • Mutation to isoleucine
655	642	642	• Mutation to tyrosine • Mutation to phenylalanine • Mutation to tryptophan
658	645	645	• Mutation to serine • Mutation to threonine
677	664	664	• Mutation to histidine
679	666	666	• Mutation to lysine • Mutation to arginine • Mutation to histidine
681	668	668	• Mutation to histidine • Mutation to lysine • Mutation to arginine
682-685	669-672	669-672	• inactive furin cleavage site (See Table 1E)

[00131] In embodiments, the CoV S polypeptides contain one or more modifications to the NTD. In embodiments, the NTD has an amino acid sequence of SEQ ID NO: 118, which corresponds to amino acids 14-305 of SEQ ID NO: 1 or amino acids 1-292 of SEQ ID NO: 2.

[00132] The amino acid sequence of an NTD (SEQ ID NO: 118) is shown below.

[00133] QCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFS
NVTWFHAIHVS GTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLD SKTQSL
LIVNNATNVVIKVCE FQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQ
PFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLP I

GINITRFQTLALHRSYLTPGDSSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDA
VDCALDPLSETKCTLKS

[00134] In embodiments, the NTD has an amino acid sequence of SEQ ID NO: 45, which corresponds to amino acids 14 to 331 of SEQ ID NO: 1 or amino acids 1-318 of SEQ ID NO: 2. The amino acid sequence of an NTD (SEQ ID NO: 45) is shown below.

[00135] QCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFS
NVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSL
LIVNNATNVVIKVCEQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQ
PFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPI
GINITRFQTLALHRSYLTPGDSSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDA
VDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPN

[00136] In embodiments, the NTD and RBD overlap by up to about 1 amino acid, up to about 5 amino acids, up to about 10 amino acids, or up to about 20 amino acids.

[00137] In embodiments, an NTD as provided herein may be extended at the C-terminus by up to 5, up to 10, up to 15, up to 20, up to 25, or up to 30 amino acids.

[00138] In embodiments, the CoV S polypeptides described herein comprise a NTD with at least 95%, at least 96 %, at least 97%, at least 98 %, at least 99%, at least 99.5 %, or 100% identity to the NTD of SEQ ID NO: 1 or SEQ ID NO: 2. The NTD may have a deletion, an insertion, or mutation of up to about 1, up to about 2, up to about 3, up to about 4, up to about 5, up to about 10, up to about 15, up to about 20, up to about 25, or up to about 30 amino acids compared to the amino acid sequence of the NTD of SEQ ID NO: 1 or SEQ ID NO: 2. The NTD may have a deletion, an insertion, or mutation of between about 1 and about 5 amino acids, between about 3 and about 10 amino acids, between about 5 and 10 amino acids, between about 8 and 12 amino acids, between about 10 and 15 amino acids, between about 12 and 17 amino acids, between about 15 and 20 amino acids, between about 18 and 23 amino acids, between about 20 and 25 amino acids, between about 22 and about 27 amino acids, or between about 25 and 30 amino acids as compared to the NTD of SEQ ID NO: 1 or SEQ ID NO: 2.

[00139] In embodiments, the CoV S polypeptides contain a deletion of one or more amino acids from the N-terminal domain (NTD) (corresponding to amino acids 1-292 of SEQ ID NO: 2. In embodiments, the CoV S polypeptides contain a deletion of up to about 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 292 amino acids of the NTD.

[00140] In embodiments, the CoV S polypeptides contain a deletion of one or more amino acids from the NTD (corresponding to amino acids 1-318 of SEQ ID NO: 2). In embodiments, the CoV S polypeptides contain a deletion of amino acids 1-318 of the NTD of SEQ ID NO: 2. In embodiments, deletion of the NTD enhances protein expression of the CoV Spike (S) polypeptide. In embodiments, the CoV S polypeptides which have an NTD deletion have amino acid sequences represented by SEQ ID NOS: 46, 48, 49, 51, 52, and 54. In embodiments, the CoV S polypeptides which have an NTD deletion are encoded by an isolated nucleic acid sequence selected from the group consisting of SEQ ID NO: 47, SEQ ID NO: 50, and SEQ ID NO: 53.

[00141] In embodiments, the NTD may contain any combination of modifications shown in Table 1B. The modifications are shown with respect to SEQ ID NO: 2, the mature S polypeptide sequence for reference.

Table 1B

Modifications to NTD (SEQ ID NO: 118) * amino acids 14-305 of SEQ ID NO: 1 and amino acids 1-292 of SEQ ID NO: 2			
Position within SEQ ID NO: 1	SEQ ID NO: 2 residue	SEQ ID NO: 118 or SEQ ID NO: 45 residue	Modifications
14-305	1-292	1-292	deletion of up to about 1, 2, 3, 4, 5, 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 292 amino acids
18	5	5	- mutation to phenylalanine - mutation to tyrosine - mutation to tryptophan
19	6	6	- mutation to arginine - mutation to lysine - mutation to histidine - mutation to isoleucine
20	7	7	- mutation to asparagine - mutation to glutamine - mutation to isoleucine - mutation to valine
24	11	11	- mutation to serine - mutation to threonine

Modifications to NTD (SEQ ID NO: 118) * amino acids 14-305 of SEQ ID NO: 1 and amino acids 1-292 of SEQ ID NO: 2			
Position within SEQ ID NO: 1	SEQ ID NO: 2 residue	SEQ ID NO: 118 or SEQ ID NO: 45 residue	Modifications
			deletion
25	12	12	- insertion of amino acids proline-proline-alanine (PPA) after amino acid 25 - deletion
26	13	13	- mutation to serine - mutation to threonine - deletion
27	14	14	- mutation to serine - mutation to threonine
52	39	39	- mutation to arginine - mutation to lysine - mutation to histidine
64	51	51	- mutation to histidine - mutation to lysine - mutation to arginine
66	53	53	- mutation to tryptophan - mutation to tyrosine - mutation to phenylalanine
67	54	54	- mutation to valine - mutation to isoleucine - mutation to leucine
69	56	56	Deletion of amino acid
70	57	57	- Deletion of amino acid - Mutation to phenylalanine - Mutation to tyrosine - Mutation to tryptophan
75	62	62	- Mutation to valine - Mutation to leucine - Mutation to isoleucine
76	63	63	- Mutation to isoleucine - Mutation to valine - Mutation to leucine
80	67	67	- mutation to alanine - mutation to glycine
83	70	70	mutation to alanine
95	82	82	- mutation to beta branched amino acid - mutation to isoleucine

Modifications to NTD (SEQ ID NO: 118) * amino acids 14-305 of SEQ ID NO: 1 and amino acids 1-292 of SEQ ID NO: 2			
Position within SEQ ID NO: 1	SEQ ID NO: 2 residue	SEQ ID NO: 118 or SEQ ID NO: 45 residue	Modifications
			• mutation to valine
138	125	125	• mutation to tyrosine • mutation to phenylalanine • mutation to tryptophan
142	129	129	• mutation to aspartic acid • mutation to glutamic acid
143	130	130	• deletion of amino acid
144	131	131	• Deletion of amino acid • Mutation to serine
145	132	132	• Deletion of amino acid • Mutation to histidine • Mutation to asparagine • Mutation to glutamine
146	133	133	• mutation to aromatic amino acid • mutation to tyrosine • mutation to phenylalanine • mutation to tryptophan • mutation to glutamine • mutation to asparagine
147	134	134	• mutation to glutamic acid • mutation to aspartic acid
152	139	139	• mutation to cysteine • mutation to methionine • mutation to serine • mutation to threonine • mutation to arginine • mutation to lysine
156	143	143	• mutation to glycine • mutation to alanine
157	144	144	• deletion of amino acid • mutation to leucine
158	145	145	• deletion of amino acid
180	167	167	• mutation to isoleucine • mutation to valine • mutation to leucine • mutation to beta branched amino acid
183	170	170	• mutation to glutamic acid

Modifications to NTD (SEQ ID NO: 118) * amino acids 14-305 of SEQ ID NO: 1 and amino acids 1-292 of SEQ ID NO: 2			
Position within SEQ ID NO: 1	SEQ ID NO: 2 residue	SEQ ID NO: 118 or SEQ ID NO: 45 residue	Modifications
			• mutation to aspartic acid
190	177	177	• mutation to serine • mutation to threonine • mutation to cysteine
210	197	197	• mutation to valine • mutation to isoleucine • mutation to leucine • mutation to beta branched amino acid
211	198	198	• mutation to isoleucine • mutation to valine • mutation to leucine • mutation to beta branched amino acid • deletion of amino acid
212	199	199	• mutation to isoleucine • mutation to valine • mutation to leucine • mutation to beta branched amino acid • deletion of amino acid
213	200	200	• mutation to valine • mutation to leucine • mutation to isoleucine • mutation to beta branched amino acid • mutation to proline • mutation to glycine • mutation to glutamic acid • mutation to aspartic acid
214	201	201	• mutation to arginine • mutation to lysine • mutation to histidine • mutation to aspartic acid • mutation to glutamic acid • insertion of amino acids glutamic acid-proline-glutamic acid (EPE) after 214
215	202	202	• mutation to glycine • mutation to alanine • insertion of amino acids glutamic acid-proline-glutamic acid (EPE) after 215
222	209	209	• mutation to valine

Modifications to NTD (SEQ ID NO: 118) * amino acids 14-305 of SEQ ID NO: 1 and amino acids 1-292 of SEQ ID NO: 2			
Position within SEQ ID NO: 1	SEQ ID NO: 2 residue	SEQ ID NO: 118 or SEQ ID NO: 45 residue	Modifications
			• mutation to leucine • mutation to isoleucine
241-244	228-231	228-231	• deletion of 1, 2, 3, or 4 amino acids •
242	229	229	• mutation to histidine • mutation to lysine • mutation to arginine
246	233	233	• mutation to beta-branched amino acid • mutation to isoleucine • mutation to valine • mutation to threonine • mutation to asparagine
247	234	234	• deletion of amino acid
248	235	235	• deletion of amino acid
249	236	236	• deletion of amino acid
250	237	237	• deletion of amino acid
251	238	238	• deletion of amino acid
252	239	239	• deletion of amino acid • mutation to valine • mutation to isoleucine • mutation to leucine • mutation to beta branched amino acid • mutation to glycine
253	240	240	• mutation to glycine • deletion of amino acid
257	244	244	• mutation to serine • mutation to threonine
258	245	245	• mutation to isoleucine • mutation to valine • mutation to leucine • mutation to beta branched amino acid

[00142] In embodiments, the CoV S polypeptides contain one or more modifications to the RBD.

[00143] In embodiments, the RBD has an amino acid sequence of SEQ ID NO: 126, which corresponds to amino acids 331-527 of SEQ ID NO: 1 or amino acids 318-514 of SEQ ID NO: 2.

[00144] The amino acid sequence of the RBD (SEQ ID NO: 126) is shown below:

[00145] NITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLVNSASFSTFKCYGVSPSTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGNGYNYLYRLFRKSNLKPFRDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGP

[00146] In embodiments, the RBD has an amino acid sequence of SEQ ID NO: 116, which corresponds to amino acids 335-530 of SEQ ID NO: 1 or amino acids 322-517 of SEQ ID NO: 2.

[00147] The amino acid sequence of the RBD (SEQ ID NO: 116) is shown below.

[00148] LCPFGEVFNATRFASVYAWNRKRISNCVADYSVLVNSASFSTFKCYGVSPSTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGNGYNYLYRLFRKSNLKPFRDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKS

[00149] In embodiments, an RBD as provided herein may be extended at the N-terminus or C-terminus by up to 1 amino acid, up to 5 amino acids, up to 10 amino acids, up to 15 amino acids, up to 20 amino acids, up to 25 amino acids, or up to 30 amino acids.

[00150] In embodiments, the CoV S polypeptides described herein comprise a RBD with at least 95%, at least 96 %, at least 97%, at least 98 %, at least 99%, at least 99.5 %, or 100% identity to the RBD of SEQ ID NO: 1 or SEQ ID NO: 2. The RBD may have a deletion, an insertion, or mutation of up to about 1, up to about 2, up to about 3, up to about 4, up to about 5, up to about 10, up to about 15, up to about 20, up to about 25, or up to about 30 amino acids compared to the amino acid sequence of the RBD of SEQ ID NO: 1 or SEQ ID NO: 2. The RBD may have a deletion, an insertion, or mutation of from about 1 to about 50 amino acids, from about 1 to about 5 amino acids, from about 3 to about 10 amino acids, from about 5 to about 10 amino acids, from about 8 to about 12 amino acids, from about 10 to about 15 amino acids, from about 12 to about 17 amino acids, from about 15 to about 20 amino acids, from about 18 to about 23 amino acids, from about 20 to about 25 amino acids, from about 22 to about 27 amino acids, or from about 25 to about 30 amino acids as compared to the RBD of SEQ ID NO: 1 or SEQ ID NO: 2.

[00151] In embodiments, the CoV S polypeptide has at least one, at least two, at least three, at least four, at least four, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or at least 20 modifications in the RBD. In aspects, there are up to about 20, up to about 25, up to about 30, up to about 35, up to about 40, up to about 45, or up to about 50 modifications in the RBD. In embodiments, the RBD may contain any combination of modifications as shown in Table 1C.

Table 1C

Modifications to RBD (SEQ ID NO: 126)			
* amino acids 331-527 of SEQ ID NO: 1 and amino acids 318-514 of SEQ ID NO: 2			
Position within SEQ ID NO: 1	Position within SEQ ID NO: 2	Position within SEQ ID NO: 126	Potential Modifications
339	326	9	• mutation to aspartic acid • mutation to glutamic acid • mutation to histidine
346	333	16	• mutation to lysine • mutation to arginine • mutation to histidine • mutation to threonine • mutation to serine
368	355	38	• mutation to isoleucine • mutation to leucine • mutation to valine • mutation to beta-branched amino acid
371	358	41	• mutation to leucine • mutation to isoleucine • mutation to valine • mutation to phenylalanine • mutation to tyrosine • mutation to tryptophan
373	360	43	• mutation to proline
375	362	45	• mutation to phenylalanine • mutation to tyrosine • mutation to tryptophan
376	363	46	• mutation to alanine • mutation to glycine
405	392	75	• mutation to asparagine • mutation to glutamine
408	395	78	• mutation to serine

Modifications to RBD (SEQ ID NO: 126)			
* amino acids 331-527 of SEQ ID NO: 1 and amino acids 318-514 of SEQ ID NO: 2			
Position within SEQ ID NO: 1	Position within SEQ ID NO: 2	Position within SEQ ID NO: 126	Potential Modifications
			• mutation to threonine
417	404	87	• mutation to asparagine • mutation to threonine • mutation to isoleucine • mutation to valine • mutation to serine • mutation to glutamine • mutation to beta-branched amino acid
432	419	102	• mutation to lysine • mutation to arginine • mutation to histidine
439	426	109	• mutation to lysine • mutation to arginine • mutation to histidine •
440	427	110	• Mutation to lysine • Mutation to arginine • Mutation to histidine
444	431	114	• Mutation to threonine • Mutation to serine
445	432	115	• Mutation to proline
446	433	116	• Mutation to serine • Mutation to threonine
452	439	122	• mutation to arginine • mutation to lysine • mutation to histidine • mutation to glutamine • mutation to asparagine
453	440	123	• mutation to phenylalanine • mutation to tryptophan
456	443	126	• mutation to leucine • mutation to isoleucine • mutation to valine
460	447	130	• mutation to lysine • mutation to arginine
477	464	147	• mutation to asparagine • mutation to glutamine

Modifications to RBD (SEQ ID NO: 126)			
* amino acids 331-527 of SEQ ID NO: 1 and amino acids 318-514 of SEQ ID NO: 2			
Position within SEQ ID NO: 1	Position within SEQ ID NO: 2	Position within SEQ ID NO: 126	Potential Modifications
478	465	148	• mutation to lysine • mutation to arginine • mutation to histidine
484	471	154	• mutation to alanine • mutation to lysine • mutation to arginine • mutation to histidine • mutation to glutamine • mutation to asparagine
486	473	156	• mutation to valine • mutation to leucine • mutation to isoleucine • mutation to serine • mutation to threonine
490	477	160	• mutation to serine • mutation to threonine
493	480	163	• mutation to lysine • mutation to arginine • mutation to histidine
494	481	164	• mutation to proline
496	483	166	• mutation to serine • mutation to threonine
498	485	168	• mutation to lysine • mutation to arginine • mutation to histidine
501	488	171	• mutation to tyrosine • mutation to phenylalanine • mutation to tryptophan
505	492	175	• mutation to histidine
521	508	191	• mutation to serine • mutation to threonine

[00152] In embodiments, the CoV S polypeptides contain one or more modifications to the SD1/2 having an amino acid sequence of SEQ ID NO: 122, which corresponds to amino acids 542-681 of SEQ ID NO: 1 or amino acids 529-668 of SEQ ID NO: 2.

[00153] The amino acid sequence of the SD1/2 (SEQ ID NO: 122) is shown below.

[00154] NFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITP
CSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQT
RAGCLIGAEHVNNSYECDIPIGAGICASYQTQTNSP

[00155] In embodiments, the CoV S polypeptides described herein comprise a SD1/2 with at least 95%, at least 96 %, at least 97%, at least 98 %, at least 99%, at least 99.5%, or 100 % identity to the SD1/2 of SEQ ID NO: 1 or SEQ ID NO: 2. The SD1/2 may have a deletion, an insertion, or mutation of up to about 1, up to about 2, up to about 3, up to about 4, up to about 5, up to about 10, up to about 15, up to about 20, up to about 25, or up to about 30 amino acids compared to the amino acid sequence of the SD1/2 of SEQ ID NO: 1 or SEQ ID NO: 2. The SD1/2 may have a deletion, an insertion, or mutation of between about 1 and about 5 amino acids, between about 3 and about 10 amino acids, between about 5 and 10 amino acids, between about 8 and 12 amino acids, between about 10 and 15 amino acids, between about 12 and 17 amino acids, between about 15 and 20 amino acids, between about 18 and 23 amino acids, between about 20 and 25 amino acids, between about 22 and about 27 amino acids, or between about 25 and 30 amino acids as compared to the SD1/2 of SEQ ID NO: 1 or SEQ ID NO: 2.

[00156] In embodiments, the CoV S polypeptide has at least one, at least two, at least three, at least four, at least four, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or at least 20 modifications in the SD1/2. In aspects, there are up to about 20, up to about 25, up to about 30, up to about 35, up to about 40, up to about 45, or up to about 50 modifications in the SD1/2. In embodiments, the SD1/2 may contain any combination of modifications as shown in Table 1D.

Table 1D

Modifications to SD1/2 (SEQ ID NO: 122)			
* amino acids 542-681 of SEQ ID NO: 1 or amino acids 529-668 of SEQ ID NO: 2			
Position within SEQ ID NO: 1	Position within SEQ ID NO: 2	Position within SEQ ID NO: 122	Potential Modifications
547	534	6	• mutation to lysine • mutation to arginine • mutation to histidine

Modifications to SD1/2 (SEQ ID NO: 122)			
* amino acids 542-681 of SEQ ID NO: 1 or amino acids 529-668 of SEQ ID NO: 2			
Position within SEQ ID NO: 1	Position within SEQ ID NO: 2	Position within SEQ ID NO: 122	Potential Modifications
570	557	29	• Mutation to aspartic acid • Mutation to glutamic acid
604	591	63	• Mutation to isoleucine • Mutation to leucine • Mutation to valine • Mutation to beta-branched amino acid
613	600	72	• Mutation to histidine • Mutation to lysine • Mutation to arginine
614	601	73	• Mutation to glycine • Mutation to alanine
639	626	98	• Mutation to valine • Mutation to leucine • Mutation to isoleucine
655	642	114	• Mutation to tyrosine • Mutation to phenylalanine • Mutation to tryptophan
658	645	117	• Mutation to serine
677	664	136	• Mutation to histidine
679	666	138	• Mutation to lysine • Mutation to arginine • Mutation to histidine
681	668	140	• Mutation to histidine • Mutation to lysine • Mutation to arginine
547	534	6	• mutation to lysine • mutation to arginine • mutation to histidine
570	557	29	• Mutation to aspartic acid • Mutation to glutamic acid
613	600	72	• Mutation to histidine • Mutation to lysine • Mutation to arginine
614	601	73	• Mutation to glycine • Mutation to alanine
655	642	114	• Mutation to tyrosine • Mutation to phenylalanine • Mutation to tryptophan
677	664	136	• Mutation to histidine

Modifications to SD1/2 (SEQ ID NO: 122)			
* amino acids 542-681 of SEQ ID NO: 1 or amino acids 529-668 of SEQ ID NO: 2			
Position within SEQ ID NO: 1	Position within SEQ ID NO: 2	Position within SEQ ID NO: 122	Potential Modifications
679	666	138	• Mutation to lysine • Mutation to arginine • Mutation to histidine

[00157] In embodiments, the CoV S polypeptides contain a furin site (RRAR), which corresponds to amino acids 682-685 of SEQ ID NO: 1 or amino acids 669-672 of SEQ ID NO: 2, that is inactivated by one or more mutations. Inactivation of the furin cleavage site prevents furin from cleaving the CoV S polypeptide. In embodiments, the CoV S polypeptides described herein which contain an inactivated furin cleavage site are expressed as a single chain.

[00158] In embodiments, one or more of the amino acids comprising the native furin cleavage site is mutated to any natural amino acid. In embodiments, the amino acids are L-amino acids. Non-limiting examples of amino acids include alanine, arginine, glycine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, serine, threonine, histidine, lysine, methionine, proline, valine, isoleucine, leucine, tyrosine, tryptophan, and phenylalanine.

[00159] In embodiments, one or more of the amino acids comprising the native furin cleavage site is mutated to glutamine. In embodiments, 1, 2, 3, or 4 amino acids may be mutated to glutamine. In embodiments, one of the arginines comprising the native furin cleavage site is mutated to glutamine. In embodiments, two of the arginines comprising the native furin cleavage site are mutated to glutamine. In embodiments, three of the arginines comprising the native furin cleavage site are mutated to glutamine.

[00160] In embodiments, one or more of the amino acids comprising the native furin cleavage site, is mutated to alanine. In embodiments, 1, 2, 3, or 4 amino acids may be mutated to alanine. In embodiments, one of the arginines comprising the native furin cleavage site is mutated to alanine. In embodiments, two of the arginines comprising the native furin cleavage site are mutated to alanine. In embodiments, three of the arginines comprising the native furin cleavage site are mutated to alanine.

[00161] In embodiments, one or more of the amino acids comprising the native furin cleavage site is mutated to glycine. In embodiments, 1, 2, 3, or 4 amino acids may be mutated to glycine. In embodiments, one of the arginines of the native furin cleavage site is mutated to glycine. In embodiments, two of the arginines comprising the native furin cleavage site are mutated to glycine. In embodiments, three of the arginines comprising the native furin cleavage site are mutated to glycine.

[00162] In embodiments, one or more of the amino acids comprising the native furin cleavage site, is mutated to asparagine. For example 1, 2, 3, or 4 amino acids may be mutated to asparagine. In embodiments, one of the arginines comprising the native furin cleavage site is mutated to asparagine. In embodiments, two of the arginines comprising the native furin cleavage site are mutated to asparagine. In embodiments, three of the arginines comprising the native furin cleavage site are mutated to asparagine.

[00163] In embodiments, in lieu of an active furin cleavage site (SEQ ID NO: 6) the CoV S polypeptides described herein contain an inactivated furin cleavage site. In embodiments, the amino acid sequence of the inactivated furin cleavage site is represented by any one of SEQ ID NO: 7-34 or SEQ ID NO: 97. In embodiments, the amino acid sequence of the inactivated furin cleavage site is QQAQ (SEQ ID NO: 7). In embodiments, the amino acid sequence of the inactivated furin cleavage site is GSAS (SEQ ID NO: 97). In embodiments, the amino acid sequence of the inactivated furin cleavage site is GSGA (SEQ ID NO: 111). In embodiments, the amino acid sequence of the inactivated furin cleavage site is GG, GGG (SEQ ID NO: 127), GGGG (SEQ ID NO: 128), or GGGGG (SEQ ID NO: 129).

[00164] Non-limiting examples of the amino acid sequences of the inactivated furin sites contained within the CoV S polypeptides are found in Table 1E.

Table 1E

Amino Acid Sequence of Furin Cleavage Site	Active or Inactive Furin Cleavage Site
RRAR (SEQ ID NO: 6)	Active
QQAQ (SEQ ID NO: 7)	Inactive
QRAR (SEQ ID NO: 8)	Inactive
RQAR (SEQ ID NO: 9)	Inactive

Amino Acid Sequence of Furin Cleavage Site	Active or Inactive Furin Cleavage Site
RRAQ (SEQ ID NO: 10)	Inactive
QQAR (SEQ ID NO: 11)	Inactive
RQAQ (SEQ ID NO: 12)	Inactive
QRAQ (SEQ ID NO: 13)	Inactive
NNAN (SEQ ID NO: 14)	Inactive
NRAR (SEQ ID NO: 15)	Inactive
RNAR (SEQ ID NO: 16)	Inactive
RRAN (SEQ ID NO: 17)	Inactive
NNAR (SEQ ID NO: 18)	Inactive
RNAN (SEQ ID NO: 19)	Inactive
NRAN (SEQ ID NO: 20)	Inactive
AAAA (SEQ ID NO: 21)	Inactive
ARAR (SEQ ID NO: 22)	Inactive
RAAR (SEQ ID NO: 23)	Inactive
RRAA (SEQ ID NO: 24)	Inactive
AAAR (SEQ ID NO: 25)	Inactive
RAAA (SEQ ID NO: 26)	Inactive
ARAA (SEQ ID NO: 27)	Inactive
GGAG (SEQ ID NO: 28)	Inactive
GRAR (SEQ ID NO: 29)	Inactive
RGAR (SEQ ID NO: 30)	Inactive
RRAG (SEQ ID NO: 31)	Inactive
GGAR (SEQ ID NO: 32)	Inactive
RGAG (SEQ ID NO: 33)	Inactive

Amino Acid Sequence of Furin Cleavage Site	Active or Inactive Furin Cleavage Site
GRAG (SEQ ID NO: 34)	Inactive
GSAS (SEQ ID NO: 97)	Inactive
GSGA (SEQ ID NO: 111)	Inactive

[00165] In embodiments, the CoV S polypeptides contain one or more modifications to the S2 subunit having an amino acid sequence of SEQ ID NO: 120, which corresponds to amino acids 686-1273 of SEQ ID NO: 1 or amino acids 673-1260 of SEQ ID NO: 2.

[00166] The amino acid sequence of the S2 subunit (SEQ ID NO: 120) is shown below.

[00167] SVASQSIIAYTMSLGAENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTS
VDCTMYICGDSTECNLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKT
PIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLI
CAQKFNGLTVLPPLLTDEMAQYTSALLAGTITSGWTFGAGAALQIPFAMQMAYRFN
GIGVTQNVLYENQKLIANQFNQSAIGKIQDSLSSTASALGKLQDVVNQNAQALNTLVK
QLSSNFGAISSVLNDILSRDKVEAEVQIDRLITGRLQSLQTYVTQQILIRAAEIRASAN
LAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNTTAA
PAICHGKAHFPREGVFVSNNGTHWFVTQRNFYEPQIITDNTFVSGNCDVVIGIVNN
TVYDPLQPELDSFKEELDKYFKNHTSPDVLGDISGINASVVNIQKEIDRLNEVAKNL
NESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCCLKGCCSC
GSCCKFDEDDSEPVCLKGVKLHYT

[00168] In embodiments, the CoV S polypeptides described herein comprise an S2 subunit with at least 95%, at least 96 %, at least 97%, at least 98 %, at least 99%, at least 99.5 %, at least 99.7 %, at least 99.9 %, or 100% identity to the S2 subunit of SEQ ID NO: 1 or SEQ ID NO: 2. The S2 subunit may have a deletion, an insertion, or mutation of up to about 1, up to about 2, up to about 3, up to about 4, up to about 5, up to about 10, up to about 15, up to about 20, up to about 25, or up to about 30 amino acids compared to the amino acid sequence of the S2 subunit of SEQ ID NO: 1 or SEQ ID NO: 2. The S2 subunit may have a deletion, an insertion, or mutation of from about 1 to about 50 amino acids, from about 1 to about 5 amino acids, from about 3 to about 10 amino acids, from about 5 to 10 amino acids, from about 8 to 12 amino acids, from about 10 to 15 amino acids, from about 12 to 17 amino acids, from about

15 to 20 amino acids, from about 18 to 23 amino acids, from about 20 to 25 amino acids, from about 22 to about 27 amino acids, or from about 25 to 30 amino acids as compared to the S2 subunit of SEQ ID NO: 1 or SEQ ID NO: 2.

[00169] In embodiments, the CoV S polypeptides contain a mutation at Lys-973 of the native CoV Spike (S) polypeptide (SEQ ID NO: 2). In embodiments, Lys-973 is mutated to any natural amino acid. In embodiments, Lys-973 is mutated to proline. In embodiments, Lys-973 is mutated to glycine. In embodiments, the CoV S polypeptides containing a mutation at amino acid 973 are selected from the group consisting of SEQ ID NO: 84-89, 105-106, and 109-110.

[00170] In embodiments, the CoV S polypeptides contain a mutation at Val-974 of the native CoV Spike (S) polypeptide (SEQ ID NO: 2). In embodiments, Val-974 is mutated to any natural amino acid. In embodiments, Val-974 is mutated to proline. In embodiments, Val-974 is mutated to glycine. In embodiments, the CoV S polypeptides containing a mutation at amino acid 974 are selected from the group consisting of SEQ ID NO: 84-89, 105-106, and 109-110.

[00171] In embodiments, the CoV S polypeptides contain a mutation at Lys-973 and Val-974 of the native CoV Spike (S) polypeptide (SEQ ID NO: 2). In embodiments, Lys-973 and Val-974 are mutated to any natural amino acid. In embodiments, Lys-973 and Val-974 are mutated to proline. In embodiments, the CoV S polypeptides containing a mutation at amino acids 973 and 974 are selected from SEQ ID NOS: 84-89, 105-106, 109-110, 175, 220, and 217-228.

[00172] In embodiments, the S2 subunit may contain any combination of modifications as shown in Table 1F.

Table 1F

Modifications to S2 (SEQ ID NO: 120) * amino acids 686-1273 of SEQ ID NO: 1 and amino acids 673-1260 of SEQ ID NO: 2			
Position within SEQ ID NO: 1	Position within SEQ ID NO: 2	Position within SEQ ID NO: 120	Possible Modifications
689-698	676-685	4-13	• Deletion of up to about 1, up to about 2, up to about 3, up to about 4, up to about 5, up to about 6, up to about 7, up to about 8, up to about 9, or up to about 10 amino acids

Modifications to S2 (SEQ ID NO: 120)			
* amino acids 686-1273 of SEQ ID NO: 1 and amino acids 673-1260 of SEQ ID NO: 2			
Position within SEQ ID NO: 1	Position within SEQ ID NO: 2	Position within SEQ ID NO: 120	Possible Modifications
701	688	16	• Mutation to beta-branched amino acid • Mutation to valine • Mutation to isoleucine • Mutation to threonine
704	691	19	• Mutation to leucine • Mutation to isoleucine • Mutation to valine
715-724	702-711	30-39	• Deletion of up to about 1, up to about 2, up to about 3, up to about 4, up to about 5, up to about 6, up to about 7, up to about 8, up to about 9, or up to about 10 amino acids
716	703	31	• Mutation to beta-branched amino acid • Mutation to valine • Mutation to isoleucine
764	751	79	• Mutation to lysine • Mutation to arginine • Mutation to histidine
788-806	775-793	103-121	• Deletion of up to about 1, up to about 2, up to about 3, up to about 4, up to about 5, up to about 6, up to about 7, up to about 8, up to about 9, up to about 10, up to about 11, up to about 12, up to about 13, up to about 14, up to about 15, up to about 16, up to about 17, up to about 18, or up to about 19 amino acids
796	783	111	• Mutation to tyrosine • Mutation to phenylalanine • Mutation to tryptophan
819-828	806-815	134-143	• Deletion of up to about 1, up to about 2, up to about 3, up to about 4, up to about 5, up to about 6, up to about 7, up to about 8, up to about 9, or up to about 10 amino acids
856	843	171	• Mutation to lysine • Mutation to arginine • Mutation to histidine
859	846	174	• Mutation to asparagine • Mutation to glutamine
888	875	203	• Mutation to leucine • Mutation to isoleucine • Mutation to valine

Modifications to S2 (SEQ ID NO: 120)			
* amino acids 686-1273 of SEQ ID NO: 1 and amino acids 673-1260 of SEQ ID NO: 2			
Position within SEQ ID NO: 1	Position within SEQ ID NO: 2	Position within SEQ ID NO: 120	Possible Modifications
950	937	265	• Mutation to asparagine • Mutation to glutamine
954	941	269	• Mutation to histidine • Mutation to lysine • Mutation to arginine
969	956	284	• Mutation to lysine • Mutation to arginine • Mutation to histidine
981	968	296	• Mutation to phenylalanine • Mutation to tyrosine • Mutation to tryptophan
982	969	297	• Mutation to alanine • Mutation to glycine • Mutation to threonine
986	973	301	• Mutation to proline • Mutation to glycine
987	974	302	• Mutation to proline • Mutation to glycine
1027	1014	342	• Mutation to isoleucine • Mutation to valine • Mutation to serine
1071	1058	386	• Mutation to histidine • Mutation to arginine • Mutation to lysine
1118	1105	433	• Mutation to histidine • Mutation to lysine • Mutation to arginine • Mutation to asparagine • Mutation to glutamine
1176	1163	491	• Mutation to phenylalanine • Mutation to tyrosine • Mutation to tryptophan
1199	1186	514	• Mutation to asparagine • Mutation to glutamine

Modifications to S2 (SEQ ID NO: 120) * amino acids 686-1273 of SEQ ID NO: 1 and amino acids 673-1260 of SEQ ID NO: 2			
Position within SEQ ID NO: 1	Position within SEQ ID NO: 2	Position within SEQ ID NO: 120	Possible Modifications
1214-1237	1201-1224	1-24	• Deletion of one or more amino acids of TM
1238-1273	1225-1260	1-36	• Deletion of one or more amino acids of CD

[00173] In embodiments, the immunogenic compositions comprise 1, 2, 3, 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45, about 46, about 47, about 48, about 49, or about 50 SARS-CoV-2 S glycoproteins, including all values and ranges therebetween.

[00174] Multiple examples of SARS-CoV-2 S glycoproteins are described in the following patent documents, which are each incorporated by reference herein in their entirety for all purposes: International Publication No. 2021/015220, International Publication No. 2022/020974, International Publication No. 2022/235662, International Publication No. 2023/102448.

Nanoparticles comprising Viral Glycoproteins

[00175] In embodiments, the immunogenic compositions and vaccine compositions described herein comprise nanoparticles. The nanoparticles of the present disclosure are non-naturally occurring products, the components of which do not occur together in nature. In embodiments, the nanoparticles are detergent-core nanoparticles comprising a viral glycoprotein and a non-ionic detergent. In embodiments, provided herein are compositions comprising detergent-core nanoparticles comprising an RSV F glycoprotein and a non-ionic detergent. In embodiments, provided herein are compositions comprising detergent-core nanoparticles comprising a HA glycoprotein and a non-ionic detergent. In

embodiments, provided herein are compositions comprising detergent-core nanoparticles comprising a CoV S glycoprotein and a non-ionic detergent.

[00176] In embodiments, a single nanoparticle comprises one or more different viral glycoproteins. In embodiments, provided herein are detergent-core nanoparticles comprising an RSV F glycoprotein and a HA glycoprotein. In embodiments, provided herein are detergent-core nanoparticles comprising an RSV F glycoprotein and a CoV S glycoprotein. In embodiments, provided herein are detergent-core nanoparticles comprising an RSV F glycoprotein, a HA glycoprotein, and a CoV S glycoprotein.

[00177] In embodiments, the detergent-core nanoparticle comprises a viral glycoprotein with a transmembrane domain, wherein the viral glycoprotein is anchored to the detergent core via the transmembrane domain of the viral glycoprotein. In embodiments, the viral glycoproteins form trimers.

[00178] In embodiments, to produce detergent-core nanoparticles, a detergent exchange approach is utilized, wherein a first detergent is used to isolate a viral glycoprotein and then that first detergent is exchanged for a second detergent to form the nanoparticles.

[00179] The viral glycoproteins contained in the nanoparticles are typically produced by recombinant expression in host cells. Standard recombinant techniques may be used. In embodiments, the viral glycoproteins are expressed in insect host cells using a baculovirus system. In embodiments, from 1-50 viral glycoproteins are co-expressed in a host cell. In embodiments, the baculovirus is a cathepsin-L knock-out baculovirus, a chitinase knock-out baculovirus. Optionally, the baculovirus is a double knock-out for both cathepsin-L and chitinase. High level expression may be obtained in insect cell expression systems. Non limiting examples of insect cells are, *Spodoptera frugiperda* (Sf) cells, e.g. Sf9, Sf21, *Trichoplusia ni* cells, e.g. High Five cells, and *Drosophila* S2 cells. In embodiments, the viral glycoproteins described herein are produced in any suitable host cell. In embodiments, the host cell is an insect cell. In embodiments, the insect cell is an Sf9 cell.

[00180] Typical transfection and cell growth methods can be used to culture the cells. Vectors, e.g., vectors comprising polynucleotides that encode fusion proteins, can be transfected into host cells according to methods well known in the art. For example, introducing nucleic acids into eukaryotic cells can be achieved by calcium phosphate co-precipitation, electroporation, microinjection, lipofection, and

transfection employing polyamine transfection reagents. In one embodiment, the vector is a recombinant baculovirus.

[00181] Methods to grow host cells include, but are not limited to, batch, batch-fed, continuous and perfusion cell culture techniques. Cell culture means the growth and propagation of cells in a bioreactor (a fermentation chamber) where cells propagate and express protein (e.g. recombinant proteins) for purification and isolation. Typically, cell culture is performed under sterile, controlled temperature and atmospheric conditions in a bioreactor. A bioreactor is a chamber used to culture cells in which environmental conditions such as temperature, atmosphere, agitation and/or pH can be monitored. In one embodiment, the bioreactor is a stainless steel chamber. In another embodiment, the bioreactor is a pre-sterilized plastic bag (e.g. Cellbag®, Wave Biotech, Bridgewater, N.J.). In other embodiment, the pre-sterilized plastic bags are about 50 L to 3500 L bags.

[00182] After growth of the host cells, the protein may be harvested from the host cells using detergents and purification protocols. In embodiments, multiple viral glycoproteins are purified simultaneously. In embodiments, host cells expressing multiple viral glycoproteins are pooled together. Once the host cells have grown for 48 to 96 hours, the cells are isolated from the media and a detergent-containing solution is added to solubilize the cell membrane, releasing the protein in a detergent extract. Triton X-100 and TERGITOL® nonylphenol ethoxylate, also known as NP-9, are each preferred detergents for extraction. The detergent may be added to a final concentration of about 0.1% to about 1.0%. For example, the concentration may be about 0.1%, about 0.2%, about 0.3%, about 0.5%, about 0.7%, about 0.8%, or about 1.0 %. The range may be about 0.1% to about 0.3%. In aspects, the concentration is about 0.5%.

[00183] In other aspects, different first detergents may be used to isolate the protein from the host cell. For example, the first detergent may be Bis(polyethylene glycol bis[imidazolylcarbonyl]), nonoxynol-9, Bis(polyethylene glycol bis[imidazolyl carbonyl]), BRIJ® Polyethylene glycol dodecyl ether 35, BRIJ® Polyethylene glycol (3) cetyl ether 56, BRIJ® alcohol ethoxylate 72, BRIJ® Polyoxyl 2 stearyl ether 76, BRIJ® polyethylene glycol monooleyl ether 92V, BRIJ® Polyoxyethylene (10) oleyl ether 97, BRIJ® Polyethylene glycol hexadecyl ether 58P, CREMOPHOR® EL Macrogolglycerol ricinoleate, Decaethyleneglycol monododecyl ether, N-Decanoyl-N-methylglucamine, n-Decyl alpha-Dglucopyranoside, Decyl beta-D-maltopyranoside, n-

Dodecanoyl-N-methylglucamide, nDodecyl alpha-D-maltoside, n-Dodecyl beta-D-maltoside, n-Dodecyl beta-D-maltoside, Heptaethylene glycol monodecyl ether, Heptaethylene glycol monododecyl ether, Heptaethylene glycol monotetradecyl ether, n-Hexadecyl beta-D-maltoside, Hexaethylene glycol monododecyl ether, Hexaethylene glycol monohexadecyl ether, Hexaethylene glycol monooctadecyl ether, Hexaethylene glycol monotetradecyl ether, Igepal CA-630, Igepal CA -630, Methyl-6-O-(N -heptylcarbamoyl)-alpha-D-glucopyranoside, Nonaethylene glycol monododecyl ether, N-Nonanoyl-N-methylglucamine, N-Nonanoyl-N-methylglucamine, Octaethylene glycol monodecyl ether, Octaethylene glycol monododecyl ether, Octaethylene glycol monohexadecyl ether, Octaethylene glycol monooctadecyl ether, Octaethylene glycol monotetradecyl ether, Octyl-beta-D glucopyranoside, Pentaethylene glycol monodecyl ether, Pentaethylene glycol monododecyl ether, Pentaethylene glycol monohexadecyl ether, Pentaethylene glycol monohexyl ether, Pentaethylene glycol monooctadecyl ether, Pentaethylene glycol monoethyl ether, Polyethylene glycol diglycidyl ether, Polyethylene glycol ether W-1, Polyoxyethylene 10 tridecyl ether, Polyoxyethylene 100 stearate, Polyoxyethylene 20 isohexadecyl ether, Polyoxyethylene 20 oleyl ether, Polyoxyethylene 40 stearate, Polyoxyethylene 50 stearate, Polyoxyethylene 8 stearate, Polyoxyethylene bis(imidazolyl carbonyl), Polyoxyethylene 25 propylene glycol stearate, Saponin from Quillaja bark, SPAN® 20 sorbitan laurate, SPAN® 40 sorbitan monopalmitate, SPAN® 60 sorbitan stearate, SPAN® 65 sorbitan tristearate, SPAN® 80 sorbitane monooleate, SPAN® 85 sorbitane trioleate, TERGITOL® secondary alcohol ethoxylate Type 15-S-12, TERGITOL® secondary alcohol ethoxylate Type 15-S-30, TERGITOL® secondary alcohol ethoxylate Type 15-S-5, TERGITOL® secondary alcohol ethoxylate Type 15-S-7, TERGITOL® secondary alcohol ethoxylate Type 15-S-9, TERGITOL® nonylphenol ethoxylate Type NP-10, TERGITOL® nonylphenol ethoxylate Type NP-4, TERGITOL® nonylphenol ethoxylate Type NP-40, TERGITOL® nonylphenol ethoxylate Type NP-7, TERGITOL® nonylphenol ethoxylate Type NP-9, TERGITOL® branched secondary alcohol ethoxylate Type TMN-10, TERGITOL® branched secondary alcohol ethoxylate Type TMN-6, TRITONTM X-100 Polyethylene glycol tert-octylphenyl ether or combinations thereof.

[00184] The nanoparticles may then be isolated from cellular debris using centrifugation. In embodiments, gradient centrifugation, such as using cesium chloride, sucrose and iodixanol, may be used. Other techniques may be used as

alternatives or in addition, such as standard purification techniques including, e.g., ion exchange, affinity, and gel filtration chromatography.

[00185] For example, the first column may be an ion exchange chromatography resin, such as FRACTOGEL® EMD methacrylate based polymeric beads TMAE (EMD Millipore), the second column may be a lentil (*Lens culinaris*) lectin affinity resin, and the third column may be a cation exchange column such as a FRACTOGEL® EMD methacrylate based polymeric beads SO3 (EMD Millipore) resin. In other aspects, the cation exchange column may be an MMC column or a Nuvia C Prime column (Bio-Rad Laboratories, Inc). Preferably, the methods disclosed herein do not use a detergent extraction column; for example a hydrophobic interaction column. Such a column is often used to remove detergents during purification but may negatively impact the methods disclosed here.

[00186] To form detergent-core nanoparticles, the first detergent, used to extract the protein from the host cell is substantially replaced with a second detergent to arrive at the nanoparticle structure. In embodiments, the first detergent is NP-9. Typically, the nanoparticles do not contain detectable NP-9 when measured by HPLC. The second detergent is typically selected from the group consisting of PS20, PS40, PS60, PS65, and PS80. In embodiments, the second detergent is PS80.

[00187] In particular aspects, detergent exchange is performed using affinity chromatography to bind glycoproteins via their carbohydrate moiety. For example, the affinity chromatography may use a legume lectin column. Legume lectins are proteins originally identified in plants and found to interact specifically and reversibly with carbohydrate residues. See, for example, Sharon and Lis, "Legume lectins--a large family of homologous proteins," *FASEB J.* 1990 Nov;4(14):3198-208; Liener, "The Lectins: Properties, Functions, and Applications in Biology and Medicine," Elsevier, 2012. Suitable lectins include concanavalin A (con A), pea lectin, sainfoin lect, and lentil lectin. Lentil lectin is a preferred column for detergent exchange due to its binding properties. Lectin columns are commercially available; for example, Capto Lentil Lectin, is available from GE Healthcare. In certain aspects, the lentil lectin column may use a recombinant lectin. At the molecular level, it is thought that the carbohydrate moieties bind to the lentil lectin, freeing the amino acids of the protein to coalesce around the detergent resulting in the formation of a detergent core providing nanoparticles having multiple copies of the antigen, e.g., glycoprotein oligomers which can be dimers, trimers, or tetramers anchored in the detergent. In embodiments, the

viral glycoproteins form trimers. In embodiments, the viral glycoprotein trimers are anchored in detergent. In embodiments, each viral glycoprotein nanoparticle contains at least one trimer associated with a non-ionic core.

[00188] The detergent, when incubated with the protein to form the nanoparticles during detergent exchange, may be present at up to about 0.1% (w/v) during early purifications steps and this amount is lowered to achieve the final nanoparticles having optimum stability. For example, the non-ionic detergent (e.g., PS80) may be about 0.005% (v/v) to about 0.1% (v/v), for example, about 0.005 % (v/v), about 0.006 % (v/v), about 0.007 % (v/v), about 0.008 % (v/v), about 0.009 % (v/v), about 0.01 % (v/v), about 0.015 % (v/v), about 0.02 % (v/v), about 0.025 % (v/v), about 0.03 % (v/v), about 0.035 % (v/v), about 0.04 % (v/v), about 0.045 % (v/v), about 0.05 % (v/v), about 0.055 % (v/v), about 0.06 % (v/v), about 0.065 % (v/v), about 0.07 % (v/v), about 0.075 % (v/v), about 0.08 % (v/v), about 0.085 % (v/v), about 0.09 % (v/v), about 0.095 % (v/v), or about 0.1 % (v/v) PS80. In embodiments, the nanoparticle contains about 0.03% to about 0.05% PS80. In embodiments, the nanoparticle contains about 0.01 % (v/v) PS80.

[00189] In embodiments, purified viral glycoproteins are dialyzed. In embodiments, dialysis occurs after purification. In embodiments, the viral glycoproteins are dialyzed in a solution comprising sodium phosphate, NaCl, and PS80. In embodiments, the dialysis solution comprising sodium phosphate contains from about 5 mM to about 100 mM of sodium phosphate, for example, about 5 mM, about 10 mM, about 15 mM, about 20 mM, about 25 mM, about 30 mM, about 35 mM, about 40 mM, about 45 mM, about 50 mM, about 55 mM, about 60 mM, about 65 mM, about 70 mM, about 75 mM, about 80 mM, about 85 mM, about 90 mM, about 95 mM, or about 100 mM sodium phosphate. In embodiments, the pH of the solution comprising sodium phosphate is about 6.5, about 6.6, about 6.7, about 6.8, about 6.9, about 7.0, about 7.1, about 7.2, about 7.3, about 7.4, or about 7.5. In embodiments, the dialysis solution comprising sodium chloride comprises about 50 mM NaCl to about 750 mM NaCl, for example, about 50 mM, about 60 mM, about 70 mM, about 80 mM, about 90 mM, about 100 mM, about 110 mM, about 120 mM, about 130 mM, about 140 mM, about 150 mM, about 160 mM, about 170 mM, about 180 mM, about 190 mM, about 200 mM, about 210 mM, about 220 mM, about 230 mM, about 240 mM, about 250 mM, about 260 mM, about 270 mM, about 280 mM, about 290 mM, about 300 mM, about 310 mM, about 320 mM, about 330 mM, about 340 mM, about 350

mM, about 360 mM, about 370 mM, about 380 mM, about 390 mM, about 400 mM, about 410 mM, about 420 mM, about 430 mM, about 440 mM, about 450 mM, about 460 mM, about 470 mM, about 480 mM, about 490 mM, about 500 mM, about 510 mM, about 520 mM, about 530 mM, about 540 mM, about 550 mM, about 560 mM, about 570 mM, about 580 mM, about 590 mM, about 600 mM, about 610 mM, about 620 mM, about 630 mM, about 640 mM, about 650 mM, about 660 mM, about 670 mM, about 680 mM, about 690, about 700 mM, about 710 mM, about 720 mM, about 730 mM, about 740 mM, or about 750 mM NaCl. In embodiments, the dialysis solution comprising PS80 comprises about 0.005 % (v/v), about 0.006 % (v/v), about 0.007 % (v/v), about 0.008 % (v/v), about 0.009 % (v/v), about 0.01 % (v/v), about 0.015 % (v/v), about 0.02 % (v/v), about 0.025 % (v/v), about 0.03 % (v/v), about 0.035 % (v/v), about 0.04 % (v/v), about 0.045 % (v/v), about 0.05 % (v/v), about 0.055 % (v/v), about 0.06 % (v/v), about 0.065 % (v/v), about 0.07 % (v/v), about 0.075 % (v/v), about 0.08 % (v/v), about 0.085 % (v/v), about 0.09 % (v/v), about 0.095 % (v/v), or about 0.1 % (v/v) PS80. In embodiments, the dialysis solution comprises about 25 mM sodium phosphate (pH 7.2), about 300 mM NaCl, and about 0.01% (v/v) PS80.

[00190] In embodiments, the pharmaceutically acceptable buffer comprises 10 mM sodium phosphate, 150 mM NaCl, 100 mM arginine, 5 % trehalose, and 0.03 % PS80 at a pH of 7.5. In embodiments, the pharmaceutically acceptable buffer comprises 25 mM sodium phosphate, 300 mM NaCl, and 0.03 % PS80 at a pH of 7.2. In embodiments, the pharmaceutically acceptable buffer comprises 25 mM sodium phosphate, 600 mM NaCl, and 0.01 % PS80 at a pH of 6.8.

[00191] Detergent exchange may be performed with proteins purified as discussed above and purified, frozen for storage, and then thawed for detergent exchange.

[00192] Stability of compositions disclosed herein may be measured in a variety of ways. In one approach, a peptide map may be prepared to determine the integrity of the antigen protein after various treatments designed to stress the nanoparticles by mimicking harsh storage conditions. Thus, a measure of stability is the relative abundance of antigen peptides in a stressed sample compared to a control sample. For example, the stability of nanoparticles containing the viral glycoproteins may be evaluated by exposing the nanoparticles to various pHs, proteases, salt, oxidizing agents, including but not limited to hydrogen peroxide, various temperatures, freeze/thaw cycles, and agitation. It is thought that the position of the glycoprotein

anchored into the detergent core provides enhanced stability by reducing undesirable interactions. For example, the improved protection against protease-based degradation may be achieved through a shielding effect whereby anchoring the glycoproteins into the core at the molar ratios disclosed herein results in steric hindrance blocking protease access. Stability may also be measured by monitoring intact proteins.

[00193] In embodiments, provided herein are immunogenic compositions and vaccine compositions containing nanoparticles comprising RSV F glycoproteins, HA glycoproteins, SARS-CoV-2 S glycoproteins, or a combination thereof.

[00194] In embodiments, the mature RSV F glycoproteins are used to produce a vaccine comprising RSV F glycoprotein nanoparticles. In embodiments, nanoparticles of the present disclosure comprise the RSV F glycoproteins described herein. In embodiments, immunogenic compositions and vaccine compositions provided herein comprise nanoparticles comprising CoV S glycoproteins, influenza HA glycoproteins, RSV F glycoproteins, or any combination thereof.

[00195] In embodiments, the nanoparticles of the present disclosure comprise viral glycoproteins (e.g., RSV F, CoV S, influenza HA glycoproteins, or a combination thereof) associated with a detergent core. The presence of the detergent facilitates formation of the nanoparticles by forming a core that organizes and presents the antigens. In embodiments, the nanoparticles may contain the viral glycoprotein assembled into multi-oligomeric glycoprotein-detergent (e.g. PS80) nanoparticles with the head regions projecting outward and hydrophobic regions and detergent forming a central core surrounded by the glycoprotein. In embodiments, the viral glycoprotein inherently contains or is adapted to contain a transmembrane domain to promote association of the protein into a detergent core. In embodiments, the viral glycoprotein contains a head domain. Primarily the transmembrane domains of a viral glycoprotein trimer associate with detergent; however, other portions of the polypeptide may also interact. Advantageously, the nanoparticles have improved resistance to environmental stresses such that they provide enhanced stability and/or improved presentation to the immune system due to organization of multiple copies of the protein around the detergent.

[00196] In embodiments, the detergent core is a non-ionic detergent core. In embodiments, RSV F glycoprotein is associated with the non-ionic detergent core. In embodiments, the detergent is selected from the group consisting of polysorbate-20

(PS20), polysorbate-40 (PS40), polysorbate-60 (PS60), polysorbate-65 (PS65) and polysorbate-80 (PS80).

[00197] In embodiments, the detergent is PS80.

[00198] In embodiments, the RSV F glycoprotein forms a trimer. In embodiments, the RSV F glycoprotein nanoparticles are composed of multiple polypeptide trimers surrounding a non-ionic detergent core. In embodiments, the nanoparticles contain at least about 1 trimer or more. In embodiments, the nanoparticles contain at least about 5 trimers to about 30 trimers of the Spike protein. In embodiments, each nanoparticle may contain 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 15, 20, 25, or 30 trimers, including all values and ranges in between. Compositions disclosed herein may contain nanoparticles having different numbers of trimers. For example, a composition may contain nanoparticles where the number of trimers ranges from 2-9; in embodiments, the nanoparticles in a composition may contain from 2-6 trimers. In embodiments, the compositions contain a heterogeneous population of nanoparticles having 2 to 6 trimers per nanoparticle, or 2 to 9 trimers per nanoparticle. In embodiments, the compositions may contain a substantially homogenous population of nanoparticles. For example, the population may contain about 95% nanoparticles having 5 trimers.

[00199] The nanoparticles disclosed herein range in particle size. In embodiments, the nanoparticles disclosed herein range in particle size from a Z-ave size from about 20 nm to about 60 nm, about 20 nm to about 50 nm, about 20 nm to about 45 nm, about 20 nm to about 35 nm, about 20 nm to about 30 nm, about 25 nm to about 35 nm, about 25 nm to about 45 nm, about 30 nm to about 120 nm, about 30 nm to about 80 nm, about 30 nm to about 60 nm, about 30 nm to about 65 nm, or from about 30 nm to about 50 nm. Particle size (Z-ave) is measured by dynamic light scattering (DLS) using a Zetasizer NanoZS (Malvern, UK), unless otherwise specified.

[00200] Several nanoparticle types may be included in vaccine compositions disclosed herein. In aspects, the nanoparticle type is in the form of an anisotropic rod, which may be a dimer or a monomer. In other aspects, the nanoparticle type is a spherical oligomer. In yet other aspects, the nanoparticle may be described as an intermediate nanoparticle, having sedimentation properties intermediate between the first two types. Formation of nanoparticle types may be regulated by controlling detergent and protein concentration during the production process. Nanoparticle type may be determined by measuring sedimentation co-efficient.

Amounts of Viral Glycoproteins in Immunogenic Compositions and Vaccine Compositions

[00201] In embodiments, the compositions described herein comprise from about 1 µg to about 400 µg, from 1 µg to about 350 µg, from 1 µg to about 300 µg, from 1 µg to about 250 µg, from 1 µg to about 200 µg, from 1 µg to about 150 µg, from 1 µg to about 100 µg, from 1 µg to about 50 µg, or from 1 µg to about 25 µg of each viral glycoprotein. In embodiments, the compositions comprise about 1 µg, about 2 µg, about 3 µg, about 4 µg, about 5 µg, about 6 µg, about 7 µg, about 8 µg, about 9 µg, about 10 µg, about 11 µg, about 12 µg, about 13 µg, about 14 µg, about 15 µg, about 16 µg, about 17 µg, about 18 µg, about 19 µg, about 20 µg, about 21 µg, about 22 µg, about 23 µg, about 24 µg, about 25 µg, about 26 µg, about 27 µg, about 28 µg, about 29 µg, about 30 µg, about 31 µg, about 32 µg, about 33 µg, about 34 µg, about 35 µg, about 36 µg, about 37 µg, about 38 µg, about 39 µg, about 40 µg, about 41 µg, about 42 µg, about 43 µg, about 44 µg, about 45 µg, about 46 µg, about 47 µg, about 48 µg, about 49 µg, about 50 µg, about 51 µg, about 52 µg, about 53 µg, about 54 µg, about 55 µg, about 56 µg, about 57 µg, about 58 µg, about 59 µg, about 60 µg, about 61 µg, about 62 µg, about 63 µg, about 64 µg, about 65 µg, about 66 µg, about 67 µg, about 68 µg, about 69 µg, about 70 µg, about 71 µg, about 72 µg, about 73 µg, about 74 µg, about 75 µg, about 76 µg, about 77 µg, about 78 µg, about 79 µg, about 80 µg, about 81 µg, about 82 µg, about 83 µg, about 84 µg, about 85 µg, about 86 µg, about 87 µg, about 88 µg, about 89 µg, about 90 µg, about 91 µg, about 92 µg, about 93 µg, about 94 µg, about 95 µg, about 96 µg, about 97 µg, about 98 µg, about 99 µg, about 100 µg, about 101 µg, about 102 µg, about 103 µg, about 104 µg, about 105 µg, about 106 µg, about 107 µg, about 108 µg, about 109 µg, about 110 µg, about 111 µg, about 112 µg, about 113 µg, about 114 µg, about 115 µg, about 116 µg, about 117 µg, about 118 µg, about 119 µg, about 120 µg, about 121 µg, about 122 µg, about 123 µg, about 124 µg, about 125 µg, about 126 µg, about 127 µg, about 128 µg, about 129 µg, about 130 µg, about 131 µg, about 132 µg, about 133 µg, about 134 µg, about 135 µg, about 136 µg, about 137 µg, about 138 µg, about 139 µg, about 140 µg, about 141 µg, about 142 µg, about 143 µg, about 144 µg, about 145 µg, about 146 µg, about 147 µg, about 148 µg, about 149 µg, about 150 µg, about 151 µg, about 152 µg, about 153 µg, about 154 µg, about 155 µg, about 156 µg, about 157 µg, about 158 µg, about 159 µg, about 160 µg, about 161 µg, about 162 µg, about 163 µg, about 164 µg, about 165 µg, about 166 µg, about 167 µg, about 168 µg, about 169 µg, about 170 µg, about 171 µg, about 172

[illegible]

µg, about 377 µg, about 378 µg, about 379 µg, about 380 µg, about 381 µg, about 382 µg, about 383 µg, about 384 µg, about 385 µg, about 386 µg, about 387 µg, about 388 µg, about 389 µg, about 390 µg, about 391 µg, about 392 µg, about 393 µg, about 394 µg, about 395 µg, about 396 µg, about 397 µg, about 398 µg, about 399 µg, or about 400 µg of each viral glycoprotein.

[00202] In embodiments, the immunogenic compositions comprise about 1 µg of each viral glycoprotein. In embodiments, the immunogenic compositions comprise about 5 µg of each viral glycoprotein. In embodiments, the immunogenic compositions comprise about 25 µg of each viral glycoprotein. In embodiments, the immunogenic compositions comprise about 60 µg of each viral glycoprotein. In embodiments, the immunogenic compositions comprise about 240 µg of each viral glycoprotein.

[00203] In embodiments, the immunogenic compositions described herein comprise from about 1 µg to about 300 µg of each RSV F glycoprotein. In embodiments, the immunogenic compositions described herein comprise from about 5 µg to about 60 µg of each RSV F glycoprotein. In embodiments, the immunogenic compositions described herein comprise from about 24 µg to about 40 µg of each RSV F glycoprotein. In embodiments, the immunogenic compositions described herein comprise from about 30 µg to about 40 µg of each RSV F glycoprotein. In embodiments, the immunogenic compositions described herein comprise from about 30 µg to about 60 µg of each RSV F glycoprotein. In embodiments, the immunogenic compositions described herein comprise from about 33 µg to about 39 µg of each RSV F glycoprotein. In embodiments, the immunogenic compositions described herein comprise from about 20 µg to about 240 µg of each RSV F glycoprotein. In embodiments, the immunogenic compositions described herein comprise from about 30 µg to about 60 µg of each RSV F glycoprotein. In embodiments, the immunogenic compositions described herein comprise from about 24 µg to about 40 µg of each RSV F glycoprotein. In embodiments, the immunogenic compositions comprise about 1 µg, about 2 µg, about 3 µg, about 4 µg, about 5 µg, about 6 µg, about 7 µg, about 8 µg, about 9 µg, about 10 µg, about 11 µg, about 12 µg, about 13 µg, about 14 µg, about 15 µg, about 16 µg, about 17 µg, about 18 µg, about 19 µg, about 20 µg, about 21 µg, about 22 µg, about 23 µg, about 24 µg, about 25 µg, about 26 µg, about 27 µg, about 28 µg, about 29 µg, about 30 µg, about 31 µg, about 32 µg, about 33 µg, about 34 µg, about 35 µg, about 36 µg, about 37 µg, about 38 µg, about 39 µg, about 40 µg, about 41 µg, about 42 µg, about 43 µg, about 44 µg, about 45 µg, about 46 µg, about 47 µg,

about 48 µg, about 49 µg, about 50 µg, about 51 µg, about 52 µg, about 53 µg, about 54 µg, about 55 µg, about 56 µg, about 57 µg, about 58 µg, about 59 µg, about 60 µg, about 61 µg, about 62 µg, about 63 µg, about 64 µg, about 65 µg, about 66 µg, about 67 µg, about 68 µg, about 69 µg, about 70 µg, about 71 µg, about 72 µg, about 73 µg, about 74 µg, about 75 µg, about 76 µg, about 77 µg, about 78 µg, about 79 µg, about 80 µg, about 81 µg, about 82 µg, about 83 µg, about 84 µg, about 85 µg, about 86 µg, about 87 µg, about 88 µg, about 89 µg, about 90 µg, about 91 µg, about 92 µg, about 93 µg, about 94 µg, about 95 µg, about 96 µg, about 97 µg, about 98 µg, about 99 µg, about 100 µg, about 101 µg, about 102 µg, about 103 µg, about 104 µg, about 105 µg, about 106 µg, about 107 µg, about 108 µg, about 109 µg, about 110 µg, about 111 µg, about 112 µg, about 113 µg, about 114 µg, about 115 µg, about 116 µg, about 117 µg, about 118 µg, about 119 µg, about 120 µg, about 121 µg, about 122 µg, about 123 µg, about 124 µg, about 125 µg, about 126 µg, about 127 µg, about 128 µg, about 129 µg, about 130 µg, about 131 µg, about 132 µg, about 133 µg, about 134 µg, about 135 µg, about 136 µg, about 137 µg, about 138 µg, about 139 µg, about 140 µg, about 141 µg, about 142 µg, about 143 µg, about 144 µg, about 145 µg, about 146 µg, about 147 µg, about 148 µg, about 149 µg, about 150 µg, about 151 µg, about 152 µg, about 153 µg, about 154 µg, about 155 µg, about 156 µg, about 157 µg, about 158 µg, about 159 µg, about 160 µg, about 161 µg, about 162 µg, about 163 µg, about 164 µg, about 165 µg, about 166 µg, about 167 µg, about 168 µg, about 169 µg, about 170 µg, about 171 µg, about 172 µg, about 173 µg, about 174 µg, about 175 µg, about 176 µg, about 177 µg, about 178 µg, about 179 µg, about 180 µg, about 181 µg, about 182 µg, about 183 µg, about 184 µg, about 185 µg, about 186 µg, about 187 µg, about 188 µg, about 189 µg, about 190 µg, about 191 µg, about 192 µg, about 193 µg, about 194 µg, about 195 µg, about 196 µg, about 197 µg, about 198 µg, about 199 µg, about 200 µg, about 201 µg, about 202 µg, about 203 µg, about 204 µg, about 205 µg, about 206 µg, about 207 µg, about 208 µg, about 209 µg, about 210 µg, about 211 µg, about 212 µg, about 213 µg, about 214 µg, about 215 µg, about 216 µg, about 217 µg, about 218 µg, about 219 µg, about 220 µg, about 221 µg, about 222 µg, about 223 µg, about 224 µg, about 225 µg, about 226 µg, about 227 µg, about 228 µg, about 229 µg, about 230 µg, about 231 µg, about 232 µg, about 233 µg, about 234 µg, about 235 µg, about 236 µg, about 237 µg, about 238 µg, about 239 µg, about 240 µg, about 241 µg, about 242 µg, about 243 µg, about 244 µg, about 245 µg, about 246 µg, about 247 µg, about 248 µg, about 249 µg, about 250 µg, about 251 µg, about 252 µg, about 253 µg, about 254 µg, about 255

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[00204] In embodiments, the immunogenic compositions described herein comprise from about 1 μg to about 1000 μg of total RSV F glycoproteins. In embodiments, the immunogenic compositions comprise about 1 μg, about 2 μg, about 3 μg, about 4 μg, about 5 μg, about 6 μg, about 7 μg, about 8 μg, about 9 μg, about 10 μg, about 11 μg, about 12 μg, about 13 μg, about 14 μg, about 15 μg, about 16 μg, about 17 μg, about 18 μg, about 19 μg, about 20 μg, about 21 μg, about 22 μg, about 23 μg, about 24 μg, about 25 μg, about 26 μg, about 27 μg, about 28 μg, about 29 μg, about 30 μg, about 31 μg, about 32 μg, about 33 μg, about 34 μg, about 35 μg, about 36 μg, about 37 μg, about 38 μg, about 39 μg, about 40 μg, about 41 μg, about 42 μg, about 43 μg, about 44 μg, about 45 μg, about 46 μg, about 47 μg, about 48 μg, about 49 μg, about 50 μg, about 51 μg, about 52 μg, about 53 μg, about 54 μg, about 55 μg, about 56 μg, about 57 μg, about 58 μg, about 59 μg, about 60 μg, about 61 μg, about 62 μg, about 63 μg, about 64 μg, about 65 μg, about 66 μg, about 67 μg, about 68 μg, about 69 μg, about 70 μg, about 71 μg, about 72 μg, about 73 μg, about 74 μg, about 75 μg, about 76 μg, about 77 μg, about 78 μg, about 79 μg, about 80 μg, about 81 μg, about 82 μg, about 83 μg, about 84 μg, about 85 μg, about 86 μg, about 87 μg, about 88 μg, about 89 μg, about 90 μg, about 91 μg, about 92 μg, about 93 μg, about 94 μg, about 95 μg, about 96 μg, about 97 μg, about 98 μg, about 99 μg, about 100 μg, about 101 μg, about 102 μg, about 103 μg, about 104 μg, about 105 μg, about 106 μg, about 107 μg, about 108 μg, about 109 μg, about 110 μg, about 111 μg, about 112 μg, about 113 μg, about 114 μg, about 115 μg, about 116 μg, about 117 μg, about 118 μg, about 119 μg, about 120 μg, about 121 μg, about 122 μg, about 123 μg, about 124 μg, about

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[00205] In embodiments, the immunogenic compositions described herein comprise from about 1 µg to about 300 µg of each HA glycoprotein. In embodiments, the immunogenic compositions described herein comprise from about 5 µg to about 60 µg of each HA glycoprotein. In embodiments, the immunogenic compositions described herein comprise from about 24 µg to about 40 µg of each HA glycoprotein. In embodiments, the immunogenic compositions described herein comprise from about 30 µg to about 40 µg of each HA glycoprotein. In embodiments, the immunogenic compositions described herein comprise from about 30 µg to about 60 µg of each HA glycoprotein. In embodiments, the immunogenic compositions described herein comprise from about 33 µg to about 39 µg of each HA glycoprotein. In embodiments, the immunogenic compositions described herein comprise from about 20 µg to about 240 µg of each HA glycoprotein. In embodiments, the immunogenic compositions described herein comprise from about 30 µg to about 60 µg of each HA glycoprotein. In embodiments, the immunogenic compositions described herein comprise from about 24 µg to about 40 µg of each HA glycoprotein. In embodiments, the immunogenic compositions comprise about 1 µg, about 2 µg, about 3 µg, about 4 µg, about 5 µg, about 6 µg, about 7 µg, about 8 µg, about 9 µg, about 10 µg, about 11 µg, about 12 µg, about 13 µg, about 14 µg, about 15 µg, about 16 µg, about 17 µg, about 18 µg, about 19 µg, about 20 µg, about 21 µg, about 22 µg, about 23 µg, about 24 µg, about 25 µg, about 26 µg, about 27 µg, about 28 µg, about 29 µg, about 30 µg, about 31 µg, about 32 µg, about 33 µg, about 34 µg, about 35 µg, about 36 µg, about 37 µg, about 38 µg, about 39 µg, about 40 µg, about 41 µg, about 42 µg, about 43 µg, about 44 µg, about 45 µg, about 46 µg, about 47 µg, about 48 µg,

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[00206] In embodiments, the immunogenic compositions described herein comprise from about 1 μg to about 1000 μg of total HA glycoproteins. In embodiments, the immunogenic compositions comprise about 1 μg, about 2 μg, about 3 μg, about 4 μg, about 5 μg, about 6 μg, about 7 μg, about 8 μg, about 9 μg, about 10 μg, about 11 μg, about 12 μg, about 13 μg, about 14 μg, about 15 μg, about 16 μg, about 17 μg, about 18 μg, about 19 μg, about 20 μg, about 21 μg, about 22 μg, about 23 μg, about 24 μg, about 25 μg, about 26 μg, about 27 μg, about 28 μg, about 29 μg, about 30 μg, about 31 μg, about 32 μg, about 33 μg, about 34 μg, about 35 μg, about 36 μg, about 37 μg, about 38 μg, about 39 μg, about 40 μg, about 41 μg, about 42 μg, about 43 μg, about 44 μg, about 45 μg, about 46 μg, about 47 μg, about 48 μg, about 49 μg, about 50 μg, about 51 μg, about 52 μg, about 53 μg, about 54 μg, about 55 μg, about 56 μg, about 57 μg, about 58 μg, about 59 μg, about 60 μg, about 61 μg, about 62 μg, about 63 μg, about 64 μg, about 65 μg, about 66 μg, about 67 μg, about 68 μg, about 69 μg, about 70 μg, about 71 μg, about 72 μg, about 73 μg, about 74 μg, about 75 μg, about 76 μg, about 77 μg, about 78 μg, about 79 μg, about 80 μg, about 81 μg, about 82 μg, about 83 μg, about 84 μg, about 85 μg, about 86 μg, about 87 μg, about 88 μg, about 89 μg, about 90 μg, about 91 μg, about 92 μg, about 93 μg, about 94 μg, about 95 μg,

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[00207] In embodiments, the immunogenic compositions described herein comprise from about 1 μg to about 100 μg of each CoV S glycoprotein. In embodiments, the immunogenic compositions described herein comprise from about 2.5 μg to about 35 μg of each CoV S glycoprotein. In embodiments, the immunogenic compositions described herein comprise from about 5 μg to about 25 μg of each CoV S glycoprotein. In embodiments, the immunogenic compositions described herein comprise from about 14 μg to about 15 μg of each CoV S glycoprotein. In embodiments, the immunogenic compositions described herein comprise from about 15 μg to about 35 μg of each CoV S glycoprotein. In embodiments, the immunogenic compositions described herein comprise from about 1 μg to about 100 μg of total CoV S glycoproteins. In embodiments, the immunogenic compositions comprise about 1 μg, about 2 μg, about 3 μg, about 4 μg, about 5 μg, about 6 μg, about 7 μg, about 8 μg, about 9 μg, about 10 μg, about 11 μg, about 12 μg, about 13 μg, about 14 μg, about 15 μg, about 16 μg, about 17 μg, about 18 μg, about 19 μg, about 20 μg, about 21 μg, about 22 μg, about 23 μg, about 24 μg, about 25 μg, about 26 μg, about 27 μg, about 28 μg, about 29 μg, about 30 μg, about 31 μg, about 32 μg, about 33 μg, about 34 μg, about 35 μg, about 36 μg, about 37 μg, about 38 μg, about 39 μg, about 40 μg, about 41 μg, about 42 μg, about 43 μg, about 44 μg, about 45 μg, about 46 μg, about

47 µg, about 48 µg, about 49 µg, about 50 µg, about 51 µg, about 52 µg, about 53 µg, about 54 µg, about 55 µg, about 56 µg, about 57 µg, about 58 µg, about 59 µg, about 60 µg, about 61 µg, about 62 µg, about 63 µg, about 64 µg, about 65 µg, about 66 µg, about 67 µg, about 68 µg, about 69 µg, about 70 µg, about 71 µg, about 72 µg, about 73 µg, about 74 µg, about 75 µg, about 76 µg, about 77 µg, about 78 µg, about 79 µg, about 80 µg, about 81 µg, about 82 µg, about 83 µg, about 84 µg, about 85 µg, about 86 µg, about 87 µg, about 88 µg, about 89 µg, about 90 µg, about 91 µg, about 92 µg, about 93 µg, about 94 µg, about 95 µg, about 96 µg, about 97 µg, about 98 µg, about 99 µg, about 100 µg, including all values and ranges therebetween, of each CoV S glycoprotein. In embodiments, the immunogenic compositions comprise about 1 µg, about 2 µg, about 3 µg, about 4 µg, about 5 µg, about 6 µg, about 7 µg, about 8 µg, about 9 µg, about 10 µg, about 11 µg, about 12 µg, about 13 µg, about 14 µg, about 15 µg, about 16 µg, about 17 µg, about 18 µg, about 19 µg, about 20 µg, about 21 µg, about 22 µg, about 23 µg, about 24 µg, about 25 µg, about 26 µg, about 27 µg, about 28 µg, about 29 µg, about 30 µg, about 31 µg, about 32 µg, about 33 µg, about 34 µg, about 35 µg, about 36 µg, about 37 µg, about 38 µg, about 39 µg, about 40 µg, about 41 µg, about 42 µg, about 43 µg, about 44 µg, about 45 µg, about 46 µg, about 47 µg, about 48 µg, about 49 µg, about 50 µg, about 51 µg, about 52 µg, about 53 µg, about 54 µg, about 55 µg, about 56 µg, about 57 µg, about 58 µg, about 59 µg, about 60 µg, about 61 µg, about 62 µg, about 63 µg, about 64 µg, about 65 µg, about 66 µg, about 67 µg, about 68 µg, about 69 µg, about 70 µg, about 71 µg, about 72 µg, about 73 µg, about 74 µg, about 75 µg, about 76 µg, about 77 µg, about 78 µg, about 79 µg, about 80 µg, about 81 µg, about 82 µg, about 83 µg, about 84 µg, about 85 µg, about 86 µg, about 87 µg, about 88 µg, about 89 µg, about 90 µg, about 91 µg, about 92 µg, about 93 µg, about 94 µg, about 95 µg, about 96 µg, about 97 µg, about 98 µg, about 99 µg, about 100 µg of total CoV S glycoproteins, including all values and ranges therebetween. In embodiments, the immunogenic compositions comprise about 1 µg of a CoV S glycoprotein. In embodiments, the immunogenic compositions comprise about 13 µg of a CoV S glycoprotein. In embodiments, the immunogenic compositions comprise about 5 µg of a CoV S glycoprotein. In embodiments, the immunogenic compositions comprise about 25 µg of a CoV S glycoprotein.

Adjuvants in Immunogenic Compositions and Vaccine Compositions

[00208] In certain embodiments, the compositions disclosed herein comprise one or more adjuvants. In embodiments, the one or more adjuvants enhance an immune response. In other embodiments, the compositions are prepared without adjuvants, and are thus available to be administered as adjuvant-free compositions. Advantageously, adjuvant-free compositions disclosed herein may provide protective immune responses when administered as a single dose.

Aluminum-based adjuvants

[00209] In embodiments, the adjuvant may be alum (e.g. AlPO_4 or $\text{Al}(\text{OH})_3$). Typically, the nanoparticle is substantially bound to the alum. For example, the nanoparticle may be at least 80% bound, at least 85% bound, at least 90% bound or at least 95% bound to the alum. Often, the nanoparticle is 92% to 97% bound to the alum in a composition. The amount of alum is present per dose is typically in a range between about 400 μg to about 1250 μg . For example, the alum may be present in a per dose amount of about 300 μg to about 900 μg , about 400 μg to about 800 μg , about 500 μg to about 700 μg , about 400 μg to about 600 μg , or about 400 μg to about 500 μg . Typically, the alum is present at about 400 μg for a dose of 120 μg of the protein nanoparticle.

Saponin Adjuvants

[00210] Adjuvants containing saponin may also be combined with the immunogens disclosed herein. Saponins are glycosides derived from the bark of the Quillaja saponaria Molina tree. Typically, saponin is prepared using a multi-step purification process resulting in multiple fractions. As used, herein, the term “a saponin fraction from Quillaja saponaria Molina” is used generically to describe a semi-purified or defined saponin fraction of Quillaja saponaria or a substantially pure fraction thereof.

Saponin Fractions

[00211] Several approaches for producing saponin fractions are suitable. Fractions A, B, and C are described in U.S. Pat. No. 6,352,697 and may be prepared as follows. A lipophilic fraction from Quil A, a crude aqueous Quillaja saponaria Molina extract, is separated by chromatography and eluted with 70% acetonitrile in water to recover the lipophilic fraction. This lipophilic fraction is then separated by semi-preparative HPLC with elution using a gradient of from 25% to 60% acetonitrile in acidic water. The fraction referred to herein as “Fraction A” or “QH-A” is, or corresponds to, the fraction, which is eluted at approximately 39% acetonitrile. The

fraction referred to herein as "Fraction B" or "QH-B" is, or corresponds to, the fraction, which is eluted at approximately 47% acetonitrile. The fraction referred to herein as "Fraction C" or "QH-C" is, or corresponds to, the fraction, which is eluted at approximately 49% acetonitrile. Additional information regarding purification of Fractions is found in U.S. Pat. No. 5,057,540. When prepared as described herein, Fractions A, B and C of *Quillaja saponaria* Molina each represent groups or families of chemically closely related molecules with definable properties. The chromatographic conditions under which they are obtained are such that the batch-to-batch reproducibility in terms of elution profile and biological activity is highly consistent.

[00212] Other saponin fractions have been described. Fractions B3, B4 and B4b are described in EP 0436620. Fractions QA1-QA22 are described EP03632279 B2, Q-VAC (Nor-Feed, AS Denmark), *Quillaja saponaria* Molina Spikoside (Isconova AB, Ultunaallén 2B, 756 51 Uppsala, Sweden). Fractions QA-1, QA-2, QA-3, QA-4, QA-5, QA-6, QA-7, QA-8, QA-9, QA-10, QA-11, QA-12, QA-13, QA-14, QA-15, QA-16, QA-17, QA-18, QA-19, QA-20, QA-21, and QA-22 of EP 0 3632 279 B2, especially QA-7, QA-17, QA-18, and QA-21 may be used. They are obtained as described in EP 0 3632 279 B2, especially at page 6 and in Example 1 on page 8 and 9.

[00213] The saponin fractions described herein and used for forming adjuvants are often substantially pure fractions; that is, the fractions are substantially free of the presence of contamination from other materials. In particular aspects, a substantially pure saponin fraction may contain up to 40% by weight, up to 30% by weight, up to 25% by weight, up to 20% by weight, up to 15% by weight, up to 10% by weight, up to 7% by weight, up to 5% by weight, up to 2% by weight, up to 1% by weight, up to 0.5% by weight, or up to 0.1% by weight of other compounds such as other saponins or other adjuvant materials.

ISCOM Structures

[00214] Saponin fractions may be administered in the form of a cage-like particle referred to as an ISCOM (Immune Stimulating COMplex). ISCOMs may be prepared as described in EP0109942B1, EP0242380B1 and EP0180546 B1. In particular embodiments a transport and/or a passenger antigen may be used, as described in EP 9600647-3 (PCT/SE97/00289).

Matrix Adjuvants

[00215] In embodiments, the ISCOM is an ISCOM matrix complex. An ISCOM matrix complex comprises at least one saponin fraction and a lipid. The lipid is at least a sterol, such as cholesterol. In particular aspects, the ISCOM matrix complex also contains a phospholipid. The ISCOM matrix complexes may also contain one or more other immunomodulatory (adjuvant-active) substances, not necessarily a glycoside, and may be produced as described in EP0436620B1, which is incorporated by reference in its entirety herein.

[00216] In other aspects, the ISCOM is an ISCOM complex. An ISCOM complex contains at least one saponin, at least one lipid, and at least one kind of antigen or epitope. The ISCOM complex contains antigen associated by detergent treatment such that that a portion of the antigen integrates into the particle. In contrast, ISCOM matrix is formulated as an admixture with antigen and the association between ISCOM matrix particles and antigen is mediated by electrostatic and/or hydrophobic interactions.

[00217] According to one embodiment, the saponin fraction integrated into an ISCOM matrix complex or an ISCOM complex, or at least one additional adjuvant, which also is integrated into the ISCOM or ISCOM matrix complex or mixed therewith, is selected from fraction A, fraction B, or fraction C of Quillaja saponaria, a semipurified preparation of Quillaja saponaria, a purified preparation of Quillaja saponaria, or any purified sub-fraction e.g., QA 1-21.

[00218] In particular aspects, each ISCOM particle may contain at least two saponin fractions. Any combinations of weight % of different saponin fractions may be used. Any combination of weight % of any two fractions may be used. For example, the particle may contain any weight % of fraction A and any weight % of another saponin fraction, such as a crude saponin fraction or fraction C, respectively. Accordingly, in particular aspects, each ISCOM matrix particle or each ISCOM complex particle may contain from 0.1 to 99.9 by weight, 5 to 95% by weight, 10 to 90% by weight 15 to 85% by weight, 20 to 80% by weight, 25 to 75% by weight, 30 to 70% by weight, 35 to 65% by weight, 40 to 60% by weight, 45 to 55% by weight, 40 to 60% by weight, or 50% by weight of one saponin fraction, e.g. fraction A and the rest up to 100% in each case of another saponin e.g. any crude fraction or any other faction e.g. fraction C. The weight is calculated as the total weight of the saponin fractions. Examples of ISCOM matrix complex and ISCOM complex adjuvants are disclosed in

U.S Published Application No. 2013/0129770, which is incorporated by reference in its entirety herein.

[00219] In particular embodiments, the ISCOM matrix or ISCOM complex comprises from 5-99% by weight of one fraction, e.g. fraction A and the rest up to 100% of weight of another fraction e.g. a crude saponin fraction or fraction C. The weight is calculated as the total weight of the saponin fractions.

[00220] In another embodiment, the ISCOM matrix or ISCOM complex comprises from 40% to 99% by weight of one fraction, e.g. fraction A and from 1% to 60% by weight of another fraction, e.g. a crude saponin fraction or fraction C. The weight is calculated as the total weight of the saponin fractions.

[00221] In yet another embodiment, the ISCOM matrix or ISCOM complex comprises from 70% to 95% by weight of one fraction e.g., fraction A, and from 30% to 5% by weight of another fraction, e.g., a crude saponin fraction, or fraction C. The weight is calculated as the total weight of the saponin fractions. In other embodiments, the saponin fraction from Quillaja saponaria Molina is selected from any one of QA 1-21.

[00222] In addition to particles containing mixtures of saponin fractions, ISCOM matrix particles and ISCOM complex particles may each be formed using only one saponin fraction. Compositions disclosed herein may contain multiple particles wherein each particle contains only one saponin fraction. That is, certain compositions may contain one or more different types of ISCOM-matrix complexes particles and/or one or more different types of ISCOM complexes particles, where each individual particle contains one saponin fraction from Quillaja saponaria Molina, wherein the saponin fraction in one complex is different from the saponin fraction in the other complex particles.

[00223] In particular aspects, one type of saponin fraction or a crude saponin fraction may be integrated into one ISCOM matrix complex or particle and another type of substantially pure saponin fraction, or a crude saponin fraction, may be integrated into another ISCOM matrix complex or particle. A composition or vaccine may comprise at least two types of complexes or particles each type having one type of saponins integrated into physically different particles.

[00224] In the compositions, mixtures of ISCOM matrix complex particles and/or ISCOM complex particles may be used in which one saponin fraction Quillaja saponaria Molina and another saponin fraction Quillaja saponaria Molina are

separately incorporated into different ISCOM matrix complex particles and/or ISCOM complex particles.

[00225] The ISCOM matrix or ISCOM complex particles, which each have one saponin fraction, may be present in composition at any combination of weight %. In particular aspects, a composition may contain 0.1% to 99.9% by weight, 5% to 95% by weight, 10% to 90% by weight, 15% to 85% by weight, 20% to 80% by weight, 25% to 75% by weight, 30% to 70% by weight, 35% to 65% by weight, 40% to 60% by weight, 45% to 55% by weight, 40 to 60% by weight, or 50% by weight, of an ISCOM matrix or complex containing a first saponin fraction with the remaining portion made up by an ISCOM matrix or complex containing a different saponin fraction. In aspects, the remaining portion is one or more ISCOM matrix or complexes where each matrix or complex particle contains only one saponin fraction. In other aspects, the ISCOM matrix or complex particles may contain more than one saponin fraction.

[00226] In particular compositions, the only saponin fraction in a first ISCOM matrix or ISCOM complex particle is Fraction A and the only saponin fraction in a second ISCOM matrix or ISCOM complex particle is Fraction C.

[00227] In embodiments, the Fraction A of *Quillaja Saponaria* Molina accounts for at least about 80 %, 81 %, 82 %, 83 %, 84 %, 85 %, 86 %, 87 %, 88 %, 89 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, or 99 % by weight, and fraction C of *Quillaja Saponaria* Molina accounts for the remainder, respectively, of the sum of the weights of fraction A of *Quillaja Saponaria* Molina and fraction C of *Quillaja Saponaria* Molina in the adjuvant.

[00228] Preferred compositions comprise a first ISCOM matrix containing Fraction A and a second ISCOM matrix containing Fraction C, wherein the Fraction A ISCOM matrix constitutes about 70% per weight of the total saponin adjuvant, and the Fraction C ISCOM matrix constitutes about 30% per weight of the total saponin adjuvant. In another preferred composition, the Fraction A ISCOM matrix constitutes about 85% per weight of the total saponin adjuvant, and the Fraction C ISCOM matrix constitutes about 15% per weight of the total saponin adjuvant. In another preferred composition, the Fraction A ISCOM matrix constitutes about 92% per weight of the total saponin adjuvant, and the Fraction C ISCOM matrix constitutes about 8% per weight of the total saponin adjuvant. Thus, in certain compositions, the Fraction A ISCOM matrix is present in a range of about 70% to about 85%, and Fraction C ISCOM matrix is present in a range of about 15% to about 30%, of the total weight amount of

saponin adjuvant in the composition. In certain compositions, the Fraction A ISCOM matrix is present in a range of about 70% to about 92%, and Fraction C ISCOM matrix is present in a range of about 8% to about 30%, of the total weight amount of saponin adjuvant in the composition. In embodiments, the Fraction A ISCOM matrix accounts for 50-96 % by weight and Fraction C ISCOM matrix accounts for the remainder, respectively, of the sums of the weights of Fraction A ISCOM matrix and Fraction C ISCOM in the adjuvant. In a particularly preferred composition, referred to herein as MATRIX-M™, the Fraction A ISCOM matrix is present at about 85 % and Fraction C ISCOM matrix is present at about 15% of the total weight amount of saponin adjuvant in the composition. MATRIX-M™ may be referred to interchangeably as Matrix-M1.

[00229] Exemplary QS-7 and QS-21 fractions, their production and their use is described in U.S. Pat. Nos. 5,057,540; 6,231,859; 6,352,697; 6,524,584; 6,846,489; 7,776,343, and 8,173,141, which are incorporated by reference herein.

[00230] In embodiments, other adjuvants may be used in addition or as an alternative. The inclusion of any adjuvant described in Vogel et al., "A Compendium of Vaccine Adjuvants and Excipients (2nd Edition)," herein incorporated by reference in its entirety for all purposes, is envisioned within the scope of this disclosure. Other adjuvants include complete Freund's adjuvant (a non-specific stimulator of the immune response containing killed *Mycobacterium tuberculosis*), incomplete Freund's adjuvants and aluminum hydroxide adjuvant. Other adjuvants comprise GMCSF, BCG, MDP compounds, such as thur-MDP and nor-MDP, CGP (MTP-PE), lipid A, and monophosphoryl lipid A (MPL), MF-59, RIBI, which contains three components extracted from bacteria, MPL, trehalose dimycolate (TDM) and cell wall skeleton (CWS) in a 2% squalene/TWEEN® polysorbate 80 emulsion. In embodiments, the adjuvant may be a paucilamellar lipid vesicle; for example, NOVASOMES®. NOVASOMES® are paucilamellar nonphospholipid vesicles ranging from about 100 nm to about 500 nm. They comprise BRIJ® alcohol ethoxylate 72, cholesterol, oleic acid and squalene. NOVASOMES® have been shown to be an effective adjuvant (see, U.S. Pat. Nos. 5,629,021, 6,387,373, and 4,911,928).

Amount of Adjuvant in Compositions

[00231] In embodiments, the immunogenic compositions described herein comprise from about 1 µg to about 400 µg, from 1 µg to about 350 µg, from 1 µg to about 300 µg, from 1 µg to about 250 µg, from 1 µg to about 200 µg, from 1 µg to about 150 µg, from 1 µg to about 100 µg, from 1 µg to about 50 µg, or from 1 µg to

about 25 µg of adjuvant. In embodiments, the immunogenic compositions comprise about 1 µg, about 2 µg, about 3 µg, about 4 µg, about 5 µg, about 6 µg, about 7 µg, about 8 µg, about 9 µg, about 10 µg, about 11 µg, about 12 µg, about 13 µg, about 14 µg, about 15 µg, about 16 µg, about 17 µg, about 18 µg, about 19 µg, about 20 µg, about 21 µg, about 22 µg, about 23 µg, about 24 µg, about 25 µg, about 26 µg, about 27 µg, about 28 µg, about 29 µg, about 30 µg, about 31 µg, about 32 µg, about 33 µg, about 34 µg, about 35 µg, about 36 µg, about 37 µg, about 38 µg, about 39 µg, about 40 µg, about 41 µg, about 42 µg, about 43 µg, about 44 µg, about 45 µg, about 46 µg, about 47 µg, about 48 µg, about 49 µg, about 50 µg, about 51 µg, about 52 µg, about 53 µg, about 54 µg, about 55 µg, about 56 µg, about 57 µg, about 58 µg, about 59 µg, about 60 µg, about 61 µg, about 62 µg, about 63 µg, about 64 µg, about 65 µg, about 66 µg, about 67 µg, about 68 µg, about 69 µg, about 70 µg, about 71 µg, about 72 µg, about 73 µg, about 74 µg, about 75 µg, about 76 µg, about 77 µg, about 78 µg, about 79 µg, about 80 µg, about 81 µg, about 82 µg, about 83 µg, about 84 µg, about 85 µg, about 86 µg, about 87 µg, about 88 µg, about 89 µg, about 90 µg, about 91 µg, about 92 µg, about 93 µg, about 94 µg, about 95 µg, about 96 µg, about 97 µg, about 98 µg, about 99 µg, about 100 µg, about 101 µg, about 102 µg, about 103 µg, about 104 µg, about 105 µg, about 106 µg, about 107 µg, about 108 µg, about 109 µg, about 110 µg, about 111 µg, about 112 µg, about 113 µg, about 114 µg, about 115 µg, about 116 µg, about 117 µg, about 118 µg, about 119 µg, about 120 µg, about 121 µg, about 122 µg, about 123 µg, about 124 µg, about 125 µg, about 126 µg, about 127 µg, about 128 µg, about 129 µg, about 130 µg, about 131 µg, about 132 µg, about 133 µg, about 134 µg, about 135 µg, about 136 µg, about 137 µg, about 138 µg, about 139 µg, about 140 µg, about 141 µg, about 142 µg, about 143 µg, about 144 µg, about 145 µg, about 146 µg, about 147 µg, about 148 µg, about 149 µg, about 150 µg, about 151 µg, about 152 µg, about 153 µg, about 154 µg, about 155 µg, about 156 µg, about 157 µg, about 158 µg, about 159 µg, about 160 µg, about 161 µg, about 162 µg, about 163 µg, about 164 µg, about 165 µg, about 166 µg, about 167 µg, about 168 µg, about 169 µg, about 170 µg, about 171 µg, about 172 µg, about 173 µg, about 174 µg, about 175 µg, about 176 µg, about 177 µg, about 178 µg, about 179 µg, about 180 µg, about 181 µg, about 182 µg, about 183 µg, about 184 µg, about 185 µg, about 186 µg, about 187 µg, about 188 µg, about 189 µg, about 190 µg, about 191 µg, about 192 µg, about 193 µg, about 194 µg, about 195 µg, about 196 µg, about 197 µg, about 198 µg, about 199 µg, about 200 µg, about 201 µg, about 202 µg, about 203 µg, about 204 µg, about 205 µg, about 206

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composition comprises about 50 µg of adjuvant. In embodiments, the immunogenic composition comprises about 75 µg of adjuvant.

Methods of Stimulating an Immune Response against RSV, Influenza, SARS-CoV-2 or a variant thereof, or a combination thereof

[00232] In embodiments, the disclosure provides a method for eliciting an immune response against one or more of RSV, influenza, and SARS-CoV-2 or a variant thereof, comprising administering an immunogenic composition described herein. In embodiments, the immunogenic composition contains one or more viral glycoproteins. In embodiments, the one or more viral glycoproteins are selected from any one of a RSV F glycoprotein, a CoV S glycoprotein, and an influenza HA glycoprotein.

[00233] In embodiments, the immunogenic compositions lack an adjuvant. In embodiments, the immunogenic compositions comprise an adjuvant. Non-limiting examples of adjuvants are described herein.

[00234] In embodiments, the immunogenic compositions described herein have an efficacy against a virus (e.g., RSV, influenza, SARS-CoV-2, a SARS-CoV-2 variant thereof, or a combination thereof) that is between about 50 % and about 99 %, between about 80 % and about 99 %, between about 75 % and about 99 %, between about 80 % and about 95 %, between about 90 % and about 98 %, between about 75 % and about 95 %, at least about 50 %, at least about 55 %, at least about 60 %, at least about 65 %, at least about 70 %, at least about 75 %, at least about 80 %, at least about 85 %, at least about 90 %, at least about 91 %, at least about 92 %, at least about 93 %, at least about 94 %, at least about 95 %, at least about 96 %, at least about 97 %, at least about 98 %, or at least about 99 %.

[00235] Compositions disclosed herein may be administered via a systemic route or a mucosal route or a transdermal route or directly into a specific tissue. As used herein, the term “systemic administration” includes parenteral routes of administration. In particular, parenteral administration includes subcutaneous, intraperitoneal, intravenous, intraarterial, intramuscular, or intrasternal injection, intravenous, or kidney dialytic infusion techniques. Typically, the systemic, parenteral administration is intramuscular injection. As used herein, the term “mucosal administration” includes oral, intranasal, intravaginal, intra-rectal, intra-tracheal, intestinal and ophthalmic administration. In embodiments, the methods comprise

administering immunogenic compositions described herein intramuscularly. In embodiments, the methods comprise administering immunogenic compositions described herein intranasally.

[00236] Compositions may be administered on a single dose schedule or a multiple dose schedule. Multiple doses may be used in a primary immunization schedule or in a booster immunization schedule. In embodiments, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 doses are administered. In a multiple dose schedule the various doses may be given by the same or different routes e.g., a parenteral prime and mucosal boost, a mucosal prime and parenteral boost, etc. In aspects, a boost dose is administered about 2 weeks, about 3 weeks, about 4 weeks, about 5 weeks, about 6 weeks, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months (1 year), about 2 years, about 3 years, about 4 years, about 5 years, about 6 years, about 7 years, about 8 years, about 9 years, or about 10 years after the first dose. In embodiments, a boost dose is administered every year after administration of the initial dose. In embodiments, the follow-on boost dose is administered 3 weeks or 4 weeks after administration of the prior dose. In embodiments, the first dose is administered at day 0, and the boost dose is administered at day 21. In embodiments, the first dose is administered at day 0, and the boost dose is administered at day 28. In embodiments, the first dose is administered at day 0, a boost dose is administered at day 21, and a second boost dose is administered about six months after administration of the first dose or second dose. In embodiments, the first dose is administered at day 0, and the boost dose is administered at day 28, and a second boost dose is administered about six months after administration of the first dose. In embodiments, the first dose is administered at day 0, a boost dose is administered at day 21, and a second boost dose is administered about six months after administration of the second dose. In embodiments, the first dose is administered at day 0, and the boost dose is administered at day 28, and a second boost dose is administered about six months after administration of the second dose.

[00237] In embodiments, the first dose is administered at day 0, a boost dose is administered at day 21, and a second boost dose is administered about 1 year after administration of the first dose or the first boost dose. In embodiments, the first dose is administered at day 0, a first boost dose is administered at day 28, and a second

boost dose is administered about 1 year after administration of the first dose. In embodiments, the first dose is administered at day 0, a boost dose is administered at day 21, and a second boost dose is administered about 1 year after administration of the second dose. In embodiments, the first dose is administered at day 0, a first boost dose is administered at day 28, and a second boost dose is administered about 1 year after administration of the second dose. In embodiments, the second boost dose is administered from 6 months to 24 months or from 12 to 24 months after the first boost dose.

[00238] In embodiments, the dose, as measured in μg , may be the total weight of the dose including the solute, or the weight of the RSV F glycoprotein nanoparticles, or the weight of the RSV F glycoprotein. Dose is measured using protein concentration assay either A280 or ELISA.

[00239] Certain populations may be administered with or without adjuvants. In certain aspects, compositions may be free of added adjuvant. In such circumstances, the dose may be increased by about 10%.

[00240] In embodiments, the immunogenic compositions described herein are provided in pre-filled syringes. When the immunogenic composition is prepared in a pre-filled syringe, the RSV F glycoproteins and adjuvant are combined in advance of administration.

[00241] In embodiments, the dose is administered in a volume of about 0.1 mL to about 1.5 mL, for example, about 0.1 mL, about 0.2 mL, about 0.25 mL, about 0.3 mL, about 0.4 mL, about 0.5 mL, about 0.6 mL, about 0.7 mL, about 0.8 mL, about 0.9 mL, about 1.0 mL, about 1.1 mL, about 1.2 mL, about 1.3 mL, about 1.4 mL, or about 1.5 mL. In embodiments, the dose is administered in a volume of 0.25 mL. In embodiments, the dose is administered in a volume of 0.5 mL. In embodiments, the dose is administered in a volume of 0.6 mL.

[00242] In embodiments, the concentration of viral glycoprotein nanoparticles (e.g., RSV F, CoV S, or HA glycoprotein nanoparticles) in the immunogenic compositions or vaccine compositions described herein is from about 1 $\mu\text{g/mL}$ to about 50 $\mu\text{g/mL}$, 10 $\mu\text{g/mL}$ to about 100 $\mu\text{g/mL}$, about 10 $\mu\text{g/mL}$ to about 50 $\mu\text{g/mL}$, about 175 $\mu\text{g/mL}$ to about 325 $\mu\text{g/mL}$, about 200 $\mu\text{g/mL}$ to about 300 $\mu\text{g/mL}$, about 220 $\mu\text{g/mL}$ to about 280 $\mu\text{g/mL}$, or about 240 $\mu\text{g/mL}$ to about 260 $\mu\text{g/mL}$.

[00243] In embodiments, the methods comprise administering from about 1 μg to about 300 μg , from about 5 μg to about 25 μg , from about 1 μg to about 300 μg ,

from about 90 µg to about 270 µg, from about 100 µg to about 160 µg, from about 110 µg to about 150 µg, from about 120 µg to about 140 µg, or from about 140 µg to about 160 of each RSV F glycoprotein. In embodiments, the methods comprise administering from about 5 µg to about 60 µg of each RSV F glycoprotein. In embodiments, the methods comprise administering from about 24 µg to about 40 µg of each RSV F glycoprotein. In embodiments, the methods comprise administering from about 30 µg to about 40 µg of each RSV F glycoprotein. In embodiments, the methods comprise administering from about 30 µg to about 60 µg of each RSV F glycoprotein. In embodiments, the methods comprise administering from about 33 µg to about 39 µg of each RSV F glycoprotein. In embodiments, the methods comprise administering from about 20 µg to about 240 µg of each RSV F glycoprotein. In embodiments, the methods comprise administering from about 30 µg to about 60 µg of each RSV F glycoprotein. In embodiments, the methods comprise administering from about 24 µg to about 40 µg of each RSV F glycoprotein. In embodiments, the methods comprise administering about 1 µg, about 2 µg, about 3 µg, about 4 µg, about 5 µg, about 6 µg, about 7 µg, about 8 µg, about 9 µg, about 10 µg, about 11 µg, about 12 µg, about 13 µg, about 14 µg, about 15 µg, about 16 µg, about 17 µg, about 18 µg, about 19 µg, about 20 µg, about 21 µg, about 22 µg, about 23 µg, about 24 µg, about 25 µg, about 26 µg, about 27 µg, about 28 µg, about 29 µg, about 30 µg, about 31 µg, about 32 µg, about 33 µg, about 34 µg, about 35 µg, about 36 µg, about 37 µg, about 38 µg, about 39 µg, about 40 µg, about 41 µg, about 42 µg, about 43 µg, about 44 µg, about 45 µg, about 46 µg, about 47 µg, about 48 µg, about 49 µg, about 50 µg, about 51 µg, about 52 µg, about 53 µg, about 54 µg, about 55 µg, about 56 µg, about 57 µg, about 58 µg, about 59 µg, about 60 µg, about 61 µg, about 62 µg, about 63 µg, about 64 µg, about 65 µg, about 66 µg, about 67 µg, about 68 µg, about 69 µg, about 70 µg, about 71 µg, about 72 µg, about 73 µg, about 74 µg, about 75 µg, about 76 µg, about 77 µg, about 78 µg, about 79 µg, about 80 µg, about 81 µg, about 82 µg, about 83 µg, about 84 µg, about 85 µg, about 86 µg, about 87 µg, about 88 µg, about 89 µg, about 90 µg, about 91 µg, about 92 µg, about 93 µg, about 94 µg, about 95 µg, about 96 µg, about 97 µg, about 98 µg, about 99 µg, about 100 µg, about 101 µg, about 102 µg, about 103 µg, about 104 µg, about 105 µg, about 106 µg, about 107 µg, about 108 µg, about 109 µg, about 110 µg, about 111 µg, about 112 µg, about 113 µg, about 114 µg, about 115 µg, about 116 µg, about 117 µg, about 118 µg, about 119 µg, about 120 µg, about 121 µg, about 122 µg, about 123 µg, about 124 µg, about 125 µg, about 126

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[00244] In embodiments, the methods comprise administering from about 1 µg to about 1000 µg of total RSV F glycoproteins. In embodiments, the methods comprise administering about 1 µg, about 2 µg, about 3 µg, about 4 µg, about 5 µg, about 6 µg, about 7 µg, about 8 µg, about 9 µg, about 10 µg, about 11 µg, about 12 µg, about 13 µg, about 14 µg, about 15 µg, about 16 µg, about 17 µg, about 18 µg, about 19 µg, about 20 µg, about 21 µg, about 22 µg, about 23 µg, about 24 µg, about 25 µg, about 26 µg, about 27 µg, about 28 µg, about 29 µg, about 30 µg, about 31 µg, about 32 µg, about 33 µg, about 34 µg, about 35 µg, about 36 µg, about 37 µg, about 38 µg, about 39 µg, about 40 µg, about 41 µg, about 42 µg, about 43 µg, about 44 µg, about 45 µg, about 46 µg, about 47 µg, about 48 µg, about 49 µg, about 50 µg, about 51 µg, about 52 µg, about 53 µg, about 54 µg, about 55 µg, about 56 µg, about 57 µg, about 58 µg, about 59 µg, about 60 µg, about 61 µg, about 62 µg, about 63 µg, about 64 µg, about 65 µg, about 66 µg, about 67 µg, about 68 µg, about 69 µg, about 70 µg, about 71 µg, about 72 µg, about 73 µg, about 74 µg, about 75 µg, about 76 µg, about 77 µg, about 78 µg, about 79 µg, about 80 µg, about 81 µg, about 82 µg, about 83 µg, about 84 µg, about 85 µg, about 86 µg, about 87 µg, about 88 µg, about 89 µg, about 90 µg, about 91 µg, about 92 µg, about 93 µg, about 94 µg, about 95 µg, about 96 µg, about 97 µg, about 98 µg, about 99 µg, about 100 µg, about 101 µg, about 102 µg, about 103 µg, about 104 µg, about 105 µg, about 106 µg, about 107 µg, about 108 µg, about 109 µg, about 110 µg, about 111 µg, about 112 µg, about 113 µg, about 114 µg, about 115 µg, about 116 µg, about 117 µg, about 118 µg, about 119 µg, about 120 µg, about 121 µg, about 122 µg, about 123 µg, about 124 µg, about 125 µg, about 126 µg, about 127 µg, about 128 µg, about 129 µg, about 130 µg, about 131 µg, about 132 µg, about 133 µg, about 134 µg, about 135 µg, about 136 µg, about 137 µg, about 138 µg, about 139 µg, about 140 µg, about 141 µg, about 142 µg, about 143 µg, about 144 µg, about 145 µg, about 146 µg, about 147 µg, about 148 µg, about 149 µg, about 150 µg, about 151 µg, about 152 µg, about 153 µg, about 154 µg, about 155 µg, about 156 µg, about 157 µg, about 158 µg, about 159 µg, about 160 µg, about 161 µg, about 162 µg, about 163 µg, about 164 µg, about 165 µg, about 166 µg, about 167 µg, about 168 µg, about 169 µg, about 170 µg, about 171 µg, about 172 µg, about 173 µg, about 174 µg, about 175 µg, about 176 µg, about 177 µg, about 178 µg, about 179 µg, about 180 µg, about 181 µg, about 182 µg, about 183 µg, about 184 µg, about 185 µg, about 186 µg, about 187 µg, about 188 µg, about 189 µg, about 190 µg, about 191 µg, about 192 µg, about 193 µg, about 194 µg, about 195 µg, about 196 µg, about 197 µg, about 198 µg, about 199

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[00245] In embodiments, the methods comprise administering from about 1 µg to about 100 µg of each CoV S glycoprotein. In embodiments, the methods comprise administering from about 2.5 µg to about 35 µg of each CoV S glycoprotein. In embodiments, the methods comprise administering from about 5 µg to about 25 µg of each CoV S glycoprotein. In embodiments, the methods comprise administering from about 14 µg to about 15 µg of each CoV S glycoprotein. In embodiments, the methods comprise administering from about 15 µg to about 35 µg of each CoV S glycoprotein. In embodiments, the methods comprise administering from about 1 µg to about 100 µg of total CoV S glycoproteins. In embodiments, the methods comprise administering about 1 µg, about 2 µg, about 3 µg, about 4 µg, about 5 µg, about 6 µg, about 7 µg, about 8 µg, about 9 µg, about 10 µg, about 11 µg, about 12 µg, about 13 µg, about 14 µg, about 15 µg, about 16 µg, about 17 µg, about 18 µg, about 19 µg, about 20 µg, about 21 µg, about 22 µg, about 23 µg, about 24 µg, about 25 µg, about 26 µg, about 27 µg, about 28 µg, about 29 µg, about 30 µg, about 31 µg, about 32 µg, about 33 µg, about 34 µg, about 35 µg, about 36 µg, about 37 µg, about 38 µg, about 39 µg, about 40 µg, about 41 µg, about 42 µg, about 43 µg, about 44 µg, about 45 µg, about 46 µg, about 47 µg, about 48 µg, about 49 µg, about 50 µg, about 51 µg, about 52 µg, about 53 µg, about 54 µg, about 55 µg, about 56 µg, about 57 µg, about 58 µg, about 59 µg, about 60 µg, about 61 µg, about 62 µg, about 63 µg, about 64 µg, about 65 µg, about 66 µg, about 67 µg, about 68 µg, about 69 µg, about 70 µg, about 71 µg, about 72 µg, about 73 µg, about 74 µg, about 75 µg, about 76 µg, about 77 µg, about 78 µg, about 79 µg, about 80 µg, about 81 µg, about 82 µg, about 83 µg, about 84 µg, about 85 µg, about 86 µg, about 87 µg, about 88 µg, about 89 µg, about 90 µg, about 91 µg, about 92 µg, about 93 µg, about 94 µg, about 95 µg, about 96 µg, about 97 µg, about 98 µg, about 99 µg, about 100 µg, including all values and ranges therebetween, of each CoV S glycoprotein. In embodiments, the methods comprise administering about 1 µg, about 2 µg, about 3 µg, about 4 µg, about 5 µg, about 6 µg, about 7 µg, about 8 µg, about 9 µg, about 10 µg, about 11 µg, about 12 µg, about 13 µg, about 14 µg, about 15 µg, about 16 µg, about 17 µg, about 18 µg, about 19 µg, about 20 µg, about 21 µg, about 22 µg, about 23 µg, about 24 µg, about 25 µg, about 26 µg, about 27 µg, about 28 µg, about 29 µg, about 30 µg, about 31 µg, about 32 µg, about 33 µg, about 34 µg, about 35 µg, about 36 µg, about 37 µg, about 38 µg, about 39 µg, about 40 µg, about 41 µg, about 42 µg, about 43 µg, about 44 µg, about 45 µg, about 46 µg, about 47 µg, about 48 µg, about 49 µg, about 50 µg, about 51 µg, about 52 µg, about 53 µg, about

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[00246] In embodiments, the methods comprise administering from about 1 µg to about 300 µg of each HA glycoprotein. In embodiments, the methods comprise administering from about 5 µg to about 60 µg of each HA glycoprotein. In embodiments, the methods comprise administering from about 24 µg to about 40 µg of each HA glycoprotein. In embodiments, the methods comprise administering from about 30 µg to about 40 µg of each HA glycoprotein. In embodiments, the methods comprise administering from about 30 µg to about 60 µg of each HA glycoprotein. In embodiments, the methods comprise administering from about 33 µg to about 39 µg of each HA glycoprotein. In embodiments, the methods comprise administering from about 20 µg to about 240 µg of each HA glycoprotein. In embodiments, the methods comprise administering from about 30 µg to about 60 µg of each HA glycoprotein. In embodiments, the methods comprise administering from about 24 µg to about 40 µg of each HA glycoprotein. In embodiments, the methods comprise administering about 1 µg, about 2 µg, about 3 µg, about 4 µg, about 5 µg, about 6 µg, about 7 µg, about 8 µg, about 9 µg, about 10 µg, about 11 µg, about 12 µg, about 13 µg, about 14 µg, about 15 µg, about 16 µg, about 17 µg, about 18 µg, about 19 µg, about 20 µg, about 21 µg, about 22 µg, about 23 µg, about 24 µg, about 25 µg, about 26 µg, about 27 µg, about 28 µg, about 29 µg, about 30 µg, about 31 µg, about 32 µg, about 33 µg, about 34 µg, about 35 µg, about 36 µg, about 37 µg, about 38 µg, about 39 µg, about 40 µg, about 41 µg, about 42 µg, about 43 µg, about 44 µg, about 45 µg, about 46 µg, about 47 µg, about 48 µg, about 49 µg, about 50 µg, about 51 µg, about 52 µg, about 53 µg,

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[00247] In embodiments, the methods comprise administering from about 1 µg to about 1000 µg of total HA glycoproteins. In embodiments, the methods comprise administering about 1 µg, about 2 µg, about 3 µg, about 4 µg, about 5 µg, about 6 µg, about 7 µg, about 8 µg, about 9 µg, about 10 µg, about 11 µg, about 12 µg, about 13 µg, about 14 µg, about 15 µg, about 16 µg, about 17 µg, about 18 µg, about 19 µg, about 20 µg, about 21 µg, about 22 µg, about 23 µg, about 24 µg, about 25 µg, about 26 µg, about 27 µg, about 28 µg, about 29 µg, about 30 µg, about 31 µg, about 32 µg, about 33 µg, about 34 µg, about 35 µg, about 36 µg, about 37 µg, about 38 µg, about 39 µg, about 40 µg, about 41 µg, about 42 µg, about 43 µg, about 44 µg, about 45 µg, about 46 µg, about 47 µg, about 48 µg, about 49 µg, about 50 µg, about 51 µg, about 52 µg, about 53 µg, about 54 µg, about 55 µg, about 56 µg, about 57 µg, about 58 µg, about 59 µg, about 60 µg, about 61 µg, about 62 µg, about 63 µg, about 64 µg, about 65 µg, about 66 µg, about 67 µg, about 68 µg, about 69 µg, about 70 µg, about 71 µg, about 72 µg, about 73 µg, about 74 µg, about 75 µg, about 76 µg, about 77 µg, about 78 µg, about 79 µg, about 80 µg, about 81 µg, about 82 µg, about 83 µg, about 84 µg, about 85 µg, about 86 µg, about 87 µg, about 88 µg, about 89 µg, about 90 µg, about 91 µg, about 92 µg, about 93 µg, about 94 µg, about 95 µg, about 96 µg, about 97 µg, about 98 µg, about 99 µg, about 100 µg, about 101 µg, about 102 µg, about 103 µg, about 104 µg, about 105 µg, about 106 µg, about 107 µg, about 108 µg, about 109

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[00248] In embodiments, the methods comprise administering from about 1 μg to about 400 μg, from 1 μg to about 350 μg, from 1 μg to about 300 μg, from 1 μg to about 250 μg, from 1 μg to about 200 μg, from 1 μg to about 150 μg, from 1 μg to about 100 μg, from 1 μg to about 50 μg, or from 1 μg to about 25 μg of each viral glycoprotein. In embodiments, the methods comprise administering about 1 μg, about 2 μg, about 3 μg, about 4 μg, about 5 μg, about 6 μg, about 7 μg, about 8 μg, about 9 μg, about 10 μg, about 11 μg, about 12 μg, about 13 μg, about 14 μg, about 15 μg, about 16 μg, about 17 μg, about 18 μg, about 19 μg, about 20 μg, about 21 μg, about 22 μg, about 23 μg, about 24 μg, about 25 μg, about 26 μg, about 27 μg, about 28 μg, about 29 μg, about 30 μg, about 31 μg, about 32 μg, about 33 μg, about 34 μg, about 35 μg, about 36 μg, about 37 μg, about 38 μg, about 39 μg, about 40 μg, about 41 μg, about 42 μg, about 43 μg, about 44 μg, about 45 μg, about 46 μg, about 47 μg, about 48 μg, about 49 μg, about 50 μg, about 51 μg, about 52 μg, about 53 μg, about 54 μg, about 55 μg, about 56 μg, about 57 μg, about 58 μg, about 59 μg, about 60 μg, about 61 μg, about 62 μg, about 63 μg, about 64 μg, about 65 μg, about 66 μg, about 67 μg, about 68 μg, about 69 μg, about 70 μg, about 71 μg, about 72 μg, about 73 μg, about 74 μg, about 75 μg, about 76 μg, about 77 μg, about 78 μg, about 79 μg, about 80 μg, about 81 μg, about 82 μg, about 83 μg, about 84 μg, about 85 μg, about 86 μg, about 87 μg, about 88 μg, about 89 μg, about 90 μg, about 91 μg, about 92 μg, about 93 μg, about 94 μg, about 95 μg, about 96 μg, about 97 μg, about 98 μg, about 99 μg, about

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304 µg, about 305 µg, about 306 µg, about 307 µg, about 308 µg, about 309 µg, about 310 µg, about 311 µg, about 312 µg, about 313 µg, about 314 µg, about 315 µg, about 316 µg, about 317 µg, about 318 µg, about 319 µg, about 320 µg, about 321 µg, about 322 µg, about 323 µg, about 324 µg, about 325 µg, about 326 µg, about 327 µg, about 328 µg, about 329 µg, about 330 µg, about 331 µg, about 332 µg, about 333 µg, about 334 µg, about 335 µg, about 336 µg, about 337 µg, about 338 µg, about 339 µg, about 340 µg, about 341 µg, about 342 µg, about 343 µg, about 344 µg, about 345 µg, about 346 µg, about 347 µg, about 348 µg, about 349 µg, about 350 µg, about 351 µg, about 352 µg, about 353 µg, about 354 µg, about 355 µg, about 356 µg, about 357 µg, about 358 µg, about 359 µg, about 360 µg, about 361 µg, about 362 µg, about 363 µg, about 364 µg, about 365 µg, about 366 µg, about 367 µg, about 368 µg, about 369 µg, about 370 µg, about 371 µg, about 372 µg, about 373 µg, about 374 µg, about 375 µg, about 376 µg, about 377 µg, about 378 µg, about 379 µg, about 380 µg, about 381 µg, about 382 µg, about 383 µg, about 384 µg, about 385 µg, about 386 µg, about 387 µg, about 388 µg, about 389 µg, about 390 µg, about 391 µg, about 392 µg, about 393 µg, about 394 µg, about 395 µg, about 396 µg, about 397 µg, about 398 µg, about 399 µg, or about 400 µg of each viral glycoprotein.

[00249] In embodiments, the methods comprise administering from about 1 µg to about 400 µg, from 1 µg to about 350 µg, from 1 µg to about 300 µg, from 1 µg to about 250 µg, from 1 µg to about 200 µg, from 1 µg to about 150 µg, from 1 µg to about 100 µg, from 1 µg to about 50 µg, or from 1 µg to about 25 µg of adjuvant. In embodiments, the immunogenic compositions comprise about 1 µg, about 2 µg, about 3 µg, about 4 µg, about 5 µg, about 6 µg, about 7 µg, about 8 µg, about 9 µg, about 10 µg, about 11 µg, about 12 µg, about 13 µg, about 14 µg, about 15 µg, about 16 µg, about 17 µg, about 18 µg, about 19 µg, about 20 µg, about 21 µg, about 22 µg, about 23 µg, about 24 µg, about 25 µg, about 26 µg, about 27 µg, about 28 µg, about 29 µg, about 30 µg, about 31 µg, about 32 µg, about 33 µg, about 34 µg, about 35 µg, about 36 µg, about 37 µg, about 38 µg, about 39 µg, about 40 µg, about 41 µg, about 42 µg, about 43 µg, about 44 µg, about 45 µg, about 46 µg, about 47 µg, about 48 µg, about 49 µg, about 50 µg, about 51 µg, about 52 µg, about 53 µg, about 54 µg, about 55 µg, about 56 µg, about 57 µg, about 58 µg, about 59 µg, about 60 µg, about 61 µg, about 62 µg, about 63 µg, about 64 µg, about 65 µg, about 66 µg, about 67 µg, about 68 µg, about 69 µg, about 70 µg, about 71 µg, about 72 µg, about 73 µg, about 74 µg, about 75 µg, about 76 µg, about 77 µg, about 78 µg, about 79 µg, about 80 µg, about 81 µg,

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287 µg, about 288 µg, about 289 µg, about 290 µg, about 291 µg, about 292 µg, about 293 µg, about 294 µg, about 295 µg, about 296 µg, about 297 µg, about 298 µg, about 299 µg, about 300 µg, about 301 µg, about 302 µg, about 303 µg, about 304 µg, about 305 µg, about 306 µg, about 307 µg, about 308 µg, about 309 µg, about 310 µg, about 311 µg, about 312 µg, about 313 µg, about 314 µg, about 315 µg, about 316 µg, about 317 µg, about 318 µg, about 319 µg, about 320 µg, about 321 µg, about 322 µg, about 323 µg, about 324 µg, about 325 µg, about 326 µg, about 327 µg, about 328 µg, about 329 µg, about 330 µg, about 331 µg, about 332 µg, about 333 µg, about 334 µg, about 335 µg, about 336 µg, about 337 µg, about 338 µg, about 339 µg, about 340 µg, about 341 µg, about 342 µg, about 343 µg, about 344 µg, about 345 µg, about 346 µg, about 347 µg, about 348 µg, about 349 µg, about 350 µg, about 351 µg, about 352 µg, about 353 µg, about 354 µg, about 355 µg, about 356 µg, about 357 µg, about 358 µg, about 359 µg, about 360 µg, about 361 µg, about 362 µg, about 363 µg, about 364 µg, about 365 µg, about 366 µg, about 367 µg, about 368 µg, about 369 µg, about 370 µg, about 371 µg, about 372 µg, about 373 µg, about 374 µg, about 375 µg, about 376 µg, about 377 µg, about 378 µg, about 379 µg, about 380 µg, about 381 µg, about 382 µg, about 383 µg, about 384 µg, about 385 µg, about 386 µg, about 387 µg, about 388 µg, about 389 µg, about 390 µg, about 391 µg, about 392 µg, about 393 µg, about 394 µg, about 395 µg, about 396 µg, about 397 µg, about 398 µg, about 399 µg, or about 400 µg of adjuvant. In embodiments, the methods comprise administering about 50 µg of adjuvant. In embodiments, the methods comprise administering about 75 µg of adjuvant.

[00250] In another embodiment, the disclosure provides a method of formulating a vaccine composition that induces immunity to an infection or at least one disease symptom thereof to a mammal, comprising adding to the composition an effective dose of a RSV F glycoprotein or RSV F glycoprotein nanoparticles. The disclosed RSV F glycoprotein and RSV F glycoprotein nanoparticles are useful for preparing compositions that stimulate an immune response that confers immunity or substantial immunity to infectious agents. Thus, in one embodiment, the disclosure provides a method of inducing immunity to infections or at least one disease symptom thereof in a subject, comprising administering at least one effective dose of a RSV F glycoprotein or RSV F glycoprotein nanoparticles.

[00251] In embodiments, the present disclosure provides a method of producing one or more of high affinity anti-RSV antibodies. The high affinity antibodies produced

by immunization with the nanoparticles disclosed herein are produced by administering an immunogenic composition comprising an RSV F glycoprotein or RSV F glycoprotein nanoparticle to an animal, collecting the serum and/or plasma from the animal, and purifying the antibody from the serum/ and or plasma. In one embodiment, the animal is a human. In embodiments, the animal is a chicken, mouse, guinea pig, rat, rabbit, goat, human, horse, sheep, or cow. In one embodiment, the animal is bovine or equine. In another embodiment, the bovine or equine animal is transgenic. In yet a further embodiment, the transgenic bovine or equine animal produces human antibodies. In embodiments, the animal produces monoclonal antibodies. In embodiments, the animal produces polyclonal antibodies. In one embodiment, the method further comprises administration of an adjuvant or immune stimulating compound. In a further embodiment, the purified high affinity antibody is administered to a human subject. In one embodiment, the human subject is at risk for infection with RSV.

[00252] In embodiments, the composition is administered intranasally. In embodiments, intranasal administration of the composition includes administration of a liquid drop of the composition directly onto the nasal epithelium. In embodiments, the methods comprise administering from 1 to 100, from 1 to 90, from 1 to 80, from 1 to 70, from 1 to 60, from 1 to 50, from 1 to 40, from 1 to 30, from 1 to 20, from 1 to 10, or from 1 to 5 drops of the immunogenic composition are intranasally. In embodiments, the liquid drop is delivered by an intranasal delivery device described herein.

[00253] In embodiments, the methods comprise administering an aerosol of an immunogenic composition described herein to the nasal epithelium. In embodiments, the aerosol is a liquid aerosol. In embodiments, the aerosol is a powder aerosol. A powder aerosol comprises a dry powder composition. In embodiments, the methods comprise administering from 1 to 100, from 1 to 90, from 1 to 80, from 1 to 70, from 1 to 60, from 1 to 50, from 1 to 40, from 1 to 30, from 1 to 20, from 1 to 10, or from 1 to 5 sprays of the aerosol intranasally. In embodiments, the aerosol is delivered by an intranasal delivery device. Intranasal delivery devices are described in International Publication No. 2012/105236 and U.S. Patent No. 10,441,436, which are incorporated by reference herein in their entireties for all purposes. An intranasal delivery device may also be a prefilled syringe, as described herein.

[00254] In embodiments, the methods comprise administering an intranasal immunogenic composition that is in solution. In embodiments, the methods comprise administering an intranasal immunogenic composition that is in suspension.

[00255] In embodiments, the methods comprise intranasally administering a mist or puff of an intranasal immunogenic composition to a patient's nostril. In embodiments, the methods comprise intranasally administering from 1 to about 100 mists or puffs of an intranasal immunogenic composition to a patient's nostril. In embodiments, the methods comprise intranasally administering about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45, about 46, about 47, about 48, about 49, about 50, about 51, about 52, about 53, about 54, about 55, about 56, about 57, about 58, about 59, about 60, about 61, about 62, about 63, about 64, about 65, about 66, about 67, about 68, about 69, about 70, about 71, about 72, about 73, about 74, about 75, about 76, about 77, about 78, about 79, about 80, about 81, about 82, about 83, about 84, about 85, about 86, about 87, about 88, about 89, about 90, about 91, about 92, about 93, about 94, about 95, about 96, about 97, about 98, about 99, or about 100 puffs or mists, including all values and ranges therebetween, of an intranasal immunogenic composition to a patient's nostril. In embodiments, a mist or puff is delivered to each of the patient's nostrils.

[00256] In embodiments, the methods described herein allow the immunogenic composition to reach the lungs of a patient. In embodiments, the methods described herein allow the immunogenic composition to reach the upper respiratory tract of a patient. In embodiments, the methods described herein allow the immunogenic composition to reach the lower respiratory tract of a patient.

[00257] In embodiments, the methods comprise intranasally administering from 0.05 mL to about 1 mL of an intranasal immunogenic composition described herein. In embodiments, the methods comprise intranasally administering from about 0.05 mL, about 0.1 mL, about 0.2 mL, about 0.3 mL, about 0.4 mL, about 0.5 mL, about 0.6 mL, about 0.7 mL, about 0.8 mL, about 0.9 mL, or about 1 mL of an intranasal immunogenic composition described herein, including all values and ranges therebetween. In embodiments the methods comprise intranasally administering 0.2 mL of an intranasal

immunogenic composition described herein. In embodiments the methods comprise intranasally administering 0.5 mL of an intranasal immunogenic composition described herein. In embodiments the methods comprise intranasally administering 0.25 mL of an intranasal immunogenic composition described herein to each nostril of a patient.

[00258] In embodiments, provided herein are methods of producing IgA and/or IgG antibodies against SARS-CoV-2, influenza, and/or RSV in a subject. In embodiments, the antibodies are neutralizing antibodies. In embodiments, provided herein are methods of inducing mucosal immunity against SARS-CoV-2, influenza, RSV, or a combination thereof in a patient comprising intranasally administering the immunogenic composition described herein. In embodiments, the antibodies are located in the bronchoalveolar lavage of the subject.

[00259] In some embodiments, the disclosure provides co-formulation (i.e., pre-filled syringes or pre-mix) strategies for immunogenic compositions comprising a RSV F glycoprotein and an adjuvant (e.g., a saponin adjuvant). Typical vaccine administration strategies currently being utilized are bedside mix formulations. That is, vaccine compositions and adjuvants are stored separately and are mixed prior to administration. Pre-mix, co-formulation, or pre-filled syringe strategies for vaccine are less common due to the concerns of the stability of the antigens (e.g., a RSV F glycoprotein) and their subsequent immunogenic capabilities. The present disclosure provides immunogenic compositions that can be pre-mixed and stored in advance. The disclosed vaccination strategies and formulations may improve the efficiency of vaccination and may reduce the risks of bedside mixing errors, while maintaining the overall safety and immunogenicity.

[00260] A variety of containers may be used to store and transport the pre-mix formulations, including syringes for single administrations and plastic ampules. In some instances, plastic ampules can be manufactured using the blow-fill-seal manufacturing technique or method. In general, the blow-fill-seal (BFS) manufacturing method includes extruding a plastic material (e.g., resin) to form a parison, which is then placed into a mold and cut to size. A filling needle or mandrel is then used to inflate the plastic, which in turn, results in a hollow ampule that substantially conforms to the shape of the mold. Once inflated, a desired volume of liquid can be injected into the ampule, the filling needle or mandrel can be removed, and the ampule can be

sealed. Accordingly, BFS can be an automated process that can be performed in a sterile environment without direct human intervention.

[00261] In some instances, the ability to aseptically manufacture sterile ampules containing a desired liquid can make BFS manufactured ampules particularly well suited for the pharmaceutical industry. BFS technology, however, has not been compatible with all pharmaceutical liquids, products, etc. For example, some known BFS manufacturing methods include delivering the liquid or product into the ampule while the plastic is still relatively hot, which can result in adverse effects to temperature sensitive liquids and/or products such as vaccines, biologics, etc. Advances in cool BFS technology, however, have increased the variety of suitable products, liquids, etc. allowing some vaccines, biologics, and/or other temperature sensitive pharmaceuticals to be contained in BFS ampules.

[00262] In some instances, a BFS ampule can have a size, shape, and/or configuration that is at least partially based on a desired use and/or a desired pharmaceutical liquid or dosage that the ampule is configured to contain. For example, some known BFS ampules can include a pierce through top, a twist-off top, a top including a male or female luer, and/or the like. Some known BFS ampules can have a size and/or shape based on volume of the liquid or dosage configured to be disposed therein. In addition, some known BFS ampules can be manufactured in a strip of multiple, temporarily connected ampules, which can increase manufacturing, packaging, and/or storing efficiencies and/or the like.

[00263] In embodiments, the immunogenic compositions described herein are provided in pre-filled syringes. When the immunogenic composition is prepared in a pre-filled syringe, an antigen and adjuvant is combined in advance of administration. In embodiments, the pre-filled syringe contains hemagglutinin, a SARS-CoV-2 S glycoprotein, an RSV F glycoprotein, or a combination thereof. In embodiments, a subject is administered an intranasal immunogenic composition from a pre-filled syringe.

[00264] In embodiments, the pre-filled syringe contains a RSV F glycoprotein, a HA glycoprotein, a CoV S glycoprotein, or a combination thereof. In embodiments, the adjuvant is a saponin adjuvant. In embodiments, the saponin adjuvant comprises at least two iscom particles, wherein the first iscom particle comprises fraction A of Quillaja Saponaria Molina and not fraction C of Quillaja Saponaria Molina; and the second iscom particle comprises fraction C of Quillaja Saponaria Molina and not

fraction A of Quillaja Saponaria Molina; wherein fraction A of Quillaja Saponaria Molina and fraction C of Quillaja Saponaria Molina account for about 85 % by weight and about 15 % by weight, respectively, of the sum of weights of fraction A of Quillaja Saponaria Molina and fraction C of Quillaja Saponaria Molina in the adjuvant. In embodiments, the pre-filled syringe contains a RSV F glycoprotein and a saponin adjuvant, wherein the adjuvant comprises at least two iscom particles, wherein the first iscom particle comprises fraction A of Quillaja Saponaria Molina and not fraction C of Quillaja Saponaria Molina; and the second iscom particle comprises fraction C of Quillaja Saponaria Molina and not fraction A of Quillaja Saponaria Molina; wherein fraction A of Quillaja Saponaria Molina and fraction C of Quillaja Saponaria Molina account for about 92 % by weight and about 8 % by weight, respectively, of the sum of weights of fraction A of Quillaja Saponaria Molina and fraction C of Quillaja Saponaria Molina in the adjuvant. In embodiments, the pre-filled syringe contains a RSV F glycoprotein and a saponin adjuvant, wherein the adjuvant comprises at least two iscom particles, wherein the first iscom particle comprises fraction A of Quillaja Saponaria Molina and not fraction C of Quillaja Saponaria Molina; and the second iscom particle comprises fraction C of Quillaja Saponaria Molina and not fraction A of Quillaja Saponaria Molina; wherein fraction A of Quillaja Saponaria Molina accounts for at least about 75 % by weight and fraction C of Quillaja Saponaria Molina accounts for the remainder of the sum of weights of fraction A of Quillaja Saponaria Molina and fraction C of Quillaja Saponaria Molina in the adjuvant.

EXAMPLES

Example 1

Expression and Purification of RSV F Polypeptide Nanoparticles

[00265] The native RSV F glycoprotein (SEQ ID NO: 329 and SEQ ID NO: 330) and RSV F glycoproteins which have amino acid sequences corresponding to SEQ ID NOS: 329-336 were expressed in a baculovirus expression system and recombinant plaques expressing the RSV F glycoproteins were picked and confirmed. In each case the signal peptide is SEQ ID NO: 360.

Table A: Selected RSV F glycoproteins

RSV F glycoprotein	SEQ ID NO.	Modifications compared to SEQ ID NO: 2
BV2279 Cav1	333	Inactive secondary furin cleavage site, P102A, I379V, M447V, deletion of amino acids 137-146 of the fusion peptide, S190F, V207L
	334	Inactive secondary furin cleavage site, P102A, I379V, M447V, deletion of amino acids 137-146 of the fusion peptide, N116Q
BV2267 DS	335	Inactive secondary furin cleavage site, P102A, I379V, M447V, deletion of amino acids 137-146 of the fusion peptide, S155C, S290C
Cav1DS	336	Inactive secondary furin cleavage site, P102A, I379V, M447V, deletion of amino acids 137-146 of the fusion peptide, S190F, V207L, S155C, S290C

RSV F glycoprotein	SEQ ID NO.	Modifications compared to SEQ ID NO: 2
BV1184	331	Inactive secondary furin cleavage site, P102A, I379V, M447V, deletion of amino acids 137-146 of the fusion peptide

Protein and Nanoparticle Production

[0266] The recombinant virus is amplified by infection of Sf9 insect cells. A culture of insect cells is infected at ~0.6 MOI (Multiplicity of infection = virus ffu or pfu/cell) with baculovirus. The culture and supernatant is harvested 48-72 hrs post-infection. The crude cell harvest, approximately 30 mL, is clarified by centrifugation for 15 minutes at approximately 800 x g. The resulting crude cell harvests containing the RSV F glycoprotein are purified as nanoparticles as described below.

[0267] To produce nanoparticles, non-ionic surfactant TERGITOL® nonylphenol ethoxylate NP-9 is used in the membrane protein extraction protocol. Crude extraction is further purified by passing through anion exchange chromatography, lentil lectin affinity/HIC and cation exchange chromatography. The washed cells are lysed by detergent treatment and then subjected to low pH treatment which leads to precipitation of BV and Sf9 host cell DNA and protein. The neutralized low pH treatment lysate is clarified and further purified on anion exchange and affinity chromatography before a second low pH treatment is performed.

[0268] Affinity chromatography is used to remove Sf9/BV proteins, DNA and NP-9, as well as to concentrate the RSV F glycoprotein. Briefly, lentil lectin is a metalloprotein containing calcium and manganese, which reversibly binds polysaccharides and glycosylated proteins containing glucose or mannose. The RSV F glycoprotein - containing anion exchange flow through fraction is loaded onto the lentil lectin affinity chromatography resin (Capto Lentil Lectin, GE Healthcare). The glycosylated RSV F protein is selectively bound to the resin while non-glycosylated proteins and DNA are removed in the column flow through. Weakly bound glycoproteins are removed by buffers containing high salt and low molar concentration of methyl α -D-mannopyranoside (MMP).

[0269] The column washes are also used to detergent exchange the NP-9 detergent with the surfactant polysorbate 80 (PS80). The RSV F glycoproteins are eluted in nanoparticle structure from the lentil lectin column with a high concentration of MMP. After elution, the RSV F glycoproteins are assembled into nanoparticles composed of RSV F glycoprotein trimers and PS80 contained in a detergent core.

Example 2

Immunogenicity of RSV F Glycoproteins Nanoparticle Vaccines in Cotton Rats

[0270] *Methods:* RSV F glycoprotein nanoparticles were produced as described in Example 1. Cotton rats were immunized intramuscularly (IM) with a composition of Table B. Each composition contained 0.01-0.0001 µg RSV F glycoprotein and saponin adjuvant. The saponin adjuvant contained 85 % w/w Fraction A ISCOM matrix and 15 % w/w Fraction C ISCOM matrix. Each composition contained 20 µg saponin adjuvant. Table B. Compositions

Compositions	RSV F glycoprotein	adjuvant
BV1184	BV1184 (SEQ ID NO: 332)	Saponin adjuvant
BV2279	BV2279 (SEQ ID NO: 333)	0.01-0.0001 µg

[0271] Animals were immunized on days 0 and 21. Blood was collected on Days 0, 21, and 42 to evaluate immunogenicity. Animals were intranasally challenged with 105 pfu RSV A/Long on Day 42, followed by tissue collection four days later.

[0272] Immune responses to RSV F compositions were measured by anti-RSV F IgG ELISA and RSVA/RSVB Neutralization Assays. Protective efficacy was evaluated by measuring lung and nose tissue viral titers using an RSV plaque assay in HEp2 cells.

[0273] Neutralization assay, and prefusion F specificity of antibodies was determined by Octet QK384 competition binning assays using antigenic site-specific antibodies to pre- and post-fusion epitopes. F protein bound to amine-reactive biosensor tips was presented in consecutive Association and Competition steps with polyclonal antibodies in sera and competing mAbs to RSV F sites II, IV, ϕ , V and p27. Competitive antibody equivalents (CAE) were calculated based on percentage competition, concentration of competing antibody, and dilution of serum.

[0274] Protective efficacy was evaluated by measuring lung and nose tissue viral titers using an RSV plaque assay in HEP2 cells.

[0275] *Results:* Octet kinetic analysis of antigenic site-specific monoclonal antibodies confirmed all pre- and post-fusion antigenic sites and neutralizing epitopes on the RSV F glycoproteins were intact in the BV2279 glycoprotein. Immunization with BV1184 or BV2279 elicited comparable anti-F IgG responses and RSV A/B neutralizing responses. Immunization with BV2279 generated greater antibody responses toward Site ϕ , IV, and VIII compared to immunization with BV1184. The BV 2279 nanoparticle vaccine provided sterilizing immunity in the lungs at the 0.01 μg dose and reduced viral load in upper airways post RSV live virus challenge in cotton rats. In contrast, the BV1184 vaccine did not reduce vaccine load in the upper airways. Together, these data show that the BV2279 vaccine is a potent immunogen eliciting a robust RSV F-specific immune response to pre- and post-fusion F epitopes.

[0276] The table below shows that the BV2279 protein retains both pre-fusion and post-fusion epitopes.

mAb (Site)	Ka (1/Ms)	Kd (1/s)	KD (M)
Palivizumab (Site II)	1.27×10^5	$<1.0 \times 10^{-7}$	$<1.0 \times 10^{-12}$
RSV.42 (Site IV)	3.32×10^4	1.12×10^{-7}	$<1.0 \times 10^{-12}$
D25 (Site ϕ)	1.11×10^5	$<1.0 \times 10^{-7}$	$<1.0 \times 10^{-12}$

hRSV90 (Site VIII)	1.50×10^5	$<1.0 \times 10^{-7}$	$<1.0 \times 10^{-12}$
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[0277]Fig. 1 shows the binding of the BV2279 glycoprotein to antibodies recognizing the site II (palivizumab), site IV (RSV.42, referred to as “RSV.42.2”), site ϕ (D25), and site VIII (hRSV90) epitopes. Fig. 2 shows anti-RSV F IgG titers in cotton rat sera 42 days after immunization with either the BV2279 (labeled “prefusion RSV F Cav1”) or BV1184 (labeled “prefusogenic RSV F”) vaccine compositions of Example 2. Fig. 3 shows the RSV neutralization titers against the RSV A (Long) and RSV B (18537) strains after immunization with either the BV2279 (labeled “prefusion RSV F Cav1”) or BV1184 (labeled “prefusogenic RSV F”) vaccine compositions of Example 2. Fig. 4 shows competing antibody equivalent titers induced by immunization with either the BV2279 (labeled “prefusion RSV F Cav1”) or BV1184 (labeled “prefusogenic RSV F”) vaccine compositions of Example 2. Fig. 5 shows the RSV viral titers in cotton rat tissue four days post viral challenge. The rats were immunized with either the BV2279 (labeled “prefusion RSV F Cav1”) or BV1184 (labeled “prefusogenic RSV F”) vaccine compositions of Example 2.

INCORPORATION BY REFERENCE

[0278] All references, articles, publications, patents, patent publications, and patent applications cited herein are incorporated by reference in their entireties for all purposes. However, mention of any reference, article, publication, patent, patent publication, and patent application cited herein is not, and should not be taken as, an acknowledgment or any form of suggestion that they constitute valid prior art or form part of the common general knowledge in any country in the world. The following patent documents are incorporated by reference herein in their entireties for all purposes: International Publication No. 2004/004762 and International Publication No. 2017/041100.

CLAIMS

1. A respiratory syncytial virus (RSV) fusion (F) glycoprotein, comprising an F1 domain, an F2 domain, and p27; wherein the glycoprotein comprises one or more modifications selected from the group consisting of:
 - (a) deletion of one or more of amino acids 137-146;
 - (b) an inactivated primary furin cleavage site;
 - (c) an inactivated secondary furin cleavage site;
 - (d) S155C;
 - (e) S290C;
 - (f) S190F;
 - (g) V207L; and
 - (h) N116Q;wherein the modifications are numbered according to the RSV F glycoprotein of SEQ ID NO: 330.
2. The RSV F glycoprotein of claim 1, comprising (a) deletion of one or more of amino acids 137-146; and (b) an inactivated primary furin cleavage site.
3. The RSV F glycoprotein of claim 1 or 2, comprising a deletion of all of amino acids 137-146.
4. The RSV F glycoprotein of any one of claims 1-3, wherein the inactivated primary furin cleavage site comprises an amino acid sequence selected from the group consisting of: SEQ ID NOS: 342-345.
5. The RSV F glycoprotein of any one of claims 1-4, wherein the inactivated primary furin cleavage site comprises an amino acid sequence of SEQ ID NO: 342.
6. The RSV F glycoprotein of any one of claims 1-5, wherein the RSV F glycoprotein comprises S155C and S290C mutations.
7. The RSV F glycoprotein of any one of claims 1-5, wherein the RSV F glycoprotein comprises S190F and V207L mutations.

8. The RSV F glycoprotein of any one of claims 1-5, wherein the RSV F glycoprotein comprises S155C, S290C, S190F, and V207L mutations.
9. The RSV F glycoprotein of any one of claims 1-8, wherein the RSV F glycoprotein comprises a N116Q mutation.
10. The RSV F glycoprotein of any one of claims 1-9, wherein the RSV F glycoprotein comprises an inactivated primary furin cleavage site having the amino acid sequence of KKQKQQ (SEQ ID NO: 342), an S190F mutation, a V207 mutation, and deletion of amino acids 137-146.
11. The RSV F glycoprotein of any one of claims 1-9, wherein the RSV F glycoprotein comprises an inactivated primary furin cleavage site having the amino acid sequence of KKQKQQ (SEQ ID NO: 342), an S190F mutation, a V207 mutation, and deletion of amino acids 137-146, wherein the RSV F glycoprotein has at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identity to an RSV F glycoprotein of SEQ ID NO: 337.
12. The RSV F glycoprotein of any one of claims 1-11, wherein the RSV F glycoprotein comprises: (i) an F1 domain having an amino acid sequence with at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identity to the F1 domain of SEQ ID NO: 352; and (ii) an F2 domain having an amino acid sequence with at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identity to the F2 domain of SEQ ID NO: 357.
13. The RSV F glycoprotein of any one of claims 1-12, wherein the F1 domain is at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91

%, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identical to a F1 domain of any one of SEQ ID NOS: 350-355.

14. The RSV F glycoprotein of any one of claims 1-13, wherein the F2 domain is at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 % at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identical to a F2 domain of any one of SEQ ID NOS: 356-357.

15. The RSV F glycoprotein of any one of claims 1-14, wherein the p27 domain is at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 % at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identical to a p27 domain of any one of SEQ ID NOS: 358-359.

16. The RSV F glycoprotein of any one of claims 1-10, wherein the RSV F glycoprotein is at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 % at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identical to a RSV F glycoprotein of any one of SEQ ID NOS: 364-366.

17. The RSV F glycoprotein of any one of claims 1-10, wherein the RSV F glycoprotein is at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 % at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identical to a RSV F glycoprotein of SEQ ID NO: 364.

18. The RSV F glycoprotein of any one of claims 1-10, wherein the RSV F glycoprotein is at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84

% at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identical to any one of SEQ ID NOS: 333-340 and 364-366.

19. The RSV F glycoprotein of any one of claims 1-18, wherein the p27 and F1 domain form a single subunit called p27-F1, and wherein p27-F1 and the F2 subunit are connected by a disulfide bond.

20. The RSV F glycoprotein of any one of claims 1-19, wherein the RSV F glycoprotein comprises a signal peptide.

21. The RSV F glycoprotein of claim 13, wherein the signal peptide comprises the sequence of MELLILKANAITTILTAVTFCFASG (SEQ ID NO: 360) or MELLIHRLSAIFLTL (SEQ ID NO: 367).

22. A nucleic acid comprising the RSV F glycoprotein of any one of claims 1-21.

23. A vector comprising the nucleic acid of claim 22.

24. A nanoparticle comprising the RSV F glycoprotein of any one of claims 1-21 and a non-ionic detergent core.

25. The nanoparticle of claim 24, wherein the non-ionic detergent is selected from the group consisting of polysorbate-20 (PS20), polysorbate-40 (PS40), polysorbate-60 (PS60), polysorbate-65 (PS65), and polysorbate-80 (PS80).

26. The nanoparticle of claim 24, wherein the non-ionic detergent is PS80.

27. A cell expressing the RSV F glycoprotein of any of claims 1-21.

28. The cell of claim 27, wherein the cell is an insect cell.

29. An immunogenic composition comprising at least one RSV F glycoprotein of any one of claims 1-21 or a nanoparticle of any one of claims 24-26 and a pharmaceutically acceptable buffer.
30. The immunogenic composition of claim 29, comprising one, two, three, four, five, six, seven, eight, nine, or ten different RSV F glycoproteins.
31. The immunogenic composition of any one of claims 29-30, comprising a
- (i) a first RSV F glycoprotein with at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identity to an RSV F glycoprotein of any one of SEQ ID NOS: 337-340; and
 - (ii) second RSV F glycoprotein with at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identity to an RSV F glycoprotein of any one of SEQ ID NOS: 364-366.
32. The immunogenic composition of any one of claims 29-30, comprising a
- (i) a first RSV F glycoprotein with at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identity to an RSV F glycoprotein of SEQ ID NO: 337; and
 - (ii) second RSV F glycoprotein with at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identity to an RSV F glycoprotein of any one of SEQ ID NOs: 364.

33. The immunogenic composition of any one of claims 29-32, comprising from 30 μ g to about 300 μ g of the RSV F glycoprotein.
34. The immunogenic composition of any one of claims 29-33, comprising about 60 μ g of the RSV F glycoprotein.
35. The immunogenic composition of any one of claims 29-34, comprising about 240 μ g of the RSV F glycoprotein.
36. The immunogenic composition of any one of claims 29-35, comprising at least one viral glycoprotein that is not an RSV F glycoprotein.
37. The immunogenic composition of any one of claims 29-36, comprising from 1 to about 20, from 1 to about 19, from 1 to about 18, from 1 to about 17, from 1 to about 16, from 1 to about 15, from 1 to about 14, from 1 to about 13, from 1 to about 12, from 1 to about 11, from 1 to about 10, from 1 to about 9, from 1 to about 8, from 1 to about 7, from 1 to about 6, from 1 to about 5, from 1 to about 4, from 1 to about 3, from 1 to about 2, from about 2 to about 20, from about 2 to about 19, from about 2 to about 18, from about 2 to about 17, from about 2 to about 16, from about 2 to about 15, from about 2 to about 14, from about 2 to about 13, from about 2 to about 12, from about 2 to about 11, from about 2 to about 10, from about 2 to about 9, from about 2 to about 8, from about 2 to about 7, from about 2 to about 6, from about 2 to about 5, from about 2 to about 4, from about 2 to about 3, from about 3 to about 20, from about 3 to about 19, from about 3 to about 18, from about 3 to about 17, from about 3 to about 16, from about 3 to about 15, from about 3 to about 14, from about 3 to about 13, from about 3 to about 12, from about 3 to about 11, from about 3 to about 10, from about 3 to about 9, from about 3 to about 8, from about 3 to about 7, from about 3 to about 6, from about 3 to about 5, from about 3 to about 4, from about 4 to about 20, from about 4 to about 19, from about 4 to about 18, from about 4 to about 17, from about 4 to about 16, from about 4 to about 15, from about 4 to about 14, from about 4 to about 13, from about 4 to about 12, from about 4 to about 11, from about 4 to about 10, from

about 4 to about 9, from about 4 to about 8, from about 4 to about 7, from about 4 to about 6, from about 4 to about 5, from about 5 to about 20, from about 5 to about 19, from about 5 to about 18, from about 5 to about 17, from about 5 to about 16, from about 5 to about 15, from about 5 to about 14, from about 5 to about 13, from about 5 to about 12, from about 5 to about 11, from about 5 to about 10, from about 5 to about 9, from about 5 to about 8, from about 5 to about 7, from about 5 to about 6, from about 6 to about 20, from about 6 to about 19, from about 6 to about 18, from about 6 to about 17, from about 6 to about 16, from about 6 to about 15, from about 6 to about 14, from about 6 to about 13, from about 6 to about 12, from about 6 to about 11, from about 6 to about 10, from about 6 to about 9, from about 6 to about 8, from about 6 to about 7, from about 7 to about 20, from about 7 to about 19, from about 7 to about 18, from about 7 to about 17, from about 7 to about 16, from about 7 to about 15, from about 7 to about 14, from about 7 to about 13, from about 7 to about 12, from about 7 to about 11, from about 7 to about 10, from about 7 to about 9, from about 7 to about 8, from about 8 to about 20, from about 8 to about 19, from about 8 to about 18, from about 8 to about 17, from about 8 to about 16, from about 8 to about 15, from about 8 to about 14, from about 8 to about 13, from about 8 to about 12, from about 8 to about 11, from about 8 to about 10, from about 8 to about 9, from about 9 to about 20, from about 9 to about 19, from about 9 to about 18, from about 9 to about 17, from about 9 to about 16, from about 9 to about 15, from about 9 to about 14, from about 9 to about 13, from about 9 to about 12, from about 9 to about 11, from about 9 to about 10, from about 10 to about 20, from about 10 to about 19, from about 10 to about 18, from about 10 to about 17, from about 10 to about 16, from about 10 to about 15, from about 10 to about 14, from about 10 to about 13, from about 10 to about 12, from about 10 to about 11, from about 11 to about 20, from about 11 to about 19, from about 11 to about 18, from about 11 to about 17, from about 11 to about 16, from about 11 to about 15, from about 11 to about 14, from about 11 to about 13, from about 11 to about 12, from about 12 to about 20, from about 12 to about 19, from about 12 to about 18, from about 12 to about 17, from about 12 to about 16, from about 12 to about 15, from about 12 to about 14, from about 12 to about 13, from about 13 to about 20, from about 13 to about 19, from about 13 to about 18,

from about 13 to about 17, from about 13 to about 16, from about 13 to about 15, from about 13 to about 14, from about 14 to about 20, from about 14 to about 19, from about 14 to about 18, from about 14 to about 17, from about 14 to about 16, from about 14 to about 15, from about 15 to about 20, from about 15 to about 19, from about 15 to about 18, from about 15 to about 17, from about 15 to about 16, from about 16 to about 20, from about 16 to about 19, from about 16 to about 18, from about 16 to about 17, from about 17 to about 20, from about 17 to about 19, from about 17 to about 18, from about 18 to about 20, from about 18 to about 19, or from about 19 to about 20 different viral glycoproteins.

38. The immunogenic composition of claim 36, wherein the at least one viral glycoprotein that is not an RSV F glycoprotein is an influenza hemagglutinin (HA) glycoprotein or a SARS-CoV-2 Spike (CoV S) glycoprotein.
39. The immunogenic composition of claim 37, wherein the at least one viral glycoprotein is an influenza HA glycoprotein.
40. The immunogenic composition of claim 39, wherein the amino acid sequence of the HA glycoprotein has 100 % identity to the amino acid sequence of a native HA glycoprotein.
41. The immunogenic composition of claim 39 or 40, wherein the HA glycoprotein has a subtype selected from the group consisting of H1, H3, H4, H5, and H7.
42. The immunogenic composition of any one of claims 39-41, comprising a first, second, and third hemagglutinin (HA) glycoprotein, wherein each HA glycoprotein is from a different influenza strain.
43. The immunogenic composition of claim 42, comprising a fourth HA glycoprotein, wherein the fourth HA glycoprotein is from a different influenza strain than the first, second, and third HA glycoprotein.
44. The immunogenic composition of any one of claims 41-43, comprising:

- (i) a detergent-core nanoparticle comprising the first HA glycoprotein and a non-ionic detergent; and
 - (ii) a HaSMaN comprising the second HA glycoprotein.
45. The immunogenic composition of claim 43, comprising:
- (i) a first detergent-core nanoparticle comprising the first HA glycoprotein and a non-ionic detergent;
 - (ii) a second detergent-core nanoparticle comprising the second HA glycoprotein and a non-ionic detergent;
 - (iii) a third detergent-core nanoparticle comprising the third HA glycoprotein and a non-ionic detergent; and
 - (iv) a fourth detergent-core nanoparticle comprising the third HA glycoprotein and a non-ionic detergent.
46. The immunogenic composition of claim 43, comprising:
- (i) a detergent-core nanoparticle comprising the first HA glycoprotein and a non-ionic detergent;
 - (ii) a first HaSMaN comprising the second HA glycoprotein;
 - (iii) a second HaSMaN comprising the third HA glycoprotein; and
 - (iv) a third HaSMaN comprising the fourth HA glycoprotein.
47. The immunogenic composition of claim 43, comprising:
- (i) a first detergent-core nanoparticle comprising the first HA glycoprotein and a non-ionic detergent;
 - (ii) a second detergent-core nanoparticle comprising the second HA glycoprotein and a non-ionic detergent;
 - (iii) a first HaSMaN comprising the third HA glycoprotein; and
 - (iv) a second HaSMaN comprising the fourth HA glycoprotein.
48. The immunogenic composition of claim 43, comprising:
- (i) a first detergent-core nanoparticle comprising the first HA glycoprotein and a non-ionic detergent;
 - (ii) a second detergent-core nanoparticle comprising the second HA glycoprotein and a non-ionic detergent;

- (iii) a third detergent-core nanoparticle comprising the third HA glycoprotein and a non-ionic detergent; and
- (iv) a HaSMaN comprising the fourth HA glycoprotein.

49. The immunogenic composition of any one of claims 44-48, wherein the HA glycoproteins of the detergent-core nanoparticles is from a Type A influenza strain.

50. The immunogenic composition of any one of claims 44-48, wherein the HA glycoproteins of the detergent-core nanoparticles is from a Type B influenza strain.

51. The immunogenic composition of any one of claims 44 and 46-48, wherein the HA glycoproteins of the HaSMaNs are from a Type A influenza strain.

52. The immunogenic composition of any one of claims 39-51, comprising from about 30 µg to about 300 µg of each HA glycoprotein.

53. The immunogenic composition of any one of claims 39-51 comprising about 60 µg of each HA glycoprotein.

54. The immunogenic composition of any one of claims 39-51, comprising about 240 µg of each HA glycoprotein.

55. The immunogenic composition of any one of claims 29-54, comprising a SARS-CoV-2 Spike (CoV S) glycoprotein.

56. The immunogenic composition of claim 55, wherein the SARS-CoV-2 S glycoprotein contains (i) an inactive furin cleavage site, and (ii) proline at amino acid positions 973 and 974, wherein the SARS-CoV-2 glycoprotein is numbered according to the polypeptide of SEQ ID NO: 2.

57. The immunogenic composition of claim 56, wherein the inactive furin cleavage site has an amino acid sequence selected from any one of: 7-34, 97, and 111.

58. The immunogenic composition of claim 56, wherein the inactive furin cleavage site has the amino acid sequence of QQAQ (SEQ ID NO: 7).

59. The immunogenic composition of any one of claims 55-58, wherein the CoV S glycoprotein has at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % to the SARS-CoV-2 S glycoprotein of SEQ ID NO: 87.

60. The immunogenic composition of any one of claims 55-58, wherein the CoV S glycoprotein has at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % to the SARS-CoV-2 S glycoprotein of SEQ ID NO: 274.

61. The immunogenic composition of any one of claims 55-58, wherein the CoV S glycoprotein has at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or at least 100 % identity to a polypeptide of any one of SEQ ID NOS: 87, 89, 106, 112, 113, 114, 115, 132, 144, 151, 153, 156, 158, 174, 175, 176, 181, 182, 183, 184, 186, 188, 190, 192, 195, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 233, 234, 235, 236, 260, 261, 262, 263, 264, 274, 276, 278, 280, 284, 288, 292, 296, 300, 304, 308, 312, 316, 320, 324, and 328.

62. The immunogenic composition of any one of claims 29-61, comprising:

(i) a first CoV S glycoprotein comprising (a) an inactive furin cleavage site, and (b) proline at amino acid positions 973 and 974, wherein the SARS-CoV-2 glycoprotein is numbered according to the polypeptide of SEQ ID NO: 2; and

(ii) a second CoV S glycoprotein comprising (a) an inactive furin cleavage site, and (b) proline at amino acid positions 973 and 974, wherein the SARS-CoV-2 glycoprotein is numbered according to the polypeptide of SEQ ID NO: 2, and (c) from

1 to about 2, from 1 to about 3, from 1 to about 4, from 1 to about 5, from 2 to about 5, from 1 to about 6, from 1 to about 7, from 1 to about 8, from 1 to about 9, from 1 to about 10, from 1 to about 11, from 1 to about 12, from 1 to about 13, from 1 to about 14, from 1 to about 15, from 1 to about 16, from 1 to about 17, from 1 to about 18, from 1 to about 18, from 1 to about 19, or from 1 to about 20, from 1 to about 25, from 1 to about 30, from 1 to about 35, from 1 to about 40, from 1 to about 45, or from 1 to about 50 modifications compared to the first CoV S glycoprotein.

63. The immunogenic composition of any one of claims 29-61, comprising:

(i) a first CoV S glycoprotein comprising (a) an inactive furin cleavage site, and (b) proline at amino acid positions 973 and 974, wherein the SARS-CoV-2 glycoprotein is numbered according to the polypeptide of SEQ ID NO: 2;

(ii) a second CoV S glycoprotein comprising (a) an inactive furin cleavage site, and (b) proline at amino acid positions 973 and 974, wherein the SARS-CoV-2 glycoprotein is numbered according to the polypeptide of SEQ ID NO: 2, and (c) from 1 to about 2, from 1 to about 3, from 1 to about 4, from 1 to about 5, from 2 to about 5, from 1 to about 6, from 1 to about 7, from 1 to about 8, from 1 to about 9, from 1 to about 10, from 1 to about 11, from 1 to about 12, from 1 to about 13, from 1 to about 14, from 1 to about 15, from 1 to about 16, from 1 to about 17, from 1 to about 18, from 1 to about 18, from 1 to about 19, or from 1 to about 20, from 1 to about 25, from 1 to about 30, from 1 to about 35, from 1 to about 40, from 1 to about 45, or from 1 to about 50 modifications compared to the first CoV S glycoprotein; and

(iii) a third CoV S glycoprotein comprising (a) an inactive furin cleavage site, and (b) proline at amino acid positions 973 and 974, wherein the SARS-CoV-2 glycoprotein is numbered according to the polypeptide of SEQ ID NO: 2, and (c) from 1 to about 2, from 1 to about 3, from 1 to about 4, from 1 to about 5, from 2 to about 5, from 1 to about 6, from 1 to about 7, from 1 to about 8, from 1 to about 9, from 1 to about 10, from 1 to about 11, from 1 to about 12, from 1 to about 13, from 1 to about 14, from 1 to about 15, from 1 to about 16, from 1 to about 17, from 1 to about 18, from 1 to about 18, from 1 to about 19, or from 1 to about 20, from 1 to about 25, from 1 to about 30, from 1 to about 35, from 1 to about 40, from 1 to about 45, or from 1 to about 50 modifications compared to the first CoV S glycoprotein.

64. The immunogenic composition of any one of claims 29-61, comprising:

(i) a first CoV S glycoprotein comprising (a) an inactive furin cleavage site, and (b) proline at amino acid positions 973 and 974, wherein the SARS-CoV-2 glycoprotein is numbered according to the polypeptide of SEQ ID NO: 2;

(ii) a second CoV S glycoprotein comprising (a) an inactive furin cleavage site, and (b) proline at amino acid positions 973 and 974, wherein the SARS-CoV-2 glycoprotein is numbered according to the polypeptide of SEQ ID NO: 2, and (c) from 1 to about 2, from 1 to about 3, from 1 to about 4, from 1 to about 5, from 2 to about 5, from 1 to about 6, from 1 to about 7, from 1 to about 8, from 1 to about 9, from 1 to about 10, from 1 to about 11, from 1 to about 12, from 1 to about 13, from 1 to about 14, from 1 to about 15, from 1 to about 16, from 1 to about 17, from 1 to about 18, from 1 to about 18, from 1 to about 19, or from 1 to about 20, from 1 to about 25, from 1 to about 30, from 1 to about 35, from 1 to about 40, from 1 to about 45, or from 1 to about 50 modifications compared to the first CoV S glycoprotein;

(iii) a third CoV S glycoprotein comprising (a) an inactive furin cleavage site, and (b) proline at amino acid positions 973 and 974, wherein the SARS-CoV-2 glycoprotein is numbered according to the polypeptide of SEQ ID NO: 2, and (c) from 1 to about 2, from 1 to about 3, from 1 to about 4, from 1 to about 5, from 2 to about 5, from 1 to about 6, from 1 to about 7, from 1 to about 8, from 1 to about 9, from 1 to about 10, from 1 to about 11, from 1 to about 12, from 1 to about 13, from 1 to about 14, from 1 to about 15, from 1 to about 16, from 1 to about 17, from 1 to about 18, from 1 to about 18, from 1 to about 19, or from 1 to about 20, from 1 to about 25, from 1 to about 30, from 1 to about 35, from 1 to about 40, from 1 to about 45, or from 1 to about 50 modifications compared to the first CoV S glycoprotein; and

(iv) a fourth CoV S glycoprotein comprising (a) an inactive furin cleavage site, and (b) proline at amino acid positions 973 and 974, wherein the SARS-CoV-2 glycoprotein is numbered according to the polypeptide of SEQ ID NO: 2, and (c) from 1 to about 2, from 1 to about 3, from 1 to about 4, from 1 to about 5, from 2 to about 5, from 1 to about 6, from 1 to about 7, from 1 to about 8, from 1 to about 9, from 1 to about 10, from 1 to about 11, from 1 to about 12, from 1 to about 13, from 1 to about 14, from 1 to about 15, from 1 to about 16, from 1 to about 17, from 1 to about 18, from 1 to about 18, from 1 to about 19, or from 1 to about 20, from 1 to about 25, from 1 to about 30, from 1 to about 35, from 1 to about 40, from 1 to about 45, or from 1 to about 50 modifications compared to the first CoV S glycoprotein.

65. The immunogenic composition of any one of claims 29-61, comprising:

(i) a first CoV S glycoprotein comprising (a) an inactive furin cleavage site, and (b) proline at amino acid positions 973 and 974, wherein the SARS-CoV-2 glycoprotein is numbered according to the polypeptide of SEQ ID NO: 2;

(ii) a second CoV S glycoprotein comprising (a) an inactive furin cleavage site, and (b) proline at amino acid positions 973 and 974, wherein the SARS-CoV-2 glycoprotein is numbered according to the polypeptide of SEQ ID NO: 2, and (c) from 1 to about 2, from 1 to about 3, from 1 to about 4, from 1 to about 5, from 2 to about 5, from 1 to about 6, from 1 to about 7, from 1 to about 8, from 1 to about 9, from 1 to about 10, from 1 to about 11, from 1 to about 12, from 1 to about 13, from 1 to about 14, from 1 to about 15, from 1 to about 16, from 1 to about 17, from 1 to about 18, from 1 to about 18, from 1 to about 19, or from 1 to about 20, from 1 to about 25, from 1 to about 30, from 1 to about 35, from 1 to about 40, from 1 to about 45, or from 1 to about 50 modifications compared to the first CoV S glycoprotein;

(iii) a third CoV S glycoprotein comprising (a) an inactive furin cleavage site, and (b) proline at amino acid positions 973 and 974, wherein the SARS-CoV-2 glycoprotein is numbered according to the polypeptide of SEQ ID NO: 2, and (c) from 1 to about 2, from 1 to about 3, from 1 to about 4, from 1 to about 5, from 2 to about 5, from 1 to about 6, from 1 to about 7, from 1 to about 8, from 1 to about 9, from 1 to about 10, from 1 to about 11, from 1 to about 12, from 1 to about 13, from 1 to about 14, from 1 to about 15, from 1 to about 16, from 1 to about 17, from 1 to about 18, from 1 to about 18, from 1 to about 19, or from 1 to about 20, from 1 to about 25, from 1 to about 30, from 1 to about 35, from 1 to about 40, from 1 to about 45, or from 1 to about 50 modifications compared to the first CoV S glycoprotein;

(iv) a fourth CoV S glycoprotein comprising (a) an inactive furin cleavage site, and (b) proline at amino acid positions 973 and 974, wherein the SARS-CoV-2 glycoprotein is numbered according to the polypeptide of SEQ ID NO: 2, and (c) from 1 to about 2, from 1 to about 3, from 1 to about 4, from 1 to about 5, from 2 to about 5, from 1 to about 6, from 1 to about 7, from 1 to about 8, from 1 to about 9, from 1 to about 10, from 1 to about 11, from 1 to about 12, from 1 to about 13, from 1 to about 14, from 1 to about 15, from 1 to about 16, from 1 to about 17, from 1 to about 18, from 1 to about 18, from 1 to about 19, or from 1 to about 20, from 1 to about 25, from 1 to about 30, from 1 to about 35, from 1 to about 40, from 1 to about 45, or from 1 to about 50 modifications compared to the first CoV S glycoprotein; and

(v) a fifth CoV S glycoprotein comprising (a) an inactive furin cleavage site, and (b) proline at amino acid positions 973 and 974, wherein the SARS-CoV-2 glycoprotein is numbered according to the polypeptide of SEQ ID NO: 2, and (c) from 1 to about 2, from 1 to about 3, from 1 to about 4, from 1 to about 5, from 2 to about 5, from 1 to about 6, from 1 to about 7, from 1 to about 8, from 1 to about 9, from 1 to about 10, from 1 to about 11, from 1 to about 12, from 1 to about 13, from 1 to about 14, from 1 to about 15, from 1 to about 16, from 1 to about 17, from 1 to about 18, from 1 to about 18, from 1 to about 19, or from 1 to about 20, from 1 to about 25, from 1 to about 30, from 1 to about 35, from 1 to about 40, from 1 to about 45, or from 1 to about 50 modifications compared to the first CoV S glycoprotein.

66. The immunogenic composition of any one of claims 29-65, wherein the first CoV S glycoprotein has at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 %, at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or at least 100 % identity to a polypeptide of any one of SEQ ID NOS: 87, 89, 106, 112, 113, 114, 115, 132, 144, 151, 153, 156, 158, 174, 175, 176, 181, 182, 183, 184, 186, 188, 190, 192, 195, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 233, 234, 235, 236, 260, 261, 262, 263, 264, 274, 276, 278, 280, 284, 288, 292, 296, 300, 304, 308, 312, 316, 320, 324, and 328.

67. The immunogenic composition of claim 65 or 66, comprising about 5 µg of the first CoV S glycoprotein and from 1 ng to about 4.9 µg of each of the second, third, fourth, and fifth CoV S glycoproteins.

68. The immunogenic composition of any one of claims 65-67, wherein one or more of the modifications in the second, third, fourth, and fifth CoV S glycoprotein occur in the receptor binding domain (RBD).

69. The immunogenic composition of any one of claims 65-67, wherein one or more of the modifications in the second, third, fourth, and fifth CoV S glycoprotein occur in the N-terminal domain (NTD).

70. The immunogenic composition of any one of claims 65-67, wherein one or more of the modifications in the second, third, fourth, and fifth CoV S glycoprotein occur in the S1 subunit.
71. The immunogenic composition of any one of claims 65-67, wherein one or more of the modifications in the second, third, fourth, and fifth CoV S glycoprotein occur in the S2 subunit.
72. The immunogenic composition of any one of claims 55-71, comprising from about 1 µg to about 175 µg or from about 1 µg to about 10 µg of SARS-CoV-2 S glycoproteins.
73. The immunogenic composition of any one of claims 55-71, comprising about 5 µg of the SARS-CoV-2 S glycoprotein.
74. The immunogenic composition of any one of claims 55-71, comprising about 25 µg of the SARS-CoV-2 S glycoprotein.
75. The immunogenic composition of any one of claims 29-74, comprising an adjuvant.
76. The immunogenic composition of claim 75, wherein the adjuvant comprises at least two iscom particles, wherein:
the first iscom particle comprises fraction A of *Quillaja Saponaria* Molina and not fraction C of *Quillaja Saponaria* Molina; and
the second iscom particle comprises fraction C of *Quillaja Saponaria* Molina and not fraction A of *Quillaja Saponaria* Molina.
77. The immunogenic composition of claim 76, wherein:
fraction A of *Quillaja Saponaria* Molina and fraction C of *Quillaja Saponaria* Molina account for about 85 % by weight and about 15 % by weight, respectively, of the sum of weights of fraction A of *Quillaja Saponaria* Molina and fraction C of *Quillaja Saponaria* Molina in the adjuvant.

78. The immunogenic composition of claim 76, wherein fraction A of *Quillaja Saponaria* Molina and fraction C of *Quillaja Saponaria* Molina account for about 92 % by weight and about 8 % by weight, respectively, of the sum of the weights of fraction A of *Quillaja Saponaria* Molina and fraction C of *Quillaja Saponaria* Molina in the adjuvant.
79. The immunogenic composition of claim 76, wherein fraction A of *Quillaja Saponaria* Molina accounts for at least about 85 % by weight, and fraction C of *Quillaja Saponaria* Molina accounts for the remainder, respectively, of the sum of the weights of fraction A of *Quillaja Saponaria* Molina and fraction C of *Quillaja Saponaria* Molina in the adjuvant.
80. The immunogenic composition of claim 76, wherein fraction A of *Quillaja Saponaria* Molina accounts for 50-96% by weight and fraction C of *Quillaja Saponaria* Molina accounts for the remainder, respectively, of the sum of the weights of fraction A of *Quillaja Saponaria* Molina and fraction C of *Quillaja Saponaria* Molina in the adjuvant.
81. The immunogenic composition of any one of claims 75-80, comprising from about 25 µg to about 100 µg, from about 1 µg to about 100 µg, or from about 50 µg to about 100 µg of adjuvant.
82. The immunogenic composition of any one of claims 75-81, comprising about 20 µg or 50 µg of adjuvant.
83. The immunogenic composition of any one of claims 75-81, wherein the adjuvant does not contain 3-O-desacyl-4'-monophosphoryl lipid A from *Salmonella Minnesota*.
84. The immunogenic composition of any one of claims 29-83, wherein the immunogenic composition is an intramuscular immunogenic composition.

85. The immunogenic composition of any one of claims 29-83, wherein the immunogenic composition is an intranasal immunogenic composition.
86. A pre-filled syringe comprising the immunogenic composition of any one of claims 29-85.
87. A method of stimulating an immune response against one or more of respiratory syncytial virus (RSV), influenza, and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in a subject comprising administering the immunogenic composition of any one of claims 29-85 to a subject.
88. The method of claim 87, comprising administering 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 doses of the immunogenic composition.
89. The method of claim 87 or 88, comprising administering a first dose and a second dose of the immunogenic composition, wherein the second dose is administered twenty one days after the first dose.
90. The method of any one of claims 87-89, comprising administering 0.5 mL of the immunogenic composition.
91. The method of any one of claims 87-90, wherein the immunogenic composition is administered intramuscularly.
92. The method of any one of claims 87-90, wherein the immunogenic composition is administered intranasally.
93. The method of any one of claims 87-92, comprising administering the immunogenic composition in a pre-filled syringe.
94. The method of any one of claims 87-93, wherein the method prevents one or more of RSV, influenza, and SARS-CoV-2 with an efficacy from about 50 % to

about 99 %, from about 50 % to about 95 %, from about 50 % to about 90 %, from about 50 % to about 85 %, from about 50 % to about 80 %, from about 60 % to about 99 %, from about 65 % to about 95 %, from about 65 % to about 90 %, from about 65 % to about 85 %, from about 69 % to about 81 %, from about 60 % to about 95 %, from about 60 % to about 90 %, from about 60 % to about 85 %, from about 60 % to about 80 %, from about 40 % to about 99 %, from about 40 % to about 95 %, from about 40 % to about 90 %, from about 40 % to about 85 %, from about 40 % to about 80 %, from about 40 % to about 75 %, from about 40 % to about 70 %, from about 40 % to about 65 %, from about 40 % to about 55 %, or from about 40 % to about 50 % for at least about 2 months, at least about 2.5 months, at least about 3 months, at least about 3.5 months, at least about 4 months, at least about 4.5 months, at least about 5 months, at least about 5.5 months, at least about 6 months, at least about 6.5 months, at least about 7 months, at least about 7.5 months, at least about 8 months, at least about 8.5 months, at least about 9 months, at least about 9.5 months, at least about 10 months, at least about 10.5 months, at least about 11 months, at least about 11.5 months, at least about 12 months, at least 13 months, at least 14 months, at least 15 months, at least 16 months, at least 17 months, at least 18 months, at least 19 months, at least 20 months, at least 21 months, at least 22 months, at least 23 months, or at least 24 months after administration of the immunogenic composition.

95. The method of any one of claims 87-93, wherein the method prevents one or more of RSV, influenza, and SARS-CoV-2 with an efficacy from about 50 % to about 99 %, from about 50 % to about 95 %, from about 50 % to about 90 %, from about 50 % to about 85 %, from about 50 % to about 80 %, from about 60 % to about 99 %, from about 65 % to about 95 %, from about 65 % to about 90 %, from about 65 % to about 85 %, from about 69 % to about 81 %, from about 60 % to about 95 %, from about 60 % to about 90 %, from about 60 % to about 85 %, from about 60 % to about 80 %, from about 40 % to about 99 %, from about 40 % to about 95 %, from about 40 % to about 90 %, from about 40 % to about 85 %, from about 40 % to about 80 %, from about 40 % to about 75 %, from about 40 % to about 70 %, from about 40 %

to about 65 %, from about 40 % to about 55 %, or from about 40 % to about 50 % for up to about 2 months, up to about 2.5 months, up to about 3 months, up to about 3.5 months, up to about 4 months, up to about 4.5 months, up to about 5 months, up to about 5.5 months, up to about 6 months, up to about 6.5 months, up to about 7 months, up to about 7.5 months, up to about 8 months, up to about 8.5 months, up to about 9 months, up to about 9.5 months, up to about 10 months, up to about 10.5 months, up to about 11 months, up to about 11.5 months, up to about 12 months, up to 13 months, up to 14 months, up to 15 months, up to 16 months, up to 17 months, up to 18 months, up to 19 months, up to 20 months, up to 21 months, up to 22 months, up to 23 months, or up to 24 months after administration of the immunogenic composition.

96. The method of any one of claims 87-95, wherein the subject is a human.
97. The method of any one of claims 87-96, wherein the subject is a pregnant female.
98. The method of any one of claims 87-97, wherein the subject is a child.
99. The method of claim 97, comprising administering the immunogenic composition to a pregnant female that has a gestational age from about 32 weeks to about 36 weeks.
100. The method of claim 97, comprising administering the immunogenic composition to a pregnant female that has a gestational age from about 28 weeks to about 33 weeks.
101. The method of any one of claims 87-100, comprising administering the immunogenic composition to a human subject that is at least sixty years old.
102. The method of claim 98, wherein the child is five years old or younger.