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(57) **Abrégé/Abstract:**
This disclosure relates to the field of RNA to prevent or treat coronavirus infection. In particular, the present disclosure relates to methods and agents for vaccination against coronavirus infection and inducing effective coronavirus antigen?specific immune responses such as antibody and/or T cell responses. Specifically, in one embodiment, the present disclosure relates to methods comprising administering to a subject RNA encoding a peptide or protein comprising an epitope of SARS-CoV-2 spike protein (S protein) for inducing an immune response against coronavirus S protein, in particular S protein of SARS-CoV-2, in the subject, i.e., vaccine RNA encoding vaccine antigen.

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Abstract:

This disclosure relates to the field of RNA to prevent or treat coronavirus infection. In particular, the present disclosure relates to methods and agents for vaccination against coronavirus infection and inducing effective coronavirus antigenspecific immune responses such as antibody and/or T cell responses. Specifically, in one embodiment, the present disclosure relates to methods comprising administering to a subject RNA encoding a peptide or protein comprising an epitope of SARSCoV2 spike protein (S protein) for inducing an immune response against coronavirus S protein, in particular S protein of SARSCoV2, in the subject, i.e., vaccine RNA encoding vaccine antigen.

DEMANDE OU BREVET VOLUMINEUX

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CORONAVIRUS VACCINE

This disclosure relates to the field of RNA to prevent or treat coronavirus infection. In particular, the present disclosure relates to methods and agents for vaccination against coronavirus infection and inducing effective coronavirus antigen-specific immune responses such as antibody and/or T cell responses. These methods and agents are, in particular, useful for the prevention or treatment of coronavirus infection. Administration of RNA disclosed herein to a subject can protect the subject against coronavirus infection. Specifically, in one embodiment, the present disclosure relates to methods comprising administering to a subject RNA encoding a peptide or protein comprising an epitope of SARS-CoV-2 spike protein (S protein) for inducing an immune response against coronavirus S protein, in particular S protein of SARS-CoV-2, in the subject, i.e., vaccine RNA encoding vaccine antigen. Administering to the subject RNA encoding vaccine antigen may provide (following expression of the RNA by appropriate target cells) vaccine antigen for inducing an immune response against vaccine antigen (and disease-associated antigen) in the subject.

Coronaviruses are positive-sense, single-stranded RNA ((+)ssRNA) enveloped viruses that encode for a total of four structural proteins, spike protein (S), envelope protein (E), membrane protein (M) and nucleocapsid protein (N). The spike protein (S protein) is responsible for receptor-recognition, attachment to the cell, infection via the endosomal pathway, and the genomic release driven by fusion of viral and endosomal membranes. Though sequences between the different family members vary, there are conserved regions and motifs within the S protein making it possible to divide the S protein into two subdomains: S1 and S2. While the S2, with its transmembrane domain, is responsible for membrane fusion, the S1 domain recognizes the virus-specific receptor and binds to the target host cell. Within several coronavirus isolates, the receptor binding domain (RBD) was identified and a general structure of the S protein defined (Figure 1).

In December 2019, a pneumonia outbreak of unknown cause occurred in Wuhan, China and it became clear that a novel coronavirus (severe acute respiratory syndrome coronavirus 2; SARS-CoV-2) was the underlying cause. The genetic sequence of SARS-CoV-2 became available to the WHO and public (MN908947.3) and the virus was categorized into the betacoronavirus subfamily. By sequence analysis, the phylogenetic tree revealed a closer relationship to severe

acute respiratory syndrome (SARS) virus isolates than to another coronavirus infecting humans, namely the Middle East respiratory syndrome (MERS) virus.

SARS-CoV-2 infections and the resulting disease COVID-19 have spread globally, affecting a growing number of countries. On 11 March 2020 the WHO characterized the COVID-19 outbreak as a pandemic. As of 01 December 2020, there have been >63 million globally confirmed COVID-19 cases and >1.4 million deaths, with 191 countries/regions affected. The ongoing pandemic remains a significant challenge to public health and economic stability worldwide.

Every individual is at risk of infection as there is no pre-existing immunity to SARS-CoV-2. Following infection some but not all individuals develop protective immunity in terms of neutralising antibody responses and cell mediated immunity. However, it is currently unknown to what extent and for how long this protection lasts. According to WHO 80% of infected individuals recover without need for hospital care, while 15% develop more severe disease and 5% need intensive care. Increasing age and underlying medical conditions are considered risk factors for developing severe disease.

The presentation of COVID-19 is generally with cough and fever, with chest radiography showing ground-glass opacities or patchy shadowing. However, many patients present without fever or radiographic changes, and infections may be asymptomatic which is relevant to controlling transmission. For symptomatic subjects, progression of disease may lead to acute respiratory distress syndrome requiring ventilation and subsequent multi-organ failure and death. Common symptoms in hospitalized patients (in order of highest to lowest frequency) include fever, dry cough, shortness of breath, fatigue, myalgias, nausea/vomiting or diarrhoea, headache, weakness, and rhinorrhoea. Anosmia (loss of smell) or ageusia (loss of taste) may be the sole presenting symptom in approximately 3% of individuals who have COVID-19.

All ages may present with the disease, but notably case fatality rates (CFR) are elevated in persons >60 years of age. Comorbidities are also associated with increased CFR, including cardiovascular disease, diabetes, hypertension, and chronic respiratory disease. Healthcare workers are overrepresented among COVID-19 patients due to occupational exposure to infected patients.

In most situations, a molecular test is used to detect SARS-CoV-2 and confirm infection. The reverse transcription polymerase chain reaction (RT-PCR) test methods targeting SARS-CoV-2

viral RNA are the gold standard in vitro methods for diagnosing suspected cases of COVID-19. Samples to be tested are collected from the nose and/or throat with a swab.

Among other things, the present disclosure provides insights into immune responses elicited by exposure to (e.g., by vaccination and/or infection) different SARS-CoV-2 variants or immunogenic polypeptides (e.g., S protein), or immunogenic fragments thereof. For example, in some embodiments, administering RNA encoding an S protein of a BA.2 and/or BA.4/5 Omicron SARS-CoV-2 variant, or an immunogenic fragment thereof, can result in an improved immune response, which includes, e.g., improved neutralization of Omicron BA.4 and/or Omicron BA.5 SARS-CoV-2 variants and/or broader cross-neutralization of Omicron variants of concern (e.g., higher neutralization titers against a larger number of Omicron variants of concern). In some embodiments, the present disclosure provides an insight that a bivalent coronavirus vaccine (e.g., a bivalent BA.4/5 vaccine comprising a first RNA encoding a SARS-CoV-2 S protein of a Wuhan strain or an immunogenic fragment thereof, and a second RNA encoding a SARS-CoV-2 S protein comprising one or more mutations that are characteristic of a BA.4/5 Omicron variant or an immunogenic fragment thereof) can provide broader cross-neutralization against SARS-CoV-2 Wuhan strain and certain variants thereof (e.g., in some embodiments variants that are prevalent and/or rapidly spreading in a relevant jurisdiction, e.g., certain Omicron variants) in certain subjects as compared to a monovalent coronavirus vaccine (e.g., a vaccine comprising RNA encoding a SARS-CoV-2 S protein of a coronavirus strain or variant thereof). In some embodiments, such broader cross-neutralization can be observed in vaccine-naïve subjects. In some embodiments, such broader cross-neutralization can be observed in subjects without a coronavirus infection (e.g., a SARS-CoV-2 infection). In some embodiments, such broader cross-neutralization can be observed in subjects who previously received a SARS-CoV-2 vaccine (e.g., in some embodiments an RNA vaccine encoding a SARS-CoV-2 S protein, e.g., in some embodiments of a Wuhan strain). In some embodiments, such broader cross-neutralization can be observed in in young pediatric subjects (e.g., subjects aged 6 months to less than 2 years, and/or 2 years to less than 5 years). In some embodiments, the present disclosure provides an insight that exposure to at least two certain SARS-CoV-2 variants or immunogenic polypeptides (e.g., S protein), or immunogenic fragments thereof can result in a synergistic improvement in immune response (e.g., higher neutralization titers, broader cross-neutralization, and/or an immune response that is less susceptible to immune escape) as compared to exposure to one SARS-CoV-2 strain

and/or other combination of SARS-CoV-2 variants. In some embodiments, the present disclosure provides an insight that exposure to a SARS-CoV-2 S protein from a Wuhan strain or an immunogenic fragment thereof (e.g., by vaccination and/or infection), and exposure to a SARS-CoV-2 S protein of an Omicron BA.1 variant or an immunogenic fragment thereof (e.g., by vaccination and/or infection) can result in a synergistic improvement in immune response (e.g., higher neutralization titers, broader cross-neutralization, and/or an immune response that is less susceptible to immune escape) as compared to exposure to one SARS-CoV-2 strain and/or other combinations of SARS-CoV-2 variants. In some embodiments, the present disclosure provides an insight that exposure to a SARS-CoV-2 S protein from a Wuhan strain or an immunogenic fragment thereof (e.g., by vaccination and/or infection), and exposure to a SARS-CoV-2 S protein of an Omicron BA.4 or BA.5 variant or an immunogenic fragment thereof (e.g., by vaccination and/or infection) can result in an synergistic improvement in immune response (e.g., higher neutralization titers, broader cross-neutralization, and/or an immune response that is less susceptible to immune escape) as compared to exposure to one SARS-CoV-2 strain and/or other combinations of SARS-CoV-2 variants. In some embodiments, the present disclosure provides an insight that (i) exposure to a SARS-CoV-2 S protein from a strain/variant selected from the group consisting of Wuhan strain, an alpha variant, beta variant, delta variant, Omicron BA.1, and sublineages derived from any of the aforementioned strains/variants, or immunogenic fragments thereof (e.g., by vaccination and/or infection), combined with (ii) exposure to a SARS-CoV-2 S protein from a strain/variant selected from the group consisting of Omicron BA.2, Omicron BA.4, Omicron BA.5, and sublineages derived from any of the aforementioned strains/variants, or immunogenic fragments thereof (e.g., by vaccination and/or infection) can result in a synergistic improvement in immune response (e.g., higher neutralization titers, broader cross-neutralization, and/or an immune response that is less susceptible to immune escape) as compared to exposure to one SARS-CoV-2 strain and/or other combinations of SARS-CoV-2 variants).

The present disclosure also provides significant insights into how an immune response develops in subjects following exposures to (e.g., vaccinations and/or infections) multiple, different SARS-CoV-2 strains. Among other things, disclosed herein is a finding that different combinations of SARS-CoV-2 variants elicit different immune responses. Specifically, the present disclosure provides an insight that exposure to certain combinations of SARS-CoV-2 variants can elicit an improved immune response (e.g., higher neutralization titers, broader

cross-neutralization, and/or an immune response that is less susceptible to immune escape). In some embodiments, an improved immune response can be produced when subjects are delivered two or more antigens (e.g., as polypeptides or RNAs encoding such polypeptides), each having few shared epitopes. In some embodiments, an improved immune response can be produced when subjects are delivered a combination of SARS-CoV-2 S proteins (e.g., as polypeptides or RNAs encoding such polypeptides) sharing no more than 50% (e.g., no more than 40%, no more than 30%, no more 20% or more) of epitopes (including, e.g., amino acid mutations) that can be bound by neutralization antibodies. In some embodiments, an improved immune response can be produced by delivering, as polypeptides or RNAs encoding such polypeptides, (a) a SARS-CoV-2 S protein from a Wuhan strain, an Alpha variant, Beta variant, or a Delta variant of SARS-CoV-2 or an immunogenic fragment thereof, and (b) an S protein from a SARS-CoV-2 Omicron variant or an immunogenic fragment thereof. In some embodiments, an improved immune response can be produced by delivering, as polypeptides or RNAs encoding such polypeptides, (a) a SARS-CoV-2 S protein from a Wuhan strain, an Alpha variant, a Beta variant, or a Delta variant of SARS-CoV-2 or an immunogenic fragment thereof, and (b) an S protein of a SARS-CoV-2 Omicron variant that is not a BA.1 Omicron variant or an immunogenic fragment thereof. In some embodiments, an improved immune response can be produced by delivering, as polypeptides or RNAs encoding such polypeptides, (a) an S protein from a Wuhan strain, an Alpha variant, a Beta Variant, a Delta SARS-CoV-2 variant, or a BA.1 Omicron variant or an immunogenic fragment thereof and (b) an S protein of a SARS-CoV-2 Omicron variant that is not a BA.1 Omicron variant or an immunogenic fragment thereof. In some embodiments, an improved immune response can be produced by delivering, as polypeptides or RNAs encoding such polypeptides, (a) a SARS-CoV-2 S protein from a Wuhan strain, an Alpha variant, a Beta variant, or a Delta variant, or an immunogenic fragment thereof and (b) an S protein of a BA.2 or a BA.4 or BA.5 SARS-CoV-2 Omicron variant or an immunogenic fragment thereof.

In some embodiments, the present disclosure also provides an insight that administration of multiple doses (e.g., at least 2, at least 3, at least 4, or more doses) of a coronavirus vaccine described herein (e.g., a bivalent vaccine described herein such as a bivalent BA.4/5 vaccine) may provide certain beneficial effect(s) on affinity of antibodies against one or more SARS-CoV-2 strain or variants thereof. In some embodiments, such beneficial effect(s) on affinity of antibodies may be observed with respect to antibodies against certain Omicron variants. By

way of example only, in some embodiments, such beneficial effect(s) on affinity of antibodies may be observed with respect to antibodies against certain Omicron variants that share at least one or more common epitopes, for example, with a Wuhan strain.

Also disclosed herein are compositions that can produce an improved immune response (e.g., an immune response having broader cross-neutralization activity, stronger neutralization, and/or which is less susceptible to immune escape). In some embodiments, a composition described herein comprises two or more antigens or nucleic acids (e.g., RNA) that encodes such antigens that have few shared epitopes. In some embodiments, a composition described herein delivers, as polypeptides or nucleic acids encoding such polypeptides, a combination of SARS-CoV-2 S proteins or immunogenic fragments thereof sharing no more than 50% (e.g., no more than 40%, no more than 30%, no more than 20% or more) of epitopes (including, e.g., amino acid mutations) that can be bound by neutralization antibodies. In some embodiments, a composition described herein comprises (a) RNA encoding a SARS-CoV-2 S protein from a Wuhan strain, an Alpha variant, a Beta variant, or a Delta variant or an immunogenic fragment thereof and (b) RNA encoding an S protein from an Omicron variant of SARS-CoV-2 (e.g., in some embodiments an S protein from a BA.1, BA.2, or BA.4/5 Omicron variant) or an immunogenic fragment thereof. In some embodiments, a composition described herein comprises (a) RNA encoding a SARS-CoV-2 S protein from a Wuhan strain, an Alpha variant, a Beta variant, or a Delta variant or an immunogenic fragment thereof and (b) RNA encoding an S protein of an Omicron variant of SARS-CoV-2 that is not a BA.1 Omicron variant or an immunogenic fragment thereof. In some embodiments, a composition described herein comprises (a) RNA encoding a SARS-CoV-2 S protein of a Wuhan strain, an Alpha variant, a Beta variant, or a Delta variant or a BA.1 Omicron variant or an immunogenic fragment thereof and (b) RNA encoding an S protein of a Omicron variant that is not a BA.1 Omicron variant or an immunogenic fragment thereof. In some embodiments, a composition described herein comprises (a) RNA encoding a SARS-CoV-2 S protein of a Wuhan strain, an Alpha variant, a Beta variant or a Delta variant of SARS-CoV-2 and (b) RNA encoding an S protein from a BA.2 or a BA.4 or BA.5 Omicron variant of SARS-CoV-2 or an immunogenic fragment thereof. In some embodiments, a composition described herein comprises RNA encoding an S protein from a BA.2 Omicron variant of SARS-CoV-2 or an immunogenic fragment thereof. In some embodiments, a composition comprises RNA encoding an S protein from a BA.4 or BA.5 Omicron variant of SARS-CoV-2 or an immunogenic fragment thereof.

SARS-CoV-2 is an RNA virus with four structural proteins. One of them, the spike protein is a surface protein which binds the angiotensin-converting enzyme 2 (ACE-2) present on host cells. Therefore, the spike protein is considered a relevant antigen for vaccine development.

BNT162b2 (SEQ ID NO: 20) is an mRNA vaccine for prevention of COVID-19 and demonstrated an efficacy of 95% or more at preventing COVID-19. The vaccine is made of a 5'capped mRNA encoding for the full-length SARS-CoV-2 spike glycoprotein (S) encapsulated in lipid nanoparticles (LNPs). The finished product is presented as a concentrate for dispersion for injection containing BNT162b2 as active substance. Other ingredients are: ALC-0315 (4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate), ALC-0159 (2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide), 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol, potassium chloride, potassium dihydrogen phosphate, sodium chloride, disodium phosphate dihydrate, sucrose and water for injection.

In some embodiments, a different buffer may be used in lieu of PBS. In some embodiments, the buffer is formulated in a Tris-buffered solution. In some embodiments, the formulation comprises ALC-0315 (4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate), ALC-0159 (2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide), DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine), cholesterol, sucrose, trometamol (Tris), trometamol hydrochloride and water.

In some embodiments, the concentration of RNA in a pharmaceutical RNA preparation is about 0.1 mg/ml. In some embodiments about 30 ug of RNA is administered by administering about 200 uL of RNA preparation. In some embodiments, RNA in a pharmaceutical RNA preparation is diluted prior to administration (e.g., diluted to a concentration of about 0.05 mg/ml). In some embodiments, administration volumes are between about 200 µl and about 300 µl. In some embodiments, RNA in a pharmaceutical RNA preparation is formulated in about 10 mM Tris buffer, and about 10% sucrose.

In some embodiments, the concentration of RNA in a pharmaceutical RNA preparation is about 0.1 mg/ml, and is formulated in about 10 mM Tris buffer, about 10% sucrose and a dose of about 10 µg or RNA is administered by diluting a pharmaceutical RNA preparation about 1:1 and administering about 200 µl of diluted pharmaceutical RNA preparation. In some embodiments, the concentration of RNA in a pharmaceutical RNA preparation is about 0.1 mg/ml, and is formulated in about 10 mM Tris buffer, about 10% sucrose and a dose of RNA

of about 10 µg is administered by diluting a pharmaceutical RNA preparation about 1:5.75 and administering about 200 µl of diluted pharmaceutical RNA preparation.

The amino acid sequence of the S protein encoded by BNT162b2 was chosen based on the sequence for the "SARS-CoV-2 isolate Wuhan-Hu -1": GenBank: MN908947.3 (complete genome) and GenBank: QHD43416.1 (spike surface glycoprotein). The BNT162b2 active substance consists of a single-stranded, 5'-capped codon-optimized mRNA that is translated into the spike antigen of SARS-CoV-2. The spike antigen protein sequence encoded by BNT162b2 contains two proline mutations, which stabilizes an antigenically improved pre-fusion confirmation (P2 S). BNT162b2 does not contain any uridines; instead of uridine the modified N1-methylpseudouridine is used in RNA synthesis. BNT162b2 mRNA is translated into a SARS-CoV-2 S protein in host cells. The S protein is then expressed on the cell surface where it induces an adaptive immune response. The S protein encoded by BNT162b2 is identified as a target for neutralising antibodies against the virus and is considered a relevant vaccine component. For adult vaccine naïve subjects (i.e., subjects 16 years and older who have not previously been administered a SARS-CoV-2 vaccine), the dosing regimen of BNT162b2 approved by the FDA comprises administering intramuscularly (IM) two 30 µg doses of the diluted vaccine solution approximately 21 days apart.

The recent emergence of novel circulating variants of SARS-CoV-2 has raised significant concerns about geographic and temporal efficacy of vaccine interventions. One of the earliest variants that emerged and rapidly became globally dominant was D614G.

The alpha variant (also known as B.1.1.7, VOC202012/01, 501Y.V1 or GRY) was initially detected in the United Kingdom. The alpha variant has a large number of mutations, including several mutations in the S gene. It has been shown to be inherently more transmissible, with a growth rate that has been estimated to be 40-70% higher than other SARS-CoV-2 lineages in multiple countries (Volz et al., 2021, Nature, https://doi.org/10.1038/s41586-021-03470-x; Washington et al., 2021, Cell https://doi.org/10.1016/j.cell.2021.03.052).

The beta variant (also known as B.1.351 or GH/501Y.V2) was first detected in South Africa. The beta variant carries several mutations in the S gene. Three of these mutations are at sites in the RBD that are associated with immune evasion: N501Y (shared with alpha) and E484K and K417N.

The gamma variant (also known as P.1 or GR/501Y.V3) was first detected in Brazil. The gamma variant carries several mutations that affect the spike protein, including two shared with beta (N501Y and E484K), as well as a different mutation at position 417 (K417T).

The delta variant (also known as B.1.617.2 or G/478K.V1) was first documented in India. The delta variant has several point mutations that affect the spike protein, including P681R (a mutation position shared with alpha and adjacent to the furin cleavage site), and L452R, which is in the RBD and has been linked with increased binding to ACE2 and neutralizing antibody resistance. There is also a deletion in the spike protein at position 156/157.

These four VOCs have circulated globally and became dominant variants in the geographic regions where they were first identified.

On 24 November 2021, the Omicron (B.1.1.529) variant was first reported to WHO from South Africa. SARS-CoV-2 Omicron and its sublineages have had a major impact on the epidemiological landscape of the COVID-19 pandemic since initial emergence in November 2021 (WHO Technical Advisory Group on SARS-CoV-2 Virus Evolution (TAG-VE): Classification of Omicron (B.1.1.259): SARS-CoV-2 Variant of Concern (2021); WHO Headquarters (HQ), WHO Health Emergencies Programme, Enhancing Response to Omicron SARS-CoV-2 variant: Technical brief and priority actions for Member States (2022)). Significant alterations in the spike (S) glycoprotein of the first Omicron variant BA.1 leading to the loss of many neutralizing antibody epitopes (M. Hoffmann et al., "The Omicron variant is highly resistant against antibody mediated neutralization: Implications for control of the COVID-19 pandemic", Cell 185, 447–456.e11 (2022)) rendered BA.1 capable of partially escaping previously established SARS-CoV-2 wild-type strain (Wuhan-Hu-1)-based immunity (V. Servellita, et al., "Neutralizing immunity in vaccine breakthrough infections from the SARS-CoV-2 Omicron and Delta variants", Cell 185, 1539–1548.e5 (2022); Y. Cao et al., "Omicron escapes the majority of existing SARS-CoV-2 neutralizing antibodies", Nature 602, 657–663 (2022)). Hence, breakthrough infection of vaccinated individuals with Omicron are more common than with previous Variants of Concern (VOCs). While Omicron BA.1 was displaced by the BA.2 variant in many countries around the globe, other variants such as BA.1.1 and BA.3 temporarily and/or locally gained momentum but did not become globally dominant (S. Xia et al., "Origin, virological features, immune evasion and intervention of SARS-CoV-2 Omicron sublineages. Signal Transduct. Target. Ther. 7, 241 (2022); H. Gruell et al., "SARS-CoV-2 Omicron sublineages exhibit distinct antibody escape patterns, Cell Host Microbe 7, 241 (2022).).

Omicron BA.2.12.1 subsequently displaced BA.2 to become dominant in the United States, whereas BA.4 and BA.5 displaced BA.2 in Europe, parts of Africa, and Asia/Pacific (H. Gruell et al., "SARS-CoV-2 Omicron sublineages exhibit distinct antibody escape patterns," Cell Host Microbe 7, 241 (2022); European Centre for Disease Prevention and Control, Weekly COVID-19 country overview -Country overview report: Week 31 2022 (2022); J. Hadfield et al., "Nextstrain: Real-time tracking of pathogen evolution," Bioinformatics 34, 4121–4123 (2018)). Currently, Omicron BA.5 is dominant globally, including in the United States (Centers for Disease Control and Prevention. COVID Data Tracker. Atlanta, GA: US Department of Health and Human Services, CDC; 2022, August 12. https://covid.cdc.gov/coviddata-tracker (2022)). Omicron has acquired numerous alterations (amino acid exchanges, insertions, or deletions) in the S glycoprotein, among which some are shared between all Omicron VOCs while others are specific to one or more Omicron sublineages. Antigenically, BA.2.12.1 exhibits high similarity with BA.2 but not BA.1, whereas BA.4 and BA.5 differ considerably from their ancestor BA.2 and even more so from BA.1, in line with their genealogy (A. Z. Mykytyn et al., "Antigenic cartography of SARS-CoV-2 reveals that Omicron BA.1 and BA.2 are antigenically distinct," Sci. Immunol. 7, eabq4450 (2022).). Major differences of BA.1 from the remaining Omicron VOCs include Δ143–145, L212I, or ins214EPE in the S glycoprotein N-terminal domain and G446S or G496S in the receptor binding domain (RBD). Amino acid changes T376A, D405N, and R408S in the RBD are in turn common to BA.2 and its descendants but not found in BA.1. In addition, some alterations are specific for individual BA.2-descendant VOCs, including L452Q for BA.2.12.1 or L452R and F486V for BA.4 and BA.5 (BA.4 and BA.5 encode for the same S sequence). Most of these shared and VOC-specific alterations were shown to play an important role in immune escape from monoclonal antibodies and polyclonal sera raised against the wild-type S glycoprotein. In particular, the BA.4/BA.5-specific alterations are strongly implicated in immune escape of these VOCs (P. Wang et al., "Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7. Nature 593, 130–135 (2021); Q. Wang et al., "Antibody evasion by SARS-CoV-2 Omicron subvariants BA.2.12.1, BA.4, & BA.5. Nature 608, 603–608 (2022)).

Summary

The present disclosure generally embraces immunotherapeutic treatment of a subject comprising administration of RNA, e.g., vaccine RNA, encoding an amino acid sequence, e.g.,

a vaccine antigen, comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof, i.e., an antigenic peptide or protein. Thus, vaccine antigen comprises an epitope of SARS-CoV-2 S protein for inducing an immune response against coronavirus S protein, in particular SARS-CoV-2 S protein, in the subject. RNA encoding vaccine antigen is administered to provide (following expression of the polynucleotide by appropriate target cells) antigen for induction, i.e., stimulation, priming and/or expansion, of an immune response, e.g., antibodies and/or immune effector cells, which is targeted to target antigen (coronavirus S protein, in particular SARS-CoV-2 S protein) or a procession product thereof. In one embodiment, the immune response which is to be induced according to the present disclosure is a B cell-mediated immune response, i.e., an antibody-mediated immune response. Additionally or alternatively, in one embodiment, the immune response which is to be induced according to the present disclosure is a T cell-mediated immune response. In one embodiment, the immune response is an anti-coronavirus, in particular anti-SARS-CoV-2 immune response.

Vaccines described herein comprise as an active principle single-stranded RNA that may be translated into protein upon entering cells of a recipient. In addition to wildtype or codon-optimized sequences encoding an antigen sequence, RNA may contain one or more structural elements optimized for maximal efficacy with respect to stability and translational efficiency (e.g., 5' cap, 5' UTR, 3' UTR, poly(A)-tail, and combinations thereof). In one embodiment, RNA contains all of these elements. In one embodiment, a cap1 structure may be utilized as specific capping structure at the 5'-end of an RNA drug substance. In one embodiment, beta-S-ARCA(D1) ($m_2^{7,2'\text{-}O}$GppSpG) or $m_2^{7,3'\text{-}O}$Gppp($m_1^{2'\text{-}O}$)ApG may be utilized as specific capping structure at the 5'-end of an RNA drug substance. As 5'-UTR sequence, the 5'-UTR sequence of the human alpha-globin mRNA, optionally with an optimized 'Kozak sequence' to increase translational efficiency (e.g., SEQ ID NO: 12) may be used. As 3'-UTR sequence, a combination of two sequence elements (FI element) derived from the "amino terminal enhancer of split" (AES) mRNA (called F) and the mitochondrial encoded 12S ribosomal RNA (called I) (e.g., SEQ ID NO: 13) placed between the coding sequence and the poly(A)-tail to assure higher maximum protein levels and prolonged persistence of the mRNA may be used. These features were identified by an *ex vivo* selection process for sequences that confer RNA stability and augment total protein expression (see WO 2017/060314, herein incorporated by reference). Alternatively, the 3'-UTR may be two re-iterated 3'-UTRs of the human beta-globin mRNA.

Additionally or alternatively, in some embodiments, a poly(A)-tail may comprise a length of at least 100 adenosine residues (including, e.g., at least 110 adenosine residues, at least 120 adenosine residues, 130 adenosine residues, or longer). In some embodiments, a poly(A)-tail may comprise a length of about 100 to about 150 adenosine residues. In some embodiments a poly(A)-tail may comprise an interrupted poly(A)-tail. For example, in some such embodiments, a poly(A)-tail measuring 110 nucleotides in length, consisting of a stretch of 30 adenosine residues, followed by a 10 nucleotide linker sequence (of random nucleotides) and another 70 adenosine residues (e.g., SEQ ID NO: 14) may be used. This poly(A)-tail sequence was designed to enhance RNA stability and translational efficiency.

Furthermore, in some embodiments, a nucleotide sequence encoding a secretory signal peptide (sec) may be fused to antigen-encoding regions of an RNA, preferably in some embodiments in a way that the sec is translated as an N terminal tag. In one embodiment, sec corresponds to the secretory signal peptide of a SARS-CoV-2 S protein (e.g., of a Wuhan strain). In some embodiments, sequences coding for short linker peptides predominantly consisting of the amino acids glycine (G) and serine (S), as commonly used for fusion proteins, may be used as GS/Linkers between sec and an antigen.

Vaccine RNA described herein may be complexed with proteins and/or lipids, preferably lipids, to generate RNA-particles for administration. If a combination of different RNAs is used, RNAs may be complexed together or complexed separately with proteins and/or lipids to generate RNA-particles for administration.

In one aspect, the present disclosure relates to a composition or medical preparation comprising RNA encoding an amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof.

In one embodiment, an immunogenic fragment of the SARS-CoV-2 S protein comprises the S1 subunit of the SARS-CoV-2 S protein, or the receptor binding domain (RBD) of the S1 subunit of the SARS-CoV-2 S protein.

In one embodiment, an amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof is able to form a multimeric complex, in particular a trimeric complex. To this end, an amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or

the immunogenic variant thereof may comprise a domain allowing the formation of a multimeric complex, in particular a trimeric complex of the amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof. In one embodiment, the domain allowing formation of a multimeric complex comprises a trimerization domain, for example, a trimerization domain as described herein, e.g., SARS-CoV-2 S protein trimerization domain. In one embodiment, trimerization is achieved by addition of a trimerization domain, e.g., a T4-fibritin-derived "foldon" trimerization domain (e.g., SEQ ID NO: 10), in particular if the amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof corresponds to a portion of a SARS-CoV-2 S protein that does not comprise the SARS-CoV-2 S protein trimerization domain.

In one embodiment, the amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof is encoded by a coding sequence which is codon-optimized and/or the G/C content of which is increased compared to wild type coding sequence, wherein the codon-optimization and/or the increase in the G/C content preferably does not change the sequence of the encoded amino acid sequence. Those skilled in the art will appreciate that codon optimization involves choosing between or among alternative codons encoding the same amino acid residue. Codon optimization typically includes consideration of codon(s) preferred by a particular host in which a sequence is to be expressed. In accordance with the present disclosure, in many embodiments, a preferred host is a human. In some embodiments, a preferred host may be a domestic animal. Alternatively or additionally, in some embodiments, selection between or among possible codons encoding the same amino acid may consider one or more other features such as, for example, overall G/C content (as noted above) and/or similarity to a particular reference. For example, in some embodiments of the present disclosure, a provided coding sequence that encodes a SARS-CoV-2 S protein or immunogenic variant thereof that differs in amino acid sequence from that encoded by a BNT162b2 construct described herein utilizes a codon, in at least one position of such difference, that preserves greater similarity to the BNT162b2 construct sequence relative to at least one alternative codon encoding the same amino acid at such position of difference.

In one embodiment,

(i) the RNA encoding a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the nucleotide sequence of nucleotides 979 to 1584 of SEQ ID NO: 2, 8 or 9, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 979 to 1584 of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of nucleotides 979 to 1584 of SEQ ID NO: 2, 8 or 9, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 979 to 1584 of SEQ ID NO: 2, 8 or 9; and/or

(ii) a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1, or an immunogenic fragment of the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1.

In one embodiment,

(i) the RNA encoding a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the nucleotide sequence of nucleotides 111 to 986 of SEQ ID NO: 30, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 111 to 986 of SEQ ID NO: 30, or a fragment of the nucleotide sequence of nucleotides 111 to 986 of SEQ ID NO: 30, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 111 to 986 of SEQ ID NO: 30; and/or

(ii) a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the amino acid sequence of amino acids 20 to 311 of SEQ ID NO: 29, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 20 to 311 of SEQ ID NO: 29, or an immunogenic fragment of the amino acid sequence of amino acids 20 to 311 of SEQ ID NO: 29, or the amino acid sequence having at least 99%, 98%,

97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 20 to 311 of SEQ ID NO: 29.

In one embodiment,

(i) the RNA encoding a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the nucleotide sequence of nucleotides 49 to 2055 of SEQ ID NO: 2, 8 or 9, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 49 to 2055 of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of nucleotides 49 to 2055 of SEQ ID NO: 2, 8 or 9, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 49 to 2055 of SEQ ID NO: 2, 8 or 9; and/or

(ii) a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the amino acid sequence of amino acids 17 to 685 of SEQ ID NO: 1, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 17 to 685 of SEQ ID NO: 1, or an immunogenic fragment of the amino acid sequence of amino acids 17 to 685 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 17 to 685 of SEQ ID NO: 1.

In one embodiment,

(i) the RNA encoding a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the nucleotide sequence of nucleotides 49 to 3819 of SEQ ID NO: 2, 8 or 9, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 49 to 3819 of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of nucleotides 49 to 3819 of SEQ ID NO: 2, 8 or 9, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 49 to 3819 of SEQ ID NO: 2, 8 or 9; and/or

(ii) a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of

amino acids 17 to 1273 of SEQ ID NO: 1 or 7, or an immunogenic fragment of the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7.

In one embodiment, the amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises a secretory signal peptide.

In one embodiment, the secretory signal peptide is fused, preferably N-terminally, to a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof.

In one embodiment,

(i) the RNA encoding the secretory signal peptide comprises the nucleotide sequence of nucleotides 1 to 48 of SEQ ID NO: 2, 8 or 9, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 1 to 48 of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of nucleotides 1 to 48 of SEQ ID NO: 2, 8 or 9, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 1 to 48 of SEQ ID NO: 2, 8 or 9; and/or

(ii) the secretory signal peptide comprises the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1, or a functional fragment of the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1.

In one embodiment,

(i) the RNA encoding a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the nucleotide sequence of SEQ ID NO: 6, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 6, or a fragment of the nucleotide sequence of SEQ ID NO: 6, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 6; and/or

(ii) a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the amino acid sequence of SEQ ID NO: 5, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 5, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 5, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 5.

In one embodiment,

(i) the RNA encoding a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the nucleotide sequence of nucleotides 54 to 986 of SEQ ID NO: 30, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 54 to 986 of SEQ ID NO: 30, or a fragment of the nucleotide sequence of nucleotides 54 to 986 of SEQ ID NO: 30, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 54 to 986 of SEQ ID NO: 30; and/or

(ii) a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the amino acid sequence of amino acids 1 to 311 of SEQ ID NO: 29, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 311 of SEQ ID NO: 29, or an immunogenic fragment of the amino acid sequence of amino acids 1 to 311 of SEQ ID NO: 29, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 311 of SEQ ID NO: 29.

In one embodiment, the RNA is a modified RNA, in particular a stabilized mRNA. In one embodiment, the RNA comprises a modified nucleoside in place of at least one uridine. In one embodiment, the RNA comprises a modified nucleoside in place of each uridine. In one embodiment, the modified nucleoside is independently selected from pseudouridine (ψ), N1-methyl-pseudouridine (m1ψ), and 5-methyl-uridine (m5U).

In one embodiment, RNA comprises a modified nucleoside in place of uridine.

In one embodiment, the modified nucleoside is selected from pseudouridine (ψ), N1-methyl-pseudouridine (m1ψ), and 5-methyl-uridine (m5U).

In one embodiment, RNA comprises a 5' cap.

In one embodiment, RNA encoding an amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises a 5' UTR comprising the nucleotide sequence of SEQ ID NO: 12, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 12.

In one embodiment, RNA encoding an amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises a 3' UTR comprising the nucleotide sequence of SEQ ID NO: 13, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 13.

In one embodiment, RNA encoding an amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises a poly-A sequence.

In one embodiment, the poly-A sequence comprises at least 100 nucleotides.

In one embodiment, the poly-A sequence comprises or consists of the nucleotide sequence of SEQ ID NO: 14.

In one embodiment, RNA is formulated or is to be formulated as a liquid, a solid, or a combination thereof.

In one embodiment, RNA is formulated or is to be formulated for injection.

In one embodiment, RNA is formulated or is to be formulated for intramuscular administration.

In one embodiment, RNA is formulated or is to be formulated as particles.

In one embodiment, particles are lipid nanoparticles (LNP) or lipoplex (LPX) particles.

In one embodiment, LNPs comprise ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate), 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide, 1,2-Distearoyl-sn-glycero-3-phosphocholine, and cholesterol.

In one embodiment, RNA lipoplex particles are obtainable by mixing RNA with liposomes. In one embodiment, RNA lipoplex particles are obtainable by mixing RNA with lipids.

In one embodiment, RNA is formulated or is to be formulated as colloid. In one embodiment, RNA is formulated or is to be formulated as particles, forming the dispersed phase of a colloid.

In one embodiment, 50% or more, 75% or more, or 85% or more of RNA is present in the

dispersed phase. In one embodiment, RNA is formulated or is to be formulated as particles comprising RNA and lipids. In one embodiment, particles are formed by exposing RNA, dissolved in an aqueous phase, with lipids, dissolved in an organic phase. In one embodiment, the organic phase comprises ethanol. In one embodiment, particles are formed by exposing RNA, dissolved in an aqueous phase, with lipids, dispersed in an aqueous phase. In one embodiment, the lipids dispersed in an aqueous phase form liposomes.

In one embodiment, RNA is mRNA or saRNA.

In one embodiment, a composition or medical preparation is a pharmaceutical composition.

In one embodiment, a composition or medical preparation is a vaccine.

In one embodiment, a pharmaceutical composition further comprises one or more pharmaceutically acceptable carriers, diluents and/or excipients.

In one embodiment, a composition or medical preparation is a kit.

In one embodiment, RNA and optionally the particle forming components are in separate vials.

In one embodiment, a kit further comprises instructions for use of a composition or medical preparation for inducing an immune response against coronavirus in a subject.

In one aspect, the present disclosure relates to a composition or medical preparation described herein for pharmaceutical use.

In one embodiment, a pharmaceutical use comprises inducing an immune response against coronavirus in a subject.

In one embodiment, a pharmaceutical use comprises a therapeutic or prophylactic treatment of a coronavirus infection.

In one embodiment, a composition or medical preparation described herein is for administration to a human.

In one embodiment, the coronavirus is a betacoronavirus.

In one embodiment, the coronavirus is a sarbecovirus.

In one embodiment, the coronavirus is SARS-CoV-2.

In one aspect, the present disclosure relates to a method of inducing an immune response against coronavirus in a subject comprising administering to the subject a composition comprising RNA encoding an amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof.

In one embodiment, an immunogenic fragment of the SARS-CoV-2 S protein comprises the S1 subunit of the SARS-CoV-2 S protein, or the receptor binding domain (RBD) of the S1 subunit of the SARS-CoV-2 S protein.

In one embodiment, an amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof is able to form a multimeric complex, in particular a trimeric complex. To this end, an amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof may comprise a domain allowing the formation of a multimeric complex, in particular a trimeric complex of the amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof. In one embodiment, the domain allowing the formation of a multimeric complex comprises a trimerization domain, for example, a trimerization domain as described herein.

In one embodiment, an amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof is encoded by a coding sequence which is codon-optimized and/or the G/C content of which is increased compared to wild type coding sequence, wherein the codon-optimization and/or the increase in the G/C content preferably does not change the sequence of the encoded amino acid sequence.

In one embodiment,

(i) the RNA encoding a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the nucleotide sequence of nucleotides 979 to 1584 of SEQ ID NO: 2, 8 or 9, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 979 to 1584 of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of nucleotides 979 to 1584 of SEQ ID NO: 2, 8 or 9, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 979 to 1584 of SEQ ID NO: 2, 8 or 9; and/or

(ii) a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1, an amino acid sequence having at least

99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1, or an immunogenic fragment of the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1.

In one embodiment,

(i) the RNA encoding a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the nucleotide sequence of nucleotides 111 to 986 of SEQ ID NO: 30, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 111 to 986 of SEQ ID NO: 30, or a fragment of the nucleotide sequence of nucleotides 111 to 986 of SEQ ID NO: 30, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 111 to 986 of SEQ ID NO: 30; and/or

(ii) a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the amino acid sequence of amino acids 20 to 311 of SEQ ID NO: 29, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 20 to 311 of SEQ ID NO: 29, or an immunogenic fragment of the amino acid sequence of amino acids 20 to 311 of SEQ ID NO: 29, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 20 to 311 of SEQ ID NO: 29.

In one embodiment,

(i) the RNA encoding a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the nucleotide sequence of nucleotides 49 to 2055 of SEQ ID NO: 2, 8 or 9, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 49 to 2055 of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of nucleotides 49 to 2055 of SEQ ID NO: 2, 8 or 9, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 49 to 2055 of SEQ ID NO: 2, 8 or 9; and/or

(ii) a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the amino acid sequence of amino acids 17 to 685 of SEQ ID NO: 1, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 17 to 685 of SEQ ID NO: 1, or an immunogenic fragment of the amino acid sequence of amino acids 17 to 685 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 17 to 685 of SEQ ID NO: 1.

In one embodiment,

(i) the RNA encoding a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the nucleotide sequence of nucleotides 49 to 3819 of SEQ ID NO: 2, 8 or 9, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 49 to 3819 of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of nucleotides 49 to 3819 of SEQ ID NO: 2, 8 or 9, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 49 to 3819 of SEQ ID NO: 2, 8 or 9; and/or

(ii) a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7, or an immunogenic fragment of the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7.

In one embodiment, an amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises a secretory signal peptide.

In one embodiment, a secretory signal peptide is fused, preferably N-terminally, to a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof.

In one embodiment,

(i) the RNA encoding the secretory signal peptide comprises the nucleotide sequence of nucleotides 1 to 48 of SEQ ID NO: 2, 8 or 9, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 1 to 48 of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of nucleotides 1 to 48 of SEQ ID NO: 2, 8 or 9, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 1 to 48 of SEQ ID NO: 2, 8 or 9; and/or

(ii) the secretory signal peptide comprises the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1, or a functional fragment of the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1.

In one embodiment,

(i) the RNA encoding a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the nucleotide sequence of SEQ ID NO: 6, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 6, or a fragment of the nucleotide sequence of SEQ ID NO: 6, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 6; and/or

(ii) a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the amino acid sequence of SEQ ID NO: 5, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 5, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 5, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 5.

In one embodiment,

(i) the RNA encoding a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the nucleotide sequence of nucleotides 54 to 986 of SEQ ID NO: 30, a nucleotide

sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 54 to 986 of SEQ ID NO: 30, or a fragment of the nucleotide sequence of nucleotides 54 to 986 of SEQ ID NO: 30, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 54 to 986 of SEQ ID NO: 30; and/or

(ii) a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the amino acid sequence of amino acids 1 to 311 of SEQ ID NO: 29, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 311 of SEQ ID NO: 29, or an immunogenic fragment of the amino acid sequence of amino acids 1 to 311 of SEQ ID NO: 29, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 311 of SEQ ID NO: 29.

In one embodiment, RNA is modified RNA, in particular stabilized mRNA. In one embodiment, RNA comprises a modified nucleoside in place of at least one uridine. In one embodiment, RNA comprises a modified nucleoside in place of each uridine. In one embodiment, a modified nucleoside is independently selected from pseudouridine (ψ), N1-methyl-pseudouridine (m1ψ), and 5-methyl-uridine (m5U).

In one embodiment, RNA comprises a modified nucleoside in place of uridine.

In one embodiment, the modified nucleoside is selected from pseudouridine (ψ), N1-methyl-pseudouridine (m1ψ), and 5-methyl-uridine (m5U).

In one embodiment, RNA comprises a cap.

In one embodiment, RNA encoding an amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises a 5' UTR comprising the nucleotide sequence of SEQ ID NO: 12, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 12.

In one embodiment, RNA encoding an amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises a 3' UTR comprising the nucleotide sequence of SEQ ID NO: 13, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 13.

In one embodiment, RNA encoding an amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises a poly-A sequence.

In one embodiment, a poly-A sequence comprises at least 100 nucleotides.

In one embodiment, a poly-A sequence comprises or consists of the nucleotide sequence of SEQ ID NO: 14.

In one embodiment, RNA is formulated as a liquid, a solid, or a combination thereof.

In one embodiment, RNA is administered by injection.

In one embodiment, RNA is administered by intramuscular administration.

In one embodiment, RNA is formulated as particles.

In one embodiment, the particles are lipid nanoparticles (LNP) or lipoplex (LPX) particles.

In one embodiment, LNPs comprise ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate), 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide, 1,2-Distearoyl-sn-glycero-3-phosphocholine, and cholesterol.

In one embodiment, RNA lipoplex particles are obtainable by mixing RNA with liposomes. In one embodiment, RNA lipoplex particles are obtainable by mixing RNA with lipids.

In one embodiment, RNA is formulated as colloid. In one embodiment, RNA is formulated as particles, forming the dispersed phase of a colloid. In one embodiment, 50% or more, 75% or more, or 85% or more of RNA are present in the dispersed phase. In one embodiment, RNA is formulated as particles comprising RNA and lipids. In one embodiment, particles are formed by exposing RNA, dissolved in an aqueous phase, with lipids, dissolved in an organic phase. In one embodiment, the organic phase comprises ethanol. In one embodiment, particles are formed by exposing RNA, dissolved in an aqueous phase, with lipids, dispersed in an aqueous phase. In one embodiment, the lipids dispersed in an aqueous phase form liposomes.

In one embodiment, RNA is mRNA or saRNA.

In one embodiment, a method disclosed herein is a method for vaccination against coronavirus.

In one embodiment, a method disclosed herein is a method for therapeutic or prophylactic treatment of a coronavirus infection.

In one embodiment, a subject is a human.

In one embodiment, the coronavirus is a betacoronavirus.

In one embodiment, the coronavirus is a sarbecovirus.

In one embodiment, the coronavirus is SARS-CoV-2.

In one embodiment of methods described herein, a composition described herein is administered to a subject.

In one aspect, the present disclosure relates to a composition or medical preparation described herein for use in a method described herein.

Among other things, the present disclosure teaches that a composition comprising a lipid nanoparticle encapsulated RNA encoding at least a portion (e.g., that is or comprises an epitope) of a SARS-CoV-2-encoded polypeptide (e.g., of a SARS-CoV-2-encoded S protein) can achieve detectable antibody titer against an epitope in serum within 7 days after administration to a population of adult human subjects according to a regimen that includes administration of at least one dose of the composition. Moreover, the present disclosure teaches persistence of such antibody titer. In some embodiments, such antibody titer is increased when a modified mRNA is used, as compared with titer achieved with a corresponding unmodified mRNA.

In some embodiments, a provided regimen includes at least one dose. In some embodiments, a provided regimen includes a first dose and at least one subsequent dose. In some embodiments, the first dose is the same amount as at least one subsequent dose. In some embodiments, the first dose is the same amount as all subsequent doses. In some embodiments, the first dose is a different amount as at least one subsequent dose. In some embodiments, the first dose is a different amount than all subsequent doses. In some embodiments, a provided regimen comprises two doses. In some embodiments, a provided regimen consists of two doses.

In particular embodiments, an immunogenic composition is formulated as a single-dose in a container, e.g., a vial. In some embodiments, an immunogenic composition is formulated as a multi-dose formulation in a vial. In some embodiments, the multi-dose formulation includes at least 2 doses per vial. In some embodiments, the multi-dose formulation includes a total of 2-20 doses per vial, such as, for example, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 doses per vial. In some embodiments, each dose in the vial is equal in volume. In some embodiments, a first dose is a different volume than a subsequent dose.

A "stable" multi-dose formulation exhibits no unacceptable levels of microbial growth, and substantially no or no breakdown or degradation of the active biological molecule component(s). As used herein, a "stable" immunogenic composition includes a formulation

that remains capable of eliciting a desired immunologic response when administered to a subject.

In some embodiments, a multi-dose formulation remains stable for a specified time with multiple or repeated inoculations/insertions into the multi-dose container. For example, in some embodiments a multi-dose formulation may be stable for at least three days with up to ten usages, when contained within a multi-dose container. In some embodiments, multi-dose formulations remain stable with 2-20 inoculations/insertions.

In some embodiments, administration of a composition comprising a lipid nanoparticle encapsulated RNA (e.g., in some embodiments mRNA) encoding at least a portion (e.g., that is or comprises an epitope) of a SARS-CoV-2-encoded polypeptide (e.g., of a SARS-CoV-2-encoded S protein), e.g., according to a regimen as described herein, may result in lymphopenia in some subjects (e.g., in all subjects, in most subjects, in about 50% or fewer, in about 40% or fewer, in about 40% or fewer, in about 25% or fewer, in about 20% or fewer, in about 15% or fewer, in about 10% or fewer, in about 5% or fewer, etc). Among other things, the present disclosure teaches that such lymphopenia can resolve over time. For example, in some embodiments, lymphopenia resolves within about 14, about 10, about 9, about 8, about 7 days or less. In some embodiments, lymphopenia is Grade 3, Grade 2, or less.

Thus, among other things, the present disclosure provides compositions comprising a lipid nanoparticle encapsulated RNA (e.g., in some embodiments mRNA) encoding at least a portion (e.g., that is or comprises an epitope) of a SARS-CoV-2-encoded polypeptide (e.g., of a SARS-CoV-2-encoded S protein) that are characterized, when administered to a relevant population of adults, to display certain characteristics (e.g., achieve certain effects) as described herein. In some embodiments, provided compositions may have been prepared, stored, transported, characterized, and/or used under conditions where temperature does not exceed a particular threshold. Alternatively or additionally, in some embodiments, provided compositions may have been protected from light (e.g., from certain wavelengths) during some or all of their preparation, storage, transport, characterization, and/or use. In some embodiments, one or more features of provided compositions (e.g., RNA stability, as may be assessed, for example, by one or more of size, presence of particular moiety or modification, etc; lipid nanoparticle stability or aggregation, pH, etc) may be or have been assessed at one or more points during preparation, storage, transport, and/or use prior to administration.

Among other things, the present disclosure documents that certain provided compositions in which nucleotides within an RNA (e.g., in some embodiments mRNA) are not modified (e.g., are naturally-occurring A, U, C, G), and/or provided methods relating to such compositions, are characterized (e.g., when administered to a relevant population, which may in some embodiments be or comprise an adult population), by an intrinsic adjuvant effect. In some embodiments, such a composition and/or method can induce an antibody and/or a T cell response. In some embodiments, such a composition and/or method can induce a higher T cell response, as compared to conventional vaccines (e.g., non-RNA vaccines such as protein vaccines).

Alternatively or additionally, the present disclosure documents that provided compositions (e.g., compositions comprising a lipid nanoparticle encapsulated RNA encoding at least a portion (e.g., that is or comprises an epitope) of a SARS-CoV-2-encoded polypeptide (e.g., of a SARS-CoV-2-encoded S protein)) in which nucleotides within an RNA are modified, and/or provided methods relating to such compositions, are characterized (e.g., when administered to a relevant population, which may in some embodiments be or comprise an adult population), by absence of an intrinsic adjuvant effect, or by a reduced intrinsic adjuvant effect as compared with an otherwise comparable composition (or method) with unmodified results. Alternatively or additionally, in some embodiments, such compositions (or methods) are characterized in that they (e.g., when administered to a relevant population, which may in some embodiments be or comprise an adult population) induce an antibody response and/or a CD4+ T cell response. Still further alternatively or additionally, in some embodiments, such compositions (or methods) are characterized in that they (e.g., when administered to a relevant population, which may in some embodiments be or comprise an adult population) induce a higher CD4+ T cell response than that observed with an alternative vaccine format (e.g., a peptide vaccine). In some embodiments involving modified nucleotides, such modified nucleotides may be present, for example, in a 3' UTR sequence, an antigen-encoding sequence, and/or a 5'UTR sequence. In some embodiments, modified nucleotides are or include one or more modified uracil residues and/or one or more modified cytosine residues.

Among other things, the present disclosure documents that provided (e.g., compositions comprising a lipid nanoparticle encapsulated RNA encoding at least a portion (e.g., that is or comprises an epitope) of a SARS-CoV-2-encoded polypeptide (e.g., of a SARS-CoV-2-encoded S protein)) and/or methods are characterized by (e.g., when administered to a relevant

population, which may in some embodiments be or comprise an adult population) sustained expression of an encoded polypeptide (e.g., of a SARS-CoV-2-encoded protein [such as an S protein] or portion thereof, which portion, in some embodiments, may be or comprise an epitope thereof). For example, in some embodiments, such compositions and/or methods are characterized in that, when administered to a human, they achieve detectable polypeptide expression in a biological sample (e.g., serum) from such human and, in some embodiments, such expression persists for a period of time that is at least at least 36 hours or longer, including, e.g., at least 48 hours, at least 60 hours, at least 72 hours, at least 96 hours, at least 120 hours, at least 148 hours, or longer.

Those skilled in the art, reading the present disclosure, will appreciate that it describes various RNA constructs (e.g., in some embodiments mRNA constructs) encoding at least a portion (e.g., that is or comprises an epitope) of a SARS-CoV-2-encoded polypeptide (e.g., of a SARS-CoV-2-encoded S protein)). Such person of ordinary skill, reading the present disclosure, will particularly appreciate that it describes various RNA constructs (e.g., in some embodiments mRNA constructs) encoding at least a portion of a SARS-CoV-2 S protein, for example at least an RBD portion of a SARS-CoV-2 S protein. Still further, such a person of ordinary skill, reading the present disclosure, will appreciate that it describes particular characteristics and/or advantages of RNA constructs (e.g., in some embodiments mRNA constructs) encoding at least a portion (e.g., that is or comprises an epitope) of a SARS-CoV-2-encoded polypeptide (e.g., of a SARS-CoV-2-encoded S protein). In some embodiments, an RNA construct (e.g., in some embodiments, an mRNA construct) may encode at least one domain of a SARS-CoV-2 encoded polypeptide (e.g., one or more domains of a SARS-CoV-2 encoded polypeptide as described in WO 2021/159040, including, e.g., an N-terminal domain (NTD) of a SARS-CoV-2 Spike protein, a receptor binding domain (RBD) of a SARS- CoV-2 Spike protein, Heptapeptide repeat sequence 1 (HR1) of a SARS-CoV-2 Spike protein, Heptapeptide repeat sequence 2 (HR1) of a SARS-CoV-2 Spike protein, and/or combinations thereof). Among other things, the present disclosure particularly documents surprising and useful characteristics and/or advantages of certain RNA constructs (e.g., in some embodiments mRNA constructs) encoding a SARS-CoV-2 RBD portion and, in some embodiments, not encoding a full length SARS-CoV-2 S protein. Without wishing to be bound by any particular theory, the present disclosure suggests that provided RNA constructs (e.g., in some embodiments mRNA constructs) that encode less than a full-length SARS-CoV-2 S protein, and particularly those that encode at least an RBD portion

of such SARS-CoV-2 S protein may be particularly useful and/or effective for use as or in an immunogenic composition (e.g., a vaccine), and/or for achieving immunological effects as described herein (e.g., generation of SARS-CoV-2 neutralizing antibodies, and/or T cell responses (e.g., CD4+ and/or CD8+ T cell responses)).

In some embodiments, the present disclosure provides an RNA (e.g., mRNA) comprising an open reading frame encoding a polypeptide that comprises a receptor-binding portion of a SARS-CoV-2 S protein, which RNA is suitable for intracellular expression of the polypeptide. In some embodiments, such an encoded polypeptide does not comprise the complete S protein. In some embodiments, an encoded polypeptide comprises the receptor binding domain (RBD), for example, as shown in SEQ ID NO: 5. In some embodiments, the encoded polypeptide comprises the peptide according to SEQ ID NO: 29 or 31. In some embodiments, such an RNA (e.g., mRNA) may be complexed by a (poly)cationic polymer, polyplex(es), protein(s) or peptide(s). In some embodiments, such an RNA may be formulated in a lipid nanoparticle (e.g., ones described herein). In some embodiments, such an RNA (e.g., mRNA) may be particularly useful and/or effective for use as or in an immunogenic composition (e.g., a vaccine), and/or for achieving immunological effects as described herein (e.g., generation of SARS-CoV-2 neutralizing antibodies, and/or T cell responses (e.g., CD4+ and/or CD8+ T cell responses)). In some embodiments, such an RNA (e.g., mRNA) may be useful for vaccinating humans (including, e.g., humans known to have been exposed and/or infected by SARS-CoV-2, and/or humans not known to have been exposed to SARS-CoV-2).

Those skilled in the art, reading the present disclosure, will further appreciate that it describes various mRNA constructs comprising a nucleic acid sequence that encodes a full-length SARS-CoV-2 Spike protein (e.g., including embodiments in which such encoded SARS-CoV-2 Spike protein may comprise at least one or more amino acid substitutions, e.g., proline substitutions as described herein, and/or embodiments in which the mRNA sequence is codon-optimized e.g., for mammalian, e.g., human, subjects). In some embodiments, such a full-length SARS-CoV-2 Spike protein may have an amino acid sequence that is or comprises that set forth in SEQ ID NO: 7. Still further, such a person of ordinary skill, reading the present disclosure, will appreciate, among other things, that it describes particular characteristics and/or advantages of certain mRNA constructs comprising a nucleic acid sequence that encodes a full-length SARS-CoV-2 Spike protein. Without wishing to be bound by any particular theory, the present disclosure suggests that provided mRNA constructs that encode a full-length SARS-CoV-2 S

protein may be particularly useful and/or effective for use as or in an immunogenic composition (e.g., a vaccine) in particular subject population (e.g., particular age populations). For example, in some embodiments, such an mRNA composition may be particularly useful in younger (e.g., less than 25 years old, 20 years old, 18 years old, 15 years, 10 years old, or lower) subjects; alternatively or additionally, in some embodiments, such an mRNA composition may be particularly useful in elderly subjects (e.g., over 55 years old, 60 years old, 65 years old, 70 years old, 75 years old, 80 years old, 85 years old, or higher). In particular embodiments, an immunogenic composition comprising such an mRNA construct provided herein exhibits a minimal to modest increase (e.g., no more than 30% increase, no more than 20% increase, or no more than 10% increase, or lower) in dose level and/or dose number-dependent systemic reactogenicity (e.g., fever, fatigue, headache, chills, diarrhea, muscle pain, and/or joint pain, etc.) and/or local tolerability (e.g., pain, redness, and/or swelling, etc.), at least in some subjects (e.g., in some subject age groups); in some embodiments, such reactogenicity and/or local tolerability is observed particularly, in in younger age group (e.g., less than 25 years old, 20 years old, 18 years old or lower) subjects, and/or in older (e.g., elderly) age group (e.g., 65-85 years old). In some embodiments, provided mRNA constructs that encode a full-length SARS-CoV-2 S protein may be particularly useful and/or effective for use as or in an immunogenic composition (e.g., a vaccine) for inducing SARS-CoV-2 neutralizing antibody response level in a population of subjects that are at high risk for severe diseases associated with SARS-CoV-2 infection (e.g., an elderly population, for example, 65-85 year-old group). In some embodiments, a person of ordinary skill, reading the present disclosure, will appreciate, among other things, that provided mRNA constructs that encode a full-length SARS-CoV-2 S protein, which exhibit a favorable reactogenicity profile (e.g., as described herein) in younger and elderly age populations, may be particularly useful and/or effective for use as or in an immunogenic composition (e.g., a vaccine) for achieving immunological effects as described herein (e.g., generation of SARS-CoV-2 neutralizing antibodies, and/or T cell responses (e.g., CD4+ and/or CD8+ T cell responses)). In some embodiments, the present disclosure also suggests that provided mRNA constructs that encode a full-length SARS-CoV-2 S protein may be particularly effective to protect against SARS-CoV-2 infection, as characterized by earlier clearance of SARS-CoV-2 viral RNA in non-human mammalian subjects (e.g., rhesus macaques) that were immunized with immunogenic compositions comprising such mRNA constructs and subsequently challenged by SARS-CoV-2 strain. In some

embodiments, such earlier clearance of SARS-CoV-2 viral RNA may be observed in the nose of non-human mammalian subjects (e.g., rhesus macaques) that were immunized with immunogenic compositions comprising such mRNA constructs and subsequently challenged by SARS-CoV-2 strain.

In some embodiments, the present disclosure provides an RNA (e.g., mRNA) comprising an open reading frame encoding a full-length SARS-CoV-2 S protein (e.g., a full-length SARS-CoV-2 S protein with one or more amino acid substitutions), which RNA is suitable for intracellular expression of the polypeptide. In some embodiments, the encoded polypeptide comprises the amino acid sequence of SEQ ID NO:7. In some embodiments, such an RNA (e.g., mRNA) may be complexed by a (poly)cationic polymer, polyplex(es), protein(s) or peptide(s). In some embodiments, such an RNA may be formulated in a lipid nanoparticle (e.g., ones described herein).

In some embodiments, an immunogenic composition provided herein may comprise a plurality of (e.g., at least two or more, including, e.g., at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, etc.) immunoreactive epitopes of a SARS-CoV-2 polypeptide or variants thereof. In some such embodiments, such a plurality of immunoreactive epitopes may be encoded by a plurality of RNAs (e.g., mRNAs). In some such embodiments, such a plurality of immunoreactive epitopes may be encoded by a single RNA (e.g., mRNA). In some embodiments, nucleic acid sequences encoding a plurality of immunoreactive epitopes may be separated from each other in a single RNA (e.g., mRNA) by a linker (e.g., a peptide linker in some embodiments). Without wishing to be bound by any particular theory, in some embodiments, provided polyepitope immunogenic compositions (including, e.g., those that encode a full-length SARS-CoV-2 spike protein) may be particularly useful, when considering the genetic diversity of SARS-CoV-2 variants, to provide protection against numerous viral variants and/or may offer a greater opportunity for development of a diverse and/or otherwise robust (e.g., persistent, e.g., detectable about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 or more days after administration of one or more doses) neutralizing antibody and/or T cell response, and in particular a particularly robust T_H1-type T cell (e.g., CD4+ and/or CD8+ T cell) response.

In some embodiments, the present disclosure documents that provided compositions and/or methods are characterized by (e.g., when administered to a relevant population, which may in some embodiments be or comprise an adult population) in that they achieve one or more

particular therapeutic outcomes (e.g., effective immune responses as described herein and/or detectable expression of encoded SARS-CoV-2 S protein or an immunogenic fragment thereof) with a single administration; in some such embodiments, an outcome may be assessed, for example, as compared to that observed in absence of RNA vaccines (e.g., mRNA vaccines) described herein. In some embodiments, a particular outcome may be achieved at a lower dose than required for one or more alternative strategies.

In some embodiments, the present disclosure provides an immunogenic composition comprising an isolated messenger ribonucleic acid (mRNA) polynucleotide, wherein the isolated mRNA polynucleotide comprises an open reading frame encoding a polypeptide that comprises a receptor-binding portion of a SARs-CoV-2 S protein, and wherein the isolated mRNA polynucleotide is formulated in at least one lipid nanoparticle. For example, in some embodiments, such a lipid nanoparticle may comprise a molar ratio of 20-60% ionizable cationic lipid, 5-25% non-cationic lipid (e.g., neutral lipid), 25-55% sterol or steroid, and 0.5-15% polymer-conjugated lipid (e.g., PEG-modified lipid). In some embodiments, a sterol or steroid included in a lipid nanoparticle may be or comprise cholesterol. In some embodiments, a neutral lipid may be or comprise 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC). In some embodiments, a polymer-conjugated lipid may be or comprise PEG2000 DMG. In some embodiments, such an immunogenic composition may comprise a total lipid content of about 1 mg to 10 mg, or 3 mg to 8 mg, or 4 mg to 6 mg. In some embodiments, such an immunogenic composition may comprise a total lipid content of about 5 mg/mL -15 mg/mL or 7.5 mg/mL-12.5 mg/mL or 9-11 mg/mL. In some embodiments, such an isolated mRNA polynucleotide is provided in an effective amount to induce an immune response in a subject administered at least one dose of the immunogenic composition. In some embodiments, a polypeptide encoded by a provided isolated mRNA polynucleotide does not comprise the complete S protein. In some embodiments, such an isolated mRNA polynucleotide provided in an immunogenic composition is not self-replicating RNA.

In some embodiments, an immune response may comprise generation of a binding antibody titer against SARS-CoV-2 protein (including, e.g., a stabilized prefusion spike trimer in some embodiments) or a fragment thereof. In some embodiments, an immune response may comprise generation of a binding antibody titer against the receptor binding domain (RBD) of the SARS-CoV-2 spike protein. In some embodiments, a provided immunogenic composition has been established to achieve a detectable binding antibody titer after administration of a

first dose, with seroconversion in at least 70% (including, e.g., at least 80%, at least 90%, at least 95% and up to 100%) of a population of subjects receiving such a provided immunogenic composition, for example, by about 2 weeks.

In some embodiments, an immune response may comprise generation of a neutralizing antibody titer against SARS-CoV-2 protein (including, e.g., a stabilized prefusion spike trimer in some embodiments) or a fragment thereof. In some embodiments, an immune response may comprise generation of a neutralizing antibody titer against the receptor binding domain (RBD) of the SARS-CoV-2 spike protein. In some embodiments, a provided immunogenic composition has been established to achieve a neutralizing antibody titer in an appropriate system (e.g., in a human infected with SARS-CoV-2 and/or a population thereof, and/or in a model system therefor). For example, in some embodiments, such neutralizing antibody titer may have been demonstrated in one or more of a population of humans, a non-human primate model (e.g., rhesus macaques), and/or a mouse model.

In some embodiments, a neutralizing antibody titer is a titer that is (e.g., that has been established to be) sufficient to reduce viral infection of B cells relative to that observed for an appropriate control (e.g., an unvaccinated control subject, or a subject vaccinated with a live attenuated viral vaccine, an inactivated viral vaccine, or a protein subunit viral vaccine, or a combination thereof). In some such embodiments, such reduction is of at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more.

In some embodiments, a neutralizing antibody titer is a titer that is (e.g., that has been established to be) sufficient to reduce the rate of asymptomatic viral infection relative to that observed for an appropriate control (e.g., an unvaccinated control subject, or a subject vaccinated with a live attenuated viral vaccine, an inactivated viral vaccine, or a protein subunit viral vaccine, or a combination thereof). In some such embodiments, such reduction is of at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more. In some embodiments, such reduction can be characterized by assessment of SARS-CoV-2 N protein serology. Significant protection against asymptomatic infection was also confirmed by real life observations (see also: Dagan N. et al., N Engl J Med. 2021, doi: 10.1056/NEJMoa2101765. Epub ahead of print. PMID: 33626250)

In some embodiments, a neutralizing antibody titer is a titer that is (e.g., that has been established to be) sufficient to reduce or block fusion of virus with epithelial cells and/or B cells of a vaccinated subject relative to that observed for an appropriate control (e.g., an

unvaccinated control subject, or a subject vaccinated with a live attenuated viral vaccine, an inactivated viral vaccine, or a protein subunit viral vaccine, or a combination thereof). In some such embodiments, such reduction is of at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more.

In some embodiments, induction of a neutralizing antibody titer may be characterized by an elevation in the number of B cells, which in some embodiments may include plasma cells, class-switched IgG1- and IgG2-positive B cells, and/or germinal center B cells. In some embodiments, a provided immunogenic composition has been established to achieve such an elevation in the number of B cells in an appropriate system (e.g., in a human infected with SARS-CoV-2 and/or a population thereof, and/or in a model system therefor). For example, in some embodiments, such an elevation in the number of B cells may have been demonstrated in one or more of a population of humans, a non-human primate model (e.g., rhesus macaques), and/or a mouse model. In some embodiments, such an elevation in the number of B cells may have been demonstrated in draining lymph nodes and/or spleen of a mouse model after (e.g., at least 7 days, at least 8 days, at least 9 days, at least 10 days, at least 11 days, at least 12 days, at least 13 days, at least 14 days, after) immunization of such a mouse model with a provided immunogenic composition.

In some embodiments, induction of a neutralizing antibody titer may be characterized by a reduction in the number of circulating B cells in blood. In some embodiments, a provided immunogenic composition has been established to achieve such a reduction in the number of circulating B cells in blood of an appropriate system (e.g., in a human infected with SARS-CoV-2 and/or a population thereof, and/or in a model system therefor). For example, in some embodiments, such a reduction in the number of circulating B cells in blood may have been demonstrated in one or more of a population of humans, a non-human primate model (e.g., rhesus macaques), and/or a mouse model. In some embodiments, such a reduction in the number of circulating B cells in blood may have been demonstrated in a mouse model after (e.g., at least 4 days, at least 5 days, at least 6 days, at least 7 days, at least 8 days, at least 9 days, at least 10 days, after) immunization of such a mouse model with a provided immunogenic composition. Without wishing to be bound by theory, a reduction in circulating B cells in blood may be due to B cell homing to lymphoid compartments.

In some embodiments, an immune response induced by a provided immunogenic composition may comprise an elevation in the number of T cells. In some embodiments, such an elevation

in the number of T cells may include an elevation in the number of T follicular helper (T_{FH}) cells, which in some embodiments may comprise one or more subsets with ICOS upregulation. One of skilled in the art will understand that proliferation of T_{FH} in germinal centres is integral for generation of an adaptive B-cell response, and also that in humans, T_{FH} occurring in the circulation after vaccination is typically correlated with a high frequency of antigen-specific antibodies. In some embodiments, a provided immunogenic composition has been established to achieve such an elevation in the number of T cells (e.g., T_{FH} cells) in an appropriate system (e.g., in a human infected with SARS-CoV-2 and/or a population thereof, and/or in a model system therefor). For example, in some embodiments, such an elevation in the number of T cells (e.g., T_{FH} cells) may have been demonstrated in one or more of a population of humans, a non-human primate model (e.g., rhesus macaques), and/or a mouse model. In some embodiments, such an elevation in the number of T cells (e.g., e.g., T_{FH} cells) may have been demonstrated in draining lymph nodes, spleen, and/or blood of a mouse model after (e.g., at least 4 days, at least 5 days, at least 6 days, at least 7 days, at least 8 days, at least 9 days, at least 10 days, at least 11 days, at least 12 days, at least 13 days, at least 14 days, after) immunization of such a mouse model with a provided immunogenic composition. In some embodiments, a protective response against SARS-CoV-2 induced by a provided immunogenic composition has been established in an appropriate model system for SARS-CoV-2. For example, in some embodiments, such a protective response may have been demonstrated in an animal model, e.g., a non-human primate model (e.g., rhesus macaques) and/or a mouse model. In some embodiments, a non-human primate (e.g., rhesus macaque) or a population thereof that has/have received at least one immunization with a provided immunogenic composition is/are challenged with SARS-CoV-2, e.g., through intranasal and/or intratracheal route. In some embodiments, such a challenge may be performed several weeks (e.g., 5-10 weeks) after at least one immunization (including, e.g., at least two immunizations) with a provided immunogenic composition. In some embodiments, such a challenge may be performed when a detectable level of a SARS-CoV-2 neutralizing titer (e.g., antibody response to SARS-CoV-2 spike protein and/or a fragment thereof, including, e.g., but not limited to a stabilized prefusion spike trimer, S-2P, and/or antibody response to receptor-binding portion of SARS-CoV-2) is achieved in non-human primate(s) (e.g., rhesus macaque(s)) that has received at least one immunization (including, e.g., at least two immunizations) with a provided immunogenic composition. In some embodiments, a protective response is

characterized by absence of or reduction in detectable viral RNA in bronchoalveolar lavage (BAL) and/or nasal swabs of challenged non-human primate(s) (e.g., rhesus macaque(s)). In some embodiments, immunogenic compositions described herein may have been characterized in that a larger percent of challenged animals, for example, non-human primates in a population (e.g., rhesus macaques), that have received at least one immunization (including, e.g., at least two immunizations) with a provided immunogenic composition display absence of detectable RNA in their BAL and/or nasal swab, as compared to a population of non-immunized animals, for example, non-human primates (e.g., rhesus macaques). In some embodiments, immunogenic compositions described herein may have been characterized in that challenged animals, for example, non-human in a population (e.g., rhesus macaques), that have received at least one immunization (including, e.g., at least two immunizations) with a provided immunogenic composition may show clearance of viral RNA in nasal swab no later than 10 days, including, e.g., no later than 8 days, no later than 6 days, no later than 4 days, etc., as compared to a population of non-immunized animals, for example, non-human primates (e.g., rhesus macaques).

In some embodiments, immunogenic compositions described herein when administered to subjects in need thereof do not substantially increase the risk of vaccine-associated enhanced respiratory disease. In some embodiments, such vaccine-associated enhanced respiratory disease may be associated with antibody-dependent enhancement of replication and/or with vaccine antigens that induced antibodies with poor neutralizing activity and Th2-biased responses. In some embodiments, immunogenic compositions described herein when administered to subjects in need thereof do not substantially increase the risk of antibody-dependent enhancement of replication.

In some embodiments, a single dose of an RNA composition (e.g., mRNA formulated in lipid nanoparticles) can induce a therapeutic antibody response in less than 10 days of vaccination. In some embodiments, such a therapeutic antibody response may be characterized in that when such an RNA vaccine can induce production of about 10-100 ug/mL IgG measured at 10 days after vaccination at a dose of 0.1 to 10 ug or 0.2- 5 ug in an animal model. In some embodiments, such a therapeutic antibody response may be characterized in that such an RNA vaccine induces about 100-1000 ug/mL IgG measured at 20 days of vaccination at a dose of 0.1 to 10 ug or 0.2- 5 ug in an animal model. In some embodiments, a single dose may induce a pseudovirus-neutralization titer, as measured in an animal model, of 10-200 pVN50

titer 15 days after vaccination. In some embodiments, a single dose may induce a pseudovirus-neutralization titer, as measured in an animal model, of 50-500 pVN50 titer 15 days after vaccination.

In some embodiments, a single dose of an RNA composition (e.g., mRNA composition) can expand antigen-specific CD8 and/or CD4 T cell response by at least at 50% or more (including, e.g., at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or more), as compared to that observed in absence of such an RNA construct encoding a SARS-COV2 immunogenic protein or fragment thereof (e.g., spike protein and/or receptor binding domain). In some embodiments, a single dose of an RNA composition can expand antigen-specific CD8 and/or CD4 T cell response by at least at 1.5-fold or more (including, e.g., at least 2-fold, at least 3-fold, at least 5-fold, at least 10-fold, at least 50-fold, at least 100-fold, at least 500-fold, at least 1000-fold, or more), as compared to that observed in absence of such an RNA construct encoding a SARS-COV2 immunogenic protein or fragment thereof (e.g., spike protein and/or receptor binding domain).

In some embodiments, a regimen (e.g., a single dose of an mRNA composition) can expand T cells that exhibit a Th1 phenotype (e.g., as characterized by expression of IFN-gamma, IL-2, IL-4, and/or IL-5) by at least at 50% or more (including, e.g., at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or more), as compared to that observed in absence of such an mRNA construct encoding a SARS-COV2 immunogenic protein or fragment thereof (e.g., spike protein and/or receptor binding domain). In some embodiments, a regimen (e.g., a single dose of an mRNA composition) can expand T cells that exhibit a Th1 phenotype (e.g., as characterized by expression of IFN-gamma, IL-2, IL-4, and/or IL-5), for example by at least at 1.5-fold or more (including, e.g., at least 2-fold, at least 3-fold, at least 5-fold, at least 10-fold, at least 50-fold, at least 100-fold, at least 500-fold, at least 1000-fold, or more), as compared to that observed in absence of such an mRNA construct encoding a SARS-COV2 immunogenic protein or fragment thereof (e.g., spike protein and/or receptor binding domain). In some embodiments, a T-cell phenotype may be or comprise a Th1-dominant cytokine profile (e.g., as characterized by INF-gamma positive and/or IL-2 positive), and/or no by or biologically insignificant IL-4 secretion.

In some embodiments, a regimen as described herein (e.g., one or more doses of an mRNA composition) induces and/or achieves production of RBD-specific CD4+ T cells. Among other things, the present disclosure documents that mRNA compositions encoding an RBD-

containing portion of a SARS-CoV-2 spike protein (e.g., and not encoding a full-length SARS-CoV-2 spike protein) may be particularly useful and/or effective in such induction and/or production of RBD-specific CD4+ T cells. In some embodiments, RBD-specific CD4+ T-cells induced by an mRNA composition described herein (e.g., by an mRNA composition that encodings an RBD-containing-portion of a SARS-CoV-2 spike protein and, in some embodiments not encoding a full-length SARS-CoV-2 spike protein) demonstrate a Th1-dominant cytokine profile (e.g., as characterized by INF-gamma positive and/or IL-2 positive), and/or by no or biologically insignificant IL-4 secretion.

In some embodiments, characterization of CD4+ and/or CD8+ T cell responses (e.g., described herein) in subjects receiving RNA compositions (e.g., as described herein) may be performed using ex vivo assays using PBMCs collected from the subjects.

In some embodiments, immunogenicity of RNA (e.g., mRNA) compositions described herein may be assessed by one of or more of the following serological immunongenicity assays: detection of IgG, IgM, and/or IgA to SARS-CoV-2 S protein present in blood samples of a subject receiving a provided RNA composition, and/or neutralization assays using SARS-CoV-2 pseudovirus and/or a wild-type SARS-CoV-2 virus.

In some embodiments, an RNA composition (e.g., as described herein) provide a relatively low adverse effect (e.g., Grade 1-Grade 2 pain, redness and/or swelling) within 7 days after vaccinations at a dose of 10 ug – 100 ug or 1 ug-50 ug. In some embodiments, RNA compositions (e.g., as described herein) provide a relatively low observation of systemic events (e.g., Grade 1-Grade 2 fever, fatigue, headache, chills, vomiting, diarrhea, muscle pain, joint pain, medication, and combinations thereof) within 7 days after vaccinations at a dose of 10 ug – 100 ug.

In some embodiments, RNA (e.g., mRNA) compositions are characterized in that when administered to subjects at 10-100 ug dose or 1 ug-50 ug, IgG directed to a SARS-CoV2 immunogenic protein or fragment thereof (e.g., spike protein and/or receptor binding domain) may be produced at a level of 100-100,000 U/mL or 500-50,000 U/mL 21 days after vaccination.

In some embodiments, an RNA (e.g., mRNA) encodes a natively-folded trimeric receptor binding protein of SARS-CoV-2. In some embodiments, an RNA (e.g., mRNA) encodes a variant of such receptor binding protein such that the encoded variant binds to ACE2 at a Kd of 10 pM or lower, including, e.g., at a Kd of 9 pM, 8 pM, 7 pM, 6 pM, 5 pM, 4 pM, or lower. In some

embodiments, an RNA (e.g., mRNA) encodes a variant of such receptor binding protein such that the encoded variant binds to ACE2 at a Kd of 5 pM. In some embodiments, an RNA (e.g., mRNA) encodes a trimeric receptor binding portion of SARS-CoV-2 that comprises an ACE2 receptor binding site. In some embodiments, an RNA (e.g., mRNA) comprises a coding sequence for a receptor-binding portion of SARS-CoV-2 and a trimerization domain (e.g., a natural trimerization domain (foldon) of T4 fibritin) such that the coding sequence directs expression of a trimeric protein that has an ACE2 receptor binding site and binds ACE2. In some embodiments, an RNA (e.g., mRNA) encodes a trimeric receptor binding portion of SARS-CoV-2 or a variant thereof such that its Kd is smaller than that for a monomeric receptor-binding domain (RBD) of SARS-CoV-2. For example, in some embodiments, an RNA (e.g., mRNA) encodes a trimeric receptor binding portion of SARS-CoV-2 or a variant thereof such that its Kd is at least 10-fold (including, e.g., at least 50-fold, at least 100-fold, at least 500-fold, at least 1000-fold, etc.) smaller than that for a RBD of SARS-CoV-2.

In some embodiments, a trimer receptor binding portion of SARS-CoV-2 encoded by an RNA (e.g., as described herein) may be determined to have a size of about 3-4 angstroms when it is complexed with ACE2 and B^0AT1 neutral amino acid transporter in a closed conformation, as characterized by electron cryomicroscopy (cryoEM). In some embodiments, geometric mean SARS-CoV-2 neutralizing titer that characterizes and/or is achieved by an RNA composition or method as described herein can reach at least 1.5-fold, including, at least 2-fold, at least 2.5-fold, at least 3-fold, or higher, that of a COVID-19 convalescent human panel (e.g., a panel of sera from COVID-19 convalescing humans obtained 20-40 days after the onset of symptoms and at least 14 days after the start of asymptomatic convalescence.

In some embodiments, RNA compositions as provided herein may be characterized in that subjects who have been treated with such compositions (e.g., with at least one dose, at least two doses, etc) may show reduced and/or more transient presence of viral RNA in relevant site(s) (e.g., nose and/or lungs, etc, and/or any other tissue susceptible to infection) as compared with an appropriate control (e.g., an established expected level for a comparable subject or population not having been so treated and having been exposed to virus under reasonably comparable exposure conditions)

In some embodiments, the RBD antigen expressed by an mRNA construct (e.g., as described herein) can be modified by addition of a T4-fibritin-derived "foldon" trimerization domain, for example, to increase its immunogenicity.

In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that certain local reactions (e.g., pain, redness, and/or swelling, etc.) and/or systemic events (e.g., fever, fatigue, headache, etc.) may appear and/or peak at Day 2 after vaccination. In some embodiments, RNA (e.g., mRNA) compositions described herein are characterized in that certain local reactions (e.g., pain, redness, and/or swelling, etc.) and/or systemic events (e.g., fever, fatigue, headache, etc.) may resolve by Day 7 after vaccination.

In some embodiments, RNA compositions (e.g., mRNA) and/or methods described herein are characterized in that no Grade 1 or greater change in routine clinical laboratory values or laboratory abnormalities are observed in subjects receiving RNA compositions (e.g., as described herein). Examples of such clinical laboratory assays may include lymphocyte count, hematological changes, etc.

In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that by 21 days after a first dose (e.g., 10-100 ug inclusive or 1 ug-50 ug inclusive), geometric mean concentrations (GMCs) of IgG directed to a SARS-CoV-2 S polypeptide or an immunogenic fragment thereof (e.g., RBD) may reach 200-3000 units/mL or 500-3000 units/mL or 500-2000 units/mL, compared to 602 units/mL for a panel of COVID-19 convalescent human sera. In some embodiments, RNA (e.g., mRNA) compositions described herein are characterized in that by 7 days after a second dose (e.g., 10-30 ug inclusive; or 1 ug-50 ug inclusive), geometric mean concentrations (GMCs) of IgG directed to a SARS-CoV-2 spike polypeptide or an immunogenic fragment thereof (e.g., RBD) may increase by at least 8-fold or higher, including, e.g., at least 9-fold, at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 30-fold, at least 35-fold, at least 40-fold, or higher. In some embodiments, RNA (e.g., mRNA) compositions described herein are characterized in that by 7 days after a second dose (e.g., 10-30 ug inclusive; or 1 ug-50 ug inclusive), geometric mean concentrations (GMCs) of IgG directed to a SARS-CoV-2 S polypeptide or an immunogenic fragment thereof (e.g., RBD) may increase to 1500 units/mL to 40,000 units/mL or 4000 units/mL to 40,000 units/mL. In some embodiments, antibody concentrations described herein can persist to at least 20 days or longer, including, e.g., at least 25 days, at least 30 days, at least 35 days, at least 40 days, at least 45 days, at least 50 days, after a first dose, or at least 10 days or longer, including, e.g., at least 15 days, at least 20 days, at least 25 days, or longer, after a second dose. In some embodiments, antibody concentrations can persist to 35 days after a first dose, or at least 14 days after a second dose.

In some embodiments, RNA (e.g., mRNA) compositions described herein are characterized in that when measured at 7 days after a second dose (e.g., 1-50 ug inclusive), GMC of IgG directed to a SARS-CoV-2 S polypeptide or an immunogenic fragment thereof (e.g., RBD) is at least 30% higher (including, e.g., at least 40% higher, at least 50% higher, at least 60%, higher, at least 70% higher, at least 80% higher, at least 90% higher, at least 95 % higher, as compared to antibody concentrations observed in a panel of COVID-19 convalescent human serum. In many embodiments, geometric mean concentration (GMC) of IgG described herein is GMCs of RBD-binding IgG.

In some embodiments, RNA (e.g., mRNA) compositions described herein are characterized in that when measured at 7 days after a second dose (e.g., 10-50 ug inclusive), GMC of IgG directed to a SARS-CoV-2 S polypeptide or an immunogenic fragment thereof (e.g., RBD) is at least 1.1-fold higher (including, e.g., at least 1.5-fold, at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold higher, at least 7-fold higher, at least 8-fold higher, at least 9-fold higher, at least 10-fold higher, at least 15-fold higher, at least 20-fold higher, at least 25-fold higher, at least 30-fold higher), as compared to antibody concentrations observed in a panel of COVID-19 convalescent human serum, In many embodiments, geometric mean concentration (GMC) of IgG described herein is GMCs of RBD-binding IgG.

In some embodiments, RNA (e.g., mRNA) compositions described herein are characterized in that when measured at 21 days after a second dose, GMC of IgG directed to a SARS-CoV-2 S polypeptide or an immunogenic fragment thereof (e.g., RBD) is at least 5-fold higher (including, e.g., at least 6-fold higher, at least 7-fold higher, at least 8-fold higher, at least 9-fold higher, at least 10-fold higher, at least 15-fold higher, at least 20-fold higher, at least 25-fold higher, at least 30-fold higher), as compared to antibody concentrations observed in a panel of COVID-19 convalescent human serum, In many embodiments, geometric mean concentration (GMC) of IgG described herein is GMCs of RBD-binding IgG.

In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that an increase (e.g., at least 30%, at least 40%, at least 50%, or more) in SARS-CoV-2 neutralizing geometric mean titers (GMTs) is observed 21 days after a first dose.

In some embodiments, RNA (e.g., mRNA) compositions described herein are characterized in that a substantially greater serum neutralizing GMTs are achieved 7 days after subjects receive a second dose (e.g., 10 μg-30 μg inclusive), reaching 150-300, compared to 94 for a COVID-19 convalescent serum panel.

In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that 7 days after administration of the second dose, the protective efficacy is at least 60%, e.g., at least 70%, at least 80%, at least 90, or at least 95%. In one embodiment, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that 7 days after administration of the second dose, the protective efficacy is at least 70%. In one embodiment, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that 7 days after administration of the second dose, the protective efficacy is at least 80%. In one embodiment, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that 7 days after administration of the second dose, the protective efficacy is at least 90%. In one embodiment, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that 7 days after administration of the second dose, the protective efficacy is at least 95%.

In some embodiments, an RNA composition provided herein is characterized in that it induces an immune response against SARS-CoV-2 after at least 7 days after a dose (e.g., after a second dose). In some embodiments, an RNA composition provided herein is characterized in that it induces an immune response against SARS-CoV-2 in less than 14 days after a dose (e.g., after a second dose). In some embodiments, an RNA composition provided herein is characterized in that it induces an immune response against SARS-CoV-2 after at least 7 days after a vaccination regimen. In some embodiments, a vaccination regimen comprises a first dose and a second dose. In some embodiments, a first dose and a second dose are administered by at least 21 days apart. In some such embodiments, an immune response against SARS-CoV-2 is induced at least after 28 days after a first dose.

In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that geometric mean concentration (GMCs) of antibodies directed to a SARS-CoV-2 spike polypeptide or an immunogenic fragment thereof (e.g., RBD), as measured in serum from subjects receiving RNA (e.g., mRNA) compositions of the present disclosure (e.g., at a dose of 10-30 ug inclusive), is substantially higher than in a convalescent serum panel (e.g., as described herein). In some embodiments where a subject may receive a second dose (e.g., 21 days after 1 first dose), geometric mean concentration (GMCs) of antibodies directed to a SARS-CoV-2 spike polypeptide or an immunogenic fragment thereof (e.g., RBD), as measured in serum from the subject, may be 8.0-fold to 50-fold higher than a convalescent serum panel GMC. In some embodiments where a subject may receive a second dose (e.g., 21

days after 1 first dose), geometric mean concentration (GMCs) of antibodies directed to a SARS-CoV-2 spike polypeptide or an immunogenic fragment thereof (e.g., RBD), as measured in serum from the subject, may be at least 8.0-fold or higher, including, e.g., at least 10-fold, at least 20-fold, at least 30-fold, at least 40-fold, at least 50-fold, at least 60-fold or higher, as compared to a convalescent serum panel GMC.

In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that the SARS-CoV-2 neutralizing geometric mean titer, as measured at 28 days after a first dose or 7 days after a second dose, may be at least 1.5-fold or higher (including, e.g., at least 2-fold, at least 2.5-fold, at least 3-fold, at least 3.5-fold or higher), as compared to a neutralizing GMT of a convalescent serum panel.

In some embodiments, a regimen administered to a subject may be or comprise a single dose. In some embodiments, a regimen administered to a subject may comprise a plurality of doses (e.g., at least two doses, at least three doses, or more). In some embodiments, a regimen administered to a subject may comprise a first dose and a second dose, which are given at least 2 weeks apart, at least 3 weeks apart, at least 4 weeks apart, or more. In some embodiments, such doses may be at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 12 months, or more apart. In some embodiments, doses may be administered days apart, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 ,10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60 or more days apart. In some embodiments, doses may be administered about 1 to about 3 weeks apart, or about 1 to about 4 weeks apart, or about 1 to about 5 weeks apart, or about 1 to about 6 weeks apart, or about 1 to more than 6 weeks apart. In some embodiments, doses may be separated by a period of about 7 to about 60 days, such as for example about 14 to about 48 days, etc. In some embodiments, a minimum number of days between doses may be about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or more. In some embodiments, a maximum number of days between doses may be about 60, 59, 58, 57, 56, 55, 54, 53, 52, 51, 50, 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, or fewer. In some embodiments, doses may be about 21 to about 28 days apart. In some embodiments, doses may be about 19 to about 42 days apart. In some embodiments, doses may be about 7 to about 28 days apart. In some embodiments,

doses may be about 14 to about 24 days. In some embodiments, doses may be about 21 to about 42 days.

In some embodiments, particularly for compositions established to achieve elevated antibody and/or T-cell titres for a period of time longer than about 3 weeks – e.g., in some embodiments, a provided composition is established to achieve elevated antibody and/or T-cell titres (e.g., specific for a relevant portion of a SARS-CoV-2 spike protein) for a period of time longer than about 3 weeks; in some such embodiments, a dosing regimen may involve only a single dose, or may involve two or more doses, which may, in some embodiments, be separated from one another by a period of time that is longer than about 21 days or three weeks. For example, in some such embodiments, such period of time may be about 4 weeks, 5 weeks, 6 weeks 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, 15 wees, 16 weeks, 17 weeks, 18 weeks, 19 weeks, 20 weeks or more, or about 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10, months, 11 months, 12 months or more, or in some embodiments about a year or more.

In some embodiments, a first dose and a second dose (and/or other subsequent dose) may be administered by intramuscular injection. In some embodiments, a first dose and a second dose may be administered in the deltoid muscle. In some embodiments, a first dose and a second dose may be administered in the same arm. In some embodiments, an RNA (e.g., mRNA) composition described herein is administered (e.g., by intramuscular injection) as a series of two doses (e.g., 0.3 mL each) 21 days part. In some embodiments, each dose is about 30 ug. In some embodiments, each dose may be higher than 30 ug, e.g., about 40 ug, about 50 ug, about 60 ug. In some embodiments, each dose may be lower than 30 ug, e.g., about 20 ug, about 10 ug, about 5 ug, etc. In some embodiments, each dose is about 3 ug or lower, e.g., about 1 ug. In some such embodiments, an RNA (e.g., mRNA) composition described herein is administered to subjects of age 16 or older (including, e.g., 16-85 years). In some such embodiments, an RNA composition (e.g., mRNA) described herein is administered to subjects of age 18-55. In some such embodiments, an RNA composition (e.g., mRNA) described herein is administered to subjects of age 56-85. In some embodiments, an RNA (e.g., mRNA) composition described herein is administered (e.g., by intramuscular injection) as a single dose.

In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that RBD-specific IgG (e.g., polyclonal response) induced by such RNA

compositions and/or methods exhibit a higher binding affinity to RBD, as compared to a reference human monoclonal antibody with SARS-CoV-2 RBD-binding affinity (e.g., CR3022 as described in J. ter Meulen et al., PLOS Med. 3, e237 (2006).)

In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity across a panel (e.g., at least 10, at least 15, or more) of SARs-CoV-2 spike variants (e.g., across a panel of variants described herein). In some embodiments, such SARs-CoV-2 spike variants include mutations in RBD (e.g., but not limited to Q321L, V341I, A348T, N354D, S359N, V367F, K378R, R408I, Q409E, A435S, N439K, K458R, I472V, G476S, S477N, V483A, Y508H, H519P, etc., as compared to SEQ ID NO: 1), and/or mutations in spike protein (e.g., but not limited to D614G, etc., as compared to SEQ ID NO: 1). Those skilled in the art are aware of various spike variants, and/or resources that document them (e.g., the Table of mutating sites in Spike maintained by the COVID-19 Viral Genome Analysis Pipeline and found at https://cov.lanl.gov/components/sequence/COV/int_sites_tbls.comp) (last accessed 24 Aug 2020), and, reading the present specification, will appreciate that RNA (e..g, mRNA) compositions and/or methods described herein can be characterized for their ability to induce sera in vaccinated subject that display neutralizing activity with respect to any or all of such variants and/or combinations thereof.

In particular embodiments, RNA (e.g., mRNA) compositions encoding RBD of a SARS-CoV-2 spike protein are characterized in that sera of vaccinated subjects display neutralizing activity across a panel (e.g., at least 10, at least 15, or more) of SARs-CoV-2 spike variants including RBD variants (e.g., but not limited to Q321L, V341I, A348T, N354D, S359N, V367F, K378R, R408I, Q409E, A435S, N439K, K458R, I472V, G476S, S477N, V483A, Y508H, H519P, etc., as compared to SEQ ID NO: 1) and spike protein variants (e.g., but not limited to D614G, as compared to SEQ ID NO: 1).

In particular embodiments, RNA (e.g., mRNA) compositions encoding a SARS-CoV-2 spike protein variant that includes two consecutive proline substitutions at amino acid positions 986 and 987, at the top of the central helix in the S2 subunit, are characterized in that sera of vaccinated subjects display neutralizing activity across a panel (e.g., at least 10, at least 15, or more) of SARs-CoV-2 spike variants including RBD variants (e.g., but not limited to Q321L, V341I, A348T, N354D, S359N, V367F, K378R, R408I, Q409E, A435S, N439K, K458R, I472V, G476S, S477N, V483A, Y508H, H519P, etc., as compared to SEQ ID NO: 1) and spike protein

variants (e.g., but not limited to D614G, as compared to SEQ ID NO: 1). For example, in some embodiments, an RNA (e.g., mRNA) composition encoding SEQ ID NO: 7 (S P2) elicits an immune response against any one of a SARs-CoV-2 spike variant including RBD variants (e.g., but not limited to Q321L, V341I, A348T, N354D, S359N, V367F, K378R, R408I, Q409E, A435S, N439K, K458R, I472V, G476S, S477N, V483A, Y508H, H519P, etc., as compared to SEQ ID NO: 1) and spike protein variants (e.g., but not limited to D614G, as compared to SEQ ID NO: 1).

In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a mutation at position 501 in spike protein as compared to SEQ ID NO: 1. In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a N501Y mutation in spike protein as compared to SEQ ID NO: 1.

Said one or more SARs-CoV-2 spike variants including a mutation at position 501 in spike protein as compared to SEQ ID NO: 1 or said one or more SARs-CoV-2 spike variants including a N501Y mutation in spike protein as compared to SEQ ID NO: 1 may include one or more further mutations as compared to SEQ ID NO: 1 (e.g., but not limited to H69/V70 deletion, Y144 deletion, A570D, D614G, P681H, T716I, S982A, D1118H, D80A, D215G, E484K, A701V, L18F, R246I, K417N, L242/A243/L244 deletion etc., as compared to SEQ ID NO: 1).

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant "Variant of Concern 202012/01" (VOC-202012/01; also known as lineage B.1.1.7). The variant had previously been named the first Variant Under Investigation in December 2020 (VUI – 202012/01) by Public Health England, but was reclassified to a Variant of Concern (VOC-202012/01). VOC-202012/01 is a variant of SARS-CoV-2 which was first detected in October 2020 during the COVID-19 pandemic in the United Kingdom from a sample taken the previous month, and it quickly began to spread by mid-December. It is correlated with a significant increase in the rate of COVID-19 infection in United Kingdom; this increase is thought to be at least partly because of change N501Y inside the spike glycoprotein's receptor-binding domain, which is needed for binding to ACE2 in human cells. The VOC-202012/01 variant is defined by 23 mutations: 13 non-synonymous mutations, 4 deletions, and 6 synonymous mutations (i.e., there are 17 mutations that change proteins and

six that do not). The spike protein changes in VOC 202012/01 include deletion 69-70, deletion 144, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H. One of the most important changes in VOC-202012/01 seems to be N501Y, a change from asparagine (N) to tyrosine (Y) at amino-acid site 501. This mutation alone or in combination with the deletion at positions 69/70 in the N terminal domain (NTD) may enhance the transmissibility of the virus.

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: deletion 69-70, deletion 144, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H as compared to SEQ ID NO: 1.

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant "501.V2". This variant was first observed in samples from October 2020, and since then more than 300 cases with the 501.V2 variant have been confirmed by whole genome sequencing (WGS) in South Africa, where in December 2020 it was the dominant form of the virus. Preliminary results indicate that this variant may have an increased transmissibility. The 501.V2 variant is defined by multiple spike protein changes including: D80A, D215G, E484K, N501Y and A701V, and more recently collected viruses have additional changes: L18F, R246I, K417N, and deletion 242-244.

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: D80A, D215G, E484K, N501Y and A701V as compared to SEQ ID NO: 1, and optionally: L18F, R246I, K417N, and deletion 242-244 as compared to SEQ ID NO: 1. Said SARs-CoV-2 spike variant may also include a D614G mutation as compared to SEQ ID NO: 1.

In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a H69/V70 deletion in spike protein as compared to SEQ ID NO: 1.

In some embodiments, one or more SARs-CoV-2 spike variants including a H69/V70 deletion in spike protein as compared to SEQ ID NO: 1 may include one or more further mutations as compared to SEQ ID NO: 1 (e.g., but not limited to Y144 deletion, N501Y, A570D, D614G,

P681H, T716I, S982A, D1118H, D80A, D215G, E484K, A701V, L18F, R246I, K417N, L242/A243/L244 deletion, Y453F, I692V, S1147L, M1229I etc., as compared to SEQ ID NO: 1),

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant "Variant of Concern 202012/01" (VOC-202012/01; also known as lineage B.1.1.7).

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: deletion 69-70, deletion 144, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H as compared to SEQ ID NO: 1.

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant "Cluster 5", also referred to as ΔFVI-spike by the Danish State Serum Institute (SSI). It was discovered in North Jutland, Denmark, and is believed to have been spread from minks to humans via mink farms. In cluster 5, several different mutations in the spike protein of the virus have been confirmed. The specific mutations include 69–70deltaHV (a deletion of the histidine and valine residues at the 69th and 70th position in the protein), Y453F (a change from tyrosine to phenylalanine at position 453), I692V (isoleucine to valine at position 692), M1229I (methionine to isoleucine at position 1229), and optionally S1147L (serine to leucine at position 1147).

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: deletion 69-70, Y453F, I692V, M1229I, and optionally S1147L, as compared to SEQ ID NO: 1.

In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a mutation at position 614 in spike protein as compared to SEQ ID NO: 1. In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a D614G mutation in spike protein as compared to SEQ ID NO: 1.

In some embodiments, one or more SARs-CoV-2 spike variants including a mutation at position 614 in spike protein as compared to SEQ ID NO: 1 or said one or more SARs-CoV-2 spike variants including a D614G mutation in spike protein as compared to SEQ ID NO: 1 may include one or more further mutations as compared to SEQ ID NO: 1 (e.g., but not limited to H69/V70 deletion, Y144 deletion, N501Y, A570D, P681H, T716I, S982A, D1118H, D80A, D215G, E484K, A701V, L18F, R246I, K417N, L242/A243/L244 deletion, Y453F, I692V, S1147L, M1229I etc., as compared to SEQ ID NO: 1).

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant "Variant of Concern 202012/01" (VOC-202012/01; also known as lineage B.1.1.7).

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: deletion 69-70, deletion 144, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H as compared to SEQ ID NO: 1.

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: D80A, D215G, E484K, N501Y, A701V, and D614G as compared to SEQ ID NO: 1, and optionally: L18F, R246I, K417N, and deletion 242-244 as compared to SEQ ID NO: 1.

In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a mutation at positions 501 and 614 in spike protein as compared to SEQ ID NO: 1. In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a N501Y mutation and a D614G mutation in spike protein as compared to SEQ ID NO: 1.

In some embodiments, one or more SARS-CoV-2 spike variants including a mutation at positions 501 and 614 in spike protein as compared to SEQ ID NO: 1 or said one or more SARs-CoV-2 spike variants including a N501Y mutation and a D614G mutation in spike protein as compared to SEQ ID NO: 1 may include one or more further mutations as compared to SEQ ID NO: 1 (e.g., but not limited to H69/V70 deletion, Y144 deletion, A570D, P681H, T716I, S982A,

D1118H, D80A, D215G, E484K, A701V, L18F, R246I, K417N, L242/A243/L244 deletion, Y453F, I692V, S1147L, M1229I etc., as compared to SEQ ID NO: 1).

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant "Variant of Concern 202012/01" (VOC-202012/01; also known as lineage B.1.1.7).

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: deletion 69-70, deletion 144, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H as compared to SEQ ID NO: 1.

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: D80A, D215G, E484K, N501Y, A701V, and D614G as compared to SEQ ID NO: 1, and optionally: L18F, R246I, K417N, and deletion 242-244 as compared to SEQ ID NO: 1.

In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a mutation at position 484 in spike protein as compared to SEQ ID NO: 1. In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a E484K mutation in spike protein as compared to SEQ ID NO: 1.

In some embodiments, one or more SARs-CoV-2 spike variants including a mutation at position 484 in spike protein as compared to SEQ ID NO: 1 or said one or more SARs-CoV-2 spike variants including a E484K mutation in spike protein as compared to SEQ ID NO: 1 may include one or more further mutations as compared to SEQ ID NO: 1 (e.g., but not limited to H69/V70 deletion, Y144 deletion, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H, D80A, D215G, A701V, L18F, R246I, K417N, L242/A243/L244 deletion, Y453F, I692V, S1147L, M1229I, T20N, P26S, D138Y, R190S, K417T, H655Y, T1027I, V1176F etc., as compared to SEQ ID NO: 1).

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant "501.V2".

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: D80A, D215G, E484K, N501Y, and A701V, as compared to SEQ ID NO: 1, and optionally: L18F, R246I, K417N, and deletion 242-244 as compared to SEQ ID NO: 1. Said SARs-CoV-2 spike variant may also include a D614G mutation as compared to SEQ ID NO: 1.

Lineage B.1.1.248, known as the Brazil(ian) variant, is one of the variants of SARS-CoV-2 which has been named P.1 lineage and has 17 unique amino acid changes, 10 of which in its spike protein, including N501Y and E484K. B.1.1.248 originated from B.1.1.28. E484K is present in both B.1.1.28 and B.1.1.248. B.1.1.248 has a number of S-protein polymorphisms [L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, H655Y, T1027I, V1176F] and is similar in certain key RBD positions (K417, E484, N501) to variant described from South Africa.

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant "B.1.1.28".

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant "B.1.1.248".

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, H655Y, T1027I, and V1176F as compared to SEQ ID NO: 1.

In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a mutation at positions 501 and 484 in spike protein as compared to SEQ ID NO: 1. In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a N501Y mutation and a E484K mutation in spike protein as compared to SEQ ID NO: 1.

In some embodiments, one or more SARs-CoV-2 spike variants including a mutation at positions 501 and 484 in spike protein as compared to SEQ ID NO: 1 or said one or more SARs-CoV-2 spike variants including a N501Y mutation and a E484K mutation in spike protein as

compared to SEQ ID NO: 1 may include one or more further mutations as compared to SEQ ID NO: 1 (e.g., but not limited to H69/V70 deletion, Y144 deletion, A570D, D614G, P681H, T716I, S982A, D1118H, D80A, D215G, A701V, L18F, R246I, K417N, L242/A243/L244 deletion, Y453F, I692V, S1147L, M1229I, T20N, P26S, D138Y, R190S, K417T, H655Y, T1027I, V1176F etc., as compared to SEQ ID NO: 1).

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant "501.V2".

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: D80A, D215G, E484K, N501Y and A701V as compared to SEQ ID NO: 1, and optionally: L18F, R246I, K417N, and deletion 242-244 as compared to SEQ ID NO: 1. Said SARs-CoV-2 spike variant may also include a D614G mutation as compared to SEQ ID NO: 1.

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant "B.1.1.248".

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, H655Y, T1027I, and V1176F as compared to SEQ ID NO: 1.

In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a mutation at positions 501, 484 and 614 in spike protein as compared to SEQ ID NO: 1. In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a N501Y mutation, a E484K mutation and a D614G mutation in spike protein as compared to SEQ ID NO: 1.

In some embodiments, one or more SARs-CoV-2 spike variants including a mutation at positions 501, 484 and 614 in spike protein as compared to SEQ ID NO: 1 or said one or more SARs-CoV-2 spike variants including a N501Y mutation, a E484K mutation and a D614G

mutation in spike protein as compared to SEQ ID NO: 1 may include one or more further mutations as compared to SEQ ID NO: 1 (e.g., but not limited to H69/V70 deletion, Y144 deletion, A570D, P681H, T716I, S982A, D1118H, D80A, D215G, A701V, L18F, R246I, K417N, L242/A243/L244 deletion, Y453F, I692V, S1147L, M1229I, T20N, P26S, D138Y, R190S, K417T, H655Y, T1027I, V1176F etc., as compared to SEQ ID NO: 1).

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: D80A, D215G, E484K, N501Y, A701V, and D614G as compared to SEQ ID NO: 1, and optionally: L18F, R246I, K417N, and deletion 242-244 as compared to SEQ ID NO: 1.

In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a L242/A243/L244 deletion in spike protein as compared to SEQ ID NO: 1.

In some embodiments, one or more SARs-CoV-2 spike variants including a L242/A243/L244 deletion in spike protein as compared to SEQ ID NO: 1 may include one or more further mutations as compared to SEQ ID NO: 1 (e.g., but not limited to H69/V70 deletion, Y144 deletion, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H, D80A, D215G, E484K, A701V, L18F, R246I, K417N, Y453F, I692V, S1147L, M1229I, T20N, P26S, D138Y, R190S, K417T, H655Y, T1027I, V1176F etc., as compared to SEQ ID NO: 1).

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant "501.V2".

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: D80A, D215G, E484K, N501Y, A701V and deletion 242-244 as compared to SEQ ID NO: 1, and optionally: L18F, R246I, and K417N, as compared to SEQ ID NO: 1. Said SARs-CoV-2 spike variant may also include a D614G mutation as compared to SEQ ID NO: 1.

In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a mutation at position 417 in spike protein as

compared to SEQ ID NO: 1. In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a K417N or K417T mutation in spike protein as compared to SEQ ID NO: 1.

In some embodiments, one or more SARs-CoV-2 spike variants including a mutation at position 417 in spike protein as compared to SEQ ID NO: 1 or said one or more SARs-CoV-2 spike variants including a K417N or K417T mutation in spike protein as compared to SEQ ID NO: 1 may include one or more further mutations as compared to SEQ ID NO: 1 (e.g., but not limited to H69/V70 deletion, Y144 deletion, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H, D80A, D215G, E484K, A701V, L18F, R246I, L242/A243/L244 deletion, Y453F, I692V, S1147L, M1229I, T20N, P26S, D138Y, R190S, H655Y, T1027I, V1176F etc., as compared to SEQ ID NO: 1).

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant "501.V2".

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: D80A, D215G, E484K, N501Y, A701V and K417N,, as compared to SEQ ID NO: 1, and optionally: L18F, R246I, and deletion 242-244 as compared to SEQ ID NO: 1. Said SARs-CoV-2 spike variant may also include a D614G mutation as compared to SEQ ID NO: 1.

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant "B.1.1.248".

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, H655Y, T1027I, and V1176F as compared to SEQ ID NO: 1.

In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a mutation at positions 417 and 484 and/or 501 in spike protein as compared to SEQ ID NO: 1. In some embodiments, RNA (e.g., mRNA)

compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a K417N or K417T mutation and a E484K and/or N501Y mutation in spike protein as compared to SEQ ID NO: 1.

In some embodiments, one or more SARs-CoV-2 spike variants including a mutation at positions 417 and 484 and/or 501 in spike protein as compared to SEQ ID NO: 1 or said one or more SARs-CoV-2 spike variants including a K417N or K417T mutation and a E484K and/or N501Y mutation in spike protein as compared to SEQ ID NO: 1 may include one or more further mutations as compared to SEQ ID NO: 1 (e.g., but not limited to H69/V70 deletion, Y144 deletion, A570D, D614G, P681H, T716I, S982A, D1118H, D80A, D215G, A701V, L18F, R246I, L242/A243/L244 deletion, Y453F, I692V, S1147L, M1229I, T20N, P26S, D138Y, R190S, H655Y, T1027I, V1176F etc., as compared to SEQ ID NO: 1).

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant "501.V2".

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: D80A, D215G, E484K, N501Y, A701V and K417N, as compared to SEQ ID NO: 1, and optionally: L18F, R246I, and deletion 242-244 as compared to SEQ ID NO: 1. Said SARs-CoV-2 spike variant may also include a D614G mutation as compared to SEQ ID NO: 1.

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant "B.1.1.248".

In particular embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, H655Y, T1027I, and V1176F as compared to SEQ ID NO: 1.

In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant of the Omicron (B.1.1.529) variant. Omicron (B.1.1.529) variant is a variant of SARS-CoV-2 which was detected in South Africa. Multiple Omicron variants or sublineages

have arisen, including e.g., the BA.1, BA.2, BA.2.12.1, BA.3, BA.4, BA.5, and BA.2.75 sublineages. As used herein, unless otherwise specified, "Omicron variant" refers to the first disclosed Omicron variant (BA.1) or any variant thereof that has since arisen (e.g., Omicron variants described herein). In some embodiments, the spike protein changes in Omicron (B.1.1.529) BA.1 variant include A67V, Δ69-70, T95I, G142D, Δ143-145, Δ211, L212I, ins214EPE (insertion of EPE following amino acid 214), G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, and L981F. In some embodiments, the spike protein changes in Omicron (B.1.1.529) variant include A67V, Δ69-70, T95I, G142D, Δ143-145, Δ211, L212I, ins214EPE (insertion of EPE following amino acid 214), G339D, S371L, S373P, S375F, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, and L981F. In some embodiments, the spike changes in Omicron BA.2 variant include T19I, Δ24-26, A27S, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K. In some embodiments BA.4 and BA.5 have the same Spike protein amino acid sequence, in which case "BA.4/5" is used to either Omicron variant. In some embodiments, the spike changes in Omicron BA.4/5 include: T19I, Δ24-26, A27S, Δ69/70, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452R, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, and N969K. In some embodiments, the spike changes in Omicron BA.2.75 include T19I, Δ24-26, A27S, G142D, K147E, W152R, F157L, I210V, V213G, G257S, G339H, N354D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, G446S, N460K, S477N, T478K, E484A, Q498R, N501Y,, Y505H D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, and N969K.

In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including at least 10, at least 15, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at least 32, at least 33, at least 34, at least 35, at least 36, or at least 37 of the following mutations: T547K, H655Y, D614G, N679K, P681H, N969K, S373P, S371L, N440K, G339D, G446S, N856K, N764K, K417N, D796Y, Q954H, T95I, A67V, L981F, S477N, G496S, T478K, Q498R, Q493R, E484A, N501Y, S375F, Y505H, V143del, H69del, V70del, N211del, L212I,

ins214EPE, G142D, Y144del, Y145del, L141del, Y144F, Y145D, G142del, as compared to SEQ ID NO: 1.

In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including at least 10, at least 15, at least 20, at least 21, at least 22, at least 23, at least 24, or all of the following mutations: T547K, H655Y, D614G, N679K, P681H, N969K, S373P, S371L, N440K, G339D, G446S, N856K, N764K, K417N, D796Y, Q954H, T95I, A67V, L981F, S477N, G496S, T478K, Q498R, Q493R, E484A, as compared to SEQ ID NO: 1. Said SARs-CoV-2 spike variant may include at least 1, at least 2, at least 3, at least 4, at least 5, or all of the following mutations: N501Y, S375F, Y505H, V143del, H69del, V70del, as compared to SEQ ID NO: 1, and/or may include at least 1, at least 2, at least 3, at least 4, at least 5, or all of the following mutations: N211del, L212I, ins214EPE, G142D, Y144del, Y145del, as compared to SEQ ID NO: 1. In some embodiments, said SARs-CoV-2 spike variant may include at least 1, at least 2, at least 3, or all of the following mutations: L141del, Y144F, Y145D, G142del, as compared to SEQ ID NO: 1.

In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against a SARs-CoV-2 spike variant including at least 10, at least 15, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at least 32, at least 33 of the following mutations: A67V, Δ69-70, T95I, G142D, Δ143-145, Δ211, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, and L981F, as compared to SEQ ID NO: 1.

In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against a SARS-CoV-2 spike variant including at least 10, at least 15, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, or at least 31, of the following mutations: T19I, Δ24-26, A27S, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K, as compared to SEQ ID NO: 1.

In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against a SARS-CoV-2 spike variant including at least 10, at least 15, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at least 32, at least 33, or at least 34 of the following mutations: T19I, Δ24-26, A27S, Δ69/70, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452R, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, and N969K, as compared to SEQ ID NO: 1.

In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against a SARs-CoV-2 spike variant including the following mutations: A67V, Δ69-70, T95I, G142D, Δ143-145, Δ211, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, and L981F, as compared to SEQ ID NO: 1.

In some emdbodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizating against a SARS-CoV-2 spike variant including the following mutations: T19I, Δ24-26, A27S, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K. In some emdbodiments, RNA (e.g., mRNA) compositions and/or methods described herein are charcterized in that sera of vaccinated subjects display neutralizating against SARS-CoV2 spike variant including the following mutations: T19I, Δ24-26, A27S, Δ69/70, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452R, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, and N969K, as compared to SEQ ID NO: 1.

In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: A67V, Δ69-70, T95I, G142D, Δ143-145, Δ211, L212I, ins214EPE, G339D, S371L, S373P, S375F, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, and L981F, as compared to SEQ ID NO: 1.

In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: T19I, Δ24-26, A27S, G142D, K147E, W152R, F157L, I210V, V213G, G257S, G339H, N354D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, G446S, N460K, S477N, T478K, E484A, Q498R, N501Y, Y505H D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, and N969K, as compared to SEQ ID NO: 1.

SARs-CoV-2 spike proteins encoded by RNA described herein may or may not include a D614G mutation as compared to SEQ ID NO: 1.

In some embodiments, SARS-CoV-2 spike proteins encoded by RNA described herein comprise a mutation in a furin cleavage site (e.g., in some embodiments residues 682-685 of SEQ ID NO: 1). In some embodiments, SARS-CoV-2 spike proteins encoded by RNA described herein comprise a mutation in the furin cleavage site that prevents cleavage by a furin protease (e.g., a human furin protease). In some embodiments, a SARS-CoV-2 protein described herein comprises a furin mutation disclosed in WO2021163365 or WO2021243122 (e.g., a GSAS mutation), the contents of both of which are incorporated by reference herein in their entirety.

In some embodiments, RNA (e.g., mRNA) compositions and/or methods described herein can provide protection against SARS-CoV-2 and/or decrease severity of SARS-CoV-2 infection in at least 50% of subjects receiving such RNA compositions and/or methods.

In some embodiments, populations to be treated with RNA (e.g., mRNA) compositions described herein include subjects of age 18-55. In some embodiments, populations to be treated with RNA (e.g., mRNA) compositions described herein include subjects of age 56-85. In some embodiments, populations to be treated with RNA (e.g., mRNA) compositions described herein include older subjects (e.g., over age 60, 65, 70, 75, 80, 85, etc, for example subjects of age 65-85). In some embodiments, populations to be treated with RNA (e.g., mRNA) compositions described herein include subjects of age 18-85. In some embodiments, populations to be treated with RNA (e.g., mRNA) compositions described herein include subjects of age 18 or younger. In some embodiments, populations to be treated with RNA (e.g., mRNA) compositions described herein include subjects of age 12 or younger. In some embodiments, populations to be treated with RNA (e.g., mRNA) compositions described herein include subjects of age 10 or younger. In some embodiments, populations to be treated with RNA (e.g., mRNA) compositions described herein may include adolescent populations

(e.g., individuals approximately 12 to approximately 17 years of age). In some embodiments, populations to be treated with RNA (e.g., mRNA) compositions described herein may include pediatric populations (e.g., as described herein). In some embodiments, populations to be treated with RNA (e.g., mRNA) compositions described herein include infants (e.g., less than 1 year old). In some embodiments, populations to be treated with RNA (e.g., mRNA) compositions described herein do not include infants (e.g., less than 1 year) whose mothers have received such RNA (e.g., mRNA) compositions described herein during pregnancy. Without wishing to be bound by any particular theory, a rat study has suggested that a SARS-CoV-2 neutralizing antibody response induced in female rats given such RNA compositions during pregnancy can pass onto fetuses. In some embodiments, populations to be treated with RNA (e.g., mRNA) compositions described herein include infants (e.g., less than 1 year) whose mothers did not receive such RNA compositions described herein during pregnancy. In some embodiments, populations to be treated with RNA (e.g., mRNA) compositions described herein may include pregnant women; in some embodiments, infants whose mothers were vaccinated during pregnancy (e.g., who received at least one dose, or alternatively only who received both doses), are not vaccinated during the first weeks, months, or even years (e.g., 1, 2, 3, 4, 5, 6, 7, 8 weeks or more, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 months or more, or 1, 2, 3, 4, 5 years or more) post-birth. Alternatively or additionally, in some embodiments, infants whose mothers were vaccinated during pregnancy (e.g., who received at least one dose, or alternatively only who received both doses), receive reduced vaccination (e.g., lower doses and/or smaller numbers of administrations – e.g., boosters – and/or lower total exposure over a given period of time) after birth, for example during the first weeks, months, or even years (e.g., 1, 2, 3, 4, 5, 6, 7, 8 weeks or more, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 months or more, or 1, 2, 3, 4, 5 years or more) post-birth or may need reduced vaccination (e.g., lower doses and/or smaller numbers of administrations – e.g., boosters – over a given period of time), In some embodiments, compositions as provided herein are administered to populations that do not include pregnant women.

In some particular embodiments, compositions as provided herein are administered to pregnant women according to a regimen that includes a first dose administered after about 24 weeks of gestation (e.g., after about 22, 23, 24, 25, 26, 27, 28 or more weeks of gestation); in some embodiments, compositions as provided herein are administered to pregnant women

according to a regimen that includes a first dose administered before about 34 weeks of gestation (e.g., before about 30, 31, 32, 33, 34, 35, 36, 37, 38 weeks of gestation). In some embodiments, compositions as provided herein are administered to pregnant women according to a regimen that includes a first dose administered after about 24 weeks (e.g., after about 27 weeks of gestation, e.g., between about 24 weeks and 34 weeks, or between about 27 weeks and 34 weeks) of gestation and a second dose administered about 21 days later; in some embodiments both doses are administered prior to delivery. Without wishing to be bound by any particular theory, it is proposed that such a regimen (e.g., involving administration of a first dose after about 24 weeks, or 27 weeks of gestation and optionally before about 34 weeks of gestation), and optionally a second dose within about 21 days, ideally before delivery, may have certain advantages in terms of safety (e.g., reduced risk of premature delivery or of fetal morbidity or mortality) and/or efficacy (e.g., carryover vaccination imparted to the infant) relative to alternative dosing regimens (e.g., dosing at any time during pregnancy, refraining from dosing during pregnancy, and/or dosing later in pregnancy for example so that only one dose is administered during gestation. In some embodiments, infants born of mothers vaccinated during pregnancy, e.g., according to a particular regimen as described herein, may not need further vaccination, or may need reduced vaccination (e.g., lower doses and/or smaller numbers of administrations – e.g., boosters –, and/or lower overall exposure over a given period of time), for a period of time (e.g., as noted herein) after birth.

In some embodiments, compositions as provided herein are administered to populations in which women are advised against becoming pregnant for a period of time after receipt of the vaccine (e.g., after receipt of a first dose of the vaccine, after receipt of a final dose of the vaccine, etc.); in some such embodiments, the period of time may be at least 1 week, at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 5 weeks, at least 6 weeks, at least 7 weeks, at least 8 weeks, at least 9 weeks, at least 10 weeks or more, or may be at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, or more.

In some embodiments, populations to be treated with RNA (e.g., mRNA) compositions described herein may include one or more populations with one or more particularly high risk conditions or history, e.g., as noted herein. For example, in some embodiments, populations to be treated with RNA compositions described herein may include subjects whose profession

and/or environmental exposure may dramatically increase their risk of getting SARS-CoV-2 infection (including, e.g., but not limited to mass transportation, prisoners, grocery store workers, residents in long-term care facilities, butchers or other meat processing workers, healthcare workers, and/or first responders, e.g., emergency responders). In particular embodiments, populations to be treated with RNA (e.g., mRNA) compositions described herein may include healthcare workers and/or first responders, e.g., emergency responders. In some embodiments, populations to be treated with RNA (e.g., mRNA) compositions described herein may include those with a history of smoking or vaping (e.g., within 6 months, 12 months or more, including a history of chronic smoking or vaping). In some embodiments, populations to be treated with RNA (e.g., mRNA) compositions described herein may include certain ethnic groups that have been determined to be more susceptible to SARS-CoV-2 infection.

In some embodiments, populations to be treated with RNA (e.g., mRNA) compositions described herein may include certain populations with a blood type that may have been determined to more susceptible to SARS-CoV-2 infection. In some embodiments, populations to be treated with RNA (e.g., mRNA) compositions described herein may include immunocompromised subjects (e.g., those with HIV/AIDS; cancer patients (e.g., receiving antitumor treatment); patients who are taking certain immunosuppressive drugs (e.g., transplant patients, cancer patients, etc.); autoimmune diseases or other physiological conditions expected to warrant immunosuppressive therapy (e.g., within 3 months, within 6 months, or more); and those with inherited diseases that affect the immune system (e.g., congenital agammaglobulinemia, congenital IgA deficiency)). In some embodiments, populations to be treated with RNA (e.g., mRNA) compositions described herein may include those with an infectious disease. For example, in some embodiments, populations to be treated with RNA (e.g., mRNA) compositions described herein may include those infected with human immunodeficiency virus (HIV) and/or a hepatitis virus (e.g., HBV, HCV). In some embodiments, populations to be treated with RNA (e.g., mRNA) compositions described herein may include those with underlying medical conditions. Examples of such underlying medical conditions may include, but are not limited to hypertension, cardiovascular disease, diabetes, chronic respiratory disease, e.g., chronic pulmonary disease, asthma, etc., cancer, and other chronic diseases such as, e.g., lupus, rheumatoid arthritis, chronic liver diseases, chronic kidney diseases (e.g., Stage 3 or worse such as in some embodiments as characterized

by a glomerular filtration rate (GFR) of less than 60 mL/min/1.73m^2). In some embodiments, populations to be treated with RNA (e.g., mRNA) compositions described herein may include overweight or obese subjects, e.g., specifically including those with a body mass index (BMI) above about 30 kg/m^2. In some embodiments, populations to be treated with RNA (e.g., mRNA) compositions described herein may include subjects who have prior diagnosis of COVID-19 or evidence of current or prior SARS-CoV-2 infection, e.g., based on serology or nasal swab. In some embodiments, populations to be treated include white and/or non-Hispanic/non-Latino.

In some embodiments, certain RNA (e.g., mRNA) compositions described herein may be selected for administration to Asian populations (*e.g.*, Chinese populations), or in particular embodiments to older Asian populations (*e.g.*, 60 years old or over, e.g., 60-85 or 65-85 years old).

In some embodiments, an RNA (e.g., mRNA) composition as provided herein is administered to and/or assessed in subject(s) who have been determined not to show evidence of prior infection, and/or of present infection, before administration; in some embodiments, evidence of prior infection and/or of present infection, may be or include evidence of intact virus, or any viral nucleic acid, protein, lipid etc. present in the subject (e.g., in a biological sample thereof, such as blood, cells, mucus, and/or tissue), and/or evidence of a subject's immune response to the same. In some embodiments, an RNA (e.g., mRNA) composition as provided herein is administered to and/or assessed in subject(s) who have been determined to show evidence of prior infection, and/or of present infection, before administration; in some embodiments, evidence of prior infection and/or of present infection, may be or include evidence of intact virus, or any viral nucleic acid, protein, lipid etc. present in the subject (e.g., in a biological sample thereof, such as blood, cells, mucus, and/or tissue), and/or evidence of a subject's immune response to the same. In some embodiments, a subject is considered to have a prior infection based on having a positive N-binding antibody test result or positive nucleic acid amplification test (NAAT) result on the day of Dose 1.

In some embodiments, an RNA (e.g., mRNA) composition as provided herein is administered to a subject who has been informed of a risk of side effects that may include one or more of, for example: chills, fever, headache, injection site pain, muscle pain, tiredness; in some embodiments, an RNA (e.g., mRNA) composition is administered to a subject who has been invited to notify a healthcare provider if one or more such side effects occurs, is experienced

as more than mild or moderate, persists for a period of more than a day or a few days, or if any serious or unexpected event is experienced that the subject reasonably considers may be associated with receipt of the composition. In some embodiments, an RNA (e.g., mRNA) composition as provided herein is administered to a subject who has been invited to notify a healthcare provider of particular medical conditions which may include, for example, one or more of allergies, bleeding disorder or taking a blood thinner medication, breastfeeding, fever, immunocompromised state or taking medication that affects the immune system, pregnancy or plan to become pregnant, etc. In some embodiments, an RNA (e.g., mRNA) composition as provided herein is administered to a subject who has been invited to notify a healthcare provider of having received another COVID-19 vaccine. In some embodiments, an RNA (e.g., mRNA) composition as provided herein is administered to a subject not having one of the following medical conditions: experiencing febrile illness, receiving immunosuppressant therapy, receiving anticoagulant therapy, suffering from a bleeding disorder (e.g., one that would contraindicate intramuscular injection), or pregnancy and/or breastfeeding/lactation. In some embodiments, an RNA (e.g., mRNA) composition as provided herein is administered to a subject not having received another COVID-19 vaccine. In some embodiments, an RNA (e.g., mRNA) composition as provided herein is administered to a subject who has not had an allergic reaction to any component of the RNA (e.g., mRNA) composition. Examples of such allergic reaction may include, but are not limited to difficulty breathing, swelling of fact and/or throat, fast heartbeat, rash, dizziness and/or weakness. In some embodiments, an RNA (e.g., mRNA) composition as provided herein is administered to a subject who received a first dose and did not have an allergic reaction (e.g., as described herein) to the first dose. In some embodiments where allergic reaction occurs in subject(s) after receiving a dose of an RNA (e.g., mRNA) composition as provided herein, such subject(s) may be administered one or more interventions such as treatment to manage and/or reduce symptom(s) of such allergic reactions, for example, fever-reducing and/or anti-inflammatory agents.

In some embodiments, a subject who has received at least one dose of an RNA (e.g., mRNA) composition as provided herein is informed of avoiding being exposed to a coronavirus (e.g., SARS-CoV-2) unless and until several days (e.g., at least 7 days, at least 8 days, 9 days, at least 10 days, at least 11 days, at least 12 days, at least 13 days, at least 14 days, etc.) have passed since administration of a second dose. For example, a subject who has received at least one dose of an RNA (e.g., mRNA) composition as provided herein is informed of taking

precautionary measures against SARS-CoV-2 infection (e.g., remaining socially distant, wearing masks, frequent hand-washing, etc.) unless and until several days (e.g., at least 7 days, at least 8 days, 9 days, at least 10 days, at least 11 days, at least 12 days, at least 13 days, at least 14 days, etc.) have passed since administration of a second dose. Accordingly, in some embodiments, methods of administering an RNA (e.g., mRNA) composition as provided herein comprise administering a second dose of such an RNA (e.g., mRNA) composition as provided herein to a subject who received a first dose and took precautionary measures to avoid being exposed to a coronavirus (e.g., SARS-CoV-2).

In some embodiments, RNA (e.g., mRNA) compositions described herein may be delivered to a draining lymph node of a subject in need thereof, for example, for vaccine priming. In some embodiments, such delivery may be performed by intramuscular administration of a provided RNA (e.g., mRNA) composition.

In some embodiments, different particular RNA (e.g., mRNA) compositions may be administered to different subject population(s); alternatively or additionally, in some embodiments, different dosing regimens may be administered to different subject populations. For example, in some embodiments, RNA (e.g., mRNA) compositions administered to particular subject population(s) may be characterized by one or more particular effects (e.g., incidence and/or degree of effect) in those subject populations. In some embodiments, such effect(s) may be or comprise, for example titer and/or persistence of neutralizing antibodies and/or T cells (e.g., T_H1-type T cells such as $CD4^+$ and/or $CD8^+$ T cells), protection against challenge (e.g., via injection and/or nasal exposure, etc), incidence, severity, and/or persistence of side effects (e.g., reactogenicity), etc.

In some embodiments, one or more RNA (e.g., mRNA) compositions described herein may be administered according to a regimen established to reduce COVID-19 incidence per 1000 person-years, e.g., based on a laboratory test such as nucleic acid amplification test (NAAT). In some embodiments, one or more RNA (e.g., mRNA) compositions described herein may be administered according to a regimen established to reduce COVID-19 incidence per 1000 person-years based on a laboratory test such as nucleic acid amplification test (NAAT) in subjects receiving at least one dose of a provided RNA (e.g., mRNA) composition with no serological or virological evidence (e.g., up to 7 days after receipt of the last dose) of past SARS-CoV-2 infection. In some embodiments, one or more RNA (e.g., mRNA) compositions described herein may be administered according to a regimen established to reduce

confirmed severe COVID-19 incidence per 1000 person-years. In some embodiments, one or more RNA (e.g., mRNA) compositions described herein may be administered according to a regimen established to reduce confirmed severe COVID-19 incidence per 1000 person-years in subjects receiving at least one dose of a provided RNA (e.g., mRNA) composition with no serological or virological evidence of past SARS-CoV-2 infection.

In some embodiments, one or more RNA (e.g., mRNA) compositions described herein may be administered according to a regimen established to produce neutralizing antibodies directed to a SARS-CoV-2 spike polypeptide and/or an immunogenic fragment thereof (e.g., RBD) as measured in serum from a subject that achieves or exceeds a reference level (e.g., a reference level determined based on human SARS-CoV-2 infection/COVID-19 convalescent sera) for a period of time and/or induction of cell-mediated immune response (e.g., a T cell response against SARS-CoV-2), including, e.g., in some embodiments induction of T cells that recognize at least one or more MHC-restricted (e.g., MHC class I-restricted) epitopes within a SARS-CoV-2 spike polypeptide and/or an immunogenic fragment thereof (e.g., RBD) for a period of time. In some such embodiments, the period of time may be at least 2 months, 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 12 months or longer. In some embodiments, one or more epitopes recognized by vaccine-induced T cells (e.g., CD8+ T cells) may be presented on a MHC class I allele that is present in at least 50% of subjects in a population, including, e.g., at least 60%, at least 70%, at least 80%, at least 90%, or more; in some such embodiments, the MHC class I allele may be HLA-B*0702, HLA-A*2402, HLA-B*3501, HLA-B*4401, or HLA-A*0201. In some embodiments, an epitope may comprise HLA-A*0201 YLQPRTFLL; HLA-A*0201 RLQSLQTYV; HLA-A*2402 QYIKWPWYI; HLA-A*2402 NYNYLYRLF; HLA-A*2402 KWPWYIWLGF; HLA-B*3501 QPTESIVRF; HLA-B*3501 IPFAMQMAY; or HLA-B*3501 LPFNDGVYF.

In some embodiments, efficacy is assessed as COVID-19 incidence per 1000 person-years in individuals without serological or virological evidence of past SARS-CoV-2 infection before and during vaccination regimen; alternatively or additionally, in some embodiments, efficacy is assessed as COVID-19 incidence per 1000 person-years in subjects with and without evidence of past SARS-CoV-2 infection before and during vaccination regimen. In some such embodiments, such incidence is of COVID-19 cases confirmed within a specific time period after the final vaccination dose (e.g., a first dose in a single-dose regimen; a second dose in a

two-dose regimen, etc); in some embodiments, such time period may be within (i.e., up to and including 7 days) a particular number of days (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 days or more). In some embodiments, such time period may be within 7 days or within 14 days or within 21 days or within 28 days. In some embodiments, such time period may be within 7 days. In some embodiments, such time period may be within 14 days.

In some embodiments (e.g., in some embodiments of assessing efficacy), a subject is determined to have experienced COVID-19 infection if one or more of the following is established: detection of SARS-CoV-2 nucleic acid in a sample from the subject, detection of antibodies that specifically recognize SARS-CoV-2 (e.g., a SARS-Co-V-2 spike protein), one or more symptoms of COVID-19 infection, and combinations thereof. In some such embodiments, detection of SARS-CoV-2 nucleic acid may involve, for example, NAAT testing on a mid-turbinatae swap sample. In some such embodiments, detection of relevant antibodies may involve serological testing of a blood sample or portion thereof. In some such embodiments, symptoms of COVID-19 infection may be or include: fever, new or increased cough, new or increased shortness of breath, chills, new or increased muscle pain, new loss of taste or smell, sore throat, diarrhea, vomiting and combinations thereof. In some such embodiments, symptoms of COVID-19 infection may be or include: fever, new or increased cough, new or increased shortness of breath, chills, new or increased muscle pain, new loss of taste or smell, sore throat, diarrhea, vomiting, fatigue, headache, nasal congestion or runny nose, nausea, and combinations thereof. In some such embodiments, a subject is determined to have experienced COVID-19 infection if such subject both has experienced one such symptom and also has received a positive test for SARS-CoV-2 nucleic acid or antibodies, or both. In some such embodiments, a subject is determined to have experienced COVID-19 infection if such subject both has experienced one such symptom and also has received a positive test for SARS-CoV-2 nucleic acid. In some such embodiments, a subject is determined to have experienced COVID-19 infection if such subject both has experienced one such symptom and also has received a positive test for SARS-CoV-2 antibodies.

In some embodiments (e.g., in some embodiments of assessing efficacy), a subject is determined to have experienced severe COVID-19 infection if such subject has experienced one or more of: clinical signs at rest indicative or severe systemic illness (e.g., one or more of respiratory rate at greater than or equal to 30 breaths per minute, heart rate at or above 125

beats per minute, SpO_2 less than or equal to 93% on room air at sea level or a PaO_2/FiO_2 below 300 m Hg), respiratory failure (e.g., one or more of needing high-flow oxygen, noninvasive ventilation, mechanical ventilation, ECMO), evidence of shock (systolic blood pressure below 90 mm Hg, diastolic blood pressure below 60mm Hg, requiring vasopressors), significant acute renal, hepatic, or neurologic dysfunction, admission to an intensive care unit, death, and combinations thereof.

In some embodiments, one or more RNA (e.g., mRNA) compositions described herein may be administered according to a regimen established to reduce the percentage of subjects reporting at least one of the following: (i) one or more local reactions (e.g., as described herein) for up to 7 days following each dose; (ii) one or more systemic events for up to 7 days following each dose; (iii) adverse events (e.g., as described herein) from a first dose to 1 month after the last dose; and/or (iv) serious adverse events (e.g., as described herein) from a first dose to 6 months after the last dose.

In some embodiments, one or more subjects who have received an RNA (e.g., mRNA) composition as described herein may be monitored (e.g., for a period of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 days or more, including, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 weeks or more, including for example 1, 2, 3, 4, 5, 6, 7, 8, 9 ,10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 months or more, including for example 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 years or more) to assess, for example, presence of an immune response to component(s) of the administered composition, evidence of exposure to and/or immune response to SARS-CoV-2 or another coronavirus, evidence of any adverse event, etc. In some embodiments, monitoring may be via tele-visit. Alternatively or additionally, in some embodiments, monitoring may be in-person.

In some embodiments, a treatment effect conferred by one or more RNA (e.g., mRNA) compositions described herein may be characterized by (i) a SARS-CoV-2 anti-S1 binding antibody level above a pre-determined threshold; (ii) a SARS-CoV-2 anti-RBD binding antibody level above a pre-determined threshold; and/or (iii) a SARS-CoV-2 serum neutralizing titer above a threshold level, e.g., at baseline, 1 month, 3 months, 6 months, 9 months, 12 months, 18 months, and/or 24 months after completion of vaccination. In some embodiments, anti-S1 binding antibody and/or anti-RBD binding antibody levels and/or serum neutralizing titers may be characterized by geometric mean concentration (GMC), geometric mean titer (GMT), or geometric mean fold-rise (GMFR).

In some embodiments, a treatment effect conferred by one or more RNA (e.g., mRNA) compositions described herein may be characterized in that percentage of treated subjects showing a SARS-CoV-2 serum neutralizing titer above a pre-determined threshold, e.g., at baseline, 1 month, 3 months, 6 months, 9 months, 12 months, 18 months, and/or 24 months after completion of vaccination, is higher than the percentage of non-treated subjects showing a SARS-CoV-2 serum neutralizing titer above such a pre-determined threshold (e.g., as described herein). In some embodiments, a serum neutralizing titer may be characterized by geometric mean concentration (GMC), geometric mean titer (GMT), or geometric mean fold-rise (GMFR).

In some embodiments, a treatment effect conferred by one or more RNA (e.g., mRNA) compositions described herein may be characterized by detection of SARS-CoV-2 NVA-specific binding antibody.

In some embodiments, a treatment effect conferred by one or more RNA (e.g., mRNA) compositions described herein may be characterized by SARS-CoV-2 detection by nucleic acid amplification test.

In some embodiments, a treatment effect conferred by one or more RNA (e.g., mRNA) compositions described herein may be characterized by induction of cell-mediated immune response (e.g., a T cell response against SARS-CoV-2), including, e.g., in some embodiments induction of T cells that recognize at least one or more MHC-restricted (e.g., MHC class I-restricted) epitopes within a SARS-CoV-2 spike polypeptide and/or an immunogenic fragment thereof (e.g., RBD). In some embodiments, one or more epitopes recognized by vaccine-induced T cells (e.g., CD8+ T cells) may be presented on a MHC class I allele that is present in at least 50% of subjects in a population, including, e.g., at least 60%, at least 70%, at least 80%, at least 90%, or more; in some such embodiments, the MHC class I allele may be HLA-B*0702, HLA-A*2402, HLA-B*3501, HLA-B*4401, or HLA-A*0201. In some embodiments, an epitope may comprise HLA-A*0201 YLQPRTFLL; HLA-A*0201 RLQSLQTYV; HLA-A*2402 QYIKWPWYI; HLA-A*2402 NYNYLYRLF; HLA-A*2402 KWPWYIWLGF; HLA-B*3501 QPTESIVRF; HLA-B*3501 IPFAMQMAY; or HLA-B*3501 LPFNDGVYF.

In some embodiments, primary vaccine efficacy (VE) of one or more RNA (e.g., mRNA) compositions described herein may be established when there is sufficient evidence (posterior probability) that either primary VE1 or both primary VE1 and primary VE2 are >30% or higher (including, e.g., greater than 40%, greater than 50%, greater than 60%, greater than

70%, greater than 80%, greater than 90%, greater than 95%, greater than 96%, greater than 97%, greater than 98%, or higher), wherein primary VE is defined as primary VE = 100 × (1 – IRR); and IRR is calculated as the ratio of COVID-19 illness rate in the vaccine group to the corresponding illness rate in the placebo group. Primary VE1 represents VE for prophylactic RNA (e.g., mRNA) compositions described herein against confirmed COVID-19 in participants without evidence of infection before vaccination, and primary VE2 represents VE for prophylactic RNA (e.g., mRNA) compositions described herein against confirmed COVID-19 in all participants after vaccination. In some embodiments, primary VE1 and VE2 can be evaluated sequentially to control the overall type I error of 2.5% (hierarchical testing). In some embodiments where one or more RNA (e.g., mRNA) compositions described herein are demonstrated to achieve primary VE endponts as discussed above, secondary VE endpoints (e.g., confirmed severe COVID-19 in participants without evidence of infection before vaccination and confirmed severe COVID-19 in all participants) can be evaluated sequentially, e.g., by the same method used for the primary VE endpoint evaluation (hierarchical testing) as discussed above. In some embodiments, evaluation of primary and/or secondary VE endpoints may be based on at least 20,000 or more subjects (e.g., at least 25,000 or more subjects) randomized in a 1:1 ratio to the vaccine or placebo group, e.g., based on the following assumptions: (i) 1.0% illness rate per year in the placebo group, and (ii) 20% of the participants being non-evaluable or having serological evidence of prior infection with SARS-CoV-2, potentially making them immune to further infection.

In some embodiments, one or more RNA (e.g., mRNA) compositions described herein may be administered according to a regimen established to achieve maintenance and/or continued enhancement of an immune response. For example, in some embodiments, an administration regimen may include a first dose optionally followed by one or more subsequent doses; in some embodiments, need for, timing of, and/or magnitude of any such subsequent dose(s) may be selected to maintain, enhance, and/or modify one or more immune responses or features thereof. In some embodiments, number, timing, and/or amount(s) of dose(s) have been established to be effective when administered to a relevant population. In some embodiments, number, timing and/or amount(s) of dose(s) may be adjusted for an individual subject; for example, in some embodiments, one or more features of an immune response in an individual subject may be assessed at least once (and optionally more than once, for example multiple times, typically spaced apart, often at pre-selected intervals) after receipt

of a first dose. For example, presence of antibodies, B cells, and/or T cells (e.g., $CD4^+$ and/or $CD8^+$ T cells), and/or of cytokines secreted thereby and/or identity of and/or extent of responses to particular antigen(s) and/or epitope(s) may be assessed. In some embodiments, need for, timing of, and/or amount of a subsequent dose may be determined in light of such assessments.

As noted hereinabove, in some embodiments, one or more subjects who have received an RNA (e.g., mRNA) composition as described herein may be monitored (e.g., for a period of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 days or more, including, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 weeks or more, including for example 1, 2, 3, 4, 5, 6, 7, 8, 9 ,10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 months or more, including for example 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 years or more) from receipt of any particular dose to assess, for example, presence of an immune response to component(s) of the administered composition, evidence of exposure to and/or immune response to SARS-CoV-2 or another coronavirus, evidence of any adverse event, etc, including to perform assessment of one or more of presence of antibodies, B cells, and/or T cells (e.g., $CD4^+$ and/or $CD8^+$ T cells), and/or of cytokines secreted thereby and/or identity of and/or extent of responses to particular antigen(s) and/or epitope(s) may be assessed. Administration of a composition as described herein may be in accordance with a regimen that includes one or more such monitoring steps.

For example, in some embodiments, need for, timing of, and/or amount of a second dose relative to a first dose (and/or of a subsequent dose relative to a prior dose) is assessed, determined, and/or selected such that administration of such second (or subsequent) dose achieves amplification or modification of an immune response (e.g., as described herein) observed after the first (or other prior) dose. In some embodiments, such amplification of an immune response (e.g., ones described herein) may be at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or higher, as compared to the level of an immune response observed after the first dose. In some embodiments, such amplification of an immune response may be at least 1.5 fold, at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, at least 10-fold, at least 20-fold, at least 30-fold, or higher, as compared to the level of an immune response observed after the first dose.

In some embodiments, need for, timing of, and/or amount of a second (or subsequent) dose relative to a first (or other prior) dose is assessed, determined, and/or selected such that

administration of the later dose extends the durability of an immune response (e.g., as described herein) observed after the earlier dose; in some such embodiments, the durability may be extended by at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, or longer. In some embodiments, an immune response observed after the first dose may be characterized by production of neutralizing antibodies directed to a SARS-CoV-2 spike polypeptide and/or an immunogenic fragment thereof (e.g., RBD) as measured in serum from a subject and/or induction of cell-mediated immune response (e.g., a T cell response against SARS-CoV-2), including, e.g., in some embodiments induction of T cells that recognize at least one or more MHC-restricted (e.g., MHC class I-restricted) epitopes within a SARS-CoV-2 spike polypeptide and/or an immunogenic fragment thereof (e.g., RBD). In some embodiments, one or more epitopes recognized by vaccine-induced T cells (e.g., CD8+ T cells) may be presented on a MHC class I allele that is present in at least 50% of subjects in a population, including, e.g., at least 60%, at least 70%, at least 80%, at least 90%, or more; in some such embodiments, the MHC class I allele may be HLA-B*0702, HLA-A*2402, HLA-B*3501, HLA-B*4401, or HLA-A*0201. In some embodiments, an epitope may comprise HLA-A*0201 YLQPRTFLL; HLA-A*0201 RLQSLQTYV; HLA-A*2402 QYIKWPWYI; HLA-A*2402 NYNYLYRLF; HLA-A*2402 KWPWYIWLGF; HLA-B*3501 QPTESIVRF; HLA-B*3501 IPFAMQMAY; or HLA-B*3501 LPFNDGVYF.

In some embodiments, need for, timing of, and/or amount of a second dose relative to a first dose (or other subsequent dose relative to a prior dose) is assessed, determined and/or selected such that administration of such second (or subsequent) dose maintains or exceeds a reference level of an immune response; in some such embodiments, the reference level is determined based on human SARS-CoV-2 infection/COVID-19 convalescent sera and/or PBMC samples drawn from subjects (e.g., at least a period of time such as at least 14 days or longer, including, e.g., 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 25 days, 30 days, 35 days, 40 days, 45 days, 50 days, 55 days, 60 days, or longer, after PCR-confirmed diagnosis when the subjects were asymptomatic. In some embodiments, an immune response may be characterized by production of neutralizing antibodies directed to a SARS-CoV-2 spike polypeptide and/or an immunogenic fragment thereof (e.g., RBD) as measured in serum from a subject and/or induction of cell-mediated immune response (e.g., a T cell response against SARS-CoV-2), including, e.g., in some embodiments induction of T cells that recognize at least

one or more MHC-restricted (e.g., MHC class I-restricted) epitopes within a SARS-CoV-2 spike polypeptide and/or an immunogenic fragment thereof (e.g., RBD). In some embodiments, one or more epitopes recognized by vaccine-induced T cells (e.g., CD8+ T cells) may be presented on a MHC class I allele that is present in at least 50% of subjects in a population, including, e.g., at least 60%, at least 70%, at least 80%, at least 90%, or more; in some such embodiments, the MHC class I allele may be HLA-B*0702, HLA-A*2402, HLA-B*3501, HLA-B*4401, or HLA-A*0201. In some embodiments, an epitope may comprise HLA-A*0201 YLQPRTFLL; HLA-A*0201 RLQSLQTYV; HLA-A*2402 QYIKWPWYI; HLA-A*2402 NYNYLYRLF; HLA-A*2402 KWPWYIWLGF; HLA-B*3501 QPTESIVRF; HLA-B*3501 IPFAMQMAY; or HLA-B*3501 LPFNDGVYF.

In some embodiments, determination of need for, timing of, and/or amount of a second (or subsequent) dose may include one or more steps of assessing, after (e.g., 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 days or longer after) a first (or other prior) dose, presence and/or expression levels of neutralizing antibodies directed to a SARS-CoV-2 spike polypeptide and/or an immunogenic fragment thereof (e.g., RBD) as measured in serum from a subject and/or induction of cell-mediated immune response (e.g., a T cell response against SARS-CoV-2), including, e.g., in some embodiments induction of T cells that recognize at least one or more MHC-restricted (e.g., MHC class I-restricted) epitopes within a SARS-CoV-2 spike polypeptide and/or an immunogenic fragment thereof (e.g., RBD). In some embodiments, one or more epitopes recognized by vaccine-induced T cells (e.g., CD8+ T cells) may be presented on a MHC class I allele that is present in at least 50% of subjects in a population, including, e.g., at least 60%, at least 70%, at least 80%, at least 90%, or more; in some such embodiments, the MHC class I allele may be HLA-B*0702, HLA-A*2402, HLA-B*3501, HLA-B*4401, or HLA-A*0201. In some embodiments, an epitope may comprise HLA-A*0201 YLQPRTFLL; HLA-A*0201 RLQSLQTYV; HLA-A*2402 QYIKWPWYI; HLA-A*2402 NYNYLYRLF; HLA-A*2402 KWPWYIWLGF; HLA-B*3501 QPTESIVRF; HLA-B*3501 IPFAMQMAY; or HLA-B*3501 LPFNDGVYF.

In some embodiments, a kit as provided herein may comprise a real-time monitoring logging device, which, for example in some embodiments, is capable of providing shipment temperatures, shipment time and/or location.

In some embodiments, an RNA (e.g., mRNA) composition as described herein may be shipped, stored, and/or utilized, in a container (such as a vial or syringe), e.g., a glass container (such

as a glass vial or syringe), which, in some embodiments, may be a single-dose container or a multi-dose container (e.g., may be arranged and constructed to hold, and/or in some embodiments may hold, a single dose, or multiple doses of a product for administration). In some embodiments, a multi-dose container (such as a multi-dose vial or syringe) may be arranged and constructed to hold, and/or may hold 2, 3, 4, 5, 6, 7, 8, 9, 10 or more doses; in some particular embodiments, it may be designed to hold and/or may hold 5 doses. In some embodiments, a single-dose or multi-dose container (such as a single-dose or multi-dose vial or syringe) may be arranged and constructed to hold and/or may hold a volume or amount greater than the indicated number of doses, e.g., in order to permit some loss in transfer and/or administration. In some embodiments, an RNA (e.g., mRNA) composition as described herein may be shipped, stored, and/or utilized, in a preservative-free glass container (e.g., a preservative-free glass vial or syringe, e.g., a single-dose or multi-dose preservative-free glass vial or syringe). In some embodiments, an RNA (e.g., mRNA) composition as described herein may be shipped, stored, and/or utilized, in a preservative-free glass container (e.g., a preservative-free glass vial or syringe, e.g., a single-dose or multi-dose preservative-free glass vial or syringe) that contains a frozen liquid, e.g., in some embodiments 0.45 ml of frozen liquid (e.g., including 5 doses). In some embodiments, an RNA (e.g., mRNA) composition as described herein and/or a container (e.g., a vial or syringe) in which it is disposed, is shipped, stored, and/or utilized may be maintained at a temperature below room temperature, at or below 4 °C, at or below 0 °C, at or below -20 °C, at or below -60 °C, at or below -70 °C, at or below -80 °C , at or below -90 °C, etc. In some embodiments, an RNA (e.g., mRNA) composition as described herein and/or a container (e.g., a viral or syringe) in which it is disposed, is shipped, stored, and/or utilized may be maintained at a temperature between -80°C and -60°C and in some embodiments protected from light. In some embodiments, an RNA (e.g., mRNA) composition as described herein and/or a container (e.g., a viral or syringe) in which it is disposed, is shipped, stored, and/or utilized may be maintained at a temperature below about 25°C, and in some embodiments protected from light. In some embodiments, an RNA (e.g., mRNA) composition as described herein and/or a container (e.g., a viral or syringe) in which it is disposed, is shipped, stored, and/or utilized may be maintained at a temperature below about 5°C (e.g., below about 4°C), and in some embodiments protected from light. In some embodiments, an RNA (e.g., mRNA) composition as described herein and/or a container (e.g., a viral or syringe) in which it is disposed, is shipped, stored, and/or utilized may be maintained

at a temperature below about -20°C, and in some embodiments protected from light. In some embodiments, an RNA (e.g., mRNA) composition as described herein and/or a container (e.g., a viral or syringe) in which it is disposed, is shipped, stored, and/or utilized may be maintained at a temperature above about -60°C (e.g., in some embodiments at or above about -20°C, and in some embodiments at or above about 4-5°C, in either case optionally below about 25°C), and in some embodiments protected from light, or otherwise without affirmative steps (e.g., cooling measures) taken to achieve a storage temperature materially below about -20°C.

In some embodiments, an RNA (e.g., mRNA) composition as described herein and/or a container (e.g., a vial or syringe) in which it is disposed is shipped, stored, and/or utilized together with and/or in the context of a thermally protective material or container and/or of a temperature adjusting material. For example, in some embodiments, an RNA (e.g., mRNA) composition as described herein and/or a container (e.g., a vial or syringe) in which it is disposed is shipped, stored, and/or utilized together with ice and/or dry ice and/or with an insulating material. In some particular embodiments, a container (e.g., a vial or syringe) in which an RNA (e.g., mRNA) composition is disposed is positioned in a tray or other retaining device and is further contacted with (or otherwise in the presence of) temperature adjusting (e.g., ice and/or dry ice) material and/or insulating material. In some embodiments, multiple containers (e.g., multiple vials or syringes such as single use or multi-use vials or syringes as described herein) in which a provided RNA (e.g., mRNA) composition is disposed are co-localized (e.g., in a common tray, rack, box, etc.) and packaged with (or otherwise in the presence of) temperature adjusting (e.g., ice and/or dry ice) material and/or insulating material. To give but one example, in some embodiments, multiple containers (e.g., multiple vials or syringes such as single use or multi-use vials or syringes as described herein) in which an RNA (e.g., mRNA) composition is disposed are positioned in a common tray or rack, and multiple such trays or racks are stacked in a carton that is surrounded by a temperature adjusting material (e.g., dry ice) in a thermal (e.g., insulated) shipper. In some embodiments, temperature adjusting material is replenished periodically (e.g., within 24 hours of arrival at a site, and/or every 2 hours, 4 hours, 6 hours, 8 hours, 10 hours, 12 hours, 14 hours, 16 hours, 18 hours, 20 hours, 22 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, etc.). Preferably, re-entry into a thermal shipper should be infrequent, and desirably should not occur more than twice a day. In some embodiments, a thermal shipper is re-closed within 5, 4, 3, 2, or 1 minute, or less, of having been opened. In some

embodiments, a provided RNA (e.g., mRNA) composition that has been stored within a thermal shipper for a period of time, optionally within a particular temperature range remains useful. For example, in some embodiments, if a thermal shipper as described herein containing a provided RNA (e.g., mRNA) composition is or has been maintained (e.g., stored) at a temperature within a range of about 15 °C to about 25 °C, the RNA (e.g., mRNA) composition may be used for up to 10 days; that is, in some embodiments, a provided RNA (e.g., mRNA) composition that has been maintained within a thermal shipper, which thermal shipper is at a temperature within a range of about 15 °C to about 25 °C, for a period of not more than 10 days is administered to a subject. Alternatively or additionally, in some embodiments, if a provided RNA (e.g., mRNA) composition is or has been maintained (e.g., stored) within a thermal shipper, which thermal shipper has been maintained (e.g., stored) at a temperature within a range of about 15 °C to about 25 °C, it may be used for up to 10 days; that is, in some embodiments, a provided RNA (e.g., mRNA) composition that has been maintained within a thermal shipper, which thermal shipper has been maintained at a temperature within a range of about 15 °C to about 25 °C for a period of not more than 10 days is administered to a subject.

In some embodiments, a provided RNA (e.g., mRNA) composition is shipped and/or stored in a frozen state. In some embodiments, a provided RNA (e.g., mRNA composition is shipped and/or stored as a frozen suspension, which in some embodiments does not contain preservative. In some embodiments, a frozen RNA (e.g., mRNA) composition is thawed. In some embodiments, a thawed RNA (e.g., mRNA) composition (e.g., a suspension) may contain white to off-white opaque amorphous particles. In some embodiments, a thawed RNA (e.g., mRNA) composition may be used for up to a small number (e.g., 1, 2, 3, 4, 5, or 6) of days after thawing if maintained (e.g., stored) at a temperature at or below room temperature (e.g., below about 30 °C, 25 °C, 20 °C, 15 °C, 10 °C, 8 °C, 4 °C, etc). In some embodiments, a thawed RNA (e.g., mRNA) composition may be used after being stored (e.g., for such small number of days) at a temperature between about 2 °C and about 8 °C; alternatively or additionally, a thawed RNA (e.g., mRNA) composition may be used within a small number (e.g., 1, 2, 3, 4, 5, 6) of hours after thawing at room temperature. Thus, in some embodiments, a provided RNA (e.g., mRNA) composition that has been thawed and maintained at a temperature at or below room temperature, and in some embodiments between about 2 °C and about 8 °C, for not more than 6, 5, 4, 3, 2, or 1 days is administered to a subject. Alternatively or additionally, in

some embodiments, a provided RNA (e.g., mRNA) composition that has been thawed and maintained at room temperature for not more than 6, 5, 4, 3, 2, or 1 hours is administered to a subject. In some embodiments, a provided RNA (e.g., mRNA) composition is shipped and/or stored in a concentrated state. In some embodiments, such a concentrated composition is diluted prior to administration. In some embodiments, a diluted composition is administered within a period of about 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 hour(s) post-dilution; in some embodiments, such administration is within 6 hours post-dilution. Thus, in some embodiments, diluted preparation of a provided RNA (e.g., mRNA) composition is administered to a subject within 6 hours post-dilution (e.g., as described herein after having been maintained at an appropriate temperature, e.g., at a temperature below room temperature, at or below 4 °C, at or below 0 °C, at or below -20 °C, at or below -60 °C, at or below -70 °C, at or below - 80 °C, etc, and typically at or above about 2 °C, for example between about 2 °C and about 8 °C or between about 2 °C and about 25 °C). In some embodiments, unused composition is discarded within several hours (e.g., about 10, about 9, about 8, about 7, about 6, about 5 or fewer hours) after dilution; in some embodiments, unused composition is discarded within 6 hours of dilution.

In some embodiments, an RNA (e.g., mRNA) composition that is stored, shipped or utilized (e.g., a frozen composition, a liquid concentrated composition, a diluted liquid composition, etc.) may have been maintained at a temperature materially above -60°C for a period of time of at least 1, 2, 3, 4, 5, 6, 7 days or more, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 weeks or more, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months or more; in some such embodiments, such composition may have been maintained at a temperature at or above about -20°C for such period of time, and/or at a temperature up to or about 4-5°C for such period of time, and/or may have been maintained at a temperature above about 4-5°C, and optionally about 25°C for a period of time up that is less than two (2) months and/or optionally up to about one (1) month. In some embodiments, such composition may not have been stored, shipped or utilized (or otherwise exposed to) a temperature materially above about 4-5°C, and in particular not at or near a temperature of about 25°C for a period of time as long as about 2 weeks, or in some embodiments 1 week. In some embodiments, such composition may not have been stored, shipped or utilized (or otherwise exposed to) a temperature materially above about -20°C, and in particular not at or near a temperature of about 4-5°C for a period of time as long as about 12 months, 11 months, 10 months, 9 months, 8 months, 7 months, 6

months, 5 months, 4 months, 3 months, 2 months, or, in some embodiments, for a period of time as long as about 8 weeks or 6 weeks or materially more than about 2 months or, in some embodiments, 3 months or, in some embodiments 4 months.

In some embodiments, an RNA (e.g., mRNA) composition that is stored, shipped or utilized (e.g., a frozen composition, a liquid concentrated composition, a diluted liquid composition, etc.) may be protected from light. In some embodiments, one or more steps may be taken to reduce or minimize exposure to light for such compositions (e.g., which may be disposed within a container such as a vial or a syringe). In some embodiments, exposure to direct sunlight and/or to ultraviolent light is avoided. In some embodiments, a diluted solution may be handled and/or utilized under normal room light conditions (e.g., without particular steps taken to minimize or reduce exposure to room light). It should be understood that strict adherence to aseptic techniques is desirable during handling (e.g., diluting and/or administration) of an RNA (e.g., mRNA) composition as described herein. In some embodiments, an RNA (e.g., mRNA) composition as described herein is not administered (e.g., is not injected) intravenously. In some embodiments, an RNA (e.g., mRNA) composition as described herein is not administered (e.g., is not injected) intradermally. In some embodiments, an RNA (e.g., mRNA) composition as described herein is not administered (e.g., is not injected) subcutaneously. In some embodiments, an RNA (e.g., mRNA) composition as described herein is not administered (e.g., is not injected) any of intravenously, intradermally, or subcutaneously. In some embodiments, an RNA (e.g., mRNA) composition as described herein is not administered to a subject with a known hypersensitivity to any ingredient thereof. In some embodiments, a subject to whom an RNA (e.g., mRNA) composition has been administered is monitored for one or more signs of anaphylaxis. In some embodiments, a subject to whom an RNA (e.g., mRNA) composition is administered had previously received at least one dose of a different vaccine for SARS-CoV-2; in some embodiments, a subject to whom an RNA (e.g., mRNA) composition is administered had not previously received a different vaccine for SARS-CoV-2. In some embodiments, a subject's temperature is taken promptly prior to administration of an RNA (e.g., mRNA) composition (e.g., shortly before or after thawing, dilution, and/or administration of such composition); in some embodiments, if such subject is determined to be febrile, administration is delayed or canceled. In some embodiments, an RNA (e.g., mRNA) composition as described herein is not administered to a subject who is receiving anticoagulant therapy or is suffering from or susceptible to a bleeding

disorder or condition that would contraindicate intramuscular injection. In some embodiments, an RNA (e.g., mRNA) composition as described herein is administered by a healthcare professional who has communicated with the subject receiving the composition information relating to side effects and risks. In some embodiments, an RNA (e.g., mRNA) composition as described herein is administered by a healthcare professional who has agreed to submit an adverse event report for any serious adverse events, which may include for example one or more of death, development of a disability or congenital anomaly/birth defect (e.g., in a child of the subject), in-patient hospitalization (including prolongation of an existing hospitalization), a life-threatening event, a medical or surgical intervention to prevent death, a persistent or significant or substantial disruption of the ability to conduct normal life functions; or another important medical event that may jeopardize the individual and may require medical or surgical intervention (treatment) to prevent one of the other outcomes.

In some embodiments, provided RNA compositions are administered to a population of individuals under 18 years of age, or under 17 years of age, or under 16 years of age, or under 15 years of age, or under 14 years of age, or under 13 years of age, for example according to a regimen established to have a rate of incidence for one or more of the local reaction events indicated below that does not exceed the rate of incidence indicated below:

- pain at the injection site (75% after a first dose and/or a second dose, and/or a lower incidence after a second dose, e.g., 65% after a second dose);
- redness at the injection site (less than 5% after a first dose and/or a second dose); and/or
- swelling at the injection site (less than 5% after a first dose and/or a second dose).

In some embodiments, provided RNA compositions are administered to a population of individuals under 18 years of age, or under 17 years of age, or under 16 years of age, or under 15 years of age, or under 14 years of age, or under 13 years of age, for example according to a regimen established to have a rate of incidence for one or more of the systemic reaction events indicated below that does not exceed the rate of incidence indicated below:

- fatigue (55% after a first dose and/or a second dose);
- headache (50% after a first dose and/or a second dose);
- muscle pain (40% after a first dose and/or a second dose);
- chills (40% after a first dose and/or a second dose);

- joint pain (20% after a first dose and/or a second dose);
- fever (25% after a first dose and/or a second dose);
- vomiting (10% after a first dose and/or a second dose); and/or
- diarrhea (10% after a first dose and/or a second dose).

In some embodiments, medication that alleviates one or more symptoms of one or more local reaction and/or systemic reaction events (e.g., described herein) are administered to individuals under 18 years of age, or under 17 years of age, or under 16 years of age, or under 15 years of age, or under 14 years of age, or under 13 years of age who have been administered with provided RNA compositions and have experienced one or more of the local and/or systemic reaction events (e.g., described herein). In some embodiments, antipyretic and/or pain medication can be administered to such individuals.

Brief description of the drawings

Figure 1. Schematic overview of the S protein organization of the SARS-CoV-2 S protein.

The sequence within the S1 subunit consists of the signal sequence (SS) and the receptor binding domain (RBD) which is the key subunit within the S protein which is relevant for binding to the human cellular receptor ACE2. The S2 subunit contains the S2 protease cleavage site (S2') followed by a fusion peptide (FP) for membrane fusion, heptad repeats (HR1 and HR2) with a central helix (CH) domain, the transmembrane domain (TM) and a cytoplasmic tail (CT).

Figure 2. Exemplary SARS-CoV-2 vaccine constructs.

Based on the full and wildtype S protein, we have designed different constructs encoding the (1) full protein with mutations in close distance to the first heptad repeat (HRP1) that include stabilizing mutations preserving neutralisation sensitive sites, the (2) S1 domain or the (3) RB domain (RBD) only. Furthermore, to stabilize the protein fragments a fibritin domain (F) was fused to the C-terminus. All constructs start with the signal peptide (SP) to ensure Golgi transport to the cell membrane.

Figure 3. General structure of the RNA.

Schematic illustration of the general structure of the RNA vaccines with 5'-cap, 5'- and 3'-untranslated regions, coding sequences with intrinsic secretory signal peptide as well as GS-

linker, and poly(A)-tail. Please note that the individual elements are not drawn exactly true to scale compared to their respective sequence lengths.

UTR = Untranslated region; sec = Secretory signal peptide; RBD = Receptor Binding Domain; GS = Glycine-serine linker.

Figure 4. General structure of the RNA.

Schematic illustration of the general structure of RNA drug substances with 5'-cap, 5'- and 3'-untranslated regions, coding sequences with intrinsic secretory signal peptide as well as GS-linker, and poly(A)-tail. Please note that the individual elements are not drawn exactly true to scale compared to their respective sequence lengths.

GS = Glycine-serine linker; UTR = Untranslated region; Sec = Secretory signal peptide; RBD = Receptor Binding Domain.

Figure 5. General structure of the RNA.

Schematic illustration of the general structure of RNA vaccines with 5'-cap, 5'- and 3'-untranslated regions, coding sequences of the Venezuelan equine encephalitis virus (VEEV) RNA-dependent RNA polymerase replicase and the SARS-CoV-2 antigen with intrinsic secretory signal peptide as well as GS-linker, and poly(A)-tail. Please note that the individual elements are not drawn exactly true to scale compared to their respective sequence lengths.

UTR = Untranslated region; Sec = Secretory signal peptide; RBD = Receptor Binding Domain; GS = Glycine-serine linker.

Figure 6. Schematic overview of the S protein organization of the SARS-CoV-2 S protein and constructs for the development of a SARS-CoV-2 vaccine.

Based on the wildtype S protein, we have designed two different transmembrane-anchored RBD-based vaccine constructs encoding the RBD fragment fused to the T4 fibritin trimerization domain (F) and the autochthonus transmembrane domain (TM). Construct (1) starts with the SARS-CoV-2-S signal peptide (SP; AA 1-19 of the S protein) whereas construct (2) starts with the human Ig heavy chain signal peptide (huSec) to ensure Golgi transport to the cell membrane.

Figure 7. Anti-S protein IgG response 6, 14 and 21 d after immunization with LNP-C12 formulated modRNA coding for transmembrane-anchored RBD-based vaccine constructs.
BALB/c mice were immunized IM once with 4 µg of LNP-C12-formulated transmembrane-anchored RBD-based vaccine constructs (surrogate to BNT162b3c/BNT162b3d). On day 6, 14 and 21 after immunization, animals were bled and the serum samples were analyzed for total amount of anti-S1 (left) and anti-RBD (right) antigen specific immunoglobulin G (IgG) measured via ELISA. For day 6 (1:50), day 14 (1:300) and day 21 (1:900) different serum dilution were included in the graph. One point in the graph stands for one mouse, every mouse sample was measured in duplicates (group size n=8; mean + SEM is included for the groups).

Figure 8. Neutralization of SARS-CoV-2 pseudovirus 6, 14 and 21 d after immunization with LNP-C12 formulated modRNA coding for transmembrane-anchored RBD-based vaccine constructs.
BALB/c mice were immunized IM once with 4 µg of LNP-C12-formulated transmembrane-anchored RBD-based vaccine constructs (surrogate to BNT162b3c/BNT162b3d). On day 6, 14 and 21 after immunization, animals were bled and the sera were tested for SARS CoV-2 pseudovirus neutralization. Graphs depict pVN_{50} serum dilutions (50% reduction of infectious events, compared to positive controls without serum). One point in the graphs stands for one mouse. Every mouse sample was measured in duplicate. Group size n=8. Mean + SEM is shown by horizontal bars with whiskers for each group. LLOQ, lower limit of quantification. ULOQ, upper limit of quantification.

Fig. 9. 50% pseudovirus neutralization titers ($pVNT_{50}$) of sera collected 21 days after the second dose and 1 month after the third dose of BNT162b2 against VSV-SARS-CoV-2-S pseudovirus bearing the Wuhan Hu-1 reference or Omicron lineage spike protein. N=19-20 sera from immunized subjects collected either 21 days after the second BNT162b2 dose or 1 months after the third BNT162b2 dose were tested. For values below the limit of detection (LOD; 10), LOD/2 values are plotted. Group GMTs (values above bars) with 95% confidence intervals are shown.

Fig. 10. CD8+ T cell epitopes in BNT162b2 vaccine remain largely unaffected by Omicron BA.1 variant mutations. Shown is the number of previously identified MHC-I

epitopes affected in various variants of concern (VOCs). Approximately 80% of previously identified CD8+ epitopes are not affected by the mutations in the BA.1 Omicron variant, suggesting that two doses of BNT162b2 may still induce protection against severe disease.

Fig. 11. Neutralization of Omicron BA.1 after two doses of BNT162b2 and variant specific booster. Shown is neutralization of the Omicron BA.1 variant from sera of patients administered two doses of BNT162b2 and (i) a third booster dose of BNT162b2, or (ii) a third booster dose of an RNA encoding a Spike protein with alpha or delta variant mutations, or a third booster dose of both a Spike protein comprising alpha mutations and a Spike protein comprising delta mutations. The values are derived from separate neutralization GMTs from the pseudovirus testing. Also shown is a schematic depicting a process for developing new SARS-CoV-2 variant specific vaccines.

Fig. 12. Longitudinal analysis of neutralizing antibody responses against VSV-SARS-CoV-2-S pseudovirus bearing the Wuhan or Omicron BA.1 variant spike protein in a subset of study participants. Sera from n=9 participants drawn at 21 days after dose 2, prior to dose 3, 1 month after dose 3 and 3 months after dose 3 were tested. Each serum was tested in duplicate and individual geometric mean 50% pseudovirus neutralizing titers (GMTs) were calculated. For values below the limit of detection (LOD), LOD/2 values were assigned. Group GMTs (values in table) and 95% confidence intervals per timepoint are indicated.

Fig. 13. Analysis of HLA class I T cell epitopes conservation between the Wuhan and Omicron BA.1 variants. HLA class I restricted Spike protein epitopes with T cell reactivity identified based on their recognition by CD8+ T cells and reported in IEDB (n=244) are plotted by their position (top row) along the Spike protein (bottom row). Epitope indications are positioned by the amino acid position of the center of the epitope; epitopes conserved in both variants are marked in light grey (n=208); while epitopes spanning an Omicron BA.1 mutation site are marked dark grey (n=36). NTD=N-terminal domain; RBD=Receptor-binding domain; FCS=Furin cleavage site. The S1 and S2 regions of the Spike protein are indicated.

Fig. 14. Schematics of an exemplary vaccination regimen.

Fig. 15. Cohorts, sampling and experimental setup for characterization of immune response in Omicron breakthrough cases. Blood samples were drawn from four cohorts: Omicron-naïve individuals double- or triple-vaccinated with BNT162b2, and individuals double- or triple-vaccinated with BNT162b2 that subsequently had a breakthrough infection with Omicron BA.1. PBMCs and sera were isolated in the Omicron-naïve cohorts at the time-points indicated following their most recent vaccination; for convalescent cohorts, the time from their most recent vaccination to Omicron BA.1 infection, and infection to PBMC and serum isolation are indicated (all values specified as median-range). Serum neutralizing capacity was assessed using a pseudovirus and live virus neutralization test; SARS-CoV-2 spike-specific B_{MEM} cells were assessed via a flow cytometry-based B cell phenotyping assay using bulk PBMCs. N/A, not applicable.

Fig. 16. Omicron breakthrough infection in BNT162b2 double- and triple-vaccinated individuals induces broad neutralization of Omicron BA.1, BA.2 and other VOCs. Serum was drawn from double-vaccinated individuals ($BNT162b2^2$) at 22 days after the second dose (open circles), from triple-vaccinated individuals ($BNT162b2^3$) at 28 days after the third dose (closed circles), from double-vaccinated individuals with an Omicron breakthrough infection ($BNT162b2^2$ + Omi) at 46 days post-infection (open triangles), and from triple-vaccinated individuals and Omicron breakthrough infection ($BNT162b2^3$ + Omi) at 44 days post-infection (closed triangles). Serum was tested in duplicate; (A) shows 50% pseudovirus neutralization (pVN_{50}) geometric mean titers (GMTs), (B) shows 50% virus neutralization (VN_{50}) GMTs, and (C) shows the geometric mean ratio of SARS-CoV-2 variant of concern (VOC) and Wuhan VN_{50} GMTs. For titer values below the limit of detection (LOD), LOD/2 values were plotted. Values above violin plots represent group GMTs. The non-parametric Friedman test with Dunn's multiple comparisons correction was used to compare Wuhan neutralizing group GMTs with titers against the indicated variants and SARS-CoV-1. Multiplicity-adjusted p values are shown. (A) pVN_{50} GMTs against Wuhan, VOC and SARS-CoV-1 pseudovirus. (B) VN_{50} GMTs against live SARS-CoV-2 Wuhan, Beta, Delta and Omicron BA.1. (C) Group geometric mean ratios with 95% confidence intervals for all cohorts shown in (B).

Fig. 17. B_{MEM} cells of individuals double- and triple-vaccinated with BNT162b2 broadly recognize VOCs and are further boosted by Omicron breakthrough infection. PBMC samples from double-vaccinated individuals (BNT162b2^2) at 22 days after the second dose (open squares) and 5 months after the second dose (open circles), from triple-vaccinated individuals (BNT162b2^3) at 84 days after the third dose (closed circles), from double-vaccinated individuals with Omicron breakthrough infection (BNT162b2^2 + Omi) at 46 days post-infection (open triangles), and from triple-vaccinated individuals with Omicron breakthrough infection (BNT162b23 + Omi) at 44 days post-infection (closed triangles) were analyzed via flow cytometry for SARS-CoV-2-specific B_{MEM} cell (B_{MEM} – CD3-CD19+CD20+IgD-CD38$^{int/low}$) frequencies via B cell bait staining. (A) Schematic of one-dimensional staining of B_{MEM} cells with fluorochrome-labeled SARS-CoV-2 S protein tetramer bait allowing discrimination of variant recognition. Frequencies of Wuhan or VOC full-length S protein- (B) and RBD- (C) specific B_{MEM} cells for the four groups of individuals were analyzed. Variant-specific B_{MEM} cell frequencies were normalized to Wuhan frequencies for S protein (D) and RBD- (E) binding. (F) Depicts the frequency ratios of RBD protein specific B_{MEM} cells over full-length S protein-specific BMEM cells.

Fig. 18. Omicron breakthrough infection of BNT162b2 double- and triple-vaccinated individuals primarily boosts B_{MEM} against conserved epitopes shared broadly between S proteins of Wuhan and other VOCs rather than strictly Omicron S-specific epitopes. PBMC samples from double-vaccinated individuals (BNT162b2^2) at 22 days after the second dose (open squares) and 5 months after the second dose (open circles), from triple-vaccinated individuals (BNT162b2^3) at 84 days after the third dose (closed circles), from double-vaccinated individuals with Omicron breakthrough infection (BNT162b2^2 + Omi) at 46 days post-infection (open triangles), and from triple-vaccinated individuals with Omicron breakthrough infection (BNT162b2^3 + Omi) at 44 days post-infection (closed triangle) were analyzed via flow cytometry for SARS-CoV-2-specific memory B cell (B_{MEM} – CD3-CD19+CD20+IgD-CD38$^{int/low}$) frequencies via B cell bait staining (schematic shown in (A)). (B) shows representative flow plots of Omicron and Wuhan S protein- and RBD-binding for each of the four groups of individuals investigated. Frequencies of B_{MEM} binding Omicron, Wuhan, or both (shared) shown for full-length S protein in (C) and RBD shown in (D) for Omicron-experienced and naïve BNT162b2 double and triple vaccinees. (E) Venn diagrams visualizing

the combinatorial (Boolean) gating strategy to identify cross-reactive B_{MEM} recognizing all four variants simultaneously (All 4 +ve) and B_{MEM} recognizing only Omicron (only Omi) or only Wuhan (only Wuhan) S proteins. Frequencies for these three B_{MEM} sub-groups were compared for full-length S protein (F) and RBD (G) in the four different groups of individuals investigated. RBD variant recognition pattern by B_{MEM} was assessed through Boolean flow cytometric gating strategy and frequencies recognizing combinations of Wuhan and/or variant RBDs are displayed in (H), for all Omicron convalescent subjects (double and triple vaccinees pooled, n=13). (I) Conserved site within the RBD domain recognized by RBD-specific B_{MEM} after Omicron break-through infection. Mean values are indicated in C, D, F, and G. n = number of individuals per group.

Fig. 19. Omicron breakthrough infection of individuals vaccinated with other approved COVID-19 vaccines or mixed regimens results in immune sera that broadly neutralize Omicron BA.1, BA.2 and other VOCs plus SARS-CoV-1. Serum was drawn from 10 individuals vaccinated with other approved COVID-19 vaccines or mixed regimens at a median of 43 days after infection (diamonds). Serum was tested in duplicate; individual 50% pseudovirus neutralization (pVN_{50}) geometric mean titers (GMTs) against SARS-CoV-2 Wuhan, Alpha, Beta, Delta and Omicron BA.1 and BA.2 variants, plus SARS-CoV-1 were plotted. For titer values below the limit of detection (LOD), LOD/2 values were plotted. Values above violin plots represent group GMTs. The non-parametric Friedman test with Dunn's multiple comparisons correction was used to compare Wuhan neutralizing group GMTs with titers against the indicated variants and SARS-CoV-1. Multiplicity-adjusted p values are shown. Approved vaccines included AZD1222, BNT162b2 (in some embodiments as part of a 4-dose series), Ad26.COV2.S, mRNA-1273 (administered as a two-dose or three-dose series), and combinations thereof.

Fig. 20. 50% neutralization titers of sera collected 1 month after a fourth dose of BNT162b2 or an Omicron BA.1-specific booster. Subjects who were previously administered two doses of BNT162b2, and a third (booster) dose of BNT162b2 (30 ug) received a dose (30 ug) of (i) an RNA encoding a SARS-CoV-2 S protein from an Omicron BA.1 variant (e.g., as described herein (referred to herein as "Omicron BA.1-specific RNA vaccine"), or (ii) BNT162b2, as a fourth (boster) dose. Serum from the subjects were collected one month after administration of the

4th (booster) dose. Group GMTs (values above bars) with 95% confidence intervals are shown. "b2" refers to sera from subjects administered Wuhan-specific RNA vaccine as the 4th (booster) dose of BNT162b2. "OMI" refers to sera from subjects administered an Omicron BA.1-specific 4th (booster) dose. Also shown is the fold-change in titer from before administration of the 4th dose to after administration of the 4th dose (Pre/Post Vax Fold-Rise), and the ratio of geometric mean ratio (GMR) and geometric mean fold rise (GMFR) observed in subjects administered a 4th dose of an Omicron BA.1-specific RNA vaccine as the 4th dose, as compared to subjects administered BNT162b2 as the 4th dose. "FFRNT" refers to fluorescent focus reduction neutralization test. Neutralization data was obtained using an FFRNT assay, with a viral particle containing a SARS-CoV-2 S protein from the variant indicated in the figures. (A) Comparison of titers of neutralizing antibodies against a SARS-CoV-2-S pseudovirus comprising a SARS-CoV-2 S protein having mutations characteristics of an Omicron BA.1 variant. Sera from subjects previously or currently infected with SARS-CoV-2 excluded. (B) Comparison of titers of neutralizing antibodies against a SARS-CoV-2 pseudovirus comprising a SARS-CoV-2 S protein having mutations characteristics of an Omicron BA.1 variant in sera from a population that includes subjects previously or currently infected with SARS-CoV-2 (as determined by an antigen assay or a PCR assay respectively). (C) Comparison of titers of neutralizing antibodies against a SARS-CoV-2 pseudovirus comprising a SARS-CoV-2 S protein from a Wuhan strain. Sera from subjects previously or currently infected with SARS-CoV-2 excluded. (D) Comparison of titers of neutralizing antibodies against a SARS-CoV-2 pseudovirus comprising a SARS-CoV-2 S protein from a Wuhan strain, in sera from a population comprising individuals previously or currently infected with SARS-CoV-2 (as determined by an antigen assay or a PCR assay, respectively. (E) Comparison of titers of neutralizing antibodies against a SARS-CoV-2 pseudovirus comprising a SARS-CoV-2 S protein having mutations characteristics of a delta variant. Sera from subjects previously or currently infected with SARS-CoV-2 excluded. (F) Comparison of titers of neutralization antibodies against a SARS-CoV-2 pseudovirus comprising a SARS-CoV-2 protein having mutations characteristic of a delta variant, in sera from a population including subjects previously or currently infected with SARS-CoV-2 (as determined by an antigen assay or a PCR assay, respectively).

Fig. 21. Neutralization of SARS-CoV-2 pseudovirus 7 days after immunization with modRNA coding for variant specific S proteins. Mice were immunized twice with LNP-formulated vaccine comprising (i) BNT162b2 (encoding a SARS-CoV-2 S protein from a Wuhan strain), (ii) RNA encoding a SARS-CoV-2 S protein having mutations characteristic of an Omicron BA.1 variant (Omi), (iii) RNA encoding an S protein having mutations characteristic of a delta variant, (iv) a combination of BNT162b2 and an RNA encoding an protein having mutations characteristic of an Omicron BA.1 variant (B2+Omi), or (v) RNA encoding a SARS-CoV-2 S protein having mutations characteristic of a delta variant and RNA encoding a SARS-CoV-2 S protein having mutations characteristic of an Omicron BA.1 variant (Delta + Omi). 7 days after the second immunization, animals were bled and sera was tested for neutralization of a SARS-CoV-2-S pseudovirus comprising a SARS-CoV-2 S protein from a Wuhan strain, or a SARS-CoV-2 S protein having mutations characteristic of a beta, delta, or Omicron BA.1 variant. Graphs depict pVN_{50} serum dilutions (50% reduction of infectious events, compared to positive controls without serum). One point in the graphs stands for one mouse. Every mouse sample was measured in duplicate. Mean + SEM is shown by horizontal bars with whiskers for each group. LLOD, lower limit of detection. ULOD, upper limit of detection.

Fig. 22. RNA encoding a SARS-CoV-2 S protein having mutations characteristic of a Beta variant increases neutralization antibody titers against SARS-CoV-2 when administered to patients previously administered two doses of a vaccine encoding a SARS-CoV-2 S protein of a Wuhan strain. Subjects previously administered two doses of an RNA vaccine encoding a SARS-CoV-2 S protein of a Wuhan strain were administered a third and a fourth dose of an RNA vaccine encoding a SARS-CoV-2 S protein having mutations characteristic of a Beta variant. Neutralization antibody titers were measured before administration of an RNA vaccine encoding a SARS-CoV-2 S protein of a Wuhan strain (D1-PreVax), one month after administration of a second dose of an RNA vaccine encoding a SARS-CoV-2 S protein of a Wuhan strain (M1PD2), one-month after administration of a third dose of an RNA vaccine encoding a SARS-CoV-2 S protein having mutations characteristic of a SARS-CoV-2 Beta variant, and one month after administration of a fourth dose of an RNA vaccine encoding a SARS-CoV-2 S protein having mutations characteristic of a SARS-CoV-2 Beta variant. The third and fourth doses were administered 1 month apart from one another. GMFR refers to the geometric mean fold rise, and is a measure of the increase in neutralization antibody titers

since the previous vaccine dose (e.g., the GMFR for Post-Dose2 (PD2) is a measure of the increase in neutralization antibody titers relative to before administration of any vaccine (pre-vax)). (A) Neutralization antibody titers measured in a viral neutralization assay that uses a viral particle comprising a SARS-CoV-2 S protein of a Wuhan strain. (B) Neutralization antibody titers measured in a viral neutralization assay that uses a viral particle comprising a SARS-CoV-2 S protein having mutations characteristic of a Beta variant.

Fig. 23. 50% neutralization titers of sera collected 7 days after a fourth dose of BNT162b2, an Omicron BA.1-specific booster, or a bivalent vaccine. Subjects who were previously administered two doses of BNT162b2 (30 ug), and a third (booster) dose of BNT162b2 (30 ug) received (i) a 30 ug dose of BNT162b2 (encoding a SARS-CoV-2 S protein from a Wuhan strain), (ii) a 60 ug dose of BNT162b2, (iii) a 30 ug dose of RNA encoding a SARS-CoV-2 S protein having mutations characteristic of an Omicron BA.1 variant (e.g., as described herein (referred to herein as "Omicron BA.1-specific RNA vaccine")), (iii) a 60 ug dose of RNA encoding a SARS-CoV-2 S protein having mutations characteristic of an Omicron BA.1 variant, (iv) a 30 ug dose of a bivalent vaccine, comprising 15 ug of BNT162b2 and 15 ug of RNA encoding a SARS-CoV-2 S protein comprising mutations characteristic of an Omicron BA.1 variant, or (v) a 60 ug dose of a bivalent vaccine, comprising 30 ug of BNT162b2 and 30 ug of RNA encoding a SARS-CoV-2 S protein comprising mutations characteristic of an Omicron BA.1 variant. Geometric mean ratio (GMR) of titers in serum from subjects were collected 7 days after administration of a 4th dose. "b2" refers to sera from subjects administered a Wuhan-specific RNA vaccine as a 4^{th} dose of BNT162b2. "OMI" refers to sera from subjects administered an Omicron BA.1-specific 4^{th} dose. "Bivalent" refers to sera from subjects administered a composition comprising BNT162b2 and an RNA encoding a SARS-CoV-2 S protein comprising mutations that are characteristic of an Omicron BA.1 variant as a 4^{th} dose. Also shown is the fold-rise in titer from before administration of a 4^{th} dose to 7 days after administration of a 4^{th} dose (*Fold-Rise). "FFRNT" refers to fluorescent focus reduction neutralization test. Neutralization data was obtained using an FFRNT assay, with a viral particle containing a SARS-CoV-2 S protein having mutations characteristic of the variant indicated in the figures. LLOQ refers to Lower Limit of Quantification and ULOQ refers to Upper Limit of Quantification. (A) Comparison of titers of neutralizing antibodies against a SARS-CoV-2 pseudovirus comprising a SARS-CoV-2 S protein having mutations characteristics of an Omicron BA.1 variant. Sera from subjects

previously or currently infected with SARS-CoV-2 excluded. (B) Comparison of titers of neutralizing antibodies against a SARS-CoV-2 pseudovirus comprising a SARS-CoV-2 S protein having mutations characteristics of an Omicron BA.1 variant in sera from a population that includes subjects previously or currently infected with SARS-CoV-2 (e.g., as determined by an antibody test or a PCR assay respectively). (C) Comparison of titers of neutralizing antibodies against a SARS-CoV-2 pseudovirus comprising a SARS-CoV-2 S protein of a Wuhan strain. Sera from subjects previously or currently infected with SARS-CoV-2 excluded. (D) Comparison of titers of neutralizing antibodies against a SARS-CoV-2 pseudovirus comprising a SARS-CoV-2 S protein of a Wuhan strain, in sera from a population that includes individuals previously or currently infected with SARS-CoV-2. (E) Comparison of titers of neutralizing antibodies against a SARS-CoV-2 pseudovirus comprising a SARS-CoV-2 S protein having mutations characteristics of a Delta variant. Sera from subjects previously or currently infected with SARS-CoV-2 excluded. (F) Comparison of titers of neutralization antibodies against a SARS-CoV-2 pseudovirus comprising a SARS-CoV-2 S protein having mutations characteristic of a Delta variant, in sera from a population including subjects previously or currently infected with SARS-CoV-2. (G) Geometric mean rise (GMR) of neutralization antibodies observed in subjects administered 60 ug of BNT162b2, 30 ug of RNA encoding a SARS-CoV-2 S protein having mutations characteristic of an Omicron BA.1 variant (OMI 30 ug), 60 ug of RNA encoding a SARS-CoV-2 S protein having mutations characteristic of an Omicron BA.1 variant (OMI 60 ug), 30 ug of a bivalent vaccine comprising 15 ug of BNT162b2 and 15 ug of RNA encoding a SARS-CoV-2 S protein having mutations characteristic of an Omicron BA.1 variant (Bivalent 30 ug), or 60 ug of a bivalent vaccine comprising 30 ug of BNT162b2 and 30 ug of RNA encoding a SARS-CoV-2 S protein having mutations characteristic of an Omicron BA.1 variant (Bivalent 60 ug), as compared to subjects administered 30 ug of BNT162b2 as a 4th dose. Results are shown both for a population pool that excludes subjects previously or currently infected with SARS-CoV-2 and a population pool that includes these subjects.

Fig. 24. Reactogenicity of certain exemplary RNA (formulated in LNP) at a given dose: subjects administered a 60 ug dose of RNA encoding a SARS-CoV-2 S protein are more likely to exhibit a higher injection site pain and exhibit similar systemic reactions as subjects administered a 30 ug dose of RNA. Subjects were administered 30 ug or 60 ug of RNA encoding a SARS-CoV-2 S protein from a Wuhan strain (BNT162b2, corresponding to groups

G1 and G2, respectively), 30 ug or 60 ug of RNA encoding a SARS-CoV-2 S protein having mutations characteristic of an Omicron BA.1 variant (BNT162b2 OMI, corresponding to groups G3 and G4, respectively), 30 ug of a bivalent vaccine comprising 15 ug of RNA encoding a SARS-CoV-2 S protein from a Wuhan strain and 15 ug of RNA encoding a SARS-CoV-2 S protein having mutations characteristic of an Omicron BA.1 variant (BNT162b2 (15 ug) + BNT162b2 OMI (15 ug), corresponding to group G5), or 60 ug of a bivalent vaccine comprising 30 ug of RNA encoding a SARS-CoV-2 S protein from a Wuhan strain and 30 ug of RNA encoding a SARS-CoV-2 S protein having mutations characteristic of an Omicron BA.1 variant (BNT162b2 (30 ug) + BNT162b2 OMI (30 ug), corresponding to group G6). (A) Local reactions, including redness, swelling, and pain at the injection site, observed within 7 days of injection. Injection site pain was found to be increased in subjects administered 60 ug of RNA encoding a SARS-CoV-2 S protein comprising mutations characteristic an Omicron BA.1 variant or a bivalent vaccine, as compared to other doses tested. (B) Systemic reactions, including fever, fatigue, headache, chills, vomiting, diarrhea, muscle pain, joint pain, and use of medication, observed within 7 days of injection. Systemic reactions through 7 days were observed to be broadly similar across different groups. Fatigue was found to trend higher after administration of 60 ug doses, as compared to 30 ug doses.

Fig. 25. 50% neutralization titers of sera collected 1 month after a fourth dose of BNT162b2, an Omicron BA.1-specific booster, or a bivalent vaccine against an Omicron BA.1 variant. Subjects who were previously administered two doses of BNT162b2 (30 ug), and a third (booster) dose of BNT162b2 (30 ug) were administered (i) a 30 ug dose of BNT162b2 (encoding a SARS-CoV-2 S protein from a Wuhan strain), (ii) a 60 ug dose of BNT162b2, (iii) a 30 ug dose of RNA encoding a SARS-CoV-2 S protein having mutations characteristic of an Omicron BA.1 variant („BNT162b2 OMI"), (iii) a 60 ug dose of BNT162b2 OMI, (iv) a 30 ug dose of a bivalent vaccine, comprising 15 ug of BNT162b2 and 15 ug of BNT162b2 OMI („Bivalent"), or (v) a 60 ug dose of a bivalent vaccine, comprising 30 ug of BNT162b2 and 30 ug of BNT162b2 OMI. Geometric mean titer (GMT) of neutralization antibodies were measured in serum from subjects collected 1 month after administration of a 4th dose (GMT indicated above each bar). Shown below the x-axis is the geometric fold rise (GMFR) in titer from before administration of a 4^{th} dose (pre-vax) to 1 month after administration of a 4^{th} dose (1MPD) for each patient

group. Neutralization data was obtained using a neutralization assay, with a viral particle containing a SARS-CoV-2 S protein having mutations characteristic of a Omicron BA.1 variant.

Fig. 26. Omicron BA.1 breakthrough infection of BNT162b2 double- and triple-vaccinated individuals induces broad neutralization of Omicron BA.1, BA.2 and other VOCs, but to a lesser extent against BA.4 and BA.5. This figure is an extension of Fig. 16, including data neutralizing activity against Omicron BA.4 and BA.5. As described in Fig. 16, serum was tested in duplicate; 50% pseudovirus neutralization (pVN_{50}) geometric mean titers (GMTs) (in A and B), and the geometric mean ratio of SARS-CoV-2 variants of concern (VOCs) and SARS-CoV-1 pVN_{50} GMTs normalized against Wuhan pVN_{50} GMTs (in C) were plotted. For titer values below the limit of detection (LOD), LOD/2 values were plotted. Values above violin plots represent the group GMTs. The nonparametric Friedman test with Dunn's multiple comparisons correction was used to compare Wuhan neutralizing group GMTs with titers against the indicated variants and SARS-CoV-1. Multiplicity adjusted p values are shown. (A) pVN_{50} GMTs against Wuhan, VOC and SARS-CoV-1 pseudovirus in patients who received two doses or three doses of BNT162b2. (B) pVN_{50} GMTs against Wuhan, VOC and SARS-CoV-1 pseudovirus in patients who received two doses or three doses of BNT162b2 and who have been previously infected with an Omicron BA.1 variant of SARS-CoV-2. (C) Group geometric mean ratios with 95% confidence intervals for all cohorts shown in (A) and (B).

Fig. 27. Omicron BA.1 breakthrough infection of individuals vaccinated with other approved COVID-19 vaccines or mixed regimens results in immune sera that broadly neutralize Omicron BA.1, BA.2 and other VOCs, but to a lesser extent against BA.4 and BA.5. This figure is an extension of Fig. 19, including data neutralizing activity against Omicron BA.4 and BA.5. As described in Fig. 19, serum was tested in duplicate; individual 50% pseudovirus neutralization (pVN_{50}) geometric mean titers (GMTs) against SARS-CoV-2 Wuhan, Alpha, Beta, Delta and Omicron BA.1, BA.2 and BA.4/5 variants, plus SARS-CoV-1 were plotted. For titer values below the limit of detection (LOD), LOD/2 values were plotted. Values above violin plots represent group GMTs. The nonparametric Friedman test with Dunn's multiple comparisons correction was used to compare Wuhan neutralizing group GMTs with titers against the indicated variants and SARS-CoV-1. Multiplicity-adjusted p values are shown.

Fig. 28. Sequences of RBDs of SARS-COV-2 Wuhan strain and variants thereof. Variant-specific amino acid alterations are indicated in bold red font, with the original Wuhan amino acid highlighted in bold blue font.

Fig. 29. Cohorts and sampling for the study described in Example 14. A schematic is shown for testing immune responses in triple-vaccinated patients who are (i) Omicron naïve, (ii) have been infected with an Omicron BA.1 variant, or (iii) have been infected with an Omicron BA.2 variant. Blood samples were drawn from three cohorts: Omicron-naïve individuals triple-vaccinated with BNT162b2 (BNT162b2^3, green), and individuals vaccinated with homologous or heterologous three doses regimens that subsequently had either a breakthrough infection with Omicron at a time of BA.1 dominance (November 2021 to January 2022; all Vax + BA.1, purple) or at a time of BA.2 dominance (March to May 2022; all Vax + BA.2, blue) in Germany. Sera (droplet) were isolated in the Omicron-naïve cohort at the time-point indicated following their most recent vaccination; for convalescent cohorts, the time from their most recent vaccination to Omicron infection, and infection to serum isolation are indicated. All values specified as median-range. Serum neutralizing capacity was assessed using a pseudovirus neutralization test.

Fig. 30. 50% pseudovirus neutralization (pVN_{50}) geometric mean titers (GMTs) from the BNT162b2^3 and All Vax + Omi BA.1 breakthrough infection cohorts. Serum was drawn from Omicron-naïve BNT162b2 triple-vaccinated individuals (BNT162b2^3, circles) at 28 days after the third dose, and from vaccinated individuals with subsequent Omicron BA.1 breakthrough infection (all Vax + Omi BA.1, triangles) at a median 43 days post-infection. 50% pseudovirus neutralization (pVN_{50}) geometric mean titers (GMTs) for Omicron-naive individuals are plotted in (A) and for BA.1 breakthrough infected individuals in (B). This data was previously published in Quandt et al. („Omicron BA.1 breakthrough infection drives cross-variant neutralization and memory B cell formation against conserved epitopes." *Science immunology*, eabq2427 (2022), doi:10.1126/sciimmunol.abq2427), except for BA.2.12.1 neutralization data. Serum was tested in duplicate. For titer values below the limit of detection (LOD), LOD/2 values were plotted. Values above violin plots represent group GMTs. The non-parametric Friedman test with Dunn's multiple comparisons correction was used to compare Wuhan neutralizing group

GMTs with titers against the indicated variants and SARS-CoV-1. Multiplicity-adjusted p values are shown.

Fig. 31. Omicron BA.2 breakthrough infection of previously vaccinated individuals refocuses neutralization against Omicron BA.2 and the BA.2-derived subvariants BA.2.12.1 and BA.4/BA.5. Serum was drawn from BNT162b2 triple-vaccinated individuals with subsequent Omicron BA.1 breakthrough infection at a median 44 days post-infection (BNT162b2[3] + Omi BA.1, triangles), and from BNT162b2 triple-vaccinated individuals with subsequent Omicron BA.2 breakthrough infection at 38 days post-infection (BNT162b2[3] + Omi BA.2, squares). 50% pseudovirus neutralization (pVN_{50}) geometric mean titers (GMTs) (in A, B), and the geometric mean ratio of SARS-CoV-2 variants of concern (VOCs) and SARS-CoV-1 pVN_{50} GMTs normalized against Wuhan pVN50 GMTs (in C) were plotted. pVN_{50} GMT and geometric mean ratio data for Omicron-naïve BNT162b2 triple-vaccinated individuals (BNT162b2[3], circles) and BNT162b2 triple-vaccinated individuals with Omicron BA.1 breakthrough infection was previously published in Quandt et al. („Omicron BA.1 breakthrough infection drives cross-variant neutralization and memory B cell formation against conserved epitopes." *Science immunology*, eabq2427 (2022), doi:10.1126/sciimmunol.abq2427), except for BA.2.12.1 neutralization data. Serum was tested in duplicate. For titer values below the limit of detection (LOD), LOD/2 values were plotted. Values above violin plots represent group GMTs. The non-parametric Friedman test with Dunn's multiple comparisons correction was used to compare Wuhan neutralizing group GMTs with titers against the indicated variants and SARS-CoV-1. Multiplicity-adjusted p values are shown. (A, B) pVN_{50} GMTs against Wuhan, VOC and SARS-CoV-1 pseudovirus. (C) Group geometric mean ratios with 95% confidence intervals.

Fig. 32. Characteristics of SARS-CoV-2 S glycoproteins used in the VSV-SARS-CoV-2 pseudovirus based neutralization assays. The sequence of the Wuhan-Hu-1 isolate SARS-CoV-2 S glycoprotein (GenBank: QHD43416.1) was used as reference. Amino acid positions, amino acid descriptions (one letter code) and kind of mutations (substitutions, deletions, insertions) are indicated. NTD, N-terminal domain; RBD, Receptor-binding domain, Δ, deletion; ins, insertion; *, Cytoplasmic domain truncated for the C-terminal 19 amino acids.

Fig. 33. Alterations on the spike glycoprotein amino acid sequence of SARS-CoV-2 Omicron sub-lineages. Amino acid positions, amino acid descriptions (one letter code) and kind of mutations substitutions, deletions, insertions) are indicated. White letters in boxes indicate the amino acid substitution per sub-lineage; Δ, deletion; ins, insertion; NTD, N-terminal domain; RBD, receptor-binding domain.

Fig. 34. Immunization protocol for studies with VOC boosters. BALB/c mice were immunized according to the indicated schedule with two doses (1 ug each) of the original BNT162b2 vaccine, followed by at least one dose (1 ug total) of a monovalent, bivalent, or trivalent booster dose of either: (a) the original BNT162b2 ("BNT162b2"); (b) BNT162b2 OMI BA.1 ("OMI BA.1"); (c) BNT162b2 OMI BA.4/5 ("OMI BA.4/5"); or a combination thereof.

Fig. 35. Baseline grouped neutralizing GMTs. Sera drawn from mice immunized as depicted in Fig. 34 (day 104, pre-boost) were assessed for geometric mean titers of neutralizing antibodies against various strains. Data are presented grouped by cohort.

Fig. 36. Baseline staggered neutralizing GMTs. Sera drawn from mice immunized as depicted in Fig. 34 (day 104, pre-boost) were assessed for geometric mean titers of neutralizing antibodies against various strains. Data are presented in staggered format (i.e., by strain against which neutralization was assessed).

Fig. 37. Baseline cross-neutralization. Sera drawn from mice immunized as depicted in Fig. 34 (day 104, pre-boost) were assessed for geometric mean titers of neutralizing antibodies against various strains. Cross-neutralization results are presented as calculated variant/Wuhan reference GMT Ratios.

Fig. 38. Post-boost geometric mean fold increase in GMTs. Sera drawn from mice immunized as depicted in Fig. 34 (day 111, 7-days post-boost) were assessed for geometric mean fold increase in GMT of neutralizing antibodies against various strains.

Fig. 39. Post-boost grouped neutralizing GMTs. Sera drawn from mice immunized as depicted in Fig. 34 (day 111, 7-days post-boost) were assessed for geometric mean fold

increase in GMT of neutralizing antibodies against various strains. Data are presented grouped by cohort.

Fig. 40. Post-boost cross-neutralization. Sera drawn from mice immunized as depicted in Fig. 34 (day 111, 7-days post-boost) were assessed for geometric mean fold increase in GMT of neutralizing antibodies against various strains. Cross-neutralization results are presented as calculated variant/Wuhan reference GMT Ratios.

Fig. 41. Cohorts and sampling. Serum samples (droplet) were drawn from three cohorts: individuals triple-vaccinated with BNT162b2 that were SARS-CoV-2-naïve at the time of sampling (BNT162b2^3), and from individuals vaccinated with three doses of mRNA COVID-19 vaccine (BNT162b2/mRNA-1273 homologous or heterologous regimens) who subsequently had a breakthrough infection with Omicron either at a time of BA.1 dominance (November 2021 to January 2022; mRNA-Vax^3 + BA.1) or at a time of BA.2 dominance (March to May 2022; mRNA-Vax^3 + BA.2). For convalescent cohorts, relevant intervals between key events such as the most recent vaccination, SARS-CoV-2 infection, and serum isolation are indicated. All values specified as median-range. N/A, not applicable.

Fig. 42. Omicron BA.2 breakthrough infection of triple mRNA vaccinated individuals induces broad neutralization of SARS-CoV-2 variant pseudoviruses including Omicron BA.4/5. Cohorts and serum sampling as described in Fig. 41. (A) 50% pseudovirus neutralization (pVN_{50}) geometric mean titers (GMTs) against the indicated SARS-CoV-2 variants of concern (VOCs) or SARS-CoV-1 pseudoviruses. Values above violin plots represent group geometric mean titers (GMTs). BNT162b2^3 indicates triple vaccinated, SARS-CoV-2- naïve individuals; mRNA-Vax^3 + BA.1 indicates triple-vaccinated individuals who subsequently had a breakthrough infection with a Omicron BA.1 variant; and mRNA-Vax^3 + BA.2 indicates triple vaccinated indivuals who subsequently had a breakthrough infection with an Omicron BA.2 variant. (B) SARS-CoV-2 variant of concern (VOC) pVN_{50} GMTs normalized against the wild-type strain pVN_{50} GMT (ratio VOC to wild-type). Group geometric mean ratios with 95% confidence intervals are shown in the graph and listed in the table. Serum was tested in duplicate. For titer values below the limit of detection (LOD), LOD/2 values were plotted. The non-parametric Friedman test with Dunn's multiple comparisons correction was used to

compare the wild-type strain neutralizing group GMTs with titers against the indicated variants and SARS-CoV-1. Multiplicity-adjusted p values are shown.

Fig. 43. Omicron BA.2 breakthrough infection of previously vaccinated individuals induces broad neutralization of authentic live SARS-CoV-2 variants including Omicron BA.4/5. Cohorts and serum sampling as described in Fig. 41. (A) 50% virus neutralization (VN_{50}) geometric mean titers (GMTs) against the indicated SARS-CoV-2 variants of concern (VOCs). Values listed above violin plots represent group GMTs. (B) SARS-CoV-2 VOC VN_{50} GMTs normalized against the wild-type strain VN_{50} GMT (ratio VOC to wild-type). Group geometric mean ratios with 95% confidence intervals are shown in the graph and listed in the table. Serum was tested in duplicate. For titer values below the limit of detection (LOD), LOD/2 values were plotted. The non-parametric Friedman test with Dunn's multiple comparisons correction was used to compare the wild-type strain neutralizing group GMTs with titers against the indicated variants and SARS-CoV-1. Multiplicity-adjusted p values are shown.

Fig. 44. Neutralization of Omicron BA.2 and BA.4/5 by sera of triple mRNA vaccinated BA.2 convalescent individuals is mediated to a large extent by NTD-targeting antibodies. Cohorts and serum sampling as described in Fig. 41. (A) Serum samples (n=6 per cohort) were depleted of RBD- or NTD-binding antibodies. Relative neutralizing activity of RBD- and NTD-depleted sera (pVN_{50} titers of undepleted control sera were set to 100%) against the wild-type strain, BA.1, BA.2, and BA.4/5 was calculated and group geometric mean with 95% confidence intervals are shown. (B) 50% pseudovirus neutralization (pVN_{50}) geometric mean titers (GMTs) against Omicron BA.4/5 and Omicron BA.1-BA.4/5 hybrid pseudoviruses. Numbers above plots indicate group geometric mean titers (GMTs) and fold-change in GMTs between BA.4/5 and the hybrid pseudovirus. For titer values below the limit of detection (LOD), LOD/2 values are plotted.

Fig. 45. Omicron BA.2 breakthrough infection of BNT162b2 triple-vaccinated individuals induces broad neutralization of VOCs including Omicron BA.4/BA.5. Cohorts and serum sampling as described in Fig. 41. (A)-(B) 50% pseudovirus neutralization (pVN_{50}) geometric mean titers (GMTs) against the indicated SARS-CoV-2 variants of concern (VOCs) or SARS-CoV-1 pseudoviruses. Values listed above violin plots represent group GMTs. (C) The ratio of SARS-

CoV-2 VOC pVN_{50} GMTs normalized against the wild-type strain pVN_{50} GMT. Geometric mean ratios for the Omicron BA.2 breakthrough infected cohort were compared to BNT162b2[3] and BNT162b2[3] + BA.1. Group geometric mean ratios with 95% confidence intervals are shown. (D)-(E) 50% virus neutralization (VN_{50}) GMTs for BNT162b2[3] + BA.2 and BNT162b2[3] + BA.1. Values listed above violin plots represent group GMTs. (F) The ratio of SARS-CoV-2 VOC GMTs normalized against the wild-type strain VN_{50} GMT. Serum was tested in duplicate. For titer values below the limit of detection (LOD), LOD/2 values are plotted. The non-parametric Friedman test with Dunn's multiple comparisons correction was used to compare the group GMT against the wild-type strain with group GMTs against the indicated variants and SARS-CoV-1. Multiplicity-adjusted p values are shown.

Fig. 46. 50% pseudovirus neutralization (pVN_{50}) correlates with 50% live SARS-CoV-2 neutralization (VN_{50}) titer data. Nonparametric Spearman correlation of VSV-SARS-CoV-2 pVN_{50} with live SARS-CoV-2 VN_{50} titers for n=45 serum samples drawn from SARS-CoV-2-naïve BNT162b2 triple-vaccinated individuals (BNT162b2[3]; n=18) after the third dose, from triple mRNA vaccinated individuals with subsequent Omicron BA.1 breakthrough infection (mRNA-Vax[3] + BA.1; n=14) post-infection, and from triple mRNA vaccinated individuals with subsequent Omicron BA.2 breakthrough infection (mRNA-Vax[3] + BA.2; n=13) post-infection. Correlations are plotted per SARS-CoV-2 variant. Correlation coefficient r, two-tailed P values and the linear equation are given.

Fig. 47. RBD-binding and NTD-binding antibodies can be depleted from human serum. Serum was drawn from SARS-CoV-2-naïve BNT162b2 triple-vaccinated individuals (BNT162b2[3]; n=6), and from triple RNA vaccinated individuals with Omicron BA.1 (mRNA-Vax[3] + BA.1; n=6) or Omicron BA.2 breakthrough infection (mRNA-Vax[3] + BA.2; n=6). Magnetic bead technology was used for depleting serum of RBD- or NTD-binding antibodies, or for mock depleting. (A) Schematic of antibody depletion from serum. (B) The relative concentration of RBD-binding and NTD-binding antibodies was determined by a multiplexed electrochemiluminescence immunoassay. The relative decrease in antibody concentrations in depleted compared to mock-depleted sera are shown. Numbers above graph depict geometric mean reduction within groups.

Fig. 48. Characterization of SARS-CoV-2 S glycoproteins used in the assays based on live authentic SARS-CoV-2. The sequence of the Wuhan-Hu-1 isolate SARS-CoV-2 S glycoprotein (GenBank: QHD43416.1) was used as reference. Amino acid positions, amino acid descriptions (one letter code) and kind of alterations (substitutions, deletions, insertions) are indicated. NTD, N-terminal domain; RBD, Receptor-binding domain, Δ, deletion; ins, insertion; *, Cytoplasmic domain truncated for the C-terminal 19 amino acids.

Fig. 49. BA.4/5-Breakthrough Infection and BA.4/5-Booster Study Design. (a) The effect of Omicron BA.4/BA.5 breakthrough infection on serum neutralizing activity was evaluated in individuals vaccinated with three doses of mRNA COVID-19 vaccine (BNT162b2/mRNA-1273 homologous or heterologous regimens) who subsequently experienced an infection with Omicron BA.4 or BA.5. The intervals between vaccination, breakthrough infection and sampling are indicated as median/range. (b) Effects of Omicron BA.4/BA.5-adapted booster vaccines on serum neutralizing activity was investigated in mice vaccinated twice (with the two vaccines administered 21-days apart) with BNT162b2, followed by a booster dose of BA.4/BA.5-adapted vaccines 3.5 months later. Neutralizing activity was assessed before (pre-D3) and 7, 21, and 35 days after the booster dose (d7D3, d21D3, d35D3, respectively). (c) The effects of Omicron BA.4/BA.5-adapted vaccines on serum neutralizing activity were investigated in vaccine-naïve mice vaccinated twice (with the two vaccines administered 21-days apart) with BA.4/BA.5-adapted vaccines. Neutralizing activity was assessed 14 days after administration of the second dose (d14D2).

Fig. 50. Omicron BA.4/BA.5 breakthrough infection of triple mRNA vaccinated individuals mediates pan-Omicron neutralization. Cohorts and serum sampling as described in Figure 53. (a) 50% pseudovirus neutralization (pVN_{50}) geometric mean titers (GMTs) in sera of mRNA-Vax^3 + BA.4/BA.5 against the indicated SARS-CoV-2 variants of concern (VOCs) or SARS-CoV-1 pseudoviruses. Values above bars represent group GMTs. (b) SARS-CoV-2 VOC pVN_{50} GMTs normalized against the wild-type strain pVN_{50} GMT (ratio VOC to wild-type) of mRNA-Vax^3 + BA.4/BA.5 and the reference cohorts as outlined in Figure 53. Group geometric mean ratios with 95% confidence intervals are shown. (c) 50% virus neutralization (VN_{50}) GMTs in sera of mRNA-Vax^3 + BA.4/BA.5 against the indicated SARS-CoV-2 VOCs. (d) SARS-CoV-2 VOC VN_{50} GMTs normalized against the wild-type strain VN_{50} GMT (ratio VOC to wild-type). Serum was

tested in duplicate. For titer values below the limit of detection (LOD), LOD/2 values were plotted. The non-parametric Friedman test with Dunn's multiple comparisons correction was used to compare the wild-type strain neutralizing group GMTs with titers against the indicated variants and SARS-CoV-1. Multiplicity-adjusted p values are shown.

Fig. 51. Booster immunization with an Omicron BA.4/BA.5 S glycoprotein adapted RNA-vaccine mediates pan-Omicron neutralization in double-vaccinated mice. BALB/c mice (n=8) were injected intramuscularly with two doses of 1 μg BNT162b2 21 days apart, and a third dose of either BNT162b2 (1 μg) or the indicated monovalent (1 μg) or bivalent (0.5 μg each) Omicron BA.1 or BA.4/5-adapted vaccines 104 days after the first vaccination. (a) 50% pseudovirus neutralization (pVN_{50}) geometric mean titers (GMTs) against the indicated SARS-CoV-2 variants of concern (VOCs) in sera collected 21 days after the third vaccination (d21D3). Values above bars represent group GMTs. (b) Geometric mean fold-increase (GMFI) of pVN_{50} titers on d21D3 relative to baseline titers before the third vaccination. Values above bars represent group GMFIs. (c) SARS-CoV-2 VOC pVN_{50} GMTs normalized against the wild-type strain pVN_{50} GMT (ratio VOC to wild-type). Group geometric mean ratios are shown. (d) GMFI of pVN_{50} titers against BA.1 and BA.4/5 over time relative to baseline titers before the booster vaccination with BNT162b2/BA.1 or BNT162b2/BA.4/5 bivalent vaccines. For titer values below the limit of detection (LOD), LOD/2 values were plotted. Error bars represent 95% confidence intervals (e) 50% virus neutralization (VN_{50}) GMTs against the indicated SARS-CoV-2 VOCs on d21D3. Values above bars represent group GMTs. Error bars represent 95% confidence interval. (f) SARS-CoV-2 VOC VN_{50} GMTs normalized against the wild-type strain VN_{50} GMT (ratio VOC to wild-type). Group geometric mean ratios are shown. Serum was tested in duplicate. For titer values below the limit of detection (LOD), LOD/2 values were plotted. Error bars represent 95% confidence intervals.

Fig. 52. Immunization with an Omicron BA.4/BA.5 S glycoprotein supplemented BNT162b2 mRNA vaccine drives pan-Omicron neutralization in previously unvaccinated mice. Vaccine-naïve BALB/c mice (n=5) were injected intramuscularly with two doses of either BNT162b2 (1 μg) or the indicated monovalent (1 μg) or bivalent (0.5 μg each) Omicron BA.1 or BA.4/5-adapted vaccines, 21 days apart. 50% pseudovirus neutralization (pVN_{50})

geometric mean titers (GMTs) against the indicated SARS-CoV-2 variants of concern (VOCs) in sera collected 14 days after the second vaccination (d14D2). Values above bars represent group GMTs. Error bars represent 95% confidence interval. Serum was tested in duplicate. For titer values below the limit of detection (LOD), LOD/2 values were plotted. "Wild-type" refers to neutralization titers against a pseudovirus comprising a SARS-CoV-2 S protein of the original Wuhan variant.

Fig. 53. Cohorts and sampling. Serum samples (droplet) were drawn from four cohorts: individuals vaccinated with three doses of mRNA COVID-19 vaccine (BNT162b2/mRNA-1273 homologous or heterologous regimens) who subsequently had a breakthrough infection with Omicron BA.4/BA.5 (mRNA-Vax^3 + BA.4/BA.5). The breakthrough infections occurred at a time of BA.4/BA.5 dominance and/or were variant-confirmed by genome sequencing. Three cohorts were included in the study as references: triple-mRNA vaccinated individuals who experienced breakthrough infection at a time of either BA.2 dominance (March to May 2022; mRNA-Vax^3 + BA.2), or BA.1 dominance (November 2021 to January 2022; mRNA-Vax^3 + BA.1), or individuals triple-vaccinated with BNT162b2 that were SARS-CoV-2-naïve at the time of sampling (BNT162b2^3). For convalescent cohorts, relevant intervals between key events such as the most recent vaccination, SARS-CoV-2 infection, and serum isolation are indicated. All values specified as median-range. N/A, not applicable.

Fig. 54. Design of Omicron-adapted vaccines. Characterization of SARS-CoV-2 S glycoproteins encoded by variant-specific RNA vaccines (e.g., in some embodiments mRNA vaccines). The sequence of the Wuhan-Hu-1 isolate SARS-CoV-2 S glycoprotein (GenBank: QHD43416.1) was used as reference. Amino acid positions, amino acid descriptions (one letter code) and type of alterations (substitutions, deletions, insertions) are indicated. NTD, N-terminal domain; RBD, Receptor-binding domain, Δ, deletion; ins, insertion. In some embodiments, variant specific vaccines further comprise mutations that stabalize a pre-fusion conformation (e.g., proline mutations at positions corresponding to residues 986 and 987 of SEQ ID NO: 1) and/or do not comprise a C-terminal truncation.

Fig. 55. Omicron-specific vaccines exhibit comparable RNA purity and integrity, and *in vitro* expression of antigens. (a) Liquid capillary electropherograms of *in vitro* transcribed samples. (b) Surface expression of BNT162b2 and Omicron-adapted vaccines in HEK293T

cells measured using mFc-tagged human ACE-2 as a detection reagent with subsequent analysis in flow cytometry. HEK293T cells were transfected with BNT162b2 or Omicron-adapted vaccines formulated as lipid nanoparticles or vaccine RNAs mixed with a commercial transfection reagent, or no vaccine/RNA (non-transfected). Heights of bars indicate the means of technical replicates.

Fig. 56. Breadth and magnitude of neutralizing activity against SARS-CoV-2 variants are comparable in BNT162b2-vaccinated mice prior to booster vaccination. BALB/c mice were injected intramuscularly with two doses of 1 μg BNT162b2, administered 21 days apart. Mice were allocated to groups (n=8) prior to administration with a booster dose of the indicated vaccines. Serum was collected from mice on day 104 after the first vaccination, before booster vaccines were injected. (a) 50% pseudovirus neutralization (pVN_{50}) geometric mean titers (GMTs) against the indicated SARS-CoV-2 variants of concern (VOCs). Values above bars represent group GMTs. Error bars represent 95% confidence intervals. (b) SARS-CoV-2 VOC pVN_{50} GMTs normalized against the wild-type strain pVN_{50} GMT (ratio VOC to wild-type). Group geometric mean ratios are shown. Serum was tested in duplicate. For titer values below the limit of detection (LOD), LOD/2 values were plotted.

Fig. 57. Booster immunization with an Omicron BA.4/BA.5 S glycoprotein adapted mRNA vaccine mediates pan-Omicron neutralization in mice 7 days after administering the booster. BALB/c mice (n=8) were injected intramuscularly with two doses of 1 μg BNT162b2 (with a 21-day interval between the two doses), and a third dose of either BNT162b2 (1 μg) or the indicated monovalent (1 μg) or bivalent (0.5 μg each) Omicron BA.1 or BA.4/5-adapted vaccines 104 days after the first vaccination. (a) 50% pseudovirus neutralization (pVN_{50}) geometric mean titers (GMTs) against the indicated SARS-CoV-2 variants of concern (VOCs) in sera collected 7 days after the third vaccination (d7D3). Values above bars represent group GMTs. (b) Geometric mean fold-increase (GMFI) of pVN_{50} titers on d7D3 relative to baseline titers before the third vaccination. Values above bars represent group GMFIs. (c) SARS-CoV-2 VOC pVN_{50} GMTs normalized against the wild-type strain pVN_{50} GMT (ratio VOC to wild-type). Group geometric mean ratios are shown. Serum was tested in

duplicate. For titer values below the limit of detection (LOD), LOD/2 values were plotted. Error bars represent 95% confidence intervals.

Fig. 58. Booster immunization with an Omicron BA.4/BA.5 S glycoprotein adapted vaccine mediates pan-Omicron neutralization in mice 35 days after the booster. BALB/c mice (n=8) were injected intramuscularly with two doses of 1 µg BNT162b2 (with a 21-day interval between the two doses), and a third dose of either BNT162b2 (1 µg) or the indicated monovalent (1 µg) or bivalent (0.5 µg each) Omicron BA.1 or BA.4/5-adapted vaccines 104 days after the first vaccination. (a) 50% pseudovirus neutralization (pVN_{50}) geometric mean titers (GMTs) against the indicated SARS-CoV-2 variants of concern (VOCs) in sera collected 35 days after the third vaccination (d35D3). Values above bars represent group GMTs. (b) Geometric mean fold-increase (GMFI) of pVN_{50} titers on d35D3 relative to baseline titers before the third vaccination. Values above bars represent group GMFIs. (c) SARS-CoV-2 VOC pVN_{50} GMTs normalized against the wild-type strain pVN_{50} GMT (ratio VOC to wild-type). Group geometric mean ratios are shown. (d) 50% virus neutralization (VN_{50}) GMTs against the indicated SARS-CoV-2 VOCs on d35D3. Values above bars represent group GMTs. Error bars represent 95% confidence interval. (e) SARS-CoV-2 VOC VN_{50} GMTs normalized against the wild-type strain VN_{50} GMT (ratio VOC to wild-type). Group geometric mean ratios are shown. Serum was tested in duplicate. For titer values below the limit of detection (LOD), LOD/2 values were plotted. Error bars represent 95% confidence intervals.

Fig. 59. Omicron BA.4/BA.5 breakthrough infection of triple mRNA vaccinated individuals induces cross-neutralization of Omicron BA.4.6 and BA.2.75. Cohorts and serum sampling as described in Fig. 61. (a) 50% pseudovirus neutralization (pVN_{50}) geometric mean titers (GMTs) against the indicated SARS-CoV-2 wild-type strain or Omicron variants of concern (VOCs). Values above bar graphs represent group GMTs. For titer values below the limit of detection (LOD), LOD/2 values were plotted. The non-parametric Friedman test with Dunn's multiple comparisons correction was used to compare neutralizing titers against the Omicron BA.4/BA.5 pseudovirus (which represents currently dominating BA.5) with titers against the other pseudoviruses. Multiplicity-adjusted p values are shown. (b) SARS-CoV-2 VOC pVN_{50} GMTs normalized against the wild-type strain pVN_{50} GMT (ratio VOC to wild-

type). Group geometric mean ratios with 95% confidence intervals are shown. The non-parametric Kruskal-Wallis test with Dunn's multiple comparisons correction was used to compare the VOC GMT ratios between cohorts. ****, P<0.0001; **, P<0.01; *, P<0.05. Serum was tested in duplicate.

Fig. 60. Alterations of the spike glycoprotein amino acid sequence of SARS-CoV-2 Omicron sub-lineages. White letters in boxes indicate the amino acid substitution per sub-lineage; Δ, deletion; ins, insertion; NTD, N-terminal domain; RBD, receptor-binding domain.

Fig. 61. Cohorts and sampling. Serum samples were drawn from five cohorts: SARS-CoV-2-naïve individuals triple-vaccinated with BNT162b2 (BNT162b2^3) or quadruple-vaccinated with BNT162b2 (BNT162b2^4), and individuals with three doses of mRNA COVID-19 vaccine (BNT162b2/mRNA-1273 homologous or heterologous regimens) who subsequently had a breakthrough infection with Omicron BA.1 (mRNA-Vax^3 + BA.1), with BA.2 (mRNA-Vax^3 + BA.2) or with BA.4/BA.5 (mRNA-Vax^3 + BA.4/5). Breakthrough infections occurred at a time of respective VOC dominance (BA.1: November 2021 to January 2021, BA.2: March to May 2022, BA.4/5: mid-June to mid-July 2022) and/or were variant confirmed by genome sequencing. For convalescent cohorts, relevant intervals between key events such as the most recent vaccination, SARS-CoV-2 infection, and serum isolation are indicated. All values specified as median-range. N/A, not applicable; §, Serum draw was performed between 28 to 35 Days after vaccination as per protocol.

Fig. 62. Characterization of SARS-CoV-2 S glycoproteins used in VSV-SARS-CoV-2 variant pseudovirus neutralization assays. Mutation positions shown in reference to the sequence of the Wuhan-Hu-1 isolate SARS-CoV-2 S glycoprotein (GenBank: QHD43416.1). Amino acid positions, amino acid descriptions (one letter code) and kind of alterations (substitutions, deletions, insertions) are indicated. NTD, N-terminal domain; RBD, Receptor-binding domain, Δ, deletion; ins, insertion; *, Cytoplasmic domain truncated for the C-terminal 19 amino acids.

Fig. 63. Omicron BA.4/5, BA.4.6, BA.2.75.2, BQ.1.1, and XBB.1 neutralizing response with a bivalent (BA.4/5-adapted RNA + BNT162b2) or BNT16b2 monovalent booster. Bar heights and numbers immediately above bars indicate geometric means of neutralization titers

(GMTs). Whiskers indicate 95% CI. Bars labeled as „Bivalent" indicate neutralization titers from subjects administered as a 4th dose a bivalent vaccine (comprising RNA encoding a SARS-CoV-2 S protein of a Wuhan strain and RNA encoding a SARS-CoV-2 S protein comprising one or more mutations characteristic of a BA.4/5 Omicron variant); bars labeled as „Monovalent" indicate neutralization titers from subjects administered as a 4th dose a monovalent vaccine (comprising RNA encoding a SARS-CoV-2 S protein of a Wuhan strain). $FFRNT_{50}$s against USA-WA1/2020 spike, BA.4/5-spike, BA.4.6-spike, BA.2.75.2-spike, BQ.1.1-spike, XBB.1-spike are shown for the bivalent or monovalent booster. "Pre" samples correspond to serum samples collected on the day of booster administration; "1MPD4" samples correspond to serum samples collected one month post dose 4 (i.e., one month post booster administration). GMFR corresponds to GMT fold rise, and was calculated as a ratio of 1MPD4 GMTs to Pre GMTs. Numbers above GMFRs indicate ratios between GMFRs of bivalent and GMFRs of monovalent. (A) $FFRNT_{50}$s of all subjects regardless of infection status. p values (two-tailed, Wilcoxon matched-pairs signed-rank test) for GMFR of bivalent or monovalent booster against USA-WA1/2020, BA.4/5-spike, BA.4.6-spike, BA.2.75.2-spike, BQ.1.1-spike, XBB.1-spike: all <0.0001. p values (two-tailed, Mann-Whitney test) for GMFR ratios of bivalent to monovalent booster against USA-WA1/2020, BA.4/5-spike, BA.4.6-spike, BA.2.75.2-spike, BQ.1.1-spike, XBB.1-spike: 0.0061, <0.0001, <0.0001, <0.0001, <0.0001, <0.0001. (B) $FFRNT_{50}$s of all subjects without evidence of prior SARS-CoV-2 infection. p values (two-tailed, Wilcoxon matched-pairs signed-rank test) for GMFR of bivalent booster against USA-WA1/2020, BA.4/5-spike, BA.4.6-spike, BA.2.75.2-spike, BQ.1.1-spike, XBB.1-spike: all <0.0001. p values (two-tailed, Wilcoxon matched-pairs signed-rank test) for GMFR of monovalent booster against USA-WA1/2020, BA.4/5-spike, BA.4.6-spike, BA.2.75.2-spike, BQ.1.1-spike, XBB.1-spike: 0.013, 0.0006, <0.0001, <0.0001, 0.013, 0.016. p values (two-tailed, Mann-Whitney test) for GMFR ratios of bivalent to monovalent booster against USA-WA1/2020, BA.4/5-spike, BA.4.6-spike, BA.2.75.2-spike, BQ.1.1-spike, XBB.1-spike: 0.035, <0.0001, <0.0001, <0.0001, <0.0001, <0.0001. (C) $FFRNT_{50}$s of all subjects with evidence of prior SARS-CoV-2 infection. p values (two-tailed, Wilcoxon matched-pairs signed-rank test) for GMFR of bivalent booster against USA-WA1/2020, BA.4/5-spike, BA.4.6-spike, BA.2.75.2-spike, BQ.1.1-spike, XBB.1-spike: all <0.0001. p values (two-tailed, Wilcoxon matched-pairs signed-rank test) for GMFR of monovalent booster against USA-WA1/2020, BA.4/5-spike, BA.4.6-spike, BA.2.75.2-spike, BQ.1.1-spike, XBB.1-spike: 0.0016, 0.0003, 0.0026, 0.0011, <0.0001, <0.0001. p values (two-

tailed, Mann-Whitney test) for GMFR ratios of bivalent to monovalent booster against USA-WA1/2020, BA.4/5-spike, BA.4.6-spike, BA.2.75.2-spike, BQ.1.1-spike, XBB.1-spike: 0.065, 0.025, 0.0032, 0.0086, 0.0064, 0.0006.

Fig. 64. Distinct cross-neutralization of Omicron sublineages by vaccine-elicited and convalescent immune sera. (A) 50% pseudovirus neutralization (pVN_{50}) geometric mean titers (GMTs) against the indicated SARS-CoV-2 wild-type strain or Omicron variants of concern (VOCs) in individuals (i) triple- or (ii) quadruple-vaccinated with BNT162b2 that were SARS-CoV-2-naïve at the time of sampling, and from individuals vaccinated with three doses of mRNA COVID-19 vaccine (BNT162b2/mRNA-1273 homologous or heterologous regimens) who subsequently had a breakthrough infection with Omicron at a time of (iii) BA.1 dominance, (iv) BA.2 dominance or (v) BA.4/5 dominance. Values above bar graphs represent group GMTs. For titer values below the limit of detection (LOD), LOD/2 values were plotted. The non-parametric Friedman test with Dunn's multiple comparisons correction was used to compare neutralizing titers against the Omicron BA.4/BA.5 pseudovirus (which represents currently dominating BA.5) with titers against the other pseudoviruses. Multiplicity-adjusted p values are shown. (B) SARS-CoV-2 VOC pVN_{50} GMTs normalized against the wild-type strain pVN_{50} GMT (ratio VOC to wild-type). Group geometric mean ratios with 95% confidence intervals are shown. The non-parametric Kruskal-Wallis test with Dunn's multiple comparisons correction was used to compare the VOC GMT ratios between cohorts. ****, P<0.0001; ***, p<0.001; **, P<0.01; *, P<0.05. Serum was tested in duplicate.

Fig. 65. Alterations on the spike glycoprotein amino acid sequence of SARS-CoV-2 Omicron sub-lineages. Amino acid positions, amino acid descriptions (one letter code) and types of mutations (substitutions, deletions, insertions) are indicated. White letters in boxes indicate the amino acid substitution per sub-lineage; Δ, deletion; ins, insertion; NTD, N-terminal domain; RBD, receptor-binding domain

Fig. 66. T cell epitope and B cell epitope retention in the S protein of certain SARS-CoV-2 variants of concern. All potential T cell epitopes were retrieved from the Immune Epitope Database (IEDB) on November 11, 2022, and the percentages of unaltered S protein linear T

cell epitopes (as compared to epitopes present in BNT162b2 – 100%) present in each variant strain were calculated. Percentages of unaltered B cell neutralization epitopes (NTD and RBD) present in each variant strain compared to BNT162b2 were estimated by an automated Early Warning System (described in Beguir, Karim, et al., "Early computational detection of potential high risk SARS-CoV-2 variants," bioRxiv (2021)). (A) Radar chart and (B) line plot analysis of T-cell and neutralizing B cell epitope conservedness in the S protein across SARS-CoV-2 variants of concern. Percent unaltered T cell epitopes andpercent unaltered B cell neutralization epitopes are shown. In (B) numbers on top corresponding to each variant represent number of mutations in spike protein comparing to Wuhan strain. Variants are ranked in ascending order in terms of mutation number.

Detailed description

Although the present disclosure is described in detail below, it is to be understood that this disclosure is not limited to the particular methodologies, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present disclosure which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art.

Preferably, the terms used herein are defined as described in "A multilingual glossary of biotechnological terms: (IUPAC Recommendations)", H.G.W. Leuenberger, B. Nagel, and H. Kölbl, Eds., Helvetica Chimica Acta, CH-4010 Basel, Switzerland, (1995).

The practice of the present disclosure will employ, unless otherwise indicated, conventional methods of chemistry, biochemistry, cell biology, immunology, and recombinant DNA techniques which are explained in the literature in the field (cf., e.g., Molecular Cloning: A Laboratory Manual, 2nd Edition, J. Sambrook et al. eds., Cold Spring Harbor Laboratory Press, Cold Spring Harbor 1989).

In the following, the elements of the present disclosure will be described. These elements are listed with specific embodiments, however, it should be understood that they may be combined in any manner and in any number to create additional embodiments. The variously described examples and embodiments should not be construed to limit the present disclosure

to only the explicitly described embodiments. This description should be understood to disclose and encompass embodiments which combine the explicitly described embodiments with any number of the disclosed elements. Furthermore, any permutations and combinations of all described elements should be considered disclosed by this description unless the context indicates otherwise.

Several documents are cited throughout the text of this specification. Each of the documents cited herein (including all patents, patent applications, scientific publications, manufacturer's specifications, instructions, etc.), whether supra or infra, are hereby incorporated by reference in their entirety. Nothing herein is to be construed as an admission that the present disclosure was not entitled to antedate such disclosure.

Definitions

In the following, definitions will be provided which apply to all aspects of the present disclosure. The following terms have the following meanings unless otherwise indicated. Any undefined terms have their art recognized meanings.

The term "about" means approximately or nearly, and in the context of a numerical value or range set forth herein in one embodiment means ± 20%, ± 10%, ± 5%, or ± 3% of the numerical value or range recited or claimed.

The terms "a" and "an" and "the" and similar reference used in the context of describing the present disclosure (especially in the context of the claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. Recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it was individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as"), provided herein is intended merely to better illustrate the disclosure and does not pose a limitation on the scope of the claims. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the disclosure.

Unless expressly specified otherwise, the term "comprising" is used in the context of the present document to indicate that further members may optionally be present in addition to

the members of the list introduced by "comprising". It is, however, contemplated as a specific embodiment of the present disclosure that the term "comprising" encompasses the possibility of no further members being present, i.e., for the purpose of this embodiment "comprising" is to be understood as having the meaning of "consisting of" or "consisting essentially of".

Terms such as "reduce", "decrease", "inhibit" or "impair" as used herein relate to an overall reduction or the ability to cause an overall reduction, preferably of at least 5%, at least 10%, at least 20%, at least 50%, at least 75% or even more, in the level. These terms include a complete or essentially complete inhibition, i.e., a reduction to zero or essentially to zero.

Terms such as "increase", "enhance" or "exceed" preferably relate to an increase or enhancement by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 80%, at least 100%, at least 200%, at least 500%, or even more.

According to the present disclosure, the term "peptide" comprises oligo- and polypeptides and refers to substances which comprise about two or more, about 3 or more, about 4 or more, about 6 or more, about 8 or more, about 10 or more, about 13 or more, about 16 or more, about 20 or more, and up to about 50, about 100 or about 150, consecutive amino acids linked to one another via peptide bonds. The term "protein" or "polypeptide" refers to large peptides, in particular peptides having at least about 150 amino acids, but the terms "peptide", "protein" and "polypeptide" are used herein usually as synonyms.

A "therapeutic protein" has a positive or advantageous effect on a condition or disease state of a subject when provided to the subject in a therapeutically effective amount. In one embodiment, a therapeutic protein has curative or palliative properties and may be administered to ameliorate, relieve, alleviate, reverse, delay onset of or lessen the severity of one or more symptoms of a disease or disorder. A therapeutic protein may have prophylactic properties and may be used to delay the onset of a disease or to lessen the severity of such disease or pathological condition. The term "therapeutic protein" includes entire proteins or peptides, and can also refer to therapeutically active fragments thereof. It can also include therapeutically active variants of a protein. Examples of therapeutically active proteins include, but are not limited to, antigens for vaccination and immunostimulants such as cytokines.

"Fragment", with reference to an amino acid sequence (peptide or protein), relates to a part of an amino acid sequence, i.e. a sequence which represents the amino acid sequence shortened at the N-terminus and/or C-terminus. A fragment shortened at the C-terminus (N-

terminal fragment) is obtainable e.g. by translation of a truncated open reading frame that lacks the 3'-end of the open reading frame. A fragment shortened at the N-terminus (C-terminal fragment) is obtainable e.g. by translation of a truncated open reading frame that lacks the 5'-end of the open reading frame, as long as the truncated open reading frame comprises a start codon that serves to initiate translation. A fragment of an amino acid sequence comprises e.g. at least 50 %, at least 60 %, at least 70 %, at least 80%, at least 90% of the amino acid residues from an amino acid sequence. A fragment of an amino acid sequence preferably comprises at least 6, in particular at least 8, at least 12, at least 15, at least 20, at least 30, at least 50, or at least 100 consecutive amino acids from an amino acid sequence.

By "variant" herein is meant an amino acid sequence that differs from a parent amino acid sequence by virtue of at least one amino acid modification. The parent amino acid sequence may be a naturally occurring or wild type (WT) amino acid sequence, or may be a modified version of a wild type amino acid sequence. Preferably, the variant amino acid sequence has at least one amino acid modification compared to the parent amino acid sequence, e.g., from 1 to about 20 amino acid modifications, and preferably from 1 to about 10 or from 1 to about 5 amino acid modifications compared to the parent.

By "wild type" or "WT" or "native" herein is meant an amino acid sequence that is found in nature, including allelic variations. A wild type amino acid sequence, peptide or protein has an amino acid sequence that has not been intentionally modified.

In some embodiments, the present disclosure refers to a SARS-CoV-2 variant that is prevalent and/or rapidly spreading in a relevant jurisdiction. In some embodiments, such variants may be identified based on publicly available data (e.g., data provided in the GISAID Initiative database: https://www.gisaid.org, and/or data provided by the World Health Organization WHO (e.g., as provided at https://www.who.int/activities/tracking-SARS-CoV-2-variants). In some embodiments, such a variant refers to a variant disclosed herein.

For the purposes of the present disclosure, "variants" of an amino acid sequence (peptide, protein or polypeptide) comprise amino acid insertion variants, amino acid addition variants, amino acid deletion variants and/or amino acid substitution variants. The term "variant" includes all mutants, splice variants, posttranslationally modified variants, conformations, isoforms, allelic variants, species variants, and species homologs, in particular those which are

naturally occurring. The term "variant" includes, in particular, fragments of an amino acid sequence.

Amino acid insertion variants comprise insertions of single or two or more amino acids in a particular amino acid sequence. In the case of amino acid sequence variants having an insertion, one or more amino acid residues are inserted into a particular site in an amino acid sequence, although random insertion with appropriate screening of the resulting product is also possible. Amino acid addition variants comprise amino- and/or carboxy-terminal fusions of one or more amino acids, such as 1, 2, 3, 5, 10, 20, 30, 50, or more amino acids. Amino acid deletion variants are characterized by the removal of one or more amino acids from the sequence, such as by removal of 1, 2, 3, 5, 10, 20, 30, 50, or more amino acids. The deletions may be in any position of the protein. Amino acid deletion variants that comprise the deletion at the N-terminal and/or C-terminal end of the protein are also called N-terminal and/or C-terminal truncation variants. Amino acid substitution variants are characterized by at least one residue in the sequence being removed and another residue being inserted in its place. Preference is given to the modifications being in positions in the amino acid sequence which are not conserved between homologous proteins or peptides and/or to replacing amino acids with other ones having similar properties. Preferably, amino acid changes in peptide and protein variants are conservative amino acid changes, i.e., substitutions of similarly charged or uncharged amino acids. A conservative amino acid change involves substitution of one of a family of amino acids which are related in their side chains. Naturally occurring amino acids are generally divided into four families: acidic (aspartate, glutamate), basic (lysine, arginine, histidine), non-polar (alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), and uncharged polar (glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine) amino acids. Phenylalanine, tryptophan, and tyrosine are sometimes classified jointly as aromatic amino acids. In one embodiment, conservative amino acid substitutions include substitutions within the following groups:

glycine, alanine;

valine, isoleucine, leucine;

aspartic acid, glutamic acid;

asparagine, glutamine;

serine, threonine;

lysine, arginine; and

phenylalanine, tyrosine.

Preferably the degree of similarity, preferably identity between a given amino acid sequence and an amino acid sequence which is a variant of said given amino acid sequence will be at least about 60%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%. The degree of similarity or identity is given preferably for an amino acid region which is at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90% or about 100% of the entire length of the reference amino acid sequence. For example, if the reference amino acid sequence consists of 200 amino acids, the degree of similarity or identity is given preferably for at least about 20, at least about 40, at least about 60, at least about 80, at least about 100, at least about 120, at least about 140, at least about 160, at least about 180, or about 200 amino acids, in some embodiments continuous amino acids. In some embodiments, the degree of similarity or identity is given for the entire length of the reference amino acid sequence. The alignment for determining sequence similarity, preferably sequence identity can be done with art known tools, preferably using the best sequence alignment, for example, using Align, using standard settings, preferably EMBOSS::needle, Matrix: Blosum62, Gap Open 10.0, Gap Extend 0.5.

"Sequence similarity" indicates the percentage of amino acids that either are identical or that represent conservative amino acid substitutions. "Sequence identity" between two amino acid sequences indicates the percentage of amino acids that are identical between the sequences. "Sequence identity" between two nucleic acid sequences indicates the percentage of nucleotides that are identical between the sequences.

The terms "% identical", "% identity" or similar terms are intended to refer, in particular, to the percentage of nucleotides or amino acids which are identical in an optimal alignment between the sequences to be compared. Said percentage is purely statistical, and the differences between the two sequences may be but are not necessarily randomly distributed over the entire length of the sequences to be compared. Comparisons of two sequences are usually carried out by comparing the sequences, after optimal alignment, with respect to a segment or "window of comparison", in order to identify local regions of corresponding sequences. The optimal alignment for a comparison may be carried out manually or with the aid of the local homology algorithm by Smith and Waterman, 1981, Ads App. Math. 2, 482, with the aid of the local homology algorithm by Neddleman and Wunsch, 1970, J. Mol. Biol.

48, 443, with the aid of the similarity search algorithm by Pearson and Lipman, 1988, Proc. Natl Acad. Sci. USA 88, 2444, or with the aid of computer programs using said algorithms (GAP, BESTFIT, FASTA, BLAST P, BLAST N and TFASTA in Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive, Madison, Wis.). In some embodiments, percent identity of two sequences is determined using the BLASTN or BLASTP algorithm, as available on the United States National Center for Biotechnology Information (NCBI) website (e.g., at blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch&BLAST_SPEC=blast2seq&LINK_LOC=align2seq). In some embodiments, the algorithm parameters used for BLASTN algorithm on the NCBI website include: (i) Expect Threshold set to 10; (ii) Word Size set to 28; (iii) Max matches in a query range set to 0; (iv) Match/Mismatch Scores set to 1, -2; (v) Gap Costs set to Linear; and (vi) the filter for low complexity regions being used. In some embodiments, the algorithm parameters used for BLASTP algorithm on the NCBI website include: (i) Expect Threshold set to 10; (ii) Word Size set to 3; (iii) Max matches in a query range set to 0; (iv) Matrix set to BLOSUM62; (v) Gap Costs set to Existence: 11 Extension: 1; and (vi) conditional compositional score matrix adjustment.

Percentage identity is obtained by determining the number of identical positions at which the sequences to be compared correspond, dividing this number by the number of positions compared (e.g., the number of positions in the reference sequence) and multiplying this result by 100.

In some embodiments, the degree of similarity or identity is given for a region which is at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90% or about 100% of the entire length of the reference sequence. For example, if the reference nucleic acid sequence consists of 200 nucleotides, the degree of identity is given for at least about 100, at least about 120, at least about 140, at least about 160, at least about 180, or about 200 nucleotides, in some embodiments continuous nucleotides. In some embodiments, the degree of similarity or identity is given for the entire length of the reference sequence.

Homologous amino acid sequences exhibit according to the present disclosure at least 40%, in particular at least 50%, at least 60%, at least 70%, at least 80%, at least 90% and preferably at least 95%, at least 98 or at least 99% identity of the amino acid residues.

The amino acid sequence variants described herein may readily be prepared by the skilled person, for example, by recombinant DNA manipulation. The manipulation of DNA sequences for preparing peptides or proteins having substitutions, additions, insertions or deletions, is

described in detail in Sambrook et al. (1989), for example. Furthermore, the peptides and amino acid variants described herein may be readily prepared with the aid of known peptide synthesis techniques such as, for example, by solid phase synthesis and similar methods.

In one embodiment, a fragment or variant of an amino acid sequence (peptide or protein) is preferably a "functional fragment" or "functional variant". The term "functional fragment" or "functional variant" of an amino acid sequence relates to any fragment or variant exhibiting one or more functional properties identical or similar to those of the amino acid sequence from which it is derived, i.e., it is functionally equivalent. With respect to antigens or antigenic sequences, one particular function is one or more immunogenic activities displayed by the amino acid sequence from which the fragment or variant is derived. The term "functional fragment" or "functional variant", as used herein, in particular refers to a variant molecule or sequence that comprises an amino acid sequence that is altered by one or more amino acids compared to the amino acid sequence of the parent molecule or sequence and that is still capable of fulfilling one or more of the functions of the parent molecule or sequence, e.g., inducing an immune response. In one embodiment, the modifications in the amino acid sequence of the parent molecule or sequence do not significantly affect or alter the characteristics of the molecule or sequence. In different embodiments, the function of the functional fragment or functional variant may be reduced but still significantly present, e.g., immunogenicity of the functional variant may be at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% of the parent molecule or sequence. However, in other embodiments, immunogenicity of the functional fragment or functional variant may be enhanced compared to the parent molecule or sequence.

An amino acid sequence (peptide, protein or polypeptide) "derived from" a designated amino acid sequence (peptide, protein or polypeptide) refers to the origin of the first amino acid sequence. Preferably, the amino acid sequence which is derived from a particular amino acid sequence has an amino acid sequence that is identical, essentially identical or homologous to that particular sequence or a fragment thereof. Amino acid sequences derived from a particular amino acid sequence may be variants of that particular sequence or a fragment thereof. For example, it will be understood by one of ordinary skill in the art that the antigens suitable for use herein may be altered such that they vary in sequence from the naturally occurring or native sequences from which they were derived, while retaining the desirable activity of the native sequences.

As used herein, an "instructional material" or "instructions" includes a publication, a recording, a diagram, or any other medium of expression which can be used to communicate the usefulness of the compositions and methods of the present disclosure. The instructional material of the kit of the present disclosure may, for example, be affixed to a container which contains the compositions of the present disclosure or be shipped together with a container which contains the compositions. Alternatively, the instructional material may be shipped separately from the container with the intention that the instructional material and the compositions be used cooperatively by the recipient.

"Isolated" means altered or removed from the natural state. For example, a nucleic acid or a peptide naturally present in a living animal is not "isolated", but the same nucleic acid or peptide partially or completely separated from the coexisting materials of its natural state is "isolated". An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell.

The term "recombinant" in the context of the present disclosure means "made through genetic engineering". Preferably, a "recombinant object" such as a recombinant nucleic acid in the context of the present disclosure is not occurring naturally.

The term "naturally occurring" as used herein refers to the fact that an object can be found in nature. For example, a peptide or nucleic acid that is present in an organism (including viruses) and can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory is naturally occurring.

"Physiological pH" as used herein refers to a pH of about 7.5.

The term "genetic modification" or simply "modification" includes the transfection of cells with nucleic acid. The term "transfection" relates to the introduction of nucleic acids, in particular RNA, into a cell. For purposes of the present disclosure, the term "transfection" also includes the introduction of a nucleic acid into a cell or the uptake of a nucleic acid by such cell, wherein the cell may be present in a subject, e.g., a patient. Thus, according to the present disclosure, a cell for transfection of a nucleic acid described herein can be present *in vitro* or *in vivo*, e.g. the cell can form part of an organ, a tissue and/or an organism of a patient. According to the disclosure, transfection can be transient or stable. For some applications of transfection, it is sufficient if the transfected genetic material is only transiently expressed. RNA can be transfected into cells to transiently express its coded protein. Since the nucleic acid introduced in the transfection process is usually not integrated into the nuclear genome,

the foreign nucleic acid will be diluted through mitosis or degraded. Cells allowing episomal amplification of nucleic acids greatly reduce the rate of dilution. If it is desired that the transfected nucleic acid actually remains in the genome of the cell and its daughter cells, a stable transfection must occur. Such stable transfection can be achieved by using virus-based systems or transposon-based systems for transfection. Generally, nucleic acid encoding antigen is transiently transfected into cells. RNA can be transfected into cells to transiently express its coded protein.

The term "seroconversion" includes a ≥4-fold rise from before vaccination to 1-month post Dose 2.

Coronavirus

Coronaviruses are enveloped, positive-sense, single-stranded RNA ((+) ssRNA) viruses. They have the largest genomes (26–32 kb) among known RNA viruses and are phylogenetically divided into four genera (α, β, γ, and δ), with betacoronaviruses further subdivided into four lineages (A, B, C, and D). Coronaviruses infect a wide range of avian and mammalian species, including humans. Some human coronaviruses generally cause mild respiratory diseases, although severity can be greater in infants, the elderly, and the immunocompromised. Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus (SARS-CoV), belonging to betacoronavirus lineages C and B, respectively, are highly pathogenic. Both viruses emerged into the human population from animal reservoirs within the last 15 years and caused outbreaks with high case-fatality rates. The outbreak of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) that causes atypical pneumonia (coronavirus disease 2019; COVID-19) has raged in China since mid-December 2019, and has developed to be a public health emergency of international concern. SARS-CoV-2 (MN908947.3) belongs to betacoronavirus lineage B. It has at least 70% sequence similarity to SARS-CoV.

In general, coronaviruses have four structural proteins, namely, envelope (E), membrane (M), nucleocapsid (N), and spike (S). The E and M proteins have important functions in the viral assembly, and the N protein is necessary for viral RNA synthesis. The critical glycoprotein S is responsible for virus binding and entry into target cells. The S protein is synthesized as a single-chain inactive precursor that is cleaved by furin-like host proteases in the producing cell into two noncovalently associated subunits, S1 and S2. The S1 subunit contains the receptor-

binding domain (RBD), which recognizes the host-cell receptor. The S2 subunit contains the fusion peptide, two heptad repeats, and a transmembrane domain, all of which are required to mediate fusion of the viral and host-cell membranes by undergoing a large conformational rearrangement. The S1 and S2 subunits trimerize to form a large prefusion spike.

The S precursor protein of SARS-CoV-2 can be proteolytically cleaved into S1 (685 aa) and S2 (588 aa) subunits. The S1 subunit comprises the receptor-binding domain (RBD), which mediates virus entry into sensitive cells through the host angiotensin-converting enzyme 2 (ACE2) receptor.

Antigen

The present disclosure comprises the use of RNA encoding an amino acid sequence comprising SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof. Thus, the RNA encodes a peptide or protein comprising at least an epitope SARS-CoV-2 S protein or an immunogenic variant thereof for inducing an immune response against coronavirus S protein, in particular SARS-CoV-2 S protein in a subject. The amino acid sequence comprising SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof (i.e., the antigenic peptide or protein) is also designated herein as "vaccine antigen", "peptide and protein antigen", "antigen molecule" or simply "antigen". The SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof is also designated herein as "antigenic peptide or protein" or "antigenic sequence".

The SARS-CoV-2 coronavirus full length spike (S) protein from the first detected SARS-CoV-2 strain (referred to as the Wuhan strain herein) consists of 1273 amino acids and has the amino acid sequence according to SEQ ID NO: 1:

MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNG
TKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNK
SWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEP
LVDLPIGINITRFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK
CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFS
TFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYN
YLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVC

GPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITP
GTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSYECDIPIGAGICASYQ
TQTNSPRRARSVASQSIIAYTMSLGAENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECS
NLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNK
VTLADAGFIKQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAM
QMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISS
VLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLM
SFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSGN
CDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDL
QELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCCSCGSCCKFDEDDSEPVLKGVKLHYT
(SEQ ID NO: 1)

For purposes of the present disclosure, the above sequence is considered the wildtype or Wuhan SARS-CoV-2 S protein amino acid sequence. Unless otherwise indicated, position numberings in a SARS-CoV-2 S protein given herein are in relation to the amino acid sequence according to SEQ ID NO: 1. One of skill in the art reading the present disclosure will understand the corresponding positions in SARS-CoV-2 S protein variants based on the position numbering relative to the amino acid sequence of SEQ ID NO: 1.

In specific embodiments, full length spike (S) protein encoded by an RNA described herein can be modified in such a way that the prototypical prefusion conformation is stabilized. Certain mutations that stabilize a prefusion confirmation are known in the art, e.g., as disclosed in WO 2021243122 A2 and Hsieh, Ching-Lin, et al. ("Structure-based design of prefusion-stabilized SARS-CoV-2 spikes," Science 369.6510 (2020): 1501-1505), the contents of each which are incorporated by reference herein in their entirety. In some embodiments, a SARS-CoV-2 S protein may be stabilized by introducing one or more proline mutations. In some embodiments, a SARS-CoV-2 S protein comprises a proline substitution at positions corresponding to residues 986 and/or 987 of SEQ ID NO: 1. In some embodiments, a SARS-CoV-2 S protein comprises a proline substitution at one or more positions corresponding to residues 817, 892, 899, and 942 of SEQ ID NO: 1. In some embodiments, a SARS-CoV-2 S protein comprises a proline substitution at positions corresponding to each of residues 817, 892, 899, and 942 of SEQ ID NO: 1. In some embodiments, a SARS-CoV-2 S protein comprises a proline substitution at positions corresponding to each of residues 817, 892, 899, 942, 986, and 987 of SEQ ID NO: 1.

In some embodiments, stabilization of the prefusion conformation may be obtained by introducing two consecutive proline substitutions at residues 986 and 987 in the full length spike protein. Specifically, spike (S) protein stabilized protein variants are obtained in a way that the amino acid residue at position 986 is exchanged to proline and the amino acid residue at position 987 is also exchanged to proline. In one embodiment, a SARS-CoV-2 S protein variant wherein the prototypical prefusion conformation is stabilized comprises the amino acid sequence shown in SEQ ID NO: 7:

MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNG
TKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNK
SWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEP
LVDLPIGINITRFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK
CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFS
TFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYN
YLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVC
GPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITP
GTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSYECDIPIGAGICASYQ
TQTNSPRRARSVASQSIIAYTMSLGAENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECS
NLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNK
VTLADAGFIKQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAM
QMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISS
VLNDILSRLDPPEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLM
SFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSGN
CDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDL
QELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCCSCGSCCKFDEDDSEPVLKGVKLHYT
(SEQ ID NO: 7)

Those skilled in the art are aware of various spike variants, and/or resources that document them. For example, the following strains, their SARS-CoV-2 S protein amino acid sequences and, in particular, modifications thereof compared to wildtype SARS-CoV-2 S protein amino acid sequence, e.g., as compared to SEQ ID NO: 1, are useful herein.

B.1.1.7 ("Variant of Concern 202012/01" (VOC-202012/01)

B.1.1.7 (the "alpha variant") is a variant of SARS-CoV-2 which was first detected in October 2020 during the COVID-19 pandemic in the United Kingdom from a sample taken the previous month, and it quickly began to spread by mid-December. It is correlated with a significant

increase in the rate of COVID-19 infection in United Kingdom; this increase is thought to be at least partly because of change N501Y inside the spike glycoprotein's receptor-binding domain, which is needed for binding to ACE2 in human cells. The B.1.1.7 variant is defined by 23 mutations: 13 non-synonymous mutations, 4 deletions, and 6 synonymous mutations (i.e., there are 17 mutations that change proteins and six that do not). The spike protein changes in B.1.1.7 include deletion 69-70, deletion 144, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H.

B.1.351 (501.V2)

B.1.351 lineage (the "Beta variant") and colloquially known as South African COVID-19 variant, is a variant of SARS-CoV-2. Preliminary results indicate that this variant may have an increased transmissibility. The B.1.351 variant is defined by multiple spike protein changes including: L18F, D80A, D215G, deletion 242-244, R246I, K417N, E484K, N501Y, D614G and A701V. There are three mutations of particular interest in the spike region of the B.1.351 genome: K417N, E484K, N501Y.

B.1.1.298 (Cluster 5)

B.1.1.298 was discovered in North Jutland, Denmark, and is believed to have been spread from minks to humans via mink farms. Several different mutations in the spike protein of the virus have been confirmed. The specific mutations include deletion 69–70, Y453F, D614G, I692V, M1229I, and optionally S1147L.

P.1 (B.1.1.248)

Lineage B.1.1.248 (the "gamma variant"), known as the Brazil(ian) variant, is one of the variants of SARS-CoV-2 which has been named P.1 lineage. P.1 has a number of S-protein modifications [L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I, V1176F] and is similar in certain key RBD positions (K417, E484, N501) to variant B.1.351 from South Africa.

B.1.427/B.1.429 (CAL.20C)

Lineage B.1.427/B.1.429 (the "epsilon variant"), also known as CAL.20C, is defined by the following modifications in the S-protein: S13I, W152C, L452R, and D614G of which the L452R modification is of particular concern. CDC has listed B.1.427/B.1.429 as "variant of concern".

B.1.525

B.1.525 (the "eta variant") carries the same E484K modification as found in the P.1, and B.1.351 variants, and also carries the same ΔH69/ΔV70 deletion as found in B.1.1.7, and B.1.1.298. It also carries the modifications D614G, Q677H and F888L.

B.1.526

B.1.526 (the "iota variant") was detected as an emerging lineage of viral isolates in the New York region that shares mutations with previously reported variants. The most common sets of spike mutations in this lineage are L5F, T95I, D253G, E484K, D614G, and A701V.

The following table shows an overview of circulating SARS-CoV-2 strains which are VOI/VOC.

Table 1: Overview of certain circulating SARS-CoV-2 strains which have been/are VOI/VOC

| Lineage | Amino acid substitution | | |

B.1.1.529

B.1.529 ("Omicron variant") was first detected in South Africa in November 2021. Omicron multiplies around 70 times faster than Delta variants, and quickly became the dominant strain of SARS-CoV-2 worldwide. Since its initial detection, a number of Omicron sublineages have arisen. Listed below are the current Omicron variants of concern, along with certain characteristic mutations associated with the S protein of each. The S protein of BA.4 and BA.5 have the same set of characteristic mutations, which is why the below table has a single row for "BA.4 or BA.5", and why the present disclosure refers to a "BA.4/5" S protein in some embodiments. Similarly, the S proteins of the BA.4.6 and BF.7 Omicron variants have the same set of characteristic mutations, which is why the below table has a single row for "BA.4.6 or BF.7").

Table 2: Omicron Variants of Concern and Characteristic mutations

Subvariant	Common mutations
BA.1	A67V, Δ69-70, T95I, G142D, Δ143-145, Δ211, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F
BA.2	T19I, Δ24-26, A27S, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K
BA.2.12.1	T19I, Δ24-26, A27S, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452Q, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, S704L, N764K, D796Y, Q954H, N969K
BA.4 or BA.5	T19I, Δ24-26, A27S, Δ69/70, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452R, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K

BA.2.75	T19I, Δ24-26, A27S, G142D, K147E, W152R, F157L, I210V, V213G, G257S, G339H, N354D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, G446S, N460K, S477N, T478K, E484A, Q498R, N501Y, Y505H D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, and N969K
BA.2.75.2	T19I, Δ24-26, A27S, G142D, K147E, W152R, F157L, I210V, V213G, G257S, G339H, R346T, N354D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, G446S, N460K, S477N, T478K, E484A, F486S, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K, and D1199N
BJ.1	T19I, Δ24-26, A27S, V83A, G142D, Δ144, H146Q, Q183E, V213E, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, S477N, T478K, V483A, E484A, F490V, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, G798D, Q954H, N969K, and S1003I
BA.4.6 or BF.7	T19I, Δ24-26, A27S, Δ69/70, G142D, V213G, G339D, R346T, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452R, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N658S, N679K, P681H, N764K, D796Y, Q954H, and N969K
XBB	T19I, Δ24-26, A27S, V83A, G142D, Δ144, H146Q, Q183E, V213E, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478K, E484A, F486S, F490S, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, and N969K
XBB.1	T19I, Δ24-26, A27S, V83A, G142D, Δ144, H146Q, Q183E, V213E, G252V, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478K, E484A, F486S, F490S, Q493R, Q498R, N501Y, Y505H,

	D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, and N969K
XBB.2	T19I, Δ24-26, A27S, V83A, G142D, Δ144, H146Q, Q183E, V213E, D253G, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478K, E484A, F486S, F490S, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, and N969K
XBB.1.3	T19I, Δ24-26, A27S, V83A, G142D, Δ144, H146Q, Q183E, V213E, G252V, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478K, A484T, F486S, F490S, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, and N969K
BA.2.3.20	T19I, Δ24-26, A27S, G142D, M153T, N164K, V213G, H245N, G257D, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, K444R, E484R N450D, L452M, N460K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, and N969K
BQ.1.1	T19I, Δ24-26, A27S, Δ69/70, G142D, V213G, G339D, R346T, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, K444T, L452R, N460K, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K

In some embodiments, RNA described herein comprises a nucleotide sequence encoding a SARS-CoV-2 S protein comprising one or more mutations (including, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) that are characteristic of an Omicron variant (e.g., one or more mutations of an Omicron variant listed in Table 2). In some embodiments, such RNA further comprises one or more mutations that stabilize the S protein in a pre-fusion

confirmation (e.g., in some embodiments, such RNA further comprises proline residues at positions corresponding to residues 986 and 987 of SEQ ID NO: 1). In some embodiments, an RNA comprises a nucleotide sequence encoding a SARS-CoV-2 S protein comprising one or more mutations (including, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) listed in Table 2. In some such embodiments, one or more mutations may come from two or more variants as listed in Table 2. In some embodiments, an RNA comprises a nucleotide sequence encoding a SARS-CoV-2 S protein comprising each of the mutations identified in Table 2 as being characteristic of a certain Omicron variant (e.g., in some embodiments, an RNA comprises a nucleotide sequence encoding a SARS-CoV-2 S protein comprising each of the mutations listed in Table 2 as being characteristic of an Omicron BA.1, BA.2, BA.2.12.1, BA.4/5, BA.2.75, BA.2.75.1, BA.4.6, BQ.1.1, XBB, XBB.1, XBB.2, or XBB.1.3 variant).

In some embodiments, an RNA disclosed herein comprises a nucleotide sequence that encodes an immunogenic fragment of the SARS-Cov-2 S protein (e.g., the RBD) and which comprises one or more mutations that are characteristic of a SARS-CoV-2 variant (e.g., an Omicron variant described herein). For example, in some embodiments, an RNA comprises a nucleotide sequence encoding the RBD of an S protein of a SARS-CoV-2 variant (e.g., a region of the S protein corresponding to amino acids 327 to 528 of SEQ ID NO: 1, and comprising one or more mutations that are characteristic of a variant of concern that lie within this region of the S protein).

In some embodiments, an RNA encodes a SARS-CoV-2 S protein comprising a subset of the mutations listed in Table 2. In some embodiments, an RNA encodes a SARS-CoV-2 S protein comprising the mutations listed in Table 2 that are most prevalent in a certain variant (e.g., mutations that have been detected in at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of sequences collected to date for a given variant sequenced). Mutation prevalence can be determined, e.g., based on published sequences (e.g., sequences that are collected and made available to the public by GISAID).

In some embodiments, RNA described herein encodes a SARS-CoV-2 S protein comprising one or more mutations that are characteristic of a BA.4/5 variant. In some embodiments, the one

or more mutations characteristic of a BA.4/5 variant include T19I, Δ24-26, A27S, Δ69/70, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, K417N, N440K, L452R, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, and N969K. In some embodiments, RNA described herein encodes a SARS-CoV-2 S protein comprising one or more mutations that are characteristic of a BA.4/5 variant and excludes R408S. In some embodiments, RNA described herein encodes a SARS-CoV-2 S protein comprising one or more mutations (including, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) that are characteristic of a BA.4/5 variant and excludes R408S.

In some embodiments, RNA described herein encodes a SARS-CoV-2 S protein comprising one or more (including, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) mutations characteristic of a BA.2.75 variant. In some embodiments, the one or more mutations characteristic of a BA.2.75 variant include T19I, Δ24-26, A27S, G142D, K147E, W152R, F157L, I210V, V213G, G257S, G339H, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, G446S, N460K, S477N, T478K, E484A, Q498R, N501Y, Y505H D614G, H655Y, N679K, P681H, N764K, Q954H, and N969K. In some embodiments, RNA described herein encodes a SARS-CoV-2 S protein comprising one or more mutations (including, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) that are characteristic of a BA.2.75 variant, and which excludes N354D. In some embodiments, RNA described herein encodes a SARS-CoV-2 S protein comprising one or more mutations (including, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) that are characteristic of a BA.2.75 variant, and which excludes D796Y. In some embodiments, RNA described herein encodes a SARS-CoV-2 S protein comprising one or more mutations (including, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) that are characteristic of a BA.2.75 variant, and which excludes D796Y and N354D.

In some embodiments, RNA described herein encodes a SARS-CoV-2 S protein comprising one or more mutations characteristic of a BA.2.75.2 variant. In some embodiments, the one or more mutations characteristic of a BA.2.75.2 variant include T19I, Δ24-26, A27S, G142D, K147E, W152R, F157L, I210V, V213G, G257S, G339H, R346T, N354D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, G446S, N460K, S477N, T478K, E484A, F486S, Q498R,

N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K, and D1199N. In some embodiments, RNA described herein encodes a SARS-CoV-2 S protein comprising one or more mutations (including, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) that are characteristic of a BA.2.75.2 variant, and which excludes R346T.

In some embodiments, RNA described herein encodes a SARS-CoV-2 S protein comprising one or more mutations characteristic of a BA.4.6 or BF.7 variant. In some embodiments, the one or more mutations characteristic of a BA.4.6 or BF.7 variant include T19I, Δ24-26, A27S, Δ69/70, G142D, V213G, G339D, R346T, S371F, S373P, S375F, T376A, D405N, K417N, N440K, L452R, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, and N969K. In some embodiments, RNA described herein encodes a SARS-CoV-2 S protein comprising one or more mutations (including, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) that are characteristic of a BA.4.6 or BF.7 variant, and which exclude R408S. In some embodiments, RNA described herein encodes a SARS-CoV-2 S protein comprising one or more mutations (including, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) that are characteristic of a BA.4.6 or BF.7 variant, and which exclude N658S. In some embodiments, RNA described herein encodes a SARS-CoV-2 S protein comprising one or more mutations (including, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) that are characteristic of a BA.4.6 or BF.7 variant, and which exclude N658S and R408S.

In some embodiments, RNA described herein encodes a SARS-CoV-2 S protein comprising one or more mutations characteristic of an Omicron XBB variant. In some embodiments, the one or more mutations characteristic of an Omicron XBB variant include T19I, Δ24-26, A27S, V83A, G142D, Δ144, H146Q, Q183E, V213E, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478K, E484A, F486S, F490S, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, and N969K.

In some embodiments, RNA described herein encodes a SARS-CoV-2 S protein comprising one or more mutations characteristic of an Omicron XBB.1 variant. In some embodiments, the one or more mutations characteristic of an Omicron XBB.1 variant include G252V. In some embodiments, the one or more mutations characteristic of an Omicron XBB.1 variant include T19I, Δ24-26, A27S, V83A, G142D, Δ144, H146Q, Q183E, V213E, G252V, G339H, R346T, L368I,

S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478K, E484A, F486S, F490S, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, and N969K. In some embodiments, RNA described herein encodes a SARS-CoV-2 S protein comprising one or more mutations (including, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) that are characteristic of an Omicron XBB.1 variant and which exclude Q493R. In some embodiments, RNA described herein encodes a SARS-CoV-2 S protein comprising one or more mutations (including, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) that are characteristic of an Omicron XBB variant and which exclude Q493R and G252V.

In some embodiments, RNA described herein encodes a SARS-CoV-2 S protein comprising one or more mutations characteristic of an Omicron XBB.2 variant. In some embodiments, the one or more mutations characteristic of an Omicron XBB.2 variant include D253G. In some embodiments, the one or more mutations characteristic of an Omicron-XBB.2 variant include T19I, Δ24-26, A27S, V83A, G142D, Δ144, H146Q, Q183E, V213E, D253G, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478K, E484A, F486S, F490S, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, and N969K.

In some embodiments, RNA described herein encodes a SARS-CoV-2 S protein comprising one or more mutations characteristic of an Omicron XBB.1.3 variant. In some embodiments, the one or more mutations characteristic of an Omicron XBB.1.3 variant include G252V and A484T. In some embodiments, the one or more mutations characteristic of an Omicron XBB.1.3 variant include T19I, Δ24-26, A27S, V83A, G142D, Δ144, H146Q, Q183E, V213E, G252V, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478K, A484T, F486S, F490S, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, and N969K.

In some embodiments, RNA described herein encodes a SARS-CoV-2 S protein comprising one or more mutations that are characteristic of a BQ.1.1 variant. In some embodiments, the one or more mutations characteristic of a BQ.1.1 variant include T19I, Δ24-26, A27S, Δ69/70, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, K444T, L452R, N463K, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K,

P681H, N764K, D796Y, Q954H, and N969K. In some embodiments, RNA described herein encodes a SARS-CoV-2 S protein comprising one or more mutations (including, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) that are characteristic of a BQ.1.1 variant.

In one embodiment, the vaccine antigen described herein comprises, consists essentially of or consists of a spike protein (S) of SARS-CoV-2, a variant thereof, or an immunogenic fragment thereof (e.g., but not limited to RBD).

In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7, or an immunogenic fragment of the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7. In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of nucleotides 49 to 3819 of SEQ ID NO: 2, 8 or 9, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 49 to 3819 of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of nucleotides 49 to 3819 of SEQ ID NO: 2, 8 or 9, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 49 to 3819 of SEQ ID NO: 2, 8 or 9; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7, or an immunogenic fragment of the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of nucleotides 49 to 3819 of SEQ ID NO: 2, 8 or

9; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7.

In one embodiment, the vaccine antigen comprises, consists essentially of or consists of SARS-CoV-2 spike S1 fragment (S1) (the S1 subunit of a spike protein (S) of SARS-CoV-2), a variant thereof, or a fragment thereof.

In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 17 to 683 of SEQ ID NO: 1, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 17 to 683 of SEQ ID NO: 1, or an immunogenic fragment of the amino acid sequence of amino acids 17 to 683 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 17 to 683 of SEQ ID NO: 1. In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 17 to 683 of SEQ ID NO: 1.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of nucleotides 49 to 2049 of SEQ ID NO: 2, 8 or 9, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 49 to 2049 of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of nucleotides 49 to 2049 of SEQ ID NO: 2, 8 or 9, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 49 to 2049 of SEQ ID NO: 2, 8 or 9; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 17 to 683 of SEQ ID NO: 1, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 17 to 683 of SEQ ID NO: 1, or an immunogenic fragment of the amino acid sequence of amino acids 17 to 683 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 17 to 683 of SEQ ID NO: 1. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of nucleotides 49 to 2049 of SEQ ID NO: 2, 8 or 9; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 17 to 683 of SEQ ID NO: 1.

In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 17 to 685 of SEQ ID NO: 1, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 17 to 685 of SEQ ID NO: 1, or an immunogenic fragment of the amino acid sequence of amino acids 17 to 685 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 17 to 685 of SEQ ID NO: 1. In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 17 to 685 of SEQ ID NO: 1.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of nucleotides 49 to 2055 of SEQ ID NO: 2, 8 or 9, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 49 to 2055 of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of nucleotides 49 to 2055 of SEQ ID NO: 2, 8 or 9, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 49 to 2055 of SEQ ID NO: 2, 8 or 9; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 17 to 685 of SEQ ID NO: 1, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 17 to 685 of SEQ ID NO: 1, or an immunogenic fragment of the amino acid sequence of amino acids 17 to 685 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 17 to 685 of SEQ ID NO: 1. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of nucleotides 49 to 2055 of SEQ ID NO: 2, 8 or 9; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 17 to 685 of SEQ ID NO: 1.

In one embodiment, a vaccine antigen comprises, consists essentially of or consists of the receptor binding domain (RBD) of the S1 subunit of a spike protein (S) of SARS-CoV-2, a variant thereof, or a fragment thereof. The amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1, a variant thereof, or a fragment thereof is also referred to herein as "RBD" or "RBD domain".

In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1, or an immunogenic fragment of the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1. In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of nucleotides 979 to 1584 of SEQ ID NO: 2, 8 or 9, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 979 to 1584 of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of nucleotides 979 to 1584 of SEQ ID NO: 2, 8 or 9, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 979 to 1584 of SEQ ID NO: 2, 8 or 9; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1, or an immunogenic fragment of the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of nucleotides 979 to 1584 of SEQ ID NO: 2, 8 or 9; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1.

According to certain embodiments, a signal peptide is fused, either directly or through a linker, to a SARS-CoV-2 S protein, a variant thereof, or a fragment thereof, i.e., the antigenic peptide or protein. Accordingly, in one embodiment, a signal peptide is fused to the above described amino acid sequences derived from SARS-CoV-2 S protein or immunogenic fragments thereof (antigenic peptides or proteins) comprised by the vaccine antigens described above.

Such signal peptides are sequences, which typically exhibit a length of about 15 to 30 amino acids and are preferably located at the N-terminus of the antigenic peptide or protein, without being limited thereto. Signal peptides as defined herein preferably allow the transport of the antigenic peptide or protein as encoded by the RNA into a defined cellular compartment, preferably the cell surface, the endoplasmic reticulum (ER) or the endosomal-lysosomal compartment. In one embodiment, the signal peptide sequence as defined herein includes, without being limited thereto, the signal peptide sequence of SARS-CoV-2 S protein, in particular a sequence comprising the amino acid sequence of amino acids 1 to 16 or 1 to 19 of SEQ ID NO: 1 or a functional variant thereof.

In one embodiment, a signal sequence comprises the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1, or a functional fragment of the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1. In one embodiment, a signal sequence comprises the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1.

In one embodiment, RNA encoding a signal sequence (i) comprises the nucleotide sequence of nucleotides 1 to 48 of SEQ ID NO: 2, 8 or 9, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 1 to 48 of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of nucleotides 1 to 48 of SEQ ID NO: 2, 8 or 9, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 1 to 48 of SEQ ID NO: 2, 8 or 9; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1, or a functional fragment of the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1. In one embodiment, RNA encoding a signal sequence (i) comprises the nucleotide sequence of

nucleotides 1 to 48 of SEQ ID NO: 2, 8 or 9; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1.

In one embodiment, a signal sequence comprises the amino acid sequence of amino acids 1 to 19 of SEQ ID NO: 1, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 19 of SEQ ID NO: 1, or a functional fragment of the amino acid sequence of amino acids 1 to 19 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 19 of SEQ ID NO: 1. In one embodiment, a signal sequence comprises the amino acid sequence of amino acids 1 to 19 of SEQ ID NO: 1.

In one embodiment, RNA encoding a signal sequence (i) comprises the nucleotide sequence of nucleotides 1 to 57 of SEQ ID NO: 2, 8 or 9, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 1 to 57 of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of nucleotides 1 to 57 of SEQ ID NO: 2, 8 or 9, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 1 to 57 of SEQ ID NO: 2, 8 or 9; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 1 to 19 of SEQ ID NO: 1, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 19 of SEQ ID NO: 1, or a functional fragment of the amino acid sequence of amino acids 1 to 19 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 19 of SEQ ID NO: 1. In one embodiment, RNA encoding a signal sequence (i) comprises the nucleotide sequence of nucleotides 1 to 57 of SEQ ID NO: 2, 8 or 9; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 1 to 19 of SEQ ID NO: 1.

In some embodiments, a signal peptide sequence as defined herein includes, without being limited thereto, the signal peptide sequence of an immunoglobulin, e.g., the signal peptide sequence of an immunoglobulin heavy chain variable region, wherein the immunoglobulin may be human immunoglobulin. In particular, in some embodiments the signal peptide sequence as defined herein includes a sequence comprising the amino acid sequence of amino acids 1 to 22 of SEQ ID NO: 31 or a functional variant thereof.

In some embodiments, a signal peptide sequence is functional in mammalian cells.

In some embodiments, a utilized signal sequence is "intrinsic" in that it is , in nature, it is associated with (e.g., linked to) the encoded polypeptide.

In some embodiments, a utilized signal sequence is heterologous to the encoded polypeptide – e.g., is not naturally part of a polypeptide (e.g., protein) whose sequences are included in the encoded polypeptide.

In some embodiments, signal peptides are sequences, which are typically characterized by a length of about 15 to 30 amino acids.

In many embodiments, signal peptides are positioned at the N-terminus of an encoded polypeptide as described herein, without being limited thereto. In some embodiments, signal peptides preferably allow the transport of the polypeptide encoded by RNAs of the present disclosure with which they are associated into a defined cellular compartment, preferably the cell surface, the endoplasmic reticulum (ER) or the endosomal-lysosomal compartment.

In some embodiments, a signal sequence is selected from an S1S2 signal peptide (aa 1-19), an immunoglobulin secretory signal peptide (aa 1-22), an HSV-1 gD signal peptide (MGGAAARLGAVILFVVIVGLHGVRSKY), an HSV-2 gD signal peptide (MGRLTSGVGTAALLVVAVGLRVVCA); a human SPARC signal peptide, a human insulin isoform 1 signal peptide, a human albumin signal peptide, etc. Those skilled in the art will be aware of other secretory signal peptides such as, for example, as disclosed in WO2017/081082 (e.g., SEQ ID NOs: 1-1115 and 1728, or fragments variants thereof) and WO2019008001.

In some embodiments, an RNA sequence encodes an epitope that may comprise or otherwise be linked to a signal sequence (e.g., secretory sequence), such as those listed in Table A, or at least a sequence having 1, 2, 3, 4, or 5 amino acid differences relative thereto. In some embodiments, a signal sequence such as MFVFLVLLPLVSSQCVNLT, or at least a sequence having 1, 2, 3, 4, or at the most 5 amino acid differences relative thereto is utilized. In some embodiments, a sequence such as MFVFLVLLPLVSSQCVNLT, or a sequence having 1, 2, 3, 4, or at the most 5 amino acid differences relative thereto, is utilized.

In some embodiments, a signal sequence is selected from those included in the Table A below and/or those encoded by the sequences in Table B below:

Table A: Exemplary signal sequences

SEQ ID NO:	Signal	Sequence (Amino Acid)
105	HSV-1 gD SP	MGGAAARLGAVILFVVIVGLHGVRSKY
106	HSV-2 gD SP	MGRLTSGVGTAALLVVAVGLRVVCA
107	HSV-2	MGRLTSGVGTAALLVVAVGLRVVCAKYA
108	SARS-CoV-2-S	MFVFLVLLPLVSSQCVNLT
109	human Ig heavy chain signal peptide (huSec)	MDWIWRILFLVGAATGAHSQM
110	HuIgGk signal peptide	METPAQLLFLLLLWLPDTTG
111	IgE heavy chain epsilon-1signal peptide	MDWTWILFLVAAATRVHS
112	Japanese encephalitis PRM signal sequence	MLGSNSGQRVVFTILLLLVAPAYS
113	VSVg protein signal sequence	MKCLLYLAFLFIGVNCA
114		MDWTWILFLVAAATRVHS
115		ETPAQLLFLLLLWLPDTTG
116		MLGSNSGQRVVFTILLLLVAPAYS
117		MKCLLYLAFLFIGVNCA
118		MWLVSLAIVTACAGA

119		MFVFLVLLPLVSSQC

Table B: Exemplary nucleotide sequences encoding signal sequences

SEQ ID NO:	Signal	Sequence (Nucleotide)
120	HSV-1 gD SP wild-type	ATGGGGGGGGCTGCCGCCAGGTTGGGGGCCGTGATTTTGTTTGTCGTC ATAGTGGGCCTCCATGGGGTCCGCAGCAAATAT
121	HSV-1 gD SP optimized nucleotide sequence	ATGggaggagccGCCGCCagactgggaGCCGTGatcctgttcgtggtgatcGTGgga ctgCATggagtgagaAGCaagtac
122	SARS-CoV-2-S	ATGTTTGTGTTTCTTGTGCTGCTGCCTCTTGTGTCTTCTCAGTGTGTGAAT TTGACA

123	human Ig heavy chain signal peptide (huSec)	ATGGATTGGATTTGGAGAATCCTGTTCCTCGTGGGAGCCGCTACAGGA GCCCACTCCCAGATG

In some embodiments, an RNA utilized as described herein encodes a multimerization element (e.g., a heterologous multimerization element). In some embodiments, a heterologous multimerization element comprises a dimerization, trimerization or tetramerization element.

In some embodiments, a multimerization element is one described in WO2017/081082 (e.g., SEQ ID NOs: 1116-1167, or fragments or variants thereof).

Exemplary trimerization and tetramerization elements include, but are not limited to, engineered leucine zippers, fibritin foldon domain from enterobacteria phage T4, GCN4pII, GCN4-pII, and p53.

In some embodiments, a provided encoded polypeptide(s) is able to form a trimeric complex. For example, a utilized encoded polypeptide(s) may comprise a domain allowing formation of a multimeric complex, such as for example particular a trimeric complex of an amino acid sequence comprising an encoded polypeptide(s) as described herein. In some embodiments, a domain allowing formation of a multimeric complex comprises a trimerization domain, for example, a trimerization domain as described herein.

In some embodiments, an encoded polypeptide(s) can be modified by addition of a T4-fibritin-derived "foldon" trimerization domain, for example, to increase its immunogenicity.

[298] In some embodiments, an RNAs described herein encodes a membrane association element (e.g., a heterologous membrane association element), such as a transmembrane domain.

A transmembrane domain can be N-terminal, C-terminal, or internal to an encoded polypeptide. A coding sequence of a transmembrane element is typically placed in frame (i.e., in the same reading frame), 5', 3', or internal to coding sequences of sequences (e.g., sequences encoding polypeptide(s)) with which it is to be linked.

In some embodiments, a transmembrane domain comprises or is a transmembrane domain of Hemagglutinin (HA) of Influenza virus, Env of HIV-1, equine infectious anaemia virus (EIAV),

murine leukaemia virus (MLV), mouse mammary tumor virus, G protein of vesicular stomatitis virus (VSV), Rabies virus, or a seven transmembrane domain receptor.

In one embodiment, a signal sequence comprises the amino acid sequence of amino acids 1 to 22 of SEQ ID NO: 31, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 22 of SEQ ID NO: 31, or a functional fragment of the amino acid sequence of amino acids 1 to 22 of SEQ ID NO: 31, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 22 of SEQ ID NO: 31. In one embodiment, a signal sequence comprises the amino acid sequence of amino acids 1 to 22 of SEQ ID NO: 31.

In one embodiment, RNA encoding a signal sequence (i) comprises the nucleotide sequence of nucleotides 54 to 119 of SEQ ID NO: 32, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 54 to 119 of SEQ ID NO: 32, or a fragment of the nucleotide sequence of nucleotides 54 to 119 of SEQ ID NO: 32, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 54 to 119 of SEQ ID NO: 32; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 1 to 22 of SEQ ID NO: 31, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 22 of SEQ ID NO: 31, or a functional fragment of the amino acid sequence of amino acids 1 to 22 of SEQ ID NO: 31, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 22 of SEQ ID NO: 31. In one embodiment, RNA encoding a signal sequence (i) comprises the nucleotide sequence of nucleotides 54 to 119 of SEQ ID NO: 32; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 1 to 22 of SEQ ID NO: 31.

Such signal peptides are preferably used in order to promote secretion of the encoded antigenic peptide or protein. More preferably, a signal peptide as defined herein is fused to an encoded antigenic peptide or protein as defined herein.

Accordingly, in particularly preferred embodiments, the RNA described herein comprises at least one coding region encoding an antigenic peptide or protein and a signal peptide, said

signal peptide preferably being fused to the antigenic peptide or protein, more preferably to the N-terminus of the antigenic peptide or protein as described herein.

In one embodiment, a vaccine antigen comprises the amino acid sequence of SEQ ID NO: 1 or 7, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 1 or 7, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 1 or 7, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 1 or 7. In one embodiment, a vaccine antigen comprises the amino acid sequence of SEQ ID NO: 1 or 7.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 2, 8 or 9, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of SEQ ID NO: 2, 8 or 9, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 2, 8 or 9; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 1 or 7, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 1 or 7, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 1 or 7, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 1 or 7. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 2, 8 or 9; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 1 or 7.

In one embodiment, a vaccine antigen comprises the amino acid sequence of SEQ ID NO: 7, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 7, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 7, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 7. In one embodiment, a vaccine antigen comprises the amino acid sequence of SEQ ID NO: 7.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 15, 16, 19, 20, 24, or 25, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 15, 16, 19, 20,

24, or 25, or a fragment of the nucleotide sequence of SEQ ID NO: 15, 16, 19, 20, 24, or 25, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 15, 16, 19, 20, 24, or 25; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 7, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 7, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 7, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 7. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 15, 16, 19, 20, 24, or 25; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 7.

In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 1 to 683 of SEQ ID NO: 1, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 683 of SEQ ID NO: 1, or an immunogenic fragment of the amino acid sequence of amino acids 1 to 683 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 683 of SEQ ID NO: 1. In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 1 to 683 of SEQ ID NO: 1.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of nucleotides 1 to 2049 of SEQ ID NO: 2, 8 or 9, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 1 to 2049 of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of nucleotides 1 to 2049 of SEQ ID NO: 2, 8 or 9, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 1 to 2049 of SEQ ID NO: 2, 8 or 9; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 1 to 683 of SEQ ID NO: 1, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 683 of SEQ ID NO: 1, or an immunogenic fragment of the amino acid sequence of amino acids 1 to 683 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%,

90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 683 of SEQ ID NO: 1. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of nucleotides 1 to 2049 of SEQ ID NO: 2, 8 or 9; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 1 to 683 of SEQ ID NO: 1.

In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 1 to 685 of SEQ ID NO: 1, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 685 of SEQ ID NO: 1, or an immunogenic fragment of the amino acid sequence of amino acids 1 to 685 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 685 of SEQ ID NO: 1. In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 1 to 685 of SEQ ID NO: 1.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of nucleotides 1 to 2055 of SEQ ID NO: 2, 8 or 9, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 1 to 2055 of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of nucleotides 1 to 2055 of SEQ ID NO: 2, 8 or 9, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 1 to 2055 of SEQ ID NO: 2, 8 or 9; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 1 to 685 of SEQ ID NO: 1, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 685 of SEQ ID NO: 1, or an immunogenic fragment of the amino acid sequence of amino acids 1 to 685 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 685 of SEQ ID NO: 1. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of nucleotides 1 to 2055 of SEQ ID NO: 2, 8 or 9; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 1 to 685 of SEQ ID NO: 1.

In one embodiment, a vaccine antigen comprises the amino acid sequence of SEQ ID NO: 3, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 3, or an immunogenic fragment of the amino acid

sequence of SEQ ID NO: 3, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 3. In one embodiment, a vaccine antigen comprises the amino acid sequence of SEQ ID NO: 3.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 4, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 4, or a fragment of the nucleotide sequence of SEQ ID NO: 4, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 4; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 3, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 3, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 3, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 3. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 4; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 3.

In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 1 to 221 of SEQ ID NO: 29, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 221 of SEQ ID NO: 29, or an immunogenic fragment of the amino acid sequence of amino acids 1 to 221 of SEQ ID NO: 29, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 221 of SEQ ID NO: 29. In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 1 to 221 of SEQ ID NO: 29.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of nucleotides 54 to 716 of SEQ ID NO: 30, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 54 to 716 of SEQ ID NO: 30, or a fragment of the nucleotide sequence of nucleotides 54 to 716 of SEQ ID NO: 30, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 54 to 716 of SEQ ID NO: 30; and/or (ii)

encodes an amino acid sequence comprising the amino acid sequence of amino acids 1 to 221 of SEQ ID NO: 29, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 221 of SEQ ID NO: 29, or an immunogenic fragment of the amino acid sequence of amino acids 1 to 221 of SEQ ID NO: 29, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 221 of SEQ ID NO: 29. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of nucleotides 54 to 716 of SEQ ID NO: 30; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 1 to 221 of SEQ ID NO: 29.

In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 1 to 224 of SEQ ID NO: 31, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 224 of SEQ ID NO: 31, or an immunogenic fragment of the amino acid sequence of amino acids 1 to 224 of SEQ ID NO: 31, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 224 of SEQ ID NO: 31. In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 1 to 224 of SEQ ID NO: 31.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of nucleotides 54 to 725 of SEQ ID NO: 32, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 54 to 725 of SEQ ID NO: 32, or a fragment of the nucleotide sequence of nucleotides 54 to 725 of SEQ ID NO: 32, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 54 to 725 of SEQ ID NO: 32; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 1 to 224 of SEQ ID NO: 31, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 224 of SEQ ID NO: 31, or an immunogenic fragment of the amino acid sequence of amino acids 1 to 224 of SEQ ID NO: 31, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 224 of SEQ ID NO: 31. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of

nucleotides 54 to 725 of SEQ ID NO: 32; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 1 to 224 of SEQ ID NO: 31.

According to certain embodiments, a trimerization domain is fused, either directly or through a linker, e.g., a glycine/serine linker, to a SARS-CoV-2 S protein, a variant thereof, or a fragment thereof, i.e., the antigenic peptide or protein. Accordingly, in one embodiment, a trimerization domain is fused to the above described amino acid sequences derived from SARS-CoV-2 S protein or immunogenic fragments thereof (antigenic peptides or proteins) comprised by the vaccine antigens described above (which may optionally be fused to a signal peptide as described above).

Such trimerization domains are preferably located at the C-terminus of the antigenic peptide or protein, without being limited thereto. Trimerization domains as defined herein preferably allow the trimerization of the antigenic peptide or protein as encoded by the RNA. Examples of trimerization domains as defined herein include, without being limited thereto, foldon, the natural trimerization domain of T4 fibritin. The C-terminal domain of T4 fibritin (foldon) is obligatory for the formation of the fibritin trimer structure and can be used as an artificial trimerization domain. In one embodiment, the trimerization domain as defined herein includes, without being limited thereto, a sequence comprising the amino acid sequence of amino acids 3 to 29 of SEQ ID NO: 10 or a functional variant thereof. In one embodiment, the trimerization domain as defined herein includes, without being limited thereto, a sequence comprising the amino acid sequence of SEQ ID NO: 10 or a functional variant thereof.

In one embodiment, a trimerization domain comprises the amino acid sequence of amino acids 3 to 29 of SEQ ID NO: 10, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 3 to 29 of SEQ ID NO: 10, or a functional fragment of the amino acid sequence of amino acids 3 to 29 of SEQ ID NO: 10, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 3 to 29 of SEQ ID NO: 10. In one embodiment, a trimerization domain comprises the amino acid sequence of amino acids 3 to 29 of SEQ ID NO: 10.

In one embodiment, RNA encoding a trimerization domain (i) comprises the nucleotide sequence of nucleotides 7 to 87 of SEQ ID NO: 11, a nucleotide sequence having at least 99%,

98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 7 to 87 of SEQ ID NO: 11, or a fragment of the nucleotide sequence of nucleotides 7 to 87 of SEQ ID NO: 11, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 7 to 87 of SEQ ID NO: 11; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 3 to 29 of SEQ ID NO: 10, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 3 to 29 of SEQ ID NO: 10, or a functional fragment of the amino acid sequence of amino acids 3 to 29 of SEQ ID NO: 10, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 3 to 29 of SEQ ID NO: 10. In one embodiment, RNA encoding a trimerization domain (i) comprises the nucleotide sequence of nucleotides 7 to 87 of SEQ ID NO: 11; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 3 to 29 of SEQ ID NO: 10.

In one embodiment, a trimerization domain comprises the amino acid sequence SEQ ID NO: 10, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 10, or a functional fragment of the amino acid sequence of SEQ ID NO: 10, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 10. In one embodiment, a trimerization domain comprises the amino acid sequence of SEQ ID NO: 10.

In one embodiment, RNA encoding a trimerization domain (i) comprises the nucleotide sequence of SEQ ID NO: 11, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 11, or a fragment of the nucleotide sequence of SEQ ID NO: 11, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 11; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 10, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 10, or a functional fragment of the amino acid sequence of SEQ ID NO: 10, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 10. In one embodiment, RNA encoding a trimerization domain (i) comprises the nucleotide sequence of

SEQ ID NO: 11; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 10.

Such trimerization domains are preferably used in order to promote trimerization of the encoded antigenic peptide or protein. More preferably, a trimerization domain as defined herein is fused to an antigenic peptide or protein as defined herein.

Accordingly, in particularly preferred embodiments, the RNA described herein comprises at least one coding region encoding an antigenic peptide or protein and a trimerization domain as defined herein, said trimerization domain preferably being fused to the antigenic peptide or protein, more preferably to the C-terminus of the antigenic peptide or protein.

In one embodiment, a vaccine antigen comprises the amino acid sequence of SEQ ID NO: 5, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 5, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 5, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 5. In one embodiment, a vaccine antigen comprises the amino acid sequence of SEQ ID NO: 5.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 6, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 6, or a fragment of the nucleotide sequence of SEQ ID NO: 6, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 6; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 5, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 5, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 5, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 5. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 6; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 5.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 17, 21, or 26, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%,

90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 17, 21, or 26, or a fragment of the nucleotide sequence of SEQ ID NO: 17, 21, or 26, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 17, 21, or 26; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 5, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 5, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 5, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 5. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 17, 21, or 26; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 5.

In one embodiment, a vaccine antigen comprises the amino acid sequence of SEQ ID NO: 18, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 18, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 18, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 18. In one embodiment, a vaccine antigen comprises the amino acid sequence of SEQ ID NO: 18.

In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 1 to 257 of SEQ ID NO: 29, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 257 of SEQ ID NO: 29, or an immunogenic fragment of the amino acid sequence of amino acids 1 to 257 of SEQ ID NO: 29, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 257 of SEQ ID NO: 29. In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 1 to 257 of SEQ ID NO: 29.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of nucleotides 54 to 824 of SEQ ID NO: 30, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 54 to 824 of SEQ ID NO: 30, or a fragment of the nucleotide sequence of nucleotides 54 to 824 of SEQ ID NO: 30, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or

80% identity to the nucleotide sequence of nucleotides 54 to 824 of SEQ ID NO: 30; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 1 to 257 of SEQ ID NO: 29, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 257 of SEQ ID NO: 29, or an immunogenic fragment of the amino acid sequence of amino acids 1 to 257 of SEQ ID NO: 29, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 257 of SEQ ID NO: 29. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of nucleotides 54 to 824 of SEQ ID NO: 30; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 1 to 257 of SEQ ID NO: 29.

In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 1 to 260 of SEQ ID NO: 31, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 260 of SEQ ID NO: 31, or an immunogenic fragment of the amino acid sequence of amino acids 1 to 260 of SEQ ID NO: 31, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 260 of SEQ ID NO: 31. In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 1 to 260 of SEQ ID NO: 31.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of nucleotides 54 to 833 of SEQ ID NO: 32, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 54 to 833 of SEQ ID NO: 32, or a fragment of the nucleotide sequence of nucleotides 54 to 833 of SEQ ID NO: 32, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 54 to 833 of SEQ ID NO: 32; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 1 to 260 of SEQ ID NO: 31, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 260 of SEQ ID NO: 31, or an immunogenic fragment of the amino acid sequence of amino acids 1 to 260 of SEQ ID NO: 31, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 260 of SEQ ID NO: 31. In one

embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of nucleotides 54 to 833 of SEQ ID NO: 32; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 1 to 260 of SEQ ID NO: 31.

In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 20 to 257 of SEQ ID NO: 29, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 20 to 257 of SEQ ID NO: 29, or an immunogenic fragment of the amino acid sequence of amino acids 20 to 257 of SEQ ID NO: 29, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 20 to 257 of SEQ ID NO: 29. In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 20 to 257 of SEQ ID NO: 29.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of nucleotides 111 to 824 of SEQ ID NO: 30, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 111 to 824 of SEQ ID NO: 30, or a fragment of the nucleotide sequence of nucleotides 111 to 824 of SEQ ID NO: 30, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 111 to 824 of SEQ ID NO: 30; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 20 to 257 of SEQ ID NO: 29, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 20 to 257 of SEQ ID NO: 29, or an immunogenic fragment of the amino acid sequence of amino acids 20 to 257 of SEQ ID NO: 29, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 20 to 257 of SEQ ID NO: 29.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of nucleotides 111 to 824 of SEQ ID NO: 30; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 20 to 257 of SEQ ID NO: 29.

In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 23 to 260 of SEQ ID NO: 31, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 23 to 260 of SEQ ID NO: 31, or an immunogenic fragment of the amino acid sequence of amino acids 23 to 260 of SEQ

ID NO: 31, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 23 to 260 of SEQ ID NO: 31. In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 23 to 260 of SEQ ID NO: 31.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of nucleotides 120 to 833 of SEQ ID NO: 32, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 120 to 833 of SEQ ID NO: 32, or a fragment of the nucleotide sequence of nucleotides 120 to 833 of SEQ ID NO: 32, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 120 to 833 of SEQ ID NO: 32; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 23 to 260 of SEQ ID NO: 31, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 23 to 260 of SEQ ID NO: 31, or an immunogenic fragment of the amino acid sequence of amino acids 23 to 260 of SEQ ID NO: 31, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 23 to 260 of SEQ ID NO: 31. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of nucleotides 120 to 833 of SEQ ID NO: 32; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 23 to 260 of SEQ ID NO: 31.

According to certain embodiments, a transmembrane domain is fused, either directly or through a linker, e.g., a glycine/serine linker, to a SARS-CoV-2 S protein, a variant thereof, or a fragment thereof, i.e., the antigenic peptide or protein. Accordingly, in one embodiment, a transmembrane domain is fused to the above described amino acid sequences derived from SARS-CoV-2 S protein or immunogenic fragments thereof (antigenic peptides or proteins) comprised by the vaccine antigens described above (which may optionally be fused to a signal peptide and/or trimerization domain as described above).

Such transmembrane domains are preferably located at the C-terminus of the antigenic peptide or protein, without being limited thereto. Preferably, such transmembrane domains are located at the C-terminus of the trimerization domain, if present, without being limited thereto. In one embodiment, a trimerization domain is present between the SARS-CoV-2 S

protein, a variant thereof, or a fragment thereof, i.e., the antigenic peptide or protein, and the transmembrane domain.

Transmembrane domains as defined herein preferably allow the anchoring into a cellular membrane of the antigenic peptide or protein as encoded by the RNA.

In one embodiment, the transmembrane domain sequence as defined herein includes, without being limited thereto, the transmembrane domain sequence of SARS-CoV-2 S protein, in particular a sequence comprising the amino acid sequence of amino acids 1207 to 1254 of SEQ ID NO: 1 or a functional variant thereof.

In one embodiment, a transmembrane domain sequence comprises the amino acid sequence of amino acids 1207 to 1254 of SEQ ID NO: 1, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1207 to 1254 of SEQ ID NO: 1, or a functional fragment of the amino acid sequence of amino acids 1207 to 1254 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1207 to 1254 of SEQ ID NO: 1. In one embodiment, a transmembrane domain sequence comprises the amino acid sequence of amino acids 1207 to 1254 of SEQ ID NO: 1.

In one embodiment, RNA encoding a transmembrane domain sequence (i) comprises the nucleotide sequence of nucleotides 3619 to 3762 of SEQ ID NO: 2, 8 or 9, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 3619 to 3762 of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of nucleotides 3619 to 3762 of SEQ ID NO: 2, 8 or 9, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 3619 to 3762 of SEQ ID NO: 2, 8 or 9; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 1207 to 1254 of SEQ ID NO: 1, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1207 to 1254 of SEQ ID NO: 1, or a functional fragment of the amino acid sequence of amino acids 1207 to 1254 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1207 to 1254 of SEQ ID NO: 1. In one embodiment, RNA encoding a transmembrane domain sequence (i) comprises the nucleotide

sequence of nucleotides 3619 to 3762 of SEQ ID NO: 2, 8 or 9; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 1207 to 1254 of SEQ ID NO: 1.

In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 1 to 311 of SEQ ID NO: 29, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 311 of SEQ ID NO: 29, or an immunogenic fragment of the amino acid sequence of amino acids 1 to 311 of SEQ ID NO: 29, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 311 of SEQ ID NO: 29. In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 1 to 311 of SEQ ID NO: 29.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of nucleotides 54 to 986 of SEQ ID NO: 30, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 54 to 986 of SEQ ID NO: 30, or a fragment of the nucleotide sequence of nucleotides 54 to 986 of SEQ ID NO: 30, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 54 to 986 of SEQ ID NO: 30; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 1 to 311 of SEQ ID NO: 29, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 311 of SEQ ID NO: 29, or an immunogenic fragment of the amino acid sequence of amino acids 1 to 311 of SEQ ID NO: 29, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 311 of SEQ ID NO: 29. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of nucleotides 54 to 986 of SEQ ID NO: 30; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 1 to 311 of SEQ ID NO: 29.

In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 1 to 314 of SEQ ID NO: 31, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 314 of SEQ ID NO: 31, or an immunogenic fragment of the amino acid sequence of amino acids 1 to 314 of SEQ ID NO:

31, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 314 of SEQ ID NO: 31. In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 1 to 314 of SEQ ID NO: 31.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of nucleotides 54 to 995 of SEQ ID NO: 32, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 54 to 995 of SEQ ID NO: 32, or a fragment of the nucleotide sequence of nucleotides 54 to 995 of SEQ ID NO: 32, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 54 to 995 of SEQ ID NO: 32; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 1 to 314 of SEQ ID NO: 31, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 314 of SEQ ID NO: 31, or an immunogenic fragment of the amino acid sequence of amino acids 1 to 314 of SEQ ID NO: 31, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 314 of SEQ ID NO: 31. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of nucleotides 54 to 995 of SEQ ID NO: 32; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 1 to 314 of SEQ ID NO: 31.

In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 20 to 311 of SEQ ID NO: 29, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 20 to 311 of SEQ ID NO: 29, or an immunogenic fragment of the amino acid sequence of amino acids 20 to 311 of SEQ ID NO: 29, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 20 to 311 of SEQ ID NO: 29. In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 20 to 311 of SEQ ID NO: 29.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of nucleotides 111 to 986 of SEQ ID NO: 30, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 111 to

986 of SEQ ID NO: 30, or a fragment of the nucleotide sequence of nucleotides 111 to 986 of SEQ ID NO: 30, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 111 to 986 of SEQ ID NO: 30; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 20 to 311 of SEQ ID NO: 29, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 20 to 311 of SEQ ID NO: 29, or an immunogenic fragment of the amino acid sequence of amino acids 20 to 311 of SEQ ID NO: 29, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 20 to 311 of SEQ ID NO: 29. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of nucleotides 111 to 986 of SEQ ID NO: 30; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 20 to 311 of SEQ ID NO: 29.

In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 23 to 314 of SEQ ID NO: 31, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 23 to 314 of SEQ ID NO: 31, or an immunogenic fragment of the amino acid sequence of amino acids 23 to 314 of SEQ ID NO: 31, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 23 to 314 of SEQ ID NO: 31. In one embodiment, a vaccine antigen comprises the amino acid sequence of amino acids 23 to 314 of SEQ ID NO: 31.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of nucleotides 120 to 995 of SEQ ID NO: 32, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 120 to 995 of SEQ ID NO: 32, or a fragment of the nucleotide sequence of nucleotides 120 to 995 of SEQ ID NO: 32, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 120 to 995 of SEQ ID NO: 32; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 23 to 314 of SEQ ID NO: 31, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 23 to 314 of SEQ ID NO: 31, or an immunogenic fragment of the amino acid sequence of amino acids 23 to 314

of SEQ ID NO: 31, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 23 to 314 of SEQ ID NO: 31. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of nucleotides 120 to 995 of SEQ ID NO: 32; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of amino acids 23 to 314 of SEQ ID NO: 31.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 30, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 30, or a fragment of the nucleotide sequence of SEQ ID NO: 30, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 30; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 29, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 29, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 29, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 29. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 30; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 29.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 32, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 32, or a fragment of the nucleotide sequence of SEQ ID NO: 32, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 32; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 31, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 31, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 31, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 31. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ

ID NO: 32; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 31.

In one embodiment, a vaccine antigen comprises the amino acid sequence of SEQ ID NO: 28, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 28, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 28, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 28. In one embodiment, a vaccine antigen comprises the amino acid sequence of SEQ ID NO: 28.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 27, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 27, or a fragment of the nucleotide sequence of SEQ ID NO: 27, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 27; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 28, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 28, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 28, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 28. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 27; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 28.

In one embodiment, a vaccine antigen comprises the amino acid sequence of SEQ ID NO: 49, an amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 49, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 49, or the amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 49. In one embodiment, a vaccine antigen comprises the amino acid sequence of SEQ ID NO: 49. The amino acid sequence of SEQ ID NO: 49 corresponds to the amino acid sequence of the full-length S protein from Omicron BA.1, which includes proline residues at positions corresponding to residues 986 and 987 of SEQ ID NO: 1 (residues 983 and 984 of SEQ ID NO: 49).

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 50, a nucleotide sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the nucleotide sequence of SEQ ID NO: 50, or a fragment of the nucleotide sequence of SEQ ID NO: 50, or the nucleotide sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the nucleotide sequence of SEQ ID NO: 50; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 49, an amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 49, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 49, or the amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 49. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 50; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 49. The nucleotide sequence of SEQ ID NO: 50 is a nucleotide sequence designed to encode the amino acid sequence of the full-length S protein from Omicron BA.1 with proline residues at positions corresponding to residues 986 and 987 of SEQ ID NO: 1 (residues 983 and 984 of SEQ ID NO: 49).

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 51, a nucleotide sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the nucleotide sequence of SEQ ID NO: 51, or a fragment of the nucleotide sequence of SEQ ID NO: 51, or the nucleotide sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the nucleotide sequence of SEQ ID NO: 51; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 49, an amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 49, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 49, or the amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 49. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 51; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 49. The nucleotide sequence of SEQ ID NO: 51 corresponds to an RNA construct (e.g., comprising a 5' UTR, a S-protein-encoding sequence, a 3' UTR, and a poly-A tail), which encodes the amino acid

sequence of the full-length S protein from Omicron BA.1 with proline residues at positions corresponding to residues 986 and 987 of SEQ ID NO: 1 (corresponding to residues 983 and 984 of SEQ ID NO: 49).

In one embodiment, a vaccine antigen comprises the amino acid sequence of SEQ ID NO: 55, an amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 55, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 55, or the amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 55. In one embodiment, a vaccine antigen comprises the amino acid sequence of SEQ ID NO: 55.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 56, a nucleotide sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the nucleotide sequence of SEQ ID NO: 56, or a fragment of the nucleotide sequence of SEQ ID NO: 56, or the nucleotide sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the nucleotide sequence of SEQ ID NO: 56; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 55, an amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 55, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 55, or the amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 55. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 56; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 55.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 57, a nucleotide sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the nucleotide sequence of SEQ ID NO: 57, or a fragment of the nucleotide sequence of SEQ ID NO: 57, or the nucleotide sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the nucleotide sequence of SEQ ID NO: 57; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 55, an amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 55, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 55, or the amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97%

identity to the amino acid sequence of SEQ ID NO: 55. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 57; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 55.

In one embodiment, a vaccine antigen comprises the amino acid sequence of SEQ ID NO: 58, an amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 58, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 58, or the amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 58. In one embodiment, a vaccine antigen comprises the amino acid sequence of SEQ ID NO: 58.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 59, a nucleotide sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the nucleotide sequence of SEQ ID NO: 59, or a fragment of the nucleotide sequence of SEQ ID NO: 59, or the nucleotide sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the nucleotide sequence of SEQ ID NO: 59; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 58, an amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 58, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 58, or the amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 58. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 59; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 58.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 60, a nucleotide sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the nucleotide sequence of SEQ ID NO: 60, or a fragment of the nucleotide sequence of SEQ ID NO: 60, or the nucleotide sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the nucleotide sequence of SEQ ID NO: 60; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 58, an amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 58, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 58, or the amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97%

identity to the amino acid sequence of SEQ ID NO: 58. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 60; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 58.

In one embodiment, a vaccine antigen comprises the amino acid sequence of SEQ ID NO: 61, an amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 61, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 61, or the amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 61. In one embodiment, a vaccine antigen comprises the amino acid sequence of SEQ ID NO: 61.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 62a, a nucleotide sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the nucleotide sequence of SEQ ID NO: 62a, or a fragment of the nucleotide sequence of SEQ ID NO: 62a, or the nucleotide sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the nucleotide sequence of SEQ ID NO: 62a; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 61, an amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 61, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 61, or the amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 61. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 62a; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 61.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 63a, a nucleotide sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the nucleotide sequence of SEQ ID NO: 63a, or a fragment of the nucleotide sequence of SEQ ID NO: 63a, or the nucleotide sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the nucleotide sequence of SEQ ID NO: 63a; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 61, an amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 61, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 61, or the amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97%

identity to the amino acid sequence of SEQ ID NO: 61. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 63a; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 61.

In one embodiment, a vaccine antigen comprises the amino acid sequence of SEQ ID NO: 52, an amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 52, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 52, or the amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 52. In one embodiment, a vaccine antigen comprises the amino acid sequence of SEQ ID NO: 52.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 53, a nucleotide sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the nucleotide sequence of SEQ ID NO: 53, or a fragment of the nucleotide sequence of SEQ ID NO: 53, or the nucleotide sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the nucleotide sequence of SEQ ID NO: 53; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 52, an amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 52, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 52, or the amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 52. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 53; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 52.

In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 54, a nucleotide sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the nucleotide sequence of SEQ ID NO: 54, or a fragment of the nucleotide sequence of SEQ ID NO: 54, or the nucleotide sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the nucleotide sequence of SEQ ID NO: 54; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 52, an amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 52, or an immunogenic fragment of the amino acid sequence of SEQ

ID NO: 52, or the amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 52. In one embodiment, RNA encoding a vaccine antigen (i) comprises the nucleotide sequence of SEQ ID NO: 54; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 52.

In one embodiment, the vaccine antigens described above comprise a contiguous sequence of SARS-CoV-2 coronavirus spike (S) protein that consists of or essentially consists of the above described amino acid sequences derived from SARS-CoV-2 S protein or immunogenic fragments thereof (antigenic peptides or proteins) comprised by the vaccine antigens described above. In one embodiment, the vaccine antigens described above comprise a contiguous sequence of SARS-CoV-2 coronavirus spike (S) protein of no more than 220 amino acids, 215 amino acids, 210 amino acids, or 205 amino acids.

In one embodiment, RNA encoding a vaccine antigen is nucleoside modified messenger RNA (modRNA) described herein as BNT162b1 (RBP020.3), BNT162b2 (RBP020.1 or RBP020.2), or BNT162b3 (e.g., BNT162b3c). In one embodiment, RNA encoding a vaccine antigen is nucleoside modified messenger RNA (modRNA) described herein as RBP020.2. In one embodiment, RNA encoding a vaccine antigen is nucleoside modified messenger RNA (modRNA) described herein as BNT162b3 (e.g., BNT162b3c).

In one embodiment, RNA encoding a vaccine antigen is nucleoside modified messenger RNA (modRNA) and (i) comprises the nucleotide sequence of SEQ ID NO: 21, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 21, and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 5, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 5. In one embodiment, RNA encoding a vaccine antigen is nucleoside modified messenger RNA (modRNA) and (i) comprises the nucleotide sequence of SEQ ID NO: 21; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 5.

In one embodiment, RNA encoding a vaccine antigen is nucleoside modified messenger RNA (modRNA) and (i) comprises the nucleotide sequence of SEQ ID NO: 19, or 20, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 19, or 20, and/or (ii) encodes an amino acid sequence

comprising the amino acid sequence of SEQ ID NO: 7, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 7. In one embodiment, RNA encoding a vaccine antigen is nucleoside modified messenger RNA (modRNA) and (i) comprises the nucleotide sequence of SEQ ID NO: 19, or 20; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 7.

In one embodiment, RNA encoding a vaccine antigen is nucleoside modified messenger RNA (modRNA) and (i) comprises the nucleotide sequence of SEQ ID NO: 20, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 20, and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 7, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 7. In one embodiment, RNA encoding a vaccine antigen is nucleoside modified messenger RNA (modRNA) and (i) comprises the nucleotide sequence of SEQ ID NO: 20; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 7.

In one embodiment, RNA encoding a vaccine antigen is nucleoside modified messenger RNA (modRNA) and (i) comprises the nucleotide sequence of SEQ ID NO: 30, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 30, and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 29, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 29. In one embodiment, RNA encoding a vaccine antigen is nucleoside modified messenger RNA (modRNA) and (i) comprises the nucleotide sequence of SEQ ID NO: 30; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 29.

In one embodiment, RNA encoding a vaccine antigen is nucleoside modified messenger RNA (modRNA) and (i) comprises the nucleotide sequence of SEQ ID NO: 50, a nucleotide sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the nucleotide sequence of SEQ ID NO: 50, and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 49, or an amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 49. In one embodiment,

RNA encoding a vaccine antigen is nucleoside modified messenger RNA (modRNA) and (i) comprises the nucleotide sequence of SEQ ID NO: 50; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 49.

In one embodiment, RNA encoding a vaccine antigen is nucleoside modified messenger RNA (modRNA) and (i) comprises the nucleotide sequence of SEQ ID NO: 51, a nucleotide sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the nucleotide sequence of SEQ ID NO: 51, and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 49, or an amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 49. In one embodiment, RNA encoding a vaccine antigen is nucleoside modified messenger RNA (modRNA) and (i) comprises the nucleotide sequence of SEQ ID NO: 51; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 49.

In one embodiment, RNA encoding a vaccine antigen is nucleoside modified messenger RNA (modRNA) and (i) comprises the nucleotide sequence of SEQ ID NO: 57, a nucleotide sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the nucleotide sequence of SEQ ID NO: 57, and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 55, or an amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 55. In one embodiment, RNA encoding a vaccine antigen is nucleoside modified messenger RNA (modRNA) and (i) comprises the nucleotide sequence of SEQ ID NO: 57; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 55.

In one embodiment, RNA encoding a vaccine antigen is nucleoside modified messenger RNA (modRNA) and (i) comprises the nucleotide sequence of SEQ ID NO: 60, a nucleotide sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the nucleotide sequence of SEQ ID NO: 60, and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 58, or an amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 58. In one embodiment, RNA encoding a vaccine antigen is nucleoside modified messenger RNA (modRNA) and (i) comprises the nucleotide sequence of SEQ ID NO: 60; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 58.

In one embodiment, RNA encoding a vaccine antigen is nucleoside modified messenger RNA (modRNA) and (i) comprises the nucleotide sequence of SEQ ID NO: 63a, a nucleotide sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the nucleotide sequence of SEQ ID NO: 63a, and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 61, or an amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 61. In one embodiment, RNA encoding a vaccine antigen is nucleoside modified messenger RNA (modRNA) and (i) comprises the nucleotide sequence of SEQ ID NO: 63a; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 61.

In one embodiment, RNA encoding a vaccine antigen is nucleoside modified messenger RNA (modRNA) and (i) comprises the nucleotide sequence of SEQ ID NO: 53, a nucleotide sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the nucleotide sequence of SEQ ID NO: 53, and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 52, or an amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 52. In one embodiment, RNA encoding a vaccine antigen is nucleoside modified messenger RNA (modRNA) and (i) comprises the nucleotide sequence of SEQ ID NO: 53; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 52.

In one embodiment, RNA encoding a vaccine antigen is nucleoside modified messenger RNA (modRNA) and (i) comprises the nucleotide sequence of SEQ ID NO: 54, a nucleotide sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the nucleotide sequence of SEQ ID NO: 54, and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 52, or an amino acid sequence having at least 99.5%, 99%, 98.5%, 98%, 98.5% or 97% identity to the amino acid sequence of SEQ ID NO: 52. In one embodiment, RNA encoding a vaccine antigen is nucleoside modified messenger RNA (modRNA) and (i) comprises the nucleotide sequence of SEQ ID NO: 54; and/or (ii) encodes an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 52.

As used herein, the term "vaccine" refers to a composition that induces an immune response upon inoculation into a subject. In some embodiments, the induced immune response provides protective immunity.

In one embodiment, the RNA encoding the antigen molecule is expressed in cells of the subject to provide the antigen molecule. In one embodiment, expression of the antigen molecule is at the cell surface or into the extracellular space. In one embodiment, the antigen molecule is presented in the context of MHC. In one embodiment, the RNA encoding the antigen molecule is transiently expressed in cells of the subject. In one embodiment, after administration of the RNA encoding the antigen molecule, in particular after intramuscular administration of the RNA encoding the antigen molecule, expression of the RNA encoding the antigen molecule in muscle occurs. In one embodiment, after administration of the RNA encoding the antigen molecule, expression of the RNA encoding the antigen molecule in spleen occurs. In one embodiment, after administration of the RNA encoding the antigen molecule, expression of the RNA encoding the antigen molecule in antigen presenting cells, preferably professional antigen presenting cells occurs. In one embodiment, the antigen presenting cells are selected from the group consisting of dendritic cells, macrophages and B cells. In one embodiment, after administration of the RNA encoding the antigen molecule, no or essentially no expression of the RNA encoding the antigen molecule in lung and/or liver occurs. In one embodiment, after administration of the RNA encoding the antigen molecule, expression of the RNA encoding the antigen molecule in spleen is at least 5-fold the amount of expression in lung.

In some embodiments, the methods and agents, e.g., RNA compositions, described herein following administration, in particular following intramuscular administration, to a subject result in delivery of the RNA encoding a vaccine antigen to lymph nodes and/or spleen. In some embodiments, RNA encoding a vaccine antigen is detectable in lymph nodes and/or spleen 6 hours or later following administration and preferably up to 6 days or longer.

In some embodiments, the methods and agents, e.g., mRNA compositions, described herein following administration, in particular following intramuscular administration, to a subject result in delivery of the RNA encoding a vaccine antigen to B cell follicles, subcapsular sinus, and/or T cell zone, in particular B cell follicles and/or subcapsular sinus of lymph nodes.

In some embodiments, the methods and agents, e.g., mRNA compositions, described herein following administration, in particular following intramuscular administration, to a subject result in delivery of the RNA encoding a vaccine antigen to B cells (CD19+), subcapsular sinus macrophages (CD169+) and/or dendritic cells (CD11c+) in the T cell zone and intermediary

sinus of lymph nodes, in particular to B cells (CD19+) and/or subcapsular sinus macrophages (CD169+) of lymph nodes.

In some embodiments, the methods and agents, e.g., mRNA compositions, described herein following administration, in particular following intramuscular administration, to a subject result in delivery of the RNA encoding a vaccine antigen to white pulp of spleen.

In some embodiments, the methods and agents, e.g., mRNA compositions, described herein following administration, in particular following intramuscular administration, to a subject result in delivery of the RNA encoding a vaccine antigen to B cells, DCs (CD11c+), in particular those surrounding the B cells, and/or macrophages of spleen, in particular to B cells and/or DCs (CD11c+).

In one embodiment, the vaccine antigen is expressed in lymph node and/or spleen, in particular in the cells of lymph node and/or spleen described above.

The peptide and protein antigens suitable for use according to the present disclosure typically include a peptide or protein comprising an epitope of SARS-CoV-2 S protein or a functional variant thereof for inducing an immune response. The peptide or protein or epitope may be derived from a target antigen, i.e. the antigen against which an immune response is to be elicited. For example, the peptide or protein antigen or the epitope contained within the peptide or protein antigen may be a target antigen or a fragment or variant of a target antigen. The target antigen may be a coronavirus S protein, in particular SARS-CoV-2 S protein.

The antigen molecule or a procession product thereof, e.g., a fragment thereof, may bind to an antigen receptor such as a BCR or TCR carried by immune effector cells, or to antibodies.

A peptide and protein antigen which is provided to a subject according to the present disclosure by administering RNA encoding the peptide and protein antigen, i.e., a vaccine antigen, preferably results in the induction of an immune response, e.g., a humoral and/or cellular immune response in the subject being provided the peptide or protein antigen. Said immune response is preferably directed against a target antigen, in particular coronavirus S protein, in particular SARS-CoV-2 S protein. Thus, a vaccine antigen may comprise the target antigen, a variant thereof, or a fragment thereof. In one embodiment, such fragment or variant is immunologically equivalent to the target antigen. In the context of the present disclosure, the term "fragment of an antigen" or "variant of an antigen" means an agent which

results in the induction of an immune response which immune response targets the antigen, i.e. a target antigen. Thus, the vaccine antigen may correspond to or may comprise the target antigen, may correspond to or may comprise a fragment of the target antigen or may correspond to or may comprise an antigen which is homologous to the target antigen or a fragment thereof. Thus, according to the present disclosure, a vaccine antigen may comprise an immunogenic fragment of a target antigen or an amino acid sequence being homologous to an immunogenic fragment of a target antigen. An "immunogenic fragment of an antigen" according to the present disclosure preferably relates to a fragment of an antigen which is capable of inducing an immune response against the target antigen. The vaccine antigen may be a recombinant antigen.

The term "immunologically equivalent" means that the immunologically equivalent molecule such as the immunologically equivalent amino acid sequence exhibits the same or essentially the same immunological properties and/or exerts the same or essentially the same immunological effects, e.g., with respect to the type of the immunological effect. In the context of the present disclosure, the term "immunologically equivalent" is preferably used with respect to the immunological effects or properties of antigens or antigen variants used for immunization. For example, an amino acid sequence is immunologically equivalent to a reference amino acid sequence if said amino acid sequence when exposed to the immune system of a subject induces an immune reaction having a specificity of reacting with the reference amino acid sequence.

"Activation" or "stimulation", as used herein, refers to the state of an immune effector cell such as T cell that has been sufficiently stimulated to induce detectable cellular proliferation. Activation can also be associated with initiation of signaling pathways, induced cytokine production, and detectable effector functions. The term "activated immune effector cells" refers to, among other things, immune effector cells that are undergoing cell division.

The term "priming" refers to a process wherein an immune effector cell such as a T cell has its first contact with its specific antigen and causes differentiation into effector cells such as effector T cells.

The term "clonal expansion" or "expansion" refers to a process wherein a specific entity is multiplied. In the context of the present disclosure, the term is preferably used in the context

of an immunological response in which immune effector cells are stimulated by an antigen, proliferate, and the specific immune effector cell recognizing said antigen is amplified. Preferably, clonal expansion leads to differentiation of the immune effector cells.

The term "antigen" relates to an agent comprising an epitope against which an immune response can be generated. The term "antigen" includes, in particular, proteins and peptides. In one embodiment, an antigen is presented by cells of the immune system such as antigen presenting cells like dendritic cells or macrophages. An antigen or a procession product thereof such as a T-cell epitope is in one embodiment bound by a T- or B-cell receptor, or by an immunoglobulin molecule such as an antibody. Accordingly, an antigen or a procession product thereof may react specifically with antibodies or T lymphocytes (T cells). In one embodiment, an antigen is a viral antigen, such as a coronavirus S protein, e.g., SARS-CoV-2 S protein, and an epitope is derived from such antigen.

The term "viral antigen" refers to any viral component having antigenic properties, i.e. being able to provoke an immune response in an individual. The viral antigen may be coronavirus S protein, e.g., SARS-CoV-2 S protein.

The term "expressed on the cell surface" or "associated with the cell surface" means that a molecule such as an antigen is associated with and located at the plasma membrane of a cell, wherein at least a part of the molecule faces the extracellular space of said cell and is accessible from the outside of said cell, e.g., by antibodies located outside the cell. In this context, a part is preferably at least 4, preferably at least 8, preferably at least 12, more preferably at least 20 amino acids. The association may be direct or indirect. For example, the association may be by one or more transmembrane domains, one or more lipid anchors, or by the interaction with any other protein, lipid, saccharide, or other structure that can be found on the outer leaflet of the plasma membrane of a cell. For example, a molecule associated with the surface of a cell may be a transmembrane protein having an extracellular portion or may be a protein associated with the surface of a cell by interacting with another protein that is a transmembrane protein.

"Cell surface" or "surface of a cell" is used in accordance with its normal meaning in the art, and thus includes the outside of the cell which is accessible to binding by proteins and other

molecules. An antigen is expressed on the surface of cells if it is located at the surface of said cells and is accessible to binding by e.g. antigen-specific antibodies added to the cells.

The term "extracellular portion" or "exodomain" in the context of the present disclosure refers to a part of a molecule such as a protein that is facing the extracellular space of a cell and preferably is accessible from the outside of said cell, e.g., by binding molecules such as antibodies located outside the cell. Preferably, the term refers to one or more extracellular loops or domains or a fragment thereof.

The term "epitope" refers to a part or fragment of a molecule such as an antigen that is recognized by the immune system. For example, the epitope may be recognized by T cells, B cells or antibodies. An epitope of an antigen may include a continuous or discontinuous portion of the antigen and may be between about 5 and about 100, such as between about 5 and about 50, more preferably between about 8 and about 30, most preferably between about 8 and about 25 amino acids in length, for example, the epitope may be preferably 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 amino acids in length. In one embodiment, an epitope is between about 10 and about 25 amino acids in length. The term "epitope" includes T cell epitopes.

The term "T cell epitope" refers to a part or fragment of a protein that is recognized by a T cell when presented in the context of MHC molecules. The term "major histocompatibility complex" and the abbreviation "MHC" includes MHC class I and MHC class II molecules and relates to a complex of genes which is present in all vertebrates. MHC proteins or molecules are important for signaling between lymphocytes and antigen presenting cells or diseased cells in immune reactions, wherein the MHC proteins or molecules bind peptide epitopes and present them for recognition by T cell receptors on T cells. The proteins encoded by the MHC are expressed on the surface of cells, and display both self-antigens (peptide fragments from the cell itself) and non-self-antigens (e.g., fragments of invading microorganisms) to a T cell. In the case of class I MHC/peptide complexes, the binding peptides are typically about 8 to about 10 amino acids long although longer or shorter peptides may be effective. In the case of class II MHC/peptide complexes, the binding peptides are typically about 10 to about 25 amino acids long and are in particular about 13 to about 18 amino acids long, whereas longer and shorter peptides may be effective.

The peptide and protein antigen can be 2-100 amino acids, including for example, 5 amino acids, 10 amino acids, 15 amino acids, 20 amino acids, 25 amino acids, 30 amino acids, 35 amino acids, 40 amino acids, 45 amino acids, or 50 amino acids in length. In some embodiments, a peptide can be greater than 50 amino acids. In some embodiments, the peptide can be greater than 100 amino acids.

The peptide or protein antigen can be any peptide or protein that can induce or increase the ability of the immune system to develop antibodies and T cell responses to the peptide or protein.

In one embodiment, vaccine antigen is recognized by an immune effector cell. Preferably, the vaccine antigen if recognized by an immune effector cell is able to induce in the presence of appropriate co-stimulatory signals, stimulation, priming and/or expansion of the immune effector cell carrying an antigen receptor recognizing the vaccine antigen. In the context of the embodiments of the present disclosure, the vaccine antigen is preferably presented or present on the surface of a cell, preferably an antigen presenting cell. In one embodiment, an antigen is presented by a diseased cell such as a virus-infected cell. In one embodiment, an antigen receptor is a TCR which binds to an epitope of an antigen presented in the context of MHC. In one embodiment, binding of a TCR when expressed by T cells and/or present on T cells to an antigen presented by cells such as antigen presenting cells results in stimulation, priming and/or expansion of said T cells. In one embodiment, binding of a TCR when expressed by T cells and/or present on T cells to an antigen presented on diseased cells results in cytolysis and/or apoptosis of the diseased cells, wherein said T cells preferably release cytotoxic factors, e.g. perforins and granzymes.

In one embodiment, an antigen receptor is an antibody or B cell receptor which binds to an epitope in an antigen. In one embodiment, an antibody or B cell receptor binds to native epitopes of an antigen.

Nucleic acids

The term "polynucleotide" or "nucleic acid", as used herein, is intended to include DNA and RNA such as genomic DNA, cDNA, mRNA, recombinantly produced and chemically synthesized molecules. A nucleic acid may be single-stranded or double-stranded. RNA includes *in vitro*

transcribed RNA (IVT RNA) or synthetic RNA. According to the present disclosure, a polynucleotide is preferably isolated.

Nucleic acids may be comprised in a vector. The term "vector" as used herein includes any vectors known to the skilled person including plasmid vectors, cosmid vectors, phage vectors such as lambda phage, viral vectors such as retroviral, adenoviral or baculoviral vectors, or artificial chromosome vectors such as bacterial artificial chromosomes (BAC), yeast artificial chromosomes (YAC), or P1 artificial chromosomes (PAC). Said vectors include expression as well as cloning vectors. Expression vectors comprise plasmids as well as viral vectors and generally contain a desired coding sequence and appropriate DNA sequences necessary for the expression of the operably linked coding sequence in a particular host organism (e.g., bacteria, yeast, plant, insect, or mammal) or in *in vitro* expression systems. Cloning vectors are generally used to engineer and amplify a certain desired DNA fragment and may lack functional sequences needed for expression of the desired DNA fragments.

In one embodiment of all aspects of the present disclosure, the RNA encoding the vaccine antigen is expressed in cells such as antigen presenting cells of the subject treated to provide the vaccine antigen.

The nucleic acids described herein may be recombinant and/or isolated molecules.

In the present disclosure, the term "RNA" relates to a nucleic acid molecule which includes ribonucleotide residues. In preferred embodiments, the RNA contains all or a majority of ribonucleotide residues. As used herein, "ribonucleotide" refers to a nucleotide with a hydroxyl group at the 2'-position of a β-D-ribofuranosyl group. RNA encompasses without limitation, double stranded RNA, single stranded RNA, isolated RNA such as partially purified RNA, essentially pure RNA, synthetic RNA, recombinantly produced RNA, as well as modified RNA that differs from naturally occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides. Such alterations may refer to addition of non-nucleotide material to internal RNA nucleotides or to the end(s) of RNA. It is also contemplated herein that nucleotides in RNA may be non-standard nucleotides, such as chemically synthesized nucleotides or deoxynucleotides. For the present disclosure, these altered RNAs are considered analogs of naturally-occurring RNA.

In certain embodiments of the present disclosure, the RNA is messenger RNA (mRNA) that relates to a RNA transcript which encodes a peptide or protein. As established in the art, mRNA generally contains a 5' untranslated region (5'-UTR), a peptide coding region and a 3' untranslated region (3'-UTR). In some embodiments, the RNA is produced by *in vitro* transcription or chemical synthesis. In one embodiment, the mRNA is produced by *in vitro* transcription using a DNA template where DNA refers to a nucleic acid that contains deoxyribonucleotides.

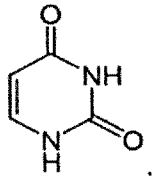
In one embodiment, RNA is *in vitro* transcribed RNA (IVT-RNA) and may be obtained by *in vitro* transcription of an appropriate DNA template. The promoter for controlling transcription can be any promoter for any RNA polymerase. A DNA template for *in vitro* transcription may be obtained by cloning of a nucleic acid, in particular cDNA, and introducing it into an appropriate vector for *in vitro* transcription. The cDNA may be obtained by reverse transcription of RNA.

In certain embodiments of the present disclosure, the RNA is "replicon RNA" or simply a "replicon", in particular "self-replicating RNA" or "self-amplifying RNA". In one particularly preferred embodiment, the replicon or self-replicating RNA is derived from or comprises elements derived from a ssRNA virus, in particular a positive-stranded ssRNA virus such as an alphavirus. Alphaviruses are typical representatives of positive-stranded RNA viruses. Alphaviruses replicate in the cytoplasm of infected cells (for review of the alphaviral life cycle see José et al., Future Microbiol., 2009, vol. 4, pp. 837–856). The total genome length of many alphaviruses typically ranges between 11,000 and 12,000 nucleotides, and the genomic RNA typically has a 5'-cap, and a 3' poly(A) tail. The genome of alphaviruses encodes non-structural proteins (involved in transcription, modification and replication of viral RNA and in protein modification) and structural proteins (forming the virus particle). There are typically two open reading frames (ORFs) in the genome. The four non-structural proteins (nsP1–nsP4) are typically encoded together by a first ORF beginning near the 5' terminus of the genome, while alphavirus structural proteins are encoded together by a second ORF which is found downstream of the first ORF and extends near the 3' terminus of the genome. Typically, the first ORF is larger than the second ORF, the ratio being roughly 2:1. In cells infected by an alphavirus, only the nucleic acid sequence encoding non-structural proteins is translated from the genomic RNA, while the genetic information encoding structural proteins is translatable

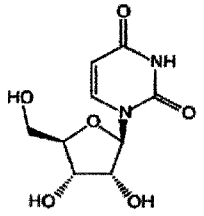
from a subgenomic transcript, which is an RNA molecule that resembles eukaryotic messenger RNA (mRNA; Gould et al., 2010, Antiviral Res., vol. 87 pp. 111–124). Following infection, i.e. at early stages of the viral life cycle, the (+) stranded genomic RNA directly acts like a messenger RNA for the translation of the open reading frame encoding the non-structural poly-protein (nsP1234). Alphavirus-derived vectors have been proposed for delivery of foreign genetic information into target cells or target organisms. In simple approaches, the open reading frame encoding alphaviral structural proteins is replaced by an open reading frame encoding a protein of interest. Alphavirus-based trans-replication systems rely on alphavirus nucleotide sequence elements on two separate nucleic acid molecules: one nucleic acid molecule encodes a viral replicase, and the other nucleic acid molecule is capable of being replicated by said replicase in trans (hence the designation trans-replication system). Trans-replication requires the presence of both these nucleic acid molecules in a given host cell. The nucleic acid molecule capable of being replicated by the replicase in trans must comprise certain alphaviral sequence elements to allow recognition and RNA synthesis by the alphaviral replicase.

In one embodiment, the RNA described herein may have modified nucleosides. In some embodiments, the RNA comprises a modified nucleoside in place of at least one (e.g., every) uridine.

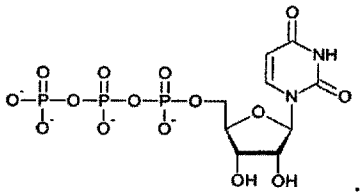
The term "uracil," as used herein, describes one of the nucleobases that can occur in the nucleic acid of RNA. The structure of uracil is:



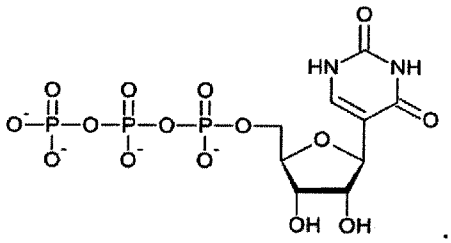
The term "uridine," as used herein, describes one of the nucleosides that can occur in RNA. The structure of uridine is:



UTP (uridine 5'-triphosphate) has the following structure:

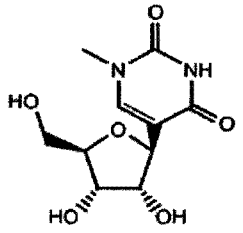


Pseudo-UTP (pseudouridine 5'-triphosphate) has the following structure:

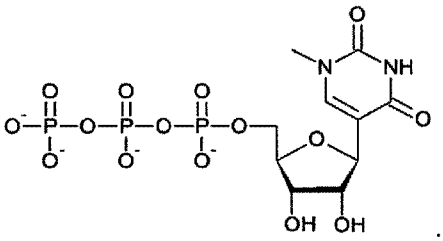


"Pseudouridine" is one example of a modified nucleoside that is an isomer of uridine, where the uracil is attached to the pentose ring via a carbon-carbon bond instead of a nitrogen-carbon glycosidic bond.

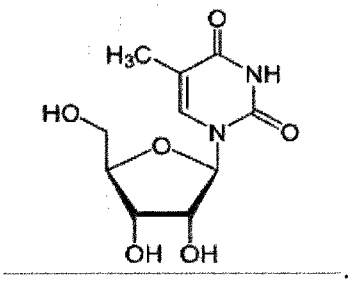
Another exemplary modified nucleoside is N1-methyl-pseudouridine (m1Ψ), which has the structure:



N1-methyl-pseudo-UTP has the following structure:



Another exemplary modified nucleoside is 5-methyl-uridine (m5U), which has the structure:



In some embodiments, one or more uridine in the RNA described herein is replaced by a modified nucleoside. In some embodiments, the modified nucleoside is a modified uridine. In some embodiments, RNA comprises a modified nucleoside in place of at least one uridine. In some embodiments, RNA comprises a modified nucleoside in place of each uridine.

In some embodiments, the modified nucleoside is independently selected from pseudouridine (ψ), N1-methyl-pseudouridine (m1ψ), and 5-methyl-uridine (m5U). In some embodiments, the modified nucleoside comprises pseudouridine (ψ). In some embodiments, the modified nucleoside comprises N1-methyl-pseudouridine (m1ψ). In some embodiments, the modified nucleoside comprises 5-methyl-uridine (m5U). In some embodiments, RNA may comprise more than one type of modified nucleoside, and the modified nucleosides are independently selected from pseudouridine (ψ), N1-methyl-pseudouridine (m1ψ), and 5-methyl-uridine (m5U). In some embodiments, the modified nucleosides comprise pseudouridine (ψ) and N1-methyl-pseudouridine (m1ψ). In some embodiments, the modified nucleosides comprise pseudouridine (ψ) and 5-methyl-uridine (m5U). In some embodiments, the modified nucleosides comprise N1-methyl-pseudouridine (m1ψ) and 5-methyl-uridine (m5U). In some embodiments, the modified nucleosides comprise pseudouridine (ψ), N1-methyl-pseudouridine (m1ψ), and 5-methyl-uridine (m5U).

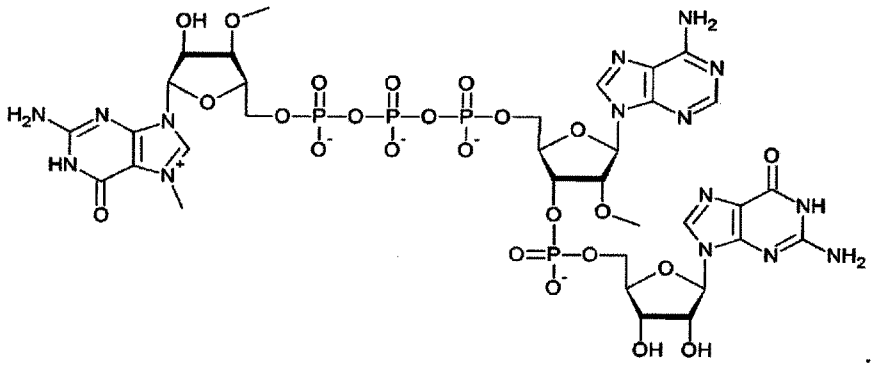
In some embodiments, the modified nucleoside replacing one or more, e.g., all, uridine in the RNA may be any one or more of 3-methyl-uridine (m^3U), 5-methoxy-uridine (mo^5U), 5-aza-uridine, 6-aza-uridine, 2-thio-5-aza-uridine, 2-thio-uridine (s^2U), 4-thio-uridine (s^4U), 4-thio-pseudouridine, 2-thio-pseudouridine, 5-hydroxy-uridine (ho^5U), 5-aminoallyl-uridine, 5-halo-uridine (e.g., 5-iodo-uridine or 5-bromo-uridine), uridine 5-oxyacetic acid (cmo^5U), uridine 5-oxyacetic acid methyl ester ($mcmo^5U$), 5-carboxymethyl-uridine (cm^5U), 1-carboxymethyl-pseudouridine, 5-carboxyhydroxymethyl-uridine (chm^5U), 5-carboxyhydroxymethyl-uridine methyl ester ($mchm^5U$), 5-methoxycarbonylmethyl-uridine (mcm^5U), 5-methoxycarbonylmethyl-2-thio-uridine (mcm^5s^2U), 5-aminomethyl-2-thio-uridine (nm^5s^2U), 5-methylaminomethyl-uridine (mnm^5U), 1-ethyl-pseudouridine, 5-methylaminomethyl-2-thio-uridine (mnm^5s^2U), 5-methylaminomethyl-2-seleno-uridine (mnm^5se^2U), 5-carbamoylmethyl-uridine (ncm^5U), 5-carboxymethylaminomethyl-uridine ($cmnm^5U$), 5-carboxymethylaminomethyl-2-thio-uridine ($cmnm^5s^2U$), 5-propynyl-uridine, 1-propynyl-pseudouridine, 5-taurinomethyl-uridine (τm^5U), 1-taurinomethyl-pseudouridine, 5-taurinomethyl-2-thio-uridine($\tau m5s2U$), 1-taurinomethyl-4-thio-pseudouridine), 5-methyl-2-thio-uridine (m^5s^2U), 1-methyl-4-thio-pseudouridine ($m^1s^4\psi$), 4-thio-1-methyl-pseudouridine, 3-methyl-pseudouridine ($m^3\psi$), 2-thio-1-methyl-pseudouridine, 1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-1-deaza-pseudouridine, dihydrouridine (D), dihydropseudouridine, 5,6-dihydrouridine, 5-methyl-dihydrouridine (m^5D), 2-thio-dihydrouridine, 2-thio-dihydropseudouridine, 2-methoxy-uridine, 2-methoxy-4-thio-uridine, 4-methoxy-pseudouridine, 4-methoxy-2-thio-pseudouridine, N1-methyl-pseudouridine, 3-(3-amino-3-carboxypropyl)uridine (acp^3U), 1-methyl-3-(3-amino-3-carboxypropyl)pseudouridine ($acp^3\ \psi$), 5-(isopentenylaminomethyl)uridine (inm^5U), 5-(isopentenylaminomethyl)-2-thio-uridine (inm^5s^2U), α-thio-uridine, 2'-O-methyl-uridine (Um), 5,2'-O-dimethyl-uridine (m^5Um), 2'-O-methyl-pseudouridine (ψm), 2-thio-2'-O-methyl-uridine (s^2Um), 5-methoxycarbonylmethyl-2'-O-methyl-uridine (mcm^5Um), 5-carbamoylmethyl-2'-O-methyl-uridine (ncm^5Um), 5-carboxymethylaminomethyl-2'-O-methyl-uridine ($cmnm^5Um$), 3,2'-O-dimethyl-uridine (m^3Um), 5-(isopentenylaminomethyl)-2'-O-methyl-uridine (inm^5Um), 1-thio-uridine, deoxythymidine, 2'-F-ara-uridine, 2'-F-uridine, 2'-OH-ara-uridine, 5-(2-

carbomethoxyvinyl) uridine, 5-[3-(1-E-propenylamino)uridine, or any other modified uridine known in the art.

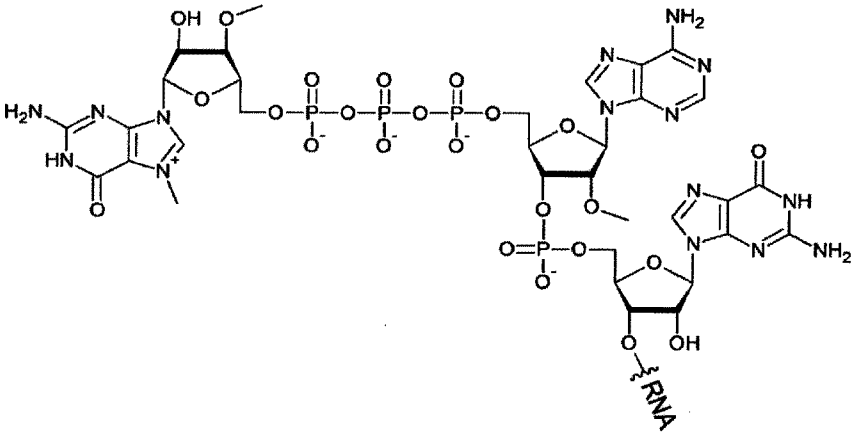
In one embodiment, the RNA comprises other modified nucleosides or comprises further modified nucleosides, e.g., modified cytidine. For example, in one embodiment, in the RNA 5-methylcytidine is substituted partially or completely, preferably completely, for cytidine. In one embodiment, the RNA comprises 5-methylcytidine and one or more selected from pseudouridine (ψ), N1-methyl-pseudouridine (m1ψ), and 5-methyl-uridine (m5U). In one embodiment, the RNA comprises 5-methylcytidine and N1-methyl-pseudouridine (m1ψ). In some embodiments, the RNA comprises 5-methylcytidine in place of each cytidine and N1-methyl-pseudouridine (m1ψ) in place of each uridine.

In some embodiments, the RNA according to the present disclosure comprises a 5'-cap. In one embodiment, the RNA of the present disclosure does not have uncapped 5'-triphosphates. In one embodiment, the RNA may be modified by a 5'- cap analog. The term "5'-cap" refers to a structure found on the 5'-end of an mRNA molecule and generally consists of a guanosine nucleotide connected to the mRNA via a 5'- to 5'-triphosphate linkage. In one embodiment, this guanosine is methylated at the 7-position. Providing an RNA with a 5'-cap or 5'-cap analog may be achieved by *in vitro* transcription, in which the 5'-cap is co-transcriptionally expressed into the RNA strand, or may be attached to RNA post-transcriptionally using capping enzymes. In some embodiments, the RNA (e.g., mRNA) comprises a cap0, cap1, or cap2, preferably cap1 or cap2, more preferably cap1. According to the present disclosure, the term "cap0" comprises the structure "m^7GpppN", wherein N is any nucleoside bearing an OH moiety at position 2'. According to the present disclosure, the term "cap1" comprises the structure "$m^7GpppNm$", wherein Nm is any nucleoside bearing an OCH_3 moiety at position 2'. According to the present disclosure, the term "cap2" comprises the structure "$m^7GpppNmNm$", wherein each Nm is independently any nucleoside bearing an OCH_3 moiety at position 2'.

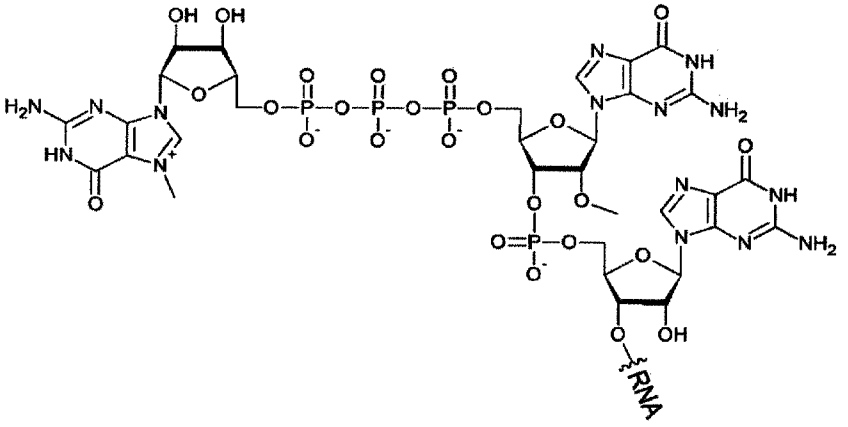
In some embodiments, the building block cap for RNA is $m_2^{7,3'-O}Gppp(m_1^{2'-O})ApG$ (also sometimes referred to as $m_2^{7,3'O}G(5')ppp(5')m^{2'-O}ApG$), which has the following structure:



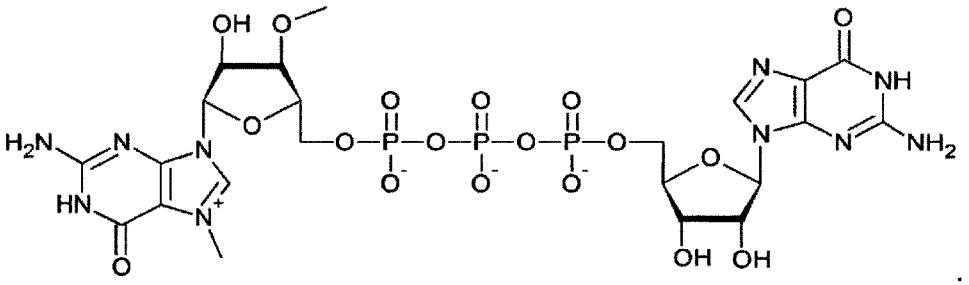
Below is an exemplary Cap1 RNA, which comprises RNA and $m_2^{7,3'-O}G(5')ppp(5')m^{2'-O}ApG$:



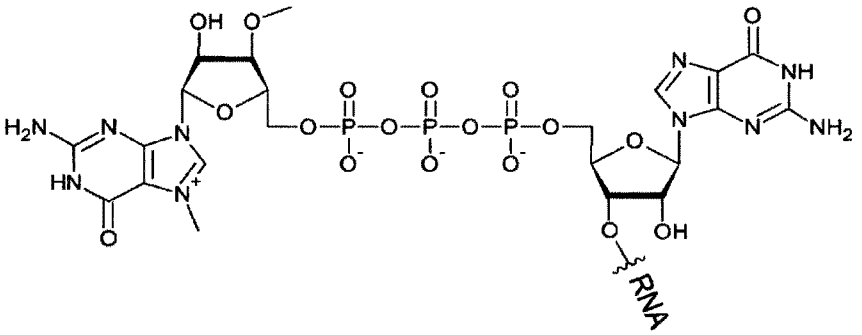
Below is another exemplary Cap1 RNA (no cap analog):



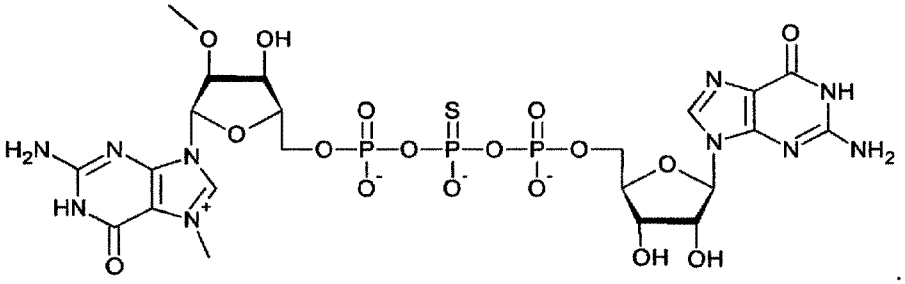
In some embodiments, the RNA is modified with "Cap0" structures using, in one embodiment, the cap analog anti-reverse cap (ARCA Cap ($m_2^{7,3'-O}G(5')ppp(5')G$)) with the structure:



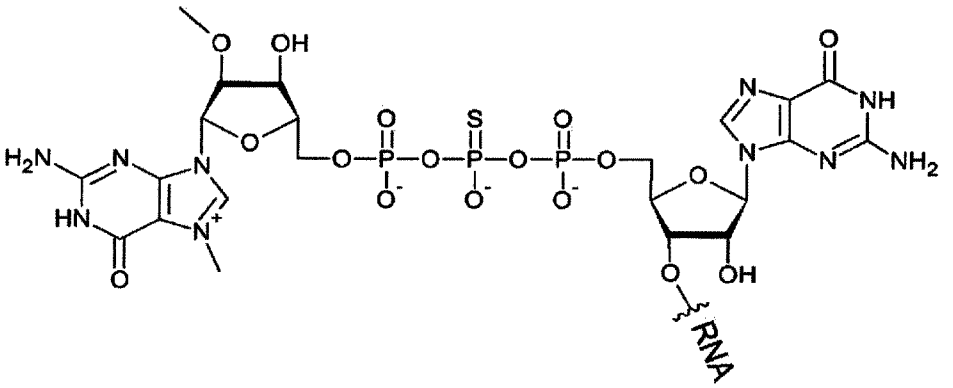
Below is an exemplary Cap0 RNA comprising RNA and $m_2^{7,3'O}G(5')ppp(5')G$:



In some embodiments, the "Cap0" structures are generated using the cap analog Beta-S-ARCA ($m_2^{7,2'O}G(5')ppSp(5')G$) with the structure:



Below is an exemplary Cap0 RNA comprising Beta-S-ARCA ($m_2^{7,2'O}G(5')ppSp(5')G$) and RNA:



The "D1" diastereomer of beta-S-ARCA or "beta-S-ARCA(D1)" is the diastereomer of beta-S-ARCA which elutes first on an HPLC column compared to the D2 diastereomer of beta-S-ARCA (beta-S-ARCA(D2)) and thus exhibits a shorter retention time (cf., WO 2011/015347, herein incorporated by reference).

A particularly preferred cap is beta-S-ARCA(D1) ($m_2^{7,2'-O}$GppSpG) or $m_2^{7,3'-O}$Gppp($m_1^{2'-O}$)ApG.

In some embodiments, RNA according to the present disclosure comprises a 5'-UTR and/or a 3'-UTR. The term "untranslated region" or "UTR" relates to a region in a DNA molecule which is transcribed but is not translated into an amino acid sequence, or to the corresponding region in an RNA molecule, such as an mRNA molecule. An untranslated region (UTR) can be present 5' (upstream) of an open reading frame (5'-UTR) and/or 3' (downstream) of an open reading frame (3'-UTR). A 5'-UTR, if present, is located at the 5' end, upstream of the start codon of a protein-encoding region. A 5'-UTR is downstream of the 5'-cap (if present), e.g. directly adjacent to the 5'-cap. A 3'-UTR, if present, is located at the 3' end, downstream of the termination codon of a protein-encoding region, but the term "3'-UTR" does preferably not include the poly(A) sequence. Thus, the 3'-UTR is upstream of the poly(A) sequence (if present), e.g. directly adjacent to the poly(A) sequence.

In some embodiments, RNA comprises a 5'-UTR comprising the nucleotide sequence of SEQ ID NO: 12, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 12.

In some embodiments, RNA comprises a 3'-UTR comprising the nucleotide sequence of SEQ ID NO: 13, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 13.

A particularly preferred 5'-UTR comprises the nucleotide sequence of SEQ ID NO: 12. A particularly preferred 3'-UTR comprises the nucleotide sequence of SEQ ID NO: 13.

In some embodiments, the RNA according to the present disclosure comprises a 3'-poly(A) sequence.

As used herein, the term "poly(A) sequence" or "poly-A tail" refers to an uninterrupted or interrupted sequence of adenylate residues which is typically located at the 3'-end of an RNA molecule. Poly(A) sequences are known to those of skill in the art and may follow the 3'-UTR in the RNAs described herein. An uninterrupted poly(A) sequence is characterized by consecutive adenylate residues. In nature, an uninterrupted poly(A) sequence is typical. RNAs disclosed herein can have a poly(A) sequence attached to the free 3'-end of the RNA by a template-independent RNA polymerase after transcription or a poly(A) sequence encoded by DNA and transcribed by a template-dependent RNA polymerase.

It has been demonstrated that a poly(A) sequence of about 120 A nucleotides has a beneficial influence on the levels of RNA in transfected eukaryotic cells, as well as on the levels of protein that is translated from an open reading frame that is present upstream (5') of the poly(A) sequence (*Holtkamp et al.*, 2006, Blood, vol. 108, pp. 4009-4017).

The poly(A) sequence may be of any length. In some embodiments, a poly(A) sequence comprises, essentially consists of, or consists of at least 20, at least 30, at least 40, at least 80, or at least 100 and up to 500, up to 400, up to 300, up to 200, or up to 150 A nucleotides, and, in particular, about 120 A nucleotides. In this context, "essentially consists of" means that most nucleotides in the poly(A) sequence, typically at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% by number of nucleotides in the poly(A) sequence are A nucleotides, but permits that remaining nucleotides are nucleotides other than A nucleotides, such as U nucleotides (uridylate), G nucleotides (guanylate), or C nucleotides (cytidylate). In this context, "consists of" means that all nucleotides in the poly(A) sequence, i.e., 100% by number of nucleotides in the poly(A) sequence, are A nucleotides. The term "A nucleotide" or "A" refers to adenylate.

In some embodiments, a poly(A) sequence is attached during RNA transcription, e.g., during preparation of *in vitro* transcribed RNA, based on a DNA template comprising repeated dT

nucleotides (deoxythymidylate) in the strand complementary to the coding strand. The DNA sequence encoding a poly(A) sequence (coding strand) is referred to as poly(A) cassette.

In some embodiments, the poly(A) cassette present in the coding strand of DNA essentially consists of dA nucleotides, but is interrupted by a random sequence of the four nucleotides (dA, dC, dG, and dT). Such random sequence may be 5 to 50, 10 to 30, or 10 to 20 nucleotides in length. Such a cassette is disclosed in WO 2016/005324 A1, hereby incorporated by reference. Any poly(A) cassette disclosed in WO 2016/005324 A1 may be used in the present disclosure. A poly(A) cassette that essentially consists of dA nucleotides, but is interrupted by a random sequence having an equal distribution of the four nucleotides (dA, dC, dG, dT) and having a length of e.g., 5 to 50 nucleotides shows, on DNA level, constant propagation of plasmid DNA in *E. coli* and is still associated, on RNA level, with the beneficial properties with respect to supporting RNA stability and translational efficiency is encompassed. Consequently, in some embodiments, the poly(A) sequence contained in an RNA molecule described herein essentially consists of A nucleotides, but is interrupted by a random sequence of the four nucleotides (A, C, G, U). Such random sequence may be 5 to 50, 10 to 30, or 10 to 20 nucleotides in length.

In some embodiments, no nucleotides other than A nucleotides flank a poly(A) sequence at its 3'-end, i.e., the poly(A) sequence is not masked or followed at its 3'-end by a nucleotide other than A.

In some embodiments, the poly(A) sequence may comprise at least 20, at least 30, at least 40, at least 80, or at least 100 and up to 500, up to 400, up to 300, up to 200, or up to 150 nucleotides. In some embodiments, the poly(A) sequence may essentially consist of at least 20, at least 30, at least 40, at least 80, or at least 100 and up to 500, up to 400, up to 300, up to 200, or up to 150 nucleotides. In some embodiments, the poly(A) sequence may consist of at least 20, at least 30, at least 40, at least 80, or at least 100 and up to 500, up to 400, up to 300, up to 200, or up to 150 nucleotides. In some embodiments, the poly(A) sequence comprises at least 100 nucleotides. In some embodiments, the poly(A) sequence comprises about 150 nucleotides. In some embodiments, the poly(A) sequence comprises about 120 nucleotides.

In some embodiments, a poly(A) sequence included in an RNA described herein is a interrupted poly(A) sequence, e.g., as described in WO2016/005324, the entire content of which is incorporated herein by reference for purposes described herein. In some embodiments, a poly(A) sequence comprises a stretch of at least 20 adenosine residues (including, e.g., at least 30, at least 40, at least 50, at least 60, at least 70, or more adenosine residues), followed by a linker sequence (e.g., in some embodiments comprising non-A nucleotides) and another stretch of at least 20 adenosine residues (including, e.g., at least 30, at least 40, at least 50, at least 60, at least 70, or more adenosine residues). In some embodiments, such a linker sequence may be 3-50 nucleotides in length, or 5-25 nucleotides in length, or 10-15 nucleotides in length. In some embodiments, a poly(A) sequence comprises a stretch of about 30 adenosine residues, followed by a linker sequence having a length of about 5-15 nucleoties (e.g., in some embodiments comprising non-A nucleotides) and another stretch of about 70 adenosine residues.

In some embodiments, RNA comprises a poly(A) sequence comprising the nucleotide sequence of SEQ ID NO: 14, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 14.

A particularly preferred poly(A) sequence comprises the nucleotide sequence of SEQ ID NO: 14.

According to the present disclosure, vaccine antigen is preferably administered as single-stranded, 5'-capped RNA (e.g., mRNA) that is translated into the respective protein upon entering cells of a subject being administered the RNA. Preferably, the RNA contains structural elements optimized for maximal efficacy of the RNA with respect to stability and translational efficiency (5'-cap, 5'-UTR, 3'-UTR, poly(A) sequence).

In one embodiment, beta-S-ARCA(D1) is utilized as specific capping structure at the 5'-end of the RNA. In one embodiment, $m_2^{7,3'-O}$Gppp($m_1^{2'-O}$)ApG is utilized as specific capping structure at the 5'-end of the RNA. In one embodiment, the 5'-UTR sequence is derived from the human alpha-globin mRNA and optionally has an optimized 'Kozak sequence' to increase translational efficiency. In one embodiment, a combination of two sequence elements (FI element) derived from the "amino terminal enhancer of split" (AES) mRNA (called F) and the mitochondrial encoded 12S ribosomal RNA (called I) are placed between the coding sequence and the poly(A)

sequence to assure higher maximum protein levels and prolonged persistence of the RNA (e.g., mRNA). In one embodiment, two re-iterated 3'-UTRs derived from the human beta-globin mRNA are placed between the coding sequence and the poly(A) sequence to assure higher maximum protein levels and prolonged persistence of the RNA (e.g., mRNA). In one embodiment, a poly(A) sequence measuring 110 nucleotides in length, consisting of a stretch of 30 adenosine residues, followed by a linker sequence (e.g., 10 nucleotide linker sequence) and another 70 adenosine residues is used. This poly(A) sequence was designed to enhance RNA stability and translational efficiency.

In one embodiment of all aspects of the present disclosure, RNA encoding a vaccine antigen is expressed in cells of the subject treated to provide the vaccine antigen. In one embodiment of all aspects of the present disclosure, the RNA is transiently expressed in cells of the subject. In one embodiment of all aspects of the present disclosure, the RNA is *in vitro* transcribed RNA. In one embodiment of all aspects of the present disclosure, expression of the vaccine antigen is at the cell surface. In one embodiment of all aspects of the present disclosure, the vaccine antigen is expressed and presented in the context of MHC. In one embodiment of all aspects of the present disclosure, expression of the vaccine antigen is into the extracellular space, i.e., the vaccine antigen is secreted.

In the context of the present disclosure, the term "transcription" relates to a process, wherein the genetic code in a DNA sequence is transcribed into RNA. Subsequently, the RNA may be translated into peptide or protein.

According to the present disclosure, the term "transcription" comprises "*in vitro* transcription", wherein the term "*in vitro* transcription" relates to a process wherein RNA, in particular mRNA, is *in vitro* synthesized in a cell-free system, preferably using appropriate cell extracts. Preferably, cloning vectors are applied for the generation of transcripts. These cloning vectors are generally designated as transcription vectors and are according to the present disclosure encompassed by the term "vector". According to the present disclosure, the RNA used in the present disclosure preferably is *in vitro* transcribed RNA (IVT-RNA) and may be obtained by *in vitro* transcription of an appropriate DNA template. The promoter for controlling transcription can be any promoter for any RNA polymerase. Particular examples of RNA polymerases are the T7, T3, and SP6 RNA polymerases. Preferably, the *in vitro*

transcription according to the disclosure is controlled by a T7 or SP6 promoter. A DNA template for *in vitro* transcription may be obtained by cloning of a nucleic acid, in particular cDNA, and introducing it into an appropriate vector for *in vitro* transcription. The cDNA may be obtained by reverse transcription of RNA.

With respect to RNA, the term "expression" or "translation" relates to the process in the ribosomes of a cell by which a strand of mRNA directs the assembly of a sequence of amino acids to make a peptide or protein.

In one embodiment, after administration of the RNA described herein, e.g., formulated as RNA lipid particles, at least a portion of the RNA is delivered to a target cell. In one embodiment, at least a portion of the RNA is delivered to the cytosol of the target cell. In one embodiment, the RNA is translated by the target cell to produce the peptide or protein it encodes. In one embodiment, the target cell is a spleen cell. In one embodiment, the target cell is an antigen presenting cell such as a professional antigen presenting cell in the spleen. In one embodiment, the target cell is a dendritic cell or macrophage. RNA particles such as RNA lipid particles described herein may be used for delivering RNA to such target cell. Accordingly, the present disclosure also relates to a method for delivering RNA to a target cell in a subject comprising the administration of the RNA particles described herein to the subject. In one embodiment, the RNA is delivered to the cytosol of the target cell. In one embodiment, the RNA is translated by the target cell to produce the peptide or protein encoded by the RNA.

"Encoding" refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (i.e., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA.

In one embodiment, the RNA encoding vaccine antigen to be administered according to the present disclosure is non-immunogenic. RNA encoding immunostimulant may be administered according to the present disclosure to provide an adjuvant effect. The RNA encoding immunostimulant may be standard RNA or non-immunogenic RNA.

The term "non-immunogenic RNA" as used herein refers to RNA that does not induce a response by the immune system upon administration, e.g., to a mammal, or induces a weaker response than would have been induced by the same RNA that differs only in that it has not been subjected to the modifications and treatments that render the non-immunogenic RNA non-immunogenic, i.e., than would have been induced by standard RNA (stdRNA). In one preferred embodiment, non-immunogenic RNA, which is also termed modified RNA (modRNA) herein, is rendered non-immunogenic by incorporating modified nucleosides suppressing RNA-mediated activation of innate immune receptors into the RNA and removing double-stranded RNA (dsRNA).

For rendering the non-immunogenic RNA non-immunogenic by the incorporation of modified nucleosides, any modified nucleoside may be used as long as it lowers or suppresses immunogenicity of the RNA. Particularly preferred are modified nucleosides that suppress RNA-mediated activation of innate immune receptors. In one embodiment, the modified nucleosides comprises a replacement of one or more uridines with a nucleoside comprising a modified nucleobase. In one embodiment, the modified nucleobase is a modified uracil. In one embodiment, the nucleoside comprising a modified nucleobase is selected from the group consisting of 3-methyl-uridine (m^3U), 5-methoxy-uridine (mo^5U), 5-aza-uridine, 6-aza-uridine, 2-thio-5-aza-uridine, 2-thio-uridine (s^2U), 4-thio-uridine (s^4U), 4-thio-pseudouridine, 2-thio-pseudouridine, 5-hydroxy-uridine (ho^5U), 5-aminoallyl-uridine, 5-halo-uridine (e.g., 5-iodo-uridine or 5-bromo-uridine), uridine 5-oxyacetic acid (cmo^5U), uridine 5-oxyacetic acid methyl ester ($mcmo^5U$), 5-carboxymethyl-uridine (cm^5U), 1-carboxymethyl-pseudouridine, 5-carboxyhydroxymethyl-uridine (chm^5U), 5-carboxyhydroxymethyl-uridine methyl ester ($mchm^5U$), 5-methoxycarbonylmethyl-uridine (mcm^5U), 5-methoxycarbonylmethyl-2-thio-uridine (mcm^5s^2U), 5-aminomethyl-2-thio-uridine (nm^5s^2U), 5-methylaminomethyl-uridine (mnm^5U), 1-ethyl-pseudouridine, 5-methylaminomethyl-2-thio-uridine (mnm^5s^2U), 5-methylaminomethyl-2-seleno-uridine (mnm^5se^2U), 5-carbamoylmethyl-uridine (ncm^5U), 5-

carboxymethylaminomethyl-uridine (cmnm^{5}U), 5-carboxymethylaminomethyl-2-thio-uridine (cmnm^{5}s^{2}U), 5-propynyl-uridine, 1-propynyl-pseudouridine, 5-taurinomethyl-uridine (τm^{5}U), 1-taurinomethyl-pseudouridine, 5-taurinomethyl-2-thio-uridine(τm5s2U), 1-taurinomethyl-4-thio-pseudouridine), 5-methyl-2-thio-uridine (m^{5}s^{2}U), 1-methyl-4-thio-pseudouridine (m^{1}s^{4}ψ), 4-thio-1-methyl-pseudouridine, 3-methyl-pseudouridine (m^{3}ψ), 2-thio-1-methyl-pseudouridine, 1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-1-deaza-pseudouridine, dihydrouridine (D), dihydropseudouridine, 5,6-dihydrouridine, 5-methyl-dihydrouridine (m^{5}D), 2-thio-dihydrouridine, 2-thio-dihydropseudouridine, 2-methoxy-uridine, 2-methoxy-4-thio-uridine, 4-methoxy-pseudouridine, 4-methoxy-2-thio-pseudouridine, N1-methyl-pseudouridine, 3-(3-amino-3-carboxypropyl)uridine (acp^{3}U), 1-methyl-3-(3-amino-3-carboxypropyl)pseudouridine (acp^3 ψ), 5-(isopentenylaminomethyl)uridine (inm^{5}U), 5-(isopentenylaminomethyl)-2-thio-uridine (inm^{5}s^{2}U), α-thio-uridine, 2'-O-methyl-uridine (Um), 5,2'-O-dimethyl-uridine (m^{5}Um), 2'-O-methyl-pseudouridine (ψm), 2-thio-2'-O-methyl-uridine (s^{2}Um), 5-methoxycarbonylmethyl-2'-O-methyl-uridine (mcm^{5}Um), 5-carbamoylmethyl-2'-O-methyl-uridine (ncm^{5}Um), 5-carboxymethylaminomethyl-2'-O-methyl-uridine (cmnm^{5}Um), 3,2'-O-dimethyl-uridine (m^{3}Um), 5-(isopentenylaminomethyl)-2'-O-methyl-uridine (inm^{5}Um), 1-thio-uridine, deoxythymidine, 2'-F-ara-uridine, 2'-F-uridine, 2'-OH-ara-uridine, 5-(2-carbomethoxyvinyl) uridine, and 5-[3-(1-E-propenylamino)uridine. In one particularly preferred embodiment, the nucleoside comprising a modified nucleobase is pseudouridine (ψ), N1-methyl-pseudouridine (m1ψ) or 5-methyl-uridine (m5U), in particular N1-methyl-pseudouridine.

In one embodiment, the replacement of one or more uridines with a nucleoside comprising a modified nucleobase comprises a replacement of at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 10%, at least 25%, at least 50%, at least 75%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% of the uridines.

During synthesis of RNA (e.g., mRNA) by *in vitro* transcription (IVT) using T7 RNA polymerase significant amounts of aberrant products, including double-stranded RNA (dsRNA) are produced due to unconventional activity of the enzyme. dsRNA induces inflammatory cytokines and activates effector enzymes leading to protein synthesis inhibition. dsRNA can be removed from RNA such as IVT RNA, for example, by ion-pair reversed phase HPLC using a

non-porous or porous C-18 polystyrene-divinylbenzene (PS-DVB) matrix. Alternatively, an enzymatic based method using E. coli RNaseIII that specifically hydrolyzes dsRNA but not ssRNA, thereby eliminating dsRNA contaminants from IVT RNA preparations can be used. Furthermore, dsRNA can be separated from ssRNA by using a cellulose material. In one embodiment, an RNA preparation is contacted with a cellulose material and the ssRNA is separated from the cellulose material under conditions which allow binding of dsRNA to the cellulose material and do not allow binding of ssRNA to the cellulose material.

As the term is used herein, "remove" or "removal" refers to the characteristic of a population of first substances, such as non-immunogenic RNA, being separated from the proximity of a population of second substances, such as dsRNA, wherein the population of first substances is not necessarily devoid of the second substance, and the population of second substances is not necessarily devoid of the first substance. However, a population of first substances characterized by the removal of a population of second substances has a measurably lower content of second substances as compared to the non-separated mixture of first and second substances.

In one embodiment, the removal of dsRNA from non-immunogenic RNA comprises a removal of dsRNA such that less than 10%, less than 5%, less than 4%, less than 3%, less than 2%, less than 1%, less than 0.5%, less than 0.3%, or less than 0.1% of the RNA in the non-immunogenic RNA composition is dsRNA. In one embodiment, the non-immunogenic RNA is free or essentially free of dsRNA. In some embodiments, the non-immunogenic RNA composition comprises a purified preparation of single-stranded nucleoside modified RNA. For example, in some embodiments, the purified preparation of single-stranded nucleoside modified RNA is substantially free of double stranded RNA (dsRNA). In some embodiments, the purified preparation is 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 at least 99.9% single stranded nucleoside modified RNA, relative to all other nucleic acid molecules (DNA, dsRNA, etc.).

In one embodiment, the non-immunogenic RNA is translated in a cell more efficiently than standard RNA with the same sequence. In one embodiment, translation is enhanced by a factor of 2-fold relative to its unmodified counterpart. In one embodiment, translation is

enhanced by a 3-fold factor. In one embodiment, translation is enhanced by a 4-fold factor. In one embodiment, translation is enhanced by a 5-fold factor. In one embodiment, translation is enhanced by a 6-fold factor. In one embodiment, translation is enhanced by a 7-fold factor. In one embodiment, translation is enhanced by an 8-fold factor. In one embodiment, translation is enhanced by a 9-fold factor. In one embodiment, translation is enhanced by a 10-fold factor. In one embodiment, translation is enhanced by a 15-fold factor. In one embodiment, translation is enhanced by a 20-fold factor. In one embodiment, translation is enhanced by a 50-fold factor. In one embodiment, translation is enhanced by a 100-fold factor. In one embodiment, translation is enhanced by a 200-fold factor. In one embodiment, translation is enhanced by a 500-fold factor. In one embodiment, translation is enhanced by a 1000-fold factor. In one embodiment, translation is enhanced by a 2000-fold factor. In one embodiment, the factor is 10-1000-fold. In one embodiment, the factor is 10-100-fold. In one embodiment, the factor is 10-200-fold. In one embodiment, the factor is 10-300-fold. In one embodiment, the factor is 10-500-fold. In one embodiment, the factor is 20-1000-fold. In one embodiment, the factor is 30-1000-fold. In one embodiment, the factor is 50-1000-fold. In one embodiment, the factor is 100-1000-fold. In one embodiment, the factor is 200-1000-fold. In one embodiment, translation is enhanced by any other significant amount or range of amounts.

In one embodiment, the non-immunogenic RNA exhibits significantly less innate immunogenicity than standard RNA with the same sequence. In one embodiment, the non-immunogenic RNA exhibits an innate immune response that is 2-fold less than its unmodified counterpart. In one embodiment, innate immunogenicity is reduced by a 3-fold factor. In one embodiment, innate immunogenicity is reduced by a 4-fold factor. In one embodiment, innate immunogenicity is reduced by a 5-fold factor. In one embodiment, innate immunogenicity is reduced by a 6-fold factor. In one embodiment, innate immunogenicity is reduced by a 7-fold factor. In one embodiment, innate immunogenicity is reduced by a 8-fold factor. In one embodiment, innate immunogenicity is reduced by a 9-fold factor. In one embodiment, innate immunogenicity is reduced by a 10-fold factor. In one embodiment, innate immunogenicity is reduced by a 15-fold factor. In one embodiment, innate immunogenicity is reduced by a 20-fold factor. In one embodiment, innate immunogenicity is reduced by a 50-fold factor. In one

embodiment, innate immunogenicity is reduced by a 100-fold factor. In one embodiment, innate immunogenicity is reduced by a 200-fold factor. In one embodiment, innate immunogenicity is reduced by a 500-fold factor. In one embodiment, innate immunogenicity is reduced by a 1000-fold factor. In one embodiment, innate immunogenicity is reduced by a 2000-fold factor.

The term "exhibits significantly less innate immunogenicity" refers to a detectable decrease in innate immunogenicity. In one embodiment, the term refers to a decrease such that an effective amount of the non-immunogenic RNA can be administered without triggering a detectable innate immune response. In one embodiment, the term refers to a decrease such that the non-immunogenic RNA can be repeatedly administered without eliciting an innate immune response sufficient to detectably reduce production of the protein encoded by the non-immunogenic RNA. In one embodiment, the decrease is such that the non-immunogenic RNA can be repeatedly administered without eliciting an innate immune response sufficient to eliminate detectable production of the protein encoded by the non-immunogenic RNA.

"Immunogenicity" is the ability of a foreign substance, such as RNA, to provoke an immune response in the body of a human or other animal. The innate immune system is the component of the immune system that is relatively unspecific and immediate. It is one of two main components of the vertebrate immune system, along with the adaptive immune system.

As used herein "endogenous" refers to any material from or produced inside an organism, cell, tissue or system.

As used herein, the term "exogenous" refers to any material introduced from or produced outside an organism, cell, tissue or system.

The term "expression" as used herein is defined as the transcription and/or translation of a particular nucleotide sequence.

As used herein, the terms "linked," "fused", or "fusion" are used interchangeably. These terms refer to the joining together of two or more elements or components or domains.

Codon-optimization / Increase in G/C content

In some embodiment, the amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or

the immunogenic variant thereof described herein is encoded by a coding sequence which is codon-optimized and/or the G/C content of which is increased compared to wild type coding sequence. This also includes embodiments, wherein one or more sequence regions of the coding sequence are codon-optimized and/or increased in the G/C content compared to the corresponding sequence regions of the wild type coding sequence. In one embodiment, the codon-optimization and/or the increase in the G/C content preferably does not change the sequence of the encoded amino acid sequence.

The term "codon-optimized" refers to the alteration of codons in the coding region of a nucleic acid molecule to reflect the typical codon usage of a host organism without preferably altering the amino acid sequence encoded by the nucleic acid molecule. Within the context of the present disclosure, coding regions are preferably codon-optimized for optimal expression in a subject to be treated using the RNA molecules described herein. Codon-optimization is based on the finding that the translation efficiency is also determined by a different frequency in the occurrence of tRNAs in cells. Thus, the sequence of RNA may be modified such that codons for which frequently occurring tRNAs are available are inserted in place of "rare codons".

In some embodiments of the present disclosure, the guanosine/cytosine (G/C) content of the coding region of the RNA described herein is increased compared to the G/C content of the corresponding coding sequence of the wild type RNA, wherein the amino acid sequence encoded by the RNA is preferably not modified compared to the amino acid sequence encoded by the wild type RNA. This modification of the RNA sequence is based on the fact that the sequence of any RNA region to be translated is important for efficient translation of that mRNA. Sequences having an increased G (guanosine)/C (cytosine) content are more stable than sequences having an increased A (adenosine)/U (uracil) content. In respect to the fact that several codons code for one and the same amino acid (so-called degeneration of the genetic code), the most favourable codons for the stability can be determined (so-called alternative codon usage). Depending on the amino acid to be encoded by the RNA, there are various possibilities for modification of the RNA sequence, compared to its wild type sequence. In particular, codons which contain A and/or U nucleotides can be modified by substituting these codons by other codons, which code for the same amino acids but contain no A and/or U or contain a lower content of A and/or U nucleotides.

In various embodiments, the G/C content of the coding region of the RNA described herein is increased by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 55%, or even more compared to the G/C content of the coding region of the wild type RNA. In some embodiments, G/C content of a coding region is increased by about 10% to about 60% (e.g., by about 20% to about 60%, about 30% to about 60%, about 40% to about 60%, about 50% to about 60%, or by about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57%, about 58%, about 59%, or about 60%) compared to the G/C content of the coding region of the wild type RNA.

In some embodiments, RNA disclosed herein comprises a sequence disclosed herein (e.g., SEQ ID NO: 9), that has been modified to encode one or more mutations characteristic of a SARS-CoV-2 variant (e.g., ones described herein including but not limited to a BA.2 or a BA.4/5 Omicron variant). In some embodiments, RNA can be modified to encode one or more mutations characteristic of a SARS-CoV-2 variant by making as few nucleotide changes as possible. In some embodiments, RNA can be modified to encode one or more mutations that are characteristic of a SARS-CoV-2 variant by introducing mutations that result in high codon-optimization and/or increased G/C content.

In some embodiments, one or more mutations characteristic of a SARS-CoV-2 variant are introduced onto a full-length S protein (e.g., an S protein comprising SEQ ID NO: 1). In some embodiments one or more mutations characteristic of a SARS-CoV-2 variant are introduced onto a full-length S protein having one or more proline mutations that increase stability of a prefusion confirmation. For example, in some embodiments, proline substitutions are made at positions corresponding to positions 986 and 987 of SEQ ID NO: 1. In some embodiments, at least 4 proline substitutions are made. In some embodiments, at least four of such proline mutations include mutations at positions corresponding to residues 817, 892, 899, and 942 of SEQ ID NO: 1, e.g., as described in WO 2021243122 A2, the entire contents of which are incorporated herein by reference in its entirety. In some embodiments, such a SARS-CoV-2 S protein comprising proline substitutions at positions corresponding to residues 817, 892, 899, and 942 of SEQ ID NO: 1, may further comprise proline substitutions at positions corresponding to residues 986 and 987 of SEQ ID NO: 1. In some embodiments, one or more

mutations characteristic of a SARS-CoV-2 variant are introduced onto an immunogenic fragment of an S protein (e.g., the RBD of SEQ ID NO: 1).

Embodiments of administered RNAs

In some embodiments, the present disclosure provides an RNA (e.g., mRNA) comprising an open reading frame encoding a polypeptide that comprises at least a portion of a SARS-CoV-2 S protein. The RNA is suitable for intracellular expression of the polypeptide. In some embodiments, such an encoded polypeptide comprises a sequence corresponding to the complete S protein. In some embodiments, such an encoded polypeptide does not comprise a sequence corresponding to the complete S protein. In some embodiments, the encoded polypeptide comprises a sequence that corresponds to the receptor binding domain (RBD). In some embodiments, the encoded polypeptide comprises a sequence that corresponds to the RBD, and further comprises a trimerization domain (e.g., a trimerization domain as disclosed herein, such as a fibritin domain). In some embodiments an RBD comprises a signaling domain (e.g., a signaling domain as disclosed herein). In some embodiments an RBD comprises a transmembrane domain (e.g., a transmembrane domain as disclosed herein). In some embodiments, an RBD comprises a signaling domain and a trimerization domain. In some embodiments, an RBD comprises a signaling domain, a trimerization domain, and transmembrane domain.

In some embodiments, the encoded polypeptide comprises a sequence that corresponds to two receptor binding domains. In some embodiments, the encoded polypeptide comprises a sequence that corresponds to two receptor binding domains in tandem in an amino acid chain, e.g., as disclosed in Dai, Lianpan, et al. "A universal design of betacoronavirus vaccines against COVID-19, MERS, and SARS," Cell 182.3 (2020): 722-733, the contents of which are incorporated by reference herein in their entirety.

In some embodiments, a SARS-CoV-2 S protein, or an immunogenic fragment thereof comprises one or more mutations to alter or remove a glycosylation site, e.g., as described in WO2022221835A2, US20220323574A1, or WO2022195351A1.

In some embodiments, compositions or medical preparations described herein comprise RNA encoding an amino acid sequence comprising SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof. Likewise, methods described herein comprise administration of such RNA.

The active platform for use herein is based on an antigen-coding RNA vaccine to induce robust neutralising antibodies and accompanying/concomitant T cell response to achieve protective immunization with preferably minimal vaccine doses. The RNA administered is preferably *in-vitro* transcribed RNA.

Three different RNA platforms are particularly preferred, namely non-modified uridine containing mRNA (uRNA), nucleoside modified mRNA (modRNA) and self-amplifying RNA (saRNA). In one particularly preferred embodiment, the RNA is in vitro transcribed RNA. In some embodiments, uRNA is mRNA. In some embodiments, modRNA is mRNA.

In the following, embodiments of these three different RNA platforms are described, wherein certain terms used when describing elements thereof have the following meanings:

S1S2 protein/S1S2 RBD: Sequences encoding the respective antigen of SARS-CoV-2.

nsP1, nsP2, nsP3, and nsP4: Wildtype sequences encoding the Venezuelan equine encephalitis virus (VEEV) RNA-dependent RNA polymerase replicase and a subgenomic promotor plus conserved sequence elements supporting replication and translation.

virUTR: Viral untranslated region encoding parts of the subgenomic promotor as well as replication and translation supporting sequence elements.

hAg-Kozak: 5'-UTR sequence of the human alpha-globin mRNA with an optimized 'Kozak sequence' to increase translational efficiency.

Sec: Sec corresponds to a secretory signal peptide (sec), which guides translocation of the nascent polypeptide chain into the endoplasmatic reticulum. In some embodiments, such a secretory signal peptide includes the intrinsic S1S2 secretory signal peptide. In some embodiments, such a secretory signal peptide is a secretory signal peptide from a non-S1S2 protein. For example, an immunoglobulin secretory signal peptide (aa 1-22), an HSV-1 gD signal peptide (MGGAAARLGAVILFVVIVGLHGVRSKY), an HSV-2 gD signal peptide

(MGRLTSGVGTAALLVVAVGLRVVCA); a human SPARC signal peptide, a human insulin isoform 1 signal peptide, a human albumin signal peptide, or any other signal peptide described herein.

Glycine-serine linker (GS): Sequences coding for short linker peptides predominantly consisting of the amino acids glycine (G) and serine (S), as commonly used for fusion proteins.

Fibritin: Partial sequence of T4 fibritin (foldon), used as artificial trimerization domain.

TM: TM sequence corresponds to the transmembrane part of a protein. A transmembrane domain can be N-terminal, C-terminal, or internal to an encoded polypeptide. A coding sequence of a transmembrane element is typically placed in frame (i.e., in the same reading frame), 5', 3', or internal to coding sequences of sequences (e.g., sequences encoding polypeptide(s)) with which it is to be linked. In some embodiments, a transmembrane domain comprises or is a transmembrane domain of Hemagglutinin (HA) of Influenza virus, Env of HIV-1, equine infectious anaemia virus (EIAV), murine leukaemia virus (MLV), mouse mammary tumor virus, G protein of vesicular stomatitis virus (VSV), Rabies virus, or a seven transmembrane domain receptor. In some embodiments, the transmembrane part of a protein is from the S1S2 protein.

FI element: The 3'-UTR is a combination of two sequence elements derived from the "amino terminal enhancer of split" (AES) mRNA (called F) and the mitochondrial encoded 12S ribosomal RNA (called I). These were identified by an *ex vivo* selection process for sequences that confer RNA stability and augment total protein expression.

A30L70: A poly(A)-tail measuring 110 nucleotides in length, consisting of a stretch of 30 adenosine residues, followed by a 10 nucleotide linker sequence and another 70 adenosine residues designed to enhance RNA stability and translational efficiency in dendritic cells.

In some embodiments, vaccine RNA described herein may comprise, from 5' to 3', one of the following structures:

Cap-5'-UTR-Vaccine Antigen-Encoding Sequence-3'-UTR-Poly(A)

or

Cap- hAg-Kozak-Vaccine Antigen-Encoding Sequence-FI-A30L70.

In some embodiments, a vaccine antigen described herein may comprise a full-length S protein or an immunogenic fragment thereof (e.g., RBD). In some embodiments where a vaccine antigen comprises a full-length S protein, its secretory signal peptide and/or transmembrane domain may be replaced by a heterologous secretory signal peptide (e.g., as described herein) and/or a heterologous transmembrane domain (e.g., as described herein).

In some embodiments, a vaccine antigen described herein may comprise, from N-terminus to C-terminus, one of the following structures:

Signal Sequence-RBD-Trimerization Domain

or

Signal Sequence-RBD-Trimerization Domain-Transmembrane Domain.

RBD and Trimerization Domain may be separated by a linker, in particular a GS linker such as a linker having the amino acid sequence GSPGSGSGS. Trimerization Domain and Transmembrane Domain may be separated by a linker, in particular a GS linker such as a linker having the amino acid sequence GSGSGS.

Signal Sequence may be a signal sequence as described herein. RBD may be a RBD domain as described herein. Trimerization Domain may be a trimerization domain as described herein. Transmembrane Domain may be a transmembrane domain as described herein.

In one embodiment,

Signal sequence comprises the amino acid sequence of amino acids 1 to 16 or 1 to 19 of SEQ ID NO: 1 or the amino acid sequence of amino acids 1 to 22 of SEQ ID NO: 31, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to this amino acid sequence,

RBD comprises the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to this amino acid sequence,

Trimerization Domain comprises the amino acid sequence of amino acids 3 to 29 of SEQ ID NO: 10 or the amino acid sequence of SEQ ID NO: 10, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to this amino acid sequence; and

Transmembrane Domain comprises the amino acid sequence of amino acids 1207 to 1254 of SEQ ID NO: 1, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to this amino acid sequence.

In one embodiment,

Signal sequence comprises the amino acid sequence of amino acids 1 to 16 or 1 to 19 of SEQ ID NO: 1 or the amino acid sequence of amino acids 1 to 22 of SEQ ID NO: 31,

RBD comprises the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1,

Trimerization Domain comprises the amino acid sequence of amino acids 3 to 29 of SEQ ID NO: 10 or the amino acid sequence of SEQ ID NO: 10; and

Transmembrane Domain comprises the amino acid sequence of amino acids 1207 to 1254 of SEQ ID NO: 1.

In some embodiments, an RNA polynucleotide comprising a sequence encoding a vaccine antigen (e.g., a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof) or comprising an open reading frame encoding a vaccine antigen (e.g., a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof) such as the nucleotide sequence of SEQ ID NO: 50 or the nucleotide sequence of SEQ ID NO: 53, a variant or fragment thereof, further comprises a 5' cap, e.g., a 5' cap comprising a Cap1 structure, a 5' UTR sequence, e.g., a 5' UTR sequence comprising the nucleotide sequence of SEQ ID NO: 12, a 3' UTR sequence, e.g., a 3' UTR sequence comprising the nucleotide sequence of SEQ ID NO: 13, and polyA sequence, e.g., a polyA sequence comprising the nucleotide sequence of SEQ ID NO: 14. In some embodiments, RNA is formulated in a composition comprising ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate), cholesterol, distearoylphosphatidylcholine, and (2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide).

RNA described herein or RNA encoding the vaccine antigen described herein may be non-modified uridine containing RNA (uRNA), nucleoside modified RNA (modRNA) or self-amplifying RNA (saRNA). In some embodiments, uRNA is mRNA. In some embodiments, modRNA is mRNA. In one embodiment, RNA described herein or RNA encoding the vaccine antigen described herein is nucleoside modified RNA (modRNA).

Variant Specific Vaccines

In some embodiments, RNA disclosed herein encodes an S protein comprising one or more mutations that are characteristic of a SARS-CoV-2 variant. In some embodiments, RNA encodes a SARS-CoV-2 S protein comprising one or more mutations characteristic of an Alpha variant. In some embodiments, RNA encodes a SARS-CoV-2 S protein comprising one or more mutations characteristic of a Beta variant. In some embodiments, RNA encodes a SARS-CoV-2 S protein comprising one or more mutations characteristic of a Delta variant. In some embodiments, RNA encodes a SARS-CoV-2 S protein comprising one or more mutations characteristic of an Omicron variant (e.g., an S protein comprising one or more mutations characteristic of a BA.1, BA.2, or BA.4/5 Omicron variant). In some embodiments, RNA encodes a SARS-CoV-2 S protein comprising one or more mutations characteristic of an BA.1 Omicron variant. In some embodiments, RNA encodes a SARS-CoV-2 S protein comprising one or more mutations characteristic of an BA.2 Omicron variant. In some embodiments, RNA encodes a SARS-CoV-2 S protein comprising one or more mutations characteristic of an BA.2.12.1 Omicron variant. In some embodiments, RNA encodes a SARS-CoV-2 S protein comprising one or more mutations characteristic of a BA.3 Omicron variant. In some embodiments, RNA encodes a SARS-CoV-2 S protein comprising one or more mutations characteristic of a BA.4 or BA.5 Omicron variant.

Non-modified uridine RNA (uRNA)

In some embodiments, a non-modified uridine RNA is a messenger RNA. In some embodiments, the active principle of non-modified messenger RNA drug substance is a single-stranded mRNA that is translated upon entering a cell. In addition to the sequence encoding the coronavirus vaccine antigen (i.e. open reading frame), each uRNA preferably contains common structural elements optimized for maximal efficacy of the RNA with respect to stability and translational efficiency (including, e.g., 5'-cap, 5'-UTR, 3'-UTR, poly(A)-tail as described herein). The preferred 5' cap structure is beta-S-ARCA(D1) ($m_2^{7,2'-O}$GppSpG). The preferred 5'-UTR and 3'-UTR comprise the nucleotide sequence of SEQ ID NO: 12 and the

nucleotide sequence of SEQ ID NO: 13, respectively. The preferred poly(A)-tail comprises the sequence of SEQ ID NO: 14.

Different embodiments of this platform are as follows:

RBL063.1 (SEQ ID NO: 15; SEQ ID NO: 7)

Structure	beta-S-ARCA(D1)-hAg-Kozak-S1S2-PP-FI-A30L70
Encoded antigen	Viral spike protein (S1S2 protein) of the SARS-CoV-2 (S1S2 full-length protein, sequence variant)

RBL063.2 (SEQ ID NO: 16; SEQ ID NO: 7)

Structure	beta-S-ARCA(D1)-hAg-Kozak-S1S2-PP-FI-A30L70
Encoded antigen	Viral spike protein (S1S2 protein) of the SARS-CoV-2 (S1S2 full-length protein, sequence variant)

BNT162a1; RBL063.3 (SEQ ID NO: 17; SEQ ID NO: 5)

Structure	beta-S-ARCA(D1)-hAg-Kozak-RBD-GS-Fibritin-FI-A30L70
Encoded antigen	Viral spike protein (S protein) of the SARS-CoV-2 (partial sequence, Receptor Binding Domain (RBD) of S1S2 protein)

Figure 3 schematizes the general structure of the antigen-encoding RNAs.

Nucleoside modified RNA (modRNA)

In some embodiments, nucleoside modified RNA is mRNA. In some embodiments, the active principle of nucleoside modified RNA (modRNA) drug substance is a single-stranded RNA (e.g., mRNA) that can be translated upon entering a cell. In addition to a sequence encoding a coronavirus vaccine antigen (i.e., open reading frame), each modRNA contains common structural elements optimized for maximal efficacy of the RNA as the uRNA (5'-cap, 5'-UTR, 3'-UTR, poly(A)-tail). Compared to uRNA, modRNA comprises at least one nucleotide modification (e.g., as described herein). In some embodiments, modRNA contains 1-methyl-pseudouridine instead of uridine. The preferred 5' cap structure is $m_2^{7,3'\text{-}O}Gppp(m_1^{2'\text{-}O})ApG$.

The preferred 5'-UTR and 3'-UTR comprise the nucleotide sequence of SEQ ID NO: 12 and the nucleotide sequence of SEQ ID NO: 13, respectively. The preferred poly(A)-tail comprises the sequence of SEQ ID NO: 14. An additional purification step is applied for modRNA to reduce dsRNA contaminants generated during the in vitro transcription reaction.

Different embodiment of this platform are as follows:

BNT162b2; RBP020.1 (SEQ ID NO: 19; SEQ ID NO: 7)

Structure $m_2^{7,3'-O}$Gppp($m_1^{2'-O}$)ApG)-hAg-Kozak-S1S2-PP-FI-A30L70

Encoded antigen Viral spike protein (S1S2 protein) of the SARS-CoV-2 (S1S2 full-length protein, sequence variant)

BNT162b2; RBP020.2 (SEQ ID NO: 20; SEQ ID NO: 7)

Structure $m_2^{7,3'-O}$Gppp($m_1^{2'-O}$)ApG)-hAg-Kozak-S1S2-PP-FI-A30L70

Encoded antigen Viral spike protein (S1S2 protein) of the SARS-CoV-2 (S1S2 full-length protein, sequence variant)

BNT162b1; RBP020.3 (SEQ ID NO: 21; SEQ ID NO: 5)

Structure $m_2^{7,3'-O}$Gppp($m_1^{2'-O}$)ApG)-hAg-Kozak-RBD-GS-Fibritin-FI-A30L70

Encoded antigen Viral spike protein (S1S2 protein) of the SARS-CoV-2 (partial sequence, Receptor Binding Domain (RBD) of S1S2 protein fused to fibritin)

Figure 4 schematizes the general structure of the antigen-encoding RNAs.

BNT162b3c (SEQ ID NO: 29; SEQ ID NO: 30)

Structure $m_2^{7,3'-O}$Gppp($m_1^{2'-O}$)ApG-hAg-Kozak-RBD-GS-Fibritin-GS-TM-FI-A30L70

Encoded antigen Viral spike protein (S1S2 protein) of the SARS-CoV-2 (partial sequence, Receptor Binding Domain (RBD) of S1S2 protein fused to Fibritin fused to Transmembrane Domain (TM) of S1S2 protein); intrinsic S1S2 protein secretory signal peptide (aa 1-19) at the N-terminus of the antigen sequence

BNT162b3d (SEQ ID NO: 31; SEQ ID NO: 32)

Structure $m_2^{7,3'\text{-}O}$Gppp($m_1^{2'\text{-}O}$)ApG-hAg-Kozak-RBD-GS-Fibritin-GS-TM-FI-A30L70

Encoded antigen Viral spike protein (S1S2 protein) of the SARS-CoV-2 (partial sequence, Receptor Binding Domain (RBD) of S1S2 protein fused to Fibritin fused to Transmembrane Domain (TM) of S1S2 protein); immunoglobulin secretory signal peptide (aa 1-22) at the N-terminus of the antigen sequence.

BNT162b2 – Beta variant; RBP020.11 (SEQ ID NO: 57; SEQ ID NO: 55)

Structure $m_2^{7,3'\text{-}O}$Gppp($m_1^{2'\text{-}O}$)ApG)-hAg-Kozak-S1S2-PP-FI-A30L70

Encoded antigen Viral spike protein (S1S2 protein) of the SARS-CoV-2 (S1S2 full-length protein, sequence variant), comprising mutations characteristic of the Beta variant of SARS-CoV-2

BNT162b2 – Alpha variant; RBP020.14 (SEQ ID NO: 60; SEQ ID NO: 58)

Structure $m_2^{7,3'\text{-}O}$Gppp($m_1^{2'\text{-}O}$)ApG)-hAg-Kozak-S1S2-PP-FI-A30L70

Encoded antigen Viral spike protein (S1S2 protein) of the SARS-CoV-2 (S1S2 full-length protein, sequence variant), comprising mutations characteristic of the Alpha variant of SARS-CoV-2

BNT162b2 – Delta variant; RBP020.16 (SEQ ID NO: 63a; SEQ ID NO: 61)

Structure $m_2^{7,3'\text{-}O}$Gppp($m_1^{2'\text{-}O}$)ApG)-hAg-Kozak-S1S2-PP-FI-A30L70

Encoded antigen Viral spike protein (S1S2 protein) of the SARS-CoV-2 (S1S2 full-length protein, sequence variant), comprising mutations characteristic of the Delta variant of SARS-CoV-2

Nucleotide Sequence of RBP020.11 (Beta-specific vaccine)

Nucleotide sequence is shown with individual sequence elements as indicated in bold letters. In addition, the sequence of the translated protein is shown in italic letters below the coding nucleotide sequence (* = stop codon). Red text indicates point mutations in the nucleotide and amino acid sequences.

```
        10         20         30         40         50   53
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                          hAg-Kozak

        63         73         83         93        103        113
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  M  F  V   F  L  V   L  L  P  L   V  S  S   Q  C  V   N  F  T  T
                          S Protein mut

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  R  T  Q   L  P  P   A  Y  T  N   S  F  T   R  G  V   Y  Y  P  D
                          S Protein mut

       183        193        203        213        223        233
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  K  V  F   R  S  S   V  L  H  S   T  Q  D   L  F  L   P  F  F  S
                          S Protein mut

       243        253        263        273        283        293
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                          S Protein mut

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  N  P  V   L  P  F   N  D  G  V   Y  F  A   S  T  E   K  S  N  I
                          S Protein mut

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  I  R  G   W  I  F   G  T  T  L   D  S  K   T  Q  S   L  L  I  V
                          S Protein mut

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  N  N  A   T  N  V   V  I  K  V   C  E  F   Q  F  C   N  D  P  F
                          S Protein mut

       483        493        503        513        523        533
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  L  G  V   Y  Y  H   K  N  N  K   S  W  M   E  S  E   F  R  V  Y
                          S Protein mut

       543        553        563        573        583        593
AGCAGCGCCA ACAACTGCAC CTTCGAGTAC GTGTCCCAGC CTTTCCTGAT GGACCTGGAA
  S  S  A   N  N  C   T  F  E  Y   V  S  Q   P  F  L   M  D  L  E
                          S Protein mut

       603        613        623        633        643        653
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  G  K  Q   G  N  F   K  N  L  R   E  F  V   F  K  N   I  D  G  Y
                          S Protein mut

       663        673        683        693        703        713
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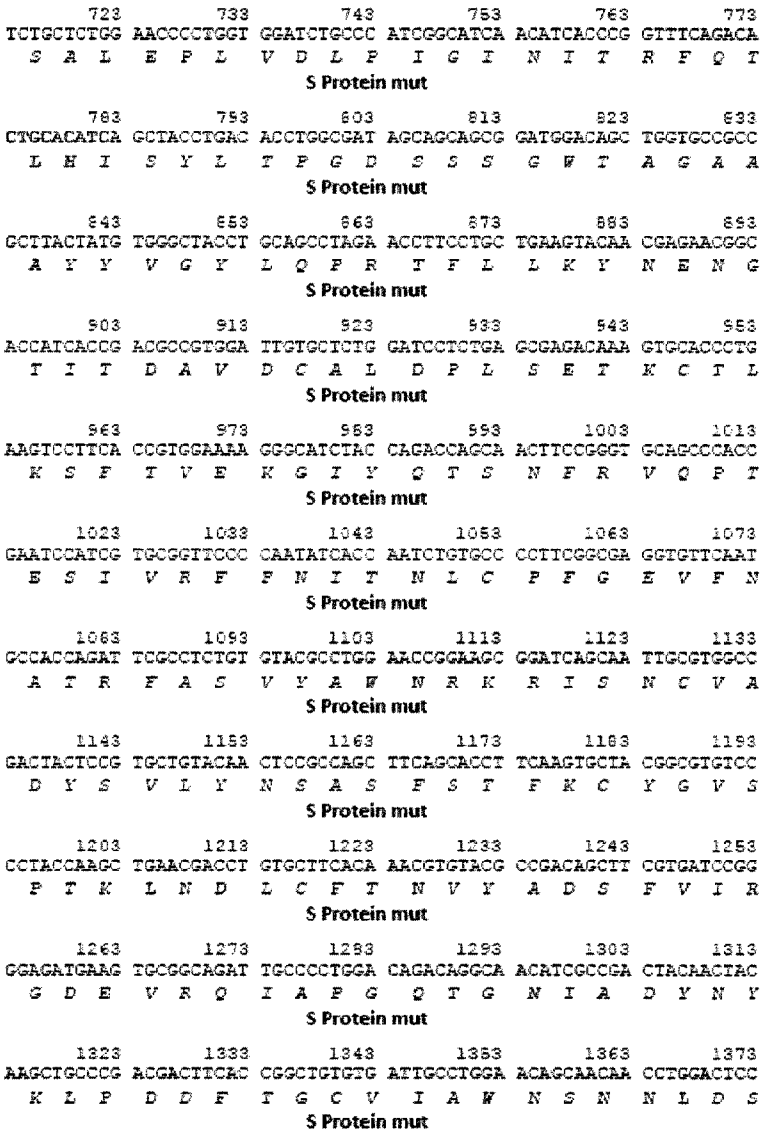
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K L P D D F T G C V I A W N S N N L D S
S Protein mut

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S Protein mut

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```

S Protein mut

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  K  G  F   N  C  Y   F  P  L  Q   S  Y  G   F  Q  P   T  Y  G  V
```

S Protein mut

```
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```

S Protein mut

```
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S Protein mut

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S Protein mut

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S Protein mut

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S Protein mut

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S Protein mut

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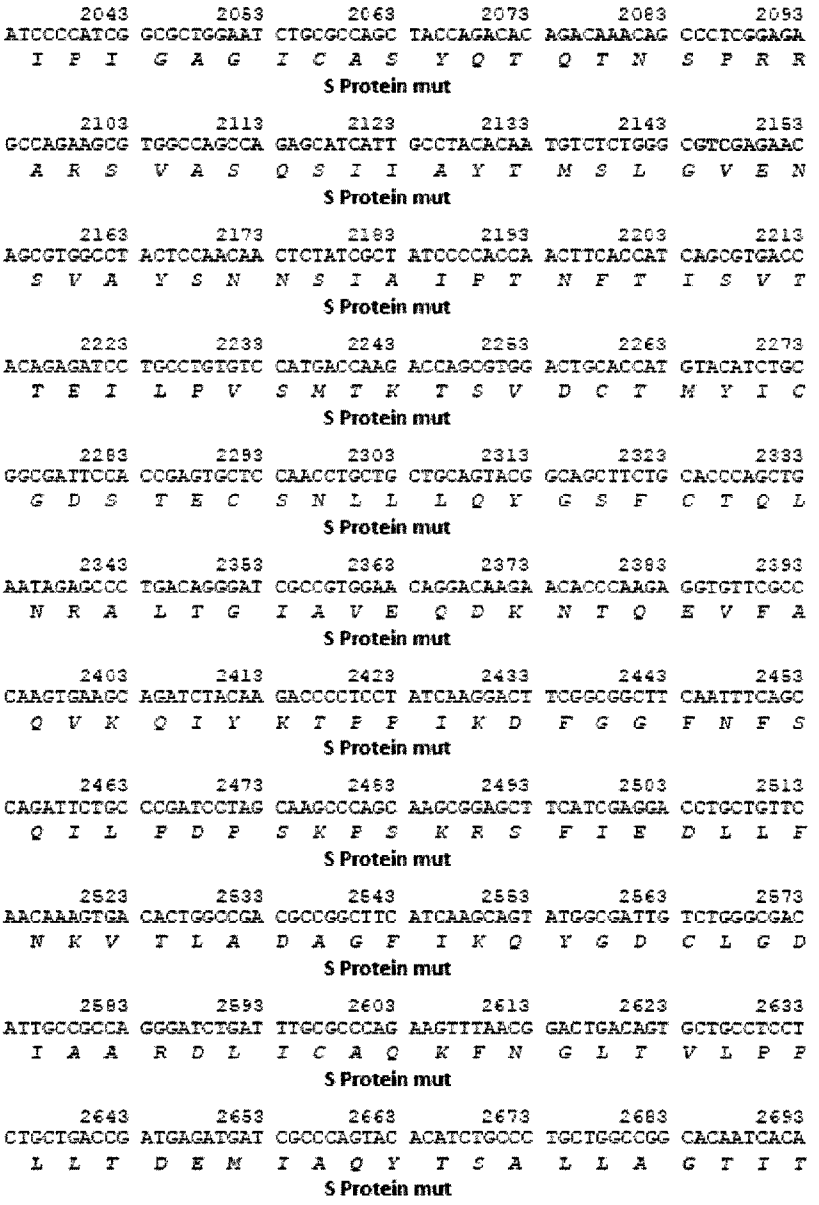
S Protein mut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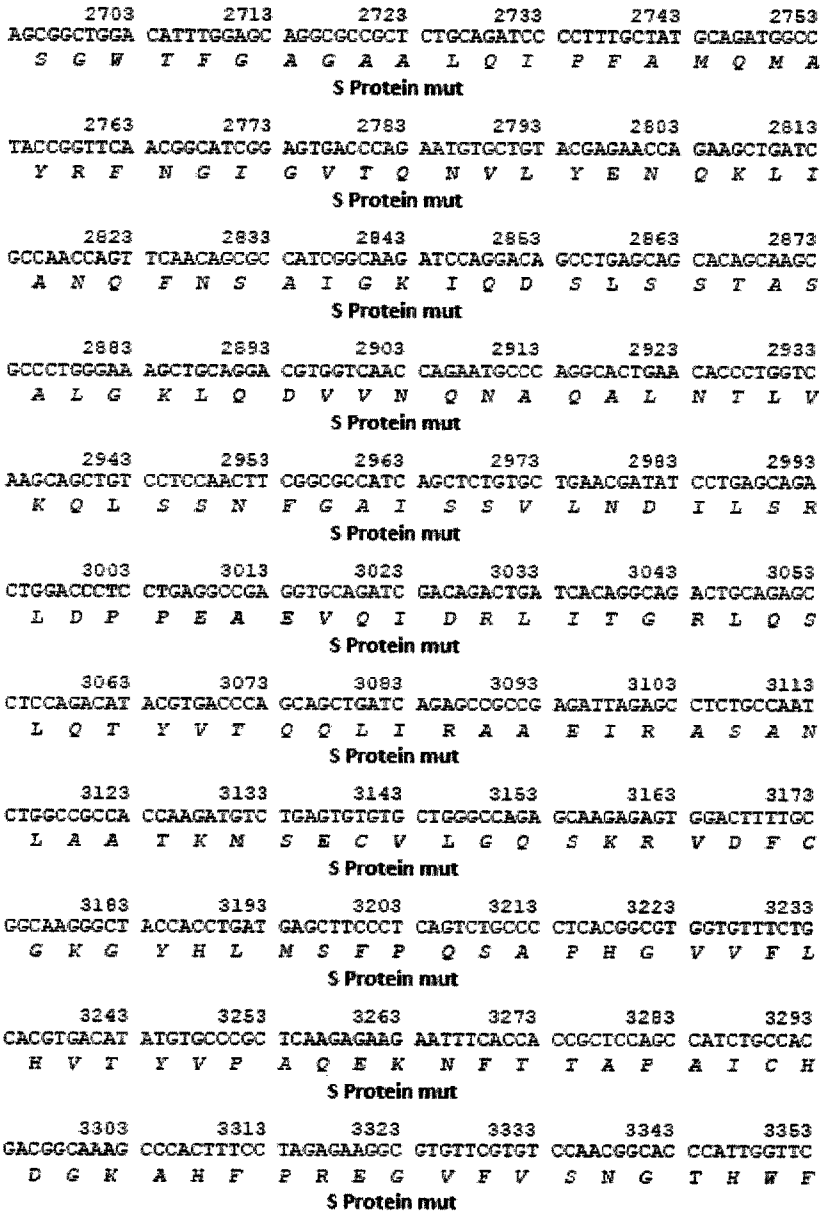
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S Protein mut

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      3363        3373        3383        3393        3403        3413
GTGACACAGC GGAACTTCTA CGAGCCCCAG ATCATCACCA CCGACAACAC CTTCGTGTCT
  V  T  Q   R  N  F   Y  E  P  Q   I  I  T   T  D  N   T  F  V  S
```

S Protein mut

```
      3423        3433        3443        3453        3463        3473
GGCAACTGCG ACGTCGTGAT CGGCATTGTG AACAATACCG TGTACGACCC TCTGCAGCCC
  G  N  C   D  V  V   I  G  I  V   N  N  T   V  Y  D   P  L  Q  P
```

S Protein mut

```
      3483        3493        3503        3513        3523        3533
GAGCTGGACA GCTTCAAAGA GGAACTGGAC AAGTACTTTA AGAACCACAC AAGCCCCGAC
  E  L  D   S  F  K   E  E  L  D   K  Y  F   K  N  H   T  S  P  D
```

S Protein mut

```
      3543        3553        3563        3573        3583        3593
GTGGACCTGG GCGATATCAG CGGAATCAAT GCCAGCGTCG TGAACATCCA GAAAGAGATC
  V  D  L   G  D  I   S  G  I  N   A  S  V   V  N  I   Q  K  E  I
```

S Protein mut

```
      3603        3613        3623        3633        3643        3653
GACCGGCTGA ACGAGGTGGC CAAGAATCTG AACGAGAGCC TGATCGACCT GCAAGAACTG
  D  R  L   N  E  V   A  K  N  L   N  E  S   L  I  D   L  Q  E  L
```

S Protein mut

```
      3663        3673        3683        3693        3703        3713
GGGAAGTACG AGCAGTACAT CAAGTGGCCC TGGTACATCT GGCTGGGCTT TATCGCCGGA
  G  K  Y   E  Q  Y   I  K  W  P   W  Y  I   W  L  G   F  I  A  G
```

S Protein mut

```
      3723        3733        3743        3753        3763        3773
CTGATTGCCA TCGTGATGGT CACAATCATG CTGTGTTGCA TGACCAGCTG CTGTAGCTGC
  L  I  A   I  V  M   V  T  I  M   L  C  C   M  T  S   C  C  S  C
```

S Protein mut

```
      3763        3793        3803        3813        3823        3833
CTGAAGGGCT GTTGTAGCTG TGGCAGCTGC TGCAAGTTCG ACGAGGACGA TTCTGAGCCC
  L  K  G   C  C  S   C  G  S  C   C  K  F   D  E  D   D  S  E  P
```

S Protein mut

```
      3843        3853        3863    3870
GTGCTGAAGG GCGTGAAACT GCACTACACA TGATGAC
  V  L  K   G  V  K   L  H  Y  T   *  *
```

S Protein mut

```
      3880        3890        3900        3910        3920        3930
TCGAGCTGGT ACTGCATGCA CGCAATGCTA GCTGCCCCTT TCCCGTCCTG GGTACCCCGA
```

FI Element

```
      3940        3950        3960        3970        3980        3990
GTCTCCCCCG ACCTCGGGTC CCAGGTATGC TCCCACCTCC ACCTGCCCCA CTCACCACCT
```

FI Element

```
      4000        4010        4020        4030        4040        4050
CTGCTAGTTC CAGACACCTC CCAAGCACGC AGCAATGCAG CTCAAAACGC TTAGCCTAGC
```

FI Element

```
      4060       4070       4080       4090       4100       4110
CACACCCCCA CGGGAAACAG CAGTGATTAA CCTTTAGCAA TAAACGAAAG TTTAACTAAG
                          FI Element

      4120       4130       4140       4150       4160 4164
CTATACTAAC CCCAGGGTTG GTCAATTTCG TGCCAGCCAC ACCCTGGAGC TAGC
                          FI Element

      4174       4184       4194       4204       4214       4224
AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA GCATATGACT AAAAAAAAAA AAAAAAAAAA
                          A30L70

      4234       4244       4254       4264       4274
AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA
                          A30L70
```

Sequences of RBP020.11 are also shown in Table 3.

Table 3: Sequences of RBP020.11 (a Beta-specific RNA vaccine)

SEQ ID NO.	Brief Description	Sequence
55	Amino acid sequence of RNA-encoded SARS-CoV-2 S protein from a Beta variant (with PRO mutations at positions corresponding to K986P and V987P of SEQ ID NO: 1; i.e., PRO mutations at positions 983 and 984 of SEQ ID NO: 55)	MFVFLVLLPLVSSQCVNFTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFH AIHVSGTNGTKRFANPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIKVCE FQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNID GYFKIYSKHTPINLVRGLPQGFSALEPLVDLPIGINITRFQTLHISYLTPGDSSSGWTAGAAAYYV GYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNIT NLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADS FVIRGDEVRQIAPGQTGNIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFE RDISTEIYQAGSTPCNGVKGFNCYFPLQSYGFQPTYGVGYQPYRVVVLSFELLHAPATVCGPKKST NLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSV ITPGTNTSNQVAVLYQGVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSYECD IPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLGVENSVAYSNNSIAIPTNFTISVTTEILPV SMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIK DFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLICAQKFNGLTVL PPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQF NSAIGKIQDSLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDPPEAEVQI DRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHG VVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSGN CDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAK NLNESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCCSCGSCCKFD EDDSEPVLKGVKLHYT**

56	RNA sequence encoding a SARS-CoV-2 S protein from a Beta variant	auguucgugu uccuggugcu gcugccucug guguccagcc aguguugaa cuucaccacc agaacacagc ugccuccagc cuacaccaac agcuuuacca gaggcgugua cuaccccgac aagguguuca gauccagcgu gcugcacucu acccaggacc uguuccugcc uuucuucagc aacgugaccu gguucccacgc cauccacgug uccggcacca auggcaccaa gaguucgcc aaccccgugc ugcccuucaa cgacggggug uacuuugcca gcaccgagaa guccaacauc aucagaggcu ggaucuucgg caccacacug gacagcaaga cccagagccu gcugaucgug aacaacgcca ccaacguggu caucaaagug ugcgaguucc aguucugcaa cgacccuuc cugggcgucu acuaccacaa gaacaacaag agcuggaugg aaagcgaguu ccggguguac agcagcgcca acaacugcac cuucgaguac guguccagc cuuuccugau ggaccuggaa ggcaagcagg gcaacuucaa gaaccugcgc gaguucgugu uuaagaacau cgacggcuac uucaagaucu acagcaagca cacccuauc aaccucgugc ggggccugcc ucagggcuuc ucugcucugg aaccccuggu ggaucugccc aucggcauca acaucacccg guuucagaca cugcacauca gcuaccugac accuggcgau agcagcagcg gauggacagc uggugccgcc gcuuacuaug ugggcuaccu gcagccuaga accuuccugc ugaaguacaa cgagaacggc accaucaccg acgccgugga uugugcucug gauccucuga gcgagacaaa gugcacccug aaguccuuca ccguggaaaa gggcaucuac cagaccagca acuucgggu gcagcccacc gaauccaucg ugcgguuccc caauaucacc aaucugugcc ccuucggcga gguguucaau gccaccagau ucgccucugu guacgccugg aaccggaagc ggaucagcaa uugcgugccc gacuacuccg ugcuguacaa cuccgccagc uucagugcua cggcgugucc ccuaccaagc ugaacgaccu gugcuucaca aacguguacg ccgacagcuu cgugauccgg ggagaugaag ugcggcagau ugccccugga cagacaggca acaucgccga cuacaacuac aagcugcccg acgacuucac cggcugugug auugccugga acagcaacaa ccuggacucc aaagucggcg gcaacuacaa uuaccuguac cggcuguucc ggaaguccaa ucugaagccc uucgagcggg acaucuccac cgagaucuau caggccggca gcacccccuug uaacggcgug aagggcuuca acugcuacuu cccacugcag uccuacggcu uucagcccac auacggcgug ggcuaucagc ccuacagagu gguggugcug agcuucgaac ugcugcaugc cccugccaca gugugcggcc cuaagaaaag caccaaucuc gugaagaaca aaugcgugaa cuucaacuuc aacggccuga ccggcaccgg cgugcugaca gagagcaaca agaaguuccu gccauuccag caguuuggcc gggauaucgc cgauaccaca gacgccguua gagaucccca gacacuggaa aucccuggaca ucacccccuug cagcuucggc ggagugucug ugaucacccc uggcaccaac accagcaauc agguggcagu gcuguaccag ggcugaacu guaccgaagu gcccguggcc auucagccgg aucagcugac accuacaugg cggguguacu ccaccggcag caauguguuu cagaccagag ccggcugucu gaucggagcc gagcaguga acaauagcua cgagugcgac aucccccaucg gcgcuggaau cugcgccagc uaccagacac agacaaacag cccucggaga gccagaagcg uggccagcca gagcaucauu gccuacacaa uguccuuggg cgucgagaac agcguggccu acuccaacaa cucuaucgcu acuccccacca acuucaccau cagcgugacc acagagaucc ugccuguguc caugaccaag accagcgugg acugcaccau guacaucugc ggcgauucca ccgagugcuc caaccugcug cugcaguacg gcagcuucug cacccagcug aauagagccc ugacagggau cgccguggaa caggacaaga acacccaaga gguguucgcc

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56a	DNA sequence encoding a SARS-CoV-2 S protein from a Beta variant	atgttcgtgt tcctggtgct gctgcctctg gtgtccagcc agtgtgtgaa cttcaccacc
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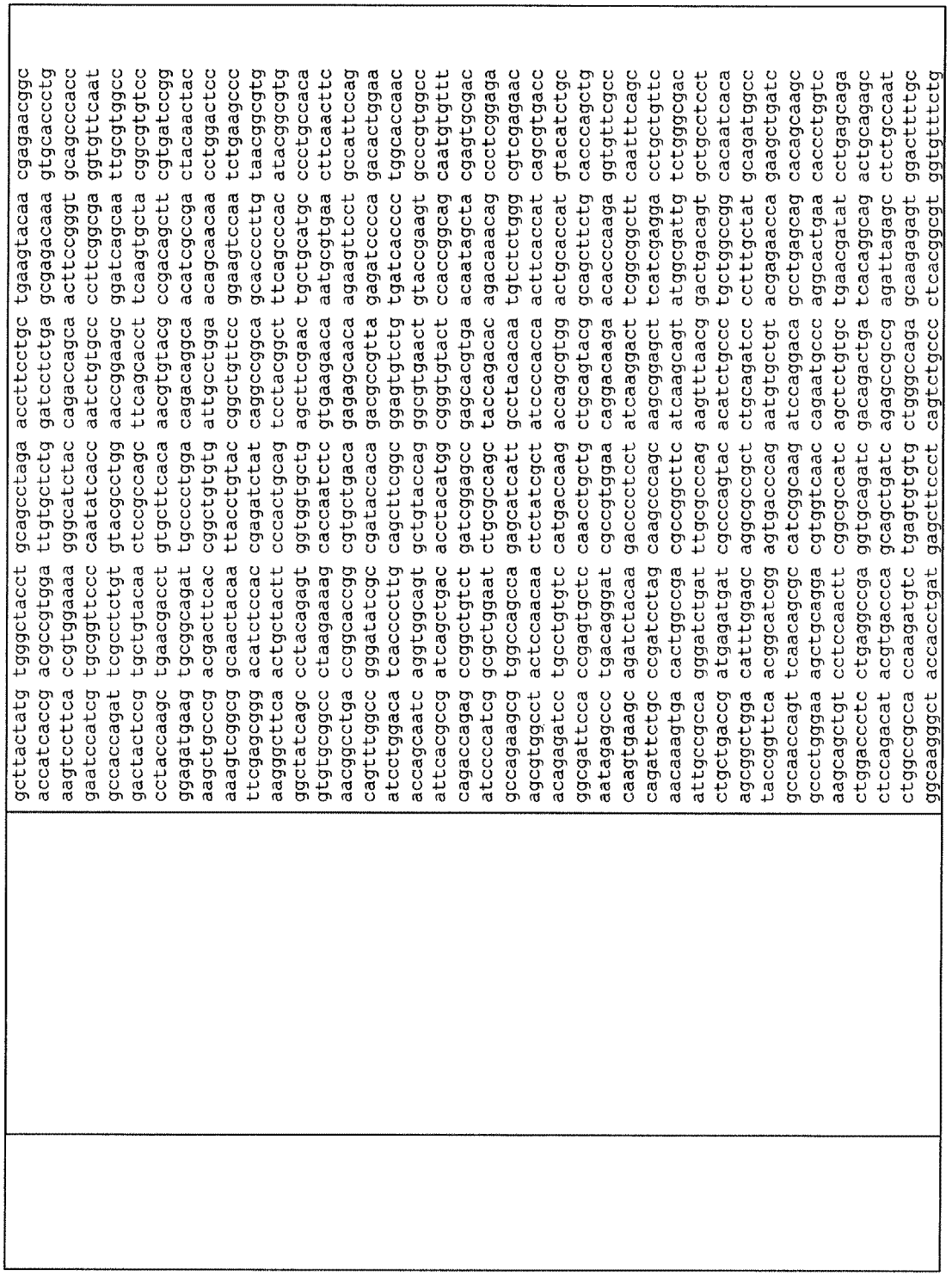

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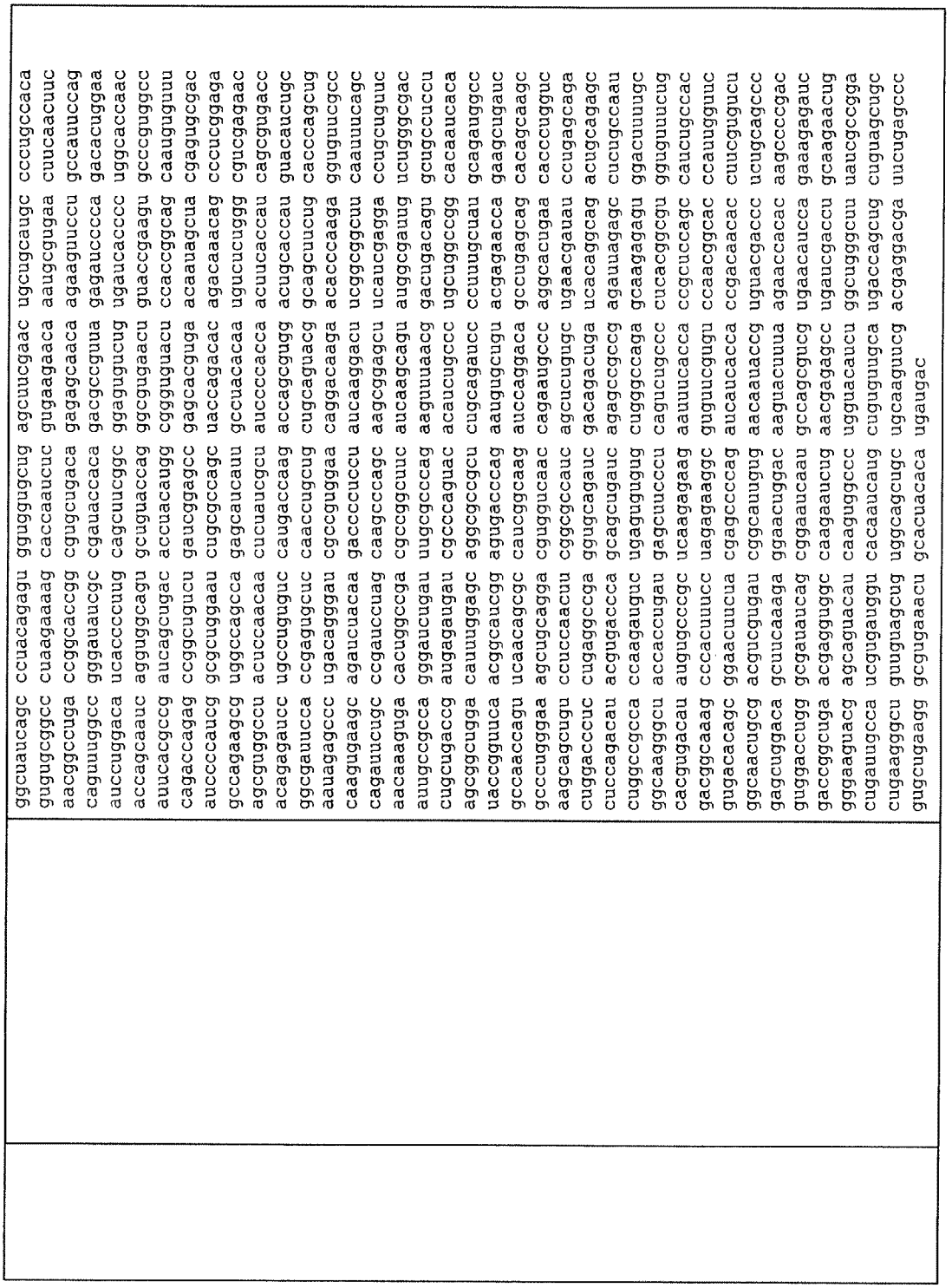
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DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 4
CONTENANT LES PAGES 1 À 219

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 4
CONTAINING PAGES 1 TO 219

NOTE: For additional volumes, please contact the Canadian Patent Office

NOM DU FICHIER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

Claims

1. A composition or medical preparation comprising RNA comprising a nucleotide sequence encoding a SARS-CoV-2 S protein comprising one or more mutations characteristic of an Omicron variant, or an immunogenic fragment thereof.

2. The composition or medical preparation of claim 1, wherein the immunogenic fragment of the SARS-CoV-2 S protein comprises the S1 subunit of the SARS-CoV-2 S protein, or the receptor binding domain (RBD) of the S1 subunit of the SARS-CoV-2 S protein.

3. The composition or medical preparation of claim 1 or 2, wherein the SARS-CoV-2 S protein or immunogenic fragment thereof comprises one or more mutations characteristic of a BA.4/5, BA.1, BA.2, XBB, XBB.1, or BQ.1.1 Omicron variant or sublineages thereof.

4. An immunogenic composition comprising: a lipid nanoparticle (LNP) comprising an RNA, wherein the RNA encodes the polypeptide of SEQ ID NO: 69 and comprises the nucleotide sequence of SEQ ID NO: 70 or a nucleotide sequence that is at least 80% (e.g., at 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 97%, at least 98%, or 99% or higher) identical to SEQ ID NO: 70, and wherein the RNA comprises:

(a) modified uridines; and

(b) a 5' cap; and

wherein the LNP comprises a cationically ionizable lipid, a neutral lipid, a sterol and a polymer-lipid conjugate.

5. The immunogenic composition of claim 4, wherein the nucleotide sequence includes modified uridines in place of all uridines.

6. The immunogenic composition of claim 4 or 5, wherein the modified uridines are each N1-methyl-pseudouridine.

7. The immunogenic composition of any one of claims 4 to 6, wherein the RNA further comprises at least one, at least two, or all of the following features:

a 5' untranslated region (UTR) comprising SEQ ID NO: 12;

a 3' untranslated region (UTR) comprising SEQ ID NO: 13; and

a poly-A sequence of at least 100 A nucleotides.

8. The immunogenic composition of claim 7, wherein the poly-A sequence comprises 30 adenine nucleotides followed by 70 adenine nucleotides, wherein the 30 adenine nucleotides and 70 adenine nucleotides are separated by a linker sequence.

9. The immunogenic composition of claim 7, wherein the poly-A sequence comprises SEQ ID NO: 14.

10. The immunogenic composition of any one of claims 4 to 9, wherein the RNA comprises SEQ ID NO: 72.

11. An immunogenic composition comprising: a lipid nanoparticle (LNP) comprising an RNA, wherein the RNA encodes the polypeptide of SEQ ID NO: 49 and comprises the nucleotide sequence of SEQ ID NO: 50 or a nucleotide sequence that is at least 80% (e.g., at 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 97%, at least 98%, or 99% or higher) identical to SEQ ID NO: 50, and wherein the RNA comprises:

(a) modified uridines; and

(b) a 5' cap; and

wherein the LNP comprises a cationically ionizable lipid, a neutral lipid, a sterol and a polymer-lipid conjugate.

12. The immunogenic composition of claim 11, wherein the nucleotide sequence includes modified uridines in place of all uridines.

13. The immunogenic composition of claim 11 or 12, wherein the modified uridines are each N1-methyl-pseudouridine.

14. The immunogenic composition of any one of claims 11 to 13, wherein the RNA further comprises at least one, at least two, or all of the following features:

a 5' untranslated region (UTR) comprising SEQ ID NO: 12;

a 3' untranslated region (UTR) comprising SEQ ID NO: 13; and

a poly-A sequence of at least 100 A nucleotides.

15. The immunogenic composition of claim 14, wherein the poly-A sequence comprises 30 adenine nucleotides followed by 70 adenine nucleotides, wherein the 30 adenine nucleotides and 70 adenine nucleotides are separated by a linker sequence.

16. The immunogenic composition of claim 15, wherein the poly-A sequence comprises SEQ ID NO: 14.

17. The immunogenic composition of any one of claims 11 to 16, wherein the RNA comprises SEQ ID NO: 51.

18. An immunogenic composition comprising a lipid nanoparticle (LNP) comprising an RNA, wherein the RNA encodes the polypeptide of SEQ ID NO: 55 and comprises the nucleotide sequence of SEQ ID NO: 56 or a nucleotide sequence that is at least 80% (e.g., at 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 97%, at least 98%, or 99% or higher) identical to SEQ ID NO: 56, and wherein the RNA comprises:

(a) modified uridines; and

(b) a 5' cap; and

wherein the LNP comprises a cationically ionizable lipid, a neutral lipid, a sterol and a polymer-lipid conjugate.

19. The immunogenic composition of claim 18, wherein the nucleotide sequence includes modified uridines in place of all uridines.

20. The immunogenic composition of claim 18 or 19, wherein the modified uridines are each N1-methyl-pseudouridine.

21. The immunogenic composition of any one of claims 18 to 20, wherein the RNA further comprises at least one, at least two, or all of the following features:

a 5' untranslated region (UTR) comprising SEQ ID NO: 12; and

a 3' untranslated region (UTR) comprising SEQ ID NO: 13; and

a poly-A sequence of at least 100 A nucleotides.

22. The immunogenic composition of claim 21, wherein the poly-A sequence comprises 30 adenine nucleotides followed by 70 adenine nucleotides, wherein the 30 adenine nucleotides and 70 adenine nucleotides are separated by a linker sequence.

23. The immunogenic composition of claim 22, wherein the poly-A sequence comprises SEQ ID NO: 14.

24. The immunogenic composition of any one of claims 18 to 23, wherein the RNA comprises SEQ ID NO: 57.

25. An immunogenic composition comprising a a lipid nanoparticle (LNP) comprising an RNA, wherein the RNA encodes the polypeptide of SEQ ID NO: 58 and comprises the nucleotide sequence of SEQ ID NO: 59 or a nucleotide sequence that is at least 80% (e.g., at 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 97%, at least 98%, or 99% or higher) identical to SEQ ID NO: 59, and wherein the RNA comprises:

(a) modified uridines; and

(b) a 5' cap; and

wherein the LNP comprises a cationically ionizable lipid, a neutral lipid, a sterol and a polymer-lipid conjugate.

26. The immunogenic composition of claim 25, wherein the nucleotide sequence includes modified uridines in place of all uridines.

27. The immunogenic composition of claim 25 or 26, wherein the modified uridines are each N1-methyl-pseudouridine.

28. The immunogenic composition of any one of claims 25 to 27, wherein the RNA further comprises at least one, at least two, or all of the following features:

a 5' untranslated region (UTR) comprising SEQ ID NO: 12;

a 3' untranslated region (UTR) comprising SEQ ID NO: 13; and

a poly-A sequence of at least 100 A nucleotides.

29. The immunogenic composition of claim 28, wherein the poly-A sequence comprises 30 adenine nucleotides followed by 70 adenine nucleotides, wherein the 30 adenine nucleotides and 70 adenine nucleotides are separated by a linker sequence.

30. The immunogenic composition of claim 29, wherein the poly-A sequence comprises SEQ ID NO: 14.

31. The immunogenic composition of any one of claims 25 to 30, wherein the RNA comprises SEQ ID NO: 60.

32. An immunogenic composition comprising a lipid nanoparticle (LNP) comprising an RNA, wherein the RNA encodes the polypeptide of SEQ ID NO: 61 and comprises the nucleotide sequence of SEQ ID NO: 62a or a nucleotide sequence that is at least 80% (e.g., at 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 97%, at least 98%, or 99% or higher) identical to SEQ ID NO: 62a, and wherein the RNA comprises:

(a) modified uridines; and

(b) a 5' cap; and

wherein the LNP comprises a cationically ionizable lipid, a neutral lipid, a sterol and a polymer-lipid conjugate .

33. The immunogenic composition of claim 32, wherein the nucleotide sequence includes modified uridines in place of all uridines.

34. The immunogenic composition of claim 32 or 33, wherein the modified uridines are each N1-methyl-pseudouridine.

35. The immunogenic composition of any one of claims 32 to 34, wherein the RNA further comprises at least one, at least two, or all of the following features:

a 5' untranslated region (UTR) comprising SEQ ID NO: 12;

a 3' untranslated region (UTR) comprising SEQ ID NO: 13; and

a poly-A sequence of at least 100 A nucleotides.

36. The immunogenic composition of claim 35, wherein the poly-A sequence comprises 30 adenine nucleotides followed by 70 adenine nucleotides, wherein the 30 adenine nucleotides and 70 adenine nucleotides are separated by a linker sequence.

37. The immunogenic composition of claim 36, wherein the poly-A sequence comprises SEQ ID NO: 14.

38. The immunogenic composition of any one of claims 32 to 37, wherein the RNA comprises SEQ ID NO: 63a.

39. An immunogenic composition comprising a first RNA and a second RNA, wherein:

the first RNA encodes the polypeptide of SEQ ID NO: 7 and comprises the nucleotide sequence of SEQ ID NO: 9 or a nucleotide sequence that is at least 80% (e.g., at 85%, at least

90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 97%, at least 98%, or 99% or higher) identical to SEQ ID NO: 9, and

the second RNA encodes the polypeptide of SEQ ID NO: 69 and comprises the nucleotide sequence of SEQ ID NO: 70 or a nucleotide sequence that is at least 80% (e.g., at 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 97%, at least 98%, or 99% or higher) identical to SEQ ID NO: 70, and

wherein each of the first RNA and the second RNA comprise:

(a) modified uridines; and

(b) a 5' cap, and

wherein the first RNA and the second RNA are formulated in lipid nanoparticles (LNPs), wherein the LNPs comprise a cationically ionizable lipid, a neutral lipid, a sterol and a polymer-lipid conjugate.

40. The immunogenic composition of claim 39, wherein the first RNA and the second RNA are formulated in the same lipid nanoparticles.

41. The immunogenic composition of claim 39, wherein the first RNA and the second RNA are formulated in seperate lipid nanoparticles.

42. The immunogenic composition of any one of claims 39 to 41, wherein each of the first RNA and the second RNA include modified uridines in place of all uridines.

43. The immunogenic composition of any one of claims 39 to 42, wherein the modified uridines are each N1-methyl-pseudouridine.

44. The immunogenic composition of any one of claims 39 to 43, wherein the first RNA and the second RNA each indepedently comprise at least one, at least two, or all of the following features:

a 5' untranslated region (UTR) comprising SEQ ID NO: 12;

a 3' untranslated region (UTR) comprising SEQ ID NO: 13; and

a poly-A sequence of at least 100 A nucleotides.

45. The immunogenic composition of claim 44, wherein the poly-A sequence comprises 30 adenine nucleotides followed by 70 adenine nucleotides, wherein the 30 adenine nucleotides and 70 adenine nucleotides are separated by a linker sequence.

46. The immunogenic composition of claim 44, wherein the poly-A sequence comprises SEQ ID NO: 14.

47. An immunogenic composition comprising a first RNA and a second RNA, wherein:

the first RNA encodes the polypeptide of SEQ ID NO: 7 and comprises the nucleotide sequence of SEQ ID NO: 9 or a nucleotide sequence that is at least 80% (e.g., at 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 97%, at least 98%, or 99% or higher) identical to SEQ ID NO: 9, and

the second RNA encodes the polypeptide of SEQ ID NO: 49 and comprises the nucleotide sequence of SEQ ID NO: 50 or a nucleotide sequence that is at least 80% (e.g., at 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 97%, at least 98%, or 99% or higher) identical to SEQ ID NO: 50, and

wherein each of the first RNA and the second RNA comprise:

(a) modified uridines; and

(b) a 5' cap, and

wherein the first RNA and the second RNA are formulated in lipid nanoparticles (LNPs), wherein the LNPs comprise a cationically ionizable lipid, a neutral lipid, a sterol and a polymer-lipid conjugate.

48. The immunogenic composition of claim 47, wherein the first RNA and the second RNA are formulated in the same lipid nanoparticles.

49. The immunogenic composition of claim 47, wherein the first RNA and the second RNA are formulated in seperate lipid nanoparticles.

50. The immunogenic composition of any one of claims 47 to 49, wherein each of the first RNA and the second RNA include modified uridines in place of all uridines.

51. The immunogenic composition of any one of claims 47 to 50, wherein the modified uridines are each N1-methyl-pseudouridine.

52. The immunogenic composition of any one of claims 47 to 51, wherein the first RNA and the second RNA each indepedently comprise at least one, at least two, or all of the following features:

a 5' untranslated region (UTR) comprising SEQ ID NO: 12;

a 3' untranslated region (UTR) comprising SEQ ID NO: 13; and

a poly-A sequence of at least 100 A nucleotides.

53. The immunogenic composition of claim 52, wherein the poly-A sequence comprises 30 adenine nucleotides followed by 70 adenine nucleotides, wherein the 30 adenine nucleotides and 70 adenine nucleotides are separated by a linker sequence.

54. The immunogenic composition of claim 52, wherein the poly-A sequence comprises SEQ ID NO: 14.

55. The immunogenic composition of any one of claims 47 to 54, wherein the first RNA comprises SEQ ID NO: 20 and the second RNA comprises SEQ ID NO: 51.

56. An immunogenic composition comprising a first RNA and a second RNA, wherein:

the first RNA comprises a nucleotide sequence that encodes the polypeptide of SEQ ID NO: 7 and comprises the nucleotide sequence of SEQ ID NO: 9 or a nucleotide sequence that is at least 80% (e.g., at 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 97%, at least 98%, or 99% or higher) identical to SEQ ID NO: 9, and

the second RNA comprises a nucleotide sequence that encodes the polypeptide of SEQ ID NO: 55, 58, or 61 and comprises the nucleotide sequence of SEQ ID NO: 56, 59, or 62a, or a nucleotide sequence that is at least 80% (e.g., at 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 97%, at least 98%, or 99% or higher) identical to SEQ ID NO: 56, 59, or 62a, and

wherein each of the first RNA and the second RNA comprise:

(a) modified uridines; and

(b) a 5' cap, and

wherein the first RNA and the second RNA are formulated in lipid nanoparticles (LNPs), wherein the LNPs comprise a cationically ionizable lipid, a neutral lipid, a sterol and a polymer-lipid conjugate.

57. The immunogenic composition of claim 56, wherein the first RNA and the second RNA are formulated in separate lipid nanoparticles.

58. The immunogenic composition of claim 56, wherein the first RNA and the second RNA are formulated in the same lipid nanoparticles.

59. The immunogenic composition of any one of claims 56 to 58, wherein the first RNA and the second RNA each include modified uridines in place of all uridines.

60. The immunogenic composition of any one of claims 56 to 59, wherein the modified uridines are each N1-methyl-pseudouridine.

61. The immunogenic composition of any one of claims 56 to 60, wherein the first RNA and the second RNA each independently further comprise at least one, at least two, or all of the following features:

a 5' untranslated region (UTR) comprising SEQ ID NO: 12;

a 3' untranslated region (UTR) comprising SEQ ID NO: 13; and

a poly-A sequence of at least 100 A nucleotides.

62. The immunogenic composition of claim 61, wherein the poly-A sequence comprises 30 adenine nucleotides followed by 70 adenine nucleotides, wherein the 30 adenine nucleotides and 70 adenine nucleotides are separated by a linker sequence.

63. The immunogenic composition of claim 62, wherein the poly-A sequence comprises SEQ ID NO: 14.

64. The immunogenic composition of any one of claims 56 to 63, wherein the first RNA comprises SEQ ID NO: 9 and the second RNA comprises SEQ ID NO: 56.

65. The immunogenic composition of any one of claims 56 to 63, wherein the first RNA comprises SEQ ID NO: 9 and the second RNA comprises SEQ ID NO: 59.

66. The immunogenic composition of any one of claims 56 to 63, wherein the first RNA comprises SEQ ID NO: 9 and the second RNA comprises SEQ ID NO: 62a.

67. The immunogenic composition of any one of claims 56 to 63, wherein the first RNA comprises SEQ ID NO: 20 and the second RNA comprises SEQ ID NO: 57.

68. The immunogenic composition of any one of claims 56 to 63, wherein the first RNA comprises SEQ ID NO: 20 and the second RNA comprises SEQ ID NO: 60.

69. The immunogenic composition of any one of claims 56 to 63, wherein the first RNA comprises SEQ ID NO: 20 and the second RNA comprises SEQ ID NO: 63a.

70. An immunogenic composition comprising a first RNA and a second RNA, wherein:

the first RNA encodes the polypeptide of SEQ ID NO: 58 and comprises the nucleotide sequence of SEQ ID NO: 59 or a nucleotide sequence that is at least 80% (e.g., at 85%, at least

90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 97%, at least 98%, or 99% or higher) identical to SEQ ID NO: 59, and

the second RNA encodes the polypeptide of SEQ ID NO: 49, 55, or 61 and comprises the nucleotide sequence of SEQ ID NO: 50, 56, or 62a, or a nucleotide sequence that is at least 80% (e.g., at 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 97%, at least 98%, or 99% or higher) identical to SEQ ID NO: 50, 56, or 62a, and

wherein each of the first RNA and the second RNA comprise:

(a) modified uridines; and

(b) a 5' cap, and

wherein the first RNA and the second RNA are formulated in lipid nanoparticles (LNPs), wherein the LNPs comprise a cationically ionizable lipid, a neutral lipid, a sterol and a polymer-lipid conjugate.

71. The immunogenic composition of claim 70, wherein the first RNA and the second RNA are formulated in separate lipid nanoparticles.

72. The immunogenic composition of claim 70, wherein the first RNA and the second RNA are formulated in the same lipid nanoparticles.

73. The immunogenic composition of any one of claims 70 to 72, wherein the first RNA and the second RNA each include modified uridines in place of all uridines.

74. The immunogenic composition of any one of claims 70 to 73, wherein the modified uridines are each N1-methyl-pseudouridine.

75. The immunogenic composition of any one of claims 70 to 74, wherein the first RNA and the second RNA each independently further comprise at least one, at least two, or all of the following features:

a 5' untranslated region (UTR) comprising SEQ ID NO: 12;

a 3' untranslated region (UTR) comprising SEQ ID NO: 13; and

a poly-A sequence of at least 100 A nucleotides.

76. The immunogenic composition of claim 75, wherein the poly-A sequence comprises 30 adenine nucleotides followed by 70 adenine nucleotides, wherein the 30 adenine nucleotides and 70 adenine nucleotides are separated by a linker sequence.

77. The immunogenic composition of claim 76, wherein the poly-A sequence comprises SEQ ID NO: 14.

78. The immunogenic composition of any one of claims 70 to 77, wherein the first RNA comprises SEQ ID NO: 59 and the second RNA comprises SEQ ID NO: 50.

79. The immunogenic composition of any one of claims 70 to 77, wherein the first RNA comprises SEQ ID NO: 59 and the second RNA comprises SEQ ID NO: 56.

80. The immunogenic composition of any one of claims 70 to 77, wherein the first RNA comprises SEQ ID NO: 59 and the second RNA comprises SEQ ID NO: 62a.

81. The immunogenic composition of any one of claims 70 to 77, wherein the first RNA comprises SEQ ID NO: 60 and the second RNA comprises SEQ ID NO: 51.

82. The immunogenic composition of any one of claims 70 to 77, wherein the first RNA comprises SEQ ID NO: 60 and the second RNA comprises SEQ ID NO: 57.

83. The immunogenic composition of any one of claims 70 to 77, wherein the first RNA comprises SEQ ID NO: 60 and the second RNA comprises SEQ ID NO: 63a.

84. An immunogenic composition comprising a first RNA and a second RNA, wherein:

the first RNA encodes the polypeptide of SEQ ID NO: 49 and comprises the nucleotide sequence of SEQ ID NO: 50 or a nucleotide sequence that is at least 80% (e.g., at 85%, at least

90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 97%, at least 98%, or 99% or higher) identical to SEQ ID NO: 50, and

the second RNA encodes the polypeptide of SEQ ID NO: 55 or 61 and comprises the nucleotide sequence of SEQ ID NO: 56 or 62a, or a nucleotide sequence that is at least 80% (e.g., at 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 97%, at least 98%, or 99% or higher) identical to SEQ ID NO: 56 or 62a, and

wherein each of the first RNA and the second RNA comprise:

(a) modified uridines; and

(b) a 5' cap, and

wherein the first RNA and the second RNA are formulated in lipid nanoparticles (LNPs), wherein the LNPs comprise a cationically ionizable lipid, a neutral lipid, a sterol and a polymer-lipid conjugate.

85. The immunogenic composition of claim 84, wherein the first RNA and the second RNA are formulated in separate lipid nanoparticles.

86. The immunogenic composition of claim 84, wherein the first RNA and the second RNA are formulated in the same lipid nanoparticles.

87. The immunogenic composition of any one of claims 84 to 86, wherein the first RNA and the second RNA each include modified uridines in place of all uridines.

88. The immunogenic composition of any one of claims 84 to 87, wherein the modified uridines are each N1-methyl-pseudouridine.

89. The immunogenic composition of any one of claims 84 to 88, wherein the first RNA and the second RNA further each independently further comprise at least one, at least two, or all of the following features:

a 5' untranslated region (UTR) comprising SEQ ID NO: 12;

a 3' untranslated region (UTR) comprising SEQ ID NO: 13; and

a poly-A sequence of at least 100 A nucleotides.

90. The immunogenic composition of claim 89, wherein the poly-A sequence comprises 30 adenine nucleotides followed by 70 adenine nucleotides, wherein the 30 adenine nucleotides and 70 adenine nucleotides are separated by a linker sequence.

91. The immunogenic composition of claim 90, wherein the poly-A sequence comprises SEQ ID NO: 14.

92. The immunogenic composition of any one of claims 84 to 91, wherein the first RNA comprises SEQ ID NO: 50 and the second RNA comprises SEQ ID NO: 56.

93. The immunogenic composition of any one of claims 84 to 91, wherein the first RNA comprises SEQ ID NO: 50 and the second RNA comprises SEQ ID NO: 62a.

94. The immunogenic composition of any one of claims 84 to 91, wherein the first RNA comprises SEQ ID NO: 51 and the second RNA comprises SEQ ID NO: 57.

95. The immunogenic composition of any one of claims 84 to 91, wherein the first RNA comprises SEQ ID NO: 51 and the second RNA comprises SEQ ID NO: 63a.

96. An immunogenic composition comprising a first RNA and a second RNA, wherein:

the first RNA encodes the polypeptide of SEQ ID NO: 55 and comprises the nucleotide sequence of SEQ ID NO: 56 or a nucleotide sequence that is at least 80% (e.g., at 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 97%, at least 98%, or 99% or higher) identical to SEQ ID NO: 56, and

the second RNA encodes the polypeptide of SEQ ID NO: 61 and comprises the nucleotide sequence of SEQ ID NO: 62a, or a nucleotide sequence that is at least 80% (e.g., at 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 97%, at least 98%, or 99% or higher) identical to SEQ ID NO: 62a, and

wherein each of the first RNA and the second RNA comprise:

(a) modified uridines; and

(b) a 5' cap, and

wherein the first RNA and the second RNA are formulated in lipid nanoparticles (LNPs), wherein the LNPs comprise a cationically ionizable lipid, a neutral lipid, a sterol and a polymer-lipid conjugate.

97. The immunogenic composition of claim 96, wherein the first RNA and the second RNA are formulated in separate lipid nanoparticles.

98. The immunogenic composition of claim 96, wherein the first RNA and the second RNA are formulated in the same lipid nanoparticles.

99. The immunogenic composition of any one of claims 96 to 98, wherein the first RNA and the second RNA each include modified uridines in place of all uridines.

100. The immunogenic composition of any one of claims 96 to 99, wherein the modified uridines are each N1-methyl-pseudouridine.

101. The immunogenic composition of any one of claims 96 to 100, wherein the first RNA and the second RNA each independently further comprise at least one, at least two, or all of the following features:

a 5' untranslated region (UTR) comprising SEQ ID NO: 12;

a 3' untranslated region (UTR) comprising SEQ ID NO: 13; and

a poly-A sequence of at least 100 A nucleotides.

102. The immunogenic composition of claim 101, wherein the poly-A sequence comprises 30 adenine nucleotides followed by 70 adenine nucleotides, wherein the 30 adenine nucleotides and 70 adenine nucleotides are separated by a linker sequence.

103. The immunogenic composition of claim 102, wherein the poly-A sequence comprises SEQ ID NO: 14.

104. The immunogenic composition of any one of claims 96 to 103, wherein the first RNA comprises SEQ ID NO: 57 and the second RNA comprises SEQ ID NO: 63a.

105. The immunogenic composition of any one of claims 4 to 104, wherein the 5'-cap is or comprises $m_2^{7,3'-O}$Gppp($m_1^{2'-O}$)ApG.

106. The immunogenic composition of any one of claims 4 to 105, wherein the LNP comprises about 40 to about 50 mole percent of a cationically ionizable lipid, about 35 to about 45 mole percent of a sterol, about 5 to about 15 mole percent of a neutral lipid, and about 1 to about 10 mole percent of a polymer-lipid conjugate.

107. The immunogenic composition of any one of claims 4 to 106, wherein the composition comprises a plurality of LNPs, wherein the average diameter of the plurality of LNPs is about 30 nm to about 200 nm or about 60 nm to about 120 nm (e.g., as determined by dynamic light scattering measurements).

108. A method of eliciting an immune response against SARS-CoV-2 comprising administering the immunogenic composition of any one of claims 4 to 107.

109. The method of claim 108, wherein the immune response is elicited against an Omicron variant of SARS-CoV-2.

110. The method of claim 108, wherein the immune response is elicited against a Beta variant of SARS-CoV-2.

111. The method of claim 108, wherein the immune response is elicited against an Alpha variant of SARS-CoV-2.

112. The method of claim 108, wherein the immune response is elicited against a Delta variant of SARS-CoV-2.

113. The method of claim 108, wherein the immune response is elicited against a Wuhan strain, an Omicron variant, a Beta variant, an Alpha variant, and a Delta variant of SARS-CoV-2.

114. A method for inducing an immune response in a subject, wherein the method comprises delivering (e.g., as a polypeptide or an RNA encoding such a polypeptide) an antigen of a SARS-CoV-2 virus that is not a BA.1 Omicron variant of SARS-CoV-2.

115. The method of claim 114, wherein the subject has previously been infected with or vaccinated against SARS-CoV-2.

116. The method of claim 114 or 115, wherein the subject has previously been delivered (e.g., as a polypeptide or an RNA encoding such a polypeptide) an antigen of a Wuhan strain of SARS-CoV-2.

117. The method of any one of claims 114 to 116, wherein the subject has previously been administered RNA encoding a SARS-CoV-2 S protein of a Wuhan strain.

118. The method of any one of claims 114 to 117, wherein the subject has previously been administered two or more doses of RNA encoding a SARS-CoV-2 S protein of a Wuhan strain.

119. The method of any one of claims 114 to 118, wherein the method comprises administering RNA encoding an antigen of a SARS-CoV-2 virus that is not a BA.1 Omicron variant.

120. The method of any one of claims 114 to 119, wherein the method comprises administering RNA encoding a SARS-CoV-2 S protein from a SARS-CoV-2 variant that is a BA.1 Omicron variant.

121. The method of any one of claims 114 to 120, wherein the method comprises administering RNA encoding an S protein of an Omicron variant of SARS-CoV-2, wherein the Omicron variant is not a BA.1 Omicron variant.

122. The method of any one of claims 114 to 121, wherein the method comprises administering RNA encoding an S protein of a BA.2 Omicron variant of SARS-CoV-2.

123. The method of any one of claims 114 to 121, wherein the method comprises administering RNA encoding an S protein of a BA.4 or BA.5 Omicron variant of SARS-CoV-2.

124. A method for inducing an immune response in a subject, wherein the method comprises administering (a) a first RNA encoding a SARS-CoV-2 S protein of a Wuhan strain, an Alpha variant, a Beta variant, or a Delta variant, and (b) a second RNA encoding a SARS-CoV-2 S protein of an Omicron variant that is not a BA.1 Omicron variant.

125. The method of claim 124, where the second RNA encodes an S protein of a BA.2 Omicron variant.

126. The method of claim 124, where the second RNA encodes an S protein of a BA.4 or BA.5 Omicron variant.

127. A method for inducing an immune response in a subject, wherein the method comprises administering (a) a first RNA encoding a SARS-CoV-2 S protein of a Wuhan strain, an Alpha variant, a Beta variant, a Delta variant, or a BA.1 Omicron variant and (b) a second RNA encoding a SARS-CoV-2 S protein that is antigenically distinct from the S protein encoded by the first RNA.

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128. The method of claim 127, wherein the second RNA encodes a SARS-CoV-2 S protein of an Omicron variant that is not a BA.1 Omicron variant.

129. The method of claim 127, wherein the second RNA encodes a SARS-CoV-2 S protein of a BA.2 Omicron variant.

130. The method of claim 127, wherein the second RNA encodes a SARS-CoV-2 S protein of a BA.4 or BA.5 Omicron variant.

131. The method of any one of claims 114 to 130, wherein the first RNA and the second RNA are encapsulated in separate LNPs.

132. The method of any one of claims 114 to 130, wherein the first RNA and the second RNA are encapsulated in the same LNP.

133. The method of any one of claims 114 to 131, wherein the first RNA and the second RNA are administered separately, e.g., at different injection sites.

134. A composition comprising (a) a first RNA encoding a SARS-CoV-2 S protein of a Wuhan strain, an Alpha variant, a Beta variant, a Delta variant, or a BA.1 Omicron variant and (b) a second RNA encoding a SARS-CoV-2 S protein that is antigenically distinct from the S protein encoded by the first RNA.

135. A composition comprising (a) a first RNA encoding a SARS-CoV-2 S protein of a Wuhan strain, an Alpha variant, a Beta variant, a Delta variant, or a BA.1 Omicron variant and (b) a second RNA encoding a SARS-CoV-2 S protein an Omicron variant that is not a BA.1 Omicron variant.

136. The composition of claim 135, wherein the second RNA encodes a SARS-CoV-2 S protein of a BA.2 Omicron variant.

137. The composition of claim 135, wherein the second RNA encodes a a SARS-CoV-2 S protein of a BA.4 or BA.5 Omicron variant.

138. The composition of claim 135, wherein the first RNA encodes a SARS-CoV-2 S protein of a Wuhan strain and the second RNA encodes a SARS-CoV-2 S protein of a BA.2 Omicron variant.

139. The composition of claim 135, wherein the first RNA encodes a SARS-CoV-2 S protein of a Wuhan strain and the second RNA encodes a SARS-CoV-2 S protein of a BA.4 or BA.5 Omicron variant.

140. The composition of claim 135, wherein the first RNA encodes a SARS-CoV-2 S protein of a BA.1 Omicron variant and the second RNA encodes a SARS-CoV-2 S protein of a BA.2 Omicron variant.

141. The composition of claim 135, wherein the first RNA encodes a SARS-CoV-2 S protein of a BA.1 Omicron variant and the second RNA encodes a SARS-CoV-2 S protein of a BA.4 or BA.5 Omicron variant.

142. The composition of any one of claims 134 to 141, wherein the first RNA and the second RNA are administered in encapsulated in the same LNP.

143. The composition of any one of claims 134 to 141, wherein the first RNA and the second RNA are encapsulated in separate LNPs.

144. A composition or medical preparation comprising RNA encoding an amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof.

145. The composition or medical preparation of claim 144, wherein an immunogenic fragment of the SARS-CoV-2 S protein comprises the S1 subunit of the SARS-CoV-2 S protein, or the receptor binding domain (RBD) of the S1 subunit of the SARS-CoV-2 S protein.

146. The composition or medical preparation of claims 144 or 145, wherein the amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof is encoded by a coding sequence which is codon-optimized and/or the G/C content of which is increased compared to wild type coding sequence, wherein the codon-optimization and/or the increase in the G/C content preferably does not change the sequence of the encoded amino acid sequence.

147. The composition or medical preparation of any one of claims 144 to 146, wherein

(i) the RNA encoding a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the nucleotide sequence of nucleotides 979 to 1584 of SEQ ID NO: 2, 8 or 9, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 979 to 1584 of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of nucleotides 979 to 1584 of SEQ ID NO: 2, 8 or 9, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 979 to 1584 of SEQ ID NO: 2, 8 or 9; and/or

(ii) a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1, or an immunogenic fragment of the amino acid sequence of

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amino acids 327 to 528 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1.

148. The composition or medical preparation of any one of claims 144 to 146, wherein

(i) the RNA encoding a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the nucleotide sequence of nucleotides 111 to 986 of SEQ ID NO: 30, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 111 to 986 of SEQ ID NO: 30, or a fragment of the nucleotide sequence of nucleotides 111 to 986 of SEQ ID NO: 30, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 111 to 986 of SEQ ID NO: 30; and/or

(ii) a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the amino acid sequence of amino acids 20 to 311 of SEQ ID NO: 29, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 20 to 311 of SEQ ID NO: 29, or an immunogenic fragment of the amino acid sequence of amino acids 20 to 311 of SEQ ID NO: 29, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 20 to 311 of SEQ ID NO: 29..

149. The composition or medical preparation of any one of claims 144 to 146, wherein

(i) the RNA encoding a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the nucleotide sequence of nucleotides 49 to 3819 of SEQ ID NO: 2, 8 or 9, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 49 to 3819 of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of nucleotides 49 to 3819 of SEQ ID NO: 2, 8 or 9, or the nucleotide

sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 49 to 3819 of SEQ ID NO: 2, 8 or 9; and/or

(ii) a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7, or an immunogenic fragment of the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7.

150. The composition or medical preparation of any one of claims 144 to 149, wherein the amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises a secretory signal peptide.

151. The composition or medical preparation of claim 150, wherein the secretory signal peptide is fused, preferably N-terminally, to a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof.

152. The composition or medical preparation of claim 150 or 151, wherein

(i) the RNA encoding the secretory signal peptide comprises the nucleotide sequence of nucleotides 1 to 48 of SEQ ID NO: 2, 8 or 9, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 1 to 48 of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of nucleotides 1 to 48 of SEQ ID NO: 2, 8 or 9, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 1 to 48 of SEQ ID NO: 2, 8 or 9; and/or

(ii) the secretory signal peptide comprises the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1, or a functional fragment of the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1.

153. The composition or medical preparation of any one of claims 144 to 152, wherein

(i) the RNA encoding a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the nucleotide sequence of SEQ ID NO: 6, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 6, or a fragment of the nucleotide sequence of SEQ ID NO: 6, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 6; and/or

(ii) a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the amino acid sequence of SEQ ID NO: 5, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 5, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 5, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 5.

154. The composition or medical preparation of any one of claims 144 to 153, wherein

(i) the RNA encoding a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the nucleotide sequence of nucleotides 54 to 986 of SEQ ID NO: 30, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 54 to 986 of SEQ ID NO: 30, or a fragment of the nucleotide sequence of nucleotides 54 to 986 of SEQ ID NO: 30, or the nucleotide sequence having at

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least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 54 to 986 of SEQ ID NO: 30; and/or

(ii) a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the amino acid sequence of amino acids 1 to 311 of SEQ ID NO: 29, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 311 of SEQ ID NO: 29, or an immunogenic fragment of the amino acid sequence of amino acids 1 to 311 of SEQ ID NO: 29, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 311 of SEQ ID NO: 29.

155. The composition or medical preparation of any one of claims 144 to 154, wherein the RNA comprises a modified nucleoside in place of uridine, in particular wherein the modified nucleoside is selected from pseudouridine (ψ), N1-methyl-pseudouridine (m1ψ), and 5-methyl-uridine (m5U), in particular wherein the modified nucleoside is N1-methyl-pseudouridine (m1ψ).

156. The composition or medical preparation of any one of claims 144 to 155, wherein the RNA comprises a 5' cap.

157. The composition or medical preparation of any one of claims 144 to 156, wherein the RNA encoding an amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises a 5' UTR comprising the nucleotide sequence of SEQ ID NO: 12, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 12.

158. The composition or medical preparation of any one of claims 144 to 157, wherein the RNA encoding an amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic

variant thereof comprises a 3' UTR comprising the nucleotide sequence of SEQ ID NO: 13, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 13.

159. The composition or medical preparation of any one of claims 144 to 158, wherein the RNA encoding an amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises a poly-A sequence.

160. The composition or medical preparation of claim 159, wherein the poly-A sequence comprises at least 100 nucleotides.

161. The composition or medical preparation of claim 159 or 160, wherein the poly-A sequence comprises or consists of the nucleotide sequence of SEQ ID NO: 14.

162. The composition or medical preparation of any one of claims 144 to 161, wherein the RNA is formulated or is to be formulated as a liquid, a solid, or a combination thereof.

163. The composition or medical preparation of any one of claims 144 to 162, wherein the RNA is formulated or is to be formulated for injection.

164. The composition or medical preparation of any one of claims 144 to 163, wherein the RNA is formulated or is to be formulated for intramuscular administration.

165. The composition or medical preparation of any one of claims 144 to 164, wherein the RNA is formulated or is to be formulated as particles.

166. The composition or medical preparation of claim 165, wherein the particles are lipid nanoparticles (LNP) or lipoplex (LPX) particles.

167. The composition or medical preparation of claim 166, wherein the LNP particles comprise a cationically ionizable lipid, a neutral lipid, a sterol and a polymer-lipid conjugate.

168. The composition or medical preparation of claim 167, wherein the RNA lipoplex particles are obtainable by mixing the RNA with liposomes.

169. The composition or medical preparation of any one of claims 144 to 168, wherein the RNA is mRNA or saRNA.

170. The composition or medical preparation of any one of claims 144 to 169, which is a pharmaceutical composition.

171. The composition or medical preparation of any one of claims 144 to 170, which is a vaccine.

172. The composition or medical preparation of claim 170 or 171, wherein the pharmaceutical composition further comprises one or more pharmaceutically acceptable carriers, diluents and/or excipients.

173. The composition or medical preparation of any one of claims 144 to 172, which is a kit.

174. The composition or medical preparation of claim 173, wherein the RNA and optionally the particle forming components are in separate vials.

175. The composition or medical preparation of claim 173 or 174, further comprising instructions for use of the composition or medical preparation for inducing an immune response against coronavirus in a subject.

176. The composition or medical preparation of any one of claims 144 to 175 for pharmaceutical use.

177. The composition or medical preparation of claim 176, wherein the pharmaceutical use comprises inducing an immune response against coronavirus in a subject.

178. The composition or medical preparation of claim 176 or 177, wherein the pharmaceutical use comprises a therapeutic or prophylactic treatment of a coronavirus infection.

179. The composition or medical preparation of any one of claims 144 to 178, which is for administration to a human.

180. The composition or medical preparation of any one of claims 175 to 179, wherein the coronavirus is a betacoronavirus.

181. The composition or medical preparation of any one of claims 175 to 180, wherein the coronavirus is a sarbecovirus.

182. The composition or medical preparation of any one of claims 175 to 181, wherein the coronavirus is SARS-CoV-2.

183. A method of inducing an immune response against coronavirus in a subject comprising administering to the subject a composition comprising RNA encoding an amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof.

184. The method of claim 183, wherein an immunogenic fragment of the SARS-CoV-2 S protein comprises the S1 subunit of the SARS-CoV-2 S protein, or the receptor binding domain (RBD) of the S1 subunit of the SARS-CoV-2 S protein.

185. The method of any one of claims 183 or 184, wherein the amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic

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fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof is encoded by a coding sequence which is codon-optimized and/or the G/C content of which is increased compared to wild type coding sequence, wherein the codon-optimization and/or the increase in the G/C content preferably does not change the sequence of the encoded amino acid sequence.

186. The method of any one of claims 183 to 185, wherein

(i) the RNA encoding a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the nucleotide sequence of nucleotides 979 to 1584 of SEQ ID NO: 2, 8 or 9, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 979 to 1584 of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of nucleotides 979 to 1584 of SEQ ID NO: 2, 8 or 9, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 979 to 1584 of SEQ ID NO: 2, 8 or 9; and/or

(ii) a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1, or an immunogenic fragment of the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1.

187. The method of any one of claims 183 to 186, wherein

(i) the RNA encoding a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the nucleotide sequence of nucleotides 111 to 986 of SEQ ID NO: 30, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 111 to 986 of SEQ ID NO: 30, or a fragment of the

nucleotide sequence of nucleotides 111 to 986 of SEQ ID NO: 30, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 111 to 986 of SEQ ID NO: 30; and/or

(ii) a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the amino acid sequence of amino acids 20 to 311 of SEQ ID NO: 29, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 20 to 311 of SEQ ID NO: 29, or an immunogenic fragment of the amino acid sequence of amino acids 20 to 311 of SEQ ID NO: 29, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 20 to 311 of SEQ ID NO: 29.

188. The method of any one of claims 183 to 187, wherein

(i) the RNA encoding a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the nucleotide sequence of nucleotides 49 to 3819 of SEQ ID NO: 2, 8 or 9, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 49 to 3819 of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of nucleotides 49 to 3819 of SEQ ID NO: 2, 8 or 9, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 49 to 3819 of SEQ ID NO: 2, 8 or 9; and/or

(ii) a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7, or an immunogenic fragment of the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7.

189. The method of any one of claims 183 to 188, wherein the amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises a secretory signal peptide.

190. The method of claim 189, wherein the secretory signal peptide is fused, preferably N-terminally, to a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof.

191. The method of claim 189 or 190, wherein

(i) the RNA encoding the secretory signal peptide comprises the nucleotide sequence of nucleotides 1 to 48 of SEQ ID NO: 2, 8 or 9 175 a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 1 to 48 of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of nucleotides 1 to 48 of SEQ ID NO: 2, 8 or 9, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 1 to 48 of SEQ ID NO: 2, 8 or 9; and/or

(ii) the secretory signal peptide comprises the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1, or a functional fragment of the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1.

192. The method of any one of claims 183 to 191, wherein

(i) the RNA encoding a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the nucleotide sequence of SEQ ID NO: 6, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 6, or a fragment of the nucleotide sequence of SEQ ID NO: 6, or the nucleotide sequence

having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 6; and/or

(ii) a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the amino acid sequence of SEQ ID NO: 5, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 5, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 5, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 5.

193. The method of any one of claims 183 to 192, wherein

(i) the RNA encoding a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the nucleotide sequence of nucleotides 54 to 986 of SEQ ID NO: 30, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 54 to 986 of SEQ ID NO: 30, or a fragment of the nucleotide sequence of nucleotides 54 to 986 of SEQ ID NO: 30, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 54 to 986 of SEQ ID NO: 30; and/or

(ii) a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the amino acid sequence of amino acids 1 to 311 of SEQ ID NO: 29, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 311 of SEQ ID NO: 29, or an immunogenic fragment of the amino acid sequence of amino acids 1 to 311 of SEQ ID NO: 29, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 311 of SEQ ID NO: 29.

194. The method of any one of claims 183 to 192, wherein the RNA comprises a modified nucleoside in place of uridine, in particular wherein the modified nucleoside is selected from

pseudouridine (ψ), N1-methyl-pseudouridine (m1ψ), and 5-methyl-uridine (m5U), in particular wherein the modified nucleoside is N1-methyl-pseudouridine (m1ψ).

195. The method of any one of claims 183 to 194, wherein the RNA comprises a cap.

196. The method of any one of claims 183 to 195, wherein the RNA encoding an amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises a 5' UTR comprising the nucleotide sequence of SEQ ID NO: 12, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 12.

197. The method of any one of claims 183 to 196, wherein the RNA encoding an amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises a 3' UTR comprising the nucleotide sequence of SEQ ID NO: 13, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 13.

198. The method of any one of claims 183 to 197, wherein the RNA encoding an amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises a poly-A sequence.

199. The method of claim 198, wherein the poly-A sequence comprises at least 100 nucleotides.

200. The method of claim 198 or 199, wherein the poly-A sequence comprises or consists of the nucleotide sequence of SEQ ID NO: 14.

201. The method of any one of claims 183 to 200, wherein the RNA is formulated as a liquid, a solid, or a combination thereof.

202. The method of any one of claims 183 to 201, wherein the RNA is administered by injection.

203. The method of any one of claims 183 to 202, wherein the RNA is administered by intramuscular administration.

204. The method of any one of claims 183 to 203, wherein the RNA is formulated as particles.

205. The method of claim 204, wherein the particles are lipid nanoparticles (LNP) or lipoplex (LPX) particles.

206. The method of claim 205, wherein the LNP particles comprise a cationically ionizable lipid, a neutral lipid, a sterol and a polymer-lipid conjugate.

207. The method of any one of claim 205, wherein the RNA lipoplex particles are obtainable by mixing the RNA with liposomes.

208. The composition or medical preparation of any one of claims 183 to 207, wherein the RNA is mRNA or saRNA.

209. The method of any one of claims 183 to 208, which is a method for vaccination against coronavirus.

210. The method of any one of claims 183 to 209, which is a method for therapeutic or prophylactic treatment of a coronavirus infection.

211. The method of any one of claims 183 to 210, wherein the subject is a human.

212. The method of any one of claims 183 to 211, wherein the coronavirus is a betacoronavirus.

213. The method of any one of claims 183 to 212, wherein the coronavirus is a sarbecovirus.

214. The method of any one of claims 183 to 213, wherein the coronavirus is SARS-CoV-2.

215. The method of any one of claims 108 to 133 or 183 to 214, wherein the composition is a composition of any one of claims 1 to 107 or 134 to 182.

216. A composition or medical preparation of any one of claims 1 to 107 or 134 to 182 for use in a method of any one of claims 108 to 133 or 183 to 215.

217. The composition or medical preparation of any one of claims 1 to 107 or 134 to 182, which is a pharmaceutical composition.

218. The composition or medical preparation of any one of claims 1 to 107 or 134 to 182, which is a vaccine.

219. The composition or medical preparation of claim 217 or 218, wherein the pharmaceutical composition or vaccine further comprises one or more pharmaceutically acceptable carriers, diluents and/or excipients.

220. The composition or medical preparation of any one of claim 1 to 107 or 134 to 182, which is a kit.

221. The composition or medical preparation of claim 220, wherein the RNA and optionally the particle forming components are in separate vials.

222. The composition or medical preparation of claim 220 or 221, further comprising instructions for use of the composition or medical preparation for inducing an immune response against coronavirus in a subject.

223. The composition or medical preparation of any one of claims 1 to 107 or 134 to 182 for pharmaceutical use.

224. The composition or medical preparation of claim 223, wherein the pharmaceutical use comprises inducing an immune response against coronavirus in a subject.

225. The composition or medical preparation of claim 223 or 224, wherein the pharmaceutical use comprises therapeutic or prophylactic treatment of a coronavirus infection.

226. The composition or medical preparation of any one of claims 1 to 107 or 134 to 182 for use in the manufacture of a medicament.

227. The composition or medical preparation of claim 226, wherein the medicament is for use in inducing an immune response against coronavirus in a subject.

228. The composition or medical preparation of claim 226 or 227, wherein the medicament is for use in therapeutic or prophylactic treatment of a coronavirus infection.

229. The composition or medical preparation of any one of claims 1 to 107 or 134 to 182, which is for administration to a human.

230. The composition or medical preparation of any one of claims 222 to 229, wherein the coronavirus is a betacoronavirus.

231. The composition or medical preparation of claim 230, wherein the coronavirus is a sarbecovirus.

232. The composition or medical preparation of claim 231, wherein the coronavirus is SARS-CoV-2.

5

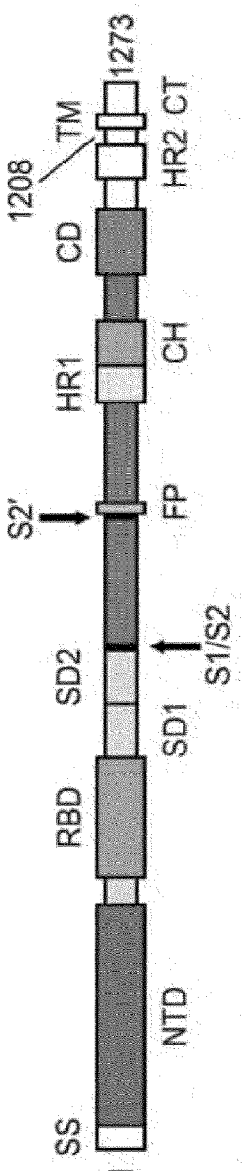


Figure 1

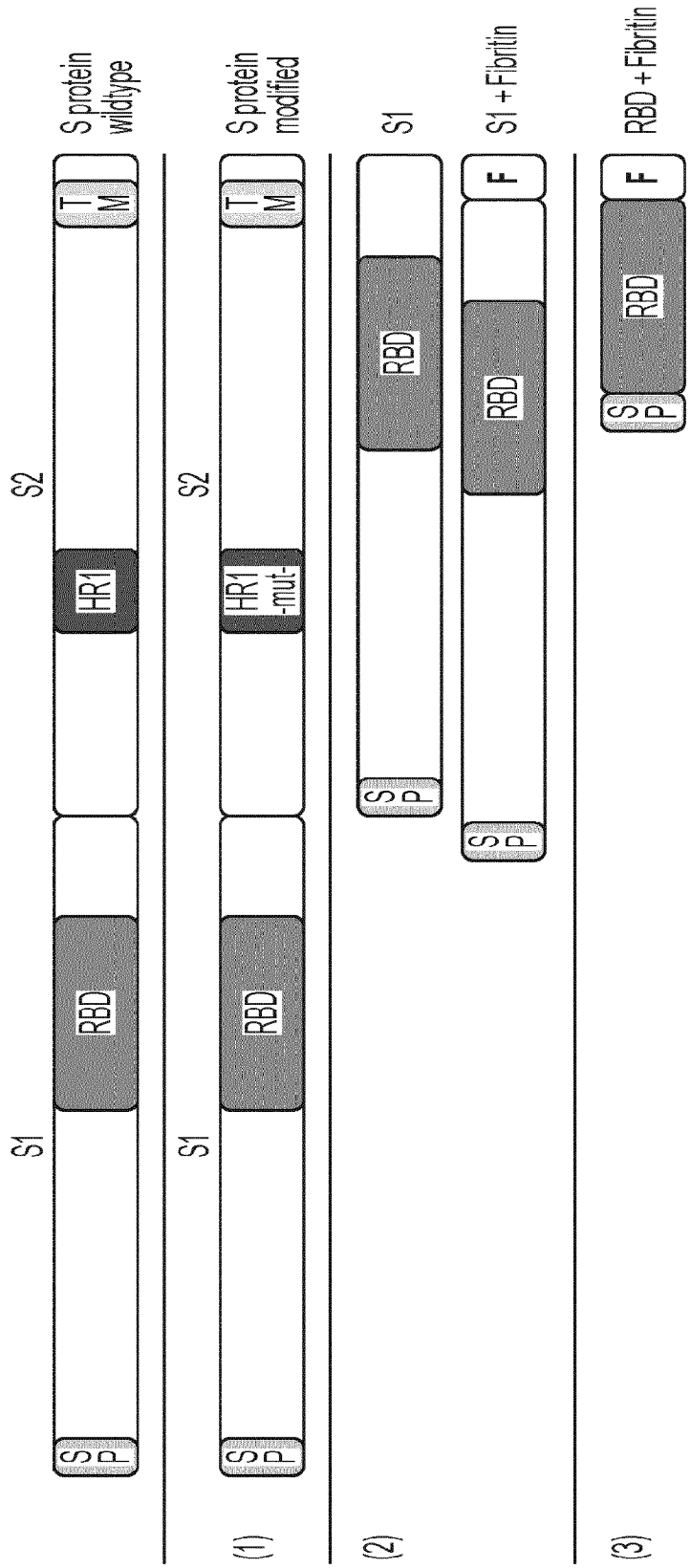

S protein wildtype
S protein modified
S1
S1 + Fibritin
RBD + Fibritin
S2
S1
RBD
S
P
HR1
HR1 -mut-
T
M
F
(1)
(2)
(3)

Figure 2

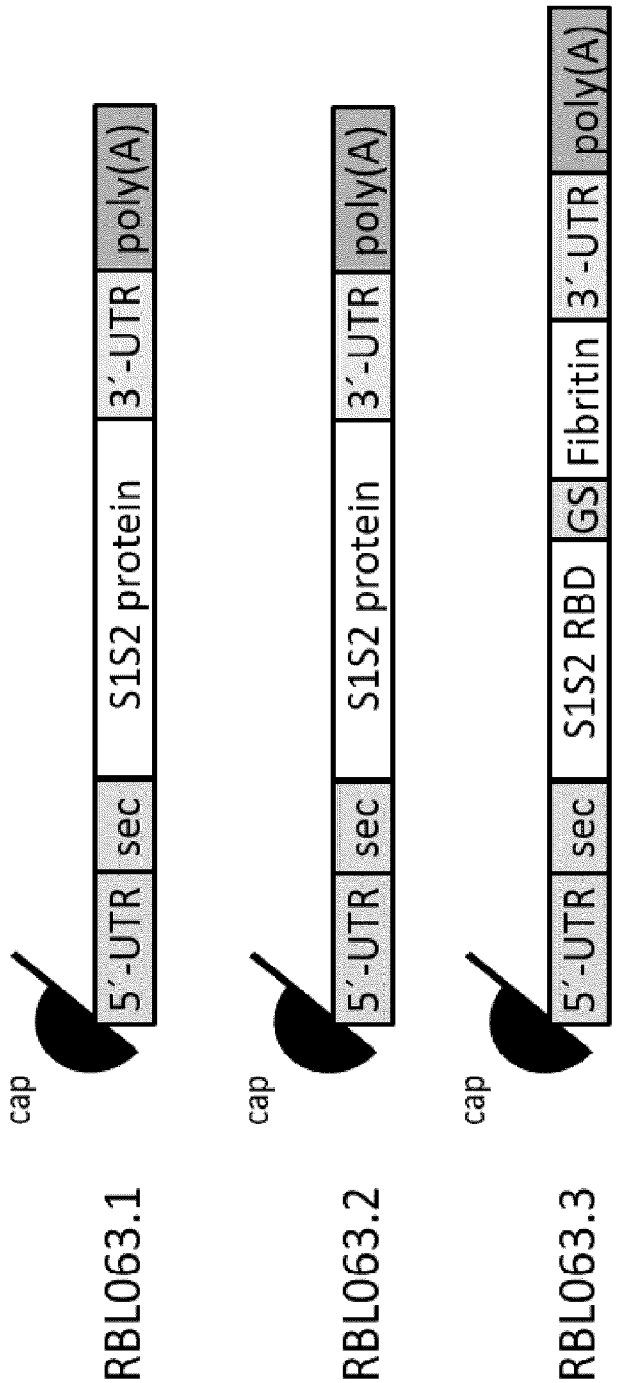

cap
5´-UTR
sec
S1S2 protein
3´-UTR
poly(A)
RBL063.1
cap
5´-UTR
sec
S1S2 protein
3´-UTR
poly(A)
RBL063.2
cap
5´-UTR
sec
S1S2 RBD
GS
Fibritin
3´-UTR
poly(A)
RBL063.3

Figure 3

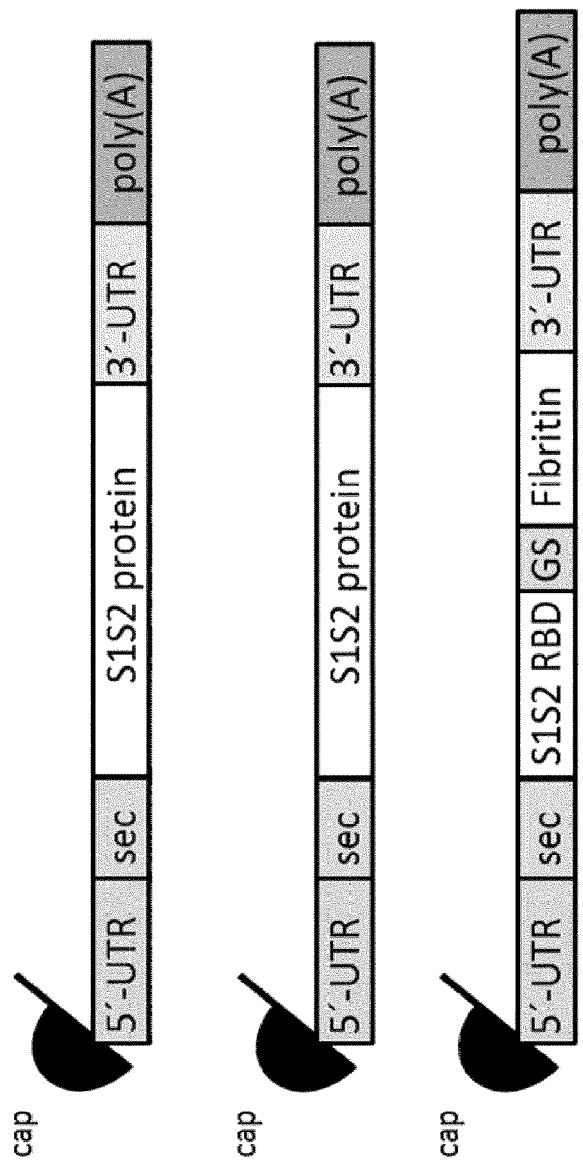

cap
5´-UTR
sec
S1S2 protein
3´-UTR
poly(A)
RBP020.1
cap
5´-UTR
sec
S1S2 protein
3´-UTR
poly(A)
RBP020.2
cap
5´-UTR
sec
S1S2 RBD
GS
Fibritin
3´-UTR
poly(A)
RBP020.3

Figure 4

Figure 5

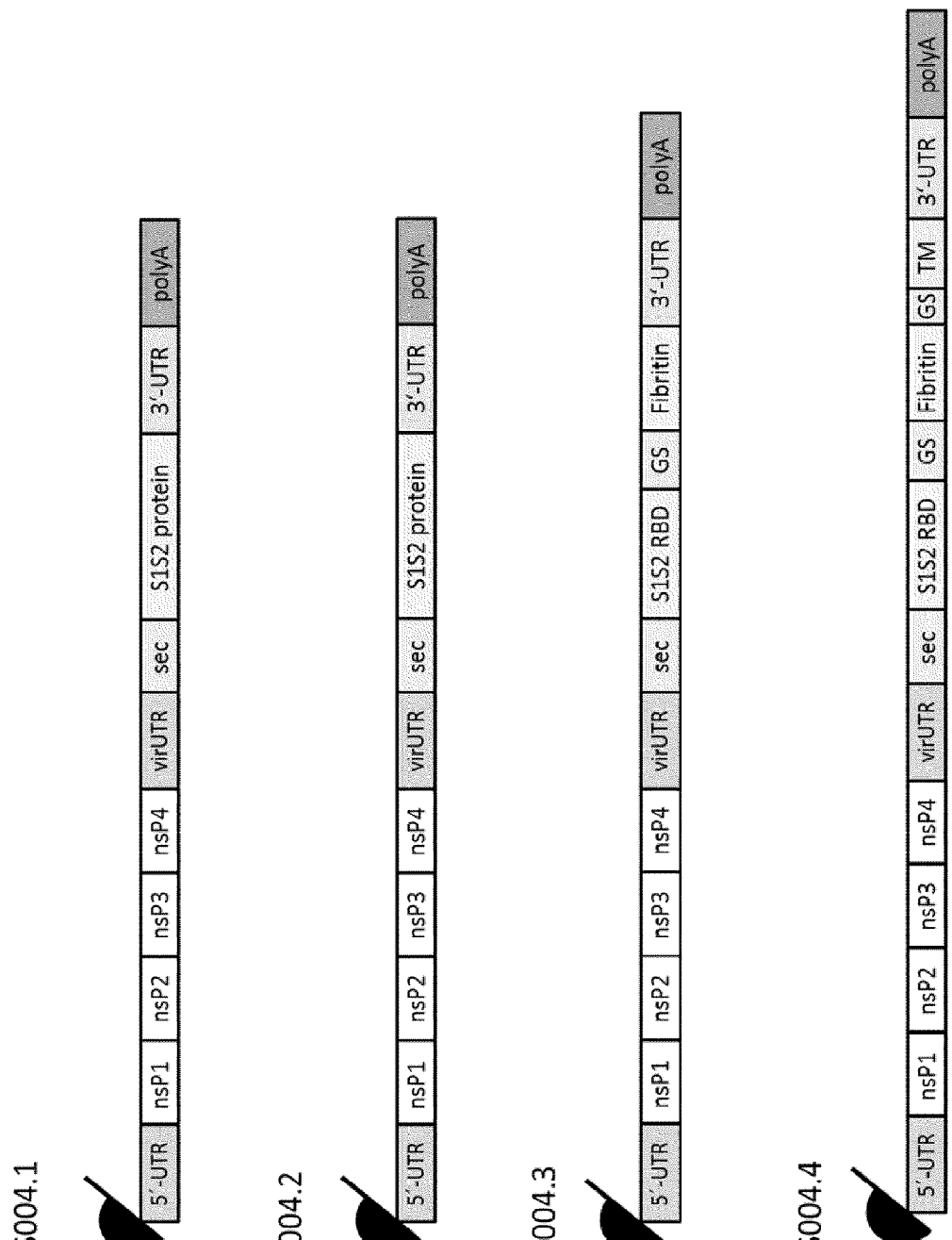

RBS004.1
cap
5'-UTR
nsP1
nsP2
nsP3
nsP4
virUTR
sec
S1S2 protein
3'-UTR
polyA
RBS004.2
cap
5'-UTR
nsP1
nsP2
nsP3
nsP4
virUTR
sec
S1S2 protein
3'-UTR
polyA
RBS004.3
cap
5'-UTR
nsP1
nsP2
nsP3
nsP4
virUTR
sec
S1S2 RBD
GS
Fibritin
3'-UTR
polyA
RBS004.4
cap
5'-UTR
nsP1
nsP2
nsP3
nsP4
virUTR
sec
S1S2 RBD
GS
Fibritin
GS
TM
3'-UTR
polyA

Figure 6

S protein wildtype
S1
S2
sec
RBD
TM
(1)
Membrane-anchored RBD+Fibritin domain with viral signal peptid
sec
RBD
F
TM
(2)
Membrane-anchored RBD+Fibritin domain with human Ig signal peptid
huSec
RBD
F
TM

Figure 7

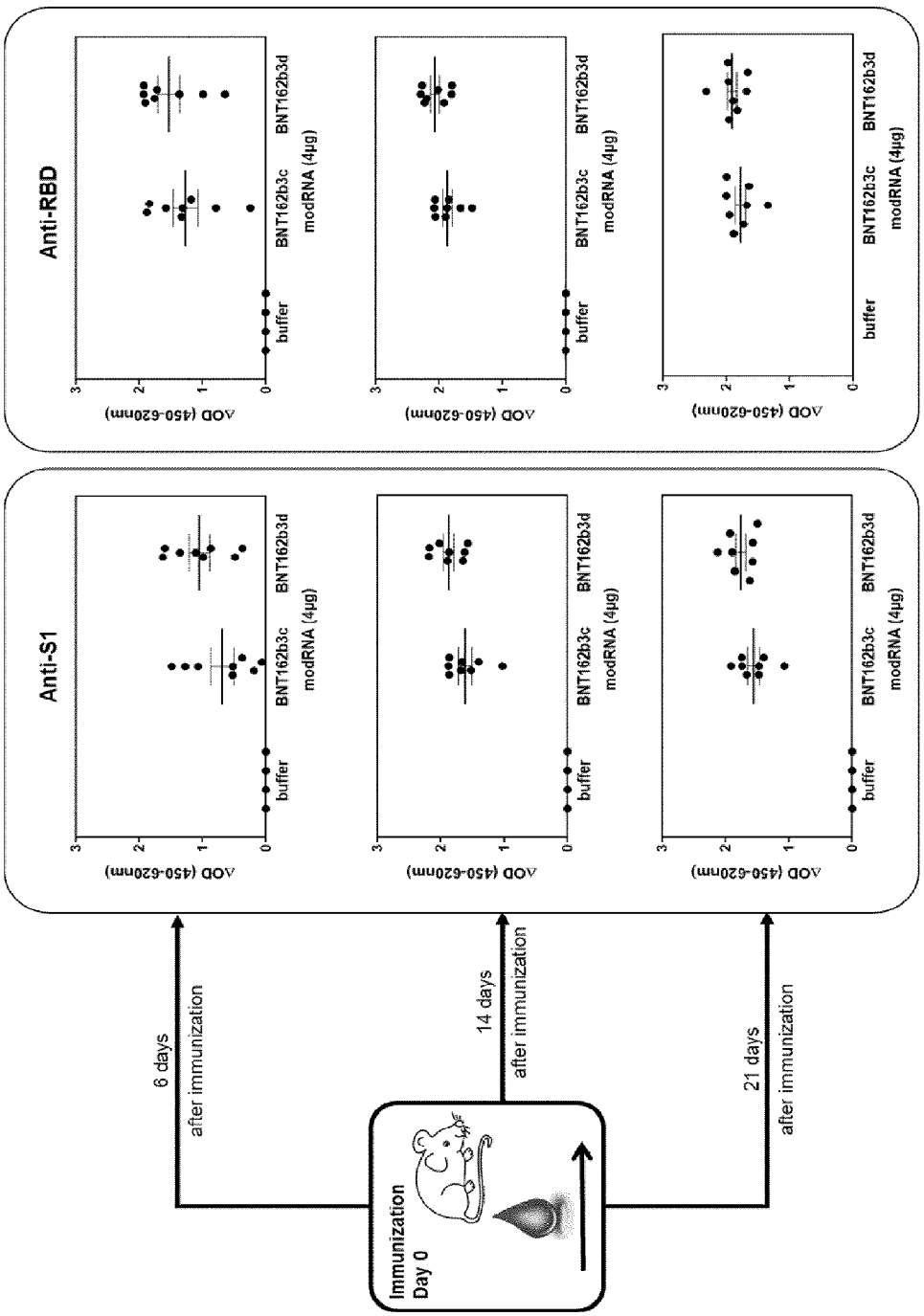

Anti-S1
Anti-RBD
ΔOD (450-620nm)
buffer
BNT162b3c
BNT162b3d
modRNA (4µg)
Immunization
Day 0
6 days
after immunization
14 days
after immunization
21 days
after immunization

Figure 8

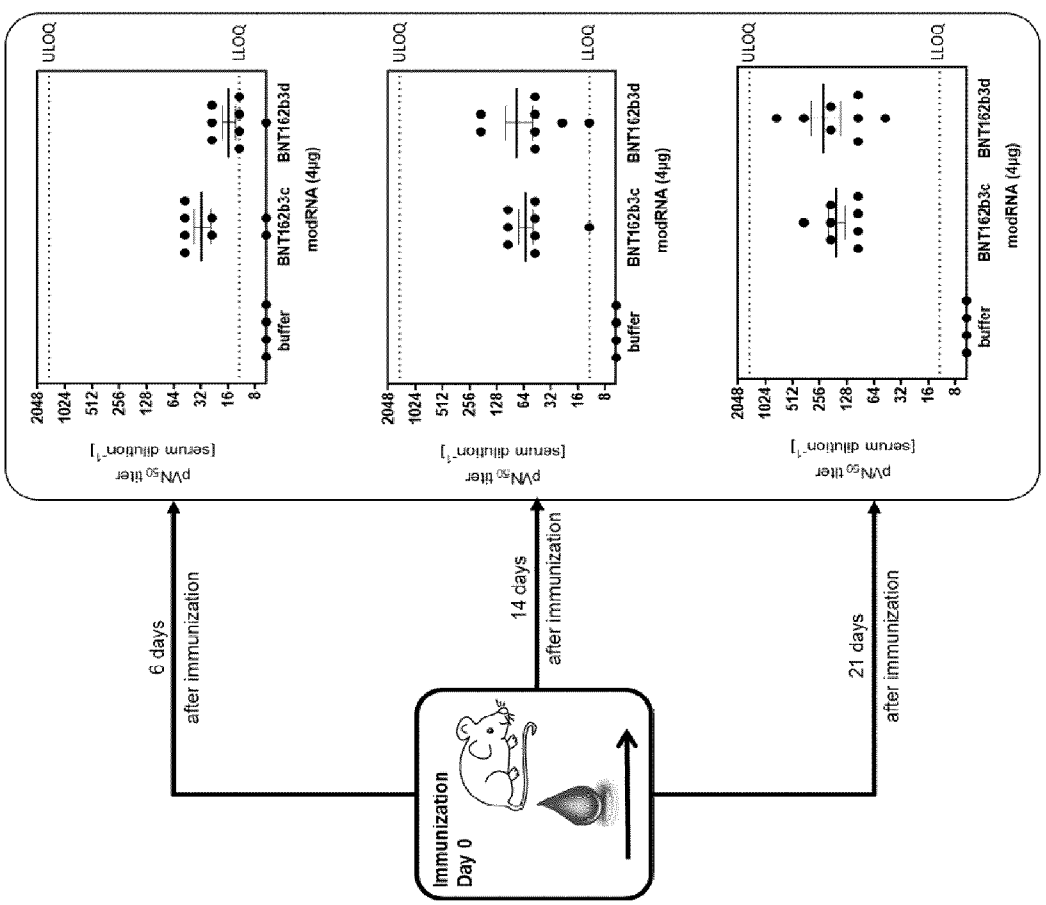

Immunization
Day 0
6 days
after immunization
14 days
after immunization
21 days
after immunization
pVN50 titer
[serum dilution-1]
2048
1024
512
256
128
64
32
16
8
ULOQ
LLOQ
buffer
BNT162b3c
BNT162b3d
modRNA (4µg)

Figure 9

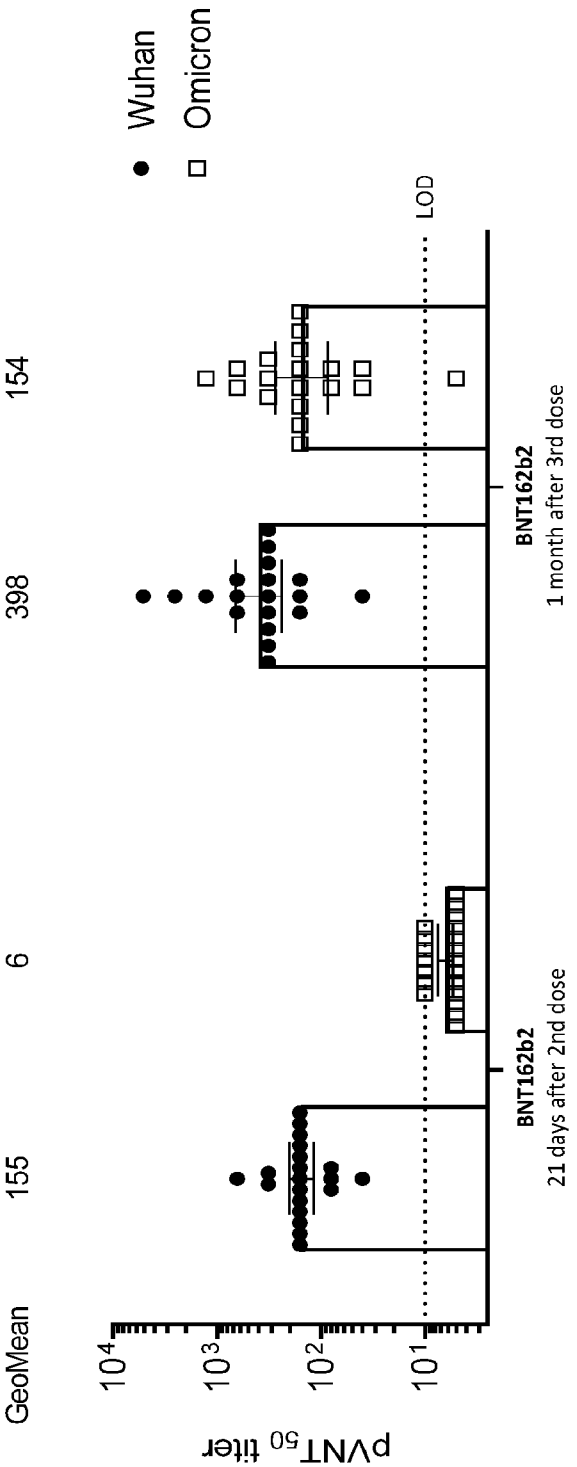
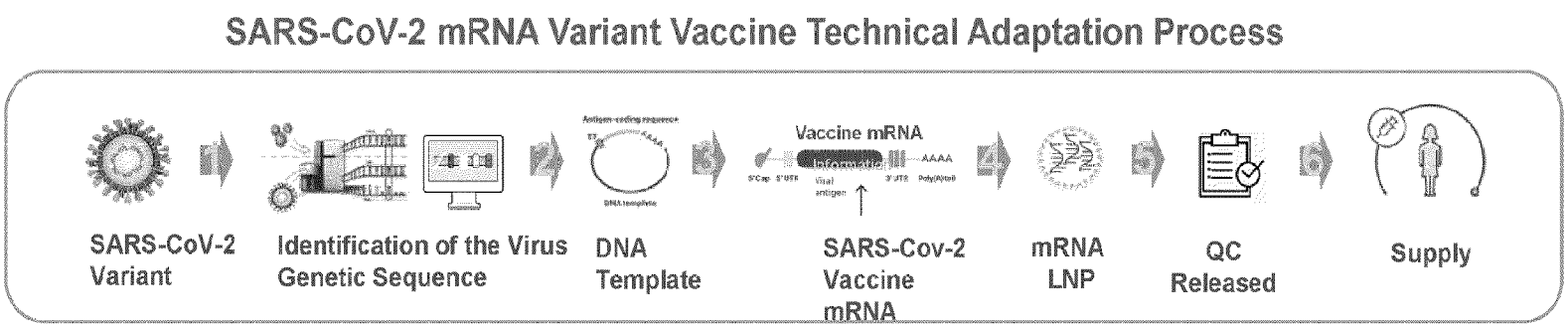

Wuhan
Omicron
LOD
pVNT50 titer
10^4
10^3
10^2
10^1
GeoMean
155
6
398
154
BNT162b2
21 days after 2nd dose
BNT162b2
1 month after 3rd dose

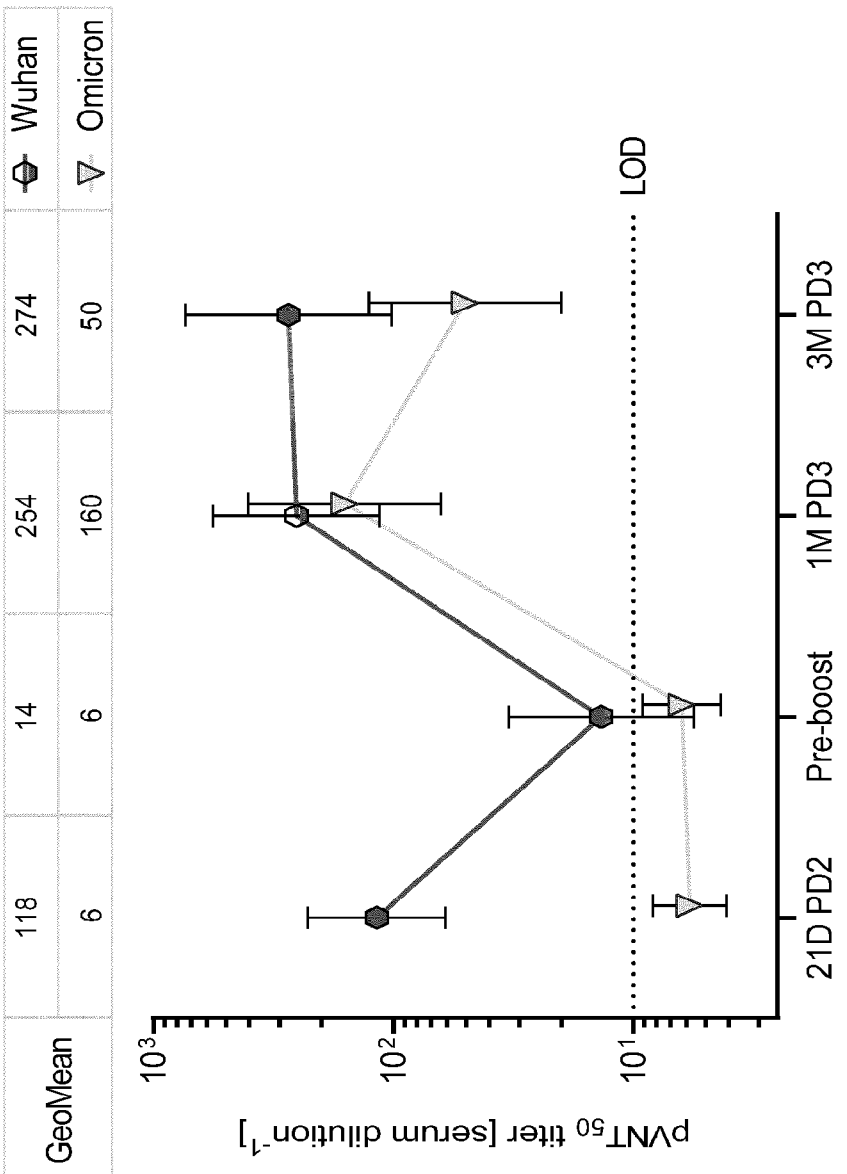
Figure 10

HLA-Allele	No of MHC-I epitopes*	No. of epitopes affected by mutations in different VOCs				
		Alpha	Beta	Gamma	Delta	Omicron
A*01:01	1	0	0	0	0	0
A*02:01	2	0	0	0	0	0
A*03:01	**2**	0	0	0	0	**1**
A*11:01	2	0	0	0	0	0
A*24:02	**5**	0	0	0	**1**	**1**
A*26:01	2	0	0	0	0	0
A*29:02	1	0	0	0	0	**1**
A*68:01	4	0	0	0	0	**1**
B*07:02	1	0	0	0	0	0
B*15:01	**3**	**1**	**2**	**1**	**1**	**2**
B*35:01	6	0	0	0	0	0
C*03:03	1	0	0	0	0	0
C*04:01	1	0	0	0	0	0
Total affected **Total unaffected**	**31**	**1 (4%)** **30 (96%)**	**2 (7%)** **26 (93%)**	**1 (4%)** **30 (96%)**	**2 (7%)** **26 (93%)**	**6 (22%)** **25 (78%)**

Figure 11

Booster vaccine	Trial ID	Omicron neutralization by variant-specific booster compared to 3rd dose BNT162b2 [%]
BNT162b2	NCT04380701	100%
Alpha Variant	NCT05004181	466%
Beta Variant	NCT04949490	Pending
Delta Variant	NCT05004181	165%
Alpha/Delta Variant Mix	NCT05004181	155%





GeoMean	118	14	254	274	
	6	6	160	50	

Figure 12

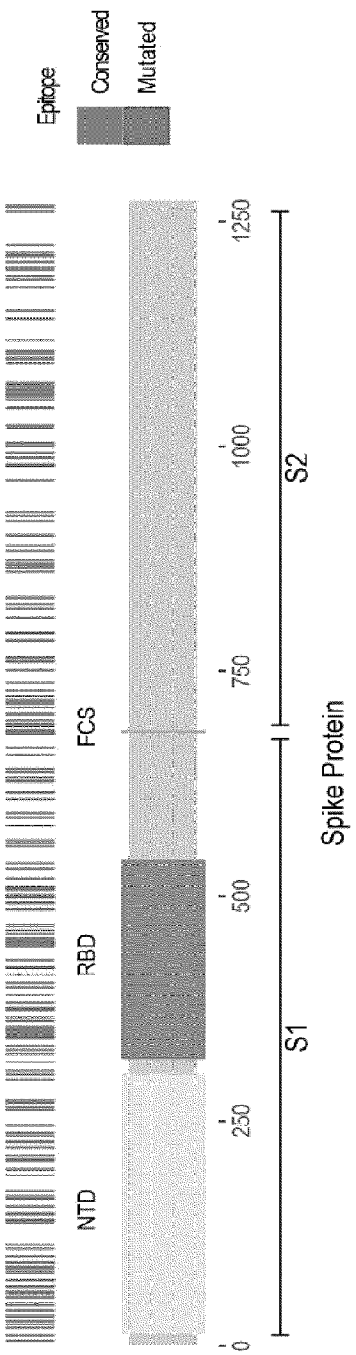

Epitope
Conserved
Mutated
NTD
RBD
FCS
S1
S2
Spike Protein
0
250
500
750
1000
1250

Figure 13

Figure 14

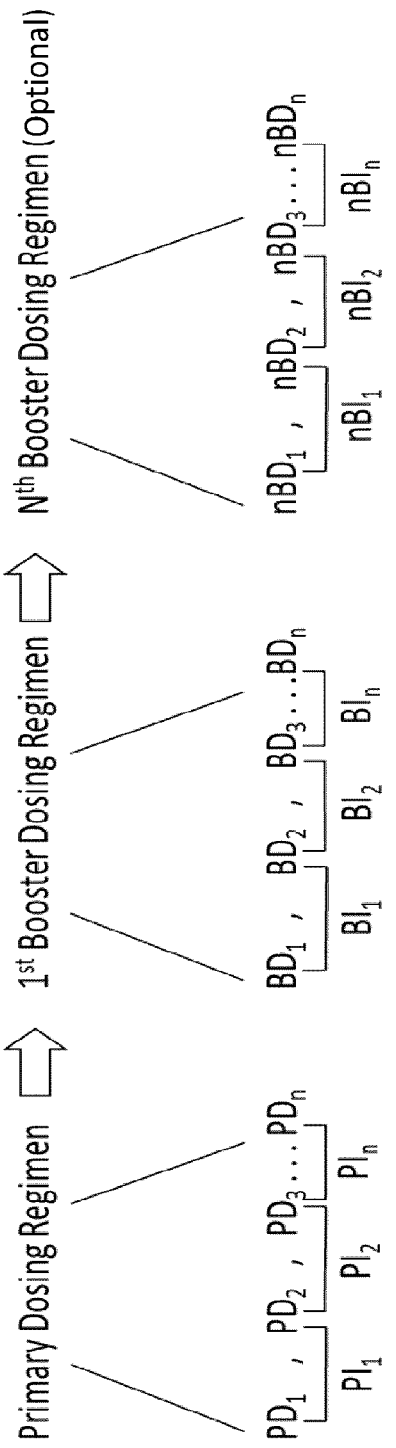

Primary Dosing Regimen
1st Booster Dosing Regimen
Nth Booster Dosing Regimen (Optional)
PD1 , PD2 , PD3 ....PDn
PI1 PI2 PIn
BD1 , BD2 , BD3 ...BDn
BI1 BI2 BIn
nBD1 , nBD2 , nBD3 ....nBDn
nBI1 nBI2 nBIn
(PD2 and subsequent doses are optional)
(BD2 and subsequent doses are optional)
(nBD2 and subsequent doses are optional)

Figure 15

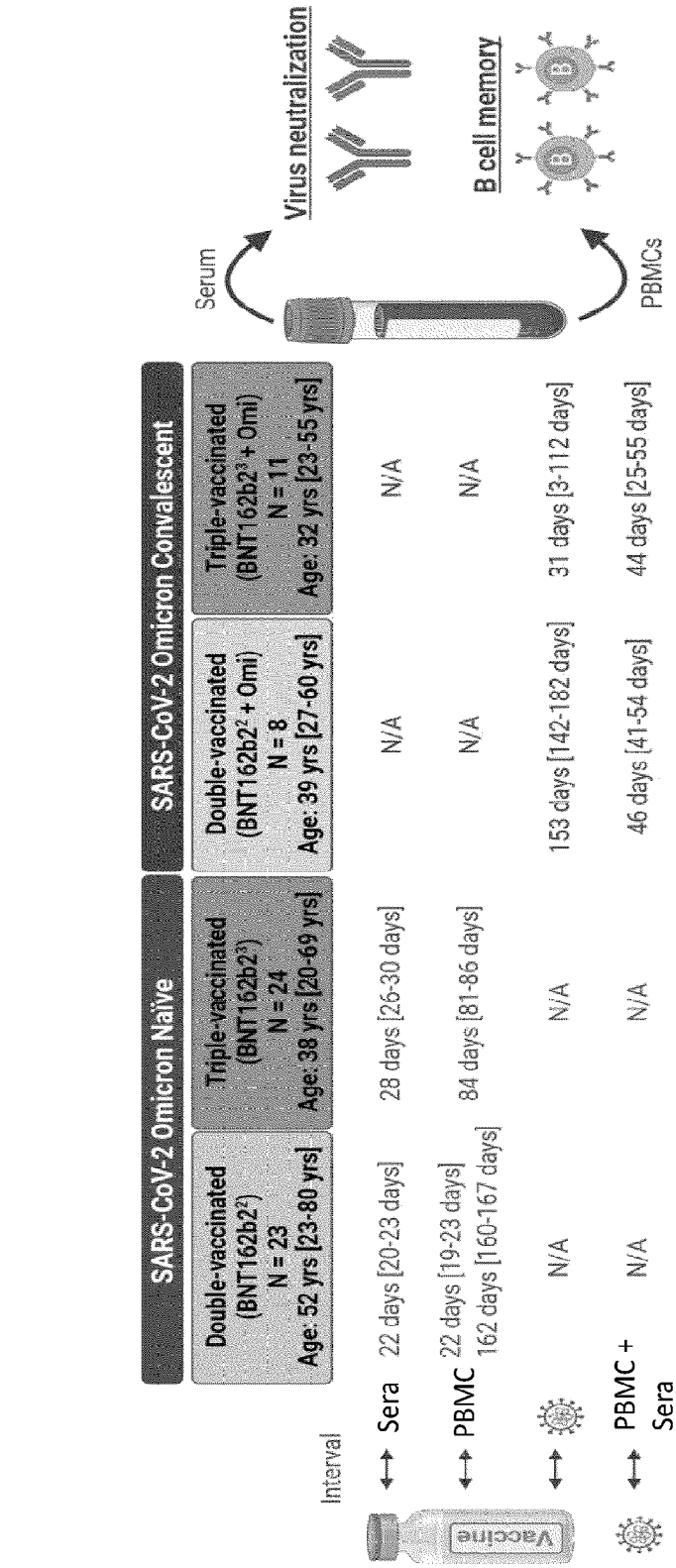

SARS-CoV-2 Omicron Naïve
SARS-CoV-2 Omicron Convalescent
Double-vaccinated (BNT162b2²) N = 23 Age: 52 yrs [23-80 yrs]
Triple-vaccinated (BNT162b2³) N = 24 Age: 38 yrs [20-69 yrs]
Double-vaccinated (BNT162b2² + Omi) N = 8 Age: 39 yrs [27-60 yrs]
Triple-vaccinated (BNT162b2³ + Omi) N = 11 Age: 32 yrs [23-55 yrs]
Interval
Sera
22 days [20-23 days]
28 days [26-30 days]
N/A
N/A
PBMC
22 days [19-23 days]
162 days [160-167 days]
84 days [81-86 days]
N/A
N/A
N/A
N/A
153 days [142-182 days]
31 days [3-112 days]
PBMC + Sera
N/A
N/A
46 days [41-54 days]
44 days [25-55 days]
Vaccine
Serum
PBMCs
Virus neutralization
B cell memory

Figure 16

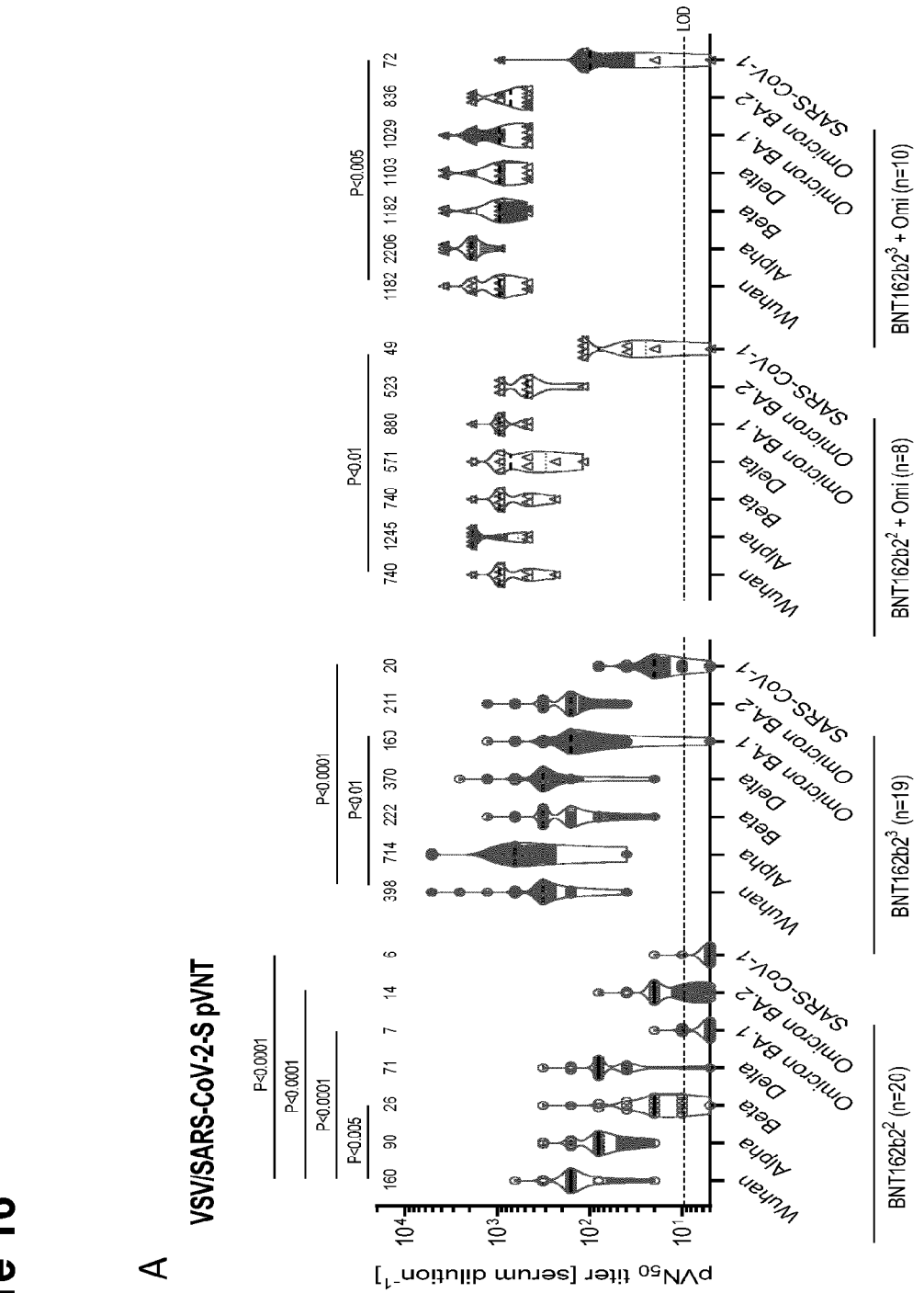

A
VSV/SARS-CoV-2-S pVNT
pVN50 titer [serum dilution-1]
LOD
P<0.0001
P<0.0001
P<0.0001
P<0.005
P<0.0001
P<0.01
P<0.01
P<0.005
160 90 26 71 7 14 6
398 714 222 370 163 211 20
740 1245 740 571 880 523 49
1182 2206 1182 1103 1029 836 72
Wuhan
Alpha
Beta
Delta
Omicron BA.1
Omicron BA.2
SARS-CoV-1
BNT162b2² (n=20)
BNT162b2³ (n=19)
BNT162b2² + Omi (n=8)
BNT162b2³ + Omi (n=10)

Figure 16 (Continued)

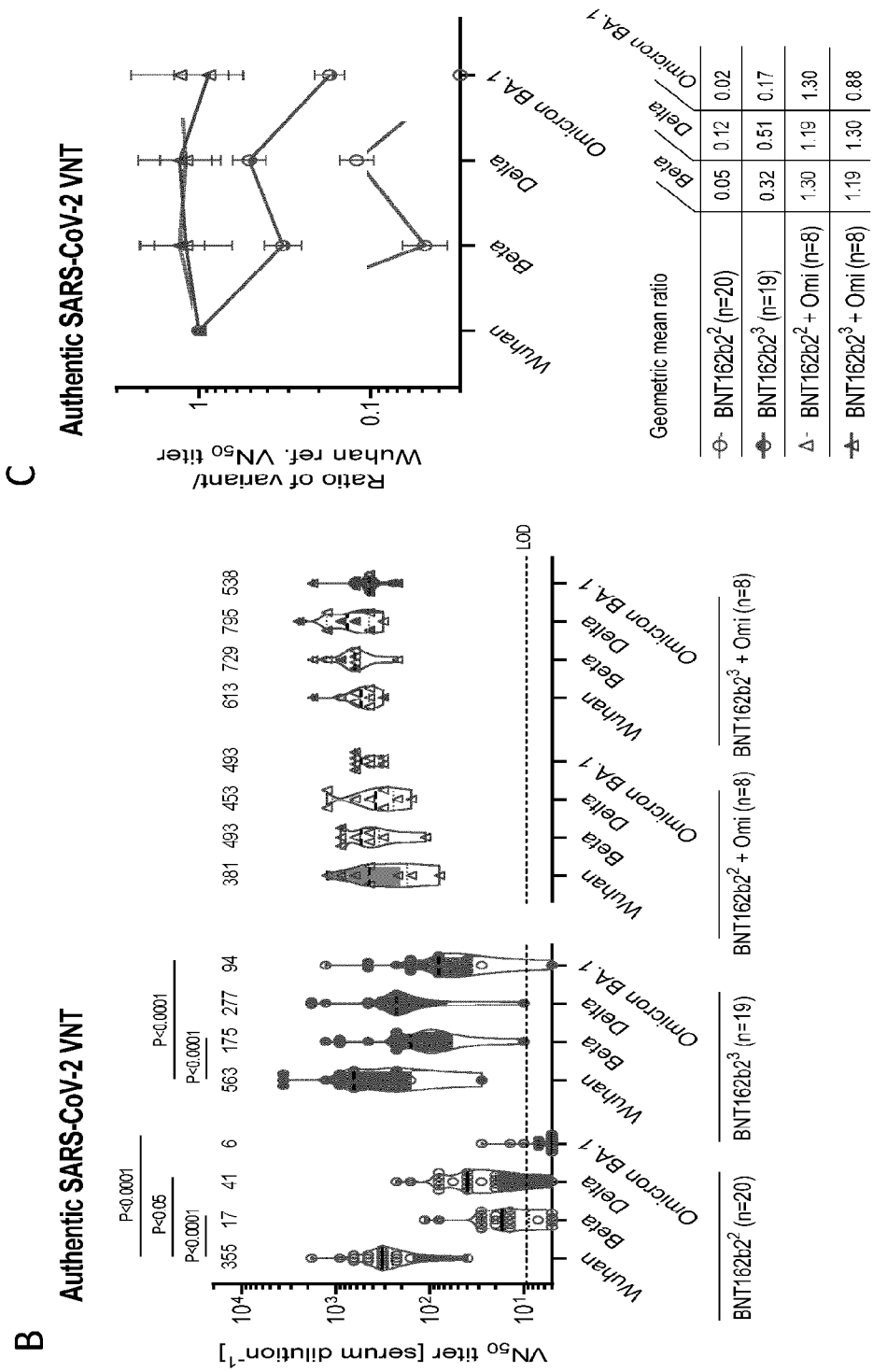


Figure 17

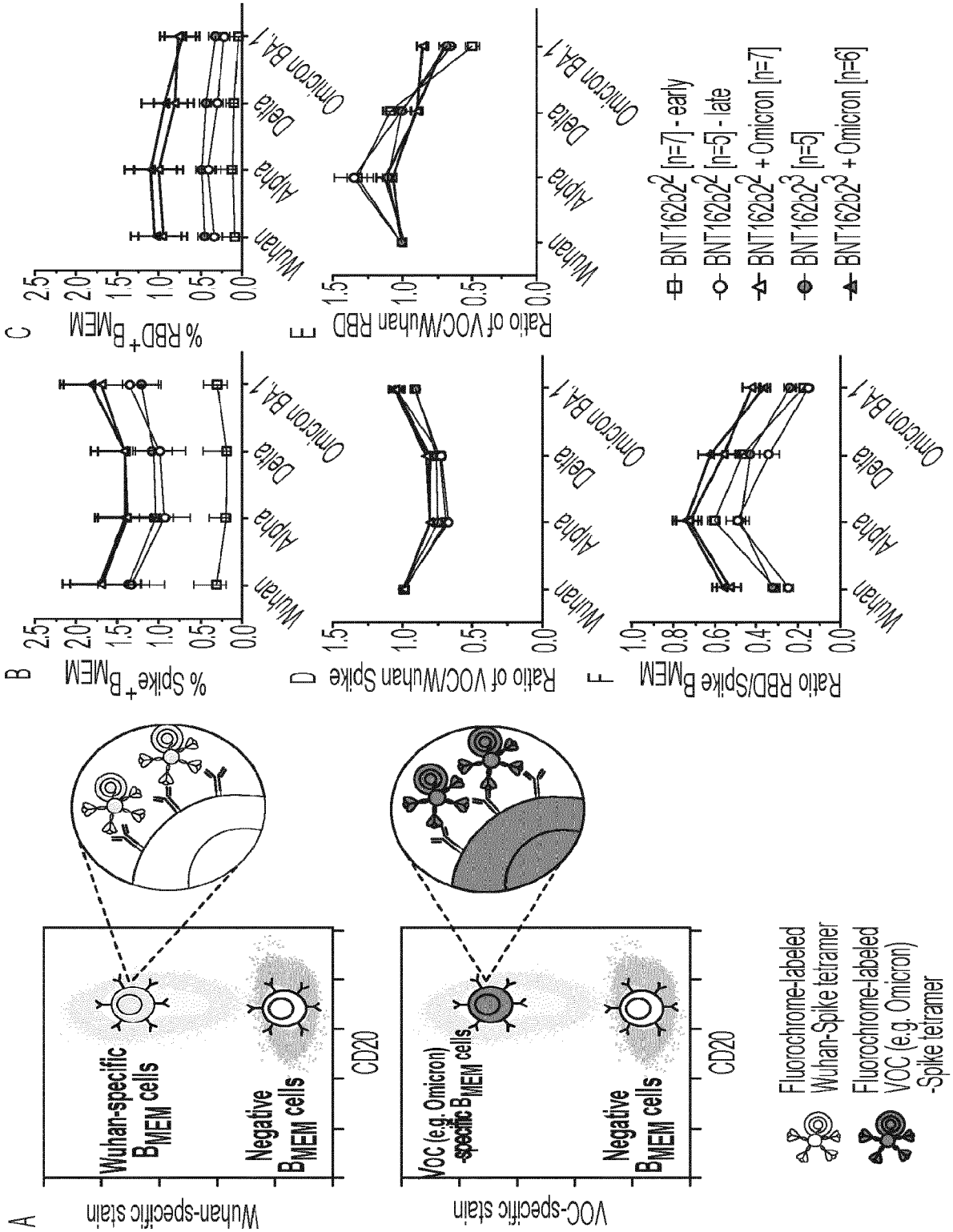

A
Wuhan-specific stain
Wuhan-specific BMEM cells
Negative BMEM cells
CD20
VOC-specific stain
VOC (e.g. Omicron) -specific BMEM cells
Negative BMEM cells
CD20
Fluorochrome-labeled Wuhan-Spike tetramer
Fluorochrome-labeled VOC (e.g. Omicron) -Spike tetramer
B
% Spike+ BMEM
C
% RBD+ BMEM
D
Ratio of VOC/Wuhan Spike
E
Ratio of VOC/Wuhan RBD
F
Ratio RBD/Spike BMEM
Wuhan
Alpha
Delta
Omicron BA.1
BNT162b2 [n=7] - early
BNT162b2 [n=5] - late
BNT162b2 + Omicron [n=7]
BNT162b3 [n=5]
BNT162b3 + Omicron [n=6]

Figure 18

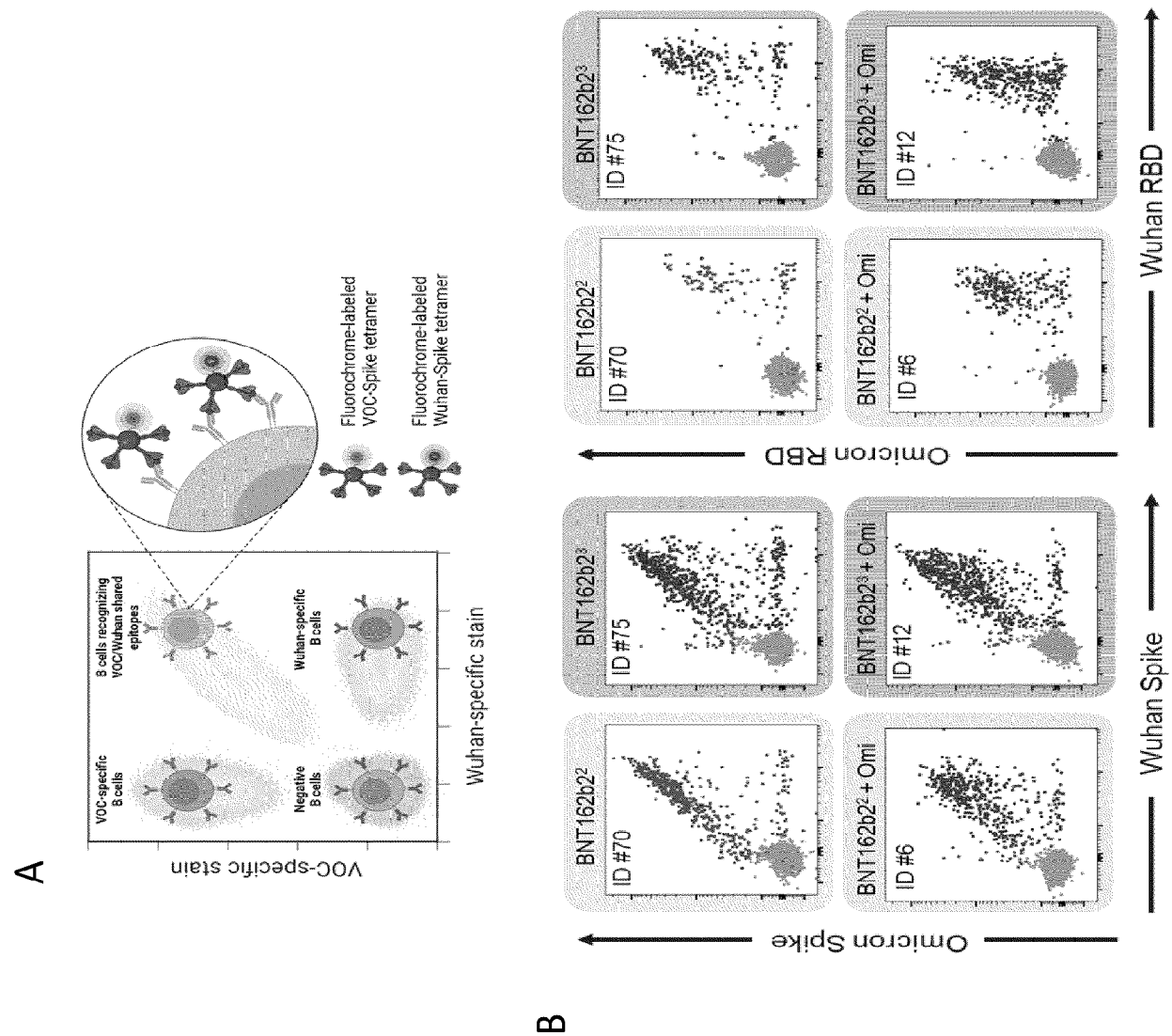

A
VOC-specific stain
Wuhan-specific stain
VOC-specific B cells
B cells recognizing VOC/Wuhan shared epitopes
Negative B cells
Wuhan-specific B cells
Fluorochrome-labeled VOC-Spike tetramer
Fluorochrome-labeled Wuhan-Spike tetramer
B
Omicron Spike
Omicron RBD
Wuhan Spike
Wuhan RBD
BNT162b2²
ID #70
BNT162b2³
ID #75
BNT162b2² + Omi
ID #6
BNT162b2³ + Omi
ID #12

Figure 18 (Continued)

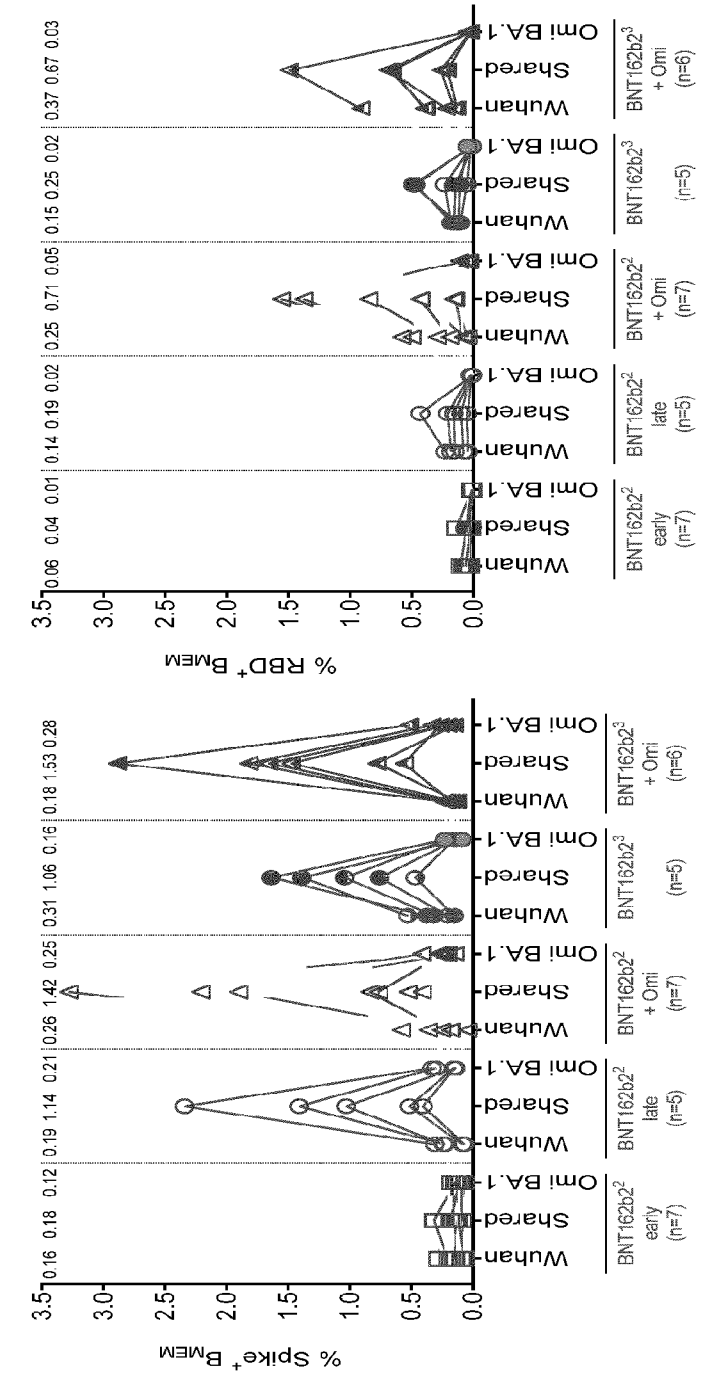
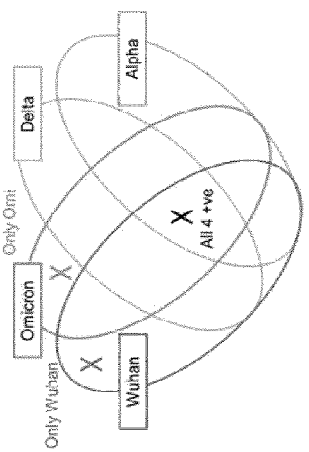


Figure 18 (Continued)



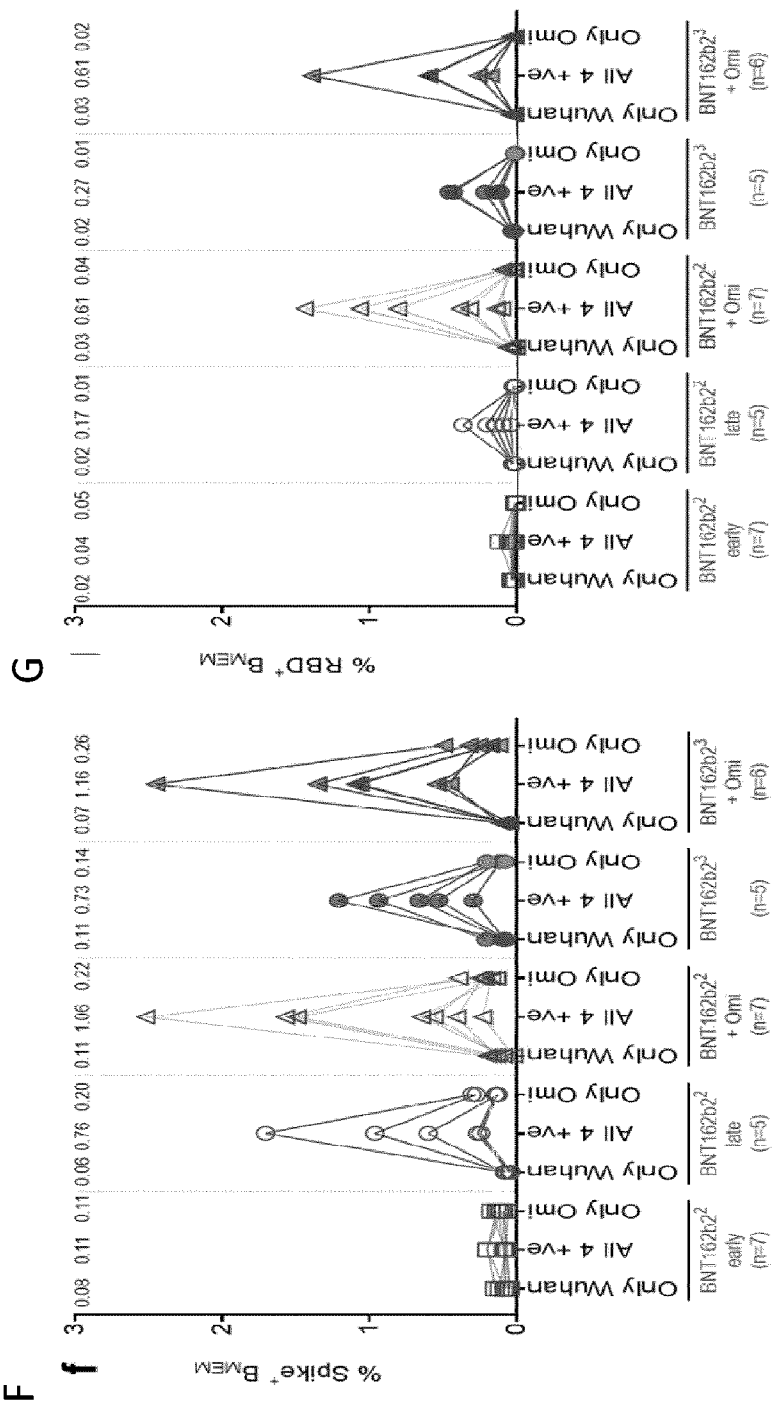
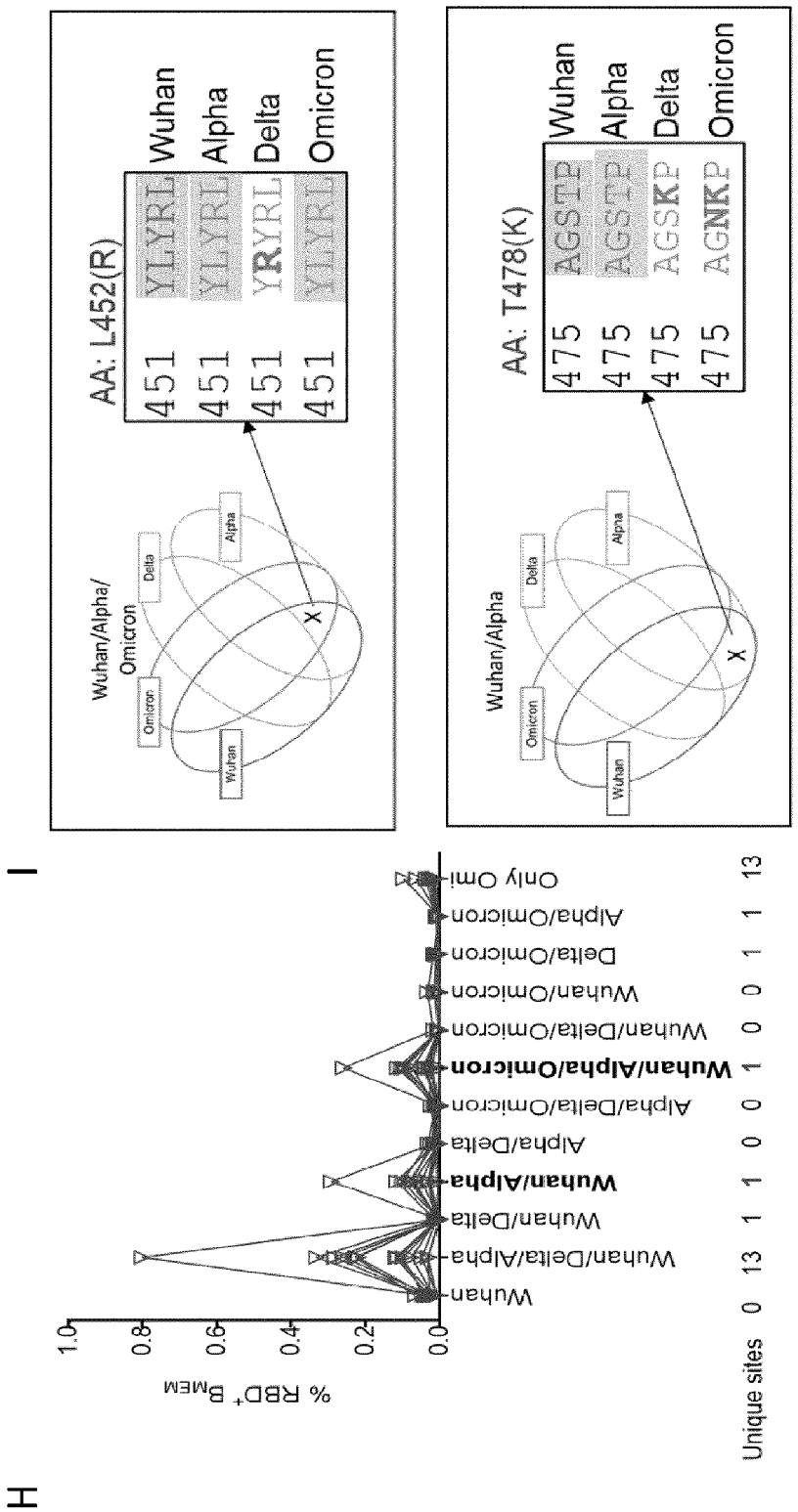


Figure 18 (Continued)



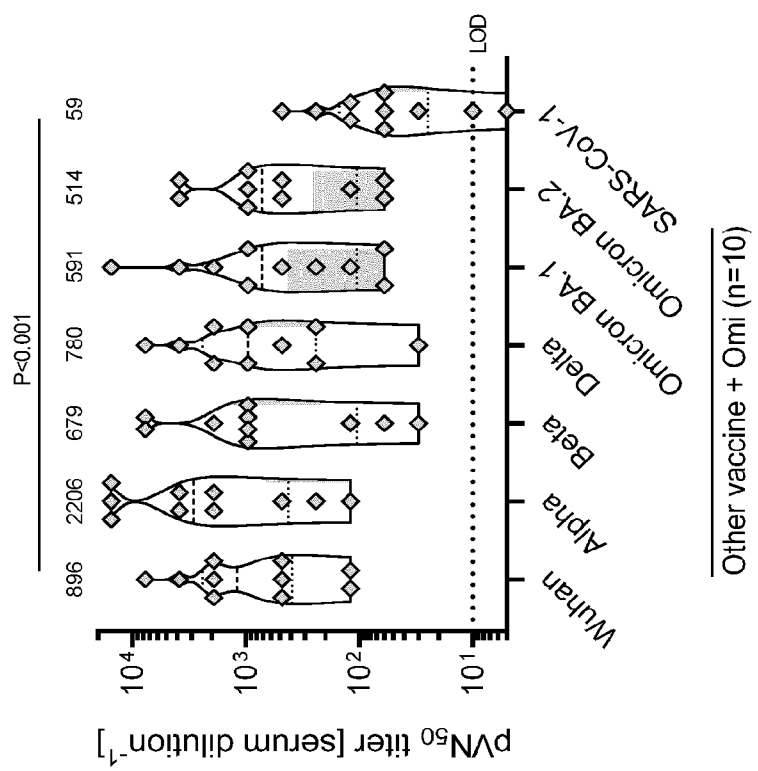

pVN50 titer [serum dilution-1]
10^4
10^3
10^2
10^1
LOD
P<0.001
896
2206
679
780
591
514
59
Wuhan
Alpha
Beta
Delta
Omicron BA.1
Omicron BA.2
SARS-CoV-1
Other vaccine + Omi (n=10)

Figure 19

Figure 20

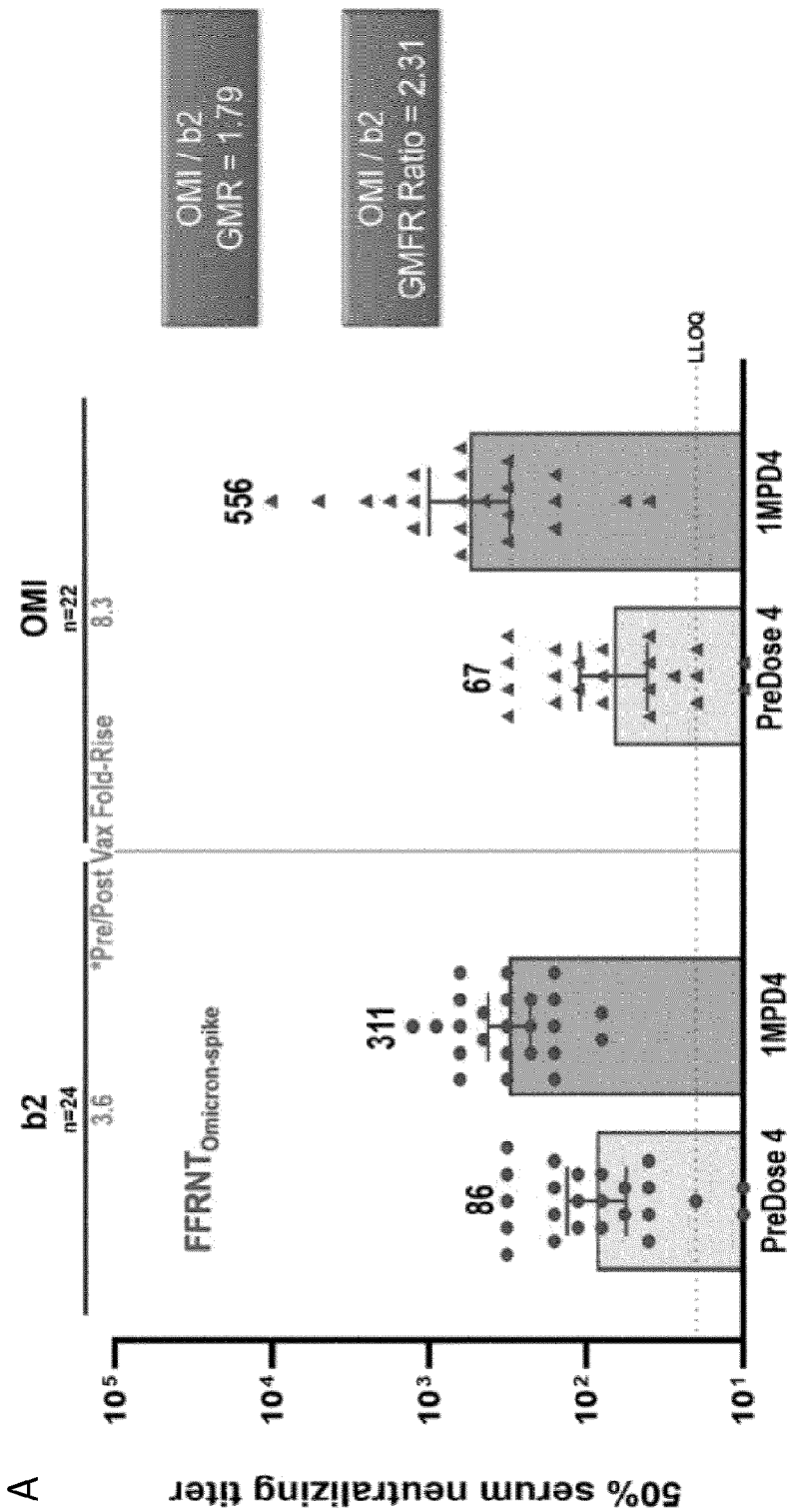

A
50% serum neutralizing titer
10^5
10^4
10^3
10^2
10^1
LLOQ
b2
n=24
3.6
*Pre/Post Vax Fold-Rise
8.3
n=22
OMI
FFRNT$_{Omicron-spike}$
86
311
67
556
PreDose 4
1MPD4
PreDose 4
1MPD4
OMI / b2
GMR = 1.79
OMI / b2
GMFR Ratio = 2.31

Figure 20 (Continued)

B

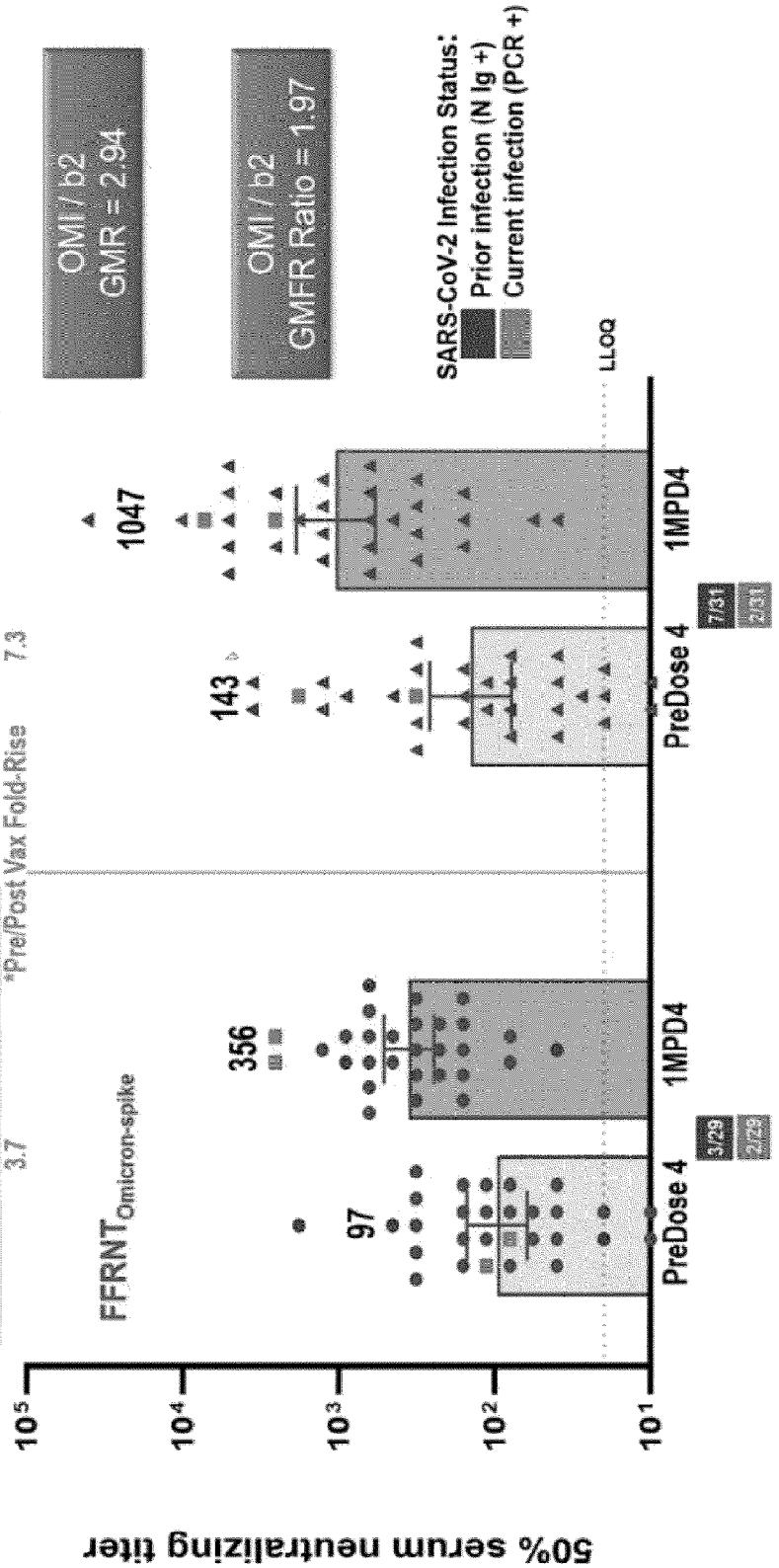


Figure 20 (Continued)

C

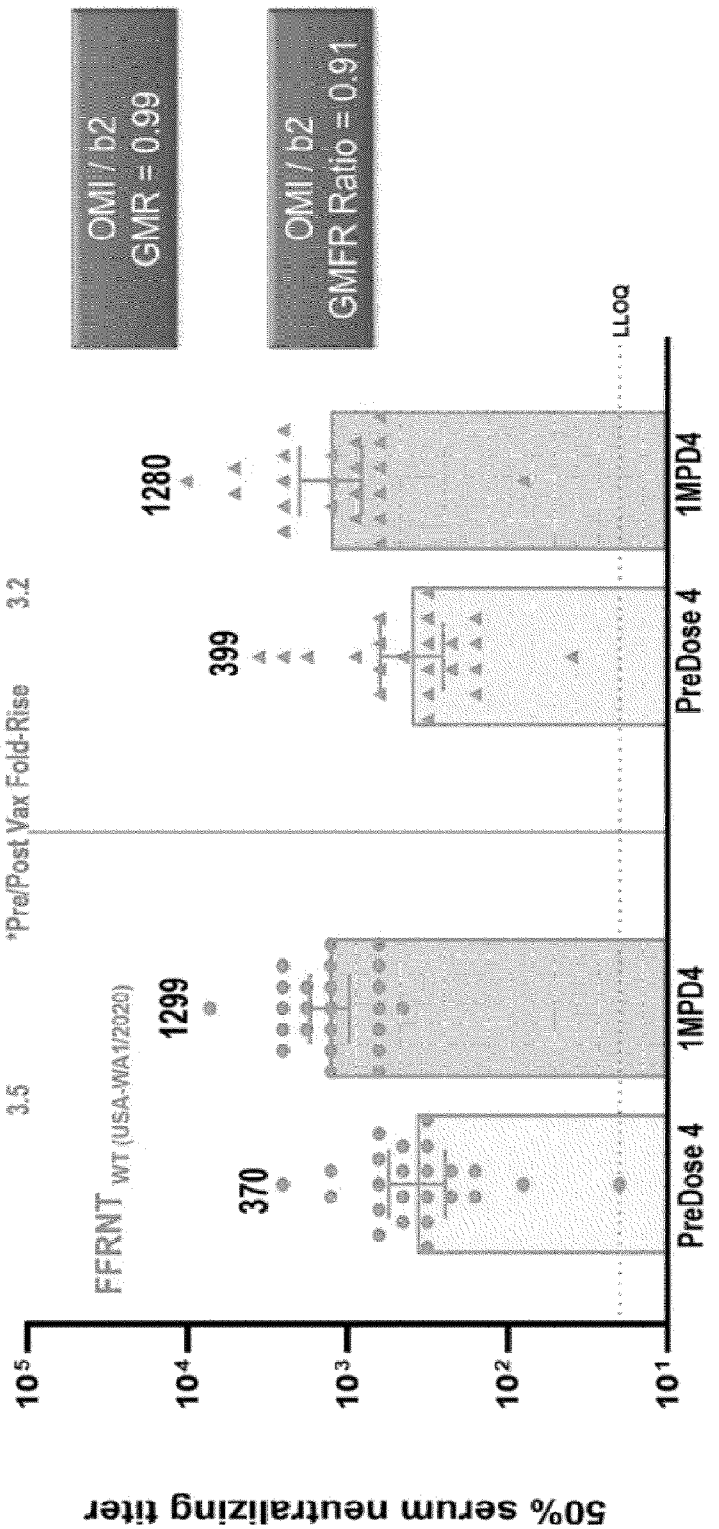


Figure 20 (Continued)

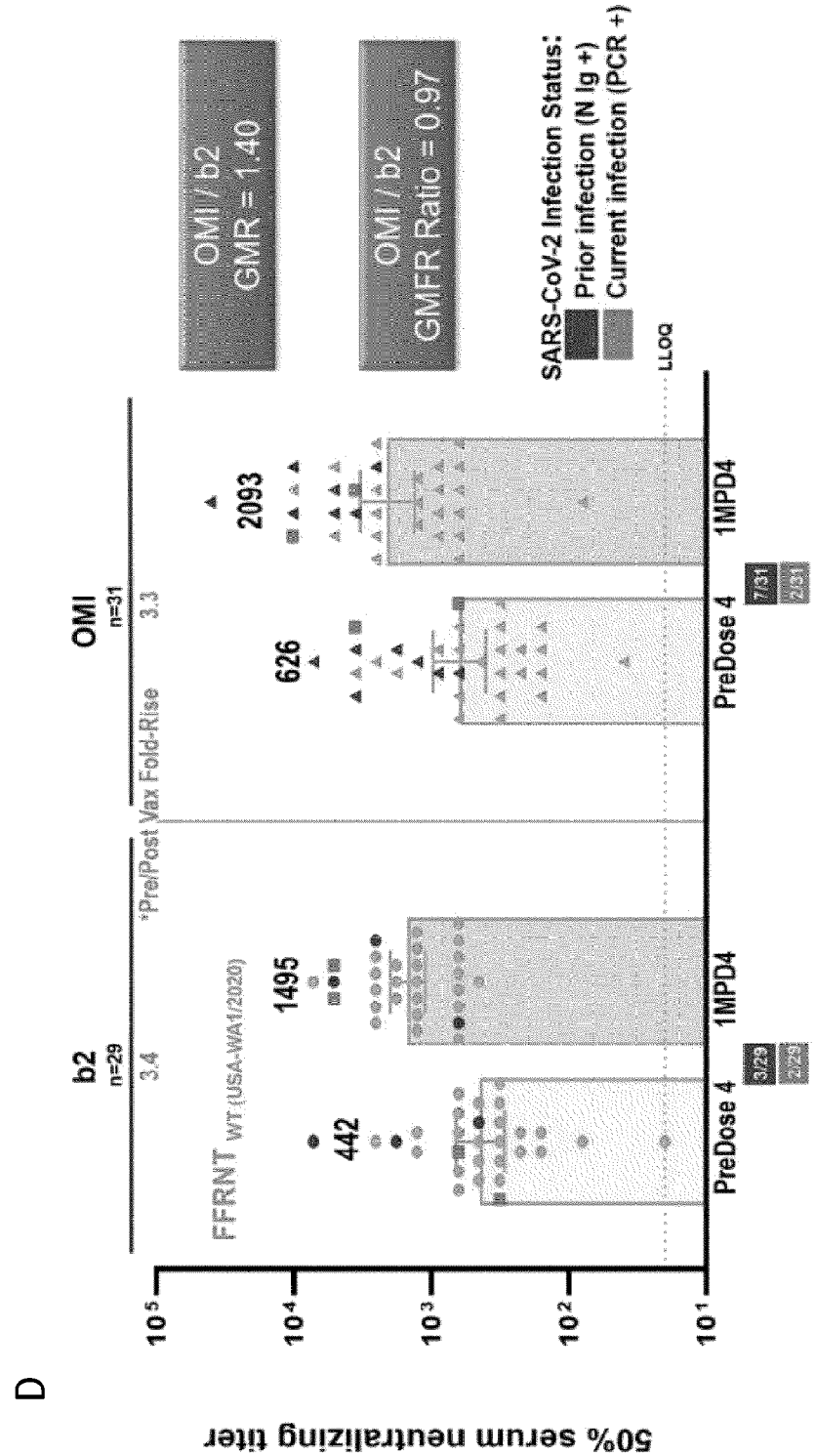


Figure 20 (Continued)

E

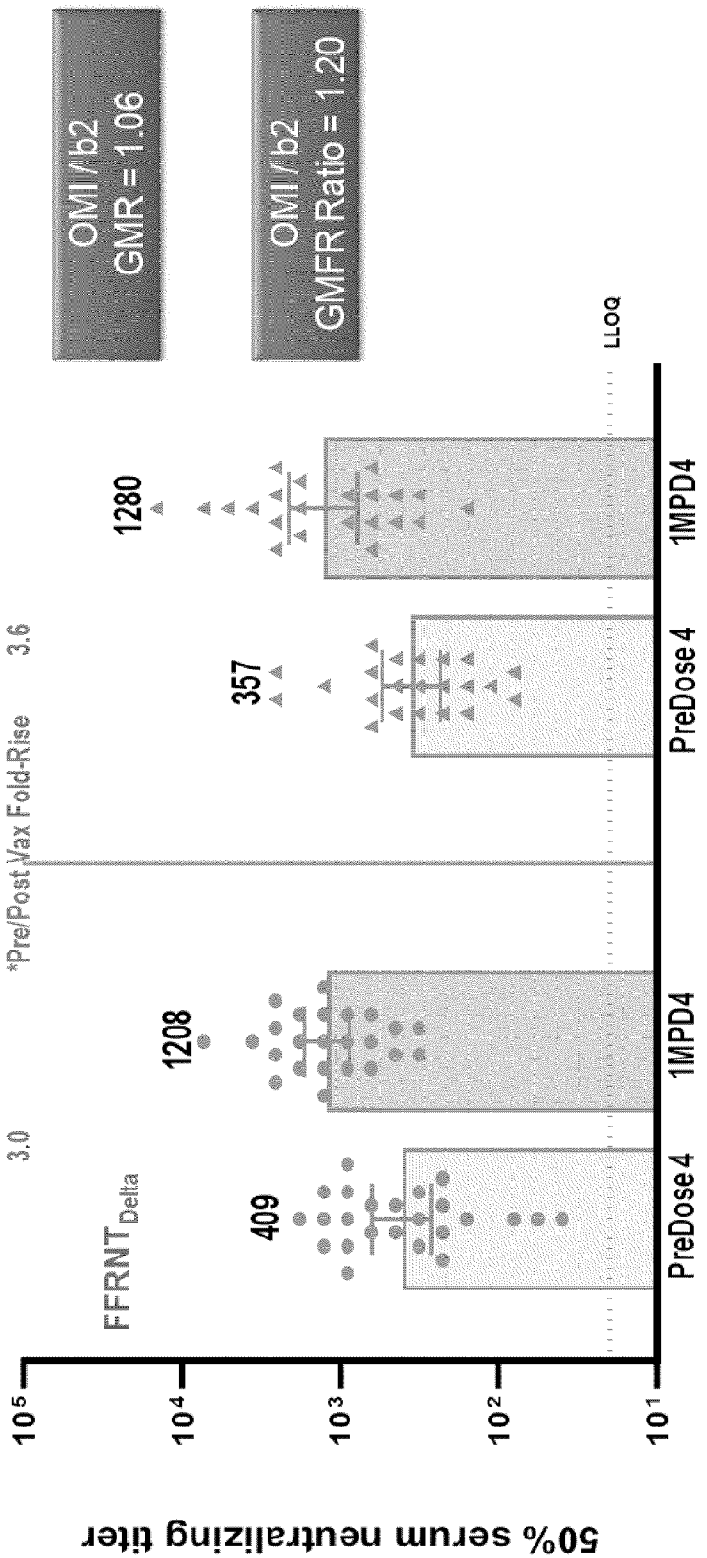
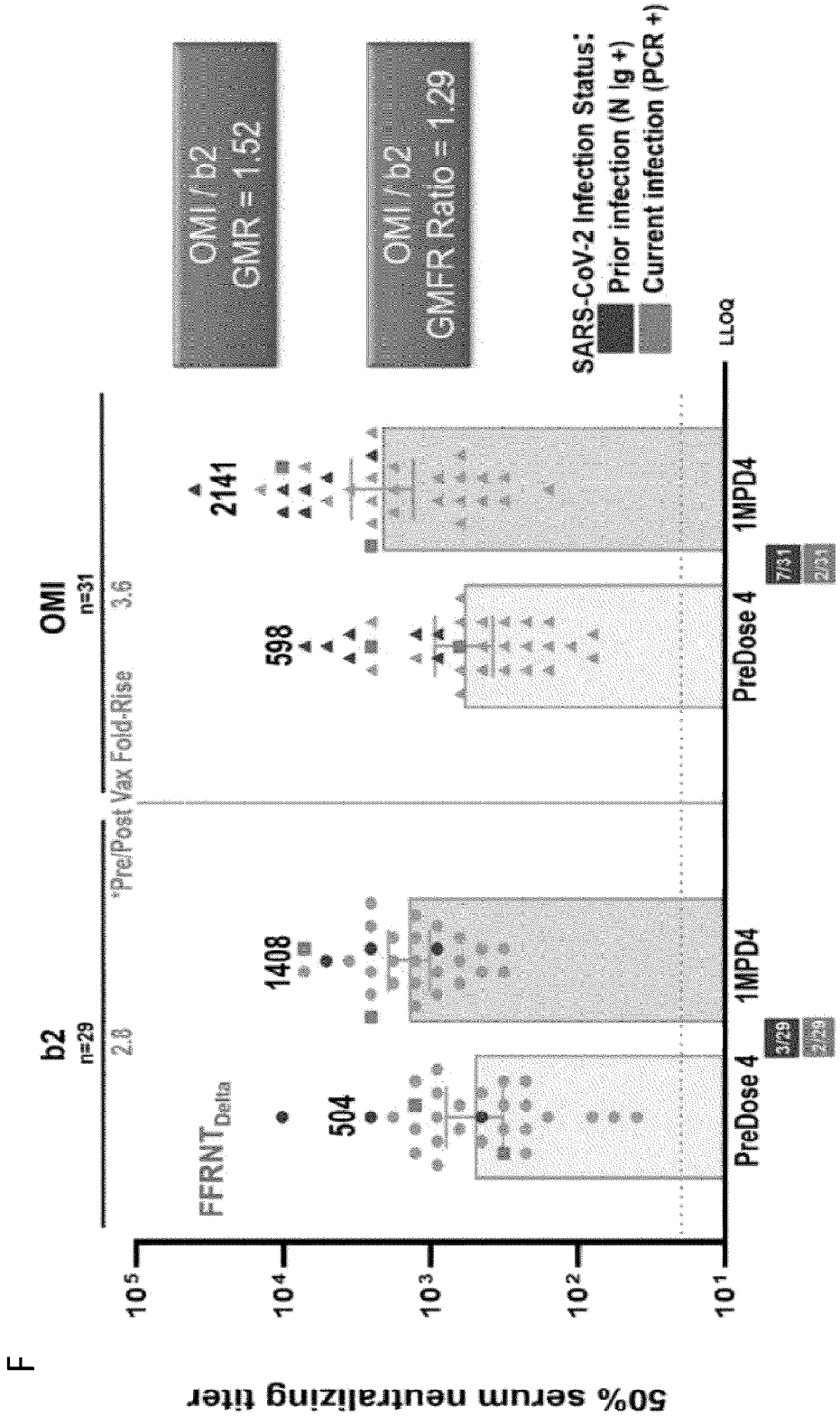


Figure 20 (Continued)



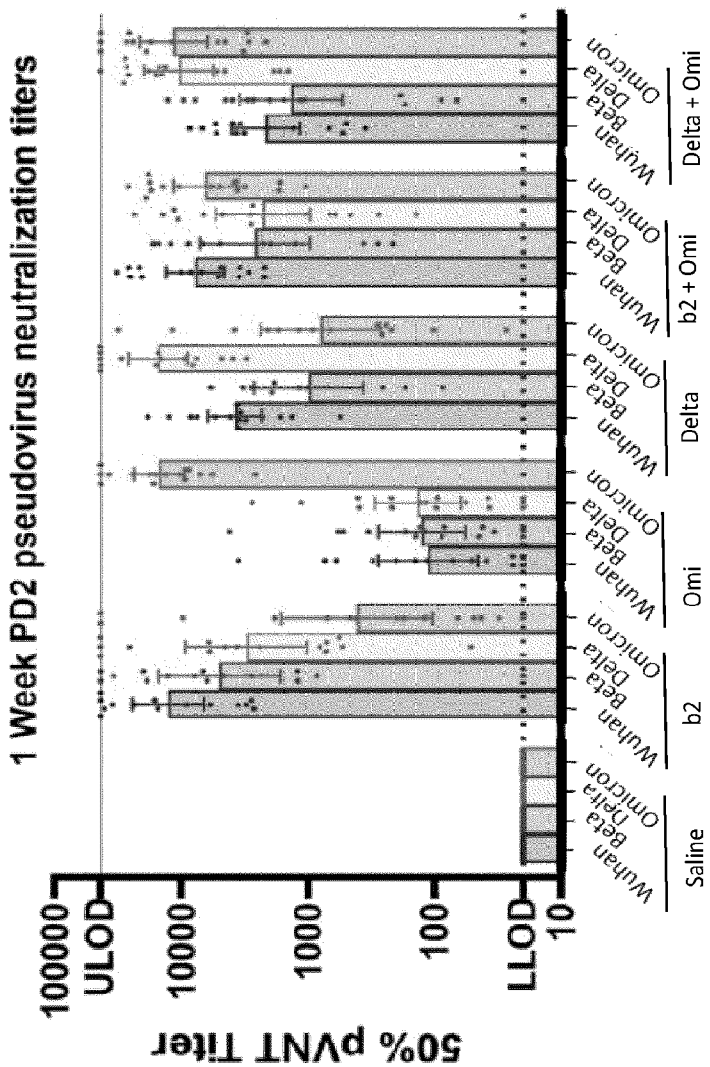

1 Week PD2 pseudovirus neutralization titers
50% pVNT Titer
100000
ULOD
10000
1000
100
LLOD
10
Saline
b2
Omi
Delta
b2 + Omi
Delta + Omi
Wuhan
Beta
Delta
Omicron

Figure 21

Figure 22

A

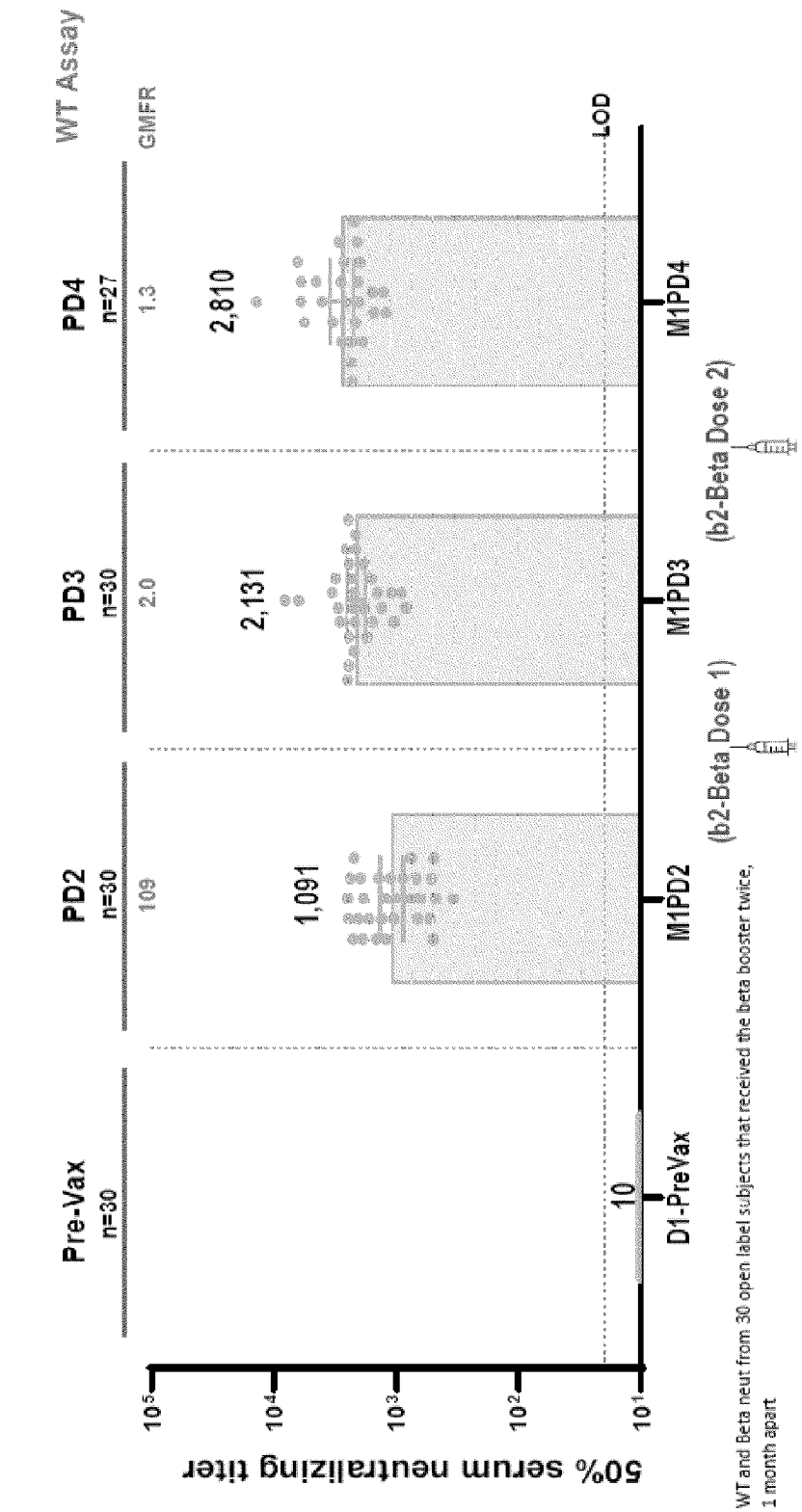

WT Assay
Pre-Vax
n=30
PD2
n=30
PD3
n=30
PD4
n=27
GMFR
109
2.0
1.3
1,091
2,131
2,810
LOD
10
D1-PreVax
M1PD2
M1PD3
M1PD4
(b2-Beta Dose 1)
(b2-Beta Dose 2)
50% serum neutralizing titer
10^5
10^4
10^3
10^2
10^1
WT and Beta neut from 30 open label subjects that received the beta booster twice, 1 month apart

Figure 22 (Continued)

B

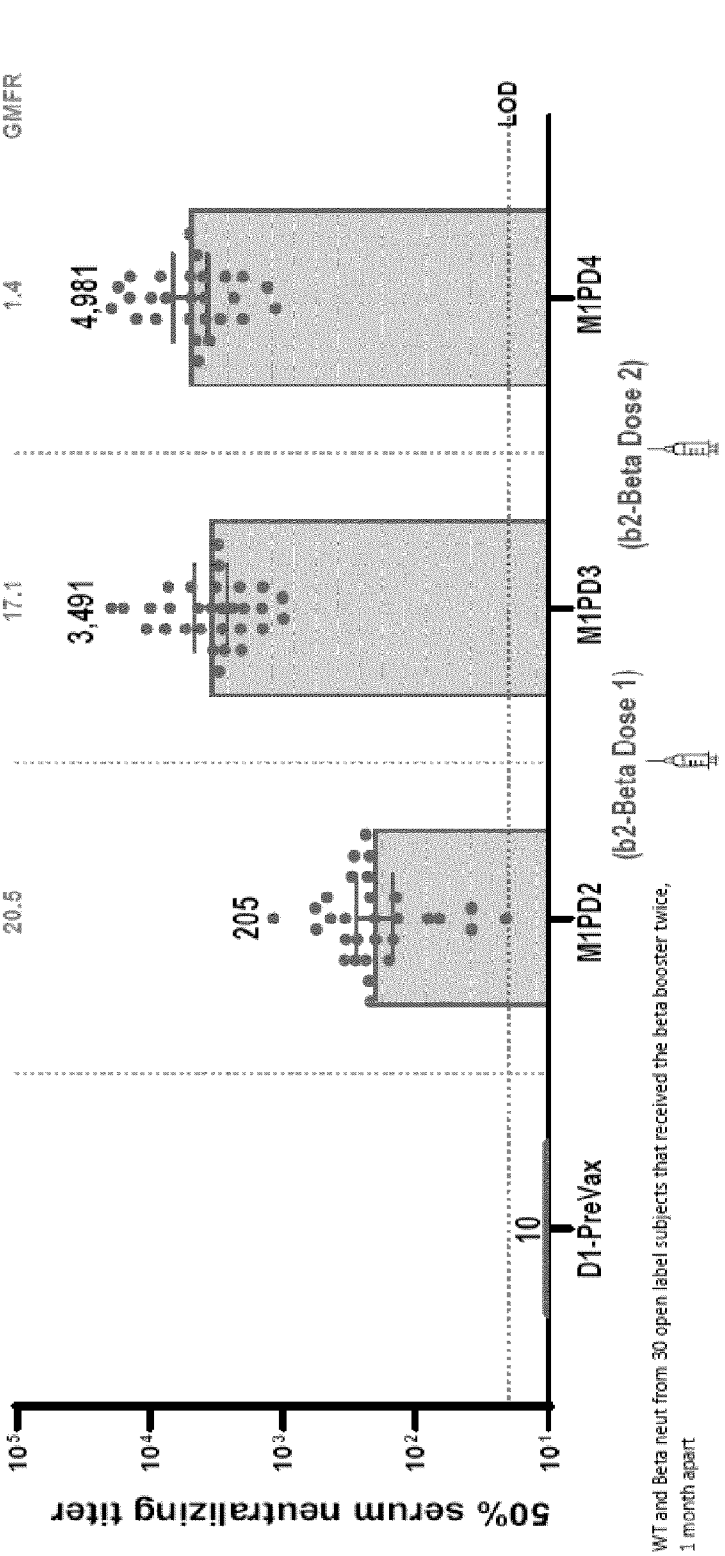


Figure 23

A

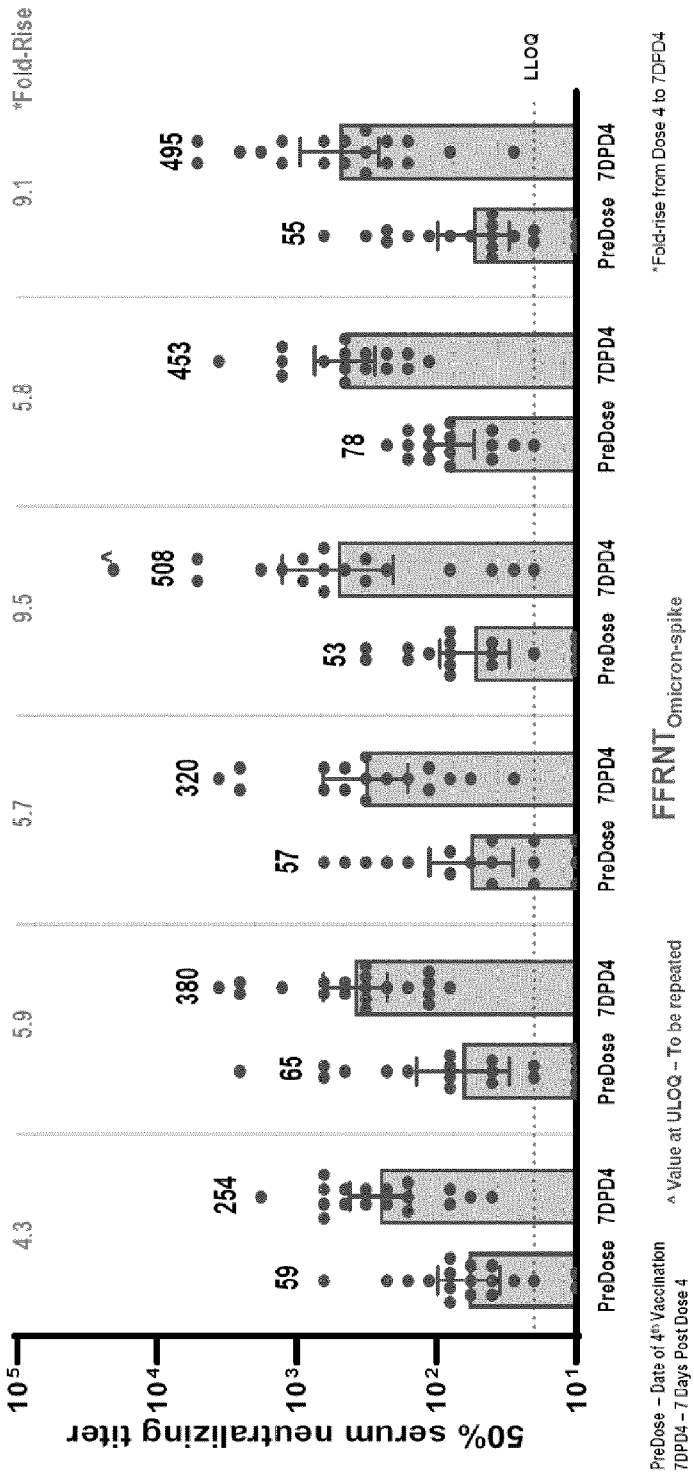

b2 30 µg
n=18
4.3
b2 60 µg
n=20
5.9
OMI 30 µg
n=17
5.7
OMI 60 µg
n=18
9.5
Bivalent 30 µg
n=16
5.8
Bivalent 60 µg
n=19
9.1
*Fold-Rise
50% serum neutralizing titer
LLOQ
PreDose
7DPD4
59
254
65
380
57
320
53
508
78
453
55
495
FFRNT Omicron-spike
*Fold-rise from Dose 4 to 7DPD4
PreDose – Date of 4th Vaccination
7DPD4 – 7 Days Post Dose 4
^ Value at ULOQ – To be repeated

Figure 23 (Continued)

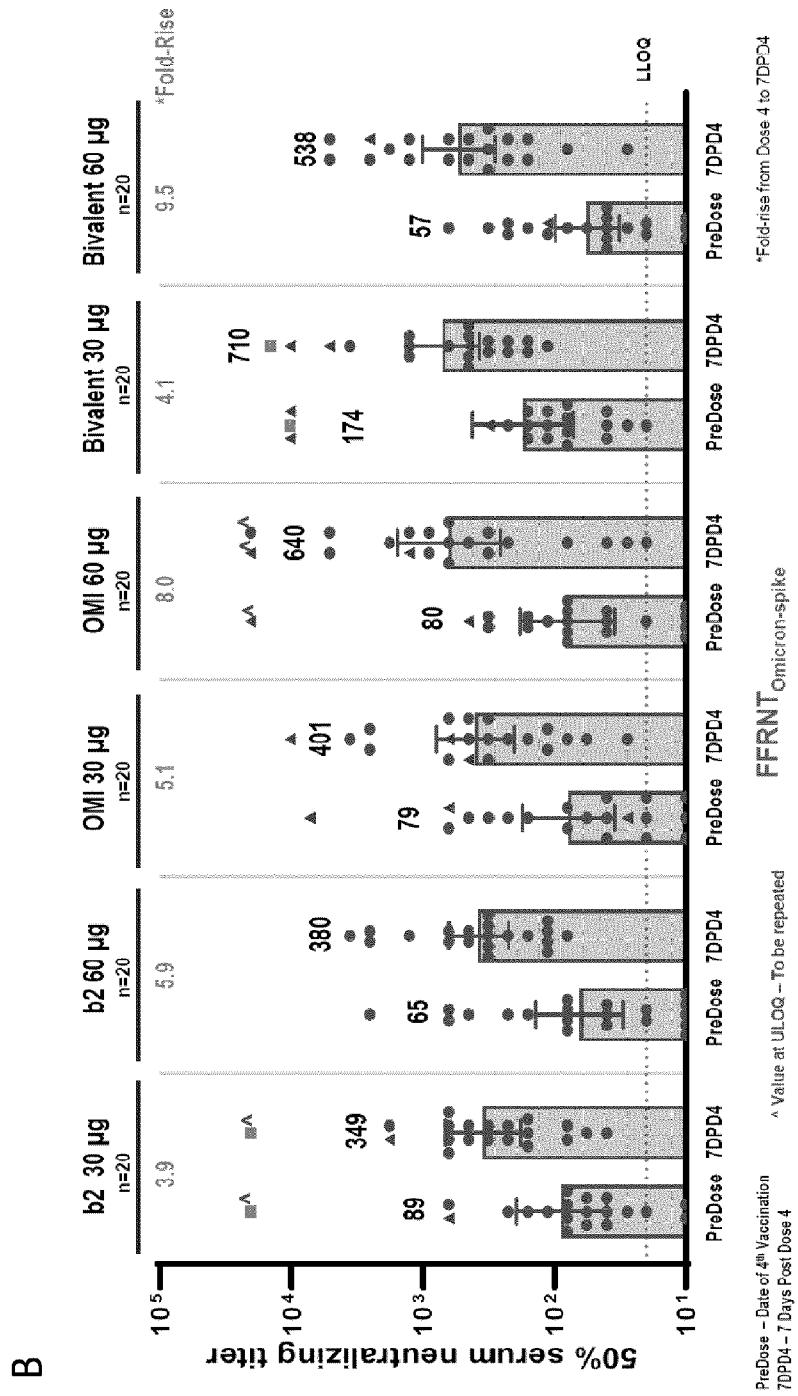


Figure 23 (Continued)

C

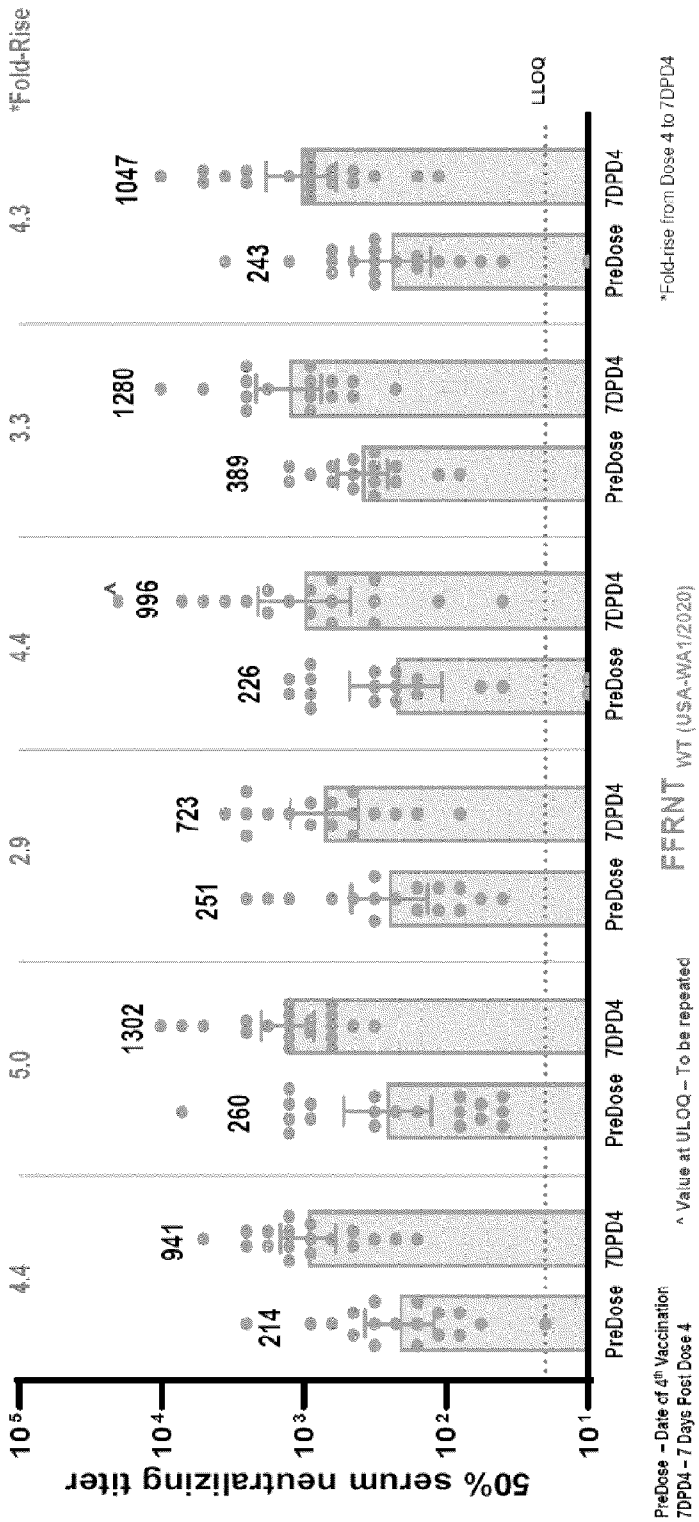


Figure 23 (Continued)

D

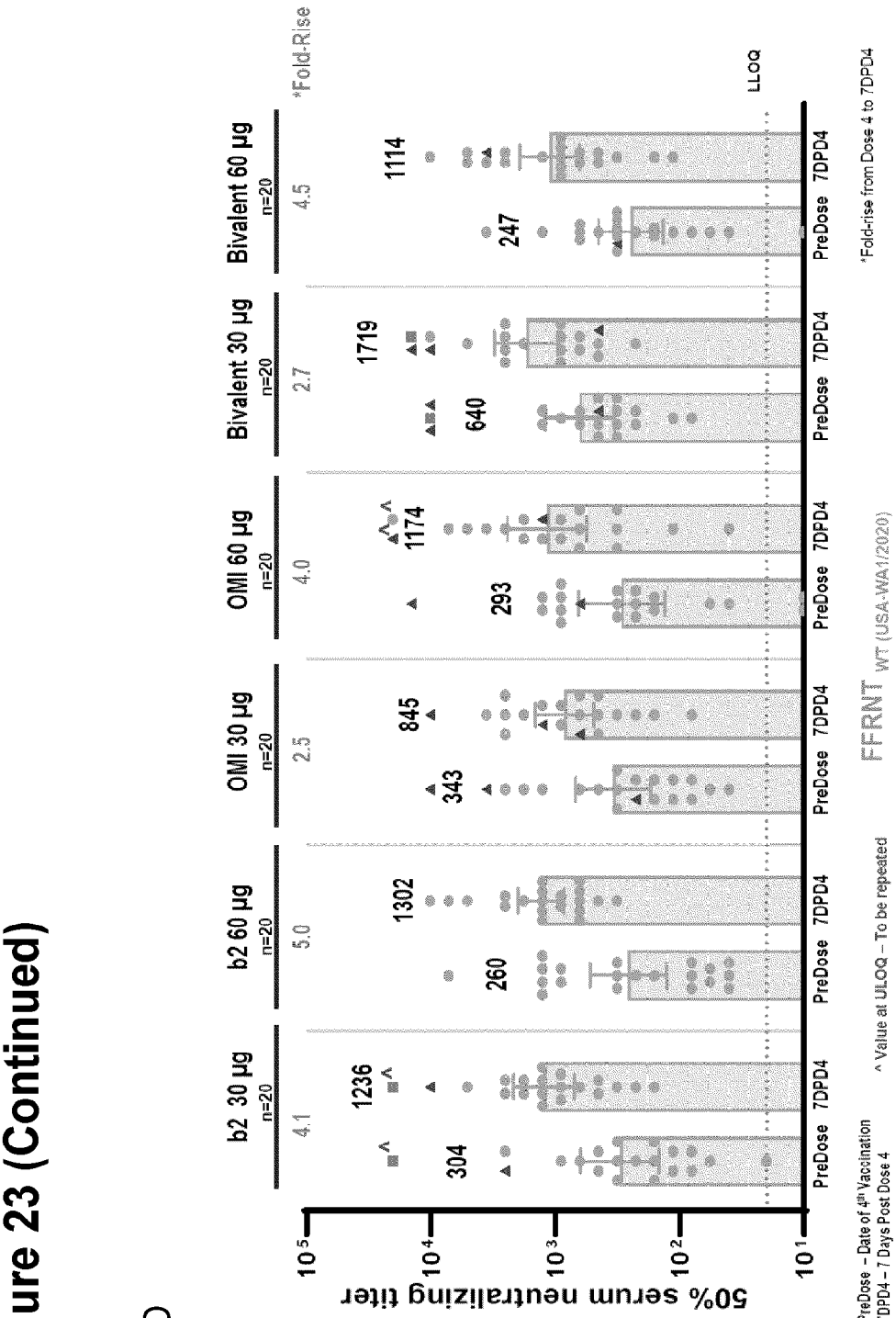


Figure 23 (Continued)

E

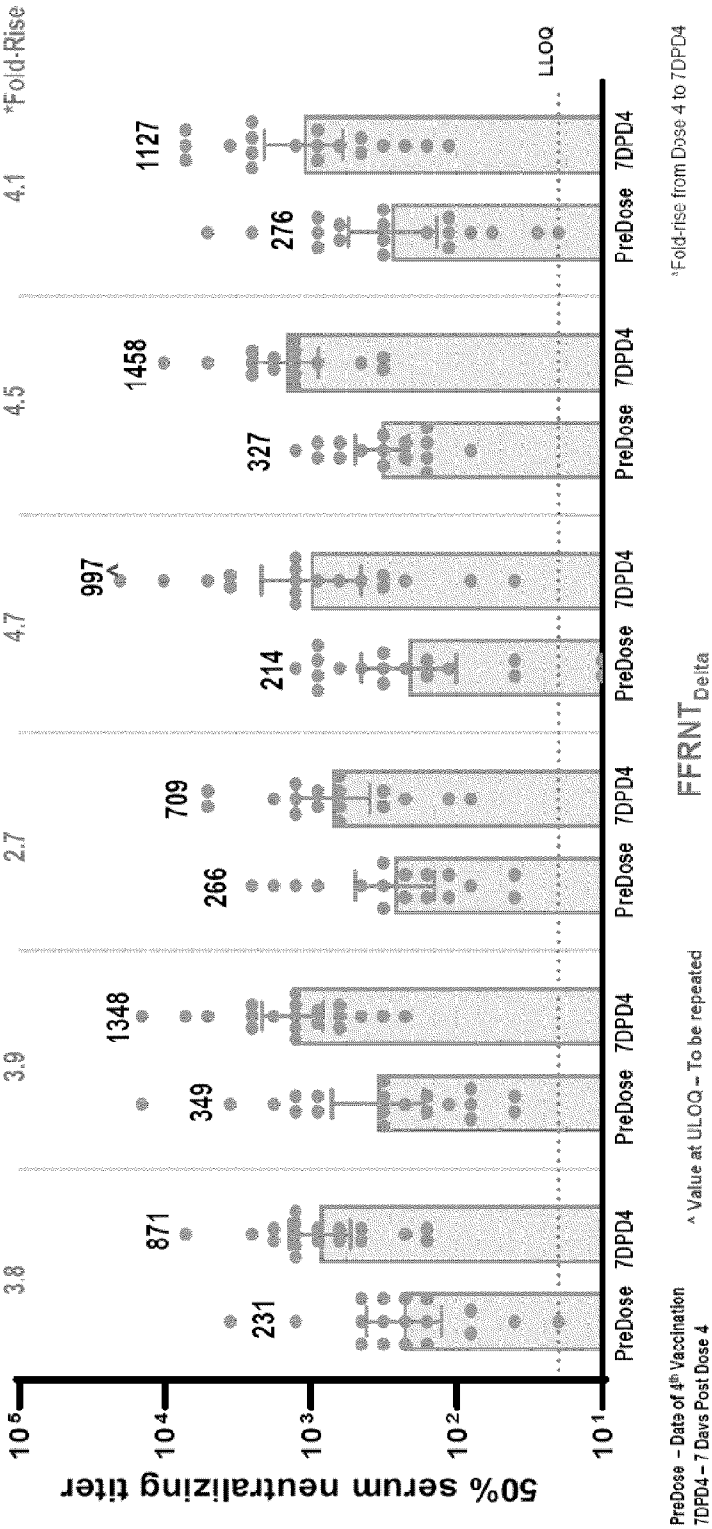


Figure 23 (Continued)

F

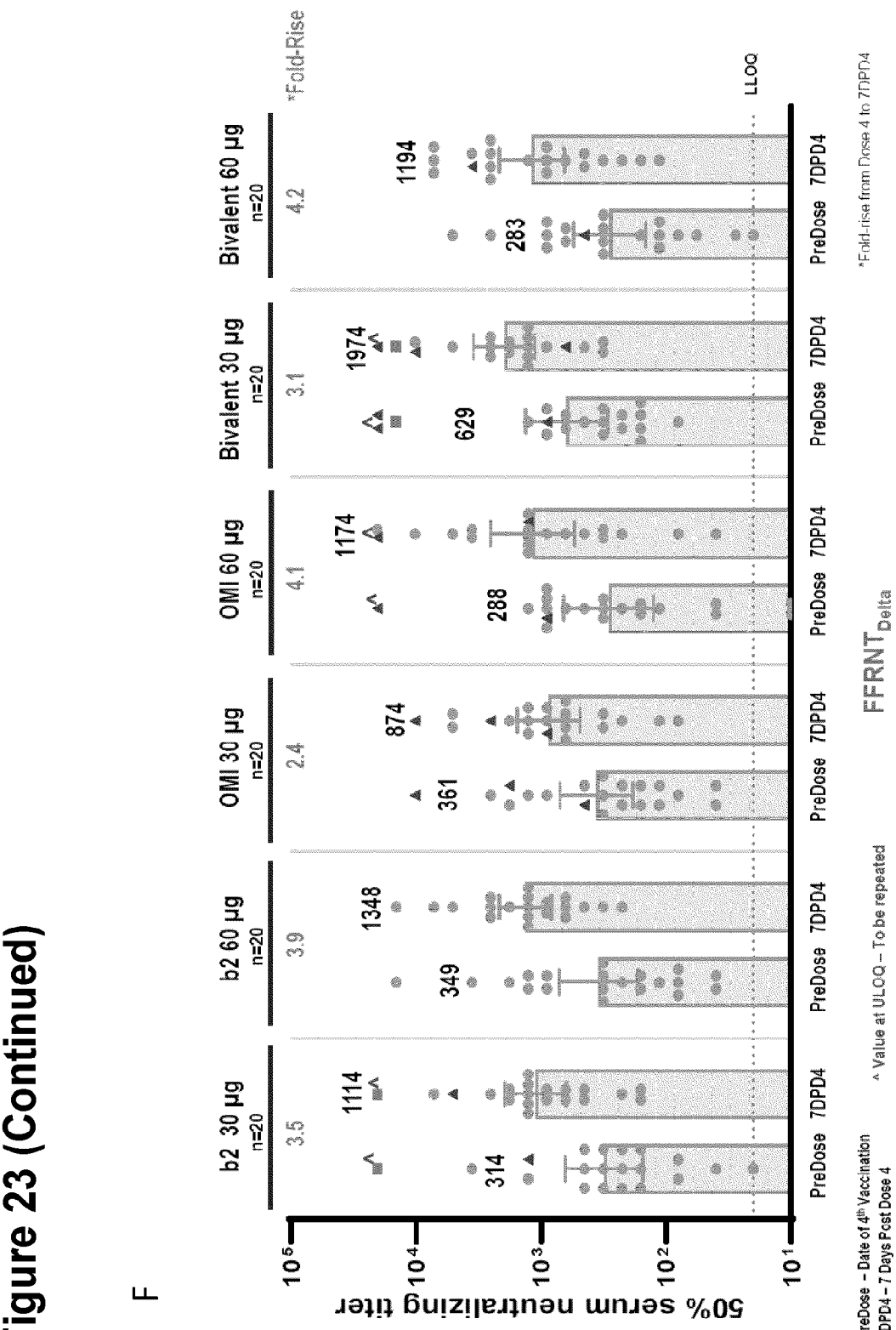


Figure 23 (Continued)

G

D7PD4 GMR vs. b2-30 µg

Subjects with evidence of existing (POS PCR) or preexisting (POS N-Ig-Binding) SARS-CoV-2 infection excluded

	b2-60 µg	OMI 30 µg	OMI 60 µg	Bivalent 30 µg	Bivalent 60 µg
WT	1.38	0.77	1.06	1.36	1.11
Delta	1.55	0.81	1.14	1.67	1.29
Omicron	1.50	1.26	2.00	1.78	1.95

All subjects included

	b2-60 µg	OMI 30 µg	OMI 60 µg	Bivalent 30 µg	Bivalent 60 µg
WT	1.05	0.68	0.95	1.39	0.90
Delta	1.21	0.78	1.05	1.77	1.07
Omicron	1.09	1.15	1.83	2.03	1.54

Figure 24

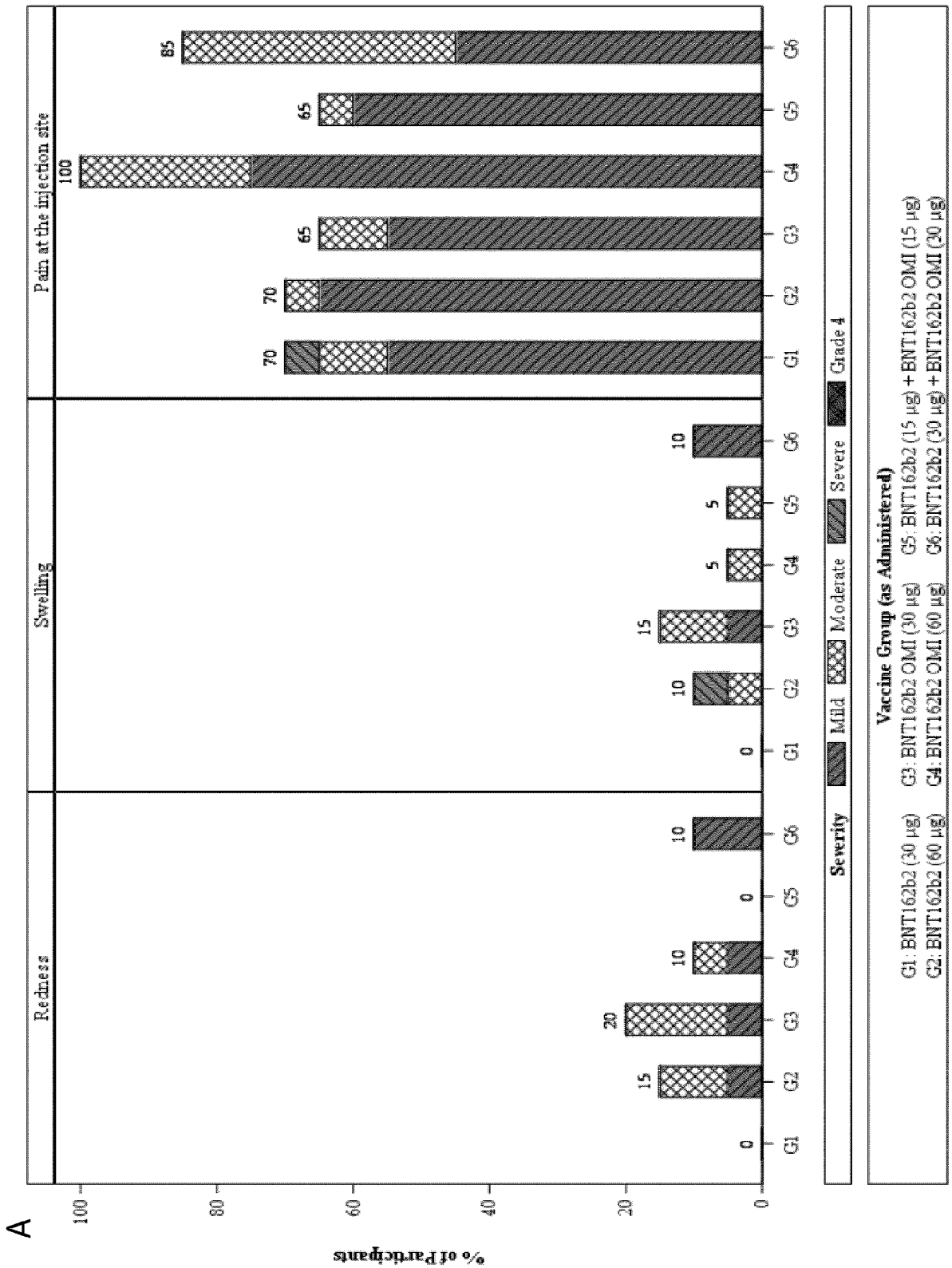
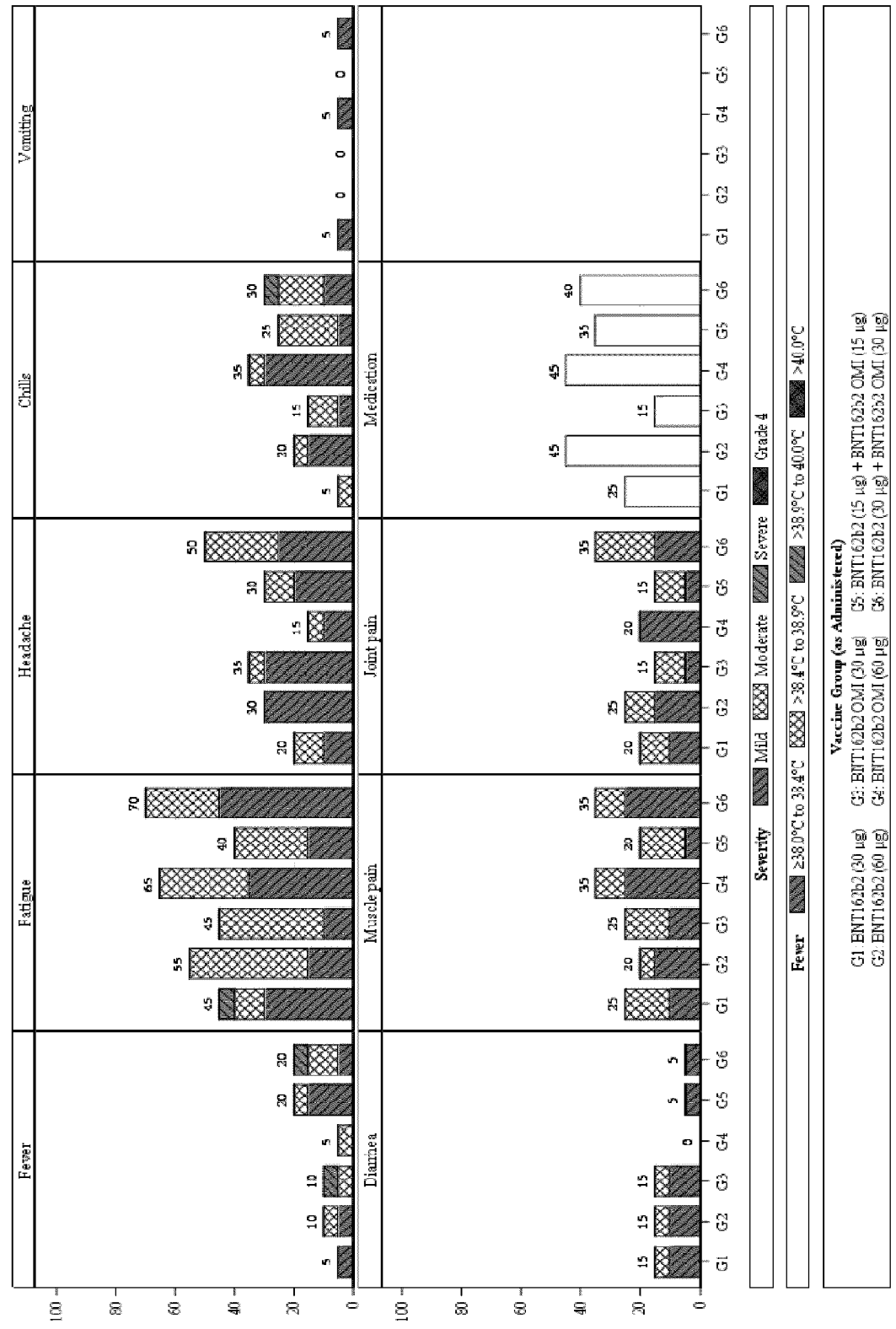

A
% of Participants
Redness
Swelling
Pain at the injection site
Severity
Mild
Moderate
Severe
Grade 4
Vaccine Group (as Administered)
G1: BNT162b2 (30 µg)
G2: BNT162b2 (60 µg)
G3: BNT162b2 OMI (30 µg)
G4: BNT162b2 OMI (60 µg)
G5: BNT162b2 (15 µg) + BNT162b2 OMI (15 µg)
G6: BNT162b2 (30 µg) + BNT162b2 OMI (30 µg)

Figure 24 (Continued)

B



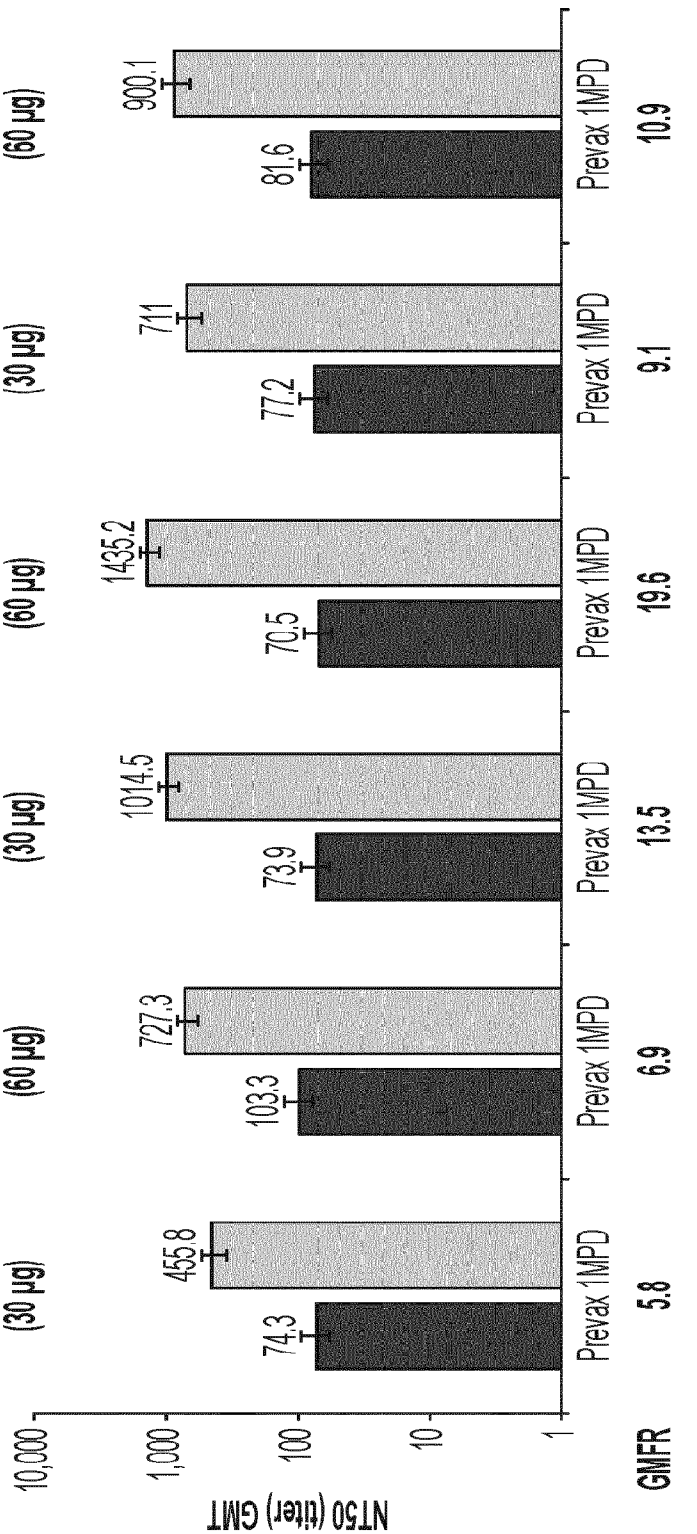


Figure 25

CA 03238757 2024-5-21

Figure 26

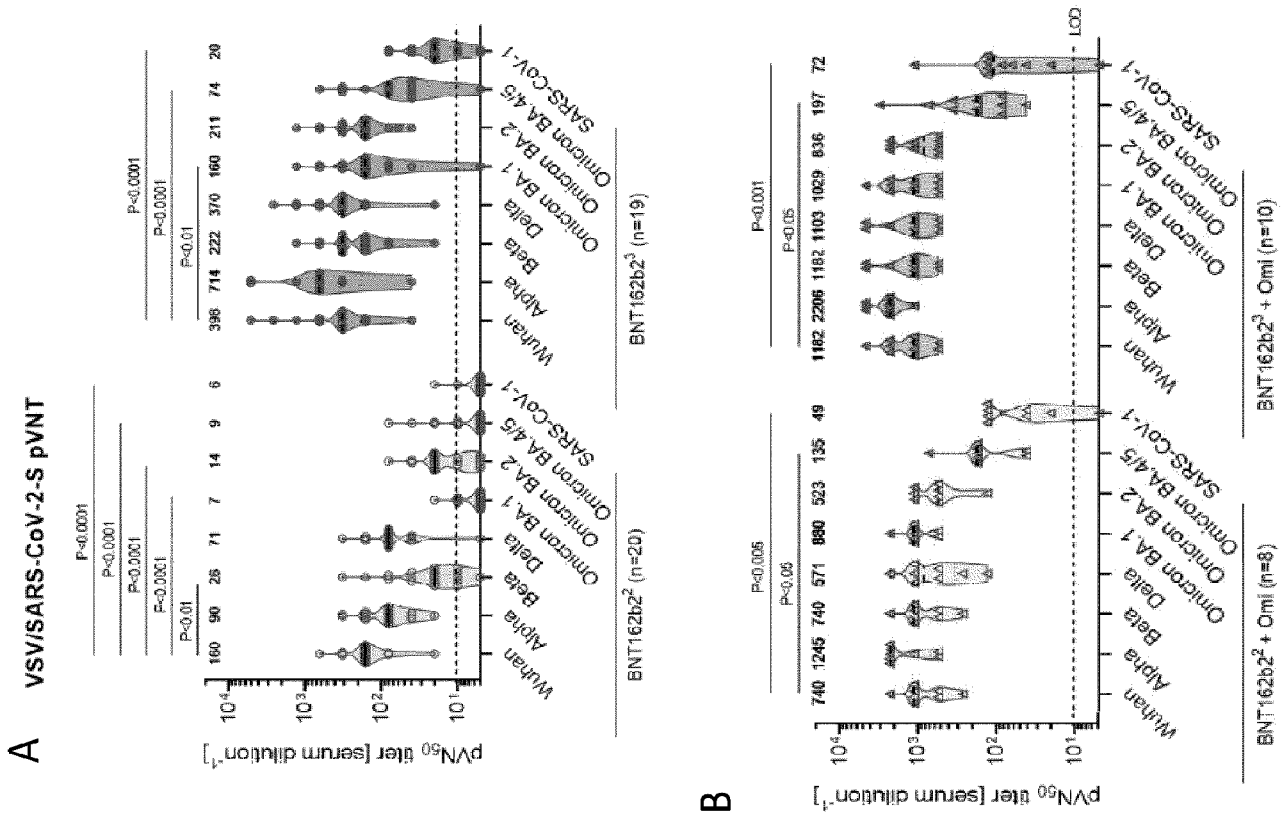
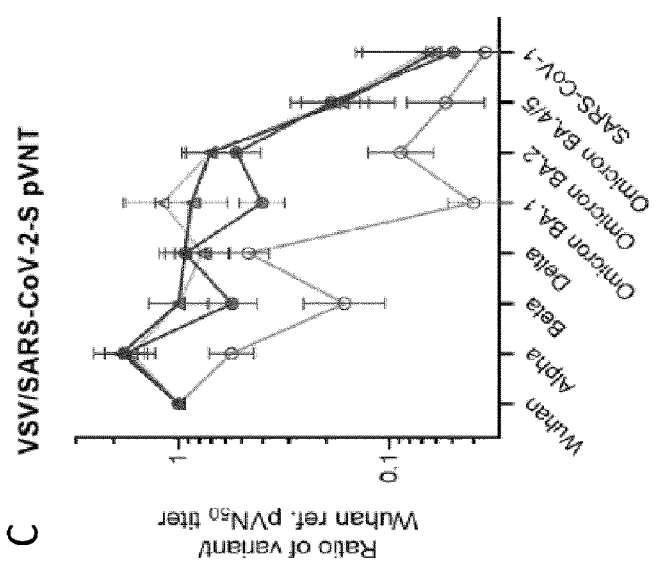

A
VSV/SARS-CoV-2-S pVNT
P<0.0001
P<0.0001
P<0.0001
P<0.0001
P<0.01
P<0.0001
P<0.0001
P<0.01
160 90 26 71 7 14 9 6 396 714 222 370 160 211 74 20
pVN50 titer [serum dilution-1]
Wuhan
Alpha
Beta
Delta
Omicron BA.1
Omicron BA.2
Omicron BA.4/5
SARS-CoV-1
BNT162b2² (n=20)
BNT162b2³ (n=19)
B
P<0.001
P<0.05
P<0.05
P<0.005
740 1245 740 571 880 523 135 49 1162 2206 1182 1103 1029 836 197 72
LOD
BNT162b2² + Omi (n=8)
BNT162b2³ + Omi (n=10)

Figure 26 (Continued)



Geometric mean ratio	Alpha	Beta	Delta	Omi BA.1	Omi BA.2	Omi BA.4/5	SARS-CoV-1
BNT162b2[2] (n=20)	0.56	0.16	0.46	0.04	0.09	0.05	0.04
BNT162b2[3] (n=19)	1.79	0.56	0.93	0.40	0.53	0.19	0.05
BNT162b2[2] + Omi (n=8)	1.68	1.00	0.77	1.19	0.71	0.18	0.07
BNT162b2[3] + Omi (n=8)	1.87	1.00	0.93	0.87	0.71	0.17	0.06

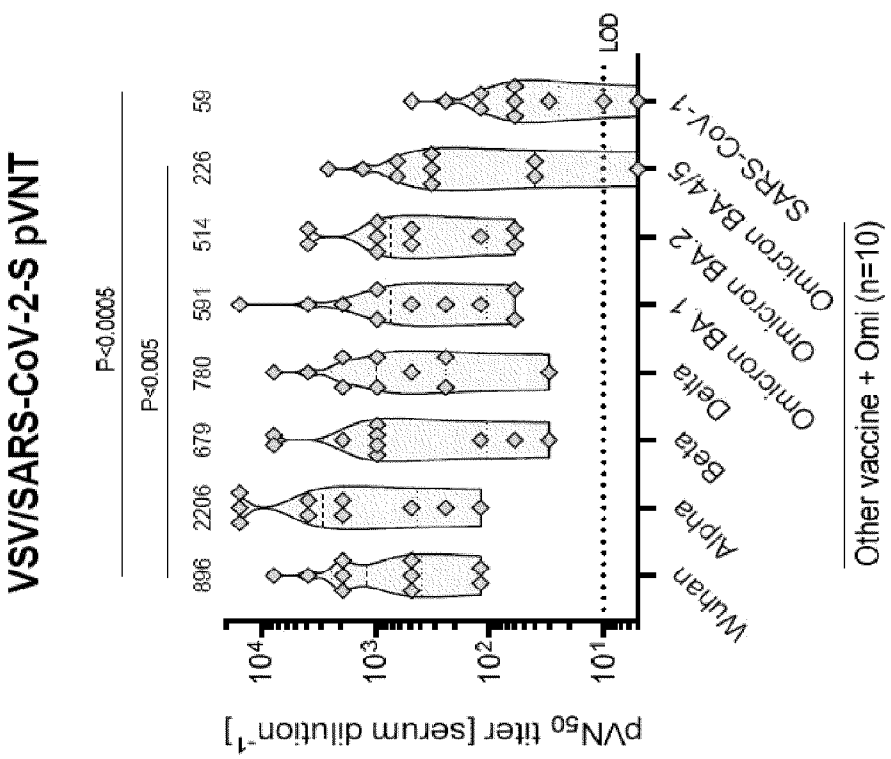

VSV/SARS-CoV-2-S pVNT
P<0.0005
P<0.005
896
2206
679
780
591
514
226
59
LOD
Wuhan
Alpha
Beta
Delta
Omicron BA.1
Omicron BA.2
Omicron BA.4/5
SARS-CoV-1
Other vaccine + Omi (n=10)
pVN50 titer [serum dilution-1]
10^4
10^3
10^2
10^1

Figure 27

Figure 28

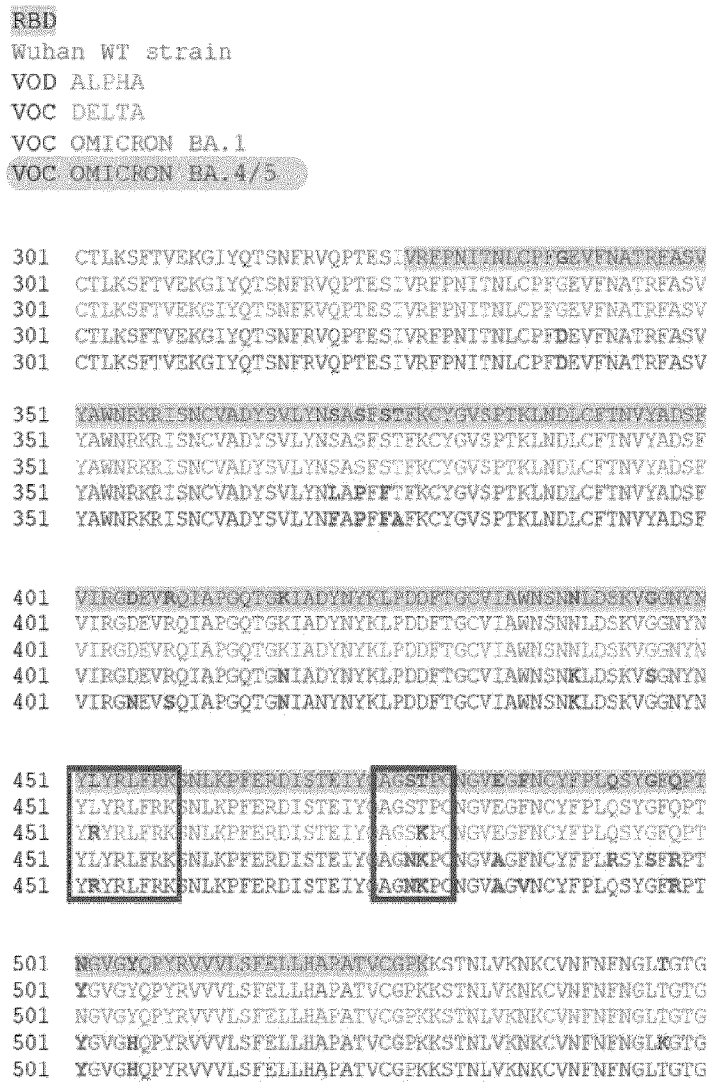

RBD
Wuhan WT strain
VOD ALPHA
VOC DELTA
VOC OMICRON BA.1
VOC OMICRON BA.4/5
301 CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASV
301 CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASV
301 CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASV
301 CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFDEVFNATRFASV
301 CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFDEVFNATRFASV
351 YAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSF
351 YAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSF
351 YAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSF
351 YAWNRKRISNCVADYSVLYNLAPFFTFKCYGVSPTKLNDLCFTNVYADSF
351 YAWNRKRISNCVADYSVLYNFAPFFAFKCYGVSPTKLNDLCFTNVYADSF
401 VIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYN
401 VIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYN
401 VIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYN
401 VIRGDEVRQIAPGQTGNIADYNYKLPDDFTGCVIAWNSNKLDSKVSGNYN
401 VIRGNEVSQIAPGQTGNIANYNYKLPDDFTGCVIAWNSNKLDSKVGGNYN
451 YLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPT
451 YLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPT
451 YRYRLFRKSNLKPFERDISTEIYQAGSKPCNGVEGFNCYFPLQSYGFQPT
451 YLYRLFRKSNLKPFERDISTEIYQAGNKPCNGVAGFNCYFPLRSYSFRPT
451 YRYRLFRKSNLKPFERDISTEIYQAGNKPCNGVAGVNCYFPLQSYGFRPT
501 NGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTG
501 YGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTG
501 NGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTG
501 YGVGHQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLKGTG
501 YGVGHQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTG

Figure 29

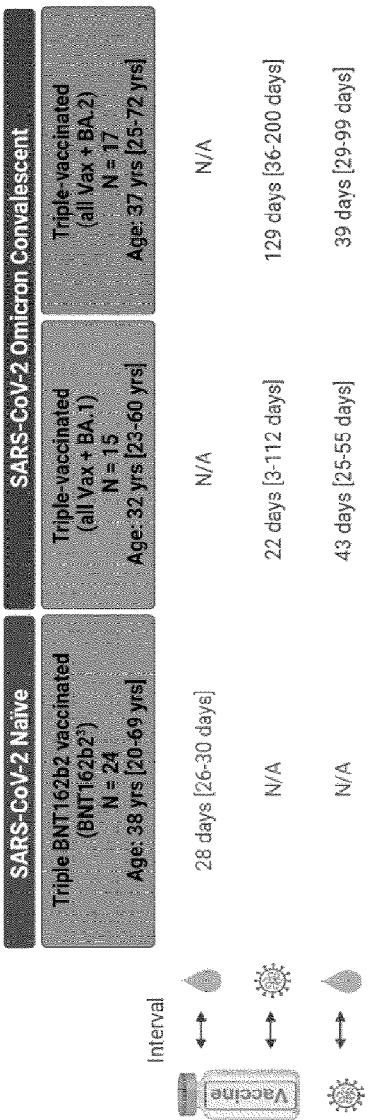

SARS-CoV-2 Naïve
SARS-CoV-2 Omicron Convalescent
Triple BNT162b2 vaccinated (BNT162b2[3])
N = 24
Age: 38 yrs [20-69 yrs]
Triple-vaccinated (all Vax + BA.1)
N = 15
Age: 32 yrs [23-60 yrs]
Triple-vaccinated (all Vax + BA.2)
N = 17
Age: 37 yrs [25-72 yrs]
Interval
Vaccine
28 days [26-30 days]
N/A
N/A
N/A
22 days [3-112 days]
129 days [36-200 days]
N/A
43 days [25-55 days]
39 days [29-99 days]

Figure 30

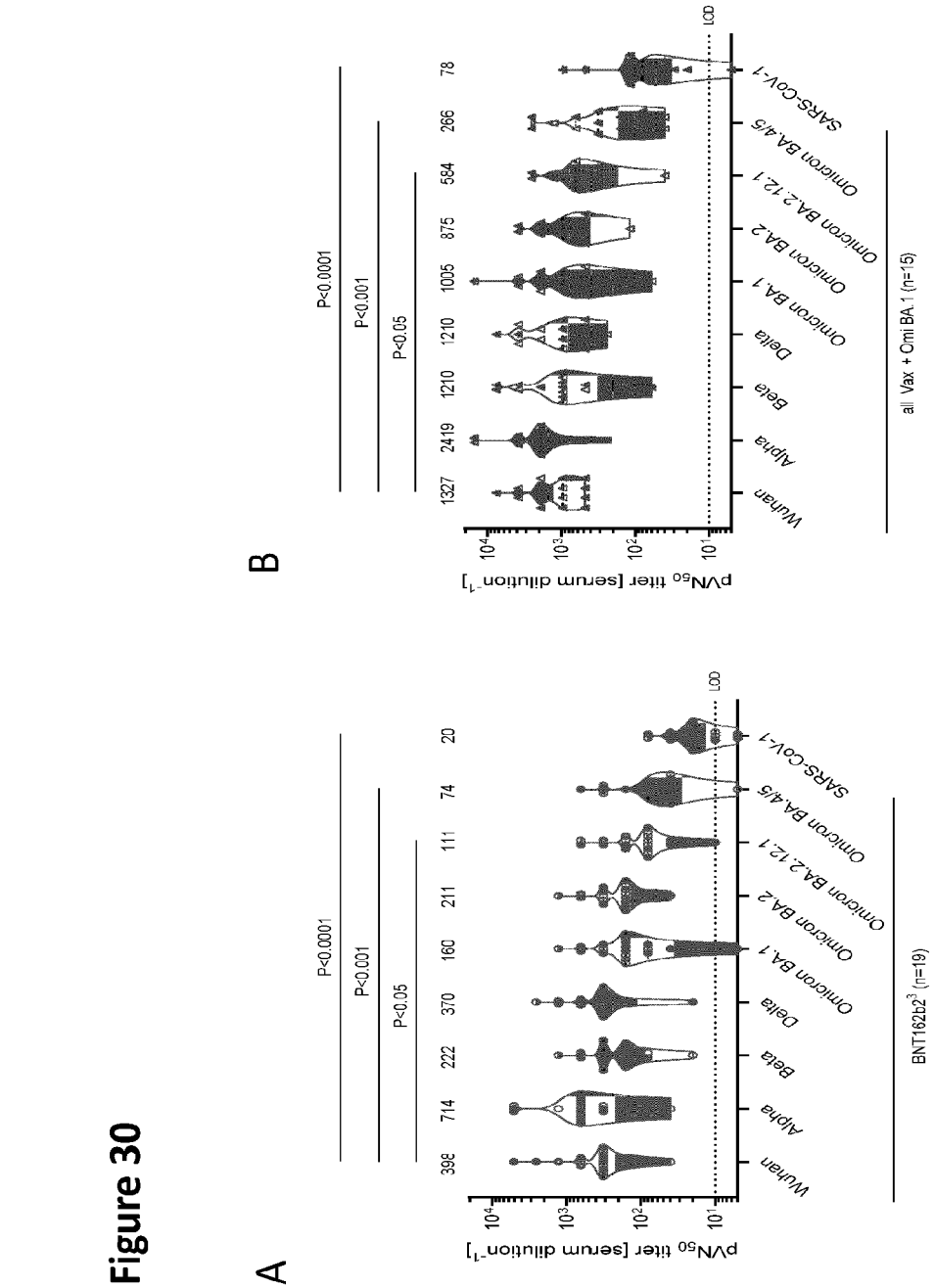

A
B
pVN50 titer [serum dilution-1]
LOD
P<0.0001
P<0.001
P<0.05
398
714
222
370
160
211
111
74
20
1327
2419
1210
1210
1005
875
584
266
78
Wuhan
Alpha
Beta
Delta
Omicron BA.1
Omicron BA.2
Omicron BA.2.12.1
Omicron BA.4/5
SARS-CoV-1
BNT162b2³ (n=19)
all Vax + Omi BA.1 (n=15)

Figure 31

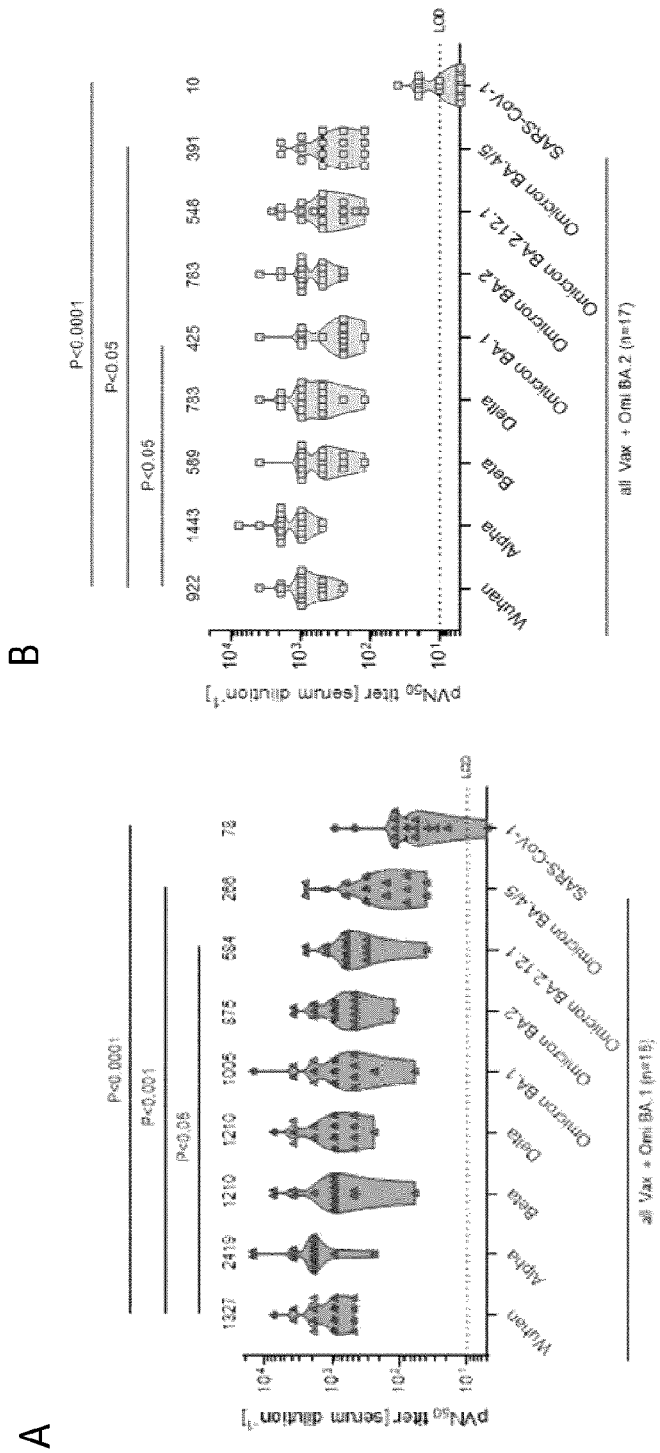

A
B
pVN50 titer [serum dilution-1]
LOD
P<0.0001
P<0.001
P<0.05
1327
2419
1210
1210
1005
875
584
256
78
922
1443
589
733
425
783
546
391
10
Wuhan
Alpha
Beta
Delta
Omicron BA.1
Omicron BA.2
Omicron BA.2.12.1
Omicron BA.4/5
SARS-CoV-1
all Vax + Omi BA.1 (n=18)
all Vax + Omi BA.2 (n=17)

Figure 31 (Continued)

C

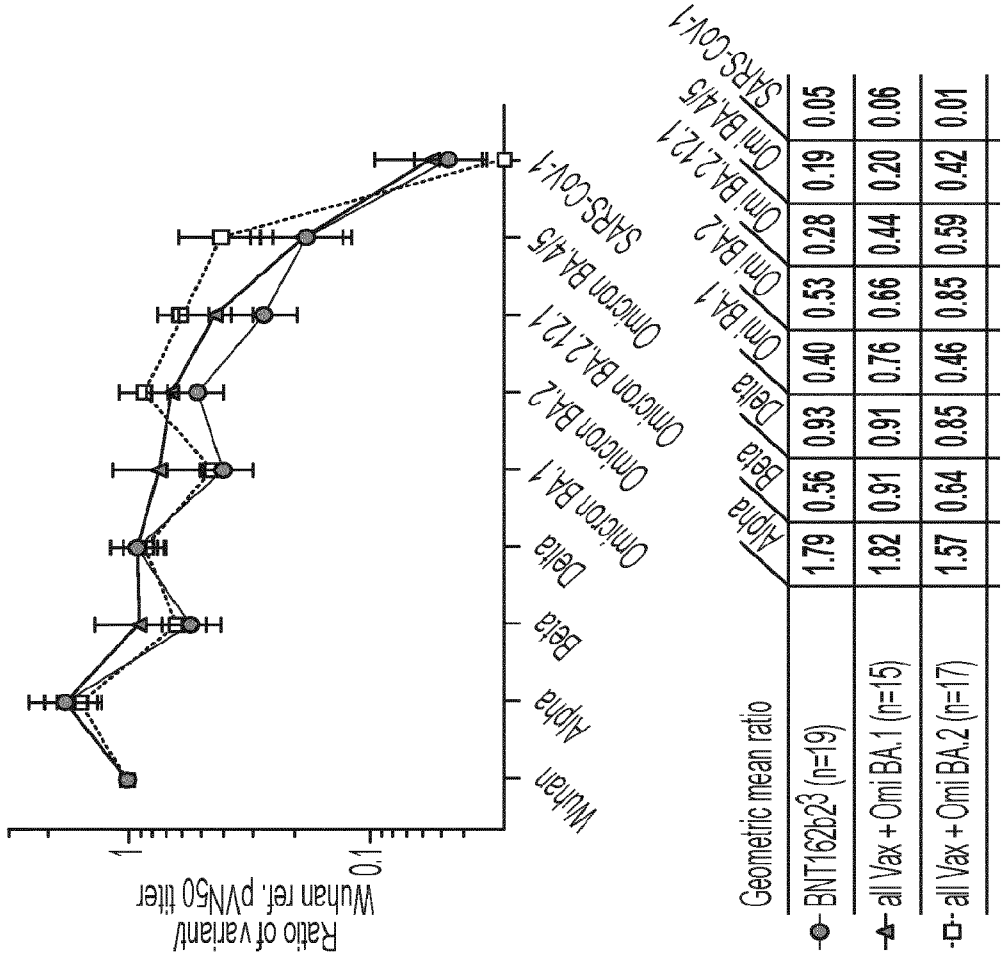


Figure 32

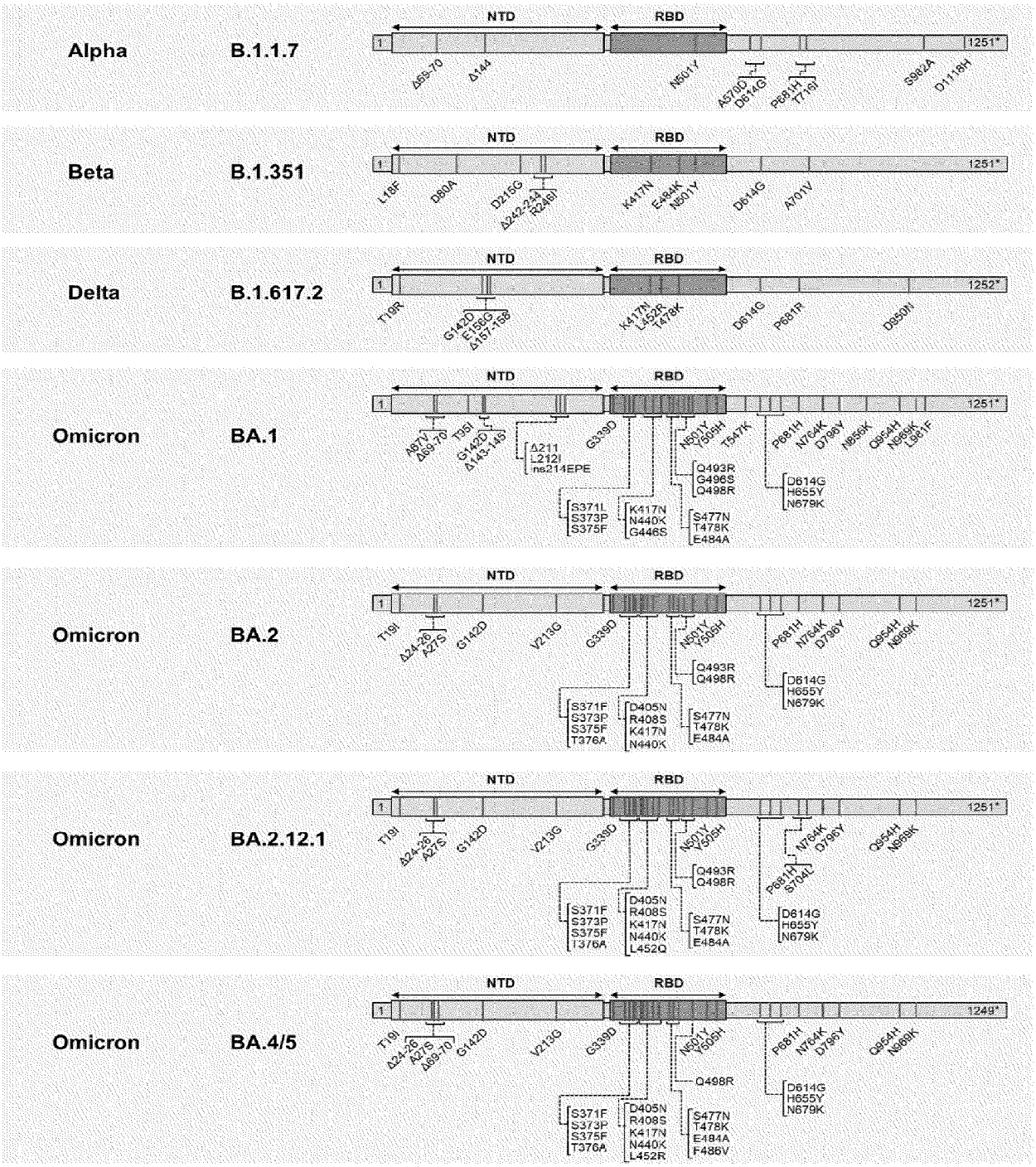

Alpha
B.1.1.7
NTD
RBD
1
1251*
Δ69-70
Δ144
N501Y
A570D
D614G
P681H
T716I
S982A
D1118H
Beta
B.1.351
1
1251*
L18F
D80A
D215G
Δ242-244
R246I
K417N
E484K
N501Y
D614G
A701V
Delta
B.1.617.2
1
1252*
T19R
G142D
E156G
Δ157-158
K417N
L452R
T478K
D614G
P681R
D950N
Omicron
BA.1
1
1251*
A67V
Δ69-70
T95I
G142D
Δ143-145
Δ211
L212I
ins214EPE
G339D
S371L
S373P
S375F
K417N
N440K
G446S
N501Y
Y505H
T547K
Q493R
G496S
Q498R
S477N
T478K
E484A
P681H
N764K
D796Y
N856K
Q954H
N969K
L981F
D614G
H655Y
N679K
Omicron
BA.2
1
1251*
T19I
Δ24-26
A27S
G142D
V213G
G339D
S371F
S373P
S375F
T376A
D405N
R408S
K417N
N440K
N501Y
Y505H
Q493R
Q498R
S477N
T478K
E484A
P681H
N764K
D796Y
Q954H
N969K
D614G
H655Y
N679K
Omicron
BA.2.12.1
1
1251*
T19I
Δ24-26
A27S
G142D
V213G
G339D
S371F
S373P
S375F
T376A
D405N
R408S
K417N
N440K
L452Q
N501Y
Y505H
Q493R
Q498R
S477N
T478K
E484A
N764K
D796Y
P681H
S704L
Q954H
N969K
D614G
H655Y
N679K
Omicron
BA.4/5
1
1249*
T19I
Δ24-26
A27S
Δ69-70
G142D
V213G
G339D
S371F
S373P
S375F
T376A
D405N
R408S
K417N
N440K
L452R
N501Y
Y505H
Q498R
S477N
T478K
E484A
F486V
P681H
N764K
D796Y
Q954H
N969K
D614G
H655Y
N679K

Figure 33

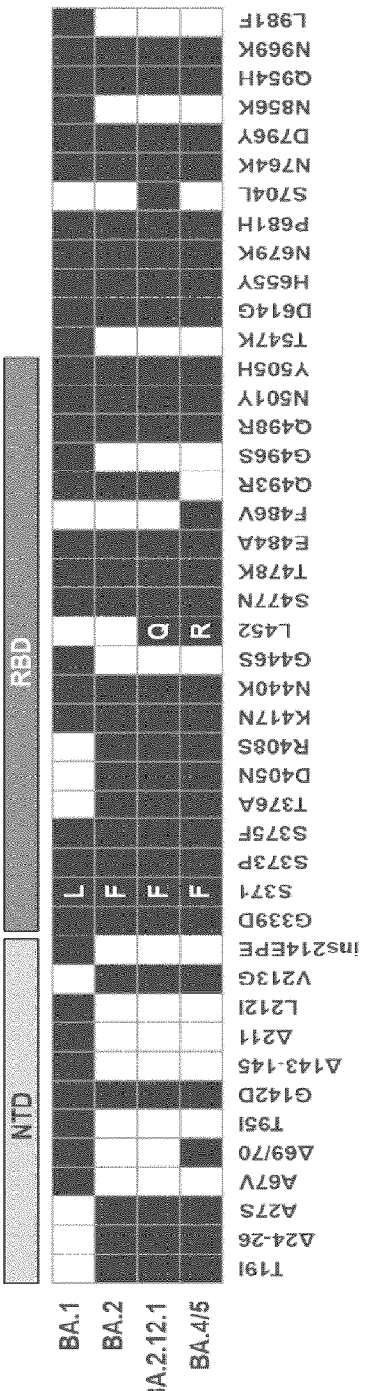
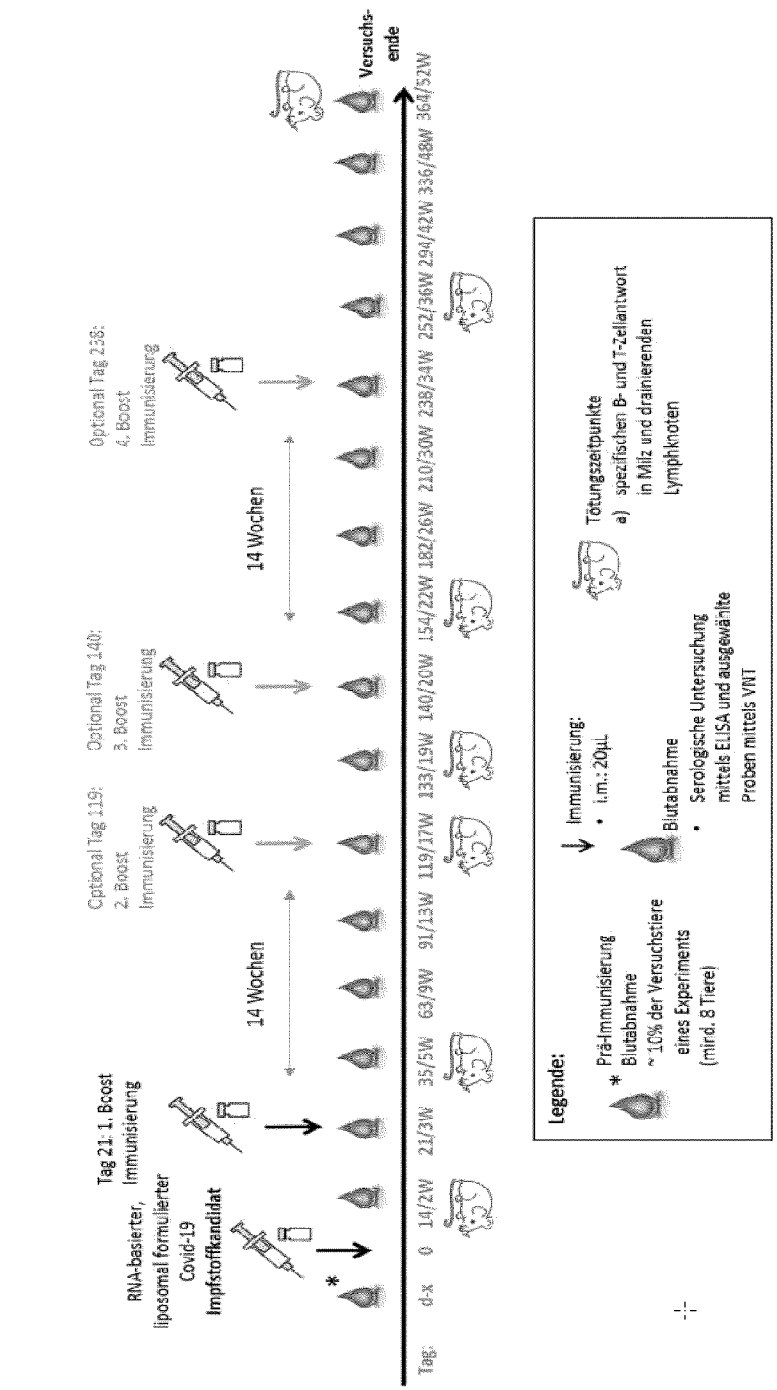

NTD
RBD
T19I
Δ24-26
A27S
A67V
Δ69/70
T95I
G142D
Δ143-145
Δ211
L212I
V213G
ins214EPE
G339D
S371 L F F F
S373P
S375F
T376A
D405N
R408S
K417N
N440K
G446S
L452 Q R
S477N
T478K
E484A
F486V
Q493R
G496S
Q498R
N501Y
Y505H
T547K
D614G
H655Y
N679K
P681H
S704L
N764K
D796Y
N856K
Q954H
N969K
L981F
BA.1
BA.2
BA.2.12.1
BA.4/5

Figure 34



Translation:

Ausgewahlte proben = selected samples

Blutabnahme = blood draw

Immunisierung = immunization

Mittels = by means of

Pra = pre

Serologische untersuchung = serological investigation

Spezifischen = specific

Versuchsende = trial end

Wochen = weeks

Figure 35

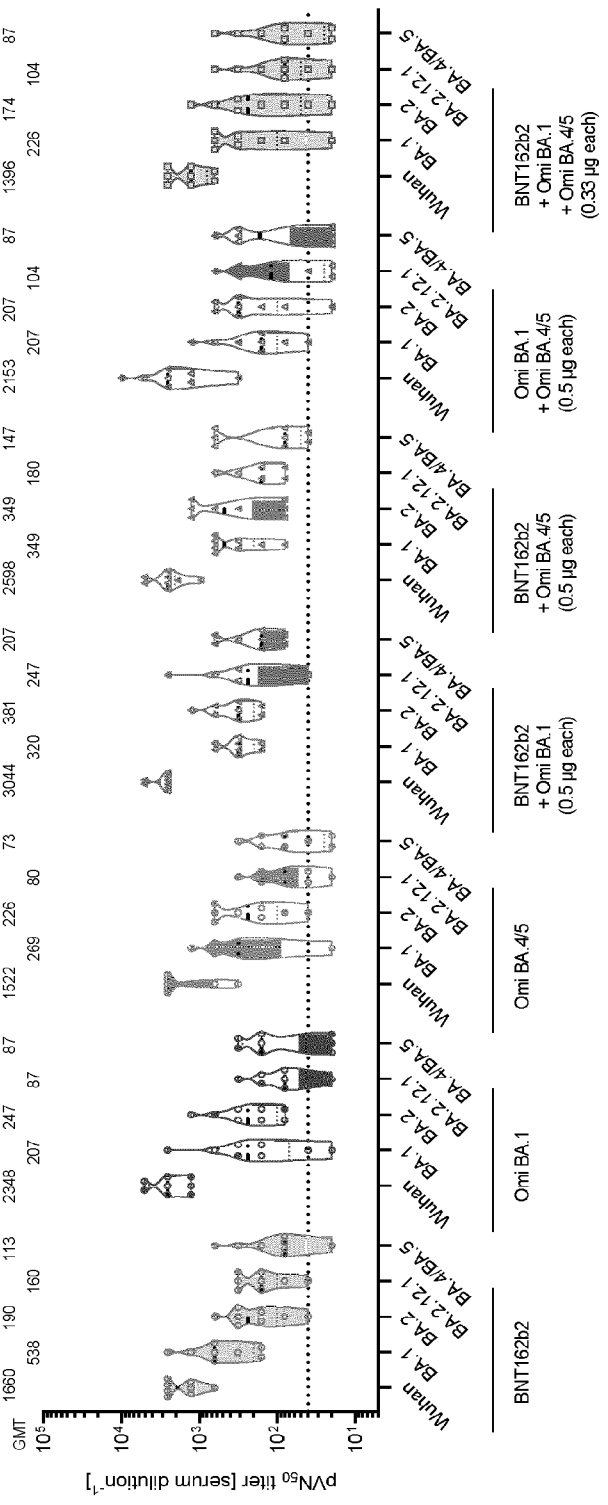

pVN50 titer [serum dilution-1]
GMT
1660 538 190 160 113 2348 207 247 87 87 1522 269 226 80 73 3044 320 381 247 207 2598 349 349 180 147 2153 207 207 104 87 1396 226 174 104 87
Wuhan BA.1 BA.2 BA.2.12.1 BA.4/BA.5
BNT162b2
Omi BA.1
Omi BA.4/5
BNT162b2 + Omi BA.1 (0.5 µg each)
BNT162b2 + Omi BA.4/5 (0.5 µg each)
Omi BA.1 + Omi BA.4/5 (0.5 µg each)
BNT162b2 + Omi BA.1 + Omi BA.4/5 (0.33 µg each)

Figure 36

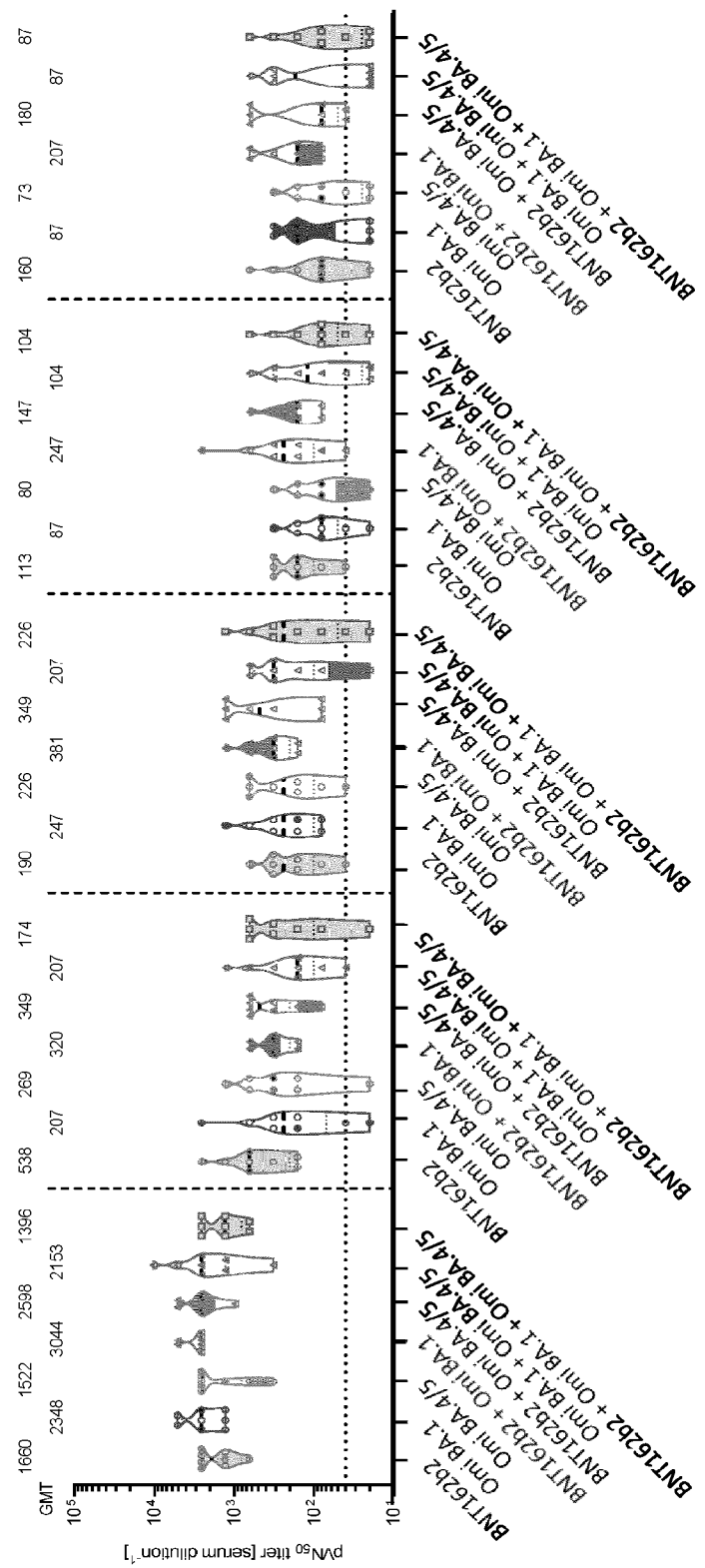
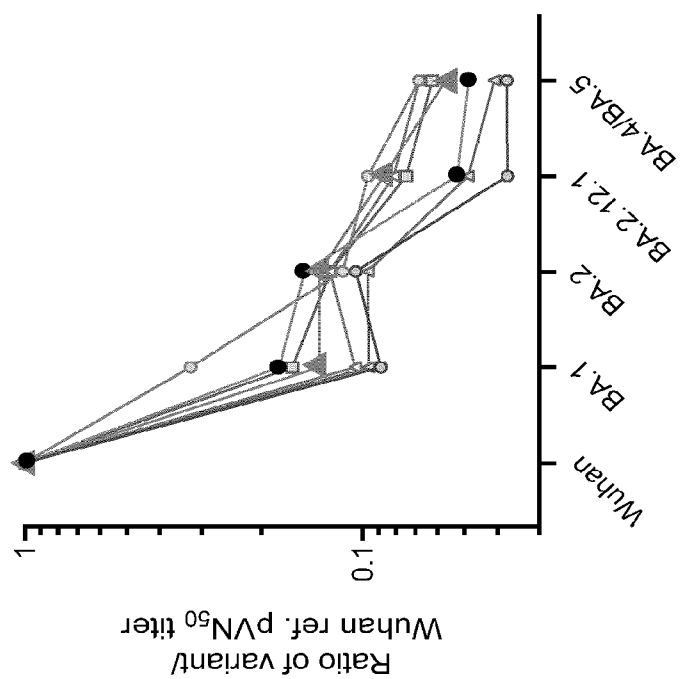

pVN50 titer [serum dilution-1]
GMT
1660
2348
1522
3044
2598
2153
1396
538
207
269
320
349
207
174
190
247
226
381
349
207
226
113
87
80
247
147
104
160
87
73
207
180
87
BNT162b2
Omi BA.1
BNT162b2 + Omi BA.4/5
Omi BA.1 + Omi BA.1
BNT162b2 + Omi BA.1 + Omi BA.4/5

Figure 37



Geometric mean ratio	Omi BA.1	Omi BA.2	Omi BA.2.12.1	Omi BA.4/BA.5
BNT162b2	0.32	0.11	0.10	0.07
Omi BA.1	0.09	0.11	0.04	0.04
Omi BA.4/5	0.18	0.15	0.05	0.05
b2 + Omi BA.1	0.11	0.13	0.08	0.07
b2 + Omi BA.4/5	0.13	0.13	0.09	0.06
Omi BA.1 + BA.4/5	0.10	0.10	0.05	0.04
b2 + Omi BA.1 + BA.4/5	0.16	0.12	0.07	0.06

Figure 38

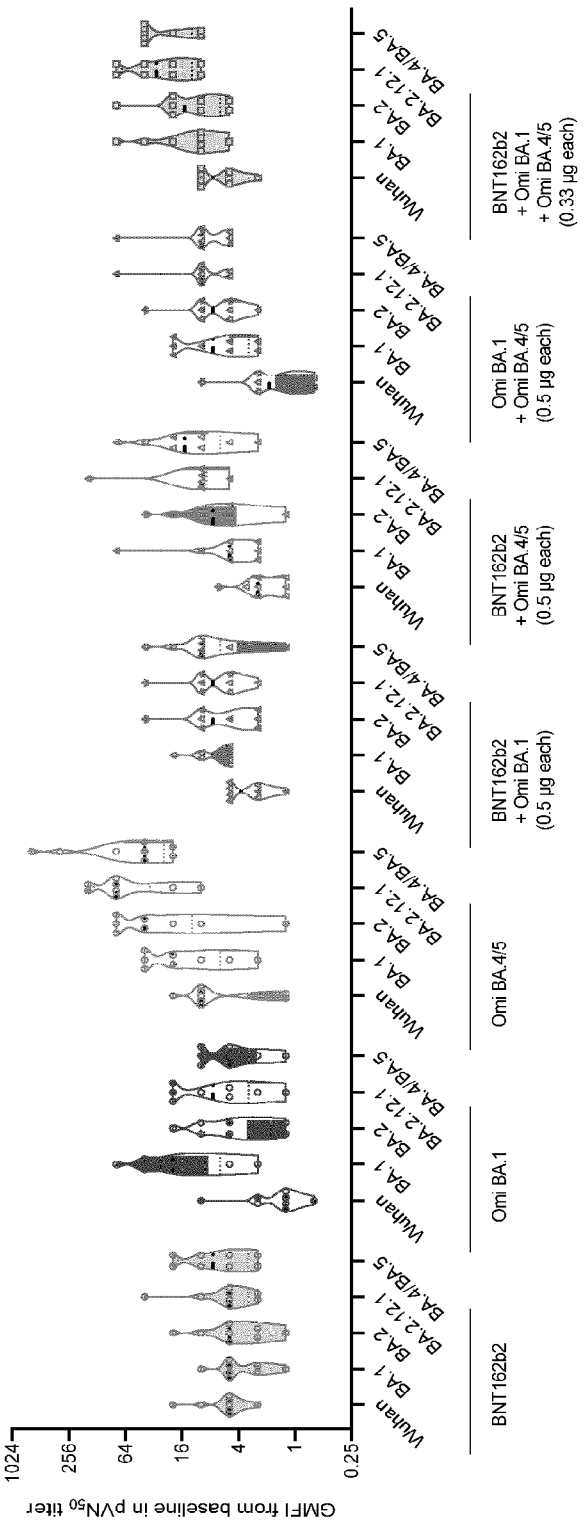

GMFI from baseline in pVN50 titer
1024
256
64
16
4
1
0.25
5.9 3.9 4.6 5.9 7.1 1.8 16.8 4.6 7.1 5.0 6.5 15.4 25.9 61.7 67.3 3.2 7.1 6.5 7.7 9.2 2.3 5.9 7.7 14.1 1.8 7.7 10.9 6.5 15.4 14.1 28.3 25.9
Wuhan BA.1 BA.2 BA.2.12.1 BA.4/BA.5
BNT162b2
Omi BA.1
Omi BA.4/5
BNT162b2 + Omi BA.1 (0.5 µg each)
BNT162b2 + Omi BA.4/5 (0.5 µg each)
Omi BA.1 + Omi BA.4/5 (0.5 µg each)
BNT162b2 + Omi BA.1 + Omi BA.4/5 (0.33 µg each)

Figure 39

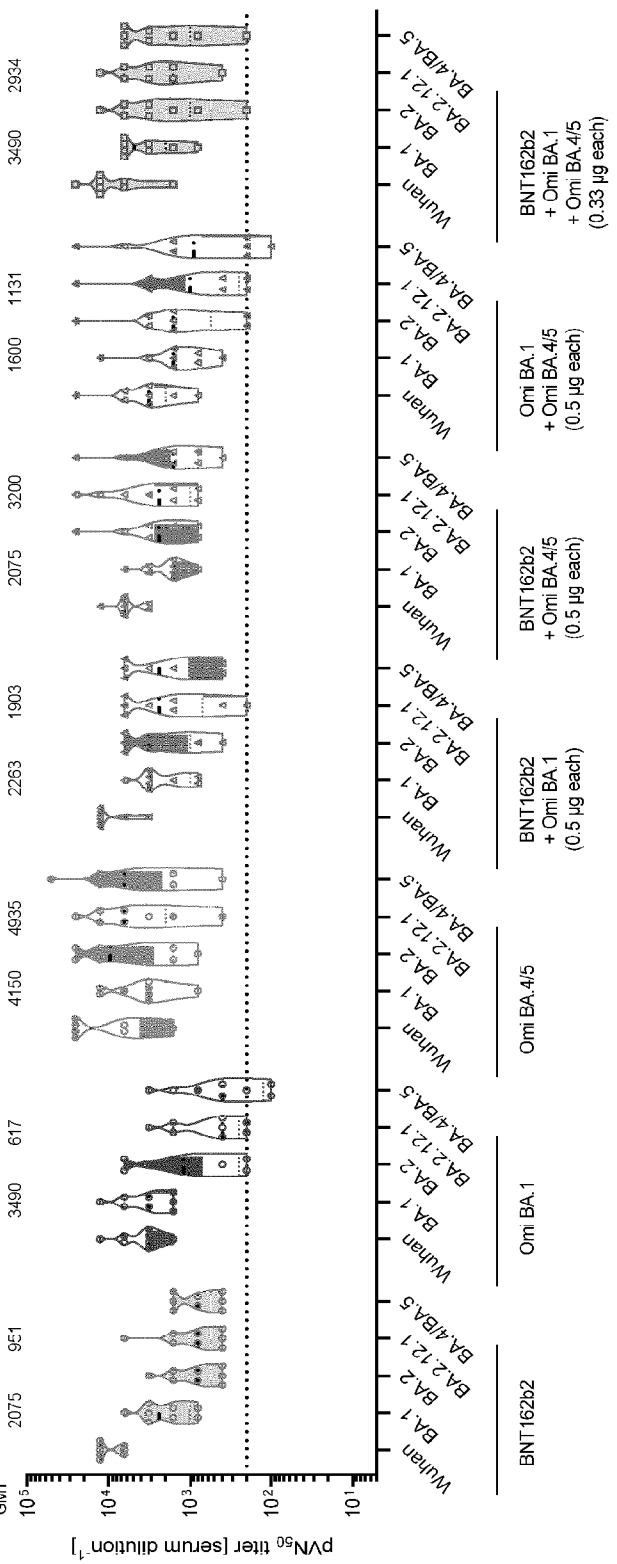
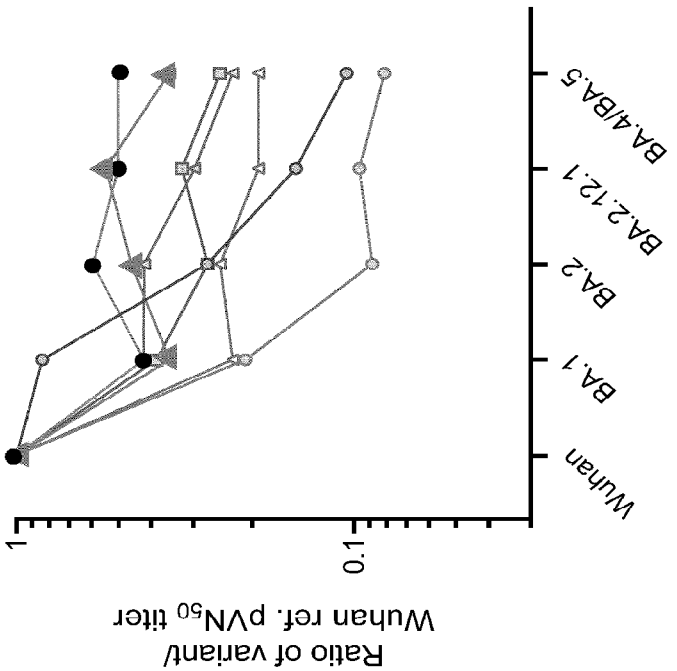

pVN50 titer [serum dilution-1]
GMT
9870 2075 872 951 800 4150 3490 1131 617 436 9670 4150 5869 4935 9670 2263 2468 1903 1963 5869 2075 2691 3200 3805 1600 1600 1131 872 9051 3490 2468 2934 2263
Wuhan BA.1 BA.2 BA.2.12.1 BA.4/BA.5
BNT162b2
Omi BA.1
Omi BA.4/5
BNT162b2 + Omi BA.1 (0.5 µg each)
BNT162b2 + Omi BA.4/5 (0.5 µg each)
Omi BA.1 + Omi BA.4/5 (0.5 µg each)
BNT162b2 + Omi BA.1 + Omi BA.4/5 (0.33 µg each)

Figure 40



Geometric mean ratio	Omi BA.1	Omi BA.2	Omi BA.2.12.1	Omi BA.4/BA.5
BNT162b2	0.21	0.09	0.10	0.08
Omi BA.1	0.84	0.27	0.15	0.11
Omi BA.4/5	0.42	0.59	0.50	0.50
b2 + Omi BA.1	0.23	0.25	0.19	0.19
b2 + Omi BA.4/5	0.35	0.46	0.55	0.35
Omi BA.1 + BA.4/5	0.42	0.42	0.30	0.23
b2 + Omi BA.1 + BA.4/5	0.39	0.27	0.32	0.25

Figure 41

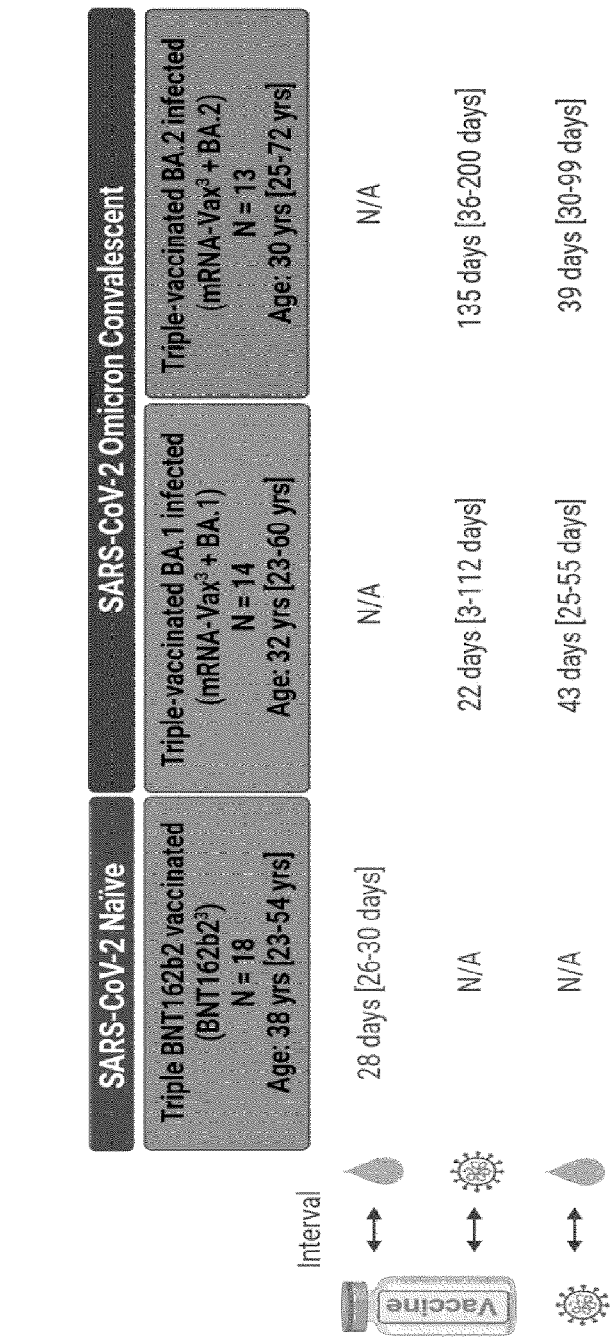

SARS-CoV-2 Naïve
SARS-CoV-2 Omicron Convalescent
Triple BNT162b2 vaccinated (BNT162b2[3])
N = 18
Age: 38 yrs [23-54 yrs]
Triple-vaccinated BA.1 infected (mRNA-Vax[3] + BA.1)
N = 14
Age: 32 yrs [23-60 yrs]
Triple-vaccinated BA.2 infected (mRNA-Vax[3] + BA.2)
N = 13
Age: 30 yrs [25-72 yrs]
Interval
Vaccine
28 days [26-30 days]
N/A
N/A
N/A
22 days [3-112 days]
135 days [36-200 days]
N/A
43 days [25-55 days]
39 days [30-99 days]

Figure 42

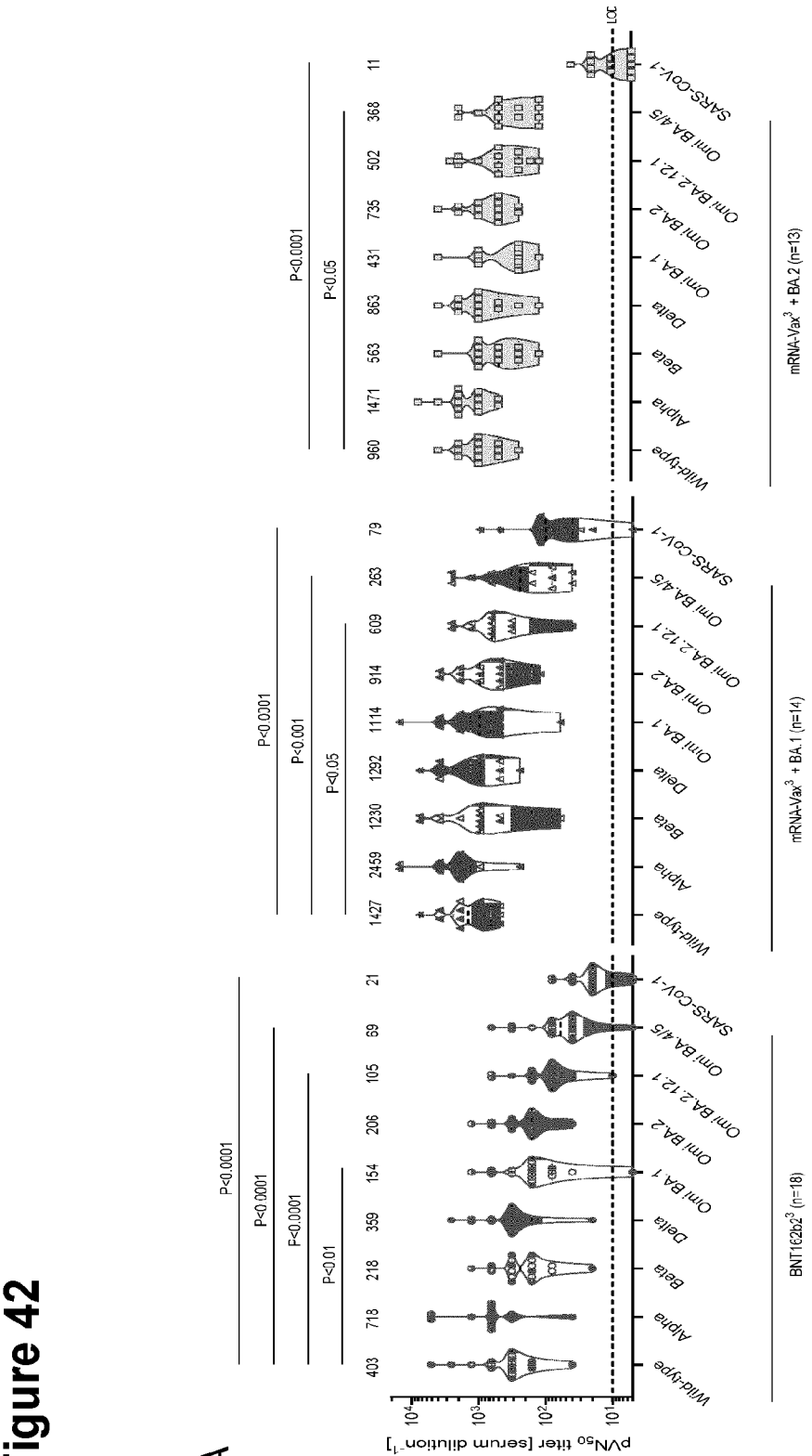
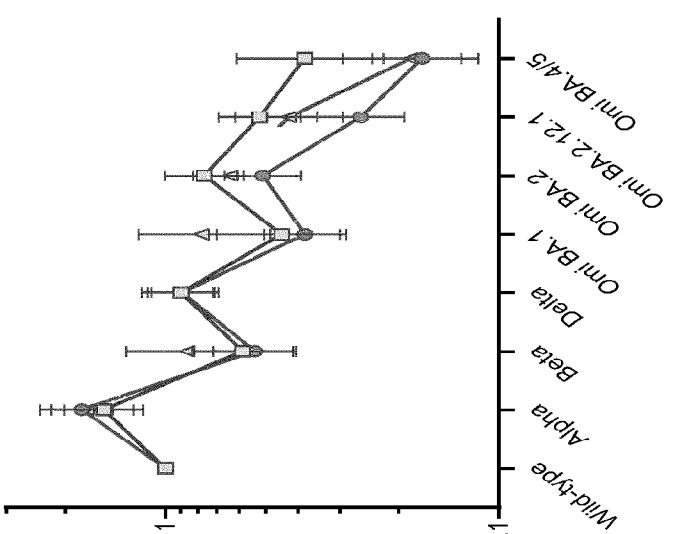

A
pVN50 titer [serum dilution-1]
LOD
P<0.0001
P<0.0001
P<0.0001
P<0.01
P<0.0301
P<0.001
P<0.05
P<0.0001
P<0.05
403 718 218 359 154 206 105 69 21
1427 2459 1230 1292 1114 914 609 263 79
960 1471 563 863 431 735 502 368 11
Wild-type
Alpha
Beta
Delta
Omi BA.1
Omi BA.2
Omi BA.2.12.1
Omi BA.4/5
SARS-CoV-1
BNT162b2[3] (n=18)
mRNA-Vax3 + BA.1 (n=14)
mRNA-Vax3 + BA.2 (n=13)

Figure 42 (Continued)

B



Geometric mean ratio	Alpha	Beta	Delta	Omi BA.1	Omi BA.2	Omi BA.2.12.1	Omi BA.4/5
BNT162b2[3] (n=18)	1.78	0.54	0.89	0.38	0.51	0.26	0.17
mRNA-Vax[3] + BA.1 (n=14)	1.72	0.86	0.91	0.78	0.64	0.43	0.18
mRNA-Vax[3] + BA.2 (n=13)	1.53	0.59	0.90	0.45	0.77	0.52	0.38

Figure 43

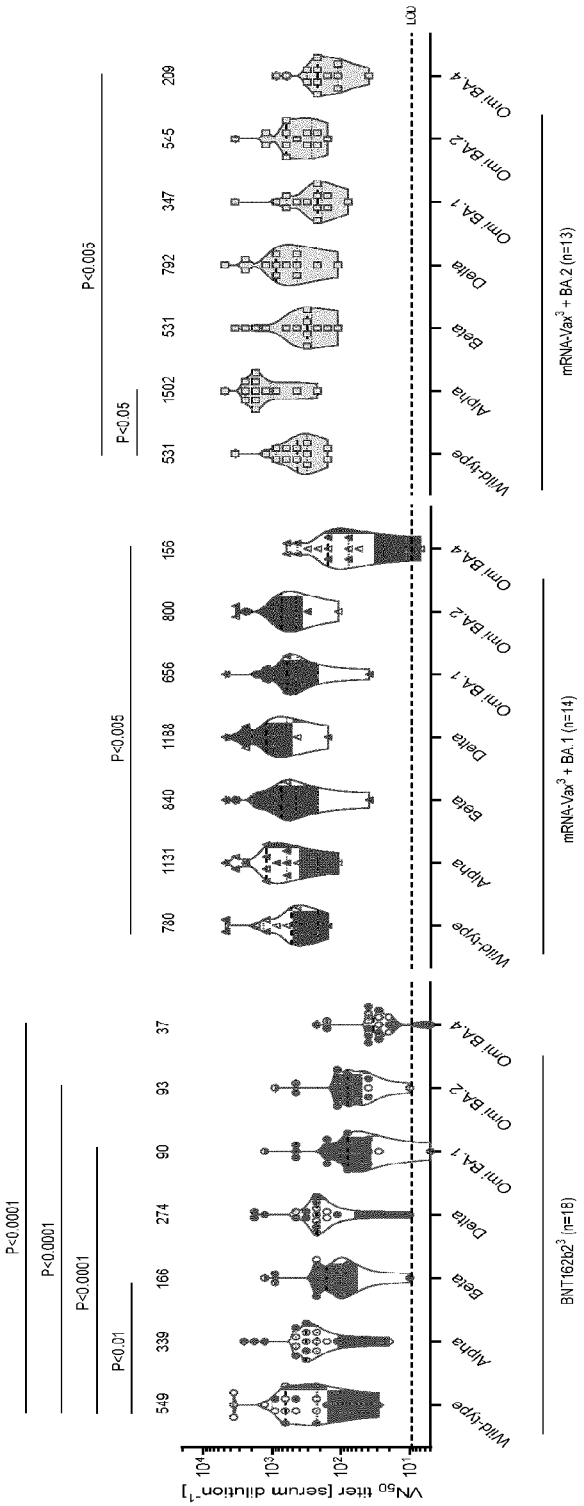
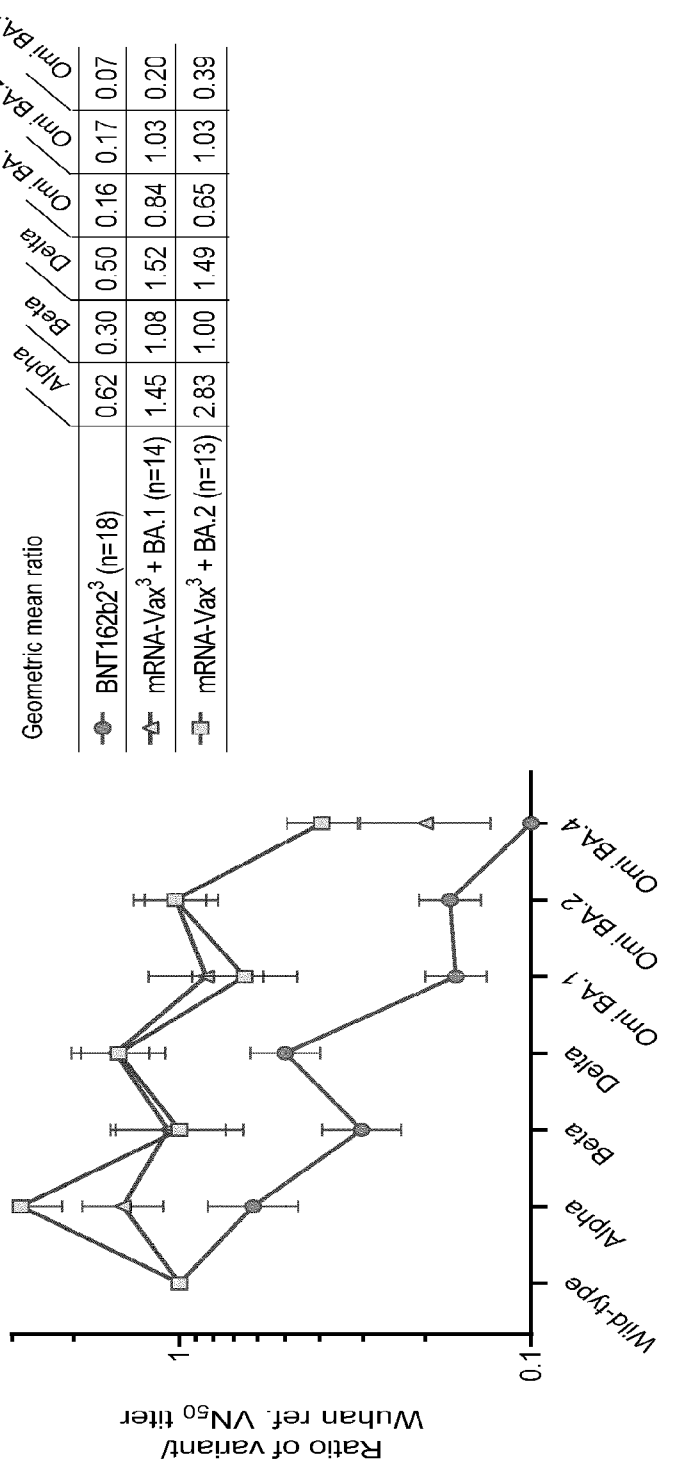

A
VN50 titer [serum dilution-1]
P<0.0001
P<0.0001
P<0.0001
P<0.01
P<0.005
P<0.005
P<0.05
549
339
166
274
90
93
37
780
1131
640
1138
656
800
156
531
1502
531
792
347
545
209
Wild-type
Alpha
Beta
Delta
Omi BA.1
Omi BA.2
Omi BA.4
BNT162b2³ (n=18)
mRNA-Vax³ + BA.1 (n=14)
mRNA-Vax³ + BA.2 (n=13)
LOD

Figure 43 (Continued)

B



Geometric mean ratio

	Alpha	Beta	Delta	Omi BA.1	Omi BA.2	Omi BA.4
BNT162b2[3] (n=18)	0.62	0.30	0.50	0.16	0.17	0.07
mRNA-Vax[3] + BA.1 (n=14)	1.45	1.08	1.52	0.84	1.03	0.20
mRNA-Vax[3] + BA.2 (n=13)	2.83	1.00	1.49	0.65	1.03	0.39

Figure 44

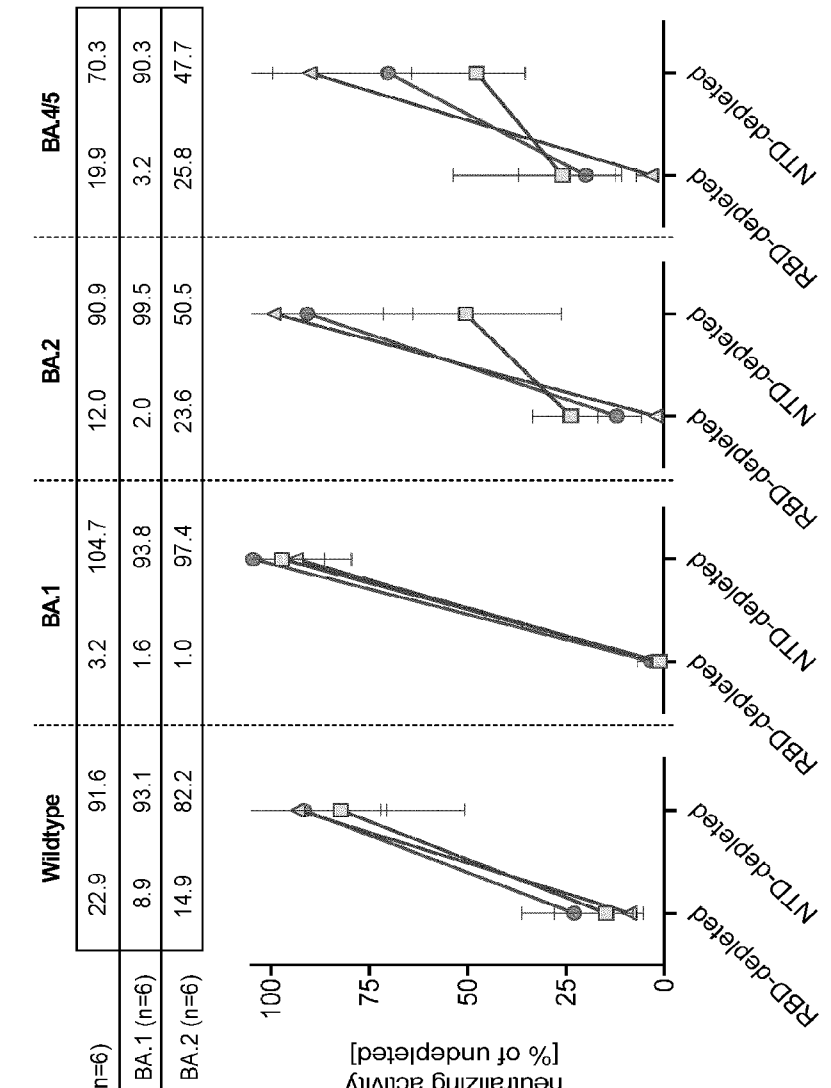

A
Wildtype
BA.1
BA.2
BA.4/5
BNT162b2[3] (n=6)
mRNA-Vax[3]+ BA.1 (n=6)
mRNA-Vax[3]+ BA.2 (n=6)
22.9 91.6 3.2 104.7 12.0 90.9 19.9 70.3
8.9 93.1 1.6 93.8 2.0 99.5 3.2 90.3
14.9 82.2 1.0 97.4 23.6 50.5 25.8 47.7
neutralizing activity
[% of undepleted]
100
75
50
25
0
RBD-depleted
NTD-depleted

Figure 44 (Continued)

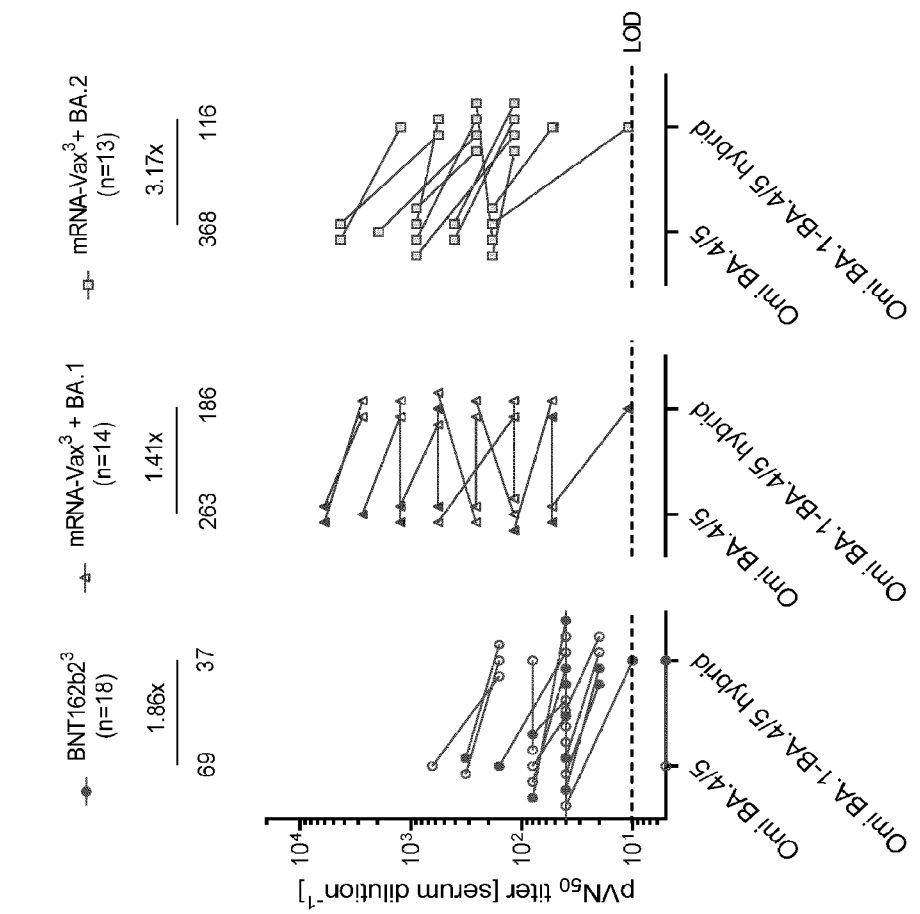


Figure 45

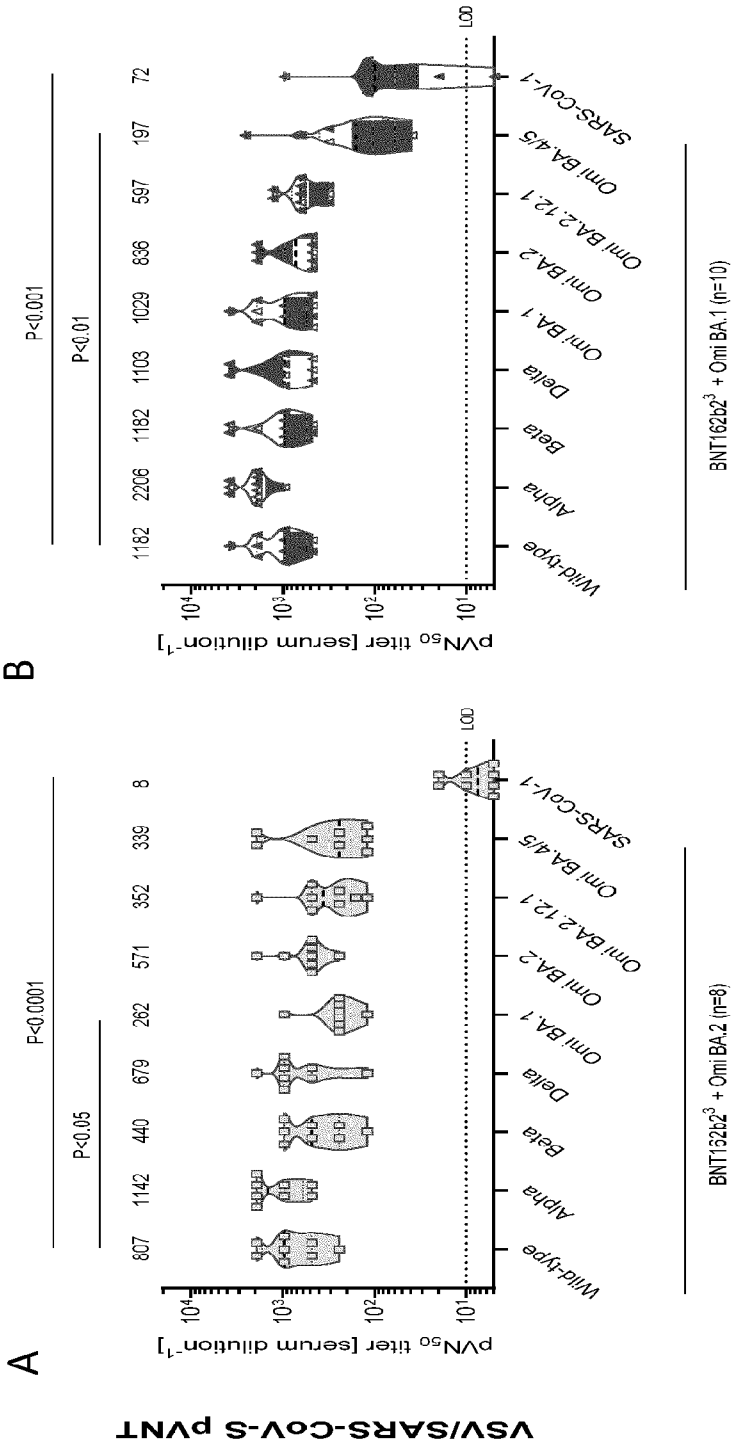
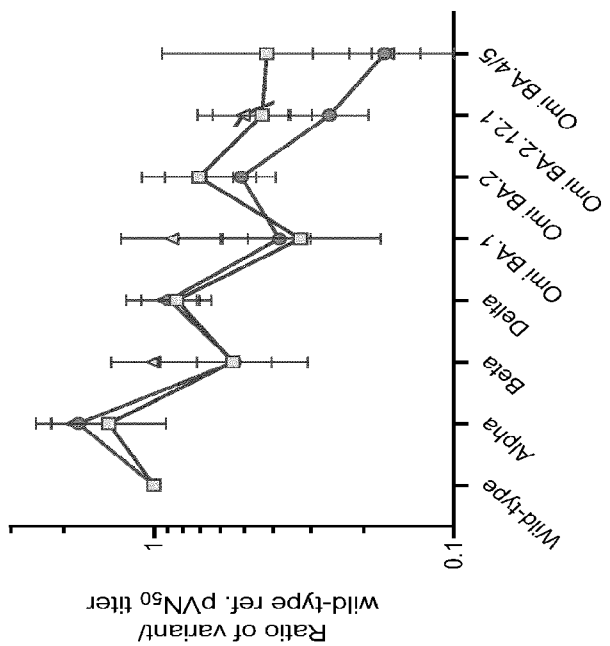

A
B
VSV/SARS-CoV-S pVNT
pVN50 titer [serum dilution-1]
LOD
P<0.0001
P<0.05
P<0.001
P<0.01
807
1142
440
679
262
571
352
339
8
1182
2206
1182
1103
1029
836
597
197
72
Wild-type
Alpha
Beta
Delta
Omi BA.1
Omi BA.2
Omi BA.2.12.1
Omi BA.4/5
SARS-CoV-1
BNT162b2[3] + Omi BA.2 (n=8)
BNT162b2[3] + Omi BA.1 (n=10)

Figure 45 (continued)

C



Geometric mean ratio	Alpha	Beta	Delta	Omi BA.1	Omi BA.2	Omi BA.2.12.1	Omi BA.4/5
BNT162b2[3] (n=18)	1.78	0.54	0.89	0.38	0.51	0.26	0.17
BNT162b2[3] + BA.1 (n=10)	1.87	1.00	0.93	0.87	0.71	0.51	0.17
BNT162b2[3] + BA.2 (n=8)	1.41	0.55	0.84	0.32	0.71	0.44	0.42

Figure 45 (continued)

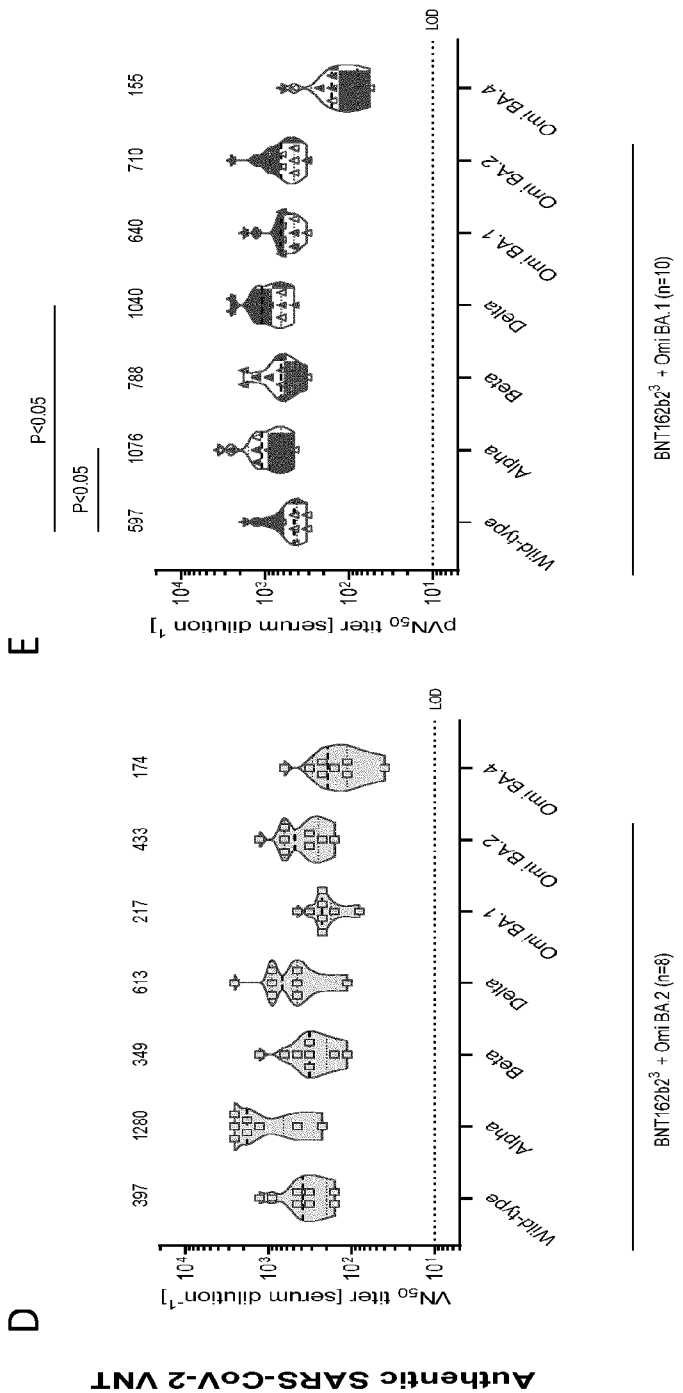
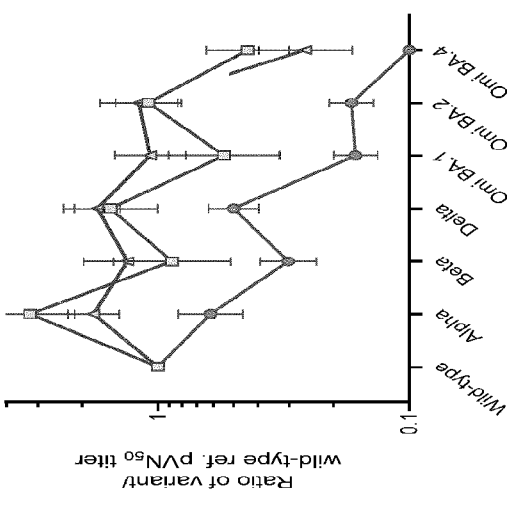


Figure 45 (continued)

F



Geometric mean ratio	Alpha	Beta	Delta	Omi BA.1	Omi BA.2	Omi BA.4
BNT162b2[3] (n=18)	0.62	0.30	0.50	0.16	0.17	0.07
BNT162b2[3] + BA.1 (n=10)	1.80	1.32	1.74	1.07	1.19	0.26
BNT162b2[3] + BA.2 (n=8)	3.22	0.88	1.54	0.55	1.09	0.44

Figure 46

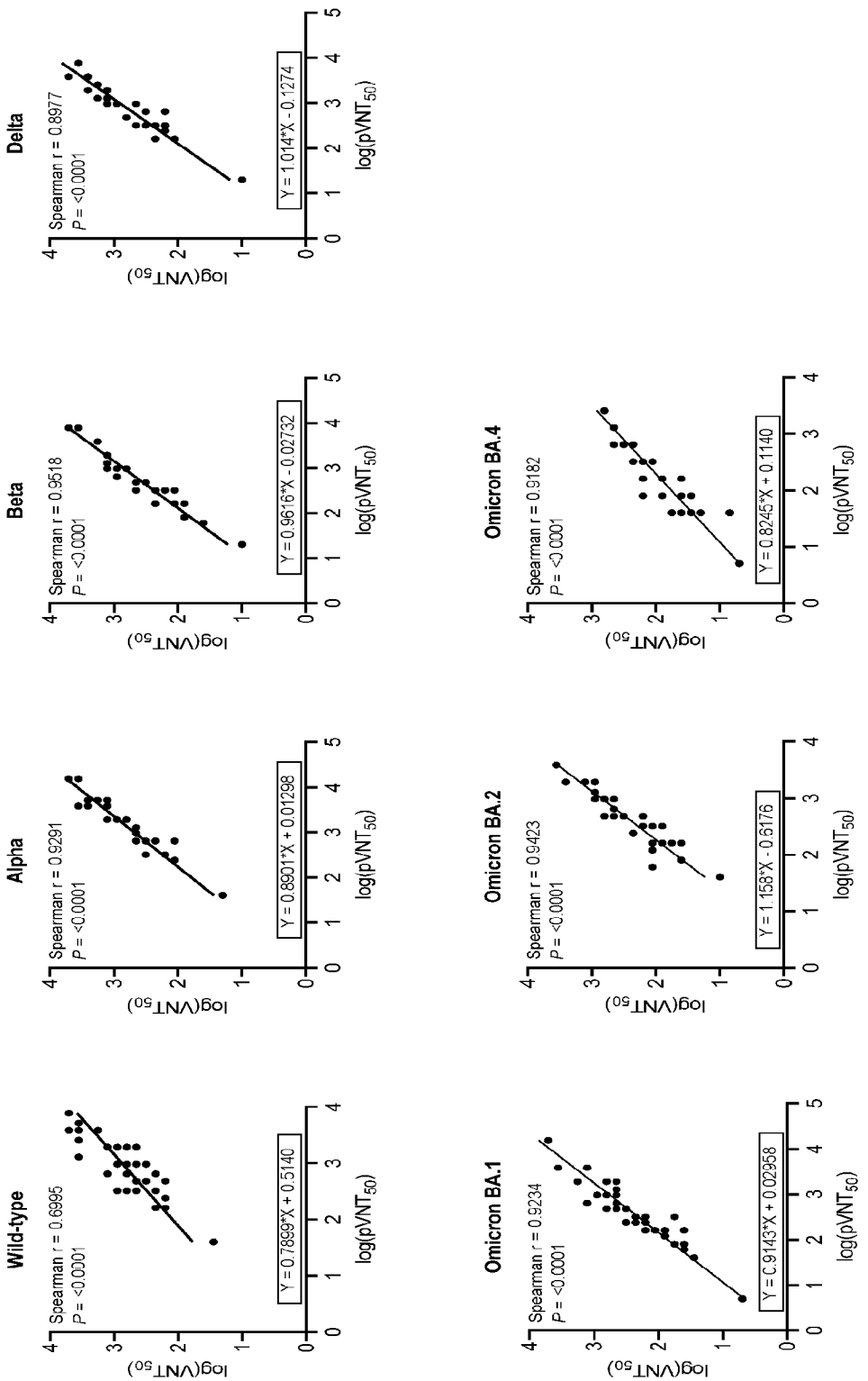
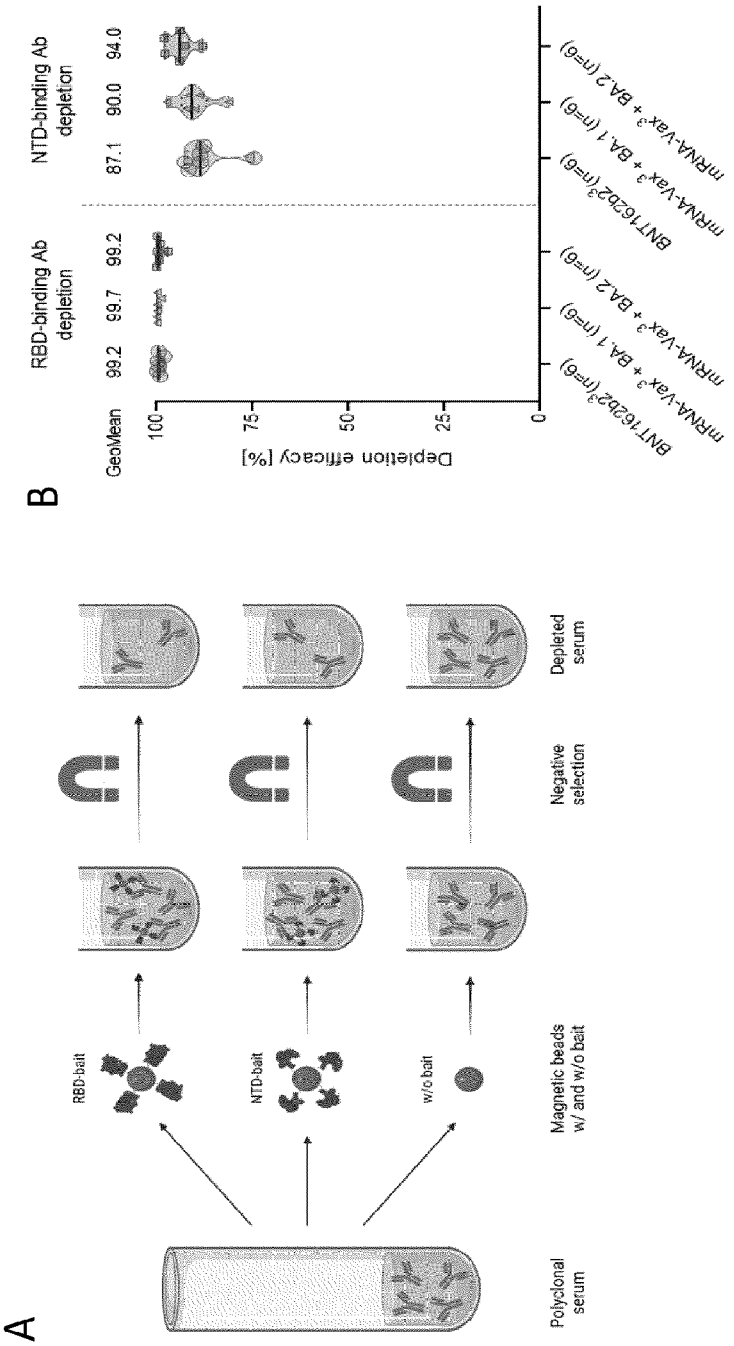

Wild-type
Spearman r = 0.6995
P = <0.0001
Y = 0.7869*X + 0.5140
Alpha
Spearman r = 0.9291
P = <0.0001
Y = 0.8931*X + 0.01298
Beta
Spearman r = 0.9518
P = <0.0001
Y = 0.9616*X - 0.02732
Delta
Spearman r = 0.8977
P = <0.0001
Y = 1.014*X - 0.1274
Omicron BA.1
Spearman r = 0.9234
P = <0.0001
Y = 0.9143*X + 0.02958
Omicron BA.2
Spearman r = 0.9423
P = <0.0001
Y = 1.158*X - 0.6176
Omicron BA.4
Spearman r = 0.9182
P = <0.0001
Y = 0.8245*X + 0.1140
log(VNT50)
log(pVNT50)

Figure 47


A
Polyclonal serum
RBD-bait
NTD-bait
w/o bait
Magnetic beads w/ and w/o bait
Negative selection
Depleted serum
B
RBD-binding Ab depletion
NTD-binding Ab depletion
GeoMean
99.2
99.7
99.2
87.1
90.0
94.0
Depletion efficacy [%]
100
75
50
25
0
BNT162b2 (n=6)
mRNA-Vax + BA.1 (n=6)
mRNA-Vax + BA.2 (n=6)
BNT162b2 (n=6)
mRNA-Vax + BA.1 (n=6)
mRNA-Vax + BA.2 (n=6)

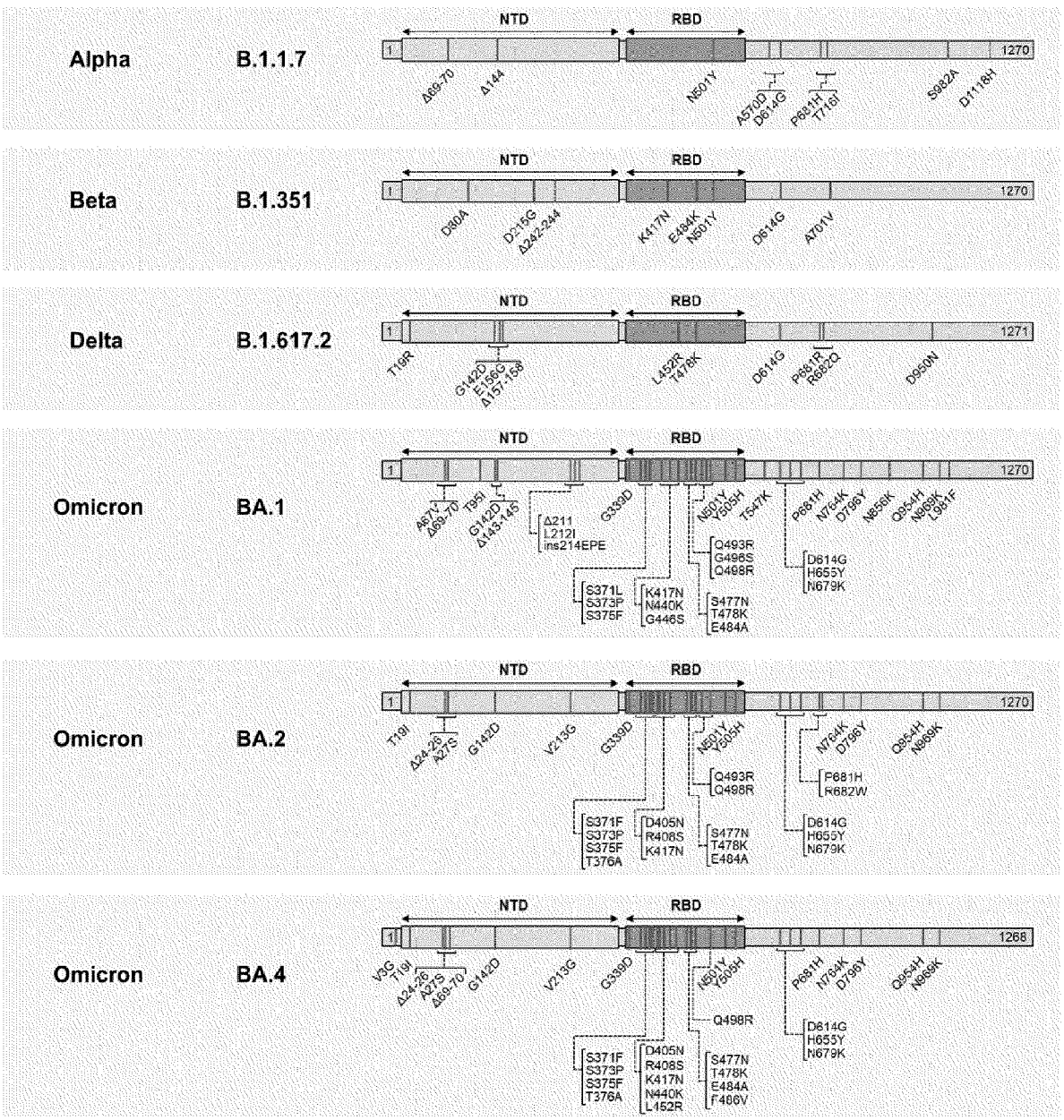

Alpha
B.1.1.7
NTD
RBD
1
1270
Δ69-70
Δ144
N501Y
A570D
D614G
P681H
T716I
S982A
D1118H
Beta
B.1.351
NTD
RBD
1
1270
D80A
D215G
Δ242-244
K417N
E484K
N501Y
D614G
A701V
Delta
B.1.617.2
NTD
RBD
1
1271
T19R
G142D
E156G
Δ157-158
L452R
T478K
D614G
P681R
R682Q
D950N
Omicron
BA.1
NTD
RBD
1
1270
A67V
Δ69-70
T95I
G142D
Δ143-145
Δ211
L212I
ins214EPE
G339D
N501Y
T505H
T547K
P681H
N764K
D796Y
N856K
Q954H
N969K
L981F
S371L
S373P
S375F
K417N
N440K
G446S
Q493R
G496S
Q498R
S477N
T478K
E484A
D614G
H655Y
N679K
Omicron
BA.2
NTD
RBD
1
1270
T19I
Δ24-26
A27S
G142D
V213G
G339D
N501Y
T505H
N764K
D796Y
Q954H
N969K
Q493R
Q498R
P681H
R682W
S371F
S373P
S375F
T376A
D405N
R408S
K417N
S477N
T478K
E484A
D614G
H655Y
N679K
Omicron
BA.4
NTD
RBD
1
1268
V3G
T19I
Δ24-26
A27S
Δ69-70
G142D
V213G
G339D
N501Y
T505H
P681H
N764K
D796Y
Q954H
N969K
Q498R
S371F
S373P
S375F
T376A
D405N
R408S
K417N
N440K
L452R
S477N
T478K
E484A
F486V
D614G
H655Y
N679K

Figure 48

Figure 49

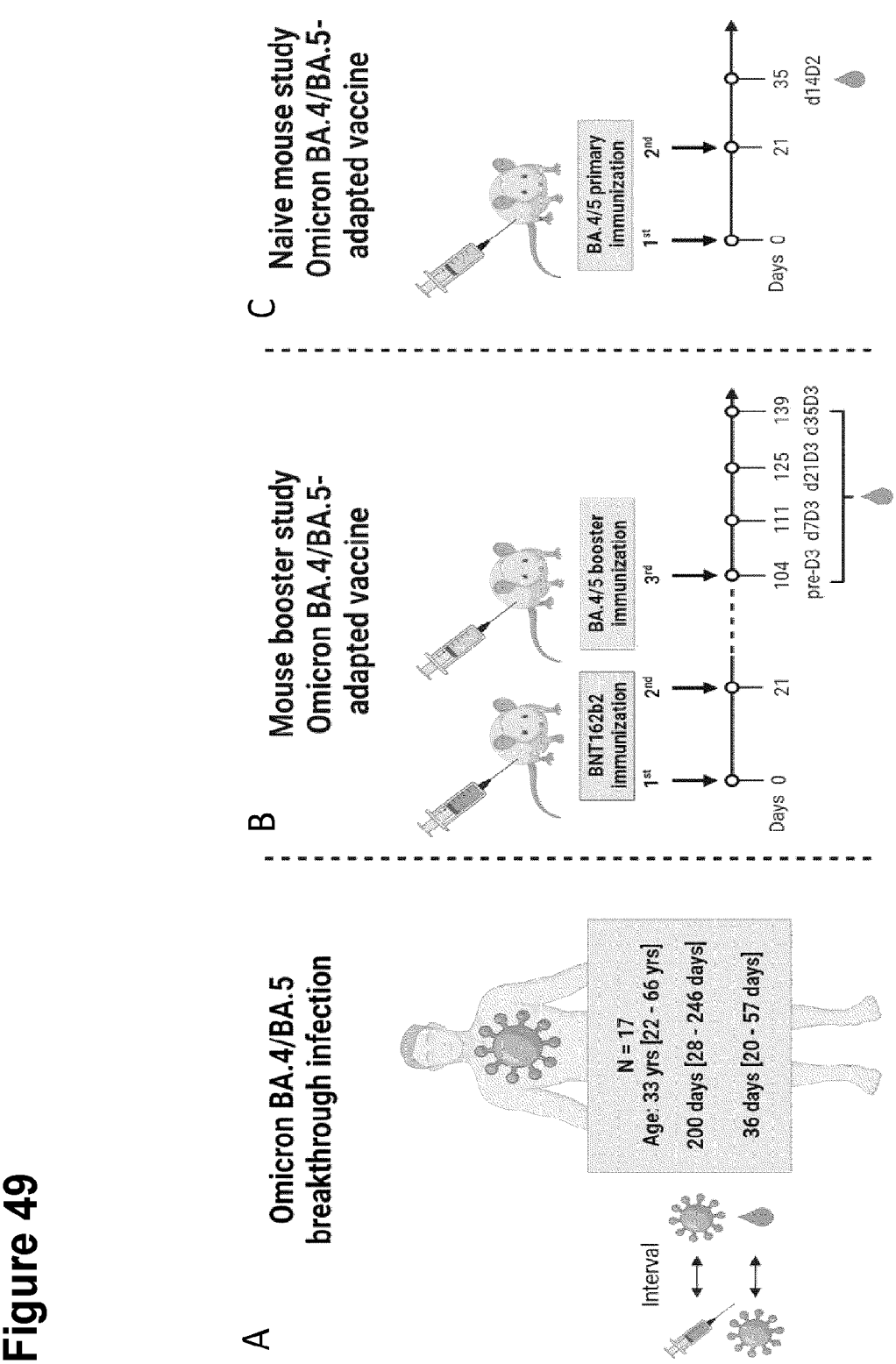
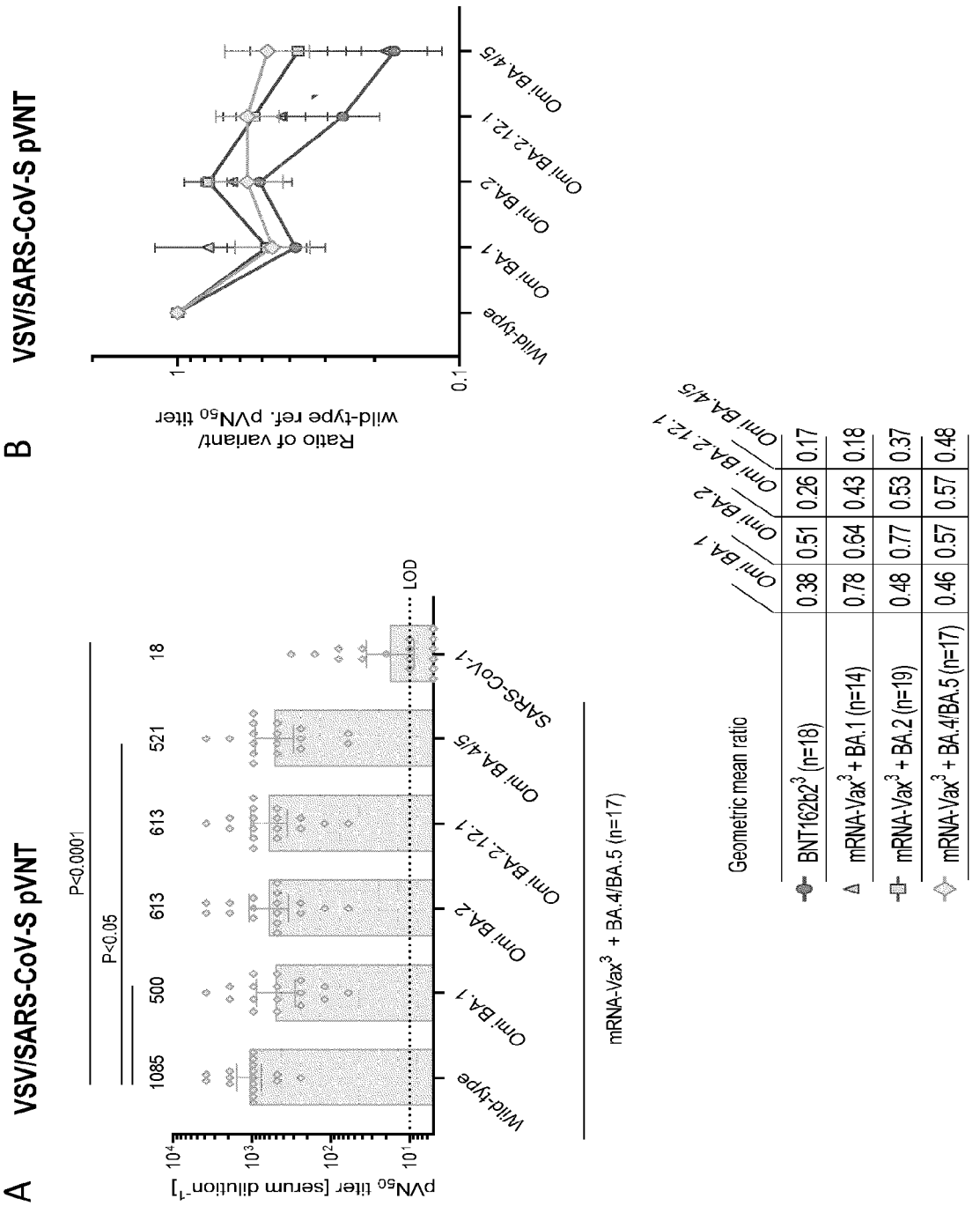

A
Omicron BA.4/BA.5 breakthrough infection
Interval
N = 17
Age: 33 yrs [22 - 66 yrs]
200 days [28 - 246 days]
36 days [20 - 57 days]
B
Mouse booster study Omicron BA.4/BA.5-adapted vaccine
BNT162b2 immunization
1st
2nd
BA.4/5 booster immunization
3rd
Days 0 21 104 111 125 139
pre-D3 d7D3 d21D3 d35D3
C
Naive mouse study Omicron BA.4/BA.5-adapted vaccine
BA.4/5 primary immunization
1st
2nd
Days 0 21 35
d14D2

Figure 50



Geometric mean ratio	Omi BA.1 /	Omi BA.2 /	Omi BA.2.12.1 /	Omi BA.4/5
BNT162b2[3] (n=18)	0.38	0.51	0.26	0.17
mRNA-Vax[3] + BA.1 (n=14)	0.78	0.64	0.43	0.18
mRNA-Vax[3] + BA.2 (n=19)	0.48	0.77	0.53	0.37
mRNA-Vax[3] + BA.4/BA.5 (n=17)	0.46	0.57	0.57	0.48

Figure 50 (Continued)

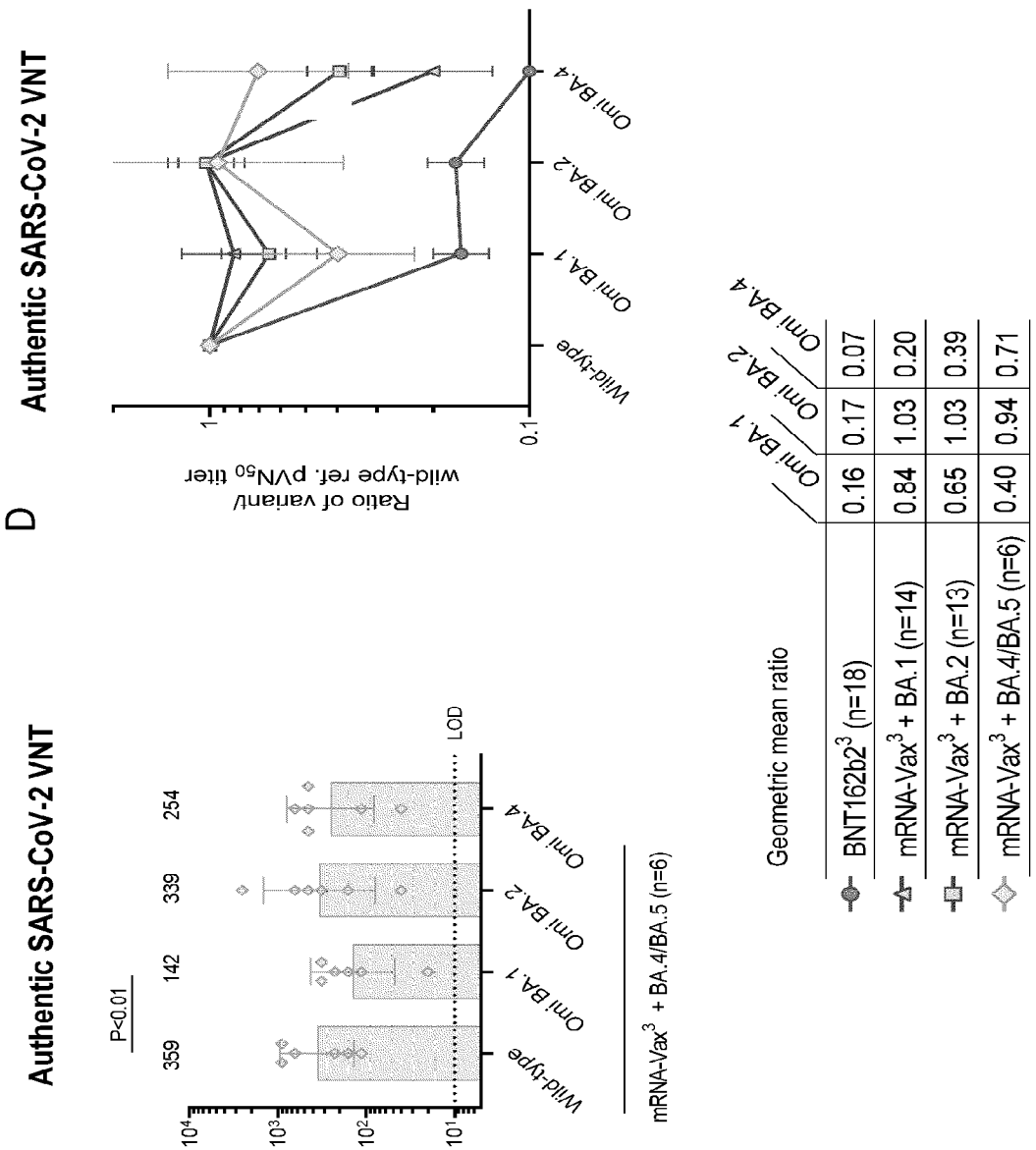


Figure 51

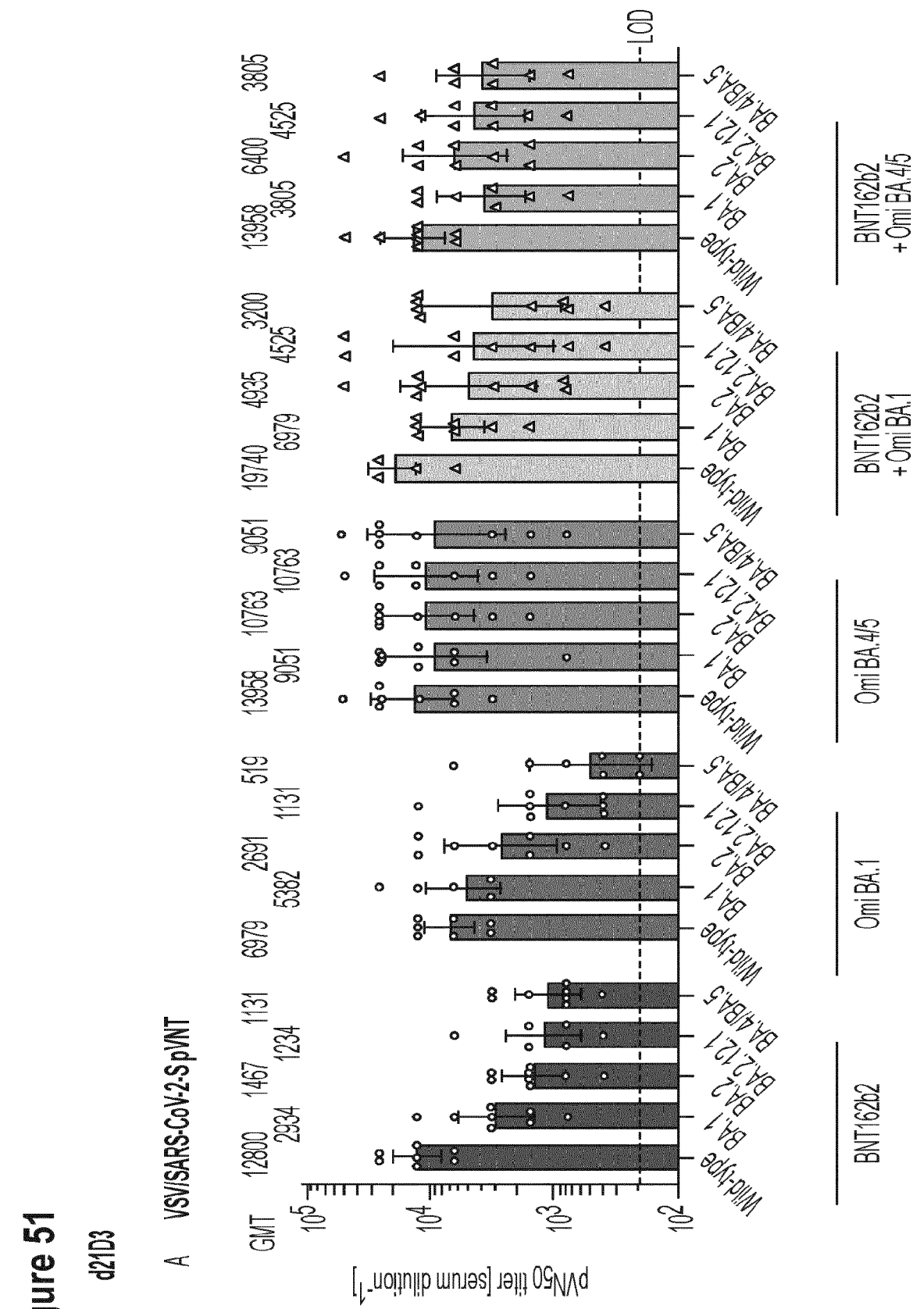

d21D3
A VSV/SARS-CoV-2-S pVNT
pVN50 titer [serum dilution-1]
GMT
LOD
Wild-type
BA.1
BA.2
BA.2.12.1
BA.4/BA.5
BNT162b2
Omi BA.1
Omi BA.4/5
BNT162b2 + Omi BA.1
BNT162b2 + Omi BA.4/5

Figure 51 (Continued)

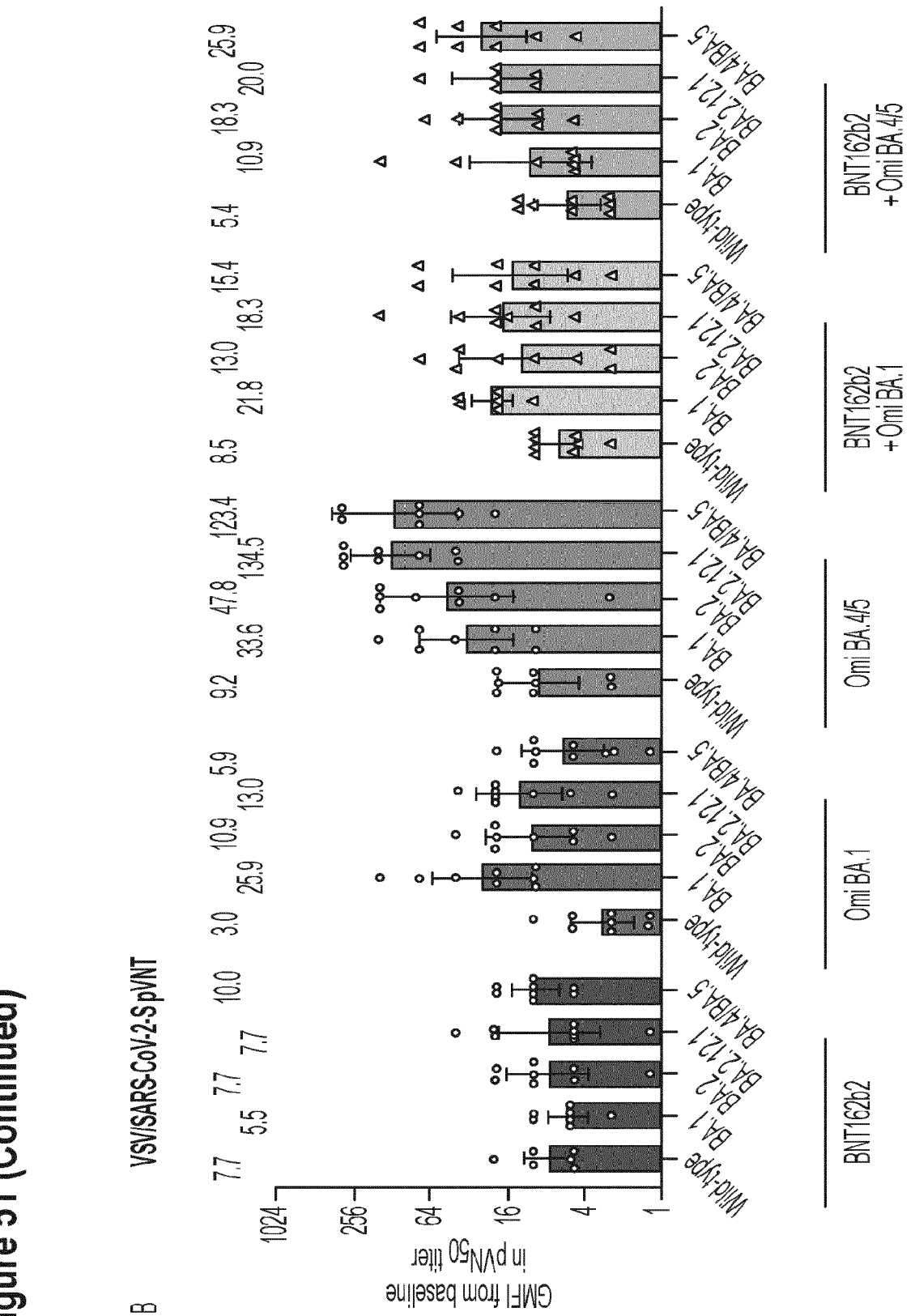
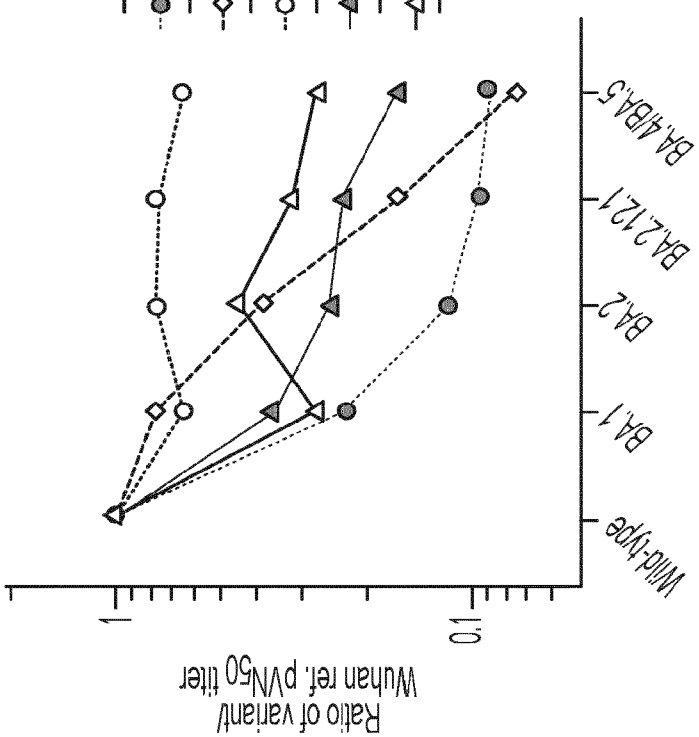


Figure 51 (Continued)

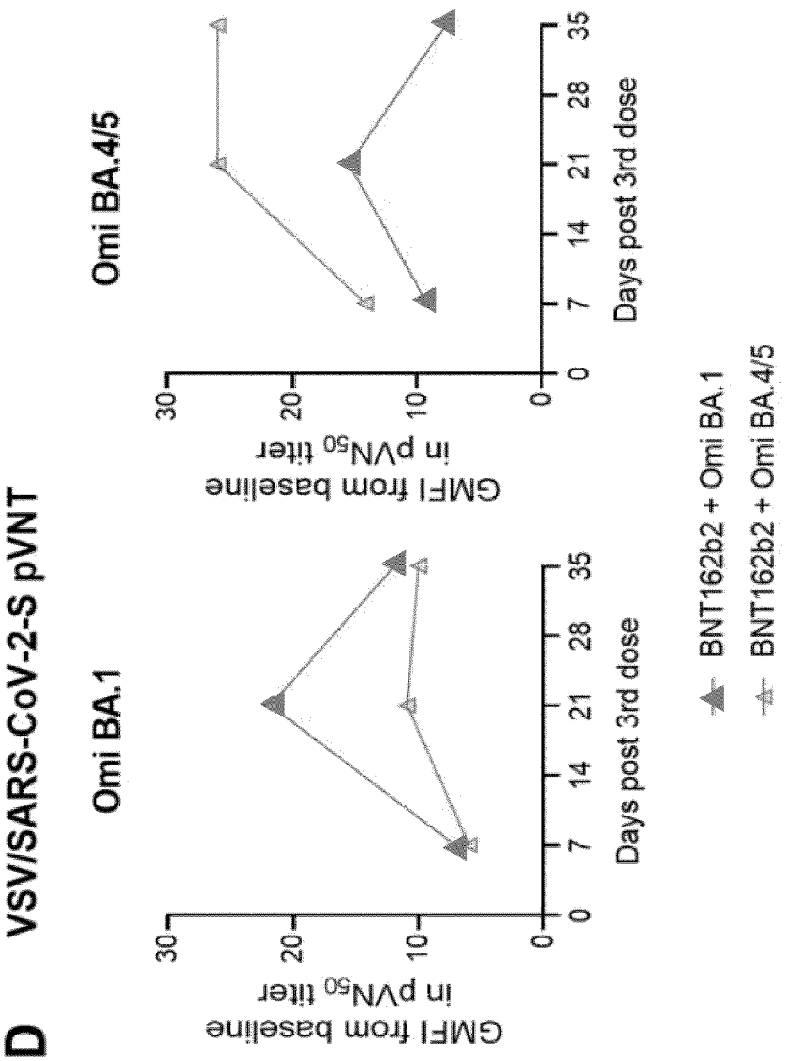
C

VSV/SARS-CoV-2-S pVNT



Geometric mean ratio	Omi BA.1	Omi BA.2	Omi BA.2.12.1	Omi BA.4/BA.5
BNT162b2	0.23	0.11	0.10	0.09
Omi BA.1	0.77	0.39	0.16	0.07
Omi BA.4/5	0.65	0.77	0.77	0.65
BNT162b2 + Omi BA.1	0.35	0.25	0.23	0.16
BNT162b2 + Omi BA.4/5	0.27	0.46	0.32	0.27

Figure 51 (Continued)



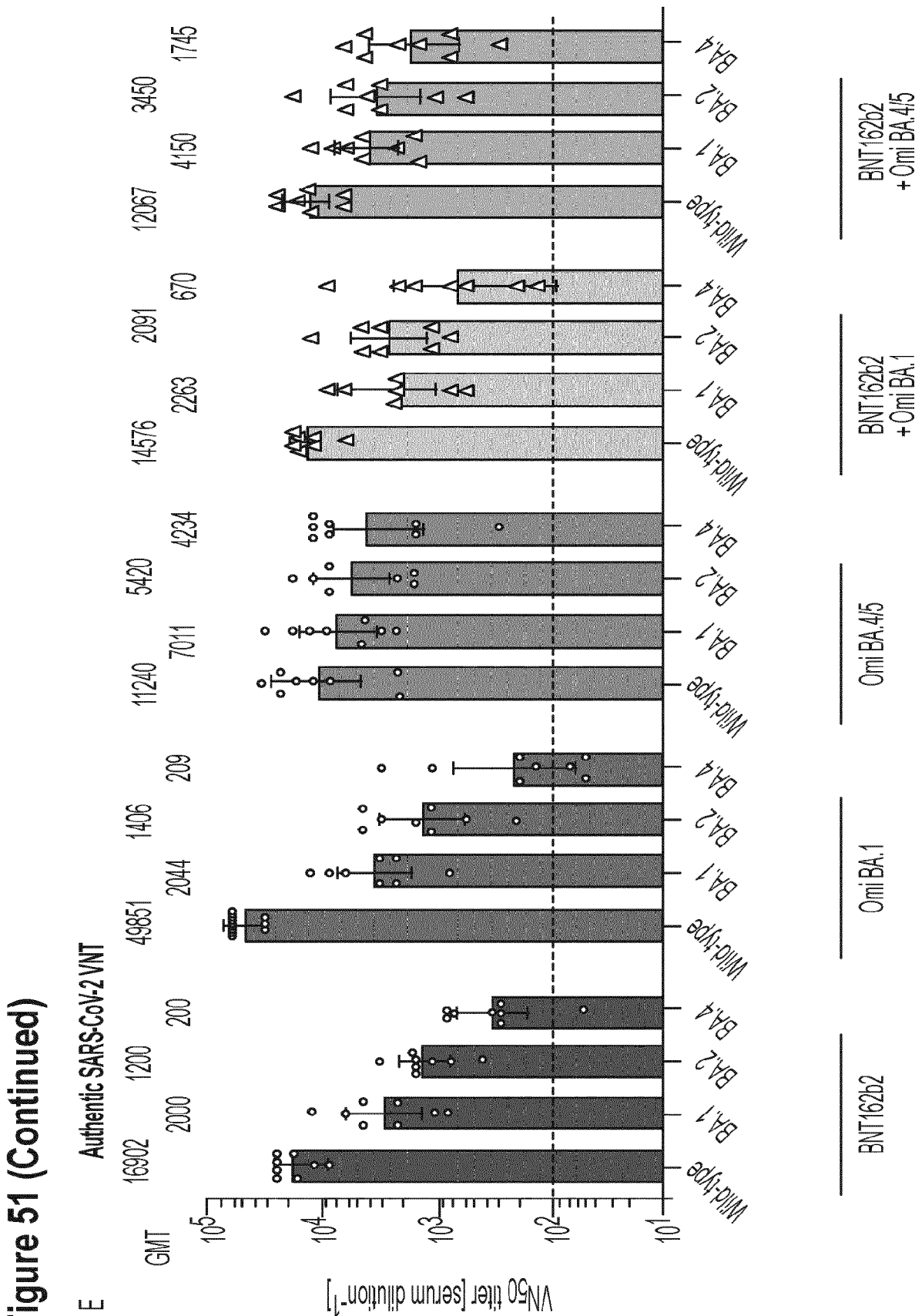
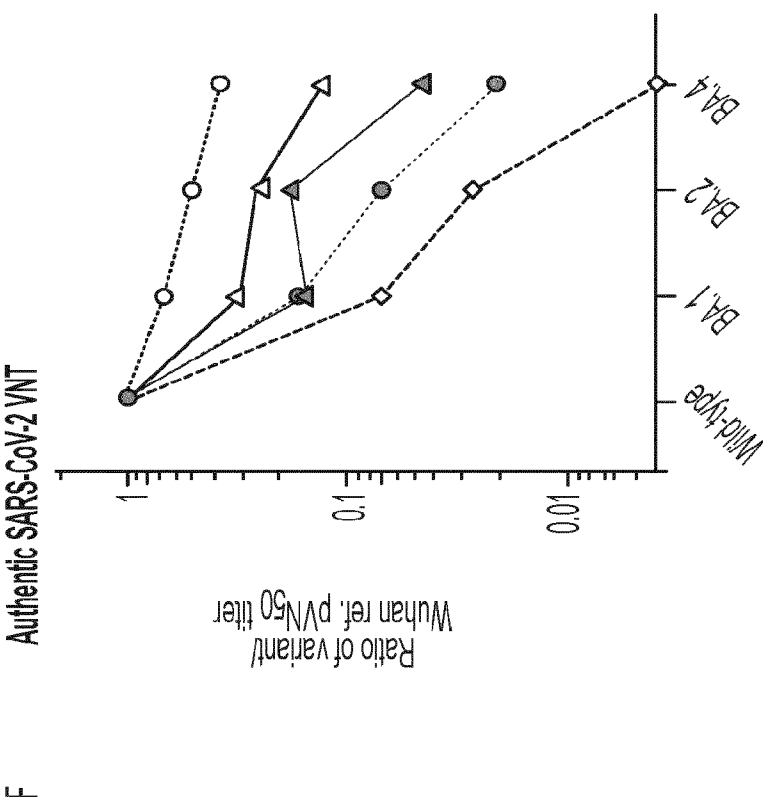


Figure 51 (Continued)
E
Authentic SARS-CoV-2 VNT
VN50 titer [serum dilution-1]
GMT
16902
2000
1200
200
49851
2044
1406
209
11240
7011
5420
4234
14576
2263
2091
670
12067
4150
3450
1745
Wild-type
BA.1
BA.2
BA.4
BNT162b2
Omi BA.1
Omi BA.4/5
BNT162b2 +Omi BA.1
BNT162b2 +Omi BA.4/5

Figure 51 (Continued)

F



Geometric mean ratio	Omi BA.1	Omi BA.2	Omi BA.4
BNT162b2	0.17	0.07	0.02
Omi BA.1	0.07	0.03	<0.01
Omi BA.4/5	0.68	0.50	0.39
BNT162b2 + Omi BA.1	0.16	0.19	0.05
BNT162b2 + Omi BA.4/5	0.31	0.26	0.13

Figure 52

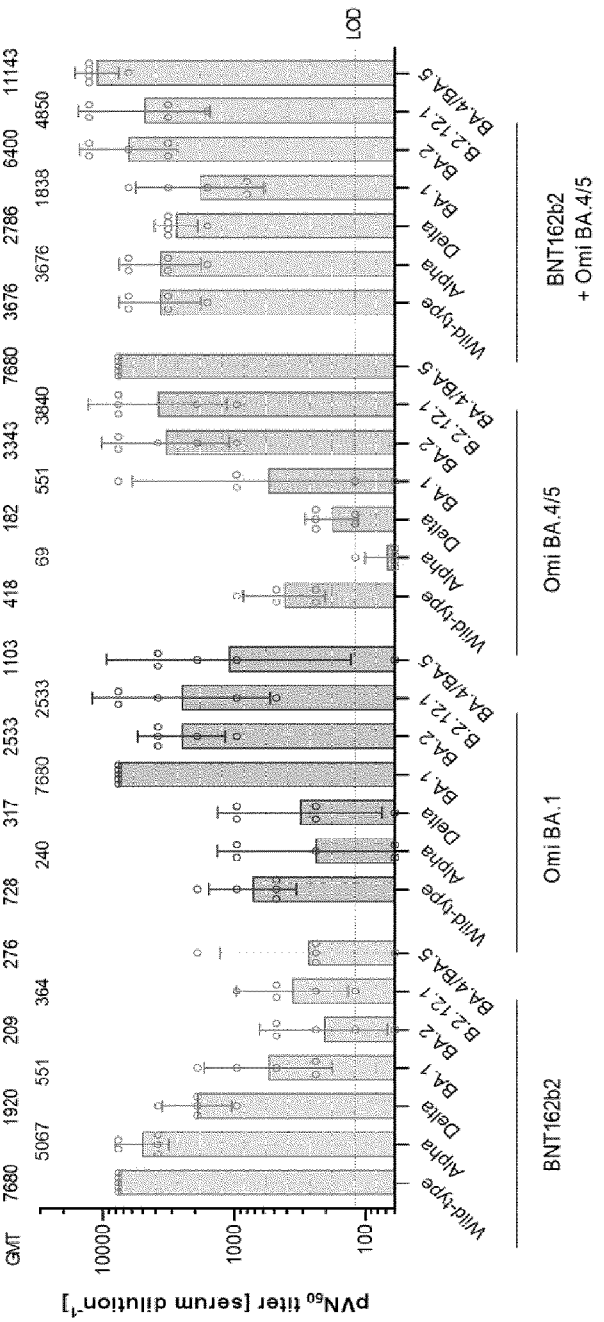

pVN50 titer [serum dilution-1]
10000
1000
100
LOD
GMT
7680 1920 551 209 364 276 728 240 317 7680 2533 2533 1103 418 69 182 551 3343 3840 7680 3676 3676 2786 1838 6400 4850 11143
5067
Wild-type
Alpha
Delta
BA.1
BA.2
B.2.12.1
BA.4/BA.5
BNT162b2
Omi BA.1
Omi BA.4/5
BNT162b2
+ Omi BA.4/5

Figure 53

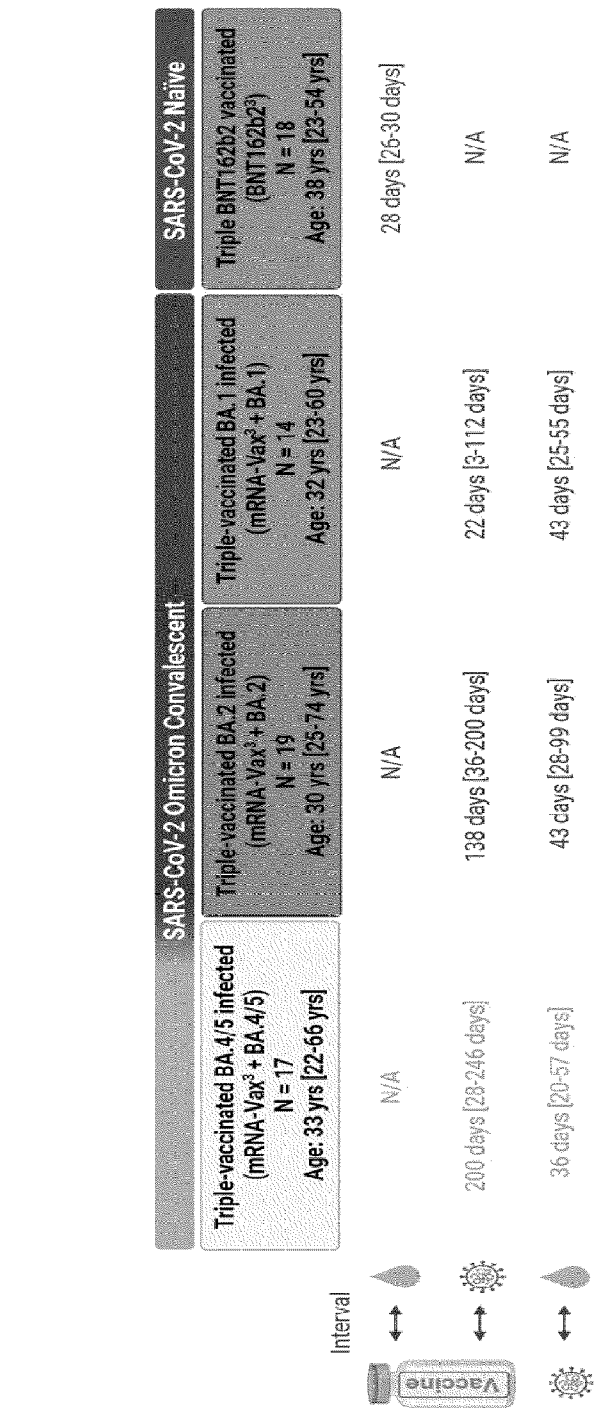

SARS-CoV-2 Omicron Convalescent
SARS-CoV-2 Naive
Triple-vaccinated BA.4/5 infected (mRNA-Vax³ + BA.4/5) N = 17 Age: 33 yrs [22-66 yrs]
Triple-vaccinated BA.2 infected (mRNA-Vax³ + BA.2) N = 19 Age: 30 yrs [25-74 yrs]
Triple-vaccinated BA.1 infected (mRNA-Vax³ + BA.1) N = 14 Age: 32 yrs [23-60 yrs]
Triple BNT162b2 vaccinated (BNT162b2³) N = 18 Age: 38 yrs [23-54 yrs]
Interval
Vaccine
N/A
200 days [28-246 days]
36 days [20-57 days]
N/A
138 days [36-200 days]
43 days [28-99 days]
N/A
22 days [3-112 days]
43 days [25-55 days]
N/A
28 days [26-30 days]

Figure 54

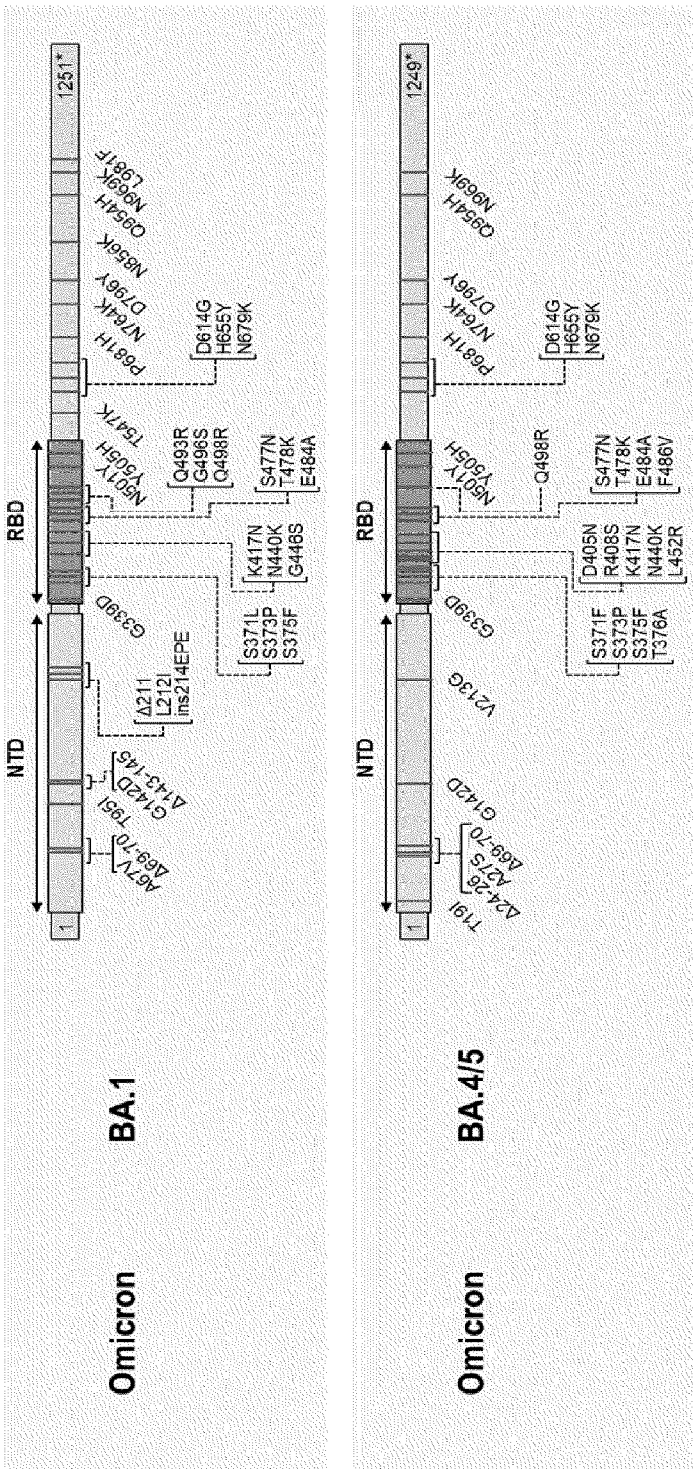

Omicron
BA.1
NTD
RBD
1
1251*
A67V
Δ69-70
T95I
G142D
Δ143-145
Δ211
L212I
ins214EPE
G339D
S371L
S373P
S375F
K417N
N440K
G446S
S477N
T478K
E484A
Q493R
G496S
Q498R
N501Y
Y505H
T547K
D614G
H655Y
N679K
P681H
N764K
D796Y
N856K
Q954H
N969K
L981F
Omicron
BA.4/5
NTD
RBD
1
1249*
T19I
Δ24-26
A27S
Δ69-70
G142D
V213G
G339D
S371F
S373P
S375F
T376A
D405N
R408S
K417N
N440K
L452R
S477N
T478K
E484A
F486V
Q498R
N501Y
Y505H
D614G
H655Y
N679K
P681H
N764K
D796Y
Q954H
N969K

Figure 55

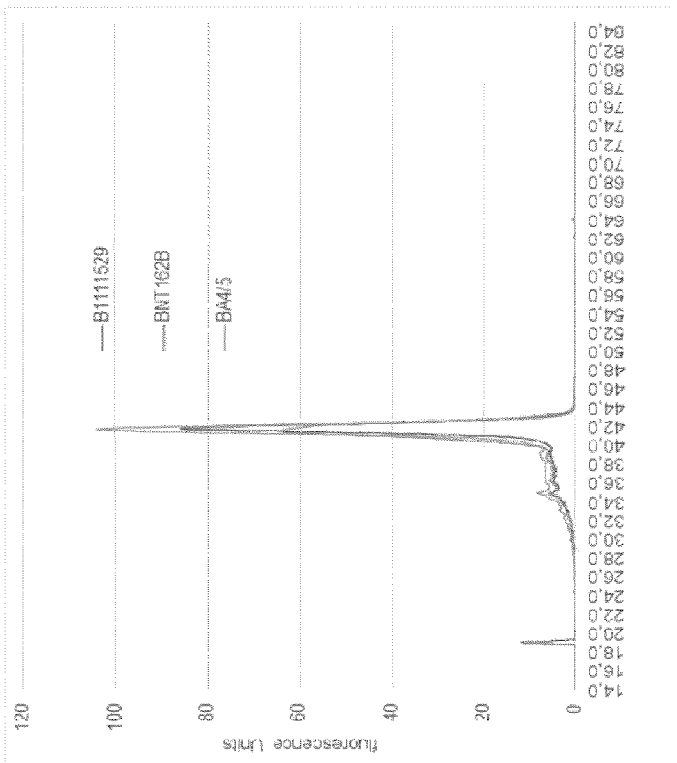
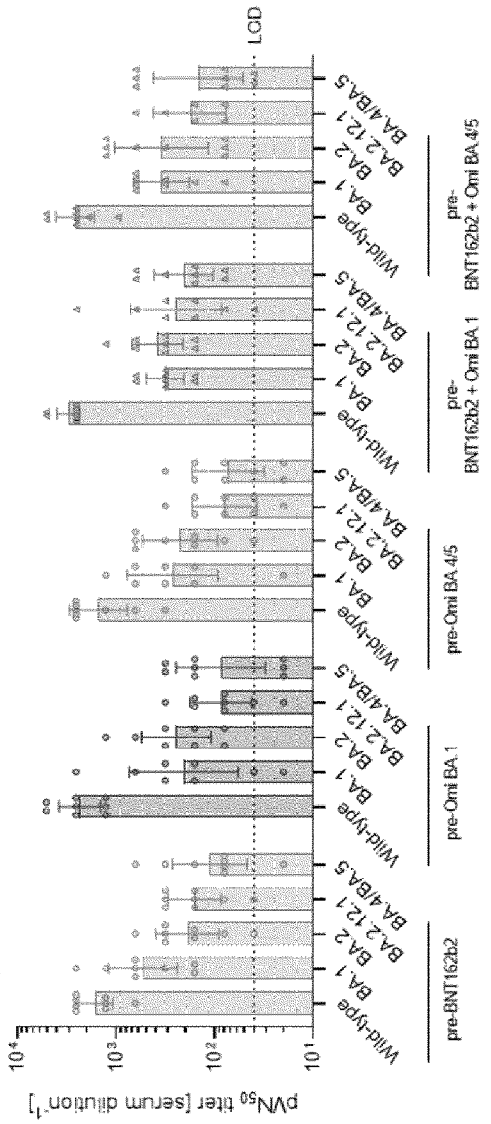

A
B1111529
BNT162B
BA4/5
fluorescence (a.u.)
B
vaccine
RNA
In vitro transfection
S1+ cells [%]
non-transfected
BNT162b2
Omi BA.1
Omi BA.4/5

Figure 56

preD3

A VSV/SARS-CoV-2-S pVNT



B VSV/SARS-CoV-2-S pVNT

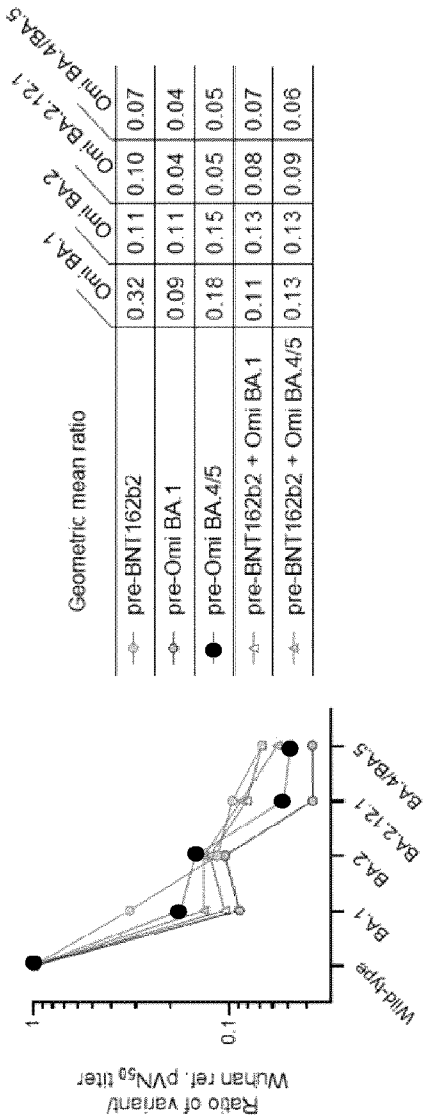


Figure 57

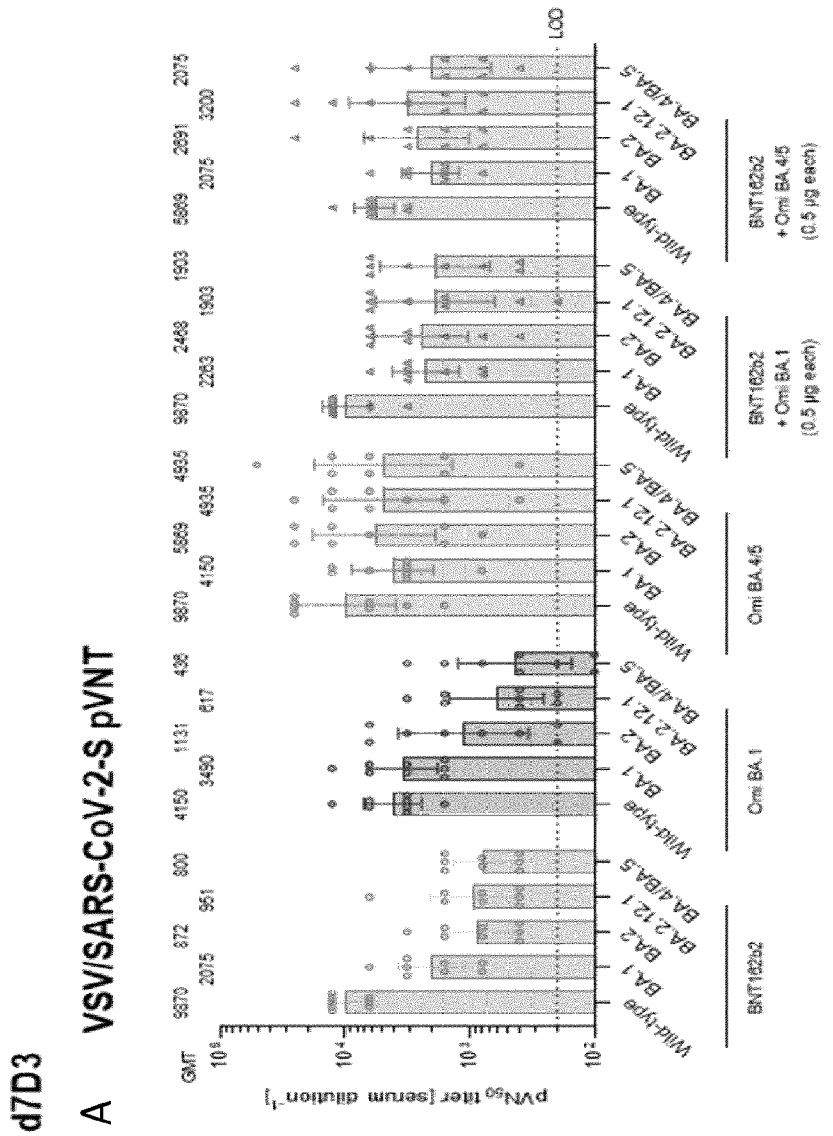

d7D3
A VSV/SARS-CoV-2-S pVNT
pVN50 titer [serum dilution-1]
LOD
GMT
BNT162b2
Omi BA.1
Omi BA.4/5
BNT162b2 + Omi BA.1 (0.5 µg each)
BNT162b2 + Omi BA.4/5 (0.5 µg each)
Wild-type
BA.1
BA.2
BA.2.12.1
BA.4/BA.5

Figure 57 (Continued)

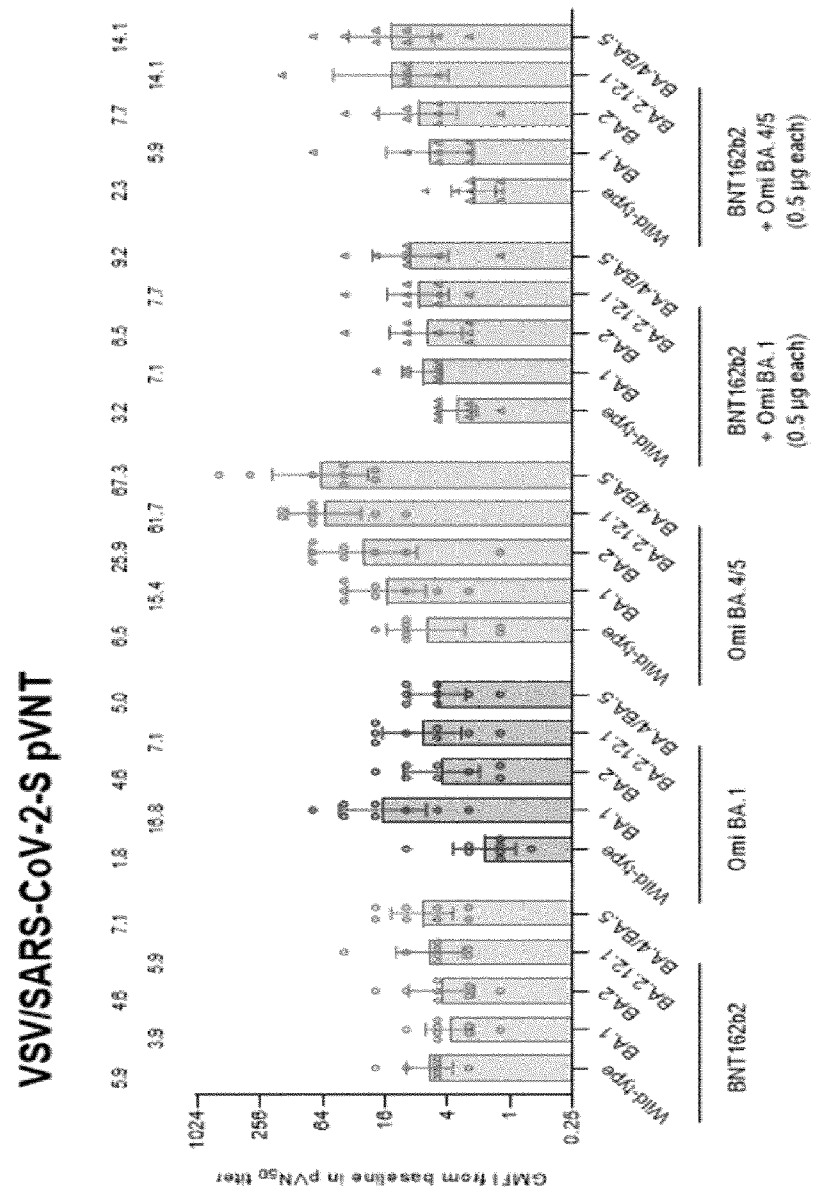


Figure 57 (Continued)

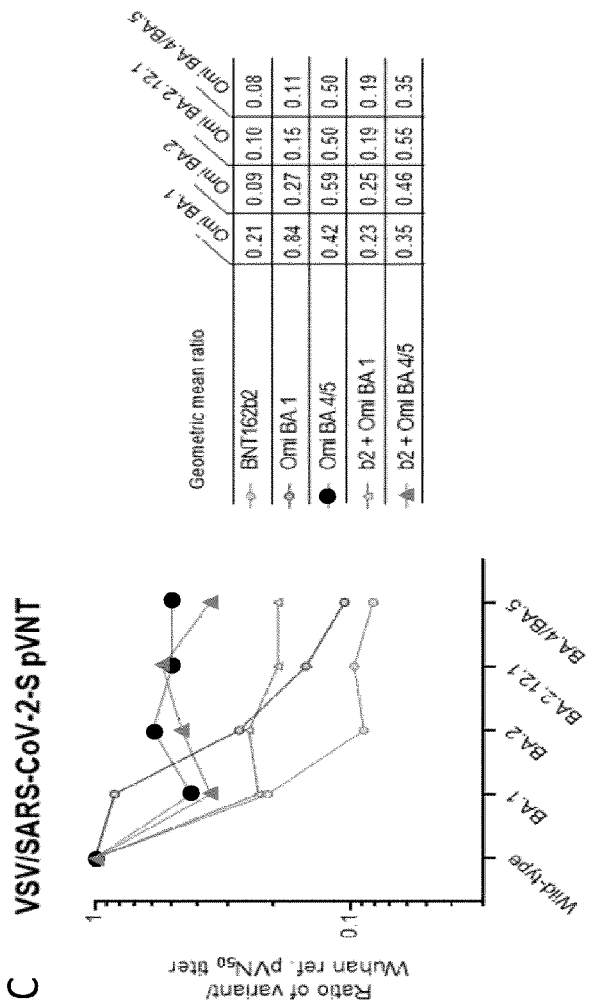


Figure 58

A VSV/SARS-CoV-2-S pVNT

d35D3

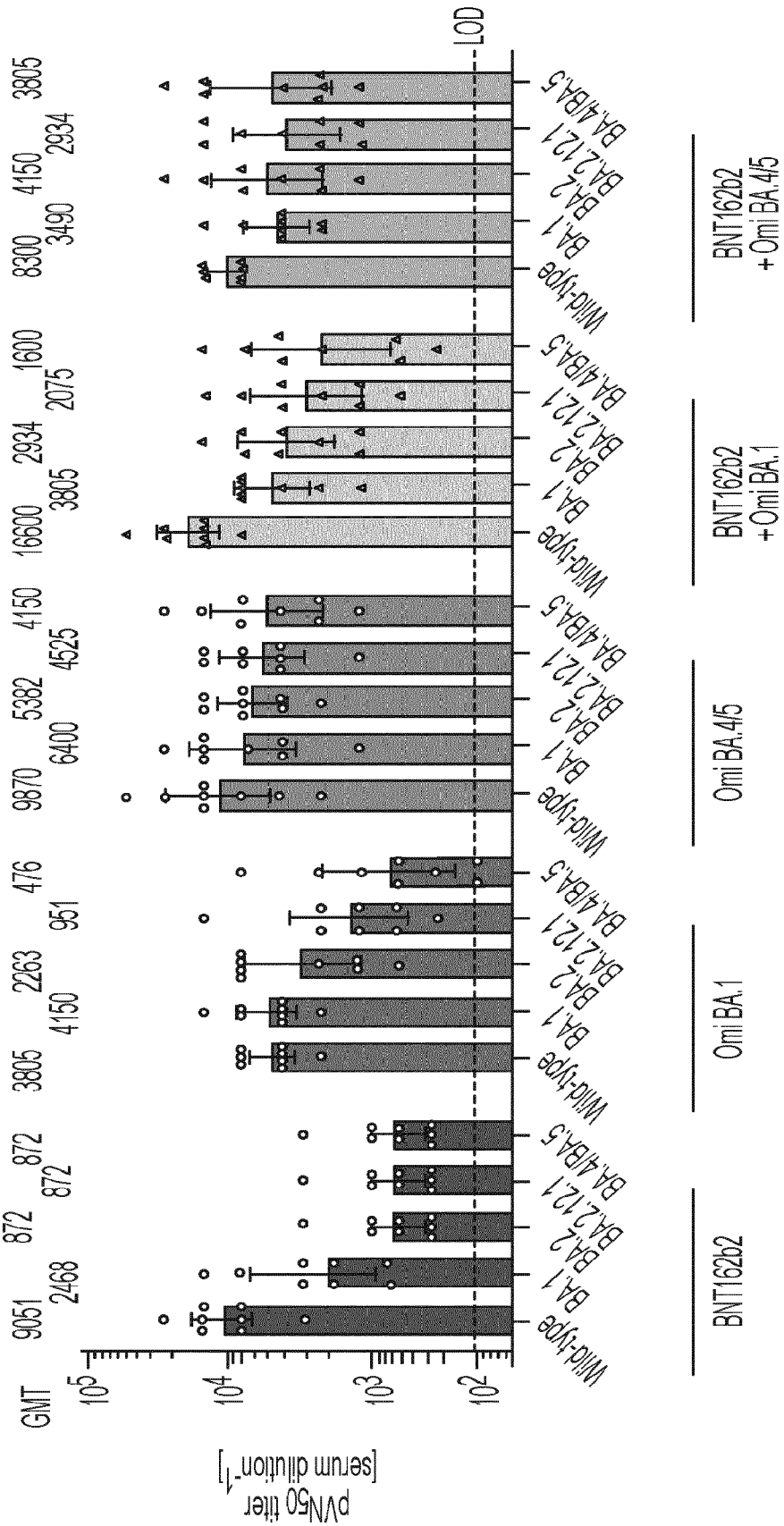


Figure 58 (Continued)

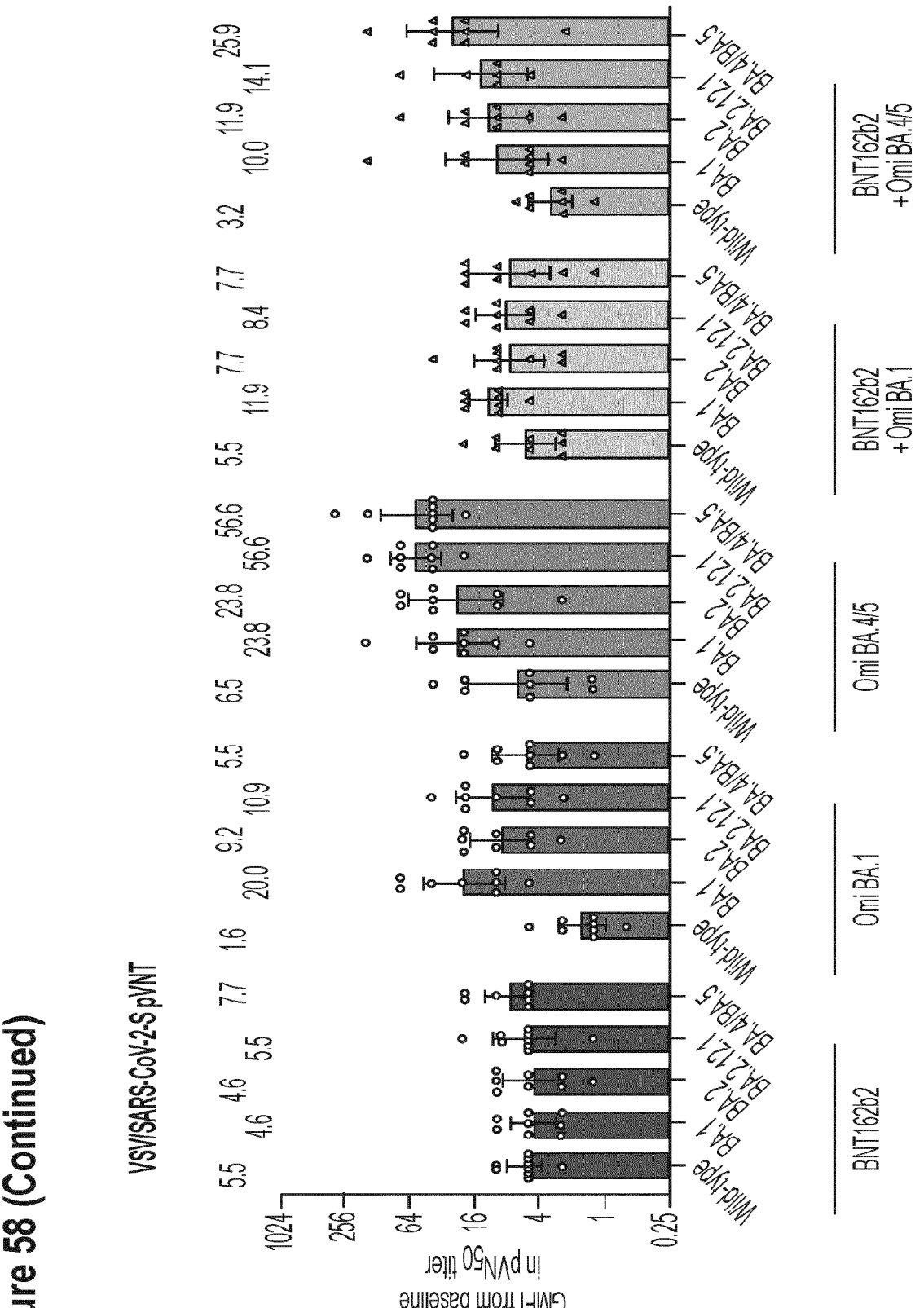
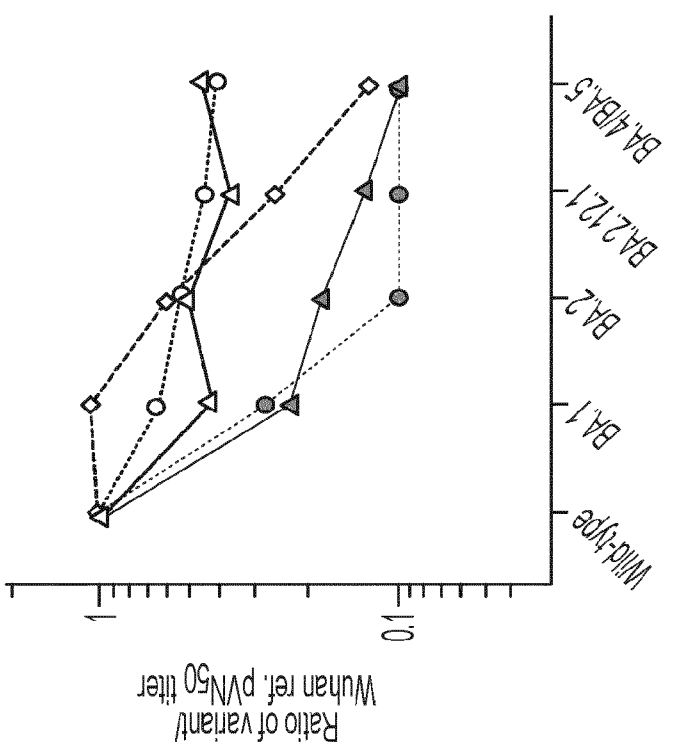


Figure 58 (Continued)

C VSV/SARS-CoV-2-SpVNT



Geometric mean ratio	Omni BA.1	Omi BA.2	Omi BA.2.12.1	Omi BA.4/BA.5
BNT162b2	0.27	0.10	0.10	0.10
Omi BA.1	1.09	0.59	0.25	0.13
Omi BA.4/5	0.65	0.55	0.46	0.42
BNT162b2 + Omi BA.1	0.23	0.18	0.13	0.10
BNT162b2 + Omi BA.4/5	0.42	0.50	0.35	0.46

Figure 58 (Continued)

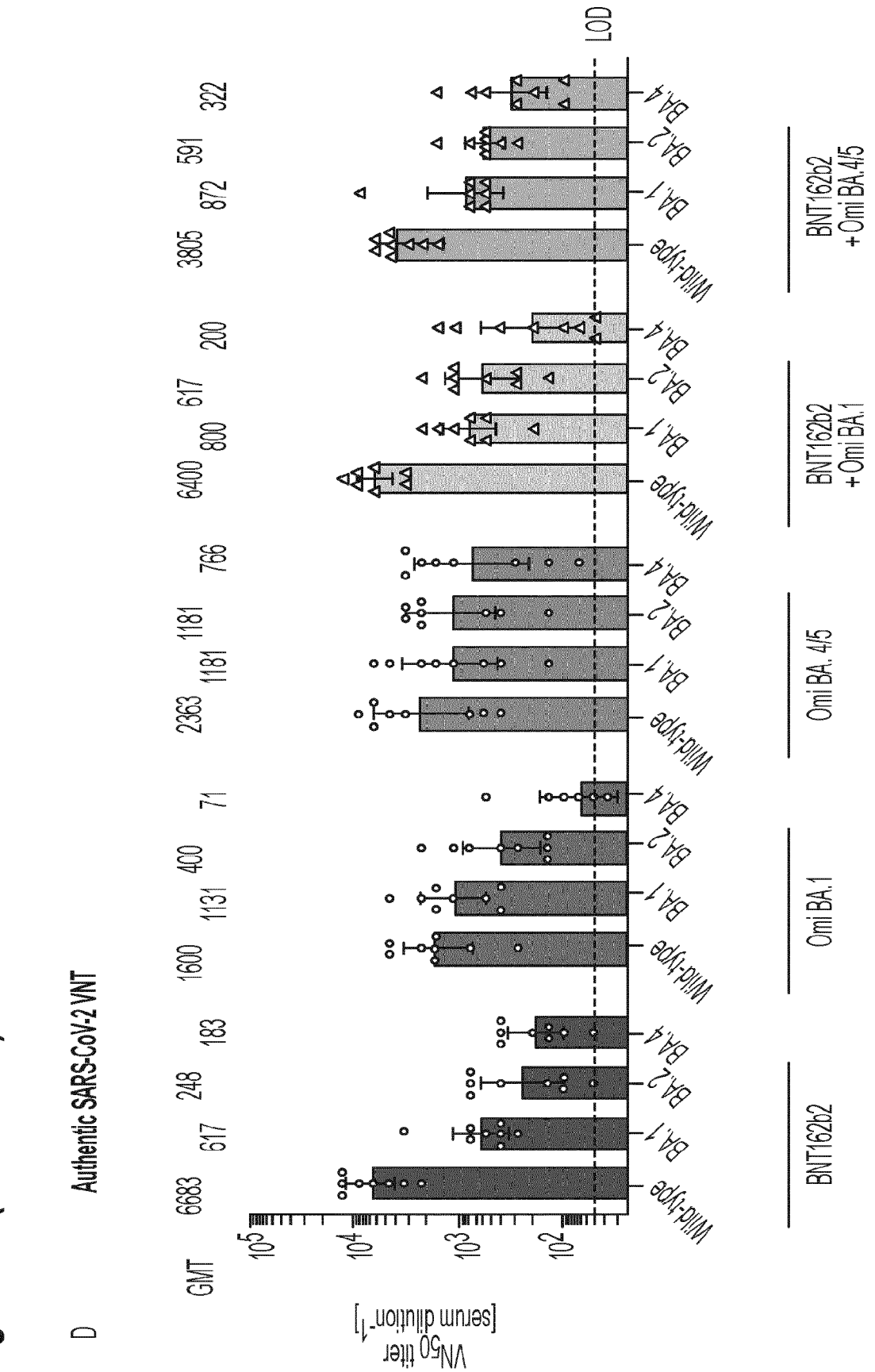
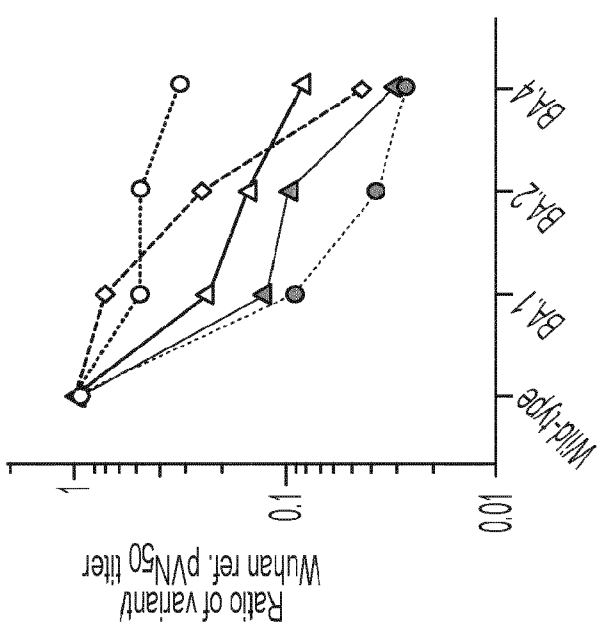


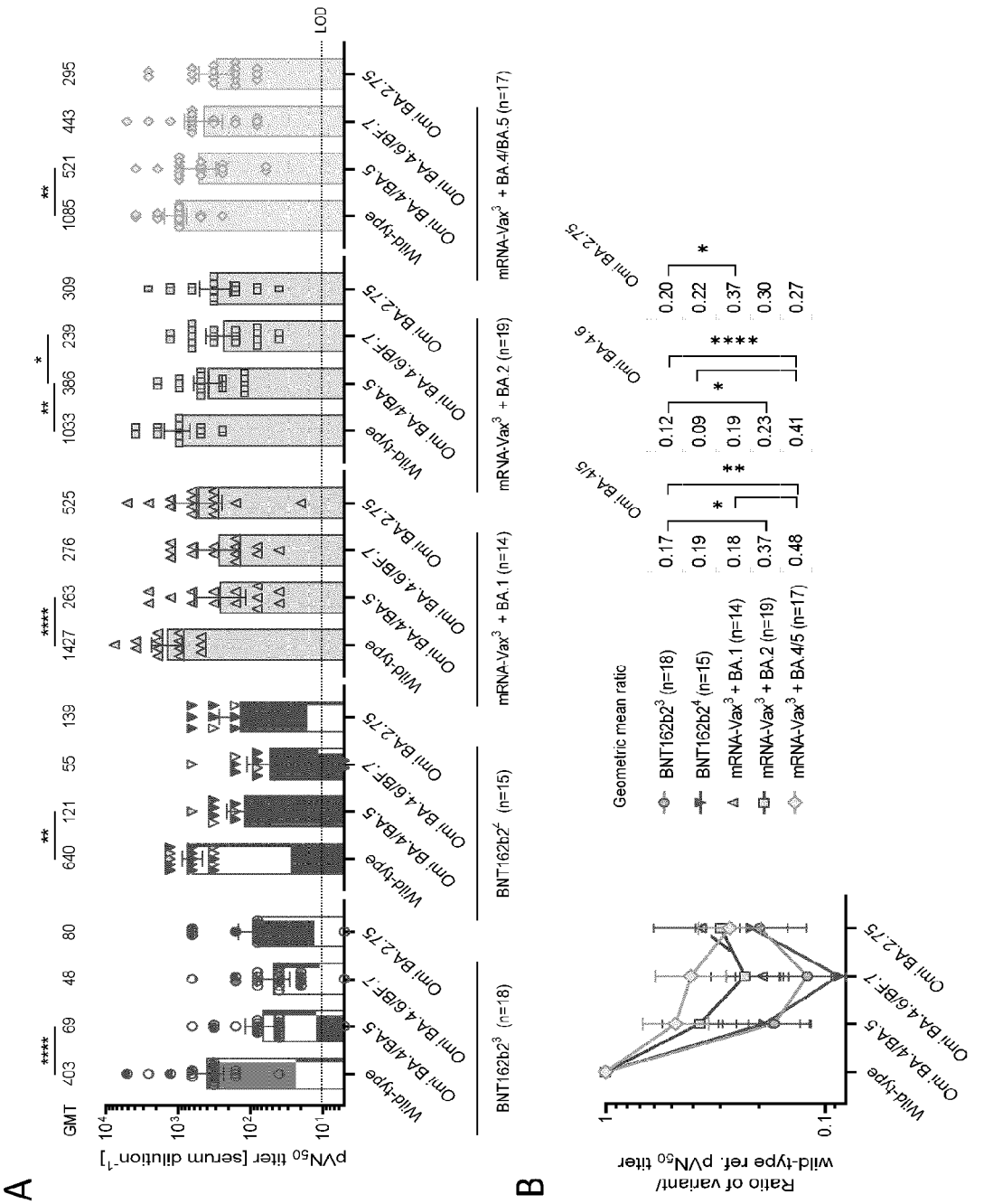
Figure 58 (Continued)

E Authentic SARS-CoV-2 VNT



Geometric mean ratio	Omi BA.1	Omi BA.2	Omi BA.4
BNT162b2	0.09	0.04	0.03
Omi BA.1	0.71	0.25	0.04
Omi BA.4/5	0.50	0.50	0.32
BNT162b2 + Omi BA.1	0.13	0.10	0.03
BNT162b2 + Omi BA.4/5	0.23	0.16	0.08

Figure 59


A
B
GMT
LOD
pVN50 titer [serum dilution-1]
Wild-type
Omi BA.4/BA.5
Omi BA.4.6/BF.7
Omi BA.2.75
BNT162b2[3] (n=18)
BNT162b2[4] (n=15)
mRNA-Vax[3] + BA.1 (n=14)
mRNA-Vax[3] + BA.2 (n=19)
mRNA-Vax[3] + BA.4/BA.5 (n=17)
Geometric mean ratio
Omi BA.4/5
Omi BA.4.6
Omi BA.2.75
Ratio of variant/ wild-type ref. pVN50 titer

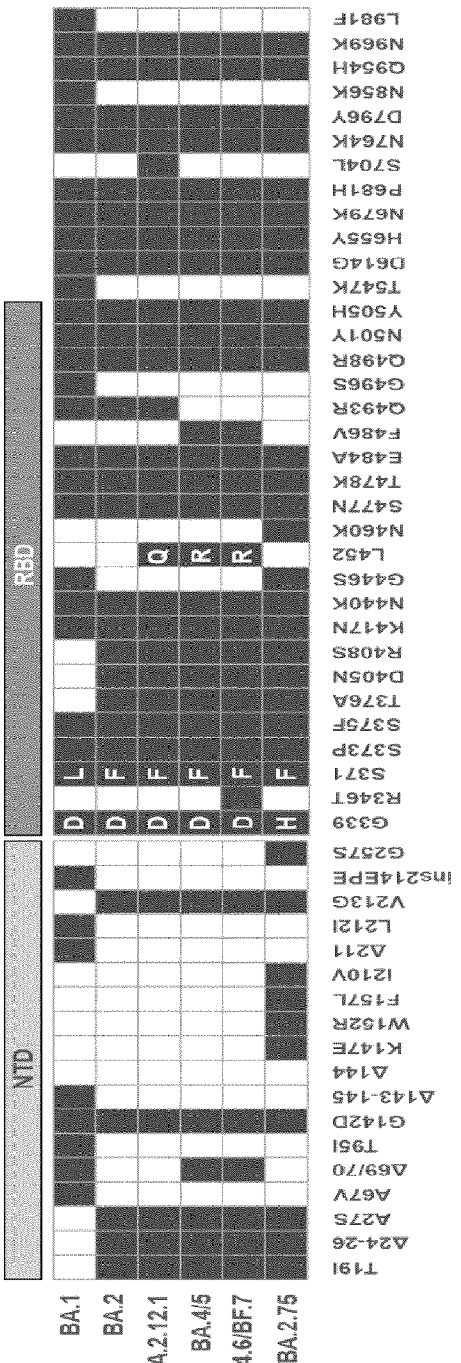
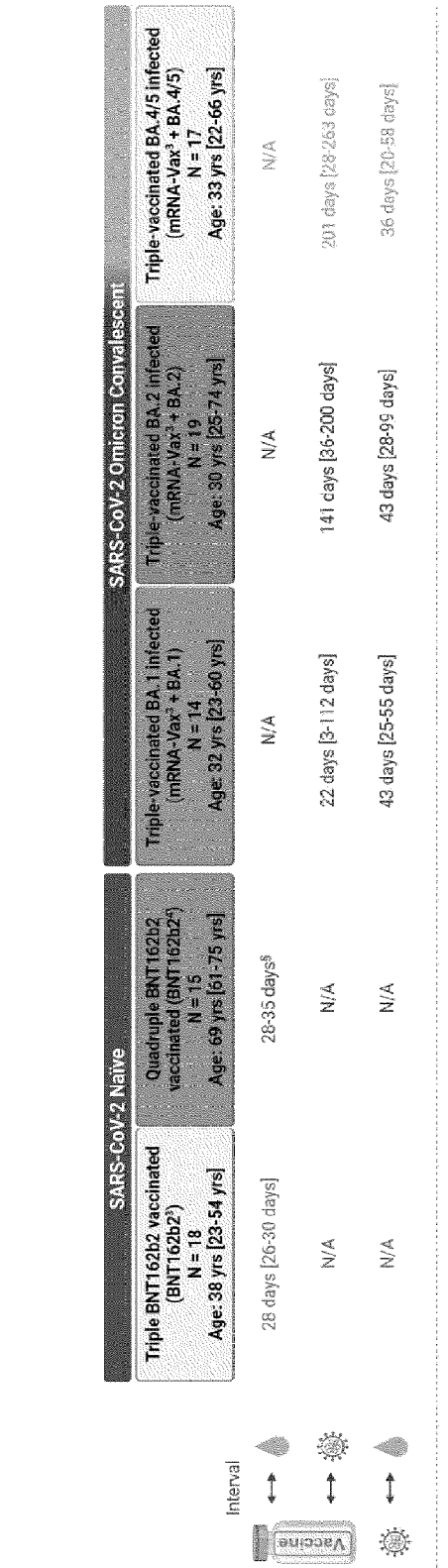

NTD
RBD
T19I
Δ24-26
A27S
A67V
Δ69/70
T95I
G142D
Δ143-145
Δ144
K147E
W152R
F157L
I210V
Δ211
L212I
V213G
ins214EPE
G257S
G339 D D D D D D H
R346T F
S371 L F F F F F F
S373P
S375F
T376A
D405N
R408S
K417N
N440K
G446S
L452 Q R R
N460K
S477N
T478K
E484A
F486V
Q493R
G496S
Q498R
N501Y
Y505H
T547K
D614G
H655Y
N679K
P681H
S704L
N764K
D796Y
N856K
Q954H
N969K
L981F
BA.1
BA.2
BA.2.12.1
BA.4/5
BA.4.6/BF.7
BA.2.75

Figure 60

Figure 61


SARS-CoV-2 Naïve
SARS-CoV-2 Omicron Convalescent
Triple BNT162b2 vaccinated (BNT162b2[a]) N = 18 Age: 38 yrs [23-54 yrs]
Quadruple BNT162b2 vaccinated (BNT162b2[a]) N = 15 Age: 69 yrs [61-75 yrs]
Triple-vaccinated BA.1 infected (mRNA-Vax[c] + BA.1) N = 14 Age: 32 yrs [23-60 yrs]
Triple-vaccinated BA.2 infected (mRNA-Vax[c] + BA.2) N = 19 Age: 30 yrs [25-74 yrs]
Triple-vaccinated BA.4/5 infected (mRNA-Vax[c] + BA.4/5) N = 17 Age: 33 yrs [22-66 yrs]
Interval
Vaccine
28 days [26-30 days]
28-35 days[g]
N/A
N/A
N/A
22 days [3-112 days]
43 days [25-55 days]
N/A
N/A
141 days [36-200 days]
43 days [28-99 days]
N/A
201 days [28-263 days]
36 days [20-58 days]
SARS-CoV-2 breakthrough infection
Serum draw

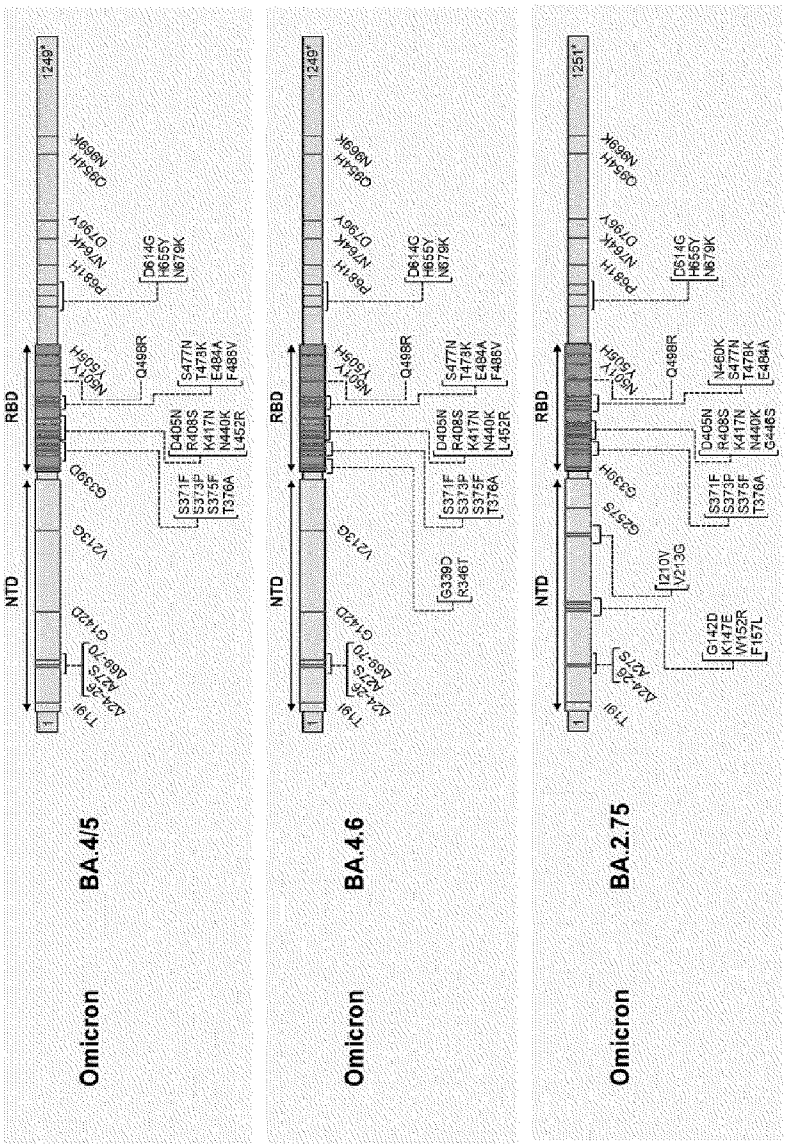

Omicron
BA.4/5
NTD
RBD
1249*
T19I
Δ24-26
A27S
Δ69-70
G142D
V213G
G339D
S371F
S373P
S375F
T376A
D405N
R408S
K417N
N440K
L452R
Q498R
N501Y
Y505H
T547K
D614G
H655Y
P681H
N764K
D796Y
Q954H
N969K
Omicron
BA.4.6
NTD
RBD
1249*
T19I
Δ24-26
A27S
Δ69-70
G142D
V213G
G339D
R346T
S371F
S373P
S375F
T376A
D405N
R408S
K417N
N440K
L452R
S477N
T478K
E484A
F486V
Q498R
N501Y
Y505H
D614G
H655Y
N679K
P681H
N764K
D796Y
Q954H
N969K
Omicron
BA.2.75
NTD
RBD
1251*
T19I
Δ24-26
A27S
G75S
I210V
V213G
G339H
G142D
K147E
W152R
F157L
S371F
S373P
S375F
T376A
D405N
R408S
K417N
N440K
G446S
N460K
S477N
T478K
E484A
Q498R
N501Y
Y505H
D614G
H655Y
N679K
P681H
N764K
D796Y
Q954H
N969K

Figure 62

Figure 63

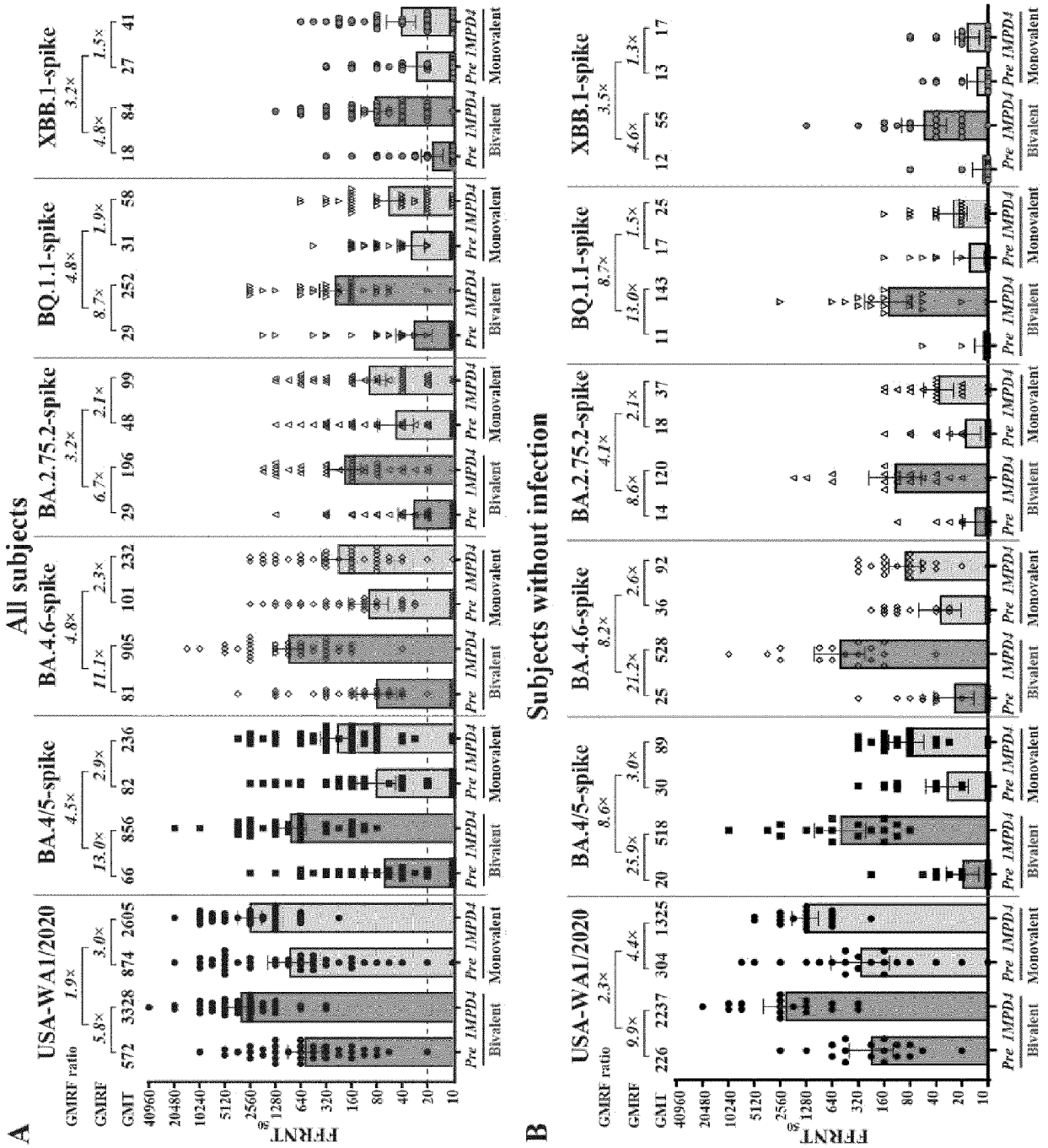

A
All subjects
B
Subjects without infection
USA-WA1/2020
BA.4/5-spike
BA.4.6-spike
BA.2.75.2-spike
BQ.1.1-spike
XBB.1-spike
GMRF ratio
GMRF
GMT
FFRNT50
Pre
1MPD4
Bivalent
Monovalent

Figure 63 (Continued)

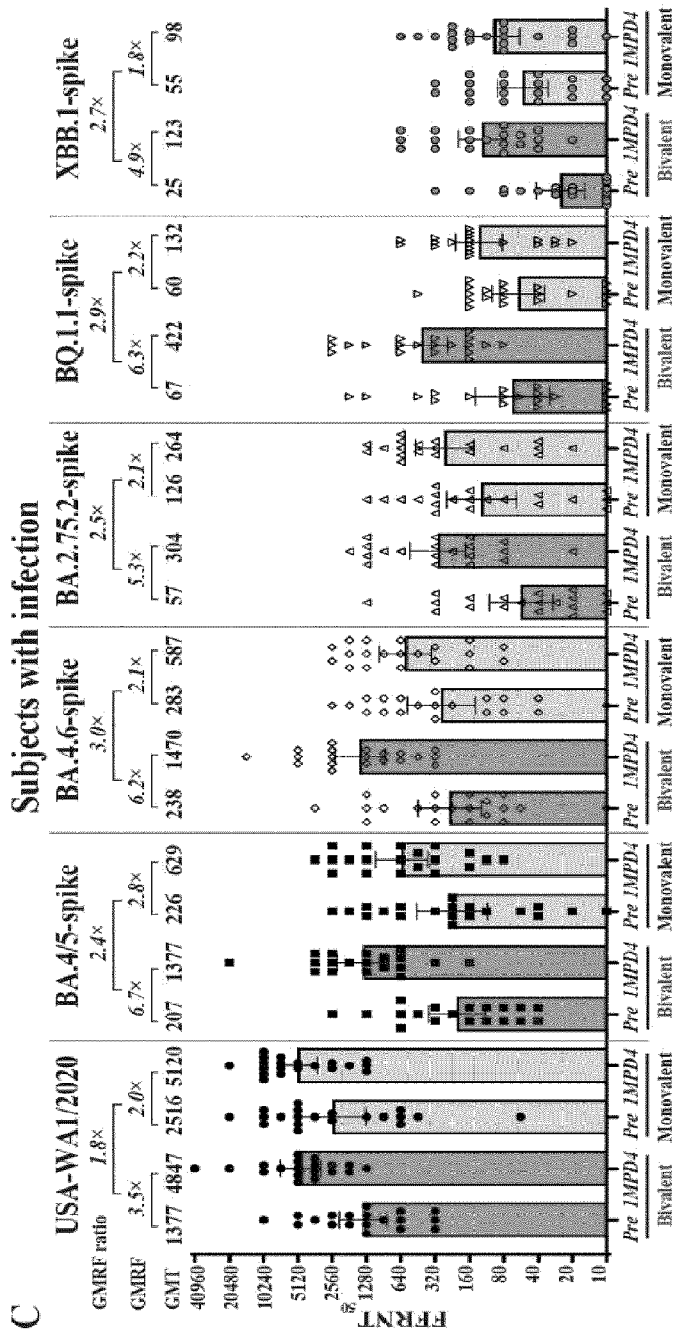


Figure 64

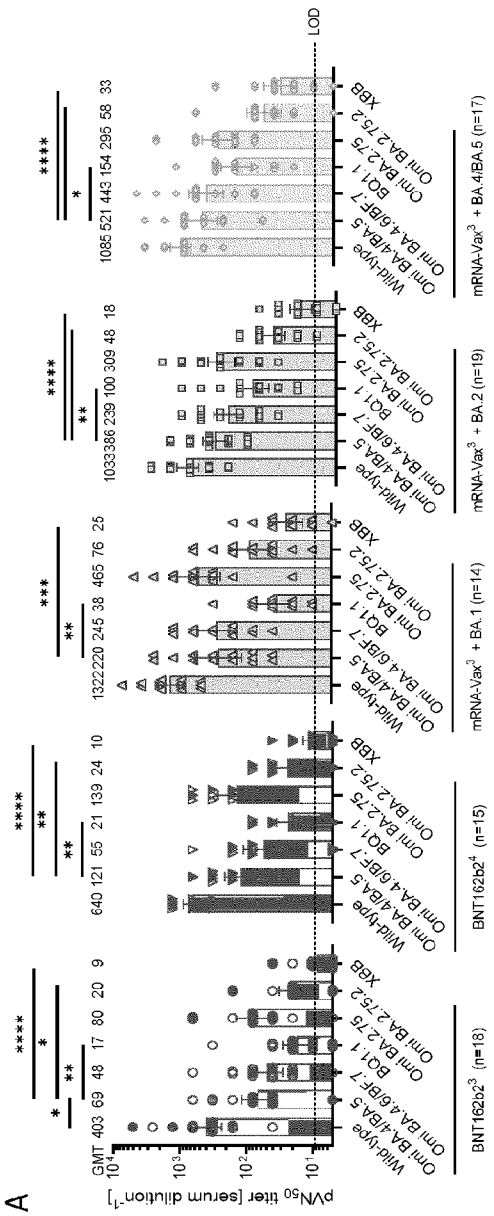
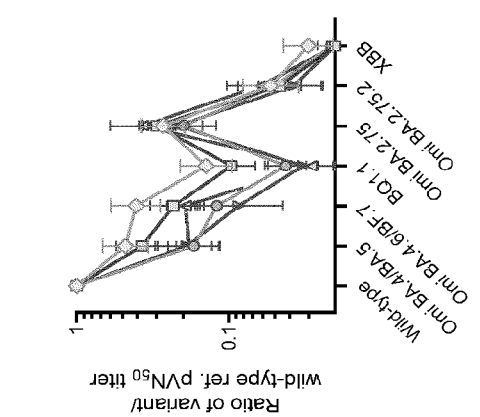

A
pVN50 titer [serum dilution-1]
LOD
GMT
403 69 48 17 80 20 9
640 121 55 21 139 24 10
1322220 245 38 465 76 25
1033386 239 100 309 48 18
1085 521 443 154 295 58 33
Wild-type
Omi BA.4/BA.5
Omi BA.4.6/BF.7
BQ1.1
Omi BA.2.75
Omi BA.2.75.2
XBB
BNT162b2^3 (n=18)
BNT162b2^4 (n=15)
mRNA-Vax^3 + BA.1 (n=14)
mRNA-Vax^3 + BA.2 (n=19)
mRNA-Vax^3 + BA.4/BA.5 (n=17)

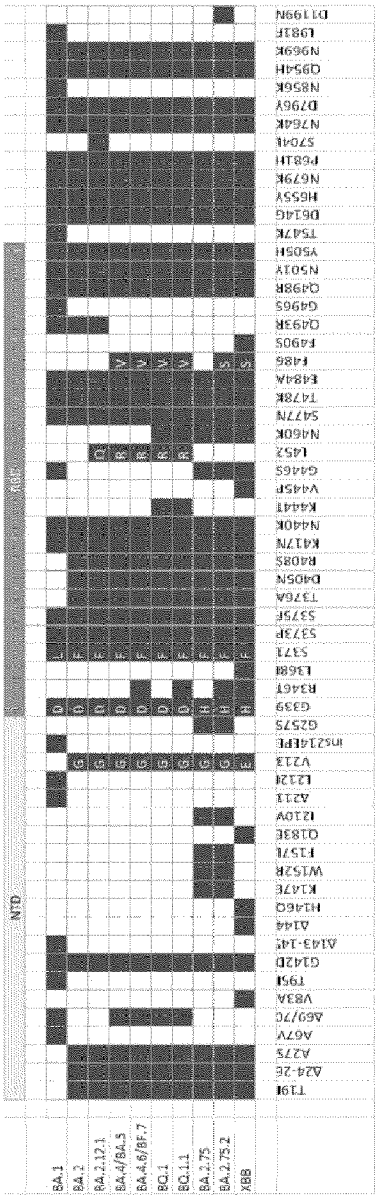
Figure 64 (Continued)

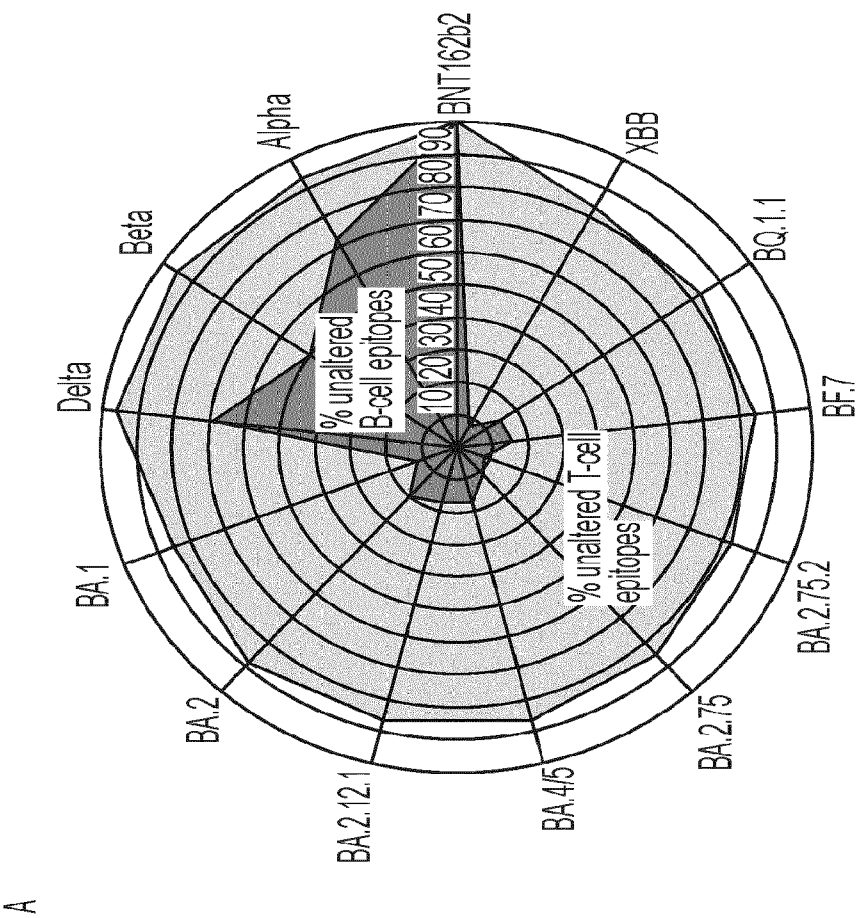
B



Geometric mean ratio	Omi BA.4/5	Omi BA.4.6/BF.7	Omi BQ1.1	Omi BA.2.75	Omi BA.2.75.2	Omi XBB
BNT162b2[3] (n=18)	0.17	0.12	0.04	0.20	0.05	0.02
BNT162b2[4] (n=15)	0.19	0.09	0.03	0.22	0.04	0.02
mRNA-Vax[3] + BA.1 (n=14)	0.18	0.19	0.03	0.37	0.06	0.02
mRNA-Vax[3] + BA.2 (n=19)	0.37	0.23	0.10	0.30	0.05	0.02
mRNA-Vax[3] + BA.4/5 (n=17)	0.48	0.41	0.14	0.27	0.05	0.03

Figure 65


NTD
RBD
BA.1
BA.2
BA.2.12.1
BA.4/BA.5
BA.4.6/BF.7
BQ.1
BQ.1.1
BA.2.75
BA.2.75.2
XBB


BNT162b2
XBB
BQ.1.1
BF.7
BA.2.75.2
BA.2.75
BA.4/5
BA.2.12.1
BA.2
BA.1
Delta
Beta
Alpha
10
20
30
40
50
60
70
80
90
% unaltered B-cell epitopes
% unaltered T-cell epitopes

A

Figure 66

Figure 66 (Continued)