

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

(19) World Intellectual Property

Organization

International Bureau

(43) International Publication Date

22 September 2022 (22.09.2022)



(10) International Publication Number

WO 2022/197720 A2

(51) International Patent Classification:

Not classified

TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(21) International Application Number:

PCT/US2022/020407

Published:

— without international search report and to be republished upon receipt of that report (Rule 48.2(g))

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

(22) International Filing Date:

15 March 2022 (15.03.2022)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/161,318 15 March 2021 (15.03.2021) US

63/256,848 18 October 2021 (18.10.2021) US

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(81) Designated States (unless otherwise indicated, for every

kind of national protection available): AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ,

CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO,

DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN,

HR, HU, ID, IL, IN, IR, IS, IT, JM, JO, JP, KE, KG, KH,

KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA,

MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI,

NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU,

RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM,

TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM,

ZW.

(84) Designated States (unless otherwise indicated, for every

kind of regional protection available): ARIPO (BW, GH,

GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ,

UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,

TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,

EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,

MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,

(54) Title: COMPOSITIONS AND METHODS FOR TREATING CORONAVIRUS INFECTION

(57) Abstract: Disclosed are immunogenic compositions and vaccines containing rationally designed coronavirus Spike proteins and polynucleotides encoding the same that can be administered to treat or inhibit a coronavirus infection. The compositions and vaccines can also be used to produce anti-coronavirus antibodies (e.g., broadly neutralizing anti-coronavirus antibodies), which can also be used for prophylactic or therapeutic purposes in the treatment of a coronavirus infection.



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COMPOSITIONS AND METHODS FOR TREATING CORONAVIRUS INFECTION

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. The ASCII copy, created on March 15, 2022, is named "01948-283WO2_Sequence_Listing_3_15_22_ST25" and is 336,865 bytes in size.

BACKGROUND

Wuhan coronavirus (2019-nCoV; also referred to as SARS-CoV-2) is a coronavirus that is responsible for a worldwide pandemic. SARS-CoV-2 and subsequently arising lineage variants thereof are known to cause respiratory symptoms and fever, which may result in death.

The World Health Organization declared the SARS-CoV-2 or a variant thereof outbreak a Public Health Emergency of International Concern on January 30, 2020 and has confirmed over 119,000,000 cases in 197 countries. Over the course of the pandemic, various SARS-CoV-2 lineages carrying different mutations in the Spike protein have developed throughout the world. Some of these lineages may be less susceptible to neutralization by antibodies produced by the original SARS-CoV-2 strain, as well as currently available vaccines directed against this strain. Accordingly, there is an unmet need in the field for therapy of SARS-CoV-2 and variants thereof, including a vaccine with enhanced breadth of antibody response (e.g., to multiple different Spike variants).

SUMMARY OF THE DISCLOSURE

The three Signature-based Epitope Targeted (SET) immunogens (e.g., SET1, SET2, SET3) (Bricault *et al.*, *Cell Host Microbe* 26:296 (2019)) and the four Epigraph (EG) designed immunogens (e.g., EG4, EG5, EG6, and EG7) (Theiler *et al.*, *Statistics in Medicine* 37:181 (2018); Theiler *et al.*, *Scientific Reports* 6:33987 (2016)) can be used to produce a vaccine composition for producing neutralizing antibodies against currently existing SARS-CoV-2 and its lineage variants, as well as newly arising variants. References describing these immunogen design approaches (e.g., Bricault *et al.*, *Cell Host Microbe* 26:296 (2019); Theiler *et al.*, *Statistics in Medicine* 37:181 (2018); and Theiler *et al.*, *Scientific Reports* 6:33987 (2016)) are each herein incorporated by reference.

A first aspect of the disclosure features an isolated nucleic acid molecule with a nucleotide sequence that encodes a polypeptide having at least 85% sequence identity to at least 500 contiguous amino acids within positions 18-1208 (e.g., positions 50-1100, 100-1000, 200-900, 300-800) of any one of SEQ ID NOs: 1 to 4 or a complementary sequence thereof, in which the polypeptide has at least one (or more) of the following mutations: S13I, L18F, T20N, P26S, Δ69-70, D80A, D80Y, L141F, Δ144, W152C, M153T, M153I, F157L, Δ242-244, D253G, S255F, A262S, V367F, K417N, K417T, N439K, L452R, Y453F, S477N, S477R, E484K, S494P, N501T, N501Y, Q613H, D614G, and P681R relative to the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 35. In some embodiments, the polypeptide is capable of eliciting an immune response in a subject; or the polypeptide has at least 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity to, or the polypeptide sequence of, any one of SEQ ID NOs: 1 to 4. In some embodiments, the polypeptide includes two, three, four, five, six, seven, eight, nine, ten,

eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, twenty-four, twenty-five, twenty-six, or twenty-seven of the mutations.

Preferably, the polypeptide includes eight to twelve of the mutations. In some embodiments, the

polypeptide has: (a) one or more of the mutations: L18F, T20N, P26S, D80A, M153T, M153I, Δ242-244,

5 K417N, Y453F, E484K, N501Y, and D614G relative to the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 35; (b) one or more of the mutations: S13I, Δ69-70, Δ144, W152C, D253G, A262S, L452R, S477N, and D614G relative to the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 35; or (c) one or more of the mutations: D80Y, L141F, F157L, S255F, V367F, K417T, N439K, S477R, S494P, N501T, Q613H, and P681R relative to the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 35. For

10 example, in some embodiments, the polypeptide of (a) includes two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve of the mutations, the polypeptide of (b) includes two, three, four, five, six, seven, eight, or nine of the mutations; or the polypeptide of (c) includes two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve of the mutations. In some embodiments, the polypeptide of (a) includes each of the mutations: L18F, T20N, P26S, D80A, M153T, Δ242-244, K417N, Y453F, E484K, N501Y, and

15 D614G relative to the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 35. In some embodiments, the polypeptide of (b) includes each of the mutations: S13I, Δ69-70, Δ144, W152C, D253G, A262S, L452R, S477N, and D614G relative to the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 35. In some embodiments, the polypeptide of (c) includes each of mutations: D80Y, L141F, F157L, S255F, V367F, K417T, N439K, S477R, S494P, N501T, Q613H, and P681R relative to the amino acid sequence

20 of SEQ ID NO: 1 or SEQ ID NO: 35. In some embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 2. In some embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 3. In some embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 4.

In some embodiments, the nucleotide sequence has at least 85% (e.g., 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to all or a portion of any
25 one of SEQ ID NOs: 7 to 9, or a complementary sequence thereof. In some embodiments, the nucleic acid molecule, or a portion thereof, is capable of eliciting an immune response in a subject. In some embodiments, the nucleic acid molecule has the nucleic acid sequence of any one of SEQ ID NOs: 7-12, such as the nucleic acid sequence of SEQ ID NO: 7, the nucleic acid sequence of SEQ ID NO: 8, or the nucleic acid sequence of SEQ ID NO: 9.

30 In some embodiments the nucleic acid molecules may also encode a Spike (S) protein of SARS-CoV-2 (e.g., SEQ ID NO: 29) or a variant thereof containing further modifications to one or more regions. For example, the nucleic acid molecule may encode a S protein, as defined herein, with a deletion of the cytoplasmic region (e.g., SEQ ID NO: 30), with a deletion of the cytoplasmic and transmembrane domains, leaving only S protein ectodomain (e.g., SEQ ID NO: 31), a deletion of the S1 domain (e.g., a
35 deletion of SEQ ID NO: 32, or variant thereof, within a S polynucleotide (e.g., SEQ ID NO: 29), or variant thereof)), a deletion of the S2 region such that only the S1 region of S remains, or a S protein with a deletion of the receptor binding domain (e.g., a deletion of SEQ ID NO: 33, or variant thereof, within a S polynucleotide (e.g., SEQ ID NO: 29), or variant thereof). The nucleic acid molecules may also feature additional modifications to regions of the S protein, including deletion of or inclusion of a signal sequence
40 (e.g., SEQ ID NO: 28), one or more stabilizing mutations (e.g., proline substitutions corresponding to amino acids K969 and V970 of, e.g., SEQ ID NO: 34), an inactivation of a furin cleavage site (e.g., SEQ

ID NO: 27), a trimerization domain (e.g., a foldon trimerization domain, e.g., SEQ ID NO: 23, or other trimerization domain known in the art), a linker or spacer sequence(s) (e.g., SEQ ID NOs: 24 and 25), and combinations thereof. In some embodiments, the nucleic acid molecules of the disclosure (e.g., SEQ ID NO: 7-9) may contain a 5' Kozak sequence, at least one or more 3' stop codons, and at least one or more 5' and/or 3' restriction enzyme sites (e.g., KpnI, HindIII, NheI, and EcoRI).

A second aspect of the disclosure features an isolated polypeptide encoded by the nucleic acid molecule of the first aspect. In some embodiments, the polypeptide has at least 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity to at least 500 contiguous amino acids within positions 18-1208 (e.g., positions 50-1100, 100-1000, 200-900, and 300-800), or the amino acid sequence of, any one of SEQ ID NOs: 1 to 4 and at least one of the following mutations: S13I, L18F, T20N, P26S, Δ69-70, D80A, D80Y, L141F, Δ144, W152C, M153T, M153I, F157L, Δ242-244, D253G, S255F, A262S, V367F, K417N, K417T, N439K, L452R, Y453F, S477N, S477R, E484K, S494P, N501T, N501Y, Q613H, D614G, and P681R relative to the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 35. In some embodiments, the polypeptide, or a portion or fragment thereof, is capable of eliciting an immune response in a subject. In some embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 2. In some embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 3. In some embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 4. In some embodiments, the polypeptides of the disclosure may also include a deletion of or an inclusion of a signal sequence (e.g., SEQ ID NO: 20), stabilizing mutations (e.g., proline substitutions corresponding to amino acids K969 and V970 of SEQ ID NO: 34), mutations to add or inactivate a furin cleavage site (e.g., SEQ ID NOs: 18 or 19), introduction of a trimerization domain (e.g., a foldon trimerization domain, e.g., SEQ ID NO: 15, or other trimerization domain known in the art), introduction of linker or spacer sequences (e.g., SEQ ID NOs: 16 and 17), and combinations thereof. A signal sequence and each of these modifications are present in the S protein of SEQ ID NO: 1, and any of SEQ ID NOs 2-4 could be similarly modified using the sequence of SEQ ID NO: 1 as a guide (e.g., by aligning the sequences of SEQ ID NO: 2-4 with SEQ ID NO: 1 and making the equivalent modifications found in SEQ ID NO: 1 at the corresponding sequence of SEQ ID NOs: 2-4).

A third aspect of the disclosure features an isolated vector including one or more of the nucleic acid molecules of the first and/or second aspects. In some embodiments, the vector is a mammalian, bacterial, or viral vector. In some embodiments, the vector is an expression vector. In some embodiments, the viral vector is a virus selected from the group consisting of a retrovirus, adenovirus, adeno-associated virus, parvovirus, coronavirus, negative strand RNA viruses, orthomyxovirus, rhabdovirus, paramyxovirus, positive strand RNA viruses, picornavirus, alphavirus, double stranded DNA viruses, herpesvirus, Epstein-Barr virus, cytomegalovirus, fowlpox, and canarypox. In some embodiments, the vector is an adenovirus. In some embodiments, the adenovirus is selected from the group consisting of Ad26, Ad52, Ad59, Ad2, Ad5, Ad11, Ad12, Ad24, Ad34, Ad35, Ad40, Ad48, Ad49, Ad50, and Pan9. Preferably, the adenovirus is Ad26. In some embodiments, the Ad52 is a rhesus Ad52 or the Ad59 is a rhesus Ad59. In some embodiments, the vector is a replication-defective vector. In some embodiments, the replication-defective vector is a viral vector (e.g., an adenoviral vector) that contains a deletion in or of one or more of the E1, E3, and/or E4 regions. In other embodiments, the viral

vector (e.g., an adenoviral vector) includes one or more of the E1, E3, and/or E4 regions and is replication-competent.

A fourth aspect of the disclosure features an isolated antibody that specifically binds to the polypeptide of any one of the foregoing aspects. In some embodiments, the antibody is generated by administering the nucleic acid molecule of the first aspect, the polypeptide of the second aspect, or the vector of the third aspect to a mammal. In some embodiments, the nucleic acid molecule includes a nucleic acid sequence of any one of SEQ ID NOs: 7 to 12 or a variant thereof with at least 85% sequence identity thereto, or a complementary sequence thereof. In some embodiments, the polypeptide includes the amino acid sequence of any one of SEQ ID NOs: 2 to 4, or a variant thereof with at least 85% sequence identity thereto. In some embodiments, the vector (e.g., an Ad26 vector) contains a nucleic acid sequence of any one of SEQ ID NOs: 7 to 12 or a variant thereof with at least 85% sequence identity thereto, or a complementary sequence thereof. In some embodiments, the mammal is a human, cow, goat, mouse, or rabbit (e.g., a human). In some embodiments, the antibody is humanized (e.g., for administration to a human). In some embodiments, the antibody is an IgG. In some embodiments, the antibody is a bis-Fab, Fv, Fab, Fab'-SH, F(ab')₂, a diabody, a linear antibody, or a scFV.

A fifth aspect of the disclosure features a method of producing an antibody including administering one or more of the nucleic acid molecules of the first aspect, one or more of the polypeptides of the second aspect, and/or one or more of the vectors of the third aspect to a subject to elicit production of neutralizing antisera in the subject (e.g., the subject is a human or a non-human mammal). In some embodiments, the one or more nucleic acid molecules includes a nucleic acid sequence of any one of SEQ ID NOs: 7 to 12 or a variant thereof with at least 85% sequence identity thereto, or a complementary sequence thereof, the one or more polypeptides comprise the amino acid sequence of any one of SEQ ID NOs: 2 to 4, or a variant thereof with at least 85% sequence identity thereto, or the one or more vectors (e.g., an Ad26 vector) contain a nucleic acid sequence of any one of SEQ ID NOs: 7 to 12 or a variant thereof with at least 85% sequence identity thereto, or a complementary sequence thereof. Preferably, the method elicits the production of neutralizing antisera directed against SARS-CoV-2 or a variant thereof after administration of the nucleic acid molecule(s), the polypeptide(s), and/or the vector(s) to the subject. In some embodiments, the antibody is produced by the method of the fourth aspect. In some embodiments, the antibody binds to an epitope within a coronavirus spike protein, such as a coronavirus spike protein that includes the amino acid sequence of any one of SEQ ID NOs: 1-4. Preferably, the antibody binds to an epitope within the N-terminal domain (NTD) or the receptor binding domain (RBD) of the coronavirus spike protein. For example, the antibody specifically binds to a coronavirus spike protein that contains one or more of the following mutations S13I, L18F, T20N, P26S, Δ69-70, D80A, D80Y, L141F, Δ144, W152C, M153T, M153I, F157L, Δ242-244, D253G, S255F, A262S, V367F, K417N, K417T, N439K, L452R, Y453F, S477N, S477R, E484K, S494P, N501T, N501Y, Q613H, D614G, and P681R, and/or the antibody neutralizes one or more of the B.1.1.7, B.1.429, B.1.1.28, B.1.351, or A23.1 lineages of SARS-CoV-2 or a variant thereof.

A sixth aspect of the disclosure features a composition containing a nucleic acid molecule of the first aspect, a polypeptide of the second aspect, a vector of the third aspect, or an antibody of the fourth or fifth aspect. In some embodiments, the composition further includes a pharmaceutically acceptable

carrier, excipient, or diluent. In some embodiments, the composition further includes an adjuvant and/or an immunostimulatory agent.

A seventh aspect of the disclosure features an immunogenic composition containing a nucleic acid molecule of the first aspect, a polypeptide of the second aspect, a vector of the third aspect, or an antibody of the fourth or fifth aspect. In some embodiments, the immunogenic composition is a vaccine. In some embodiments, the vaccine is a monovalent or a polyvalent vaccine. In some embodiments, the immunogenic composition is capable of treating or reducing the risk of a coronavirus infection, such as, for example, infection by a 2019-nCoV virus or a variant thereof, in a subject (e.g., a human) in need thereof. In some embodiments, said immunogenic composition elicits production of neutralizing anti-2019-nCoV antisera in the subject. In some embodiments, the subject is a mammal. In some embodiments, the mammal is a human. In some embodiments, the human has an underlying health condition. In some embodiments, the underlying health condition is hypertension, diabetes, or cardiovascular disease.

An eighth aspect of the disclosure features a method of identifying, diagnosing, and/or predicting the susceptibility of a subject (e.g., a human) to a coronavirus infection by determining whether the subject has a protective level of a broadly neutralizing anti-coronavirus antibody (bNAb) against two or more lineages of coronavirus (such as an anti-Spike antibody) in a sample from the subject. Preferably, the protective level is: (i) a level that is at or above a titer of at least about 70, as determined using a pseudovirus neutralization assay; or (ii) a level that is at or above a titer of at least about 25, as determined using a live virus neutralization assay; or (iii) a level that is at least 80% of a median level of an anti-coronavirus antibody in a cohort of convalescent humans, as determined by a pseudovirus neutralization assay or live virus neutralization assay. In some embodiments, the method further includes administering an effective amount of one or more of the compositions of the sixth aspect or one or more of the immunogenic compositions of the seventh aspect to the subject having less than a protective level of the bNAb. In some embodiments, the method further includes identifying a subclass and/or an effector function of the bNAb (e.g., the broadly neutralizing anti-Spike antibody). In some embodiments, (a) the subclass is IgM, IgA, IgG1, IgG2, IgG3, or FcγR2A; and/or (b) the effector function is antibody-dependent neutrophil phagocytosis (ADNP), antibody-dependent complement deposition (ADCD), antibody-dependent monocyte cellular phagocytosis (ADCP), or antibody-dependent NK cell activation. In some embodiments, the sample is a bodily fluid from the subject. Preferably, the bodily fluid is blood. In some embodiments, the coronavirus is 2019-nCoV. In some embodiments, the two or more lineages of coronavirus are selected from the group consisting of B.1.1.7, B.1.429, B.1.1.28, B.1.351, and A23.1.

A ninth aspect of the disclosure features a method of treating or reducing the risk of a coronavirus infection in a subject (e.g., a human) in need thereof, by administering a therapeutically effective amount of one or more of the compositions of the sixth aspect or one or more of the immunogenic compositions of the seventh aspect to the subject. In some embodiments, the method includes administering a therapeutically effective amount of more than one of the compositions or more than one of the immunogenic compositions to the subject. In some embodiments, the method includes administering a therapeutically effective amount of three different types of the compositions or three different types of the immunogenic compositions to the subject. In some embodiments, the method further includes administering to the subject: a) an amount of a nucleic acid molecule with the nucleotide sequence of

SEQ ID NO: 5, the nucleotide sequence of nucleotides 19-3837 of SEQ ID NO: 6, or the nucleotide sequence of SEQ ID NO: 6, and/or b) a polypeptide with the amino acid sequence of SEQ ID NO: 1 or a variant thereof with at least 85% sequence identity to amino acids 18-1208 of SEQ ID NO: 1. In some embodiments, the method further includes administering: i) an Ad26 vector including the nucleic acid molecule; and/or ii) an Ad26 vector including a nucleic acid molecule that encodes the polypeptide. In some embodiments, the method further includes measuring an anti-coronavirus antibody (e.g., an anti-Spike antibody) level in the subject. In some embodiments, the anti-coronavirus antibody level in the subject is measured before and/or after administration of the composition or the immunogenic composition. In some embodiments, the anti-coronavirus antibody level in the subject is measured one or more times over about 1, 2, 3, 4, 5, or 6 days, 1, 2, 3, 4, 5, 6, or 7 weeks, 2, 3, 4, 5, or 6 months, 1, 2, 3, 4, or 5 years after administration. In some embodiments, the anti-coronavirus antibody level of the subject is below a protective level and wherein the method further includes re-administering the composition of any one of the foregoing aspects or the immunogenic composition of any one of the foregoing aspects to said subject or administering a different anti-coronavirus composition to the subject. In some embodiments, the protective level is a level sufficient to reduce symptoms or duration of a coronavirus-mediated disease. In some embodiments, the protective level is: (i) a level that is at or above a titer of at least about 70, as determined using a pseudovirus neutralization assay; or (ii) a level that is at or above a titer of at least about 25, as determined using a live virus neutralization assay; or (iii) a level that is at least 80% of a median level of an anti-coronavirus antibody in a cohort of convalescent humans, as determined by a pseudovirus neutralization assay or live virus neutralization assay. In some embodiments, the coronavirus infection is infection by 2019-nCoV. Preferably, said 2019-nCoV is of the lineage B.1.1.7, B.1.429, B.1.1.28, B.1.351, or A23.1.

A tenth aspect of the disclosure features a method of reducing a coronavirus-mediated activity in a subject (e.g., a human) infected with a 2019-nCoV or a variant thereof, by administering a therapeutically effective amount of one or more of the compositions of the sixth aspect or one or more of the immunogenic compositions of the seventh aspect to the subject. In some embodiments, the method includes administering a therapeutically effective amount of two or more types of the composition or two or more types of the immunogenic composition to the subject. In some embodiments, the method includes administering a therapeutically effective amount of three different types of the composition or three different types of the immunogenic composition to the subject. In some embodiments the method further includes administering to the subject: a) an amount of a nucleic acid molecule with the nucleotide sequence of SEQ ID NO: 5, the nucleotide sequence of nucleotides 19-3837 of SEQ ID NO: 6, or the nucleotide sequence of SEQ ID NO: 6, and/or b) a polypeptide with the amino acid sequence of SEQ ID NO: 1 or a variant thereof with at least 85% sequence identity to amino acids 18-1208 of SEQ ID NO: 1. In some embodiments, the method includes administering: i) an Ad26 vector including the nucleic acid molecule; and/or ii) an Ad26 vector including a nucleic acid molecule that encodes the polypeptide. Preferably, the therapeutically effective amount of the composition or the immunogenic composition is sufficient to produce a log serum anti-Spike antibody titer greater than 2 in a subject (e.g., a human), as measured by an ELISA assay. In some embodiments, the therapeutically effective amount is between 15 µg and 300 µg of the one or more of compositions of any one of the foregoing aspects or the one or more immunogenic compositions of any one of the foregoing aspects. In some embodiments, the activity is

viral titer, viral spread, infection, or cell fusion. In some embodiments, the viral titer is decreased after administration of the one or more compositions or the one or more immunogenic compositions. In some embodiments, the viral titer is decreased by 25% or more. For example, the viral titer is decreased by 50% or 75% or more. In some embodiments, the coronavirus is undetectable after the administration. In some embodiments, the administering occurs prior to exposure to the coronavirus. For example, the administering occurs at least 1 hour, 1 week, 1 month, or a year prior to exposure to the coronavirus. In some embodiments, the administering occurs post-exposure to the coronavirus. For example, the administering occurs at least 15 minutes, 1 hour, 1 day, 1 week, post-exposure to the coronavirus. In some embodiments, the subject is administered at least one dose of the one or more compositions or the one or more immunogenic compositions. In some embodiments, the subject is administered at least two doses of the one or more compositions or the one or more immunogenic compositions. In some embodiments, the composition or the immunogenic composition is administered to the subject as a prime, a boost, or as a prime-boost. In some embodiments, the composition or the immunogenic composition is administered intramuscularly, intravenously, intradermally, percutaneously, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intranasally, intravitreally, intravaginally, intrarectally, topically, intratumorally, peritoneally, subcutaneously, subconjunctively, intravesicularly, mucosally, intrapericardially, intraumbilically, intraocularly, orally, topically, locally, by inhalation, by injection, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, by catheter, by lavage, by gavage, in creams, or in lipid compositions. In some embodiments, the subject is a mammal. In some embodiments, the mammal is a human. In some embodiments, the human has an underlying health condition. In some embodiments, the underlying health condition is hypertension, diabetes, or cardiovascular disease. In some embodiments, the method promotes an immune response in said subject. In some embodiments, the immune response is a humoral immune response. In some embodiments, the humoral immune response is an IgG response.

An eleventh aspect of the disclosure features a composition for use in treating or reducing the risk of a coronavirus infection, such as a SARS-CoV-2 infection or infection by a variant of SARS-CoV-2, in a subject (e.g., a human) in need thereof, containing a therapeutically effective amount of one or more of the compositions of any one of the foregoing aspects (e.g., the composition of the sixth aspect) or one or more of the immunogenic compositions of any one of the foregoing aspects (e.g., the immunogenic composition of the seventh aspect).

A twelfth aspect of the disclosure features a composition for use in reducing a coronavirus-mediated activity in a subject (e.g., a human) infected with SARS-CoV-2 or a variant thereof, including a therapeutically effective amount of the composition of any one of foregoing aspects (e.g., the composition of the sixth aspect) or one or more of the immunogenic compositions of any one of the foregoing aspects (e.g., the immunogenic composition of the seventh aspect). In some embodiments, the composition for use includes a therapeutically effective amount of two or more types of the composition or two or more types of the immunogenic composition. In some embodiments, the composition for use includes a therapeutically effective amount of three different types of the composition or three different types of the immunogenic composition. In some embodiments, the composition for use further contains a) an amount of a nucleic acid molecule including the nucleotide sequence of SEQ ID NO: 5, nucleotides 19-3837 of

SEQ ID NO: 6, or the nucleotide sequence of SEQ ID NO: 6, and/or b) a polypeptide including the amino acid sequence of SEQ ID NO: 1 or a polypeptide having at least 85% sequence identity to amino acids 18-1208 of SEQ ID NO: 1. In some embodiments, the composition includes: i) an Ad26 vector including the nucleic acid molecule of the composition or the immunogenic composition; and/or ii) an Ad26 vector including a nucleic acid molecule that encodes the polypeptide of the composition or the immunogenic composition.

A thirteenth aspect of the disclosure features a method of manufacturing an immunogenic composition for treating or reducing the risk of a coronavirus infection in a subject (e.g., a human) in need thereof. The method includes the steps of: (a) admixing at least one of the nucleic acid molecules of any one of the foregoing aspects (e.g., the first aspect), at least one of the polypeptides of any one of the foregoing aspects (e.g., the second aspect), at least one of the vectors of any one of the foregoing aspects (e.g., the third aspect), at least one of the antibodies of any one of the foregoing aspects (e.g., the fourth and fifth aspects), and at least one of the compositions of any one of the foregoing aspects (e.g., the composition of the sixth aspect and/or the immunogenic composition of the seventh aspect) with a pharmaceutically acceptable carrier, excipient, or diluent to form the immunogenic composition; and (b) placing the immunogenic composition in a container.

A fourteenth aspect of the disclosure features a kit including: (a) a first container including at least one of the nucleic acid molecules of any one of the foregoing aspects (e.g., the first aspect), at least one of the polypeptides of any one of the foregoing aspects (e.g., the second aspect), at least one of the vectors of any one of the foregoing aspects (e.g., the third aspect), at least one of the antibodies of any one of the foregoing aspects (e.g., the fourth and fifth aspects), and at least one of the compositions of any one of the foregoing aspects (e.g., the composition of the sixth aspect and/or the immunogenic composition of the seventh aspect); (b) instructions for use thereof; and optionally (c) a second container including a pharmaceutically acceptable carrier, excipient, or diluent. In some embodiments, the first container further includes a pharmaceutically acceptable carrier, excipient, or diluent. In some embodiments, the kit optionally includes an adjuvant and/or an immunostimulatory agent.

A fifteenth aspect of the disclosure features an isolated nucleic acid molecule with a nucleotide sequence that encodes a polypeptide having at least 85% sequence identity to at least 500 contiguous amino acids within positions 18-1208 (e.g., positions 50-1100, 100-1000, 200-900, and 300-800) of any one of SEQ ID NOs: 35, 40-43, and 65-68 (e.g., a EG1, EG2, EG3, and/or EG4 protein, or a variant thereof) or a complementary sequence thereof, in which the polypeptide has at least one of the following mutations: V3G, L5F, P9L, S13I, L18F, T19R, T20N, P26S, A27S, T33I, V36F, V36I, S45F, H49Y, Q52R, L54F, W64R, A67V, Δ69-70, G75V, T76I, D80A, D80G, P85S, S94F, T95I, S98F, I105V, D111N, S112L, L118F, V120L, V126A, V127F, E132Q, D138Y, G142D, Δ144, N148T, W152R, Δ156-157, F157S, R158G, S162I, T167S, S172A, L176F, D178H, G181V, L189F, R190S, V193L, D198Y, I203V, Y204H, I210T, D215G, L216F, A222V, V227A, D228H, I233V, R237K, Δ242-244, H245Y, Δ246-252, D253G, D253N, W258L, A262S, A263P, V267L, P272L, R273S, E281Q, T284I, V289L, A292S, T299I, K300M, T307I, V308L, E309Q, F318S, V320F, T323I, V227I, P330S, L335F, P337S, G339S, R346S, R346K, A348S, K356R, R357K, V362F, V367F, V367L, S371T, T376I, V382L, P384L, T385N, N394S, N394H, V395I, R403K, E406Q, R408I, Q414K, K417T, K417N, D427N, D427Y, T430I, N439K, N440S, N440K, L452M, L452R, L452Q, L461F, K462T, I468V, T470I, E471Q, T478K, E484K, F490S, S494P, N501T,

N501Y, Y505H, V510L, L513F, A520S, A522S, A522V, T531S, V534I, V534F, N540S, F543L, T547I, T549I, E554D, K558N, P561S, F565L, A570D, T572I, A575S, E583Q, E583D, L585F, G594S, V595I, I598V, T604A, T604I, Q607K, Q613H, D614G, N616S, V622F, A623S, D627G, P631S, T632S, T638I, S640F, N641S, A647S, A653V, H655Y, Y660F, I670V, A672V, Q677H, P681H, P681R, A688V, S691P, A694V, M697I, A701V, S704L, A706V, T716I, T716V, T719I, M731I, T732I, T732A, M740V, T747I, T747N, N751D, S758G, T761I, T761R, G769V, A771S, V772I, Q779K, E780D, V785I, T791I, K795R, D796H, S803A, P809S, P812S, P812L, L822F, V826L, T827I, A831V, K835R, D843N, A845S, A845V, K854N, T859N, T859I, M869I, I870V, A871V, A879S, A879V, T883I, F888L, A890V, A892V, A899S, M902I, A903S, I909V, V911I, V915I, L922F, A924S, S929I, D936Y, S939F, S940F, G946R, D950N, D950H, Q957R, T961M, V963A, S967N, V976F, S982A, K986N, K986R, R995G, I997V, T998I, Q1005H, T1006I, T1009I, R1014K, A1016V, A1020S, A1025G, T1027I, V1033A, K1038Q, V1040F, K1045N, L1049I, M1050I, A1056V, H1058Y, L1063F, T1066N, Q1071H, K1073N, A1078S, D1084Y, D1084E, A1086S, V1094F, H1101D, H1101Y, V1104L, E1111K, D1118H, D1118Y, G1124V, D1127G, I1130V, V1133F, D1139H, L1141W, D1146Y, E1150D, D1153Y, P1162L, P1162S, D1163Y, N1173S, A1174V, V1176F, E1182D, R1185H, K1191N, N1192S, E1195Q, E1202Q, K1205N, Q1208H, I1216T, G1219V, G1219C, V1228L, M1229I, V1230L, M1237I, T1238I, C1243F, C1247F, S1252F, S1252P, D1260N, P1263L, V1264L, H1271Y, T1273I, and T1273A relative to the amino acid sequence of SEQ ID NO: 1 or 35.

In some embodiments, the polypeptide is capable of eliciting an immune response in a subject. The polypeptide may have at least 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity to, or the polypeptide sequence of, any one of SEQ ID NOs: 40-43 and 65-68. In some embodiments, the polypeptide includes two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, twenty-four, twenty-five, twenty-six, twenty-seven, twenty-eight, twenty-nine, thirty, thirty-one, thirty-two, thirty-three, thirty-four, thirty-five, thirty-six, thirty-seven, thirty-eight, thirty-nine, forty, forty-one, forty-two, forty-three, forty-four, forty-five, forty-six, forty-seven, forty-eight, forty-nine, fifty, fifty-one, fifty-two, fifty-three, fifty-four, fifty-five, fifty-six, fifty-seven, fifty-eight, fifty-nine, sixty, sixty-one, sixty-two, sixty-three, sixty-four, sixty-five, sixty-six, sixty-seven, sixty-eight, sixty-nine, seventy, seventy-one, seventy-two, seventy-three, seventy-four, seventy-five, seventy-six, seventy-seven, seventy-eight, seventy-nine, eighty, eighty-one, eighty-two, eighty-three, eighty-four, eighty-five, eighty-six, eighty-seven, eighty-eight, eighty-nine, ninety, ninety-one, ninety-two, ninety-three, ninety-four, ninety-five, ninety-six, ninety-seven, ninety-eight, ninety-nine, one hundred, one hundred one, one hundred two, one hundred three, one hundred four, one hundred five, one hundred six, one hundred seven, one hundred eight, one hundred nine, one hundred ten, one hundred eleven, one hundred twelve, one hundred thirteen, one hundred fourteen, one hundred fifteen, one hundred sixteen, one hundred seventeen, one hundred eighteen, one hundred nineteen, one hundred twenty, or more of the mutations. Preferably, the polypeptide includes nine to one hundred twenty of the mutations. In some embodiments, the polypeptide has: (a) one or more of the mutations: Δ 69-70, Δ 144, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H relative to the amino acid sequence of SEQ ID NO: 1 or 35; (b) one or more of the mutations: L5F, L18F, T20N, P26S, V36F, Q52R, D80G, T95I, I105V, L118F, V127F, D138Y, Δ 156-157, R158G, T167S, D178H, R190S, I203V, D215G, A222V, I233V, Δ 242-244, D253G, A262S, P272L, T284I, T299I, V308L,

F318S, V227I, P337S, R346S, K356R, V367L, P384L, N394S, R408I, K417T, D427N, N439K, L452R, I468V, T478K, E484K, L513F, A522S, T531S, N540S, T549I, K558N, E583D, G594S, T604A, Q613H, D614G, V622F, P631S, S640F, H655Y, I670V, S691P, A701V, T732A, T747I, S758G, G769V, Q779K, D796H, P809S, L822F, A831V, A845S, T859N, I870V, A879S, F888L, A899S, I909V, L922F, S939F, D950N, T961M, I997V, T1006I, A1016V, T1027I, V1040F, L1049I, H1058Y, Q1071H, D1084Y, V1094F, V1104L, I1130V, D1139H, D1153Y, P1162S, V1176F, K1191N, Q1208H, G1219V, V1228L, M1237I, S1252F, V1264L, and T1273I relative to the amino acid sequence of SEQ ID NO: 1 or 35; (c) one or more of the mutations: P9L, T19R, T33I, H49Y, A67V, Δ 69-70, D80A, S98F, S112L, V126A, G142D, W152R, S162I, L176F, L189F, D198Y, I210T, A222V, D228H, H245Y, W258L, V267L, E281Q, A292S, T307I, T323I, L335F, R346K, R357K, V367F, T376I, T385N, V395I, E406Q, K417N, D427Y, N440K, L452Q, K462T, E471Q, E484K, F490S, N501T, V510L, A520S, V534I, T547I, P561S, A570D, T572I, E583Q, V595I, T604I, A623S, T632S, N641S, A653V, A672V, P681R, A694V, S704L, T716V, M731I, M740V, N751D, T761I, A771S, E780D, T791I, S803A, P812S, T827I, D843N, K854N, A871V, T883I, A892V, M902I, V911I, A924S, D936Y, G946R, Q957R, S967N, V976F, K986N, T998I, T1009I, A1020S, V1033A, K1045N, A1056V, T1066N, A1078S, A1086S, H1101D, E1111K, G1124V, V1133F, D1146Y, P1162L, A1174V, R1185H, E1195Q, K1205N, G1219C, M1229I, T1238I, C1247F, D1260N, and H1271Y relative to the amino acid sequence of SEQ ID NO: 1 or 35; or (d) one or more of the mutations: V3G, S13I, L18F, A27S, V36I, S45F, L54F, W64R, G75V, T76I, P85S, S94F, D111N, V120L, E132Q, N148T, F157S, S172A, G181V, V193L, Y204H, L216F, V227A, R237K, Δ 246-252, D253N, A263P, R273S, V289L, K300M, E309Q, V320F, P330S, G339S, A348S, V362F, S371T, V382L, N394H, R403K, Q414K, T430I, N440S, L452M, L461F, T470I, T478K, S494P, N501Y, Y505H, A522V, V534F, F543L, E554D, F565L, A570D, A575S, L585F, I598V, Q607K, D614G, N616S, D627G, T638I, A647S, Y660F, Q677H, A688V, M697I, A706V, T716I, T719I, T732I, T747N, T761R, V772I, V785I, K795R, P812L, V826L, K835R, A845V, T859I, M869I, A879V, A890V, A903S, V915I, S929I, S940F, D950H, V963A, V976F, S982A, K986R, R995G, Q1005H, R1014K, A1025G, K1038Q, M1050I, L1063F, K1073N, D1084E, H1101Y, D1118Y, D1127G, L1141W, E1150D, D1163Y, N1173S, E1182D, N1192S, E1202Q, I1216T, V1230L, C1243F, S1252P, P1263L, and T1273A relative to the amino acid sequence of SEQ ID NO: 1 or 35. For example, in some embodiments, the polypeptide of (a) includes two, three, four, five, six, seven, eight or nine of the mutations, the polypeptide of (b) includes two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, twenty-four, twenty-five, twenty-six, twenty-seven, twenty-eight, twenty-nine, thirty, thirty-one, thirty-two, thirty-three, thirty-four, thirty-five, thirty-six, thirty-seven, thirty-eight, thirty-nine, forty, forty-one, forty-two, forty-three, forty-four, forty-five, forty-six, forty-seven, forty-eight, forty-nine, fifty, fifty-one, fifty-two, fifty-three, fifty-four, fifty-five, fifty-six, fifty-seven, fifty-eight, fifty-nine, sixty, sixty-one, sixty-two, sixty-three, sixty-four, sixty-five, sixty-six, sixty-seven, sixty-eight, sixty-nine, seventy, seventy-one, seventy-two, seventy-three, seventy-four, seventy-five, seventy-six, seventy-seven, seventy-eight, seventy-nine, eighty, eighty-one, eighty-two, eighty-three, eighty-four, eighty-five, eighty-six, eighty-seven, eighty-eight, eighty-nine, ninety, ninety-one, ninety-two, ninety-three, ninety-four, ninety-five, ninety-six, ninety-seven, ninety-eight, ninety-nine, one hundred, one hundred one, one hundred two, one hundred three, one hundred four, one hundred five, or one hundred six of the mutations, the polypeptide of (c) includes two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen,

sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, twenty-four, twenty-five, twenty-six, twenty-seven, twenty-eight, twenty-nine, thirty, thirty-one, thirty-two, thirty-three, thirty-four, thirty-five, thirty-six, thirty-seven, thirty-eight, thirty-nine, forty, forty-one, forty-two, forty-three, forty-four, forty-five, forty-six, forty-seven, forty-eight, forty-nine, fifty, fifty-one, fifty-two, fifty-three, fifty-four, fifty-five, fifty-six, fifty-seven, fifty-eight, fifty-nine, sixty, sixty-one, sixty-two, sixty-three, sixty-four, sixty-five, sixty-six, sixty-seven, sixty-eight, sixty-nine, seventy, seventy-one, seventy-two, seventy-three, seventy-four, seventy-five, seventy-six, seventy-seven, seventy-eight, seventy-nine, eighty, eighty-one, eighty-two, eighty-three, eighty-four, eighty-five, eighty-six, eighty-seven, eighty-eight, eighty-nine, ninety, ninety-one, ninety-two, ninety-three, ninety-four, ninety-five, ninety-six, ninety-seven, ninety-eight, ninety-nine, one hundred, one hundred one, one hundred two, one hundred three, one hundred four, one hundred five, one hundred six, one hundred seven, one hundred eight, one hundred nine, one hundred ten, or one hundred eleven of the mutations, or the polypeptide of (d) includes two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, twenty-four, twenty-five, twenty-six, twenty-seven, twenty-eight, twenty-nine, thirty, thirty-one, thirty-two, thirty-three, thirty-four, thirty-five, thirty-six, thirty-seven, thirty-eight, thirty-nine, forty, forty-one, forty-two, forty-three, forty-four, forty-five, forty-six, forty-seven, forty-eight, forty-nine, fifty, fifty-one, fifty-two, fifty-three, fifty-four, fifty-five, fifty-six, fifty-seven, fifty-eight, fifty-nine, sixty, sixty-one, sixty-two, sixty-three, sixty-four, sixty-five, sixty-six, sixty-seven, sixty-eight, sixty-nine, seventy, seventy-one, seventy-two, seventy-three, seventy-four, seventy-five, seventy-six, seventy-seven, seventy-eight, seventy-nine, eighty, eighty-one, eighty-two, eighty-three, eighty-four, eighty-five, eighty-six, eighty-seven, eighty-eight, eighty-nine, ninety, ninety-one, ninety-two, ninety-three, ninety-four, ninety-five, ninety-six, ninety-seven, ninety-eight, ninety-nine, one hundred, one hundred one, one hundred two, one hundred three, one hundred four, one hundred five, one hundred six, one hundred seven, one hundred eight, one hundred nine, one hundred ten, one hundred eleven, one hundred twelve, one hundred thirteen, one hundred fourteen, one hundred fifteen, one hundred sixteen, one hundred seventeen, one hundred eighteen, one hundred nineteen, or one hundred twenty of the mutations. In some embodiments, the polypeptide of (a) includes each of the mutations: $\Delta 69-70$, $\Delta 144$, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H relative to the amino acid sequence of SEQ ID NO: 1 or 35. In some embodiments, the polypeptide of (b) includes each of the mutations: L5F, L18F, T20N, P26S, V36F, Q52R, D80G, T95I, I105V, L118F, V127F, D138Y, $\Delta 156-157$, R158G, T167S, D178H, R190S, I203V, D215G, A222V, I233V, $\Delta 242-244$, D253G, A262S, P272L, T284I, T299I, V308L, F318S, V227I, P337S, R346S, K356R, V367L, P384L, N394S, R408I, K417T, D427N, N439K, L452R, I468V, T478K, E484K, L513F, A522S, T531S, N540S, T549I, K558N, E583D, G594S, T604A, Q613H, D614G, V622F, P631S, S640F, H655Y, I670V, S691P, A701V, T732A, T747I, S758G, G769V, Q779K, D796H, P809S, L822F, A831V, A845S, T859N, I870V, A879S, F888L, A899S, I909V, L922F, S939F, D950N, T961M, I997V, T1006I, A1016V, T1027I, V1040F, L1049I, H1058Y, Q1071H, D1084Y, V1094F, V1104L, I1130V, D1139H, D1153Y, P1162S, V1176F, K1191N, Q1208H, G1219V, V1228L, M1237I, S1252F, V1264L, and T1273I relative to the amino acid sequence of SEQ ID NO: 1 or 35. In some embodiments, the polypeptide of (c) includes each of mutations: P9L, T19R, T33I, H49Y, A67V, $\Delta 69-70$, D80A, S98F, S112L, V126A, G142D, W152R, S162I, L176F, L189F, D198Y, I210T, A222V, D228H, H245Y, W258L, V267L, E281Q, A292S, T307I, T323I, L335F, R346K, R357K, V367F, T376I, T385N, V395I, E406Q,

K417N, D427Y, N440K, L452Q, K462T, E471Q, E484K, F490S, N501T, V510L, A520S, V534I, T547I, P561S, A570D, T572I, E583Q, V595I, T604I, A623S, T632S, N641S, A653V, A672V, P681R, A694V, S704L, T716V, M731I, M740V, N751D, T761I, A771S, E780D, T791I, S803A, P812S, T827I, D843N, K854N, A871V, T883I, A892V, M902I, V911I, A924S, D936Y, G946R, Q957R, S967N, V976F, K986N, T998I, T1009I, A1020S, V1033A, K1045N, A1056V, T1066N, A1078S, A1086S, H1101D, E1111K, G1124V, V1133F, D1146Y, P1162L, A1174V, R1185H, E1195Q, K1205N, G1219C, M1229I, T1238I, C1247F, D1260N, and H1271Y relative to the amino acid sequence of SEQ ID NO: 1 or 35. In some embodiments, the polypeptide of (d) includes each of mutations: V3G, S13I, L18F, A27S, V36I, S45F, L54F, W64R, G75V, T76I, P85S, S94F, D111N, V120L, E132Q, N148T, F157S, S172A, G181V, V193L, Y204H, L216F, V227A, R237K, Δ 246-252, D253N, A263P, R273S, V289L, K300M, E309Q, V320F, P330S, G339S, A348S, V362F, S371T, V382L, N394H, R403K, Q414K, T430I, N440S, L452M, L461F, T470I, T478K, S494P, N501Y, Y505H, A522V, V534F, F543L, E554D, F565L, A570D, A575S, L585F, I598V, Q607K, D614G, N616S, D627G, T638I, A647S, Y660F, Q677H, A688V, M697I, A706V, T716I, T719I, T732I, T747N, T761R, V772I, V785I, K795R, P812L, V826L, K835R, A845V, T859I, M869I, A879V, A890V, A903S, V915I, S929I, S940F, D950H, V963A, V976F, S982A, K986R, R995G, Q1005H, R1014K, A1025G, K1038Q, M1050I, L1063F, K1073N, D1084E, H1101Y, D1118Y, D1127G, L1141W, E1150D, D1163Y, N1173S, E1182D, N1192S, E1202Q, I1216T, V1230L, C1243F, S1252P, P1263L, and T1273A relative to the amino acid sequence of SEQ ID NO: 1 or 35.

In some embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 40. In some embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 41. In some embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 42. In some embodiments, the polypeptide has the amino acid of SEQ ID NO: 43. In some embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 65. In some embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 66. In some embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 67. In some embodiments, the polypeptide has the amino acid of SEQ ID NO: 68. In some embodiments, the nucleotide sequence has at least 85% (e.g., 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to all or a portion of any one of SEQ ID NOs: 44-51 and 61-64 or a complementary nucleic acid sequence thereof. In some embodiments, the nucleic acid molecule, or a portion thereof, is capable of eliciting an immune response in a subject. In some embodiments, the nucleic acid molecule has the nucleic acid sequence of any one of SEQ ID NOs: 44-51 and 61-64, such as the nucleic acid sequence of SEQ ID NO: 44, 48, or 61, the nucleic acid sequence of SEQ ID NO: 45, 49, or 62, the nucleic acid sequence of SEQ ID NO: 46, 50, or 63, or the nucleic acid sequence of SEQ ID NO: 47, 51, or 64. In some embodiments the nucleic acid molecules may also encode a Spike (S) protein of SARS-CoV-2 (e.g., SEQ ID NO: 29) or a variant thereof (e.g., SEQ ID NO: 5 or 6) containing further modifications to one or more regions. For example, the nucleic acid molecule may encode a S protein, as defined herein, with a deletion of the cytoplasmic region (e.g., SEQ ID NO: 30), with a deletion of the cytoplasmic and transmembrane domains, leaving only S protein ectodomain (e.g., SEQ ID NO: 31), a deletion of the S1 domain (e.g., a deletion of SEQ ID NO: 32, or variant thereof, within a S polynucleotide (e.g., SEQ ID NO: 29), or variant thereof)), a deletion of the S2 region such that only the S1 region of S remains, or a S protein with a deletion of the receptor binding domain (e.g., a deletion of SEQ ID NO: 33, or variant thereof, within a S polynucleotide (e.g., SEQ ID NO: 29), or variant

age site (e.g., SEQ ID NO: 26 or 27), a trimerization domain (e.g., a foldon trimerization domain, e.g., SEQ ID NO: 23, or other trimerization domain known in the art), a linker or spacer sequence(s) (e.g., SEQ ID NOs: 24 and 25), and combinations thereof. In some embodiments, the nucleic acid molecules of the disclosure (e.g., SEQ ID NO: 7-9) may contain a 5' Kozak sequence, at least one or more 3' stop codons, and at least one or more 5' and/or 3' restriction enzyme sites (e.g., KpnI, HindIII, NheI, and EcoRI).

A sixteenth aspect of the disclosure features an isolated polypeptide encoded by the nucleic acid molecule of the fifteenth aspect. In some embodiments, the polypeptide has at least 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity to at least 500 contiguous amino acids within positions 18-1208 (e.g., positions 50-1100, 100-1000, 200-900, 300-800), or the amino acid sequence of, any one of SEQ ID NOs: 39-43 and 65-68 and at least one of the following mutations: V3G, L5F, P9L, S13I, L18F, T19R, T20N, P26S, A27S, T33I, V36F, V36I, S45F, H49Y, Q52R, L54F, W64R, A67V, Δ69-70, G75V, T76I, D80A, D80G, P85S, S94F, T95I, S98F, I105V, D111N, S112L, L118F, V120L, V126A, V127F, E132Q, D138Y, G142D, Δ144, N148T, W152R, Δ156-157, F157S, R158G, S162I, T167S, S172A, L176F, D178H, G181V, L189F, R190S, V193L, D198Y, I203V, Y204H, I210T, D215G, L216F, A222V, V227A, D228H, I233V, R237K, Δ242-244, H245Y, Δ246-252, D253G, D253N, W258L, A262S, A263P, V267L, P272L, R273S, E281Q, T284I, V289L, A292S, T299I, K300M, T307I, V308L, E309Q, F318S, V320F, T323I, V227I, P330S, L335F, P337S, G339S, R346S, R346K, A348S, K356R, R357K, V362F, V367F, V367L, S371T, T376I, V382L, P384L, T385N, N394S, N394H, V395I, R403K, E406Q, R408I, Q414K, K417T, K417N, D427N, D427Y, T430I, N439K, N440S, N440K, L452M, L452R, L452Q, L461F, K462T, I468V, T470I, E471Q, T478K, E484K, F490S, S494P, N501T, N501Y, Y505H, V510L, L513F, A520S, A522S, A522V, T531S, V534I, V534F, N540S, F543L, T547I, T549I, E554D, K558N, P561S, F565L, A570D, T572I, A575S, E583Q, E583D, L585F, G594S, V595I, I598V, T604A, T604I, Q607K, Q613H, D614G, N616S, V622F, A623S, D627G, P631S, T632S, T638I, S640F, N641S, A647S, A653V, H655Y, Y660F, I670V, A672V, Q677H, P681H, P681R, A688V, S691P, A694V, M697I, A701V, S704L, A706V, T716I, T716V, T719I, M731I, T732I, T732A, M740V, T747I, T747N, N751D, S758G, T761I, T761R, G769V, A771S, V772I, Q779K, E780D, V785I, T791I, K795R, D796H, S803A, P809S, P812S, P812L, L822F, V826L, T827I, A831V, K835R, D843N, A845S, A845V, K854N, T859N, T859I, M869I, I870V, A871V, A879S, A879V, T883I, F888L, A890V, A892V, A899S, M902I, A903S, I909V, V911I, V915I, L922F, A924S, S929I, D936Y, S939F, S940F, G946R, D950N, D950H, Q957R, T961M, V963A, S967N, V976F, S982A, K986N, K986R, R995G, I997V, T998I, Q1005H, T1006I, T1009I, R1014K, A1016V, A1020S, A1025G, T1027I, V1033A, K1038Q, V1040F, K1045N, L1049I, M1050I, A1056V, H1058Y, L1063F, T1066N, Q1071H, K1073N, A1078S, D1084Y, D1084E, A1086S, V1094F, H1101D, H1101Y, V1104L, E1111K, D1118H, D1118Y, G1124V, D1127G, I1130V, V1133F, D1139H, L1141W, D1146Y, E1150D, D1153Y, P1162L, P1162S, D1163Y, N1173S, A1174V, V1176F, E1182D, R1185H, K1191N, N1192S, E1195Q, E1202Q, K1205N, Q1208H, I1216T, G1219V, G1219C, V1228L, M1229I, V1230L, M1237I, T1238I, C1243F, C1247F, S1252F, S1252P, D1260N, P1263L, V1264L, H1271Y, T1273I, and T1273A relative to the amino acid sequence of SEQ ID NO: 1 or 35.

In some embodiments, the polypeptide, or a portion or fragment thereof, is capable of eliciting an immune response in a subject. In some embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 40. In some embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 41. In some embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 42. In some

embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 43. In some embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 65. In some embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 66. In some embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 67. In some embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 68. In some embodiments, the polypeptides of the disclosure may also include a deletion of or an inclusion of a signal sequence (e.g., SEQ ID NO: 20), stabilizing mutations (e.g., proline substitutions corresponding to amino acids K969 and V970 of SEQ ID NO: 34), mutations to add or inactivate a furin cleavage site (e.g., SEQ ID NO: 19), introduction of a trimerization domain (e.g., a foldon trimerization domain, e.g., SEQ ID NO: 15, or other trimerization domain known in the art), introduction of linker or spacer sequences (e.g., SEQ ID NOs: 16 and 17), and combinations thereof. Any of SEQ ID NOs: 40-43 could be modified to include one or more of these modifications, e.g., by using the sequence of SEQ ID NO: 35 as a guide and making the modifications described by any of SEQ ID NOs: 20, 34, 22, 18, 16, and/or 17.

A seventeenth aspect of the disclosure features an isolated vector including one or more of the nucleic acid molecules of the fifteenth and/or sixteenth aspects. The isolated vector may further include one or more nucleic acid molecules of the first and/or second aspects. In some embodiments, the vector is a mammalian, bacterial, or viral vector. In some embodiments, the vector is an expression vector. In some embodiments, the viral vector is a virus selected from the group consisting of a retrovirus, adenovirus, adeno-associated virus, parvovirus, coronavirus, negative strand RNA viruses, orthomyxovirus, rhabdovirus, paramyxovirus, positive strand RNA viruses, picornavirus, alphavirus, double stranded DNA viruses, herpesvirus, Epstein-Barr virus, cytomegalovirus, fowlpox, and canarypox. In some embodiments, the vector is an adenovirus. In some embodiments, the adenovirus is selected from the group consisting of Ad26, Ad52, Ad59, Ad2, Ad5, Ad11, Ad12, Ad24, Ad34, Ad35, Ad40, Ad48, Ad49, Ad50, and Pan9. Preferably, the adenovirus is Ad26. In some embodiments, the Ad52 is a rhesus Ad52 or the Ad59 is a rhesus Ad59. In some embodiments, the vector is a replication-defective vector. In some embodiments, the replication-defective vector is a viral vector (e.g., an adenoviral vector) that contains a deletion in or of one or more of the E1, E3, and/or E4 regions. In other embodiments, the viral vector (e.g., an adenoviral vector) includes one or more of the E1, E3, and/or E4 regions and is replication-competent.

An eighteenth aspect of the disclosure features an isolated antibody that specifically binds to the polypeptide of any one of the foregoing aspects. In some embodiments, the antibody is generated by administering the nucleic acid molecule of the fifteenth aspect, the polypeptide of the sixteenth aspect, or the vector of the seventeenth aspect to a mammal. The antibody may be generated by further including the administration of one or more of the nucleic acid molecules of the first aspect, one or more of the polypeptides of the second aspect, and/or one or more of the vectors of the third aspect to a mammal. In some embodiments, the nucleic acid molecule includes a nucleic acid sequence of one or more of SEQ ID NOs: 7 to 12, SEQ ID NOs: 44 to 51, and/or SEQ ID NOs: 61-64, or a variant thereof with at least 85% sequence identity thereto, or a complementary sequence thereof. In some embodiments, the polypeptide includes the amino acid sequence of one or more of SEQ ID NOs: 2-4, SEQ ID NOs: 40-43, and/or SEQ ID NOs: 65-68, or a variant thereof with at least 85% sequence identity thereto. In some embodiments, the vector (e.g., an Ad26 vector) contains a nucleic acid sequence of one or more of SEQ ID NOs: 7-12,

SEQ ID NOs: 44-51, and/or SEQ ID NOs: 61-64, or a variant thereof with at least 85% sequence identity thereto, or a complementary sequence thereof. In some embodiments, the mammal is a human, cow, goat, mouse, or rabbit (e.g., a human). In some embodiments, the antibody is humanized (e.g., for administration to a human). In some embodiments, the antibody is an IgG. In some embodiments, the antibody is a bis-Fab, Fv, Fab, Fab'-SH, F(ab')₂, a diabody, a linear antibody, or a scFV.

A nineteenth aspect of the disclosure features a method of producing an antibody including administering one or more of the nucleic acid molecules of the fifteenth aspect, one or more of the polypeptides of the sixteenth aspect, and/or one or more of the vectors of the seventeenth aspect to a subject to elicit production of neutralizing antisera in the subject (e.g., the subject is a human or a non-human mammal). The method of producing the antibody may further include the administration of one or more of the nucleic acid molecules of the first aspect, one or more of the polypeptides of the second aspect, and/or one or more of the vectors of the third aspect to said subject. In some embodiments, the one or more nucleic acid molecules includes a nucleic acid sequence of any one of SEQ ID NOs: 7-12, SEQ ID NOs: 44-51, and/or SEQ ID NOs: 61-64, or a variant thereof with at least 85% sequence identity thereto, or a complementary sequence thereof, the one or more polypeptides comprise the amino acid sequence of one of SEQ ID NOs: 2-4, SEQ ID NOs: 40-43, and/or SEQ ID NOs: 65-68, or a variant thereof with at least 85% sequence identity thereto, or the one or more vectors (e.g., an Ad26 vector) contain a nucleic acid sequence of any one of SEQ ID NOs: 7-12, SEQ ID NOs: 44-51, and/or SEQ ID NOs: 61-64, or a variant thereof with at least 85% sequence identity thereto, or a complementary sequence thereof. Preferably, the method elicits the production of neutralizing antisera directed against SARS-CoV-2 or a variant thereof after administration of the nucleic acid molecule(s), the polypeptide(s), and/or the vector(s) to the subject. In some embodiments, the antibody is produced by the method of the fourth and/or eighteenth aspect. In some embodiments, the antibody binds to an epitope within a coronavirus spike protein, such as a coronavirus spike protein that includes the amino acid sequence of any one of SEQ ID NOs: 1, 35, 40-43, and 65-68. Neutralizing antibodies most often bind to epitopes within the N-terminal domain (NTD) or the receptor binding domain (RBD) of the coronavirus spike protein. For example, the antibody specifically binds to a coronavirus spike protein that contains one or more of the following mutations: W64R, A67V, Δ69-70, G75V, T76I, D80G, D80A, P85S, S94F, T95I, S98F, I105V, D111N, S112L, L118F, V120L, V126A, V127F, E132Q, D138Y, G142D, Δ144, N148T, W152R, Δ156-157, F157S, R158G, S162I, T167S, S172A, L176F, D178H, G181V, L189F, R190S, V193L, D198Y, I203V, Y204H, I210T, D215G, L216F, A222V, V227A, D228H, I233V, R237K, Δ242-244, H245Y, Δ246-252, D253G, D253N, W258L, V382L, P384L, T385N, N394S, N394H, V395I, R403K, E406Q, R408I, Q414K, K417T, K417N, D427N, D427Y, T430I, N439K, N440K, N440S, L452R, L452Q, L452M, L461F, K462T, I468V, T470I, E471Q, T478K, E484K, F490S, S494P, N501Y, N501T, and Y505H relative to the amino acid sequence of SEQ ID NO: 35 (e.g., the antibody may bind an epitope that contains one or more of these mutations), but may also bind to a coronavirus spike protein that contains any mutations falling outside of the NTD or RBD domains, such as those depicted in FIG 26. Neutralizing antibodies of the disclosure are intended to neutralize one or more of the B.1.1.7, B.1.429, B.1.1.28, B.1.351, A23.1, B.1.617.1, B.1.617.2, B.1.427, B.1.525, B.1.526, P.1, P.2, P.3, C.36, C.37, B.1.1.519, B.1.526.1, B.1.526.2, R.1, B.1.258.17, B.1.575, B.1.214.2, A.2.5.2, AT.1, B.1.1.523, or B.1.620 lineages of SARS-CoV-2 or a variant thereof.

A twentieth aspect of the disclosure features a composition containing a nucleic acid molecule of the fifteenth aspect, a polypeptide of the sixteenth aspect, a vector of the seventeenth aspect, or an antibody of the eighteenth or nineteenth aspect. The composition may further contain a nucleic acid molecule of the first aspect, a polypeptide of the second aspect, a vector of the third aspect, or an antibody of the fourth or fifth aspect. The vector of the composition may include nucleic acids that encode one or more of the polypeptides of the second aspect and/or one or more of the polypeptides of the sixteenth aspect in any combination. In some embodiments, the composition further includes a pharmaceutically acceptable carrier, excipient, or diluent. In some embodiments, the composition further includes an adjuvant and/or an immunostimulatory agent.

A twenty-first aspect of the disclosure features an immunogenic composition containing a nucleic acid molecule of the fifteenth aspect, a polypeptide of the sixteenth aspect, a vector of the seventeenth aspect, or an antibody of the eighteenth or nineteenth aspect. The immunogenic composition may further contain a nucleic acid molecule of the first aspect, a polypeptide of the second aspect, a vector of the third aspect, or an antibody of the fourth or fifth aspect. The vector of the immunogenic composition may include nucleic acids that encode one or more of the polypeptides of the second aspect and/or one or more of the polypeptides of the sixteenth aspect in any combination. In some embodiments, the immunogenic composition is a vaccine, such as a monovalent or a polyvalent vaccine. In some embodiments, the immunogenic composition is capable of treating or reducing the risk of a coronavirus infection, such as, for example, infection by a 2019-nCoV virus or a variant thereof, in a subject (e.g., a human) in need thereof. In some embodiments, said immunogenic composition elicits production of neutralizing anti-2019-nCoV antisera in the subject. In some embodiments, the subject is a mammal. In some embodiments, the mammal is a human. In some embodiments, the human has an underlying health condition. In some embodiments, the underlying health condition is hypertension, diabetes, or cardiovascular disease.

A twenty-second aspect of the disclosure features a method of identifying, diagnosing, and/or predicting the susceptibility of a subject (e.g., a human) to a coronavirus infection by determining whether the subject has a protective level of a broadly neutralizing anti-coronavirus antibody (bNAb) against two or more lineages of coronavirus (such as an anti-Spike antibody) in a sample from the subject. Preferably, the protective level is: (i) a level that is at or above a titer of at least about 70, as determined using a pseudovirus neutralization assay; or (ii) a level that is at or above a titer of at least about 25, as determined using a live virus neutralization assay; or (iii) a level that is at least 80% of a median level of an anti-coronavirus antibody in a cohort of convalescent humans, as determined by a pseudovirus neutralization assay or live virus neutralization assay. In some embodiments, the method further includes administering an effective amount of one or more of the compositions of the twentieth aspect or one or more of the immunogenic compositions of the twenty-first aspect to the subject having less than a protective level of the bNAb. The method may further include administering an effective amount of one or more of the compositions of the sixth aspect or one or more of the immunogenic compositions of the seventh aspect to the subject. In some embodiments, the method further includes identifying a subclass and/or an effector function of the bNAb (e.g., the broadly neutralizing anti-Spike antibody). In some embodiments, (a) the subclass is IgM, IgA, IgG1, IgG2, IgG3, or FcγR2A; and/or (b) the effector function is antibody-dependent neutrophil phagocytosis (ADNP), antibody-dependent complement deposition

(ADCD), antibody-dependent monocyte cellular phagocytosis (ADCP), or antibody-dependent NK cell activation. In some embodiments, the sample is a bodily fluid from the subject. Preferably, the bodily fluid is blood. In some embodiments, the coronavirus is 2019-nCoV. In some embodiments, the two or more lineages of coronavirus are selected from the group consisting of B.1.1.7, B.1.429, B.1.1.28, B.1.351, A23.1, B.1.617.1, B.1.617.2, B.1.427, B.1.525, B.1.526, P.1, P.2, P.3, C.36, C.37, B.1.1.519, B.1.526.1, B.1.526.2, R.1, B.1.258.17, B.1.575, B.1.214.2, A.2.5.2, AT.1, B.1.1.523, and B.1.620.

A twenty-third aspect of the disclosure features a method of treating or reducing the risk of a coronavirus infection in a subject (e.g., a human) in need thereof, by administering a therapeutically effective amount of one or more of the compositions of the twentieth aspect or one or more of the immunogenic compositions of the twenty-first aspect to the subject. The method may further include administering an effective amount of one or more of the compositions of the sixth aspect or one or more of the immunogenic compositions of the seventh aspect to the subject. In some embodiments, the method includes administering a therapeutically effective amount of more than one of the compositions or more than one of the immunogenic compositions to the subject. In some embodiments, the method includes administering a therapeutically effective amount of three different types of the compositions (e.g., a composition containing a combination of EG1, EG2, and EG3 nucleic acid molecules or polypeptides, or variants thereof as defined herein) or three different types of the immunogenic compositions (e.g., an immunogenic composition containing a combination of EG1, EG2, and EG3 nucleic acid molecules or polypeptides, or variants thereof as defined herein) to the subject. In some embodiments, the method includes administering a therapeutically effective amount of four different types of the compositions (e.g., a composition containing a combination of EG1, EG2, EG3, and EG4 nucleic acid molecules or polypeptides, or variants thereof as defined herein) or four different types of the immunogenic compositions (e.g., an immunogenic composition containing a combination of EG1, EG2, EG3, and EG4 nucleic acid molecules or polypeptides, or variants thereof as defined herein) to the subject. In some embodiments, the method further includes administering to the subject: a) an amount of a nucleic acid molecule with a nucleotide sequence of SEQ ID NO: 29, the nucleotide sequence of nucleotides 19-3837 of SEQ ID NO: 39, or the nucleotide sequence of SEQ ID NO: 39, and/or b) a polypeptide with the amino acid sequence of SEQ ID NO: 1 or 35 or a variant thereof with at least 85% sequence identity to amino acids 18-1208 of SEQ ID NO: 1 or 35. In some embodiments, the method further includes administering: i) an Ad26 vector including the nucleic acid molecule; and/or ii) an Ad26 vector including a nucleic acid molecule that encodes the polypeptide. In some embodiments, the method further includes measuring an anti-coronavirus antibody (e.g., an anti-Spike antibody) level in the subject. In some embodiments, the anti-coronavirus antibody level in the subject is measured before and/or after administration of the composition or the immunogenic composition. In some embodiments, the anti-coronavirus antibody level in the subject is measured one or more times over about 1, 2, 3, 4, 5, or 6 days, 1, 2, 3, 4, 5, 6, or 7 weeks, 2, 3, 4, 5, or 6 months, 1, 2, 3, 4, or 5 years after administration. In some embodiments, the anti-coronavirus antibody level of the subject is below a protective level and wherein the method further includes re-administering the composition of any one of the foregoing aspects or the immunogenic composition of any one of the foregoing aspects to said subject or administering a different anti-coronavirus composition to the subject. In some embodiments, the protective level is a level sufficient to reduce symptoms or duration of a coronavirus-mediated disease. In some embodiments, the protective

level is: (i) a level that is at or above a titer of at least about 70, as determined using a pseudovirus neutralization assay; or (ii) a level that is at or above a titer of at least about 25, as determined using a live virus neutralization assay; or (iii) a level that is at least 80% of a median level of an anti-coronavirus antibody in a cohort of convalescent humans, as determined by a pseudovirus neutralization assay or live virus neutralization assay. In some embodiments, the coronavirus infection is infection by 2019-nCoV. Preferably, said 2019-nCoV is of the lineage B.1.1.7, B.1.429, B.1.1.28, B.1.351, A23.1, B.1.617.1, B.1.617.2, B.1.427, B.1.525, B.1.526, P.1, P.2, P.3, C.36, C.37, B.1.1.519, B.1.526.1, B.1.526.2, R.1, B.1.258.17, B.1.575, B.1.214.2, A.2.5.2, AT.1, B.1.1.523, and B.1.620.

A twenty-fourth aspect of the disclosure features a method of reducing a coronavirus-mediated activity in a subject (e.g., a human) infected with a 2019-nCoV or a variant thereof, by administering a therapeutically effective amount of one or more of the compositions of the twentieth aspect or one or more of the immunogenic compositions of the twenty-first aspect to the subject. The method may further include administering an effective amount of one or more of the compositions of the sixth aspect or one or more of the immunogenic compositions of the seventh aspect to said subject. In some embodiments, the method includes administering a therapeutically effective amount of two or more types of the composition or two or more types of the immunogenic composition to the subject. In some embodiments, the method includes administering a therapeutically effective amount of three different types of the composition or three different types of the immunogenic composition to the subject (e.g., a composition containing a combination of EG1, EG2, and EG3 nucleic acid molecules or polypeptides, or variants thereof as defined herein). In some embodiments, the method includes administering a therapeutically effective amount of four different types of the compositions or four different types of the immunogenic compositions to the subject (e.g., an immunogenic composition containing a combination of EG1, EG2, EG3, and EG4 nucleic acid molecules or polypeptides, or variants thereof as defined herein). In some embodiments the method further includes administering to the subject: a) an amount of a nucleic acid molecule with a nucleotide sequence of SEQ ID NO: 29, the nucleotide sequence of nucleotides 19-3837 of SEQ ID NO: 39, or the nucleotide sequence of SEQ ID NO: 39, and/or b) a polypeptide with the amino acid sequence of SEQ ID NO: 1 or 35 or a variant thereof with at least 85% sequence identity to amino acids 18-1208 of SEQ ID NO: 1 or 35. In some embodiments, the method includes administering: i) an Ad26 vector including the nucleic acid molecule; and/or ii) an Ad26 vector including a nucleic acid molecule that encodes the polypeptide. Preferably, the therapeutically effective amount of the composition or the immunogenic composition is sufficient to produce a log serum anti-Spike antibody titer greater than 2 in a subject (e.g., a human), as measured by an ELISA assay. In some embodiments, the therapeutically effective amount is between 15 µg and 300 µg of the one or more of compositions of any one of the foregoing aspects or the one or more immunogenic compositions of any one of the foregoing aspects. In some embodiments, the activity is viral titer, viral spread, infection, or cell fusion. In some embodiments, the viral titer is decreased after administration of the one or more compositions or the one or more immunogenic compositions. In some embodiments, the viral titer is decreased by 25% or more. For example, the viral titer is decreased by 50% or 75% or more. In some embodiments, the coronavirus is undetectable after the administration. In some embodiments, the administering occurs prior to exposure to the coronavirus. For example, the administering occurs at least 1 hour, 1 week, 1 month, or a year prior to exposure to the coronavirus. In some embodiments, the administering occurs post-exposure to the coronavirus. For

example, the administering occurs at least 15 minutes, 1 hour, 1 day, 1 week, post-exposure to the coronavirus. In some embodiments, the subject is administered at least one dose of the one or more compositions or the one or more immunogenic compositions. In some embodiments, the subject is administered at least two doses of the one or more compositions or the one or more immunogenic compositions. In some embodiments, the composition or the immunogenic composition is administered to the subject as a prime, a boost, or as a prime-boost. In some embodiments, the composition or the immunogenic composition is administered intramuscularly, intravenously, intradermally, percutaneously, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intranasally, intravitreally, intravaginally, intrarectally, topically, intratumorally, peritoneally, subcutaneously, subconjunctively, intravesicularly, mucosally, intrapericardially, intraumbilically, intraocularly, orally, topically, locally, by inhalation, by injection, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, by catheter, by lavage, by gavage, in creams, or in lipid compositions. In some embodiments, the subject is a mammal. In some embodiments, the mammal is a human. In some embodiments, the human has an underlying health condition. In some embodiments, the underlying health condition is hypertension, diabetes, or cardiovascular disease. In some embodiments, the method promotes an immune response in said subject. In some embodiments, the immune response is a humoral immune response. In some embodiments, the humoral immune response is an IgG response.

A twenty-fifth aspect of the disclosure features a composition for use in treating or reducing the risk of a coronavirus infection, such as a SARS-CoV-2 infection or infection by a variant of SARS-CoV-2, in a subject (e.g., a human) in need thereof, containing a therapeutically effective amount of one or more of the compositions of the twentieth aspect or one or more of the immunogenic compositions of the twenty-first aspect. The composition may further contain a therapeutically effective amount of one or more of the compositions of the sixth aspect or one or more of the immunogenic compositions of the seventh aspect.

A twenty-sixth aspect of the disclosure features a composition for use in reducing a coronavirus-mediated activity in a subject (e.g., a human) infected with SARS-CoV-2 or a variant thereof, including a therapeutically effective amount of one or more of the compositions of the twentieth aspect or one or more of the immunogenic compositions of the twenty-first aspect. The composition may further include administering a therapeutically effective amount of one or more of the compositions of the sixth aspect or one or more of the immunogenic compositions of the seventh aspect. In some embodiments, the composition for use includes a therapeutically effective amount of two or more types of the composition or two or more types of the immunogenic composition. In some embodiments, the composition for use includes a therapeutically effective amount of three different types of the composition or three different types of the immunogenic composition. In some embodiments, the composition for use includes for administration a composition containing a) an amount of a nucleic acid molecule including a nucleotide sequence of SEQ ID NO: 29 nucleotides 19-3837 of SEQ ID NO: 39, or the nucleotide sequence of SEQ ID NO: 39, and/or b) a polypeptide including the amino acid sequence of SEQ ID NO: 1 or 35 or a polypeptide having at least 85% sequence identity to amino acids 18-1208 of SEQ ID NO: 1 or 35. In some embodiments, the composition includes: i) an Ad26 vector including the nucleic acid molecule of

the composition or the immunogenic composition; and/or ii) an Ad26 vector including a nucleic acid molecule that encodes the polypeptide of the composition or the immunogenic composition.

A twenty-seventh aspect of the disclosure features a method of manufacturing an immunogenic composition for treating or reducing the risk of a coronavirus infection in a subject (e.g., a human) in need thereof. The method includes the steps of: (a) admixing at least one of the nucleic acid molecules of the fifteenth aspect, at least one of the polypeptides of the sixteenth aspect, at least one of the vectors of the seventeenth aspect, at least one of the antibodies of the eighteenth or nineteenth aspect, and at least one of the compositions of the twentieth aspect and/or the immunogenic composition of the twenty-first aspect with a pharmaceutically acceptable carrier, excipient, or diluent to form the immunogenic composition; and (b) placing the immunogenic composition in a container. The method may further include the step of (c): admixing at least one of the nucleic acid molecules of the first aspect, at least one of the polypeptides of the second aspect, at least one of the vectors of the third aspect, at least one of the antibodies of the fourth or fifth aspect, and at least one of the compositions of the sixth aspect and/or the immunogenic composition of the seventh aspect with a pharmaceutically acceptable carrier, excipient, or diluent to form the immunogenic composition; and (d) placing the immunogenic composition in said container.

A twenty-eighth aspect of the disclosure features a kit including: (a) a first container including at least one of the nucleic acid molecules of the fifteenth aspect, at least one of the polypeptides of the sixteenth aspect, at least one of the vectors of the seventeenth aspect, at least one of the antibodies of any one of the eighteenth and/or nineteenth aspect, and at least one of the compositions of the twentieth aspect and/or the immunogenic composition of the twenty-first aspect; (b) instructions for use thereof; and optionally (c) a second container including a pharmaceutically acceptable carrier, excipient, or diluent. The kit may further include in the first container at least one of the nucleic acid molecules of the first aspect, at least one of the polypeptides of the second aspect, at least one of the vectors of the third aspect, at least one of the antibodies of any one of the fourth and/or fifth aspect, and at least one of the compositions of the sixth aspect and/or the immunogenic composition of the seventh aspect. In some embodiments, the first container further includes a pharmaceutically acceptable carrier, excipient, or diluent. In some embodiments, the kit optionally includes an adjuvant and/or an immunostimulatory agent.

A twenty-ninth aspect of the disclosure features an isolated nucleic acid molecule with a nucleotide sequence that encodes a polypeptide having at least 85% sequence identity to at least 100 contiguous amino acids (e.g., positions 1-100, 1-200, and 100-200) of any one of SEQ ID NOs: 75-77 or a complementary sequence thereof. In some embodiments, the nucleotide sequence has at least 85% (e.g., 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to all or a portion of any one of SEQ ID NOs: 69-71, or a complementary sequence thereof. In some embodiments, the nucleic acid molecule, or a portion thereof, is capable of eliciting an immune response in a subject. In some embodiments, the nucleic acid molecule has the nucleic acid sequence of any one of SEQ ID NOs: 69-71. In some embodiments, the nucleic acid molecule has at least 85% (e.g., 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity to all or a portion of any one of SEQ ID NOs: 69-71, but is not the sequence of SEQ ID NO: 69-71.

A thirtieth aspect of the disclosure features an isolated polypeptide encoded by the nucleic acid molecule of the twenty-ninth aspect. In some embodiments, the polypeptide has at least 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity to at least 100 contiguous amino acids (e.g., positions 1-100, 1-200, and 100-200), or the amino acid sequence of, any one of SEQ ID NOs: 75-77. In some embodiments, the polypeptide, or a portion or fragment thereof, is capable of eliciting an immune response in a subject. In some embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 75. In some embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 76. In some embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 77. In some embodiments, the polypeptide has at least 85% (e.g., 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity to all or a portion of any one of SEQ ID NOs: 75-77, but is not the sequence of SEQ ID NO: 75-77.

A thirty-first aspect of the disclosure features an isolated vector including one or more of the nucleic acid molecules of the twenty-ninth and/or thirtieth aspects. The isolated vector may further include one or more nucleic acid molecules of the first, second, fifteenth, and/or sixteenth aspects. In some embodiments, the vector is a mammalian, bacterial, or viral vector. In some embodiments, the vector is an expression vector. In some embodiments, the viral vector is a virus selected from the group consisting of a retrovirus, adenovirus, adeno-associated virus, parvovirus, coronavirus, negative strand RNA viruses, orthomyxovirus, rhabdovirus, paramyxovirus, positive strand RNA viruses, picornavirus, alphavirus, double stranded DNA viruses, herpesvirus, Epstein-Barr virus, cytomegalovirus, fowlpox, and canarypox. In some embodiments, the vector is an adenovirus. In some embodiments, the adenovirus is selected from the group consisting of Ad26, Ad52, Ad59, Ad2, Ad5, Ad11, Ad12, Ad24, Ad34, Ad35, Ad40, Ad48, Ad49, Ad50, and Pan9. Preferably, the adenovirus is Ad26. In some embodiments, the Ad52 is a rhesus Ad52 or the Ad59 is a rhesus Ad59. In some embodiments, the vector is a replication-defective vector. In some embodiments, the replication-defective vector is a viral vector (e.g., an adenoviral vector) that contains a deletion in or of one or more of the E1, E3, and/or E4 regions. In other embodiments, the viral vector (e.g., an adenoviral vector) includes one or more of the E1, E3, and/or E4 regions and is replication-competent.

A thirty-second aspect of the disclosure features an isolated antibody that specifically binds to the polypeptide of any one of the foregoing aspects. In some embodiments, the antibody is generated by administering the nucleic acid molecule of the twenty-ninth, the polypeptide of the thirtieth aspect, or the vector of the thirty-first aspect to a mammal (e.g., a human). The antibody may be generated by further including the administration of one or more of the nucleic acid molecules of the first and/or fifteenth aspect, one or more of the polypeptides of the second and/or sixteenth aspect, and/or one or more of the vectors of the third and/or seventeenth aspect to a mammal. In some embodiments, the nucleic acid molecule includes a nucleic acid sequence of one or more of SEQ ID NOs: 69-71, or a variant thereof with at least 85% sequence identity thereto, or a complementary sequence thereof. In some embodiments, the polypeptide includes the amino acid sequence of one or more of SEQ ID NOs: 75-77, or a variant thereof with at least 85% sequence identity thereto. In some embodiments, the vector (e.g., an Ad26 vector) contains a nucleic acid sequence of one or more of SEQ ID NOs: 69-71, or a variant thereof with at least 85% sequence identity thereto, or a complementary sequence thereof. In some embodiments, the mammal is a human, cow, goat, mouse, or rabbit (e.g., a human). In some

embodiments, the antibody is humanized (e.g., for administration to a human). In some embodiments, the antibody is an IgG. In some embodiments, the antibody is a bis-Fab, Fv, Fab, Fab'-SH, F(ab')₂, a diabody, a linear antibody, or a scFV.

A thirty-third aspect of the disclosure features a method of producing an antibody including administering one or more of the nucleic acid molecules of the twenty-ninth aspect, one or more of the polypeptides of the thirtieth aspect, and/or one or more of the vectors of the thirty-first aspect to a subject to elicit production of neutralizing antisera in the subject (e.g., the subject is a human or a non-human mammal). The method of producing the antibody may further include the administration of one or more of the nucleic acid molecules of the first and/or fifteenth aspect, one or more of the polypeptides of the second and/or sixteenth aspect, and/or one or more of the vectors of the third and/or seventeenth aspect to said subject. In some embodiments, the one or more nucleic acid molecules includes a nucleic acid sequence of any one of SEQ ID NOs: 69-71, or a variant thereof with at least 85% sequence identity thereto, or a complementary sequence thereof, the one or more polypeptides comprise the amino acid sequence of one of SEQ ID NOs: 75-77, or a variant thereof with at least 85% sequence identity thereto, or the one or more vectors (e.g., an Ad26 vector) contain a nucleic acid sequence of any one of SEQ ID NOs: 69-71, or a variant thereof with at least 85% sequence identity thereto, or a complementary sequence thereof. Preferably, the method elicits the production of neutralizing antisera directed against SARS-CoV-2 or a variant thereof after administration of the nucleic acid molecule(s), the polypeptide(s), and/or the vector(s) to the subject. In some embodiments, the antibody is produced by the method of the thirty-second aspect. In some embodiments, the antibody binds to an epitope within a coronavirus membrane protein, such as a coronavirus membrane protein that includes the amino acid sequence of any one of SEQ ID NOs: 75-77.

A thirty-fourth aspect of the disclosure features a composition containing a nucleic acid molecule of the twenty-ninth aspect, a polypeptide of the thirtieth aspect, a vector of the thirty-first aspect, or an antibody of the thirty-second or thirty-third aspect. The composition may further contain a nucleic acid molecule of the first and/or fifteenth aspect, a polypeptide of the second and/or sixteenth aspect, a vector of the third and/or seventeenth aspect, or an antibody of the fourth, fifth, eighteenth, and/or nineteenth aspect. In some embodiments, the composition further includes a pharmaceutically acceptable carrier, excipient, or diluent. In some embodiments, the composition further includes an adjuvant and/or an immunostimulatory agent.

A thirty-fifth aspect of the disclosure features an immunogenic composition containing a nucleic acid molecule of the twenty-ninth aspect, a polypeptide of the thirtieth aspect, a vector of the thirty-first aspect, or an antibody of the thirty-second or thirty-third aspect. The immunogenic composition may further contain a nucleic acid molecule of the first and/or fifteenth aspect, a polypeptide of the second and/or sixteenth aspect, a vector of the third and/or seventeenth aspect, or an antibody of the fourth, fifth, eighteenth, and/or nineteenth aspect. In some embodiments, the immunogenic composition is a vaccine. In some embodiments, the vaccine is a monovalent or a polyvalent vaccine. In some embodiments, the immunogenic composition is capable of treating or reducing the risk of a coronavirus infection, such as, for example, infection by a 2019-nCoV virus or a variant thereof, in a subject (e.g., a human) in need thereof. In some embodiments, said immunogenic composition elicits production of neutralizing anti-2019-nCoV antisera in the subject. In some embodiments, the subject is a mammal. In some

embodiments, the mammal is a human. In some embodiments, the human has an underlying health condition. In some embodiments, the underlying health condition is hypertension, diabetes, or cardiovascular disease.

A thirty-sixth aspect of the disclosure features a method of identifying, diagnosing, and/or predicting the susceptibility of a subject (e.g., a human) to a coronavirus infection by determining whether the subject has a protective level of a broadly neutralizing anti-coronavirus antibody (bNAb) against two or more lineages of coronavirus (such as an anti-Spike antibody) in a sample from the subject. Preferably, the protective level is: (i) a level that is at or above a titer of at least about 70, as determined using a pseudovirus neutralization assay; or (ii) a level that is at or above a titer of at least about 25, as determined using a live virus neutralization assay; or (iii) a level that is at least 80% of a median level of an anti-coronavirus antibody in a cohort of convalescent humans, as determined by a pseudovirus neutralization assay or live virus neutralization assay. In some embodiments, the method further includes administering an effective amount of one or more of the compositions of the thirty-fourth aspect or one or more of the immunogenic compositions of the thirty-fifth aspect to the subject having less than a protective level of the bNAb. The method may further include administering an effective amount of one or more of the compositions of the sixth and/or twentieth aspect or one or more of the immunogenic compositions of the seventh and/or twenty-first aspect to the subject. In some embodiments, the method further includes identifying a subclass and/or an effector function of the bNAb (e.g., the broadly neutralizing anti-Spike antibody). In some embodiments, (a) the subclass is IgM, IgA, IgG1, IgG2, IgG3, or FcγR2A; and/or (b) the effector function is antibody-dependent neutrophil phagocytosis (ADNP), antibody-dependent complement deposition (ADCD), antibody-dependent monocyte cellular phagocytosis (ADCP), or antibody-dependent NK cell activation. In some embodiments, the sample is a bodily fluid from the subject. Preferably, the bodily fluid is blood. In some embodiments, the coronavirus is 2019-nCoV. In some embodiments, the two or more lineages of coronavirus are selected from the group consisting of B.1.1.7, B.1.429, B.1.1.28, B.1.351, A23.1, B.1.617.1, B.1.617.2, B.1.427, B.1.525, B.1.526, P.1, P.2, P.3, C.36, C.37, B.1.1.519, B.1.526.1, B.1.526.2, R.1, B.1.258.17, B.1.575, B.1.214.2, A.2.5.2, AT.1, B.1.1.523, and B.1.620.

A thirty-seventh aspect of the disclosure features a method of treating or reducing the risk of a coronavirus infection in a subject (e.g., a human) in need thereof, by administering a therapeutically effective amount of one or more of the compositions of the thirty-fourth aspect or one or more of the immunogenic compositions of the thirty-fifth aspect to the subject. The method may further include administering an effective amount of one or more of the compositions of the sixth and/or twentieth aspect or one or more of the immunogenic compositions of the seventh and/or twenty-first aspect to the subject. In some embodiments, the method includes administering a therapeutically effective amount of more than one of the compositions or more than one of the immunogenic compositions to the subject. In some embodiments, the method further includes administering to the subject: a) an amount of a nucleic acid molecule with a nucleotide sequence of SEQ ID NO: 29, the nucleotide sequence of nucleotides 19-3837 of SEQ ID NO: 39, or the nucleotide sequence of SEQ ID NO: 39, and/or b) a polypeptide with the amino acid sequence of SEQ ID NO: 1 or 35 or a variant thereof with at least 85% sequence identity to amino acids 18-1208 of SEQ ID NO: 1 or 35. In some embodiments, the method further includes administering: i) an Ad26 vector including the nucleic acid molecule; and/or ii) an Ad26 vector including a nucleic acid

molecule that encodes the polypeptide. In some embodiments, the method further includes measuring an anti-coronavirus antibody (e.g., an anti-Spike antibody) level in the subject. In some embodiments, the anti-coronavirus antibody level in the subject is measured before and/or after administration of the composition or the immunogenic composition. In some embodiments, the anti-coronavirus antibody level in the subject is measured one or more times over about 1, 2, 3, 4, 5, or 6 days, 1, 2, 3, 4, 5, 6, or 7 weeks, 2, 3, 4, 5, or 6 months, 1, 2, 3, 4, or 5 years after administration. In some embodiments, the anti-coronavirus antibody level of the subject is below a protective level and wherein the method further includes re-administering the composition of any one of the foregoing aspects or the immunogenic composition of any one of the foregoing aspects to said subject or administering a different anti-coronavirus composition to the subject. In some embodiments, the protective level is a level sufficient to reduce symptoms or duration of a coronavirus-mediated disease. In some embodiments, the protective level is: (i) a level that is at or above a titer of at least about 70, as determined using a pseudovirus neutralization assay; or (ii) a level that is at or above a titer of at least about 25, as determined using a live virus neutralization assay; or (iii) a level that is at least 80% of a median level of an anti-coronavirus antibody in a cohort of convalescent humans, as determined by a pseudovirus neutralization assay or live virus neutralization assay. In some embodiments, the coronavirus infection is infection by 2019-nCoV. Preferably, said 2019-nCoV is of the lineage B.1.1.7, B.1.429, B.1.1.28, B.1.351, A23.1, B.1.617.1, B.1.617.2, B.1.427, B.1.525, B.1.526, P.1, P.2, P.3, C.36, C.37, B.1.1.519, B.1.526.1, B.1.526.2, R.1, B.1.258.17, B.1.575, B.1.214.2, A.2.5.2, AT.1, B.1.1.523, and B.1.620.

A thirty-eighth aspect of the disclosure features a method of reducing a coronavirus-mediated activity in a subject (e.g., a human) infected with a 2019-nCoV or a variant thereof, by administering a therapeutically effective amount of one or more of the compositions of the thirty-fourth aspect or one or more of the immunogenic compositions of the thirty-fifth aspect to the subject. The method may further include administering an effective amount of one or more of the compositions of the sixth and/or twentieth aspect or one or more of the immunogenic compositions of the seventh and/or twenty-first aspect to the subject. In some embodiments the method further includes administering to the subject: a) an amount of a nucleic acid molecule with a nucleotide sequence of SEQ ID NO: 29, the nucleotide sequence of nucleotides 19-3837 of SEQ ID NO: 39, or the nucleotide sequence of SEQ ID NO: 39, and/or b) a polypeptide with the amino acid sequence of SEQ ID NO: 1 or 35 or a variant thereof with at least 85% sequence identity to amino acids 18-1208 of SEQ ID NO: 1 or 35. In some embodiments, the method includes administering: i) an Ad26 vector including the nucleic acid molecule; and/or ii) an Ad26 vector including a nucleic acid molecule that encodes the polypeptide. Preferably, the therapeutically effective amount of the composition or the immunogenic composition is sufficient to produce a log serum anti-Spike antibody titer greater than 2 in a subject (e.g., a human), as measured by an ELISA assay. In some embodiments, the therapeutically effective amount is between 15 µg and 300 µg of the one or more of compositions of any one of the foregoing aspects or the one or more immunogenic compositions of any one of the foregoing aspects. In some embodiments, the activity is viral titer, viral spread, infection, or cell fusion. In some embodiments, the viral titer is decreased after administration of the one or more compositions or the one or more immunogenic compositions. In some embodiments, the viral titer is decreased by 25% or more. For example, the viral titer is decreased by 50% or 75% or more. In some embodiments, the coronavirus is undetectable after the administration. In some embodiments, the

administering occurs prior to exposure to the coronavirus. For example, the administering occurs at least 1 hour, 1 week, 1 month, or a year prior to exposure to the coronavirus. In some embodiments, the administering occurs post-exposure to the coronavirus. For example, the administering occurs at least 15 minutes, 1 hour, 1 day, 1 week, post-exposure to the coronavirus. In some embodiments, the subject is administered at least one dose of the one or more compositions or the one or more immunogenic compositions. In some embodiments, the subject is administered at least two doses of the one or more compositions or the one or more immunogenic compositions. In some embodiments, the composition or the immunogenic composition is administered to the subject as a prime, a boost, or as a prime-boost. In some embodiments, the composition or the immunogenic composition is administered intramuscularly, intravenously, intradermally, percutaneously, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intranasally, intravitreally, intravaginally, intrarectally, topically, intratumorally, peritoneally, subcutaneously, subconjunctively, intravesicularly, mucosally, intrapericardially, intraumbilically, intraocularly, orally, topically, locally, by inhalation, by injection, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, by catheter, by lavage, by gavage, in creams, or in lipid compositions. In some embodiments, the subject is a mammal. In some embodiments, the mammal is a human. In some embodiments, the human has an underlying health condition. In some embodiments, the underlying health condition is hypertension, diabetes, or cardiovascular disease. In some embodiments, the method promotes an immune response in said subject. In some embodiments, the immune response is a humoral immune response. In some embodiments, the humoral immune response is an IgG response.

A thirty-ninth aspect of the disclosure features a composition for use in treating or reducing the risk of a coronavirus infection, such as a SARS-CoV-2 infection or infection by a variant of SARS-CoV-2, in a subject (e.g., a human) in need thereof, containing a therapeutically effective amount of one or more of the compositions of the thirty-fourth aspect or one or more of the immunogenic compositions of the thirty-fifth aspect to the subject. The method may further include administering an effective amount of one or more of the compositions of the sixth and/or twentieth aspect or one or more of the immunogenic compositions of the seventh and/or twenty-first aspect to the subject.

A fortieth aspect of the disclosure features a composition for use in reducing a coronavirus-mediated activity in a subject (e.g., a human) infected with SARS-CoV-2 or a variant thereof, including a therapeutically effective amount of one or more of the compositions of the thirty-fourth aspect or one or more of the immunogenic compositions of the thirty-fifth aspect to the subject. The method may further include administering an effective amount of one or more of the compositions of the sixth and/or twentieth aspect or one or more of the immunogenic compositions of the seventh and/or twenty-first aspect to the subject. In some embodiments, the composition for use includes for administration a composition containing a) an amount of a nucleic acid molecule including a nucleotide sequence of SEQ ID NO: 29 nucleotides 19-3837 of SEQ ID NO: 39, or the nucleotide sequence of SEQ ID NO: 39, and/or b) a polypeptide including the amino acid sequence of SEQ ID NO: 1 or 35 or a polypeptide having at least 85% sequence identity to amino acids 18-1208 of SEQ ID NO: 1 or 35. In some embodiments, the composition includes: i) an Ad26 vector including the nucleic acid molecule of the composition or the immunogenic composition; and/or ii) an Ad26 vector including a nucleic acid molecule that encodes the polypeptide of the composition or the immunogenic composition.

A forty-first aspect of the disclosure features a method of manufacturing an immunogenic composition for treating or reducing the risk of a coronavirus infection in a subject (e.g., a human) in need thereof. The method includes the steps of: (a) admixing at least one of the nucleic acid molecules of the twenty-ninth aspect, at least one of the polypeptides of the thirtieth aspect, at least one of the vectors of the thirty-first aspect, at least one of the antibodies of the thirty-second or thirty-third aspect, and at least one of the compositions of the thirty-fourth aspect and/or the immunogenic composition of the thirty-fifth aspect with a pharmaceutically acceptable carrier, excipient, or diluent to form the immunogenic composition; and (b) placing the immunogenic composition in a container. The method may further include the step of (c): admixing at least one of the nucleic acid molecules of the first and/or fifteenth aspect, at least one of the polypeptides of the second and/or sixteenth aspect, at least one of the vectors of the third and/or seventeenth aspect, at least one of the antibodies of the fourth, fifth, eighteenth, and/or nineteenth aspect, and at least one of the compositions of the sixth and/or twentieth aspect, and/or at least one of the immunogenic compositions of the seventh and/or twenty-first aspect with a pharmaceutically acceptable carrier, excipient, or diluent to form the immunogenic composition; and (d) placing the immunogenic composition in said container.

A forty-second aspect of the disclosure features a kit including: (a) a first container including at least one of the nucleic acid molecules of the twenty-ninth aspect, at least one of the polypeptides of the thirtieth aspect, at least one of the vectors of the thirty-first aspect, at least one of the antibodies of any one of the thirty-second and/or thirty-third aspect, and at least one of the compositions of the thirty-fourth aspect and/or the immunogenic composition of the thirty-fifth aspect; (b) instructions for use thereof; and optionally (c) a second container including a pharmaceutically acceptable carrier, excipient, or diluent. The kit may further include in the first container at least one of the nucleic acid molecules of the first and/or fifteenth aspect, at least one of the polypeptides of the second and/or sixteenth aspect, at least one of the vectors of the third and/or seventeenth aspect, at least one of the antibodies of any one of the fourth, fifth, eighteenth, and/or nineteenth aspect, and at least one of the compositions of the sixth and/or twentieth aspect and/or the immunogenic composition of the seventh and/or twenty-first aspect. In some embodiments, the first container further includes a pharmaceutically acceptable carrier, excipient, or diluent. In some embodiments, the kit optionally includes an adjuvant and/or an immunostimulatory agent.

A forty-third aspect of the disclosure features an isolated nucleic acid molecule with a nucleotide sequence that encodes a polypeptide having at least 85% sequence identity to at least 100 contiguous amino acids (e.g., positions 1-100, 1-200, 1-300, and 1-400) of any one of SEQ ID NOs: 78-80 or a complementary sequence thereof. In some embodiments, the nucleotide sequence has at least 85% (e.g., 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to all or a portion of any one of SEQ ID NOs: 72-74, or a complementary sequence thereof. In some embodiments, the nucleic acid molecule, or a portion thereof, is capable of eliciting an immune response in a subject. In some embodiments, the nucleic acid molecule has the nucleic acid sequence of any one of SEQ ID NOs: 72-74.

A forty-fourth aspect of the disclosure features an isolated polypeptide encoded by the nucleic acid molecule of the twenty-ninth aspect. In some embodiments, the polypeptide has at least 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity to at least 100 contiguous amino acids

(e.g., positions 1-100, 1-200, 1-300, and 1-400), or the amino acid sequence of, any one of SEQ ID NOs: 78-80. In some embodiments, the polypeptide, or a portion or fragment thereof, is capable of eliciting an immune response in a subject. In some embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 78. In some embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 79.

5 In some embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 80.

A forty-fifth aspect of the disclosure features an isolated vector including one or more of the nucleic acid molecules of the forty-third and/or forty-fourth aspects. The isolated vector may further include one or more nucleic acid molecules of the first, second, fifteenth, sixteenth, twenty-ninth and/or thirtieth aspects. In some embodiments, the vector is a mammalian, bacterial, or viral vector. In some
10 embodiments, the vector is an expression vector. In some embodiments, the viral vector is a virus selected from the group consisting of a retrovirus, adenovirus, adeno-associated virus, parvovirus, coronavirus, negative strand RNA viruses, orthomyxovirus, rhabdovirus, paramyxovirus, positive strand RNA viruses, picornavirus, alphavirus, double stranded DNA viruses, herpesvirus, Epstein-Barr virus, cytomegalovirus, fowlpox, and canarypox. In some embodiments, the vector is an adenovirus. In some
15 embodiments, the adenovirus is selected from the group consisting of Ad26, Ad52, Ad59, Ad2, Ad5, Ad11, Ad12, Ad24, Ad34, Ad35, Ad40, Ad48, Ad49, Ad50, and Pan9. Preferably, the adenovirus is Ad26. In some embodiments, the Ad52 is a rhesus Ad52 or the Ad59 is a rhesus Ad59. In some embodiments, the vector is a replication-defective vector. In some embodiments, the replication-defective vector is a viral vector (e.g., an adenoviral vector) that contains a deletion in or of one or more of the E1, E3, and/or
20 E4 regions. In other embodiments, the viral vector (e.g., an adenoviral vector) includes one or more of the E1, E3, and/or E4 regions and is replication-competent.

A forty-sixth aspect of the disclosure features an isolated antibody that specifically binds to the polypeptide of any one of the foregoing aspects. In some embodiments, the antibody is generated by administering the nucleic acid molecule of the forty-third aspect, the polypeptide of the forty-fourth aspect,
25 or the vector of the forty-fifth aspect to a mammal. The antibody may be generated by further including the administration of one or more of the nucleic acid molecules of the first, fifteenth and/or twenty-ninth aspect, one or more of the polypeptides of the second, sixteenth, and/or thirtieth aspect, and/or one or more of the vectors of the third, seventeenth, and/or thirty-first aspect to a mammal. In some embodiments, the nucleic acid molecule includes a nucleic acid sequence of one or more of SEQ ID NOs:
30 72-74, or a variant thereof with at least 85% sequence identity thereto, or a complementary sequence thereof. In some embodiments, the polypeptide includes the amino acid sequence of one or more of SEQ ID NOs: 78-80, or a variant thereof with at least 85% sequence identity thereto. In some embodiments, the vector (e.g., an Ad26 vector) contains a nucleic acid sequence of one or more of SEQ ID NOs: 72-74, or a variant thereof with at least 85% sequence identity thereto, or a complementary sequence thereof. In
35 some embodiments, the mammal is a human, cow, goat, mouse, or rabbit (e.g., a human). In some embodiments, the antibody is humanized (e.g., for administration to a human). In some embodiments, the antibody is an IgG. In some embodiments, the antibody is a bis-Fab, Fv, Fab, Fab'-SH, F(ab')₂, a diabody, a linear antibody, or a scFV.

A forty-seventh aspect of the disclosure features a method of producing an antibody including
40 administering one or more of the nucleic acid molecules of the forty-third aspect, one or more of the polypeptides of the forty-fourth aspect, and/or one or more of the vectors of the forty-fifth aspect to a

subject to elicit production of neutralizing antisera in the subject (e.g., the subject is a human or a non-human mammal). The method of producing the antibody may further include the administration of one or more of the nucleic acid molecules of the first, fifteenth, and/or twenty-ninth aspect, one or more of the polypeptides of the second, sixteenth, and/or thirtieth aspect, and/or one or more of the vectors of the third, seventeenth, and/or thirty-first aspect to said subject. In some embodiments, the one or more nucleic acid molecules includes a nucleic acid sequence of any one of SEQ ID NOs: 72-74, or a variant thereof with at least 85% sequence identity thereto, or a complementary sequence thereof, the one or more polypeptides comprise the amino acid sequence of one of SEQ ID NOs: 78-80, or a variant thereof with at least 85% sequence identity thereto, or the one or more vectors (e.g., an Ad26 vector) contain a nucleic acid sequence of any one of SEQ ID NOs: 72-74, or a variant thereof with at least 85% sequence identity thereto, or a complementary sequence thereof. Preferably, the method elicits the production of neutralizing antisera directed against SARS-CoV-2 or a variant thereof after administration of the nucleic acid molecule(s), the polypeptide(s), and/or the vector(s) to the subject. In some embodiments, the antibody is produced by the method of the forty-sixth aspect. In some embodiments, the antibody binds to an epitope within a coronavirus nucleocapsid protein, such as a coronavirus membrane protein that includes the amino acid sequence of any one of SEQ ID NOs: 78-80.

A forty-eighth aspect of the disclosure features a composition containing a nucleic acid molecule of the forty-third aspect, a polypeptide of the forty-fourth aspect, a vector of the forty-fifth aspect, or an antibody of the forty-sixth or forty-seventh aspect. The composition may further contain a nucleic acid molecule of the first, fifteenth, and/or twenty-ninth aspect, a polypeptide of the second, sixteenth, and/or thirtieth aspect, a vector of the third, seventeenth, and/or thirty-first aspect, or an antibody of the fourth, fifth, eighteenth, nineteenth, thirty-second and/or thirty-third aspect. In some embodiments, the composition further includes a pharmaceutically acceptable carrier, excipient, or diluent. In some embodiments, the composition further includes an adjuvant and/or an immunostimulatory agent.

A forty-ninth aspect of the disclosure features an immunogenic composition containing a nucleic acid molecule of the forty-third aspect, a polypeptide of the forty-fourth aspect, a vector of the forty-fifth aspect, or an antibody of the forty-sixth or forty-seventh aspect. The immunogenic composition may further contain a nucleic acid molecule of the first, fifteenth, and/or twenty-ninth aspect, a polypeptide of the second, sixteenth, and/or thirtieth aspect, a vector of the third, seventeenth, and/or thirty-first aspect, or an antibody of the fourth, fifth, eighteenth, nineteenth, thirty-second, and/or thirty-third aspect. In some embodiments, the immunogenic composition is a vaccine. In some embodiments, the vaccine is a monovalent or a polyvalent vaccine. In some embodiments, the immunogenic composition is capable of treating or reducing the risk of a coronavirus infection, such as, for example, infection by a 2019-nCoV virus or a variant thereof, in a subject (e.g., a human) in need thereof. In some embodiments, said immunogenic composition elicits production of neutralizing anti-2019-nCoV antisera in the subject. In some embodiments, the subject is a mammal. In some embodiments, the mammal is a human. In some embodiments, the human has an underlying health condition. In some embodiments, the underlying health condition is hypertension, diabetes, or cardiovascular disease.

A fiftieth aspect of the disclosure features a method of identifying, diagnosing, and/or predicting the susceptibility of a subject (e.g., a human) to a coronavirus infection by determining whether the subject has a protective level of a broadly neutralizing anti-coronavirus antibody (bNAb) against two or

more lineages of coronavirus (such as an anti-Spike antibody) in a sample from the subject. Preferably, the protective level is: (i) a level that is at or above a titer of at least about 70, as determined using a pseudovirus neutralization assay; or (ii) a level that is at or above a titer of at least about 25, as determined using a live virus neutralization assay; or (iii) a level that is at least 80% of a median level of an anti-coronavirus antibody in a cohort of convalescent humans, as determined by a pseudovirus neutralization assay or live virus neutralization assay. In some embodiments, the method further includes administering an effective amount of one or more of the compositions of the forty-eighth aspect or one or more of the immunogenic compositions of the forty-ninth aspect to the subject having less than a protective level of the bNAb. The method may further include administering an effective amount of one or more of the compositions of the sixth, twentieth, and/or thirty-fourth aspect or one or more of the immunogenic compositions of the seventh, twenty-first, and/or thirty-fifth aspect to the subject. In some embodiments, the method further includes identifying a subclass and/or an effector function of the bNAb (e.g., the broadly neutralizing anti-Spike antibody). In some embodiments, (a) the subclass is IgM, IgA, IgG1, IgG2, IgG3, or FcγR2A; and/or (b) the effector function is antibody-dependent neutrophil phagocytosis (ADNP), antibody-dependent complement deposition (ADCD), antibody-dependent monocyte cellular phagocytosis (ADCP), or antibody-dependent NK cell activation. In some embodiments, the sample is a bodily fluid from the subject. Preferably, the bodily fluid is blood. In some embodiments, the coronavirus is 2019-nCoV. In some embodiments, the two or more lineages of coronavirus are selected from the group consisting of B.1.1.7, B.1.429, B.1.1.28, B.1.351, A23.1, B.1.617.1, B.1.617.2, B.1.427, B.1.525, B.1.526, P.1, P.2, P.3, C.36, C.37, B.1.1.519, B.1.526.1, B.1.526.2, R.1, B.1.258.17, B.1.575, B.1.214.2, A.2.5.2, AT.1, B.1.1.523, and B.1.620.

A fifty-first aspect of the disclosure features a method of treating or reducing the risk of a coronavirus infection in a subject (e.g., a human) in need thereof, by administering a therapeutically effective amount of one or more of the compositions of the forty-eighth aspect or one or more of the immunogenic compositions of the forty-ninth aspect to the subject. The method may further include administering an effective amount of one or more of the compositions of the sixth, twentieth, and/or thirty-fourth aspect or one or more of the immunogenic compositions of the seventh, twenty-first, and/or thirty-fifth aspect to the subject. In some embodiments, the method includes administering a therapeutically effective amount of more than one of the compositions or more than one of the immunogenic compositions to the subject. In some embodiments, the method further includes administering to the subject: a) an amount of a nucleic acid molecule with a nucleotide sequence of SEQ ID NO: 29, the nucleotide sequence of nucleotides 19-3837 of SEQ ID NO: 39, or the nucleotide sequence of SEQ ID NO: 39, and/or b) a polypeptide with the amino acid sequence of SEQ ID NO: 1 or 35 or a variant thereof with at least 85% sequence identity to amino acids 18-1208 of SEQ ID NO: 1 or 35. In some embodiments, the method further includes administering: i) an Ad26 vector including the nucleic acid molecule; and/or ii) an Ad26 vector including a nucleic acid molecule that encodes the polypeptide. In some embodiments, the method further includes measuring an anti-coronavirus antibody (e.g., an anti-Spike antibody) level in the subject. In some embodiments, the anti-coronavirus antibody level in the subject is measured before and/or after administration of the composition or the immunogenic composition. In some embodiments, the anti-coronavirus antibody level in the subject is measured one or more times over about 1, 2, 3, 4, 5, or 6 days, 1, 2, 3, 4, 5, 6, or 7 weeks, 2, 3, 4, 5, or 6 months, 1, 2, 3,

4, or 5 years after administration. In some embodiments, the anti-coronavirus antibody level of the subject is below a protective level and wherein the method further includes re-administering the composition of any one of the foregoing aspects or the immunogenic composition of any one of the foregoing aspects to said subject or administering a different anti-coronavirus composition to the subject.

5 In some embodiments, the protective level is a level sufficient to reduce symptoms or duration of a coronavirus-mediated disease. In some embodiments, the protective level is: (i) a level that is at or above a titer of at least about 70, as determined using a pseudovirus neutralization assay; or (ii) a level that is at or above a titer of at least about 25, as determined using a live virus neutralization assay; or (iii) a level that is at least 80% of a median level of an anti-coronavirus antibody in a cohort of convalescent humans,
10 as determined by a pseudovirus neutralization assay or live virus neutralization assay. In some embodiments, the coronavirus infection is infection by 2019-nCoV. Preferably, said 2019-nCoV is of the lineage B.1.1.7, B.1.429, B.1.1.28, B.1.351, A23.1, B.1.617.1, B.1.617.2, B.1.427, B.1.525, B.1.526, P.1, P.2, P.3, C.36, C.37, B.1.1.519, B.1.526.1, B.1.526.2, R.1, B.1.258.17, B.1.575, B.1.214.2, A.2.5.2, AT.1, B.1.1.523, and B.1.620.

15 A fifty-second aspect of the disclosure features a method of reducing a coronavirus-mediated activity in a subject (e.g., a human) infected with a 2019-nCoV or a variant thereof, by administering a therapeutically effective amount of one or more of the compositions of the forty-eighth aspect or one or more of the immunogenic compositions of the forty-ninth aspect to the subject. The method may further include administering an effective amount of one or more of the compositions of the sixth, twentieth,
20 and/or thirty-fourth aspect or one or more of the immunogenic compositions of the seventh, twenty-first, and/or thirty-fifth aspect to the subject. In some embodiments the method further includes administering to the subject: a) an amount of a nucleic acid molecule with a nucleotide sequence of SEQ ID NO: 29, the nucleotide sequence of nucleotides 19-3837 of SEQ ID NO: 39, or the nucleotide sequence of SEQ ID NO: 39, and/or b) a polypeptide with the amino acid sequence of SEQ ID NO: 1 or 35 or a variant thereof
25 with at least 85% sequence identity to amino acids 18-1208 of SEQ ID NO: 1 or 35. In some embodiments, the method includes administering: i) an Ad26 vector including the nucleic acid molecule; and/or ii) an Ad26 vector including a nucleic acid molecule that encodes the polypeptide. Preferably, the therapeutically effective amount of the composition or the immunogenic composition is sufficient to produce a log serum anti-Spike antibody titer greater than 2 in a subject (e.g., a human), as measured by
30 an ELISA assay. In some embodiments, the therapeutically effective amount is between 15 µg and 300 µg of the one or more of compositions of any one of the foregoing aspects or the one or more immunogenic compositions of any one of the foregoing aspects. In some embodiments, the activity is viral titer, viral spread, infection, or cell fusion. In some embodiments, the viral titer is decreased after administration of the one or more compositions or the one or more immunogenic compositions. In some
35 embodiments, the viral titer is decreased by 25% or more. For example, the viral titer is decreased by 50% or 75% or more. In some embodiments, the coronavirus is undetectable after the administration. In some embodiments, the administering occurs prior to exposure to the coronavirus. For example, the administering occurs at least 1 hour, 1 week, 1 month, or a year prior to exposure to the coronavirus. In some embodiments, the administering occurs post-exposure to the coronavirus. For example, the
40 administering occurs at least 15 minutes, 1 hour, 1 day, 1 week, post-exposure to the coronavirus. In some embodiments, the subject is administered at least one dose of the one or more compositions or the

one or more immunogenic compositions. In some embodiments, the subject is administered at least two doses of the one or more compositions or the one or more immunogenic compositions. In some embodiments, the composition or the immunogenic composition is administered to the subject as a prime, a boost, or as a prime-boost. In some embodiments, the composition or the immunogenic composition is administered intramuscularly, intravenously, intradermally, percutaneously, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intranasally, intravitreally, intravaginally, intrarectally, topically, intratumorally, peritoneally, subcutaneously, subconjunctively, intravesicularly, mucosally, intrapericardially, intraumbilically, intraocularly, orally, topically, locally, by inhalation, by injection, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, by catheter, by lavage, by gavage, in creams, or in lipid compositions. In some embodiments, the subject is a mammal. In some embodiments, the mammal is a human. In some embodiments, the human has an underlying health condition. In some embodiments, the underlying health condition is hypertension, diabetes, or cardiovascular disease. In some embodiments, the method promotes an immune response in said subject. In some embodiments, the immune response is a humoral immune response. In some embodiments, the humoral immune response is an IgG response.

A fifty-third aspect of the disclosure features a composition for use in treating or reducing the risk of a coronavirus infection, such as a SARS-CoV-2 infection or infection by a variant of SARS-CoV-2, in a subject (e.g., a human) in need thereof, containing a therapeutically effective amount of one or more of the compositions of the forty-eighth aspect or one or more of the immunogenic compositions of the forty-ninth aspect to the subject. The method may further include administering an effective amount of one or more of the compositions of the sixth, twentieth, and/or thirty-fourth aspect or one or more of the immunogenic compositions of the seventh, twenty-first, and/or thirty-fifth aspect to the subject.

A fifty-fourth aspect of the disclosure features a composition for use in reducing a coronavirus-mediated activity in a subject (e.g., a human) infected with SARS-CoV-2 or a variant thereof, including a therapeutically effective amount of one or more of the compositions of the forty-eighth aspect or one or more of the immunogenic compositions of the forty-ninth aspect to the subject. The method may further include administering an effective amount of one or more of the compositions of the sixth, twentieth, and/or thirty-fourth aspect or one or more of the immunogenic compositions of the seventh, twenty-first, and/or thirty-fifth aspect to the subject. In some embodiments, the composition for use includes for administration a composition containing a) an amount of a nucleic acid molecule including a nucleotide sequence of SEQ ID NO: 29 nucleotides 19-3837 of SEQ ID NO: 39, or the nucleotide sequence of SEQ ID NO: 39, and/or b) a polypeptide including the amino acid sequence of SEQ ID NO: 1 or 35 or a polypeptide having at least 85% sequence identity to amino acids 18-1208 of SEQ ID NO: 1 or 35. In some embodiments, the composition includes: i) an Ad26 vector including the nucleic acid molecule of the composition or the immunogenic composition; and/or ii) an Ad26 vector including a nucleic acid molecule that encodes the polypeptide of the composition or the immunogenic composition.

A fifty-fifth aspect of the disclosure features a method of manufacturing an immunogenic composition for treating or reducing the risk of a coronavirus infection in a subject (e.g., a human) in need thereof. The method includes the steps of: (a) admixing at least one of the nucleic acid molecules of the forty-third aspect, at least one of the polypeptides of the forty-fourth aspect, at least one of the vectors of

the forty-fifth aspect, at least one of the antibodies of the forty-sixth or forty-seventh aspect, and at least one of the compositions of the forty-eighth aspect and/or the immunogenic composition of the forty-ninth aspect with a pharmaceutically acceptable carrier, excipient, or diluent to form the immunogenic composition; and (b) placing the immunogenic composition in a container. The method may further

5 include the step of (c): admixing at least one of the nucleic acid molecules of the first, fifteenth and/or twenty-ninth aspect, at least one of the polypeptides of the second, sixteenth, and/or thirtieth aspect, at least one of the vectors of the third, seventeenth, and/or thirty-first aspect, at least one of the antibodies of the fourth, fifth, eighteenth, nineteenth, thirty-second, and/or thirty-third aspect, and at least one of the compositions of the sixth, twentieth, and/or thirty-fourth aspect and/or at least one of the immunogenic

10 compositions of the seventh, twenty-first, and/or thirty-fifth aspect with a pharmaceutically acceptable carrier, excipient, or diluent to form the immunogenic composition; and (d) placing the immunogenic composition in said container.

A fifty-sixth aspect of the disclosure features a kit including: (a) a first container including at least one of the nucleic acid molecules of the forty-third aspect, at least one of the polypeptides of the forty-

15 fourth aspect, at least one of the vectors of the forty-fifth aspect, at least one of the antibodies of any one of the forty-sixth and/or forty-seventh aspect, and at least one of the compositions of the forty-eighth aspect and/or the immunogenic composition of the forty-ninth aspect; (b) instructions for use thereof; and optionally (c) a second container including a pharmaceutically acceptable carrier, excipient, or diluent. The kit may further include in the first container at least one of the nucleic acid molecules of the first,

20 fifteenth, and/or twenty-ninth aspect, at least one of the polypeptides of the second, sixteenth, and/or thirtieth aspect, at least one of the vectors of the third, seventeenth, and/or thirty-first aspect, at least one of the antibodies of any one of the fourth, fifth, eighteenth, nineteenth, thirty-second, and/or thirty-third aspect, and at least one of the compositions of the sixth, twentieth, and/or thirty-fourth aspect and/or the immunogenic composition of the seventh, twenty-first, and/or thirty-fifth aspect. In some embodiments,

25 the first container further includes a pharmaceutically acceptable carrier, excipient, or diluent. In some embodiments, the kit optionally includes an adjuvant and/or an immunostimulatory agent.

BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this

30 patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

The accompanying drawings are included to illustrate embodiments of the disclosure and further an understanding of its implementations.

FIG. 1 is a graph of the global distribution of the relative number of SARS-CoV-2 spike variants,

35 through October 1, 2020, through February 20, 2021. Circle size indicates the relative sampling within each map.

FIG. 2 shows a set of graphs of entropy scores summarizing the level of diversity found in positions in the SARS-CoV-2 spike globally and in Asia, the United Kingdom, Africa, North America, South America, Europe (excluding variants present in the United Kingdom), and Oceania (e.g., the

40 islands of the central and southern Pacific, including Micronesia, Melanesia, Polynesia, and Australasia).

FIGs. 3A-3D are a set of schematics depicting the most highly variable mutations in global spike data. **FIGs. 3A and 3C** are circular plots using Cramér's V statistic or mutual information, respectively, depicting patterns of co-varying spike mutations among globally circulating strains with the residue site with the highest entropy on the top and with residue sites with decreasing entropy ordered clockwise.

Pairwise co-variation is indicated by lines connecting respective residue sites with the heat bar (blue) indicating increasing pairwise co-variation and receptor binding domain mutations are in purple text, while N-terminal domain mutations are in green text. **FIGs. 3B and 3D** are heat maps using Cramér's V statistic or mutual information, respectively, depicting covariation patterns with the most entropic residue sites of mutation at the left x-axis and y-axis. The right x-axis heat bar indicates increasing pairwise co-variation.

FIGs. 4A-4D are a set of schematics depicting the most highly variable mutations in North American spike data. **FIGs. 4A and 4C** are circular plots using Cramér's V statistic or mutual information, respectively, depicting patterns of co-varying spike mutations among globally circulating strains with the residue site with the highest entropy on the top and with residue sites with decreasing entropy ordered clockwise. Pairwise co-variation is indicated by lines connecting respective residue sites with the heat bar (blue) indicating increasing pairwise co-variation and receptor binding domain mutations are in purple text, while N-terminal domain mutations are in green text. **FIGs. 4B and 4D** are heat maps using Cramér's V statistic or mutual information, respectively, depicting covariation patterns with the most entropic residue sites of mutation at the left x-axis and y-axis. The right x-axis heat bar indicates increasing pairwise co-variation.

FIGs. 5A and 5B are a set of schematics depicting the most highly variable mutations in global spike data. **FIG. 5A** is a circular plot using Cramér's V statistic depicting patterns of co-varying spike mutations among globally circulating strains with the residue site with the highest entropy on the top and with residue sites with decreasing entropy ordered clockwise. Pairwise co-variation is indicated by lines connecting respective residue sites with the heat bar (blue) indicating increasing pairwise co-variation and receptor binding domain mutations are in purple text, while N-terminal domain mutations are in green text. **FIG. 5B** shows a mutation map aligning position-specific mutations within the spike protein of SARS-CoV-2 variants. The vertically numbered amino acid position located above each mutation is relative to amino acid positions within the Wuhan variant of SARS-CoV-2 (SEQ ID NO: 35, which is compared to deposited strains in Global Initiative on Sharing All Influenza Data (GISAID) from August 1, 2020, through February 23, 2021, in lineages B.1.1.7, B.1.1.248, B.1.429, and B.1.351. Abbreviations: NTD, N-terminal domain, RBD, receptor binding domain. In the mutation map, a dot (.) indicates the same amino acid in that position as wild type and a dash (-) indicates a deletion relative to the most common globally-observed amino acid sequence.

FIGs. 6A and 6B are a set of schematics depicting the most highly variable mutations in North American spike data. **FIG. 6A** is a circular plot using Cramér's V statistic depicting patterns of co-varying spike mutations among globally circulating strains with the residue site with the highest entropy on the top and with residue sites with decreasing entropy ordered clockwise. Pairwise co-variation is indicated by lines connecting respective residue sites with the heat bar (blue) indicating increasing pairwise co-variation and receptor binding domain mutations are in purple text, while N-terminal domain mutations are in green text. **FIG. 6B** shows a mutation map aligning position-specific mutations within the spike protein

of SARS-CoV-2 variants. The vertically numbered amino acid position located above each mutation is relative to amino acid positions within the Wuhan variant of SARS-CoV-2 (SEQ ID NO: 35). The respective count number deposited in GISAID from August 1, 2020, through February 23, 2021, in lineages B.1.1.7 and B.1.429 is indicated to the right of each row. In the mutation map, a dot (.) indicates the same amino acid in that position as wild type and a dash (-) indicates a deletion relative to the most common North American-observed amino acid sequence.

FIGs. 7A and 7B are a set of schematics depicting the most highly variable mutations in South American spike data. **FIG. 7A** is a circular plot using Cramér's V statistic depicting patterns of co-varying spike mutations among globally circulating strains with the residue site with the highest entropy on the top and with residue sites with decreasing entropy ordered clockwise. Pairwise co-variation is indicated by lines connecting respective residue sites with the heat bar (blue) indicating increasing pairwise co-variation and receptor binding domain mutations are in purple text, while N-terminal domain mutations are in green text. **FIG. 7B** shows a mutation map aligning position-specific mutations within the spike protein of SARS-CoV-2 variants. The vertically numbered amino acid position located above each mutation is relative to amino acid positions within the Wuhan variant of SARS-CoV-2 (SEQ ID NO: 35). The respective count number deposited in GISAID from August 1, 2020, through February 23, 2021, in lineages B.1.1.7 and B.1.1.248 is indicated to the right of each row. In the mutation map, a dot (.) indicates the same amino acid in that position as wild type and a dash (-) indicates a deletion relative to the most common South American-observed amino acid sequence.

FIGs. 8A and 8B are a set of schematics depicting the most highly variable mutations in Asia spike data. **FIG. 8A** is a circular plot using Cramér's V statistic depicting patterns of co-varying spike mutations among globally circulating strains with the residue site with the highest entropy on the top and with residue sites with decreasing entropy ordered clockwise. Pairwise co-variation is indicated by lines connecting respective residue sites with the heat bar (blue) indicating increasing pairwise co-variation and receptor binding domain mutations are in purple text, while N-terminal domain mutations are in green text. **FIG. 8B** shows a mutation map aligning position-specific mutations within the spike protein of SARS-CoV-2 variants. The vertically numbered amino acid position located above each mutation is relative to amino acid positions within the Wuhan variant of SARS-CoV-2 (SEQ ID NO: 35). The respective count number deposited in GISAID from August 1, 2020, through February 23, 2021, in lineages B.1.1.7, B.1.1.248, and B.1.351 is indicated to the right of each row. In the mutation map, a dot (.) indicates the same amino acid in that position as wild type and a dash (-) indicates a deletion relative to the most common Asian-observed amino acid sequence.

FIGs. 9A and 9B are a set of schematics depicting the most highly variable mutations in Oceania (e.g., the islands of the central and southern Pacific, including Micronesia, Melanesia, Polynesia, and Australasia) spike data. **FIG. 9A** is a circular plot using Cramér's V statistic depicting patterns of co-varying spike mutations among globally circulating strains with the residue site with the highest entropy on the top and with residue sites with decreasing entropy ordered clockwise. Pairwise co-variation is indicated by lines connecting respective residue sites with the heat bar (blue) indicating increasing pairwise co-variation and receptor binding domain mutations are in purple text, while N-terminal domain mutations are in green text. **FIG. 9B** shows a mutation map aligning position-specific mutations within the spike protein of SARS-CoV-2 variants. The vertically numbered amino acid position located above each

mutation is relative to amino acid positions within the Wuhan variant of SARS-CoV-2 (SEQ ID NO: 35). The respective count number deposited in GISAID from August 1, 2020, through February 23, 2021, in lineages B.1.1.7 and common derivatives is indicated to the right of each row. In the mutation map, a dot (.) indicates the same amino acid in that position as wild type and a dash (-) indicates a deletion relative to the most common Oceania-observed amino acid sequence.

FIGs. 10A and 10B are a set of schematics depicting the most highly variable mutations in African spike data. **FIG. 10A** is a circular plot using Cramér's V statistic depicting patterns of co-varying spike mutations among globally circulating strains with the residue site with the highest entropy on the top and with residue sites with decreasing entropy ordered clockwise. Pairwise co-variation is indicated by lines connecting respective residue sites with the heat bar (blue) indicating increasing pairwise co-variation and receptor binding domain mutations are in purple text, while N-terminal domain mutations are in green text. **FIG. 10B** shows a mutation map aligning position-specific mutations within the spike protein of SARS-CoV-2 variants. The vertically numbered amino acid position located above each mutation is relative to amino acid positions within the Wuhan variant of SARS-CoV-2 (SEQ ID NO: 35). The respective count number deposited in GISAID from August 1, 2020, through February 23, 2021, in lineages B.1.1.7 and B.1.351 is indicated to the right of each row. In the mutation map, a dot (.) indicates the same amino acid in that position as wild type and a dash (-) indicates a deletion relative to the most common Africa-observed amino acid sequence.

FIGs. 11A and 11B are a set of schematics depicting the most highly variable mutations in Europe (excluding the United Kingdom spike data). **FIG. 11A** is a circular plot using Cramér's V statistic depicting patterns of co-varying spike mutations among globally circulating strains with the residue site with the highest entropy on the top and with residue sites with decreasing entropy ordered clockwise. Pairwise co-variation is indicated by lines connecting respective residue sites with the heat bar (blue) indicating increasing pairwise co-variation and receptor binding domain mutations are in purple text, while N-terminal domain mutations are in green text. **FIG. 11B** shows a mutation map aligning position-specific mutations within the spike protein of SARS-CoV-2 variants. The vertically numbered amino acid position located above each mutation is relative to amino acid positions within the Wuhan variant of SARS-CoV-2 (SEQ ID NO: 35). The respective count number deposited in GISAID from August 1, 2020, through February 23, 2021, in lineages B.1.1.7 and B.1.351 is indicated to the right of each row. In the mutation map, a dot (.) indicates the same amino acid in that position as wild type and a dash (-) indicates a deletion relative to the most common Europe without the United Kingdom-observed amino acid sequence.

FIGs. 12A and 12B are a set of schematics depicting the most highly variable mutations in the United Kingdom spike data. **FIG. 12A** is a circular plot using Cramér's V statistic depicting patterns of co-varying spike mutations among globally circulating strains with the residue site with the highest entropy on the top and with residue sites with decreasing entropy ordered clockwise. Pairwise co-variation is indicated by lines connecting respective residue sites with the heat bar (blue) indicating increasing pairwise co-variation and receptor binding domain mutations are in purple text, while N-terminal domain mutations are in green text. **FIG. 12B** shows a mutation map aligning position-specific mutations within the spike protein of SARS-CoV-2 variants. The vertically numbered amino acid position located above each mutation is relative to amino acid positions within the Wuhan variant of SARS-CoV-2 (SEQ ID NO:

35). The respective count number deposited in GISAID from August 1, 2020, through February 23, 2021, in lineage B.1.1.7 is indicated to the right of each row. In the mutation map, a dot (.) indicates the same amino acid in that position as wild type and a dash (-) indicates a deletion relative to the most common United Kingdom-observed amino acid sequence.

FIG. 13 is a schematic of the SARS-CoV-2 spike protein mutation landscape, as described in FIG. 8, globally and in Asia, North America, South America, and Africa but with the mutation landscape only depicted for distinct forms of SARS-CoV-2 in lineages B.1.1.7 (clade GR and also known as 501Y.V1), B.1.1.248 (clade GH and also known as 452R), B.1.429 (clade GR and also known as 484.V2), B.1.351 (clade GH and also known as 501Y.V2), and A231 (also known as 376F.V1) with mutations in the receptor binding domain (RBD) and N-terminal domain (NTD) supersite of the spike protein.

FIG. 14 is a schematic of the SARS-CoV-2 spike protein mutation landscape, as described in FIG. 8, in the United Kingdom, Oceania (e.g., the islands of the central and southern Pacific, including Micronesia, Melanesia, Polynesia, and Australasia), and Europe (excluding the United Kingdom data), but with the mutation landscape only depicted for distinct forms of SARS-CoV-2 in lineages B.1.1.7 (clade GR and also known as 501Y.V1), B.1.1.248 (clade GH and also known as 452R), B.1.429 (clade GR and also known as 484.V2), B.1.351 (clade GH and also known as 501Y.V2), and A231 (also known as 376F.V1) with mutations in the receptor binding domain (RBD) and N-terminal domain (NTD) supersite of the spike protein.

FIG. 15 is a schematic of the amino acid mutations (e.g., substitutions and deletions) incorporated into the spike protein of the Signature-based Epitope Targeted 1 (SET1) immunogen, as well information regarding the SARS-CoV-2 lineage containing the indicated amino acid mutation and the area where the virus was observed (e.g., globally and in South America, Africa, United Kingdom, and Europe). The mutations are indicated using the amino acid one letter code. For example, "L18F" denotes the substitution of a leucine (L) residue for a phenylalanine (F) residue at position 18 of the SARS-CoV-2 Spike protein, which mutation has been observed in several different viral variants. Abbreviations: Δ: deleted residues; S. America: South America; S & N America: South and North America; UK: United Kingdom; US: United States.

FIG. 16 is a schematic of the amino acid mutations (e.g., substitutions and deletions) incorporated into the spike protein of the SET2 immunogen, as well information regarding the SARS-CoV-2 lineage containing the indicated amino acid mutation and the area where the virus was observed (e.g., globally and in South America, Africa, United Kingdom, and Europe). The mutations are indicated using the amino acid one letter code. For example, "S13I" denotes the substitution of a serine (S) residue for an isoleucine (I) residue at position 13 of the SARS-CoV-2 Spike protein, which mutation has been observed in several different viral variants. Abbreviations: Δ: deleted residues.

FIG. 17 is a schematic of the amino acid mutations (e.g., substitutions) incorporated into the spike protein of the SET3 immunogen, as well information regarding the SARS-CoV-2 lineage containing the indicated amino acid mutation and the area where the virus was observed (e.g., globally and in South America, Africa, United Kingdom, and Europe). The mutations are indicated using the amino acid one letter code. For example, "D80Y" denotes the substitution of an aspartate (D) residue for a tyrosine (Y) residue at position 80 of the SARS-CoV-2 Spike protein, which mutation has been observed in several

different viral variants. Abbreviations: N. America: North America; UK: United Kingdom; US: United States.

FIG. 18 is a schematic of the structural mapping of amino acid mutations (e.g., substitutions and deletions) in the spike protein introduced in SET1. For example, “L18F” denotes the substitution of a leucine (L) residue for a phenylalanine (F) residue at position 18. Abbreviations: del, deleted residues, RBD, receptor binding domain.

FIG. 19 is a schematic of the structural mapping of amino acid mutations (e.g., substitutions and deletions) in the spike protein introduced in SET2. For example, “S13I” denotes the substitution of a serine (S) residue for an isoleucine (I) residue at position 13. Abbreviations: del, deleted residues, RBD, receptor binding domain.

FIG. 20 is a schematic of the structural mapping of amino acid mutations (e.g., substitutions) in the spike protein introduced in SET3. For example, “D80Y” denotes the substitution of an aspartate (D) residue for a tyrosine (Y) residue at position 80. Abbreviations: del, deleted residues, RBD, receptor binding domain.

FIG. 21 is a schematic illustrating a SARS-CoV-2 haplotype of interest, A23.1, with amino acid mutations F15L, V36F, Q613H, D614D, and P681R relative to the amino acid sequence of SEQ ID NO: 34 (Wuhan SARS-CoV-2; open circles), mapped onto a parsimony tree for Europe, Asia, Oceania, North America, South America, and Africa.

Figs. 22A-22D are a set of schematics depicting key SARS-CoV-2 spike protein mutation information related to the haplotype A23.1. **FIG. 22A** is a schematic depicting the probability of amino acid variations in relevant residue positions 157, 367, 613, 614, and 681, respectively. Using the Analyze Align tool at cov.lanl.gov, data were extracted regarding the residue of interest for the A23.1 spike mutations from GISAID on February 23, 2021. The sequence at the top indicates the most common amino acid frequency in the full dataset. **FIG. 22B** is a set of exploratory plots showing SARS-CoV-2 spike protein mutation in A23.1 in Rwanda, Cambodia, Uganda, and Canada New-Brunswick, respectively, over time. **FIG 22C** is a schematic of the SARS-CoV-2 spike protein mutation landscape of variants and respective count number deposited in GISAID from August 1, 2020, through February 23, 2021. In the mutation map, a dash (-) indicates a deletion relative to the most common globally-observed amino acid sequence. Abbreviations: CA: Canada; DE: Germany; UG: Uganda; UK: United Kingdom; US: United States. **FIG 22D** is a schematic depicting the probability of amino acid variations in relevant residue positions 102, 141, 157, 367, 613, 614, and 681, respectively, of the spike protein. Using the Analyze Align tool at cov.lanl.gov, data were extracted regarding the residue of interest for the A23.1 spike mutations from GISAID on February 23, 2021. The sequence at the top indicates the most common amino acid frequency in the full dataset including data collected at residue positions.

FIG. 23 is a multiple sequence alignment (MSA) depicting exemplary N-terminal domain (NTD) fragments of the optimized spike proteins described herein (e.g., EG1, EG2, EG3, and EG4 which correspond to SEQ ID NO: 40, 41, 42, and 43, respectively) as well as globally circulating SARS-CoV-2 spike protein variants deposited in GISAID and sampled from April 9th through June 18th, 2021. All residue mutations depicted in the MSA are relative to amino acids 64-259 of SEQ ID NO: 35.

FIG. 24 is a MSA of exemplary receptor binding domain (RBD) fragments of the optimized spike proteins described herein (e.g., EG1, EG2, EG3, and EG4 which correspond to SEQ ID NO: 40, 41, 42,

and 43, respectively) as well as globally circulating SARS-CoV-2 spike protein variants deposited in GISAID and sampled from April 9th through June 18th, 2021. All residue mutations depicted in the MSA are relative to amino acids 382-509 of SEQ ID NO: 35.

FIGs. 25A and 25B are plots of linear epitope coverage for EG1, EG2, EG3, and EG4 (SEQ ID Nos: 40-43) within the SARS-CoV-2 spike protein. **FIG. 25A** is a linear plot showing the percentage of potential 9-mer linear epitopes that are missed (Y-axis) across a pseudo-position of the 1273-amino acid spike protein of SARS-CoV-2 (X-axis) within the indicated EG immunogen. The percentage of missed epitopes is, for example, illustrated for EG1, EG2, EG3, and EG4 optimized protein sequences (e.g., SEQ ID Nos: 40-43). **FIG. 25B** is a logarithmic plot of the same data from **FIG. 25A**.

FIG. 26 is a MSA of the entire protein sequence of SEQ ID NOs: 40-43 (e.g., EG1-4), relative to the Wuhan SARS-CoV-2 reference strain, SEQ ID NO: 35.

DEFINITIONS

As used herein, the term “about” means +/- 10% of the recited value.

The terms “adenovirus vector” and “adenoviral vector” are used interchangeably and refer to a genetically-engineered adenovirus that is designed to insert a polynucleotide of interest (e.g., a polynucleotide encoding a SARS-CoV-2 or a variant thereof immunogen) into a eukaryotic cell, such that the polynucleotide is subsequently expressed. Examples of adenoviruses that can be used as a viral vector include those having, or derived from, the serotypes Ad2, Ad5, Ad11, Ad12, Ad24, Ad26, Ad34, Ad35, Ad40, Ad48, Ad49, Ad50, Ad52 (e.g., RhAd52), Ad59 (e.g., RhAd59), and Pan9 (also known as AdC68); these vectors can be derived from, for example, human, chimpanzee, or rhesus adenoviruses. In some embodiments, the adenovirus is Ad26.

The term “adjuvant” refers to a pharmacological or immunological agent that modifies the effect of other agents (e.g., vaccines) while having few if any direct effects when given by itself. They are often included in vaccines to enhance the recipient's immune response to a supplied antigen while keeping the injected foreign material at a minimum.

As used herein, by “administering” is meant a method of giving a dosage of a pharmaceutical composition (e.g., an immunogenic composition (e.g., a vaccine (e.g., a monovalent or a polyvalent coronavirus vaccine (SARS-CoV-2 or a variant thereof))) to a subject. The compositions utilized in the methods described herein can be administered, for example, intramuscularly, intravenously, intradermally, percutaneously, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intranasally, intravitreally, intravaginally, intrarectally, topically, intratumorally, peritoneally, subcutaneously, subconjunctivally, intravesicularly, mucosally, intrapericardially, intraumbilically, intraocularly, orally, topically, locally, by inhalation, by injection, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, by catheter, by lavage, by gavage, in cremes, or in lipid compositions. The preferred method of administration can vary depending on various factors (e.g., the components of the composition being administered and the severity of the condition being treated).

The terms “antibody” and “immunoglobulin (Ig)” are used interchangeably in the broadest sense and include monoclonal antibodies (e.g., full-length or intact monoclonal antibodies), polyclonal antibodies, multivalent antibodies, multispecific antibodies (e.g., bispecific antibodies so long as they

exhibit the desired biological activity) and may also include certain antibody fragments. An antibody typically comprises both "light chains" and "heavy chains." The light chains of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these can be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α , δ , ϵ , γ , and μ , respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

By "broadly neutralizing antibody" or "bnAb," with respect to coronavirus (e.g., SARS-CoV-2 or a variant thereof), is meant an antibody that recognizes a specific antigen (e.g., Spike (e.g., the NTD and/or RBD of a spike protein, such as the SET1, SET2, SET3, EG1, EG2, EG3, and/or EG4 immunogens described herein)) and inhibits the effect(s) of the antigen of at least 2, 3, 4, 5, 6, 7, 8, 9 or more different strains of SARS-CoV-2 or a variant thereof, the strains belonging to the same or different clades, in the host subject (e.g., human). As used herein, the antibody can be a single antibody or a plurality of antibodies.

As used herein, the term "clade" refers to related coronaviruses classified according to their degree of genetic similarity. A clade generally refers to a distinctive branch in a phylogenetic tree. In certain exemplary embodiments, a composition described herein (e.g., a nucleic acid molecule, polypeptide, vector, and/or antibody composition described herein, such as a monovalent or polyvalent vaccine composition as described herein) can be used to elicit an immune response (e.g., the generation of neutralizing anti-coronavirus antisera) against two, three, four, five, six, seven, eight, nine, ten or more clades. In some embodiments, the two or more clades (e.g., also referred to herein as "lineages") of coronavirus are selected from the group consisting of B.1.1.7, B.1.429, B.1.1.28, B.1.351, A23.1 B.1.617.1, B.1.617.2, B.1.427, B.1.525, B.1.526, P.1, P.2, P.3, C.36, C.37, B.1.1.519, B.1.526.1, B.1.526.2, R.1, B.1.258.17, B.1.575, B.1.214.2, A.2.5.2, AT.1, B.1.1.523, and B.1.620.

The term "codon" as used herein refers to any group of three consecutive nucleotide bases in a given messenger RNA molecule, or coding strand of DNA, that specifies a particular amino acid or a starting or stopping signal for translation. The term codon also refers to base triplets in a DNA strand.

Throughout this specification and claims, the word "comprise," or variations such as "comprises" or "comprising," will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

As used herein, the terms "conservative mutation," "conservative substitution," and "conservative amino acid substitution" refer to a substitution of one or more amino acids for one or more different amino acids that exhibit similar physicochemical properties, such as polarity, electrostatic charge, and steric volume. These properties are summarized for each of the twenty naturally-occurring amino acids in Table 1 below.

Table 1. Representative physicochemical properties of naturally occurring amino acids

Amino Acid	3 Letter Code	1 Letter Code	Side-chain Polarity	Electrostatic character at physiological pH (7.4)	Steric Volume [†]
Alanine	Ala	A	nonpolar	neutral	small
Arginine	Arg	R	polar	cationic	large
Asparagine	Asn	N	polar	neutral	intermediate
Aspartic acid	Asp	D	polar	anionic	intermediate
Cysteine	Cys	C	nonpolar	neutral	intermediate
Glutamic acid	Glu	E	polar	anionic	intermediate
Glutamine	Gln	Q	polar	neutral	intermediate
Glycine	Gly	G	nonpolar	neutral	small
Histidine	His	H	polar	Both neutral and cationic forms in equilibrium at pH 7.4	large
Isoleucine	Ile	I	nonpolar	neutral	large
Leucine	Leu	L	nonpolar	neutral	large
Lysine	Lys	K	polar	cationic	large
Methionine	Met	M	nonpolar	neutral	large
Phenylalanine	Phe	F	nonpolar	neutral	large
Proline	Pro	P	non-polar	neutral	intermediate
Serine	Ser	S	polar	neutral	small
Threonine	Thr	T	polar	neutral	intermediate
Tryptophan	Trp	W	nonpolar	neutral	bulky
Tyrosine	Tyr	Y	polar	neutral	large
Valine	Val	V	nonpolar	neutral	intermediate

[†]based on volume in Å³: 50-100 is small, 100-150 is intermediate, 150-200 is large, and >200 is bulky

From this table it is appreciated that the conservative amino acid families include (i) G, A, V, L and I; (ii) D and E; (iii) C, S and T; (iv) H, K and R; (v) N and Q; and (vi) F, Y and W. A conservative mutation or substitution is therefore one that substitutes one amino acid for a member of the same amino acid family (e.g., a substitution of Ser for Thr or Lys for Arg). Table 1 also presents the one letter code for amino acids, which is used herein to designate an amino acid mutation at a particular residue position (e.g., "L18F" refers to the substitution of leucine for phenylalanine at residue position 18 of a polypeptide sequence (e.g., the sequence of a coronavirus Spike protein)).

The term "convalescent" as used herein refers to subjects who have recovered or are recovering from a coronavirus infection (e.g., SARS-CoV-2 or a variant thereof). A "cohort of convalescent humans" refers to a group of humans that share common characteristics (e.g., sex, age, weight, medical history, race, ethnicity, or environment) and have recovered or are recovering from a coronavirus infection (e.g., SARS-CoV-2 or a variant thereof). Preferably, a cohort of convalescent humans will share common characteristics with a subject having a risk of coronavirus (e.g., SARS-CoV-2 or variant thereof) infection or suspected of being susceptible to a coronavirus infection. Preferably, samples from convalescent humans will be obtained at least 7 days after documented recovery (e.g., determined with a negative nasal swab).

The terms "ectodomain" and "extracellular domain" refer to the portion of a coronavirus Spike polypeptide that extends beyond the transmembrane domain into the extracellular space. The

ectodomain mediates binding of a Spike polypeptide to one or more coronavirus receptors (e.g., ACE2). For instance, an ectodomain includes the S1 domain (e.g., SEQ ID NO: 32) and RBD (e.g., SEQ ID NO: 33) of a Spike polypeptide (e.g., SEQ ID NO: 35).

A "gene delivery vehicle" is defined as any molecule that can carry inserted polynucleotides into a host cell. Examples of gene delivery vehicles are liposomes, biocompatible polymers, including natural polymers and synthetic polymers; lipoproteins; polypeptides; polysaccharides; lipopolysaccharides; artificial viral envelopes; metal particles; and bacteria, or viruses, such as baculovirus, adenovirus and retrovirus, bacteriophage, cosmid, plasmid, fungal vectors and other recombination vehicles typically used in the art that have been described for expression in a variety of eukaryotic and prokaryotic hosts, and may be used for gene therapy as well as for simple protein expression.

"Gene delivery," "gene transfer," and the like as used herein, are terms referring to the introduction of an exogenous polynucleotide (sometimes referred to as a "transgene") into a host cell, irrespective of the method used for the introduction. Such methods include a variety of techniques such as, for example, vector-mediated gene transfer (e.g., viral infection/transfection, or various other protein-based or lipid-based gene delivery complexes) as well as techniques facilitating the delivery of "naked" polynucleotides (such as electroporation, "gene gun" delivery and various other techniques used for the introduction of polynucleotides).

The introduced polynucleotide may be stably or transiently maintained in the host cell. Stable maintenance typically requires that the introduced polynucleotide either contains an origin of replication compatible with the host cell or integrates into a replicon of the host cell such as an extrachromosomal replicon (e.g., a plasmid) or a nuclear or mitochondrial chromosome. A number of vectors are capable of mediating transfer of genes to mammalian cells.

By "gene product" is meant to include mRNAs or other nucleic acids (e.g., microRNAs) transcribed from a gene, as well as polypeptides translated from those mRNAs. In some embodiments, the gene product is from a virus (e.g., a SARS-CoV-2 or variant thereof) and may include, for example, any one or more of the viral proteins, or fragments thereof, described herein.

By "heterologous nucleic acid molecule" is meant a nucleotide sequence that may encode proteins derived or obtained from pathogenic organisms, such as viruses, which may be incorporated into a polynucleotide or vector. Heterologous nucleic acids may also encode synthetic or artificial proteins, such as immunogenic epitopes, constructed to induce immunity. An example of a heterologous nucleic acid molecule is one that encodes one or more immunogenic peptides or polypeptides derived from a coronavirus (e.g., SARS-CoV-2 or variant thereof). The heterologous nucleic acid molecule is one that is not normally associated with the other nucleic acid molecules found in the polynucleotide or vector into which the heterologous nucleic acid molecule is incorporated.

The term "host cell," refers to cells into which an exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Host cells include cells within the body of a subject (e.g., a mammalian subject (e.g., a human)) into which an exogenous nucleic acid has been introduced.

By "immunogen" is meant any polypeptide that can induce an immune response in a subject upon administration. In some embodiments, the immunogen is encoded by a nucleic acid molecule that may

be incorporated into, for example, a polynucleotide or vector, for subsequent expression of the immunogen (e.g., a gene product of interest, or fragment thereof (e.g., a polypeptide)).

The term “immunogenic composition” as used herein, is defined as material used to provoke an immune response and may confer immunity after administration of the immunogenic composition to a subject.

The term “immunostimulatory agent” refers to substances (e.g., drugs and nutrients) that stimulate the immune system by inducing activation or increasing activity of any of its components. An immunostimulatory agent includes a cytokine (e.g., the granulocyte macrophage colony-stimulating factor) and interferon (e.g., IFN- α and/or IFN- γ).

By “isolated” is meant separated, recovered, or purified from a component of its natural environment. For example, a nucleic acid molecule or polypeptide may be isolated from a component of its natural environment by 1% (e.g., by 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%) or more.

By “pharmaceutical composition” is meant any composition that contains a therapeutically or biologically active agent, such as an immunogenic composition or vaccine (e.g., a nucleic acid molecule encoding a protein of SARS-CoV-2 or a variant thereof (e.g., a Spike protein), a vector containing the nucleic acid molecule, and/or a polypeptide encoded by the nucleic acid molecule), preferably including a nucleotide sequence encoding an antigenic gene product of interest, or fragment thereof, that is suitable for administration to a subject and that treats or prevents a disease (e.g., infection by SARS-CoV-2 or a variant thereof) or reduces or ameliorates one or more symptoms of the disease (e.g., viral titer, viral spread, infection, and/or cell fusion caused by SARS-CoV-2 or a variant thereof)). For the purposes of this disclosure, pharmaceutical compositions include vaccines (e.g., monovalent vaccines and polyvalent vaccines), and pharmaceutical compositions suitable for delivering a therapeutic or biologically active agent can include, for example, tablets, gelcaps, capsules, pills, powders, granulates, suspensions, emulsions, solutions, gels, hydrogels, oral gels, pastes, eye drops, ointments, creams, plasters, drenches, delivery devices, suppositories, enemas, injectables, implants, sprays, or aerosols. Any of these formulations can be prepared by well-known and accepted methods of art. See, for example, Remington: *The Science and Practice of Pharmacy* (21st ed.), ed. A.R. Gennaro, Lippincott Williams & Wilkins, 2005, and Encyclopedia of Pharmaceutical Technology, ed. J. Swarbrick, Informa Healthcare, 2006, each of which is hereby incorporated by reference.

The terms “linked” or “links” or “link” as used herein are meant to refer to the covalent joining of two amino acid sequences or two nucleic acid sequences together through peptide or phosphodiester bonds, respectively, such joining can include any number of additional amino acid or nucleic acid sequences between the two amino acid sequences or nucleic acid sequences that are being joined.

As used herein, the term “mutation” refers to a change in the nucleotide sequence of a gene or a change in the polypeptide sequence of a protein. Mutations in a gene or protein may occur naturally as a result of, for example, errors in DNA replication, DNA repair, irradiation, and exposure to carcinogens or mutations may be induced as a result of administration of a transgene expressing a mutant gene. Mutations may result from single or multiple nucleotide insertions, deletions, or substitutions. The nomenclature for describing mutations and sequence variations uses the format “reference sequence code,” wherein the reference sequence may be “ Δ ” or “del,” designating a deletion, or may contain

reference to the substitutions occurring. The nomenclature for describing mutations resulting from amino acid substitutions uses the format “AnB,” where “A” designates the amino acid found in the wild type variant of the protein, “n” designates the number of the amino acid within the peptide chain, and “B” designates the new amino acid that resulted from the substitution. For example, the SARS-CoV-2 spike protein Signature-based Epitope Targeted (SET) 4 variant described herein contains a substitution described as N501Y, which corresponds to a change in the protein at amino acid residue #501, in which an asparagine is substituted for a tyrosine. The mutations described herein appear in the context of a Spike protein of a coronavirus (e.g., SARS-CoV-2, for example corresponding to the amino acid sequence of SEQ ID NO: 35). Thus, references herein to mutations made “relative to the amino acid sequence of SEQ ID NO: 35”, or made “relative to SEQ ID NO: 1”, indicate that the Spike protein containing one or more of the indicated mutations is in the context of a Spike (S) protein of SARS-CoV-2 or a variant thereof with the referenced sequence (e.g., the reference sequence serves as the backbone for the Spike protein with the indicated mutation(s).

“Nucleic acid molecule” or “polynucleotide,” as used interchangeably herein, refer to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase, or by a synthetic reaction. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. If present, modification to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after synthesis, such as by conjugation with a label.

A “nucleic acid vaccine” refers to a vaccine that includes a heterologous nucleic acid molecule under the control of a promoter for expression in a subject. The heterologous nucleic acid molecule can be incorporated into an expression vector, such as a plasmid. A “DNA vaccine” refers to a vaccine in which the nucleic acid is DNA. An “RNA vaccine” refers to a vaccine in which the nucleic acid is RNA (e.g., an mRNA). As used herein, a “monovalent vaccine” refers to a vaccine which contains a single strain of a single antigen (e.g., a Spike protein, or a nucleic acid molecule encoding the Spike protein, from SARS-CoV-2 or a variant thereof), whereas a “polyvalent vaccine” refers to a vaccine containing more than one antigen (e.g., multiple different Spike proteins, or nucleic acid molecules encoding multiple different Spike proteins from SARS-CoV-2 or a variant thereof). A monovalent or polyvalent vaccine as described herein may contain one or more Spike proteins, or nucleic acid molecules encoding one or more Spike proteins, from a SARS-CoV-2 variant of lineage B.1.1.7, B.1.429, B.1.1.28, B.1.351, A23.1, B.1.617.1, B.1.617.2, B.1.427, B.1.525, B.1.526, P.1, P.2, P.3, C.36, C.37, B.1.1.519, B.1.526.1, B.1.526.2, R.1, B.1.258.17, B.1.575, B.1.214.2, A.2.5.2, AT.1, B.1.1.523, and/or B.1.620 (e.g., a Spike protein encoded by the nucleic acid molecule of any one of SEQ ID NOs: 10-12). A monovalent or polyvalent vaccine as described herein can be used to induce an immune response (e.g., a neutralizing antibody response) against one or more different coronaviruses.

A nucleic acid is “operably linked” when it is placed into a structural or functional relationship with another nucleic acid sequence. For example, one segment of DNA may be operably linked to another segment of DNA if they are positioned relative to one another on the same contiguous DNA molecule and have a structural or functional relationship, such as a promoter or enhancer that is positioned relative to a

coding sequence so as to facilitate transcription of the coding sequence; a ribosome binding site that is positioned relative to a coding sequence so as to facilitate translation; or a pre-sequence or secretory leader that is positioned relative to a coding sequence so as to facilitate expression of a pre-protein (e.g., a pre-protein that participates in the secretion of the encoded polypeptide). In other examples, the operably linked nucleic acid sequences are not contiguous, but are positioned in such a way that they have a functional relationship with each other as nucleic acids or as proteins that are expressed by them. Enhancers, for example, do not have to be contiguous. Linking may be accomplished by ligation at convenient restriction sites or by using synthetic oligonucleotide adaptors or linkers.

By “optimized” is meant an immunogenic polypeptide that is not a naturally-occurring peptide, polypeptide, or protein, such as a non-naturally occurring viral polypeptide (e.g., a Spike polypeptide). Optimized viral polypeptide sequences are initially generated by modifying the amino acid sequence of one or more naturally-occurring viral gene products (e.g., peptides, polypeptides, and proteins) to increase the breadth, intensity, depth, or longevity of the antiviral immune response (e.g., cellular or humoral immune responses) generated upon immunization (e.g., when incorporated into a composition, e.g., vaccine) of a subject (e.g., a human). Thus, an optimized viral polypeptide may correspond to a “parent” viral gene sequence; alternatively, an optimized viral polypeptide may not correspond to a specific “parent” viral gene sequence but may correspond to analogous sequences from various strains or quasi-species of a virus. Modifications to the viral gene sequence that can be included in an optimized viral polypeptide include amino acid additions, substitutions, and deletions. In one embodiment, an optimized polypeptide is a Spike polypeptide from a coronavirus (e.g., from SARS-CoV-2 or a variant thereof), which has been further altered to include a leader/signal sequence (e.g., a Spike signal sequence or a tPA signal sequence) for maximal protein expression, a factor Xa site, a foldon trimerization domain (see, e.g., SEQ ID NO: 15), and/or linker or spacer (e.g., SEQ ID NOs: 16 or 17) sequences. An optimized polypeptide may, but need not, also include a cleavage site mutation(s) (e.g., a furin cleavage site mutation (e.g., SEQ ID NO: 19)). Methods of generating an optimized viral polypeptide are described in, e.g., Fisher et al. “Polyvalent Vaccine for Optimal Coverage of Potential T-Cell Epitopes in Global HIV-1 Variants,” *Nat. Med.* 13(1):100-106 (2007) and International Patent Application Publication WO 2007/024941, herein incorporated by reference. Once the optimized viral polypeptide sequence is generated, the corresponding polypeptide can be produced or administered by standard techniques (e.g., recombinant viral vectors, such as the adenoviral vectors disclosed in International Patent Application Publications WO 2006/040330 and WO 2007/104792, herein incorporated by reference) and optionally assembled to form a stabilized polypeptide trimer.

The terms “optimized codon” and “codon optimized” as used herein refer to a codon sequence that has been modified to match codon frequencies in a target (e.g., a subject) or host organism, but that does not alter the amino acid sequence of the original translated protein.

By “pharmaceutically acceptable diluent, excipient, carrier, or adjuvant” is meant a diluent, excipient, carrier, or adjuvant that is physiologically acceptable to the subject while retaining the therapeutic properties of the pharmaceutical composition with which it is administered. One exemplary pharmaceutically acceptable carrier is physiological saline. Other physiologically acceptable diluents, excipients, carriers, or adjuvants and their formulations are known to one skilled in the art (see, e.g., U.S. Pub. No. 2012/0076812).

By “portion” or “fragment” is meant a part of a whole. A portion may comprise at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of the entire length of a polynucleotide or polypeptide sequence region. For polynucleotides, for example, a portion may include at least 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800 or more contiguous nucleotides of a reference polynucleotide molecule. For polypeptides, for example, a portion may include at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 50, 75, 90, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550, 575, or 600 or more continuous amino acids of a reference polypeptide molecule.

In some instances, a fragment of a nucleic acid molecule may include at least 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700 or more consecutive nucleotides of the polynucleotide SS-Spike-dF-PP (SEQ ID NOs: 5-6 and 65-68) or one or more of the polynucleotides of SEQ ID NOs: 7-12 and 61-64.

In some instances, a fragment of a polypeptide may include at least 20, 25, 50, 75, 90, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, or more consecutive amino acids of polypeptide SS-Spike-dF-PP (SEQ ID NO: 1). In some instances, a fragment of a polypeptide may include at least 20, 25, 50, 75, 90, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, or more consecutive amino acids of a Signature-based Epitope Targeted (SET) 1 polypeptide (SEQ ID NO: 2). In some instances, a fragment of a polypeptide may include at least 20, 25, 50, 75, 90, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, or more consecutive amino acids of a SET2 polypeptide (SEQ ID NO: 3). In some instances, a fragment of a polypeptide may include at least 20, 25, 50, 75, 90, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, or more consecutive amino acids of a SET3 polypeptide (SEQ ID NO: 4).

In some instances, a fragment of a polypeptide may include at least 20, 25, 50, 75, 90, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, or more consecutive amino acids of SEQ ID NO: 35. In some instances, a fragment of a polypeptide may include at least 20, 25, 50, 75, 90, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, or more consecutive amino acids of a EG1 polypeptide (SEQ ID NO: 40). In some instances, a fragment of a polypeptide may include at least 20, 25, 50, 75, 90, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, or more consecutive amino acids of a EG2 polypeptide (SEQ ID NO: 41). In some instances, a fragment of a polypeptide may include at least 20, 25, 50, 75, 90, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, or more consecutive amino acids of a EG3 polypeptide (SEQ ID NO: 42). In some instances, a fragment of a polypeptide may include at least 20, 25, 50, 75, 90, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, or more consecutive amino acids of a EG4 polypeptide (SEQ ID NO: 43).

In some instances, administration of a fragment of a polynucleotide (e.g., SEQ ID NOs: 5-12, 55-51, and 61-64) and/or a polypeptide (e.g., SEQ ID NOs: 1-4, 35, 40-43, and 65-68) to a subject may elicit an immune response in the subject.

In some instances, administration of a fragment of a polynucleotide (e.g., SEQ ID NOs: 5-12, 45-51, and 61-64) and/or a polypeptide (e.g., SEQ ID NOs: 1-4, 35, 40-43, and 65-68) to a subject may elicit an immune response in the subject.

A “promoter” is a nucleic acid sequence enabling the initiation of the transcription of a gene sequence in a messenger RNA, such transcription being initiated with the binding of an RNA polymerase on or nearby the promoter.

By “promotes an immune response” is meant eliciting a humoral response (e.g., the production of antibodies) or a cellular response (e.g., the activation of T cells, macrophages, neutrophils, and/or natural killer cells) directed against, for example, one or more infective agents (e.g., a virus (e.g., a SARS-CoV-2 or a variant thereof)) or protein targets in a subject to which the pharmaceutical composition (e.g., an immunogenic composition or vaccine) has been administered.

The term “SARS-CoV-2 or a variant thereof-mediated disease” is used interchangeably with the terms “Coronavirus disease 2019 (COVID-19)” and “SARS-CoV-2” herein, as well as grammatical variants thereof, and refers to any pathology or sequelae known in the art to be caused by (alone or in association with other mediators), exacerbated by, or associated with SARS-CoV-2 or a variant thereof infection, including infection by a variant of SARS-CoV-2, such as those described herein or others that are later arising, or exposure in the subject having the disease. Disease can be acute (e.g., fever) or chronic (e.g., chronic fatigue), mild (e.g., hair loss) or severe (e.g., organ failure), and early-onset (e.g., 2-14 days post-infection) or late-onset (e.g., 2 weeks post-infection). Non-limiting examples of severe disease include pneumonia, acute respiratory distress syndrome (ARDS), acute respiratory failure, pulmonary edema, organ failure, or death. Non-limiting examples of symptoms include weight loss, fever, cough, difficulty breathing, fatigue, headache, loss of taste or smell, hair loss, rash, sore throat, nausea, and diarrhea. Symptoms can be mild or severe (e.g., weight loss of greater than about 5% within a week and high fever) and temporary or permanent. In some embodiments, the SARS-CoV-2 is of the lineage B.1.1.7, B.1.429, B.1.1.28, B.1.351, A23.1, B.1.617.1, B.1.617.2, B.1.427, B.1.525, B.1.526, P.1, P.2, P.3, C.36, C.37, B.1.1.519, B.1.526.1, B.1.526.2, R.1, B.1.258.17, B.1.575, B.1.214.2, A.2.5.2, AT.1, B.1.1.523, or B.1.620.

A “protective level” refers to an amount or level of a marker (e.g., an antibody, a cell (e.g., an immune cell, e.g., a T cell, a B cell, an NK cell, or a neutrophil)) that is indicative of partial or complete protection from coronavirus infection or disease. An amount or level of a marker that is above the protective level indicates protection from coronavirus infection (e.g., a SARS-CoV-2 or a variant thereof infection) or disease (e.g., a SARS-CoV-2 or a variant thereof-mediated disease, e.g., COVID-19, e.g., severe COVID-19 disease). An amount or level of a marker that is below the protective level indicates susceptibility to coronavirus infection or disease (e.g., a SARS-CoV-2 or a variant thereof-mediated disease, e.g., COVID-19, e.g., severe clinical disease). The marker may be a single measure (e.g., neutralizing antibody level) or the marker may be a combination of multiple measures (e.g., neutralizing antibody level and RBD-specific IgG2 level). In some instances, the protective level is an anti-coronavirus antibody titer of at least about 70 as measured using the pseudovirus neutralization assay described herein, an anti-coronavirus antibody titer of at least about 25 as measured using the live virus neutralization assay described herein, or an anti-coronavirus antibody titer that is above a level of at least about 80% of a median or mean level of a cohort of convalescent humans as determined by a pseudovirus neutralization assay or live virus neutralization assay as described herein. In some instances, the protective level is an anti-coronavirus antibody titer of at least about 100 as measured using the pseudovirus neutralization assay described herein.

As used herein, the term “sample” is a composition that is obtained or derived from a subject that contains a cellular and/or other molecular entity that is to be characterized and/or identified, for example based on physical, biochemical, chemical and/or physiological characteristics. A sample may be solid tissue as from a fresh, frozen, and/or preserved organ, tissue sample, biopsy, and/or aspirate; blood or any blood constituents such as plasma; bodily fluids such as cerebral spinal fluid, amniotic fluid, peritoneal fluid, or interstitial fluid. The sample may also be primary or cultured cells or cell lines. The sample may contain compounds which are not naturally intermixed with the tissue in nature such as preservatives, anticoagulants, buffers, fixatives, wax, nutrients, antibiotics, or the like.

By “sequence identity” or “sequence similarity” is meant that the identity or similarity, respectively, between two or more amino acid sequences, or two or more nucleotide sequences, is expressed in terms of the identity or similarity between the sequences. Sequence identity can be measured in terms of “percentage (%) identity,” in which a higher percentage indicates greater identity shared between the sequences. Sequence similarity can be measured in terms of percentage similarity (which takes into account conservative amino acid substitutions); the higher the percentage, the more similarity shared between the sequences. Homologs or orthologs of nucleic acid or amino acid sequences possess a relatively high degree of sequence identity/similarity when aligned using standard methods. Sequence identity may be measured using sequence analysis software on the default setting (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705). Such software may match similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications. Sequence identity/similarity can be determined across all or a defined portion of the two or more sequences compared.

By “signal peptide” is meant a short peptide (e.g., 5-30 amino acids in length, such as 17 amino acids in length, e.g., SEQ ID NO: 20) at the N-terminus of a polypeptide that directs a polypeptide towards the secretory pathway (e.g., the extracellular space). The signal peptide is typically cleaved during secretion of the polypeptide. The signal sequence may direct the polypeptide to an intracellular compartment or organelle, e.g., the Golgi apparatus. A signal sequence may be identified by homology, or biological activity, to a peptide with the known function of targeting a polypeptide to a particular region of the cell. One of ordinary skill in the art can identify a signal peptide by using readily available software (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705, BLAST, or PILEUP/PRETTYBOX programs). A signal peptide can be one that is, for example, substantially identical to the amino acid sequence of SEQ ID NO: 20. By “substantially identical” is meant that the signal peptide can have at least 80% or more (e.g., 85%, 90%, 95%, 97%, 99%, or 100%) sequence identity to the amino acid sequence of SEQ ID NO: 20.

As used herein, the phrase “specifically binds” refers to a binding reaction which is determinative of the presence of an antigen in a heterogeneous population of proteins and other biological molecules that is recognized, e.g., by an antibody or antigen-binding fragment thereof, with particularity. An antibody or antigen-binding fragment thereof that specifically binds to an antigen will bind to the antigen with a K_D of less than 100 nM. For example, an antibody or antigen-binding fragment thereof that specifically binds to an antigen will bind to the antigen with a K_D of up to 100 nM (e.g., between 1 pM and

100 nM). An antibody or antigen-binding fragment thereof that does not exhibit specific binding to a particular antigen or epitope thereof will exhibit a K_D of greater than 100 nM (e.g., greater than 500 nM, 1 μ M, 100 μ M, 500 μ M, or 1 mM) for that particular antigen or epitope thereof. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein or carbohydrate. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein or carbohydrate. See, Harlow & Lane, *Antibodies, A Laboratory Manual*, Cold Spring Harbor Press, New York (1988) and Harlow & Lane, *Using Antibodies, A Laboratory Manual*, Cold Spring Harbor Press, New York (1999), for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity.

As used herein, the term "stabilized polypeptide trimer" or "stabilized trimer" refers, but is not limited to, an oligomer that includes a protein and/or polypeptide sequence that increases the stability (e.g., via the presence of one or more oligomerization domains) of the trimeric structure (e.g., reduces dissociation of a trimer into monomeric units). The stabilized polypeptide trimer, for example, may be a homotrimer. An "oligomerization domain" refers, but is not limited to, a polypeptide sequence that can be used to increase the stability of an oligomeric envelope protein such as, e.g., to increase the stability of a Spike trimer. Oligomerization domains can be used to increase the stability of homooligomeric polypeptides as well as heterooligomeric polypeptides. Oligomerization domains are well known in the art, and include "trimerization domains." A trimerization domain refers to an oligomerization domain that stabilizes trimeric polypeptides (e.g., trimers consisting of one or more of the Spike polypeptides).

Examples of trimerization domains include, but are not limited to, the T4-fibrin "foldon" trimerization domain; the coiled-coil trimerization domain derived from GCN4 (Yang et al. (2002) *J. Virol.* 76:4634); and the catalytic subunit of *E. coli* aspartate transcarbamoylase as a trimer tag (Chen et al. (2004) *J. Virol.* 78:4508).

A "subject" is a vertebrate, such as a mammal (e.g., a primate and a human, in particular a human with underlying health conditions (e.g., hypertension, diabetes, or cardiovascular disease)). Mammals also include, but are not limited to, farm animals (such as cows), sport animals (e.g., horses), pets (such as cats, and dogs), mice, rats, bats, civets, and raccoon dogs. A subject to be treated according to the methods described herein (e.g., a subject in need of protection from a coronavirus infection (e.g., SARS-CoV-2 or a variant thereof) or having a coronavirus infection may be one who has been diagnosed by a medical practitioner as having such a need or infection. Diagnosis may be performed by any suitable means. A subject in whom the development of an infection is being prevented may or may not have received such a diagnosis. One skilled in the art will understand that a subject to be treated according to the disclosure may have been subjected to standard tests or may have been identified, without examination, as one with a suspected infection or at high risk of infection due to the presence of one or more risk factors (e.g., exposure to a coronavirus (e.g., SARS-CoV-2 or a variant thereof), for example, due to travel to an area where coronavirus infection is prevalent). Additionally, humans with underlying health conditions (e.g., hypertension, diabetes, or cardiovascular disease) are identified as subjects at high risk of infection with a coronavirus (e.g., SARS-CoV-2 or a variant thereof). The methods of treating a human subject with a composition are, therefore, particularly useful in treating, reducing, and/or preventing a coronavirus infection (e.g., SARS-CoV-2 or a variant thereof) in humans with underlying health conditions.

As used herein, the term “transfection” refers to any of a wide variety of techniques commonly used for the introduction of an exogenous nucleic acid molecule (e.g., DNA, such as an expression vector) into a prokaryotic or eukaryotic host cell, e.g., electroporation, lipofection, calcium- phosphate precipitation, DEAE- dextran transfection, and the like.

As used herein, and as well understood in the art, “treatment” is an approach for obtaining beneficial or desired results, such as clinical results. Beneficial or desired results can include, but are not limited to, alleviation or amelioration of one or more symptoms (e.g., fever, joint pain, rash, conjunctivitis, muscle pain, headache, retro-orbital pain, edema, lymphadenopathy, malaise, asthenia, sore throat, cough, nausea, vomiting, diarrhea, and hematospermia) or conditions (Zammarchi et al., *J. Clin. Virol.* 63:32-5, 2015; Waddell et al., *PLoS One* 11(5): e0156376, 2016); diminishment of the extent of disease, disorder, or condition; stabilization (e.g., not worsening) of a state of disease, disorder, or condition; prevention of spread of disease, disorder, or condition; delay or slowing the progress of the disease, disorder, or condition; amelioration or palliation of the disease, disorder, or condition; and remission (whether partial or total), whether detectable or undetectable. “Palliating” a disease, disorder, or condition means that the extent and/or undesirable clinical manifestations of the disease, disorder, or condition are lessened and/or the time course of the progression is slowed or lengthened, as compared to the extent or time course in the absence of treatment. A treatment can include one or more therapeutic agents, such as one or more of the compositions described herein and/or one or more additional therapeutic agents. Additional therapeutic agents can include agents that stimulate (e.g., interferons) or inhibit (e.g., an anti-inflammatory agent, such as corticosteroids, e.g., dexamethasone) the immune response. A treatment can include one or more therapeutic interventions, such as surgery or prone positioning.

The term “vaccine” as used herein, is defined as material used to provoke an immune response and that confers immunity for a period of time after administration of the vaccine to a subject.

By “vector” is meant a DNA construct that includes one or more polynucleotides, or fragments thereof, such as from a viral species, such as SARS-CoV-2 species. The vector can be used to infect cells of a subject, which results in the translation of the polynucleotides of the vector into a protein product. One type of vector is a “plasmid,” which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as “recombinant expression vectors” (or simply, “recombinant vectors”). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the disclosure, “plasmid” and “vector” may, at times, be used interchangeably as the plasmid is the most commonly used form of vector. Other vectors include, e.g., viral vectors, such as adenoviral vectors (e.g., an Ad26 vector), in particular, those described herein.

The term “virus,” as used herein, is defined as an infectious agent that is unable to grow or reproduce outside a host cell and that infects mammals (e.g., humans).

A “viral vector” is defined as a recombinantly produced virus or viral particle that comprises a polynucleotide to be delivered into a host cell. Examples of viral vectors include retroviral vectors,

adenovirus vectors, adeno-associated virus vectors (e.g., see PCT publication no. WO 2006/002203), alphavirus vectors and the like.

In aspects where gene transfer is mediated by a DNA viral vector, such as an adenovirus (Ad (e.g., Ad26)) or adeno-associated virus (AAV), a vector construct refers to the polynucleotide comprising the viral genome or part thereof, and a transgene. Ads are a relatively well characterized, homogenous group of viruses, including over 50 serotypes (WO 95/27071). Ads are easy to grow and do not require integration into the host cell genome. Recombinant Ad derived vectors, particularly those that reduce the potential for recombination and generation of wild-type virus, have also been constructed (WO 95/00655 and WO 95/11984). Vectors that contain both a promoter and a cloning site into which a polynucleotide can be operatively linked are known in the art. Such vectors are capable of transcribing RNA *in vitro* or *in vivo*. To optimize expression and/or *in vitro* transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation.

The term "Spike" or "S", as used herein, refers to a Spike sequence that does not contain a signal sequence (e.g., SEQ ID NO: 34, 37, or 39 and variants thereof). The term "SS", as used herein, refers to a Spike signal sequence (e.g., SEQ ID NO: 20 or 28 and variants thereof). Therefore, as an example, the term "SS-Spike" would refer to a Spike sequence that contains a signal sequence (e.g., SEQ ID NO: 29, 35, or 39). The term "dF", as used herein, refers to a mutated (i.e., dead) furin cleavage site (e.g., SEQ ID NO: 26 residing within a Spike polynucleotide sequence being mutated into, e.g., SEQ ID: 27; or SEQ ID NO: 18 residing within a Spike polypeptide sequence being mutated into, e.g., SEQ ID NO: 19). The term "PP", as used herein, refers to proline-stabilizing mutations (e.g., proline substitutions corresponding to amino acids K969 and V970 of full-length Spike (e.g., SEQ ID NO: 34)). As an example, the term "SS-Spike-dF-PP", as used herein, refers to a Spike sequence that contains a signal sequence, a mutated furin cleavage site, and proline stabilizing mutations (e.g., SEQ ID NO: 1 or 5). The term "SdCT", as used herein, refers to a Spike sequence that does not contain a signal sequence and a cytoplasmic region (e.g., SEQ ID NO: 30). As an example, the term "SS-SdCT" refers to a Spike sequence that contains a signal sequence and does not contain a cytoplasmic region (e.g., SEQ ID NO: 36). The term "S.Ecto", as used herein, refers to a Spike ectodomain sequence that does not contain a signal sequence (e.g., SEQ ID NO: 31). The term "foldon" refers to a T4-fibrin foldon trimerization domain (e.g., SEQ ID NO: 15 or 23). As an example, the term "SS-S.Ecto-dF-PP-foldon", refers to a Spike ectodomain sequence containing a signal sequence, a mutated furin cleavage site, proline stabilizing mutations, and a T4-fibrin foldon trimerization domain. The term "S1", as used herein, refers to the S1 domain of Spike (e.g., residues 18-682 of SEQ ID NO: 1 or 35). The term "transmembrane domain" refers to a hydrophobic region of a protein that can be inserted or traverse a lipid membrane (e.g., SEQ ID NO: 14 or 22). Other features and advantages will be apparent from the following Detailed Description, the drawings, and the claims.

DETAILED DESCRIPTION

Polypeptides from Wuhan coronavirus (SARS-CoV-2) and variants thereof (e.g., Signature-based Epitope Targeted (SET) and/or Epigraph (EG)) designed sequences) can be used to elicit protective and

therapeutic immune responses (e.g., humoral responses and/or cellular responses) against a coronavirus infection (e.g., infection by SARS-CoV-2 or a variant thereof) when administered to a subject (e.g., a human subject) infected with or exposed to a coronavirus (e.g., SARS-CoV-2 or a variant thereof). The compositions that can be prepared for administration to a subject can include a protein of SARS-CoV-2, such as the spike (S) protein (e.g., a SET1, SET2, and SET3 polypeptide of SEQ ID NOs: 2-4, respectively, or a EG1, EG2, and EG3 polypeptide of SEQ ID NOs: 40-43, respectively, or a spike-modified EG1, EG2, and EG3 polypeptide of SEQ ID NOs: 65-68, respectively, or any portion or variant polypeptide with at least 85%, at least 90%, at least 95%, at least 99% or more sequence identity thereto). These compositions can also include one or more of the SARS-CoV-2 nucleomembrane (MEM) protein (e.g., a MEM1, MEM2, and MEM3 polypeptide of SEQ ID NOs: 75-77, respectively, or any portion or variant polypeptide with at least 85%, at least 90%, at least 95%, at least 99% or more sequence identity thereto) and the nucleocapsid (NUL) protein (e.g., a NUL1, NUL2, and NUL3 polypeptide of SEQ ID NOs: 78-80, respectively, or any portion or variant polypeptide with at least 85%, at least 90%, at least 95%, at least 99% or more sequence identity thereto).

A composition of the disclosure can also include one or more of the SARS-CoV-2 MEM protein (e.g., a MEM1, MEM2, and MEM3 polypeptide of SEQ ID NOs: 75-77, respectively, or any portion or variant polypeptide with at least 85%, at least 90%, at least 95%, at least 99% or more sequence identity thereto) and the NUL protein (e.g., a NUL1, NUL2, and NUL3 polypeptide of SEQ ID NOs: 78-80, respectively, or any portion or variant polypeptide with at least 85%, at least 90%, at least 95%, at least 99% or more sequence identity thereto).

A composition of the disclosure also includes a vector (e.g., an expression vector, such as a plasmid, or a viral vector, such as an adenovirus (e.g., Ad26), poxvirus, adeno-associated virus, retroviral, or other viral vector, or naked or encapsulated DNA) containing a nucleic acid sequence that encodes a SARS-CoV-2 polypeptide (e.g., a S, MEM, and/or NUL protein) or a variant thereof (e.g., a nucleic acid molecule with the sequence of any one of SEQ ID NOs: 7-12, 44-51, 69-74, or a nucleic acid molecule with at least 85%, at least 90%, at least 95%, at least 99% or more sequence identity thereto). The composition may contain a vector(s) that encodes only one or more S proteins, such as those described herein (e.g., a SET1, SET2, and/or SET3 polypeptide or a EG1, EG2, and/or EG3 polypeptide, or a portion or variant thereof, as described herein). The composition may be one that contains a vector(s) that encodes only MEM and/or NUL polypeptides, such as those described herein. The composition may also contain a vector(s) that encodes a S, MEM, and NUL polypeptides, as described herein.

The generation of DNA and RNA vaccines expressing a S, MEM, and/or NUL protein of SARS-CoV-2, or a variant thereof, are described herein. The DNA and RNA vaccines can be generated by incorporating a polynucleotide (e.g., all or a fragment of any one or more of SEQ ID NOs: 7-12, 44-51, 69-74, or a variant thereof with up to 85% or more sequence identity thereto) encoding S, MEM, NUL, or a portion thereof (e.g., all or a fragment of any one or more of SEQ ID NOs: 2-4, 40-43, 65-68, and 75-80), or a variant thereof with up to 85% or more sequence identity thereto), or a complement sequence thereof, into a mammalian expression vector (e.g., pcDNA3.1+; Invitrogen, CA, USA) or a viral vector (e.g., an adenoviral vector) to generate the vaccine.

Generation of recombinant viral vectors (e.g., adenoviral vectors, such as Ad26 viral vectors) expressing a modified S, MEM, and/or NUL protein of SARS-CoV-2 or a variant thereof are also

described. A viral vector encoding a modified S, MEM, and/or NUL protein of SARS-CoV-2 or a variant thereof can be generated by incorporating a polynucleotide (e.g., all or a fragment of any one or more of SEQ ID NOs: 7-12, 44-51, 69-74, or a variant thereof with up to 85% or more sequence identity thereto) encoding S, MEM, NUL, or a portion thereof (e.g., SEQ ID NOs: 2-4, 40-43, 65-68, and 75-80), or a variant thereof with up to 85% or more sequence identity thereto (e.g., all or a fragment of any one or more of SEQ ID NOs: 2-4, 40-43, 65-68, and 75-80) into a viral vector (e.g., an Ad26 viral vector).

Anti-coronavirus antibodies (e.g., antibodies against a modified S, MEM, or NUL protein of SARS-CoV-2 or a variant thereof (e.g., anti-Spike antibodies, anti-Spike neutralizing antibodies, or broadly neutralizing anti-Spike antibodies) present in a sample from a subject (e.g., a human subject) can be used to detect and/or monitor a protective antibody response in the subject. The anti-coronavirus antibodies (e.g., anti-Spike antibodies, anti-Spike neutralizing antibodies, or broadly neutralizing anti-Spike antibodies) may be measured in a short timeframe (e.g., between 1 day post-administration and 8-weeks post-administration) or a longer timeframe (e.g., between 2 month post-administration and 15 years post-administration) after administration of a therapeutic composition (e.g., any of the compositions or immunogenic compositions described herein).

The nucleic acid molecules, polypeptides, vectors, monovalent vaccines, polyvalent vaccines, compositions, and antibodies described herein can also be used in methods of treating and/or inhibiting a SARS-CoV-2 infection in a subject (e.g., a human).

COMPOSITIONS AND METHODS

Nucleic Acid Molecules

Nucleic acid molecules of the disclosure include Signature-based Epitope Target (SET) spike (S) polynucleotide sequences (e.g., SET1-3) and Epigraph (EG) designed S (e.g., EG1-4), membrane (MEM, e.g., MEM1-3), and nucleocapsid (NUL, e.g., NUL1-3) polynucleotide sequences.

The nucleic acid molecules of SET1 (e.g., SEQ ID NOs: 7 and 10 or a variant thereof with up to 85% or more sequence identity thereto), SET2 (e.g., SEQ ID NOs: 8 and 11 or a variant thereof with up to 85% or more sequence identity thereto), and SET3 (e.g., SEQ ID NOs: 9 and 112 or a variant thereof with up to 85% or more sequence identity thereto) were designed based on a study of emerging variants around the world of the Wuhan coronavirus (SARS-CoV-2 or a variant thereof) relative to the wildtype SARS-CoV-2 S protein (e.g., SEQ ID NO: 29) and existing optimizations thereof (e.g., SEQ ID NO: 5, e.g., see U.S. Patent Application number 63/066,147, incorporated by reference herein; see also PCT/US2021/015946, incorporated by reference herein). The nucleic acid molecules described herein encode an S protein of SARS-CoV-2 or a variant thereof that has been modified to include one or more resistance mutations and includes, for example, the Signature-based Epitope Targeted (SET) 1 polypeptide, which includes the mutations: S13I, L18F, T20N, P26S, Δ69-70, D80A, D80Y, L141F, Δ144, W152C, M153T, M153I, F157L, Δ242-244, D253G, S255F, A262S, V367F, K417N, K417T, N439K, L452R, Y453F, S477N, S477R, E484K, S494P, N501T, N501Y, Q613H, D614G, and P681R, relative to the amino acid sequence of SEQ ID NO: 1 or 35; the SET2 polypeptide, which includes the mutations: L18F, T20N, P26S, D80A, M153T, M153I, Δ242-244, K417N, Y453F, E484K, N501Y, and D614G relative to the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 35; and the SET3 polypeptide, which includes the mutations: D80Y, L141F, F157L, S255F, V367F, K417T, N439K, S477R, S494P, N501T,

Q613H, and P681R, all relative to the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 35. The nucleic acid molecules may also encode a S protein of SARS-CoV-2 or a variant thereof containing further modifications to one or more regions. For example, the nucleic acid molecule may encode a S protein, as defined herein, with a deletion of the cytoplasmic region (e.g., SEQ ID NO: 30), with a deletion of the cytoplasmic and transmembrane domains, leaving only S protein ectodomain (e.g., SEQ ID NO: 31), a deletion of the S1 domain (e.g., a deletion of SEQ ID NO: 32, or variant thereof, within a S polynucleotide (e.g., SEQ ID NO: 29), or variant thereof)), a deletion of the S2 region such that only the S1 region of S remains, or a S protein with a deletion of the receptor binding domain (e.g., a deletion of SEQ ID NO: 33, or variant thereof, within a S polynucleotide (e.g., SEQ ID NO: 29), or variant thereof).

The nucleic acid molecules may also feature additional modifications to regions of S, including deletion of or inclusion of a signal sequence (e.g., SEQ ID NO: 28), one or more stabilizing mutations (e.g., proline substitutions corresponding to amino acids K969 and V970 of SEQ ID NO: 34), a mutation that inactivates a naturally occurring furin cleavage site (see, e.g., SEQ ID NO: 27 relative to the corresponding sequence in, e.g., SEQ ID NO: 35), introduction of a trimerization domain (e.g., a foldon trimerization domain, e.g., SEQ ID NO: 23), introduction of linker or spacer sequences (e.g., SEQ ID NOs: 24 and 25), and combinations thereof.

The nucleic acid molecule may also contain a nucleotide sequence that encodes a SARS-CoV-2 spike polypeptide having at least 85% sequence identity to at least 500 contiguous amino acids within positions 18-1208 (e.g., positions 50-1100, 100-1000, 200-900, and 300-800) of any one of SEQ ID NOs: 2-4 or a complementary sequence thereof, in which the polypeptide has at least one of the following mutations: S13I, L18F, T20N, P26S, Δ69-70, D80A, D80Y, L141F, Δ144, W152C, M153T, M153I, F157L, Δ242-244, D253G, S255F, A262S, V367F, K417N, K417T, N439K, L452R, Y453F, S477N, S477R, E484K, S494P, N501T, N501Y, Q613H, D614G, and P681R relative to the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 35. In some embodiments, the nucleic acid molecule contains a nucleotide sequence that encodes a coronavirus Spike polypeptide containing two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, twenty-four, twenty-five, twenty-six, or twenty-seven of the mutations in the context of a nucleic acid molecule with a nucleic acid sequence of SEQ ID NO: 1 or 34, or a variant thereof with up to 85% sequence identity thereto. In particular, the Spike polypeptide contains eight to twelve of the following mutations: S13I, L18F, T20N, P26S, Δ69-70, D80A, D80Y, L141F, Δ144, W152C, M153T, M153I, F157L, Δ242-244, D253G, S255F, A262S, V367F, K417N, K417T, N439K, L452R, Y453F, S477N, S477R, E484K, S494P, N501T, N501Y, Q613H, D614G, and P681R relative to the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 35.

In some embodiments, the nucleic acid molecule contains a nucleotide sequence that encodes a Spike polypeptide with one or more, or all, of the mutations: L18F, T20N, P26S, D80A, M153T, M153I, Δ242-244, K417N, Y453F, E484K, N501Y, and D614G relative to the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 35.

In some embodiments, the polypeptide of contains two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve of the mutations: L18F, T20N, P26S, D80A, M153T, M153I, Δ242-244, K417N, Y453F, E484K, N501Y, and D614G relative to the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 35.

In some of the embodiments, the nucleic acid molecule contains a nucleotide sequence that encodes a polypeptide with each of the mutations: L18F, T20N, P26S, D80A, M153T, M153I, Δ 242-244, K417N, Y453F, E484K, N501Y, and D614G relative to the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 35.

5 In some embodiments, the nucleic acid molecule contains a nucleotide sequence that encodes a polypeptide with the amino acid sequence of SEQ ID NO: 2, or a variant thereof with at least 85% sequence identity thereto, such as a variant that contains each of the indicated mutations.

In some embodiments, the nucleic acid molecule has the nucleic acid sequence of SEQ ID NO: 7 or 10, or a variant thereof with at least 85% sequence identity thereto, such as a variant that encodes a polypeptide that contains each of the indicated mutations.

10 A nucleic acid molecule of the disclosure may also be one that contains a nucleotide sequence that encodes a SARS-CoV-2 spike polypeptide with one or more of the mutations: S13I, Δ 69-70, Δ 144, W152C, D253G, A262S, L452R, S477N, and D614G relative to the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 35. The polypeptide may contain two, three, four, five, six, seven, eight, or nine of the mutations: S13I, Δ 69-70, Δ 144, W152C, D253G, A262S, L452R, S477N, and D614G relative to the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 35. In particular, the nucleic acid molecule may contain a nucleotide sequence that encodes a polypeptide with each of the mutations: S13I, Δ 69-70, Δ 144, W152C, D253G, A262S, L452R, S477N, and D614G relative to the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 35. For example, the nucleic acid molecule may contain a nucleotide sequence that encodes a polypeptide with the amino acid sequence of SEQ ID NO: 3, or a variant thereof with at least 85% sequence identity thereto, such as a variant that contains each of the indicated mutations. The nucleic acid molecule may have the nucleic acid sequence of SEQ ID NO: 8 or 11, or a variant thereof with at least 85% sequence identity thereto, such as a variant that encodes a polypeptide that contains each of the indicated mutations.

25 A nucleic acid molecule of the disclosure may also contain a nucleotide sequence that encodes a SARS-CoV-2 spike polypeptide with one or more of the mutations: D80Y, L141F, F157L, S255F, V367F, K417T, N439K, S477R, S494P, N501T, Q613H, and P681R relative to the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 35. The polypeptide may contain two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve of the mutations: D80Y, L141F, F157L, S255F, V367F, K417T, N439K, S477R, S494P, N501T, Q613H, and P681R relative to the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 35. In particular, the nucleic acid molecule may contain a nucleotide sequence that encodes a polypeptide with each of the mutations: D80Y, L141F, F157L, S255F, V367F, K417T, N439K, S477R, S494P, N501T, Q613H, and P681R relative to the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 35. For example, the nucleic acid molecule may contain a nucleotide sequence that encodes a polypeptide has the amino acid sequence of SEQ ID NO: 4, or a variant thereof with at least 85% sequence identity thereto, such as a variant that contains each of the indicated mutations. The nucleic acid molecule may have the nucleic acid sequence of SEQ ID NO: 9 or 12, or a variant thereof with at least 85% sequence identity thereto, such as a variant that encodes a polypeptide that contains each of the indicated mutations.

40 The nucleic acid molecules have a nucleotide sequence with at least 85% (e.g., at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%) sequence identity to,

all or a portion of any one of SEQ ID NOs: 7-12, or a complementary sequence thereof. For example, a nucleic acid molecule can have the nucleotide sequence of any one of SEQ ID NOs: 7-12. Alternatively, an isolated nucleic acid molecule has a nucleotide sequence that encodes a modified S protein of SARS-CoV-2 or a variant thereof with at least 85% (e.g., at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%) sequence identity to an amino acid sequence of any one of SEQ ID NOs: 2-4. For example, an isolated nucleic acid molecule can have a nucleotide sequence encoding a polypeptide having the amino acid sequence of any one of SEQ ID NOs: 2-4.

The nucleic acid molecules of SARS-CoV-2 spike Epigraph (EG) immunogen EG1 (e.g., SEQ ID NOs: 44, 48, and 61 or a variant thereof with up to 85% or more sequence identity thereto), EG2 (e.g., SEQ ID NOs: 45, 49, and 62 or a variant thereof with up to 85% or more sequence identity thereto), EG3 (e.g., SEQ ID NOs: 46, 50, and 63 or a variant thereof with up to 85% or more sequence identity thereto), and EG4 (e.g., SEQ ID NOs: 47, 51, and 64 or a variant thereof with up to 85% or more sequence identity thereto) were designed based on a study (e.g., Theiler *et al.*, *Statistics in Medicine* 37:181 (2018); Theiler *et al.*, *Scientific Reports* 6:33987 (2016), herein incorporated by reference) of emerging variants around the world of the Wuhan coronavirus (SARS-CoV-2 or a variant thereof) relative to the wildtype SARS-CoV-2 S protein (e.g., SEQ ID NO: 29) and existing optimizations thereof (e.g., SEQ ID NO: 5, e.g., see U.S. Patent Application number 63/066,147, incorporated by reference herein; see also PCT/US2021/015946, incorporated by reference herein). The nucleic acid molecules described herein encode an S protein of SARS-CoV-2 or a variant thereof that has been modified to include one or more resistance mutations and includes, for example, the EG1 polypeptide, which includes the mutations: Δ 69-70, Δ 144, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H, relative to the amino acid sequence of SEQ ID NO: 1 or 35; the EG2 polypeptide, which includes the mutations: L5F, L18F, T20N, P26S, V36F, Q52R, D80G, T95I, I105V, L118F, V127F, D138Y, Δ 156-157, R158G, T167S, D178H, R190S, I203V, D215G, A222V, I233V, Δ 242-244, D253G, A262S, P272L, T284I, T299I, V308L, F318S, V227I, P337S, R346S, K356R, V367L, P384L, N394S, R408I, K417T, D427N, N439K, L452R, I468V, T478K, E484K, L513F, A522S, T531S, N540S, T549I, K558N, E583D, G594S, T604A, Q613H, D614G, V622F, P631S, S640F, H655Y, I670V, S691P, A701V, T732A, T747I, S758G, G769V, Q779K, D796H, P809S, L822F, A831V, A845S, T859N, I870V, A879S, F888L, A899S, I909V, L922F, S939F, D950N, T961M, I997V, T1006I, A1016V, T1027I, V1040F, L1049I, H1058Y, Q1071H, D1084Y, V1094F, V1104L, I1130V, D1139H, D1153Y, P1162S, V1176F, K1191N, Q1208H, G1219V, V1228L, M1237I, S1252F, V1264L, and T1273I relative to the amino acid sequence of SEQ ID NO: 1 or 35; the EG3 polypeptide, which includes the mutations: P9L, T19R, T33I, H49Y, A67V, Δ 69-70, D80A, S98F, S112L, V126A, G142D, W152R, S162I, L176F, L189F, D198Y, I210T, A222V, D228H, H245Y, W258L, V267L, E281Q, A292S, T307I, T323I, L335F, R346K, R357K, V367F, T376I, T385N, V395I, E406Q, K417N, D427Y, N440K, L452Q, K462T, E471Q, E484K, F490S, N501T, V510L, A520S, V534I, T547I, P561S, A570D, T572I, E583Q, V595I, T604I, A623S, T632S, N641S, A653V, A672V, P681R, A694V, S704L, T716V, M731I, M740V, N751D, T761I, A771S, E780D, T791I, S803A, P812S, T827I, D843N, K854N, A871V, T883I, A892V, M902I, V911I, A924S, D936Y, G946R, Q957R, S967N, V976F, K986N, T998I, T1009I, A1020S, V1033A, K1045N, A1056V, T1066N, A1078S, A1086S, H1101D, E1111K, G1124V, V1133F, D1146Y, P1162L, A1174V, R1185H, E1195Q, K1205N, G1219C, M1229I, T1238I, C1247F, D1260N, and H1271Y, all relative to the amino acid sequence of SEQ ID NO: 1 or 35; and the EG4 polypeptide, which

includes the mutations: V3G, S13I, L18F, A27S, V36I, S45F, L54F, W64R, G75V, T76I, P85S, S94F, D111N, V120L, E132Q, N148T, F157S, S172A, G181V, V193L, Y204H, L216F, V227A, R237K, Δ 246-252, D253N, A263P, R273S, V289L, K300M, E309Q, V320F, P330S, G339S, A348S, V362F, S371T, V382L, N394H, R403K, Q414K, T430I, N440S, L452M, L461F, T470I, T478K, S494P, N501Y, Y505H, A522V, V534F, F543L, E554D, F565L, A570D, A575S, L585F, I598V, Q607K, D614G, N616S, D627G, T638I, A647S, Y660F, Q677H, A688V, M697I, A706V, T716I, T719I, T732I, T747N, T761R, V772I, V785I, K795R, P812L, V826L, K835R, A845V, T859I, M869I, A879V, A890V, A903S, V915I, S929I, S940F, D950H, V963A, V976F, S982A, K986R, R995G, Q1005H, R1014K, A1025G, K1038Q, M1050I, L1063F, K1073N, D1084E, H1101Y, D1118Y, D1127G, L1141W, E1150D, D1163Y, N1173S, E1182D, N1192S, E1202Q, I1216T, V1230L, C1243F, S1252P, P1263L, and T1273A, all relative to the amino acid sequence of SEQ ID NO: 1 or 35. The nucleic acid molecules may also encode a S protein of SARS-CoV-2 or a variant thereof containing further modifications to one or more regions. For example, the full-length (SEQ ID NO: 29) Spike with a deletion of the cytoplasmic region (SEQ ID NO: 30), the ectodomain (SEQ ID NO: 31), S1 (SEQ ID NO: 32), and the receptor binding domain (SEQ ID NO: 33). The nucleic acid molecules may also feature additional modifications to regions of S, including deletion of or inclusion of a signal sequence (e.g., SEQ ID NO: 28), one or more stabilizing mutations (e.g., proline substitutions corresponding to amino acids K969 and V970 of SEQ ID NO: 34), an mutation that inactivates a furin cleavage site (e.g., SEQ ID NO: 27), introduction of a trimerization domain (e.g., a foldon trimerization domain, e.g., SEQ ID NO: 23), introduction of linker or spacer sequences (e.g., SEQ ID NOs: 24 and 25), and combinations thereof.

The nucleic acid molecules may also encode a S protein of SARS-CoV-2 or a variant thereof containing further modifications to one or more regions. For example, the nucleic acid molecule may encode a S protein, as defined herein, with a deletion of the cytoplasmic region (e.g., SEQ ID NO: 30), with a deletion of the cytoplasmic and transmembrane domains, leaving only S protein ectodomain (e.g., SEQ ID NO: 31), a deletion of the S1 domain (e.g., a deletion of SEQ ID NO: 32, or variant thereof, within a S polynucleotide (e.g., SEQ ID NO: 29), or variant thereof)), a deletion of the S2 region such that only the S1 region of S remains, or a S protein with a deletion of the receptor binding domain (e.g., a deletion of SEQ ID NO: 33, or variant thereof, within a S polynucleotide (e.g., SEQ ID NO: 29), or variant thereof). The nucleic acid molecules may also feature additional modifications to regions of S protein, including deletion of or inclusion of a signal sequence (e.g., SEQ ID NO: 28), one or more stabilizing mutations (e.g., proline substitutions corresponding to amino acids K969 and V970 of SEQ ID NO: 34), a mutation that inactivates a furin cleavage site (e.g., SEQ ID NO: 27), introduction of a trimerization domain (e.g., a foldon trimerization domain, e.g., SEQ ID NO: 23), introduction of linker or spacer sequences (e.g., SEQ ID NOs: 24 and 25), and combinations thereof. The nucleic acid molecule may also contain a nucleotide sequence that encodes a polypeptide having at least 85% sequence identity to at least 500 contiguous amino acids within positions 18-1208 (e.g., positions 50-1100, 100-1000, 200-900, and 300-800) of any one of SEQ ID NOs: 35, 40-43, and 65-68 or a complementary sequence thereof, in which the polypeptide has at least one of the following mutations: V3G, L5F, P9L, S13I, L18F, T19R, T20N, P26S, A27S, T33I, V36F, V36I, S45F, H49Y, Q52R, L54F, W64R, A67V, Δ 69-70, G75V, T76I, D80A, D80G, P85S, S94F, T95I, S98F, I105V, D111N, S112L, L118F, V120L, V126A, V127F, E132Q, D138Y, G142D, Δ 144, N148T, W152R, Δ 156-157, F157S, R158G, S162I, T167S, S172A, L176F, D178H, G181V, L189F,

R190S, V193L, D198Y, I203V, Y204H, I210T, D215G, L216F, A222V, V227A, D228H, I233V, R237K, Δ242-244, H245Y, Δ246-252, D253G, D253N, W258L, A262S, A263P, V267L, P272L, R273S, E281Q, T284I, V289L, A292S, T299I, K300M, T307I, V308L, E309Q, F318S, V320F, T323I, V227I, P330S, L335F, P337S, G339S, R346S, R346K, A348S, K356R, R357K, V362F, V367F, V367L, S371T, T376I, V382L, P384L, T385N, N394S, N394H, V395I, R403K, E406Q, R408I, Q414K, K417T, K417N, D427N, D427Y, T430I, N439K, N440S, N440K, L452M, L452R, L452Q, L461F, K462T, I468V, T470I, E471Q, T478K, E484K, F490S, S494P, N501T, N501Y, Y505H, V510L, L513F, A520S, A522S, A522V, T531S, V534I, V534F, N540S, F543L, T547I, T549I, E554D, K558N, P561S, F565L, A570D, T572I, A575S, E583Q, E583D, L585F, G594S, V595I, I598V, T604A, T604I, Q607K, Q613H, D614G, N616S, V622F, A623S, D627G, P631S, T632S, T638I, S640F, N641S, A647S, A653V, H655Y, Y660F, I670V, A672V, Q677H, P681H, P681R, A688V, S691P, A694V, M697I, A701V, S704L, A706V, T716I, T716V, T719I, M731I, T732I, T732A, M740V, T747I, T747N, N751D, S758G, T761I, T761R, G769V, A771S, V772I, Q779K, E780D, V785I, T791I, K795R, D796H, S803A, P809S, P812S, P812L, L822F, V826L, T827I, A831V, K835R, D843N, A845S, A845V, K854N, T859N, T859I, M869I, I870V, A871V, A879S, A879V, T883I, F888L, A890V, A892V, A899S, M902I, A903S, I909V, V911I, V915I, L922F, A924S, S929I, D936Y, S939F, S940F, G946R, D950N, D950H, Q957R, T961M, V963A, S967N, V976F, S982A, K986N, K986R, R995G, I997V, T998I, Q1005H, T1006I, T1009I, R1014K, A1016V, A1020S, A1025G, T1027I, V1033A, K1038Q, V1040F, K1045N, L1049I, M1050I, A1056V, H1058Y, L1063F, T1066N, Q1071H, K1073N, A1078S, D1084Y, D1084E, A1086S, V1094F, H1101D, H1101Y, V1104L, E1111K, D1118H, D1118Y, G1124V, D1127G, I1130V, V1133F, D1139H, L1141W, D1146Y, E1150D, D1153Y, P1162L, P1162S, D1163Y, N1173S, A1174V, V1176F, E1182D, R1185H, K1191N, N1192S, E1195Q, E1202Q, K1205N, Q1208H, I1216T, G1219V, G1219C, V1228L, M1229I, V1230L, M1237I, T1238I, C1243F, C1247F, S1252F, S1252P, D1260N, P1263L, V1264L, H1271Y, T1273I, and T1273A (or any mutations shown in Figure 26) relative to the amino acid sequence of SEQ ID NO: 1 or 35.

In some embodiments, the nucleic acid molecule contains a nucleotide sequence that encodes a coronavirus Spike polypeptide containing two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, twenty-four, twenty-five, twenty-six, twenty-seven, twenty-eight, twenty-nine, thirty, thirty-one, thirty-two, thirty-three, thirty-four, thirty-five, thirty-six, thirty-seven, thirty-eight, thirty-nine, forty, forty-one, forty-two, forty-three, forty-four, forty-five, forty-six, forty-seven, forty-eight, forty-nine, fifty, fifty-one, fifty-two, fifty-three, fifty-four, fifty-five, fifty-six, fifty-seven, fifty-eight, fifty-nine, sixty, sixty-one, sixty-two, sixty-three, sixty-four, sixty-five, sixty-six, sixty-seven, sixty-eight, sixty-nine, seventy, seventy-one, seventy-two, seventy-three, seventy-four, seventy-five, seventy-six, seventy-seven, seventy-eight, seventy-nine, eighty, eighty-one, eighty-two, eighty-three, eighty-four, eighty-five, eighty-six, eighty-seven, eighty-eight, eighty-nine, ninety, ninety-one, ninety-two, ninety-three, ninety-four, ninety-five, ninety-six, ninety-seven, ninety-eight, ninety-nine, one hundred, one hundred one, one hundred two, one hundred three, one hundred four, one hundred five, one hundred six, one hundred seven, one hundred eight, one hundred nine, one hundred ten, one hundred eleven, one hundred twelve, one hundred thirteen, one hundred fourteen, one hundred fifteen, one hundred sixteen, one hundred seventeen, one hundred eighteen, one hundred nineteen, one hundred twenty of the mutations in the context of a polypeptide molecule with a polypeptide sequence of SEQ ID NO: 1 or 35, or a variant thereof with up to 85% sequence identity

thereto. In particular, the Spike polypeptide contains nine to one hundred twenty of the following mutations: V3G, L5F, P9L, S13I, L18F, T19R, T20N, P26S, A27S, T33I, V36F, V36I, S45F, H49Y, Q52R, L54F, W64R, A67V, Δ 69-70, G75V, T76I, D80A, D80G, P85S, S94F, T95I, S98F, I105V, D111N, S112L, L118F, V120L, V126A, V127F, E132Q, D138Y, G142D, Δ 144, N148T, W152R, Δ 156-157, F157S, R158G, S162I, T167S, S172A, L176F, D178H, G181V, L189F, R190S, V193L, D198Y, I203V, Y204H, I210T, D215G, L216F, A222V, V227A, D228H, I233V, R237K, Δ 242-244, H245Y, Δ 246-252, D253G, D253N, W258L, A262S, A263P, V267L, P272L, R273S, E281Q, T284I, V289L, A292S, T299I, K300M, T307I, V308L, E309Q, F318S, V320F, T323I, V227I, P330S, L335F, P337S, G339S, R346S, R346K, A348S, K356R, R357K, V362F, V367F, V367L, S371T, T376I, V382L, P384L, T385N, N394S, N394H, V395I, R403K, E406Q, R408I, Q414K, K417T, K417N, D427N, D427Y, T430I, N439K, N440S, N440K, L452M, L452R, L452Q, L461F, K462T, I468V, T470I, E471Q, T478K, E484K, F490S, S494P, N501T, N501Y, Y505H, V510L, L513F, A520S, A522S, A522V, T531S, V534I, V534F, N540S, F543L, T547I, T549I, E554D, K558N, P561S, F565L, A570D, T572I, A575S, E583Q, E583D, L585F, G594S, V595I, I598V, T604A, T604I, Q607K, Q613H, D614G, N616S, V622F, A623S, D627G, P631S, T632S, T638I, S640F, N641S, A647S, A653V, H655Y, Y660F, I670V, A672V, Q677H, P681H, P681R, A688V, S691P, A694V, M697I, A701V, S704L, A706V, T716I, T716V, T719I, M731I, T732I, T732A, M740V, T747I, T747N, N751D, S758G, T761I, T761R, G769V, A771S, V772I, Q779K, E780D, V785I, T791I, K795R, D796H, S803A, P809S, P812S, P812L, L822F, V826L, T827I, A831V, K835R, D843N, A845S, A845V, K854N, T859N, T859I, M869I, I870V, A871V, A879S, A879V, T883I, F888L, A890V, A892V, A899S, M902I, A903S, I909V, V911I, V915I, L922F, A924S, S929I, D936Y, S939F, S940F, G946R, D950N, D950H, Q957R, T961M, V963A, S967N, V976F, S982A, K986N, K986R, R995G, I997V, T998I, Q1005H, T1006I, T1009I, R1014K, A1016V, A1020S, A1025G, T1027I, V1033A, K1038Q, V1040F, K1045N, L1049I, M1050I, A1056V, H1058Y, L1063F, T1066N, Q1071H, K1073N, A1078S, D1084Y, D1084E, A1086S, V1094F, H1101D, H1101Y, V1104L, E1111K, D1118H, D1118Y, G1124V, D1127G, I1130V, V1133F, D1139H, L1141W, D1146Y, E1150D, D1153Y, P1162L, P1162S, D1163Y, N1173S, A1174V, V1176F, E1182D, R1185H, K1191N, N1192S, E1195Q, E1202Q, K1205N, Q1208H, I1216T, G1219V, G1219C, V1228L, M1229I, V1230L, M1237I, T1238I, C1243F, C1247F, S1252F, S1252P, D1260N, P1263L, V1264L, H1271Y, T1273I, and T1273A relative to the amino acid sequence of SEQ ID NO: 1 or 35.

A nucleic acid molecule of the disclosure may also be one that contains a nucleotide sequence that encodes a SARS-CoV-2 spike polypeptide with one or more of the mutations Δ 69-70, Δ 144, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H relative to the amino acid sequence of SEQ ID NO: 1 or 35. The polypeptide may contain two, three, four, five, six, seven, eight, or nine of the mutations: Δ 69-70, Δ 144, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H relative to the amino acid sequence of SEQ ID NO: 1 or 35. In particular, the nucleic acid molecule may contain a nucleotide sequence that encodes a polypeptide with each of the mutations: Δ 69-70, Δ 144, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H relative to the amino acid sequence of SEQ ID NO: 1 or 35. For example, the nucleic acid molecule may contain a nucleotide sequence that encodes a polypeptide with the amino acid sequence of SEQ ID NO: 40, or a variant thereof with at least 85% sequence identity thereto, such as a variant that contains each of the indicated mutations. For example, the nucleic acid molecule may contain a nucleotide sequence that encodes a polypeptide with the amino acid sequence of

SEQ ID NO: 65, or a variant thereof with at least 85% sequence identity thereto, such as a variant that contains each of the indicated mutations. The nucleic acid molecule may have the nucleic acid sequence of SEQ ID NOs: 44 or 61, or a variant thereof with at least 85% sequence identity thereto, such as a variant that encodes a polypeptide that contains each of the indicated mutations.

5 A nucleic acid molecule of the disclosure may also be one that contains a nucleotide sequence that encodes a SARS-CoV-2 spike polypeptide with one or more of the mutations: L5F, L18F, T20N, P26S, V36F, Q52R, D80G, T95I, I105V, L118F, V127F, D138Y, Δ 156-157, R158G, T167S, D178H, R190S, I203V, D215G, A222V, I233V, Δ 242-244, D253G, A262S, P272L, T284I, T299I, V308L, F318S, V227I, P337S, R346S, K356R, V367L, P384L, N394S, R408I, K417T, D427N, N439K, L452R, I468V, T478K, E484K, L513F, A522S, T531S, N540S, T549I, K558N, E583D, G594S, T604A, Q613H, D614G, V622F, P631S, S640F, H655Y, I670V, S691P, A701V, T732A, T747I, S758G, G769V, Q779K, D796H, P809S, L822F, A831V, A845S, T859N, I870V, A879S, F888L, A899S, I909V, L922F, S939F, D950N, T961M, I997V, T1006I, A1016V, T1027I, V1040F, L1049I, H1058Y, Q1071H, D1084Y, V1094F, V1104L, I1130V, D1139H, D1153Y, P1162S, V1176F, K1191N, Q1208H, G1219V, V1228L, M1237I, S1252F, V1264L, and T1273I, relative to the amino acid sequence of SEQ ID NO: 1 or 35. The polypeptide may contain two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, twenty-four, twenty-five, twenty-six, twenty-seven, twenty-eight, twenty-nine, thirty, thirty-one, thirty-two, thirty-three, thirty-four, thirty-five, thirty-six, thirty-seven, thirty-eight, thirty-nine, forty, forty-one, forty-two, forty-three, forty-four, forty-five, forty-six, forty-seven, forty-eight, forty-nine, fifty, fifty-one, fifty-two, fifty-three, fifty-four, fifty-five, fifty-six, fifty-seven, fifty-eight, fifty-nine, sixty, sixty-one, sixty-two, sixty-three, sixty-four, sixty-five, sixty-six, sixty-seven, sixty-eight, sixty-nine, seventy, seventy-one, seventy-two, seventy-three, seventy-four, seventy-five, seventy-six, seventy-seven, seventy-eight, seventy-nine, eighty, eighty-one, eighty-two, eighty-three, eighty-four, eighty-five, eighty-six, eighty-seven, eighty-eight, eighty-nine, ninety, ninety-one, ninety-two, ninety-three, ninety-four, ninety-five, ninety-six, ninety-seven, ninety-eight, ninety-nine, one hundred, one hundred one, one hundred two, one hundred three, one hundred four, one hundred five, or one hundred six of the mutations: L5F, L18F, T20N, P26S, V36F, Q52R, D80G, T95I, I105V, L118F, V127F, D138Y, Δ 156-157, R158G, T167S, D178H, R190S, I203V, D215G, A222V, I233V, Δ 242-244, D253G, A262S, P272L, T284I, T299I, V308L, F318S, V227I, P337S, R346S, K356R, V367L, P384L, N394S, R408I, K417T, D427N, N439K, L452R, I468V, T478K, E484K, L513F, A522S, T531S, N540S, T549I, K558N, E583D, G594S, T604A, Q613H, D614G, V622F, P631S, S640F, H655Y, I670V, S691P, A701V, T732A, T747I, S758G, G769V, Q779K, D796H, P809S, L822F, A831V, A845S, T859N, I870V, A879S, F888L, A899S, I909V, L922F, S939F, D950N, T961M, I997V, T1006I, A1016V, T1027I, V1040F, L1049I, H1058Y, Q1071H, D1084Y, V1094F, V1104L, I1130V, D1139H, D1153Y, P1162S, V1176F, K1191N, Q1208H, G1219V, V1228L, M1237I, S1252F, V1264L, and T1273I, relative to the amino acid sequence of SEQ ID NO: 1 or 35. In particular, the nucleic acid molecule may contain a nucleotide sequence that encodes a polypeptide with each of the mutations: L5F, L18F, T20N, P26S, V36F, Q52R, D80G, T95I, I105V, L118F, V127F, D138Y, Δ 156-157, R158G, T167S, D178H, R190S, I203V, D215G, A222V, I233V, Δ 242-244, D253G, A262S, P272L, T284I, T299I, V308L, F318S, V227I, P337S, R346S, K356R, V367L, P384L, N394S, R408I, K417T, D427N, N439K, L452R, I468V, T478K, E484K, L513F, A522S, T531S, N540S, T549I, K558N, E583D, G594S, T604A, Q613H, D614G, V622F, P631S, S640F,

H655Y, I670V, S691P, A701V, T732A, T747I, S758G, G769V, Q779K, D796H, P809S, L822F, A831V, A845S, T859N, I870V, A879S, F888L, A899S, I909V, L922F, S939F, D950N, T961M, I997V, T1006I, A1016V, T1027I, V1040F, L1049I, H1058Y, Q1071H, D1084Y, V1094F, V1104L, I1130V, D1139H, D1153Y, P1162S, V1176F, K1191N, Q1208H, G1219V, V1228L, M1237I, S1252F, V1264L, and T1273I, relative to the amino acid sequence of SEQ ID NO: 1 or 35. For example, the nucleic acid molecule may contain a nucleotide sequence that encodes a polypeptide with the amino acid sequence of SEQ ID NO: 41, or a variant thereof with at least 85% sequence identity thereto, such as a variant that contains each of the indicated mutations. For example, the nucleic acid molecule may contain a nucleotide sequence that encodes a polypeptide with the amino acid sequence of SEQ ID NO: 66, or a variant thereof with at least 85% sequence identity thereto, such as a variant that contains each of the indicated mutations. The nucleic acid molecule may have the nucleic acid sequence of SEQ ID NOs: 45 or 62, or a variant thereof with at least 85% sequence identity thereto, such as a variant that encodes a polypeptide that contains each of the indicated mutations.

A nucleic acid molecule of the disclosure may also contain a nucleotide sequence that encodes a SARS-CoV-2 spike polypeptide with one or more of the mutations: P9L, T19R, T33I, H49Y, A67V, Δ69-70, D80A, S98F, S112L, V126A, G142D, W152R, S162I, L176F, L189F, D198Y, I210T, A222V, D228H, H245Y, W258L, V267L, E281Q, A292S, T307I, T323I, L335F, R346K, R357K, V367F, T376I, T385N, V395I, E406Q, K417N, D427Y, N440K, L452Q, K462T, E471Q, E484K, F490S, N501T, V510L, A520S, V534I, T547I, P561S, A570D, T572I, E583Q, V595I, T604I, A623S, T632S, N641S, A653V, A672V, P681R, A694V, S704L, T716V, M731I, M740V, N751D, T761I, A771S, E780D, T791I, S803A, P812S, T827I, D843N, K854N, A871V, T883I, A892V, M902I, V911I, A924S, D936Y, G946R, Q957R, S967N, V976F, K986N, T998I, T1009I, A1020S, V1033A, K1045N, A1056V, T1066N, A1078S, A1086S, H1101D, E1111K, G1124V, V1133F, D1146Y, P1162L, A1174V, R1185H, E1195Q, K1205N, G1219C, M1229I, T1238I, C1247F, D1260N, and H1271Y, relative to the amino acid sequence of SEQ ID NO: 1 or 35. The polypeptide may contain two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, twenty-four, twenty-five, twenty-six, twenty-seven, twenty-eight, twenty-nine, thirty, thirty-one, thirty-two, thirty-three, thirty-four, thirty-five, thirty-six, thirty-seven, thirty-eight, thirty-nine, forty, forty-one, forty-two, forty-three, forty-four, forty-five, forty-six, forty-seven, forty-eight, forty-nine, fifty, fifty-one, fifty-two, fifty-three, fifty-four, fifty-five, fifty-six, fifty-seven, fifty-eight, fifty-nine, sixty, sixty-one, sixty-two, sixty-three, sixty-four, sixty-five, sixty-six, sixty-seven, sixty-eight, sixty-nine, seventy, seventy-one, seventy-two, seventy-three, seventy-four, seventy-five, seventy-six, seventy-seven, seventy-eight, seventy-nine, eighty, eighty-one, eighty-two, eighty-three, eighty-four, eighty-five, eighty-six, eighty-seven, eighty-eight, eighty-nine, ninety, ninety-one, ninety-two, ninety-three, ninety-four, ninety-five, ninety-six, ninety-seven, ninety-eight, ninety-nine, one hundred, one hundred one, one hundred two, one hundred three, one hundred four, one hundred five, one hundred six, one hundred seven, one hundred eight, one hundred nine, one hundred ten, or one hundred eleven of the mutations: P9L, T19R, T33I, H49Y, A67V, Δ69-70, D80A, S98F, S112L, V126A, G142D, W152R, S162I, L176F, L189F, D198Y, I210T, A222V, D228H, H245Y, W258L, V267L, E281Q, A292S, T307I, T323I, L335F, R346K, R357K, V367F, T376I, T385N, V395I, E406Q, K417N, D427Y, N440K, L452Q, K462T, E471Q, E484K, F490S, N501T, V510L, A520S, V534I, T547I, P561S, A570D, T572I, E583Q, V595I, T604I, A623S, T632S, N641S, A653V, A672V, P681R,

A694V, S704L, T716V, M731I, M740V, N751D, T761I, A771S, E780D, T791I, S803A, P812S, T827I, D843N, K854N, A871V, T883I, A892V, M902I, V911I, A924S, D936Y, G946R, Q957R, S967N, V976F, K986N, T998I, T1009I, A1020S, V1033A, K1045N, A1056V, T1066N, A1078S, A1086S, H1101D, E1111K, G1124V, V1133F, D1146Y, P1162L, A1174V, R1185H, E1195Q, K1205N, G1219C, M1229I, T1238I, C1247F, D1260N, and H1271Y, relative to the amino acid sequence of SEQ ID NO: 1 or 35. In particular, the nucleic acid molecule may contain a nucleotide sequence that encodes a polypeptide with each of the mutations: P9L, T19R, T33I, H49Y, A67V, Δ 69-70, D80A, S98F, S112L, V126A, G142D, W152R, S162I, L176F, L189F, D198Y, I210T, A222V, D228H, H245Y, W258L, V267L, E281Q, A292S, T307I, T323I, L335F, R346K, R357K, V367F, T376I, T385N, V395I, E406Q, K417N, D427Y, N440K, L452Q, K462T, E471Q, E484K, F490S, N501T, V510L, A520S, V534I, T547I, P561S, A570D, T572I, E583Q, V595I, T604I, A623S, T632S, N641S, A653V, A672V, P681R, A694V, S704L, T716V, M731I, M740V, N751D, T761I, A771S, E780D, T791I, S803A, P812S, T827I, D843N, K854N, A871V, T883I, A892V, M902I, V911I, A924S, D936Y, G946R, Q957R, S967N, V976F, K986N, T998I, T1009I, A1020S, V1033A, K1045N, A1056V, T1066N, A1078S, A1086S, H1101D, E1111K, G1124V, V1133F, D1146Y, P1162L, A1174V, R1185H, E1195Q, K1205N, G1219C, M1229I, T1238I, C1247F, D1260N, and H1271Y, relative to the amino acid sequence of SEQ ID NO: 1 or 35. For example, the nucleic acid molecule may contain a nucleotide sequence that encodes a polypeptide has the amino acid sequence of SEQ ID NO: 42, or a variant thereof with at least 85% sequence identity thereto, such as a variant that contains each of the indicated mutations. For example, the nucleic acid molecule may contain a nucleotide sequence that encodes a polypeptide has the amino acid sequence of SEQ ID NO: 67, or a variant thereof with at least 85% sequence identity thereto, such as a variant that contains each of the indicated mutations. The nucleic acid molecule may have the nucleic acid sequence of SEQ ID NOs: 46 or 63, or a variant thereof with at least 85% sequence identity thereto, such as a variant that encodes a polypeptide that contains each of the indicated mutations.

A nucleic acid molecule of the disclosure may also be one that contains a nucleotide sequence that encodes a SARS-CoV-2 spike polypeptide with one or more of the mutations: V3G, S13I, L18F, A27S, V36I, S45F, L54F, W64R, G75V, T76I, P85S, S94F, D111N, V120L, E132Q, N148T, F157S, S172A, G181V, V193L, Y204H, L216F, V227A, R237K, Δ 246-252, D253N, A263P, R273S, V289L, K300M, E309Q, V320F, P330S, G339S, A348S, V362F, S371T, V382L, N394H, R403K, Q414K, T430I, N440S, L452M, L461F, T470I, T478K, S494P, N501Y, Y505H, A522V, V534F, F543L, E554D, F565L, A570D, A575S, L585F, I598V, Q607K, D614G, N616S, D627G, T638I, A647S, Y660F, Q677H, A688V, M697I, A706V, T716I, T719I, T732I, T747N, T761R, V772I, V785I, K795R, P812L, V826L, K835R, A845V, T859I, M869I, A879V, A890V, A903S, V915I, S929I, S940F, D950H, V963A, V976F, S982A, K986R, R995G, Q1005H, R1014K, A1025G, K1038Q, M1050I, L1063F, K1073N, D1084E, H1101Y, D1118Y, D1127G, L1141W, E1150D, D1163Y, N1173S, E1182D, N1192S, E1202Q, I1216T, V1230L, C1243F, S1252P, P1263L, and T1273A relative to the amino acid sequence of SEQ ID NO: 1 or 35. The polypeptide may contain two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, twenty-four, twenty-five, twenty-six, twenty-seven, twenty-eight, twenty-nine, thirty, thirty-one, thirty-two, thirty-three, thirty-four, thirty-five, thirty-six, thirty-seven, thirty-eight, thirty-nine, forty, forty-one, forty-two, forty-three, forty-four, forty-five, forty-six, forty-seven, forty-eight, forty-nine, fifty, fifty-one, fifty-two, fifty-

three, fifty-four, fifty-five, fifty-six, fifty-seven, fifty-eight, fifty-nine, sixty, sixty-one, sixty-two, sixty-three, sixty-four, sixty-five, sixty-six, sixty-seven, sixty-eight, sixty-nine, seventy, seventy-one, seventy-two, seventy-three, seventy-four, seventy-five, seventy-six, seventy-seven, seventy-eight, seventy-nine, eighty, eighty-one, eighty-two, eighty-three, eighty-four, eighty-five, eighty-six, eighty-seven, eighty-eight, eighty-nine, ninety, ninety-one, ninety-two, ninety-three, ninety-four, ninety-five, ninety-six, ninety-seven, ninety-eight, ninety-nine, one hundred, one hundred one, one hundred two, one hundred three, one hundred four, one hundred five, one hundred six, one hundred seven, one hundred eight, one hundred nine, one hundred ten, one hundred eleven, one hundred twelve, one hundred thirteen, one hundred fourteen, one hundred fifteen, one hundred sixteen, one hundred seventeen, one hundred eighteen, one hundred nineteen, or one hundred twenty of the mutations: V3G, S13I, L18F, A27S, V36I, S45F, L54F, W64R, G75V, T76I, P85S, S94F, D111N, V120L, E132Q, N148T, F157S, S172A, G181V, V193L, Y204H, L216F, V227A, R237K, Δ246-252, D253N, A263P, R273S, V289L, K300M, E309Q, V320F, P330S, G339S, A348S, V362F, S371T, V382L, N394H, R403K, Q414K, T430I, N440S, L452M, L461F, T470I, T478K, S494P, N501Y, Y505H, A522V, V534F, F543L, E554D, F565L, A570D, A575S, L585F, I598V, Q607K, D614G, N616S, D627G, T638I, A647S, Y660F, Q677H, A688V, M697I, A706V, T716I, T719I, T732I, T747N, T761R, V772I, V785I, K795R, P812L, V826L, K835R, A845V, T859I, M869I, A879V, A890V, A903S, V915I, S929I, S940F, D950H, V963A, V976F, S982A, K986R, R995G, Q1005H, R1014K, A1025G, K1038Q, M1050I, L1063F, K1073N, D1084E, H1101Y, D1118Y, D1127G, L1141W, E1150D, D1163Y, N1173S, E1182D, N1192S, E1202Q, I1216T, V1230L, C1243F, S1252P, P1263L, ad T1273A, relative to the amino acid sequence of SEQ ID NO: 1 or 35. In particular, the nucleic acid molecule may contain a nucleotide sequence that encodes a polypeptide with each of the mutations: V3G, S13I, L18F, A27S, V36I, S45F, L54F, W64R, G75V, T76I, P85S, S94F, D111N, V120L, E132Q, N148T, F157S, S172A, G181V, V193L, Y204H, L216F, V227A, R237K, Δ246-252, D253N, A263P, R273S, V289L, K300M, E309Q, V320F, P330S, G339S, A348S, V362F, S371T, V382L, N394H, R403K, Q414K, T430I, N440S, L452M, L461F, T470I, T478K, S494P, N501Y, Y505H, A522V, V534F, F543L, E554D, F565L, A570D, A575S, L585F, I598V, Q607K, D614G, N616S, D627G, T638I, A647S, Y660F, Q677H, A688V, M697I, A706V, T716I, T719I, T732I, T747N, T761R, V772I, V785I, K795R, P812L, V826L, K835R, A845V, T859I, M869I, A879V, A890V, A903S, V915I, S929I, S940F, D950H, V963A, V976F, S982A, K986R, R995G, Q1005H, R1014K, A1025G, K1038Q, M1050I, L1063F, K1073N, D1084E, H1101Y, D1118Y, D1127G, L1141W, E1150D, D1163Y, N1173S, E1182D, N1192S, E1202Q, I1216T, V1230L, C1243F, S1252P, P1263L, ad T1273A, relative to the amino acid sequence of SEQ ID NO: 1 or 35. For example, the nucleic acid molecule may contain a nucleotide sequence that encodes a polypeptide with the amino acid sequence of SEQ ID NO: 43, or a variant thereof with at least 85% sequence identity thereto, such as a variant that contains each of the indicated mutations. For example, the nucleic acid molecule may contain a nucleotide sequence that encodes a polypeptide with the amino acid sequence of SEQ ID NO: 68, or a variant thereof with at least 85% sequence identity thereto, such as a variant that contains each of the indicated mutations. The nucleic acid molecule may have the nucleic acid sequence of SEQ ID NOs: 47 or 64, or a variant thereof with at least 85% sequence identity thereto, such as a variant that encodes a polypeptide that contains each of the indicated mutations.

A nucleic acid molecule of the disclosure may also be one that contains a nucleotide sequence that encodes a SARS-CoV-2 membrane (MEM) polypeptide having at least 85% sequence identity to at

least 100 contiguous amino acids (e.g., positions 1-100, 1-200, and 100-200) of any one of SEQ ID NOs: 75-77 (e.g., MEM1-3, respectively) or a complementary sequence thereof. In some embodiments, the nucleotide sequence has at least 85% (e.g., 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to all or a portion of any one of SEQ ID NOs: 69-71, or a complementary sequence thereof. In some embodiments, the nucleic acid molecule, or a portion thereof, is capable of eliciting an immune response in a subject. In some embodiments, the nucleic acid molecule has the nucleic acid sequence of any one of SEQ ID NOs: 69-71. In some embodiments, the nucleic acid molecule has at least 85% (e.g., 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity to all or a portion of any one of SEQ ID NOs: 69-71 but does not encode the sequence of SEQ ID NOs: 75-77.

A nucleic acid molecule of the disclosure may also be one that contains a nucleotide sequence that encodes a SARS-CoV2 nucleocapsid (NUL) polypeptide having at least 85% sequence identity to at least 100 contiguous amino acids (e.g., positions 1-100, 1-200, and 100-200) of any one of SEQ ID NOs: 78-80 (e.g., NUL1-3, respectively) or a complementary sequence thereof. In some embodiments, the nucleotide sequence has at least 85% (e.g., 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to all or a portion of any one of SEQ ID NOs: 72-74, or a complementary sequence thereof. In some embodiments, the nucleic acid molecule, or a portion thereof, is capable of eliciting an immune response in a subject. In some embodiments, the nucleic acid molecule has the nucleic acid sequence of any one of SEQ ID NOs: 72-74. In some embodiments, the nucleic acid molecule has at least 85% (e.g., 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity to all or a portion of any one of SEQ ID NOs: 69-71, but does not encode the sequence of SEQ ID NO: 78-80.

The nucleic acid molecules of the disclosure may contain a spike, membrane, or nucleocapsid nucleotide sequence that has been modified relative to a wild-type or natural variant of SARS-CoV-2 for improved expression in host cells (e.g., mammalian (e.g., human) host cells). Optimization can include the addition of a leader sequence, restriction site, and/or a Kozak sequence.

The nucleic acid molecules may be further modified, such as by codon optimization, for expression in a targeted mammalian subject (e.g., human or a non-human animal for vaccine production).

The nucleic acid molecules may also be inserted into expression vectors, such as a plasmid, or a viral vector, such as an adenovirus (e.g., Ad26 vector), poxvirus, adeno-associated virus, retroviral, or other viral vector, or prepared as naked or encapsulated DNA and incorporated into compositions.

Polypeptides

The polypeptides of the disclosure have been rationally designed using information from naturally occurring spike (S), membrane (MEM), and nucleocapsid (NUL) polypeptides from SARS-CoV-2 lineage variants.

The S polypeptides of the disclosure are modified S proteins containing one or more mutations relative to the Wuhan spike protein (e.g., relative to SEQ ID NO: 35) and existing optimizations thereof (e.g., SEQ ID NO: 1) that were designed based on a study of emerging variants of the Wuhan coronavirus around the world. The polypeptides described herein have been modified to include one or more resistance mutations identified in one or more of these lineage variants.

The polypeptides disclosed herein may be less than the full-length S protein (i.e., they may contain one or more regions of the S protein from SARS-CoV-2 or a variant thereof, such as the NTD and/or RBD). The polypeptides may also include, in addition to one or more of the resistance mutations described herein, most or all of the regions of the S protein, such that the S protein of the disclosure is capable of forming into a structurally and/or functionally stable S protein.

The S protein of the disclosure may have the amino acid sequence of any one of SEQ ID NOs: 2-4 or a variant thereof with at least 85% (e.g., at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%) sequence identity to all or a portion of any one of SEQ ID NOs: 2-4. The polypeptides may include at least 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, or 1300 or more continuous or non-continuous amino acids of any one of SEQ ID NOs: 2-4.

The S protein of the disclosure may have the amino acid sequence of any one of SEQ ID NOs: 40-43 and 65-68, or a variant thereof with at least 85% (e.g., at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%) sequence identity to all or a portion of any one of SEQ ID NOs: 40-43 and 65-68. The polypeptides may include at least 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, or 1300 or more continuous or non-continuous amino acids of any one of SEQ ID NOs: 40-43 and 65-68.

Polypeptides of the disclosure may also include a deletion of or an inclusion of a signal sequence (e.g., SEQ ID NO: 20), stabilizing mutations (e.g., proline substitutions corresponding to amino acids K969 and V970 of SEQ ID NO: 34), mutations to a furin cleavage site (e.g., SEQ ID NO: 19), introduction of a trimerization domain (e.g., a foldon trimerization domain, e.g., SEQ ID NO: 15, or other trimerization domain known in the art), introduction of linker or spacer sequences (e.g., SEQ ID NOs: 16 and 17), and combinations thereof. A signal sequence and each of these modifications are present in the S protein of SEQ ID NO: 1, and any of SEQ ID NOs 2-4 could be similarly modified using the sequence of SEQ ID NO: 1 as a guide (e.g., by aligning the sequences of SEQ ID NO: 2-4 with SEQ ID NO: 1 and making the equivalent modifications found in SEQ ID NO: 1 at the corresponding sequence of SEQ ID NOs: 2-4. Furthermore, any of SEQ ID NOs: 40-43 could be modified to include one or more of these modifications, e.g., by using the sequence of SEQ ID NO: 35 as a guide.

An isolated polypeptide of the disclosure may also be one that contains a nucleotide sequence that encodes a SARS-CoV-2 membrane polypeptide. In some embodiments, the polypeptide has at least 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity to at least 100 contiguous amino acids (e.g., positions 1-100, 1-200, and 100-200), or the amino acid sequence of, any one of SEQ ID NOs: 75-77 (e.g., MEM1-3, respectively). In some embodiments, the polypeptide, or a portion or fragment thereof, is capable of eliciting an immune response in a subject. In some embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 75. In some embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 76. In some embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 77. In some embodiments, the polypeptide has at least 85% (e.g., 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity to all or a portion of any one of SEQ ID NOs: 75-77, but is not the sequence of SEQ ID NO: 75-77.

An isolated polypeptide of the disclosure may also be one that contains a nucleotide sequence that encodes a SARS-CoV2 nucleocapsid polypeptide. In some embodiments, the polypeptide has at

least 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity to at least 100 contiguous amino acids (e.g., positions 1-100, 1-200, and 100-200), or the amino acid sequence of, any one of SEQ ID NOs: 78-80 (e.g., NUL1-3, respectively). In some embodiments, the polypeptide, or a portion or fragment thereof, is capable of eliciting an immune response in a subject. In some
5 embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 78. In some embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 79. In some embodiments, the polypeptide has the amino acid sequence of SEQ ID NO: 80. In some embodiments, the polypeptide has at least 85% (e.g., 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity to all or a portion of any one of SEQ ID NOs: 78-80, but is not the sequence of SEQ ID NO: 78-
10 80.

The polypeptides of the disclosure may be produced (e.g., recombinant methods) and may be purified during the production (e.g., isolated from other components, such as components with which the polypeptides are natively associated). The purified polypeptides may then be incorporated into a composition of the disclosure (e.g., an immunogenic composition or a vaccine composition).

Vectors

The disclosure also features recombinant vectors (e.g., an Ad26 viral vector) including any one or more of the S, MEM, and/or NUL polynucleotides described above.

The vectors can be used to deliver a nucleic acid expressing an immunogen (e.g., one of more of
20 SEQ ID NOs: 2-4 or variants thereof, having at least 85-99% sequence identity thereto, for example at least greater than 90% sequence identity thereto), and include mammalian, viral, and bacterial expression vectors. For example, a vector can be used to deliver a nucleic acid (e.g., a nucleic acid containing the nucleotide sequence of SEQ ID NOs: 7-12, or a variant thereof with up to 85% sequence identity thereto) expressing an immunogen with the amino acid sequence of any one of SEQ ID NOs: 2-4, or a variant
25 thereof with up to 85% sequence identity thereto. For example, a vector can be used to deliver a nucleic acid (e.g., a nucleic acid containing the nucleotide sequence of SEQ ID NO: 7 or 10, or a variant thereof with up to 85% sequence identity thereto), expressing an immunogen with the amino acid sequence of SEQ ID NO: 2, or a variant thereof with up to 85% sequence identity thereto. For example, a vector can be used to deliver a nucleic acid (e.g., a nucleic acid containing the nucleotide sequence of SEQ ID NO: 8
30 or 11, or a variant thereof with up to 85% sequence identity thereto) expressing an immunogen with the amino acid sequence of SEQ ID NO: 3, or a variant thereof with up to 85% sequence identity thereto. For example, a vector can be used to deliver a nucleic acid (e.g., a nucleic acid containing the nucleotide sequence of SEQ ID NO: 9 or 12, or a variant thereof with up to 85% sequence identity thereto) expressing an immunogen with the amino acid sequence of SEQ ID NO: 4, or a variant thereof with up to
35 85% sequence identity thereto. The mammalian, viral, and bacterial vectors can be genetically modified to contain one or more nucleic acid sequences set forth in SEQ ID NOs: 7-12 or variants thereof having at least 85-99% sequence identity thereto, for example at least greater than 90% sequence identity thereto, as well as complementary sequences thereof.

If desired, one or more of the vectors can also include a nucleic acid molecule containing the
40 nucleotide sequence of SEQ ID NO: 6, or a variant thereof with up to 85% sequence identity thereto, that

encodes an immunogen with the amino acid sequence of SEQ ID NO: 1, or a variant thereof with up to 85% sequence identity thereto, or a complementary sequence thereof.

The vectors of the disclosure may contain a nucleic acid molecule with combinations of different nucleic acid molecules (e.g., a vector may contain a nucleic acid molecule with the nucleotide sequence of SEQ ID NOs: 7 and 8, SEQ ID NOs: 7 and 9, SEQ ID NOs: 8 and 9, or SEQ ID NOs: 7-9, or variants thereof with up to 85% sequence identity thereto. The nucleic acid molecule of the vector may encode two or more immunogens, such as immunogens with the amino acid sequences of SEQ ID NOs: 2 and 3 SEQ ID NOs: 2 and 4, SEQ ID NOs: 3 and 4, or each of SEQ ID NOs: 2-4, or variants thereof with up to 85% sequence identity thereto. The vector may also include a nucleic acid molecule containing the nucleotide sequence of SEQ ID NO: 6, or a variant thereof with up to 85% sequence identity thereto, that encodes an immunogen with the amino acid sequence of SEQ ID NO: 1, or a variant thereof with up to 85% sequence identity thereto.

The recombinant vectors of the disclosure can be used to deliver any one or more nucleic acids expressing a SARS-CoV-2 S immunogen (e.g., one of more of SEQ ID NOs: 1-4, 35, 40-43, and/or 65-68, or variants thereof with at least 85-99% sequence identity thereto, for example at least greater than 90% sequence identity thereto), and include mammalian, viral, and bacterial expression vectors. For example, a vector can be used to deliver a nucleic acid (e.g., a nucleic acid containing the nucleotide sequence of one or more of SEQ ID NOs: 44-51 and 61-64, or a variant thereof with up to 85% sequence identity thereto) expressing an immunogen with the amino acid sequence of any one of SEQ ID NOs: 40-43 and 65-68, or a variant thereof with up to 85% sequence identity thereto. For example, a vector can be used to deliver a nucleic acid (e.g., a nucleic acid containing the nucleotide sequence of SEQ ID NOs: 44, 48, or 61, or a variant thereof with up to 85% sequence identity thereto), expressing an immunogen with the amino acid sequence of SEQ ID NO: 40 or 65, or a variant thereof with up to 85% sequence identity thereto. For example, a vector can be used to deliver a nucleic acid (e.g., a nucleic acid containing the nucleotide sequence of SEQ ID NOs: 45, 49, or 62, or a variant thereof with up to 85% sequence identity thereto) expressing an immunogen with the amino acid sequence of SEQ ID NO: 41 or 66, or a variant thereof with up to 85% sequence identity thereto. For example, a vector can be used to deliver a nucleic acid (e.g., a nucleic acid containing the nucleotide sequence of SEQ ID NOs: 46, 50, or 63, or a variant thereof with up to 85% sequence identity thereto) expressing an immunogen with the amino acid sequence of SEQ ID NO: 42 or 67, or a variant thereof with up to 85% sequence identity thereto. For example, a vector can be used to deliver a nucleic acid (e.g., a nucleic acid containing the nucleotide sequence of SEQ ID NOs: 47, 51 or 64, or a variant thereof with up to 85% sequence identity thereto) expressing an immunogen with the amino acid sequence of SEQ ID NO: 43 or 68, or a variant thereof with up to 85% sequence identity thereto. The mammalian, viral, and bacterial vectors can be genetically modified to contain one or more nucleic acid sequences set forth in SEQ ID NOs: 44-51 and 61-64 or variants thereof having at least 85-99% sequence identity thereto, for example at least greater than 90% sequence identity thereto, as well as complementary sequences thereof.

The recombinant vectors of the disclosure can be used to deliver any one or more nucleic acids expressing a SARS-CoV-2 MEM immunogen (e.g., one of more of SEQ ID NOs: 75-77, or variants thereof with at least 85-99% sequence identity thereto, for example at least greater than 90% sequence identity thereto), and/or a SARS-CoV-2 NUL immunogen (e.g., one of more of SEQ ID NOs: 78-80, or

variants thereof with at least 85-99% sequence identity thereto, for example at least greater than 90% sequence identity thereto). The recombinant vectors of the disclosure can include mammalian, viral, and bacterial expression vectors. For example, a vector can be used to deliver a nucleic acid (e.g., a nucleic acid containing the nucleotide sequence of one or more of SEQ ID NOs: 69-74, or a variant thereof with up to 85% sequence identity thereto) expressing an immunogen with the amino acid sequence of any one of SEQ ID NOs: 75-80, or a variant thereof with up to 85% sequence identity thereto. For example, a vector can be used to deliver a nucleic acid (e.g., a nucleic acid containing the nucleotide sequence of SEQ ID NO: 69, or a variant thereof with up to 85% sequence identity thereto), expressing an immunogen with the amino acid sequence of SEQ ID NO: 75 (e.g., MEM1), or a variant thereof with up to 85% sequence identity thereto. For example, a vector can be used to deliver a nucleic acid (e.g., a nucleic acid containing the nucleotide sequence of SEQ ID NO: 70, or a variant thereof with up to 85% sequence identity thereto) expressing an immunogen with the amino acid sequence of SEQ ID NO: 76 (e.g., MEM2) or a variant thereof with up to 85% sequence identity thereto. For example, a vector can be used to deliver a nucleic acid (e.g., a nucleic acid containing the nucleotide sequence of SEQ ID NO: 71 or a variant thereof with up to 85% sequence identity thereto) expressing an immunogen with the amino acid sequence of SEQ ID NO: 77 (e.g., MEM3), or a variant thereof with up to 85% sequence identity thereto. For example, a vector can be used to deliver a nucleic acid (e.g., a nucleic acid containing the nucleotide sequence of SEQ ID NOs: 72, or a variant thereof with up to 85% sequence identity thereto) expressing an immunogen with the amino acid sequence of SEQ ID NO: 78 (e.g., NUL1), or a variant thereof with up to 85% sequence identity thereto. For example, a vector can be used to deliver a nucleic acid (e.g., a nucleic acid containing the nucleotide sequence of SEQ ID NOs: 73, or a variant thereof with up to 85% sequence identity thereto) expressing an immunogen with the amino acid sequence of SEQ ID NO: 79 (e.g., NUL2), or a variant thereof with up to 85% sequence identity thereto. For example, a vector can be used to deliver a nucleic acid (e.g., a nucleic acid containing the nucleotide sequence of SEQ ID NOs: 74, or a variant thereof with up to 85% sequence identity thereto) expressing an immunogen with the amino acid sequence of SEQ ID NO: 80 (e.g., NUL3), or a variant thereof with up to 85% sequence identity thereto. The mammalian, viral, and bacterial vectors can be genetically modified to contain one or more nucleic acid sequences set forth in SEQ ID NOs: 69-74 or variants thereof having at least 85-99% sequence identity thereto, for example at least greater than 90% sequence identity thereto, as well as complementary sequences thereof.

If desired, one or more of the vectors can also include a nucleic acid molecule containing the nucleotide sequence of SEQ ID NO: 29, or a variant thereof with up to 85% sequence identity thereto, that encodes an immunogen with the amino acid sequence of SEQ ID NO: 35, or a variant thereof with up to 85% sequence identity thereto, or a complementary sequence thereof.

The vectors of the disclosure may contain a nucleic acid molecule with combinations of different nucleic acid molecules (e.g., a vector may contain a nucleic acid molecule with the nucleotide sequence of SEQ ID NOs: 44 and 45, SEQ ID NOs: 44 and 46, SEQ ID NOs: 44 and 47, SEQ ID NOs: 45 and 46, SEQ ID NOs: 45 and 47, SEQ ID NOs: 46 and 47, or each of SEQ ID NOs: 44-51, or variants thereof with up to 85% sequence identity thereto. The nucleic acid molecule of the vector may encode two or more immunogens, such as immunogens with the amino acid sequences of SEQ ID NOs: 40 and 41, SEQ ID NOs: 40 and 42, SEQ ID NOs: 40 and 43, SEQ ID NOs: 41 and 42, SEQ ID NOs: 41 and 43,

SEQ ID NOs: 42 and 43, or each of SEQ ID NOs: 40-43, or variants thereof with up to 85% sequence identity thereto. The nucleic acid molecule of the vector may encode three or more immunogens, such as immunogens with the amino acid sequences of SEQ ID NOs: 40, 41, and 42. The vector may also include a nucleic acid molecule containing the nucleotide sequence of SEQ ID NO: 29, or a variant thereof with up to 85% sequence identity thereto, that encodes an immunogen with the amino acid sequence of SEQ ID NO: 35, or a variant thereof with up to 85% sequence identity thereto. The vector may further include one or more nucleic acid molecules containing the nucleotide sequence of SEQ ID NOs: 69-71, or a variant thereof with up to 85% sequence identity thereto, that encodes one or more immunogens with the amino acid sequence of SEQ ID NOs: 75-77, or a variant thereof with up to 85% sequence identity thereto. The vector may further include one or more nucleic acid molecules containing the nucleotide sequence of SEQ ID NOs: 72-74, or a variant thereof with up to 85% sequence identity thereto, that encodes one or more immunogens with the amino acid sequence of SEQ ID NOs: 78-80, or a variant thereof with up to 85% sequence identity thereto.

The vectors may be, for example, plasmids, artificial chromosomes (e.g., BAG, PAC, YAC), and virus or phage vectors, and may optionally include a promoter, enhancer, or regulator for the expression of the polynucleotide. The vectors may also contain one or more selectable marker genes, for example an ampicillin, neomycin, and/or kanamycin resistance gene in the case of a bacterial plasmid or a resistance gene for a fungal vector. Vectors may be used *in vitro*, for example, for the production of DNA or RNA or used to transfect or transform a host cell, for example, a mammalian host cell, e.g., for the production of protein encoded by the vector. The vectors may also be adapted to be used *in vivo*, for example in a method of DNA vaccination, RNA vaccination, or gene therapy.

Promoters and other expression regulation signals may be selected to be compatible with the host cell for which expression is designed. For example, mammalian promoters include the metallothionein promoter, which can be induced in response to heavy metals, such as cadmium, and the β -actin promoter. A viral promoter, which can be obtained from the genome of a virus, such as, for example, polyoma virus, fowlpox virus, adenovirus (A), bovine papilloma virus, avian sarcoma virus, cytomegalovirus (CMV), a retrovirus, hepatitis-B virus, and Simian Virus 40 (SV40), and human papillomavirus (HPV), may also be used. These promoters are well known and readily available in the art.

A preferred promoter element is the CMV immediate early promoter. In some embodiments, the expression plasmid is pcDNA3.1+ (Invitrogen, CA, USA). In some embodiments, the expression vector is a viral vector, such as a vector derived from adenovirus or poxvirus.

Viral genomes provide a rich source of vectors that can be used for the efficient delivery of exogenous genes into the genome of a cell (e.g., a eukaryotic or prokaryotic cell). Viral genomes are particularly useful vectors for gene delivery because the polynucleotides contained within such genomes are typically incorporated into the genome of a target cell by generalized or specialized transduction. These processes occur as part of the natural viral replication cycle, and do not require added proteins or reagents in order to induce gene integration. Examples of viral vectors that can be used to deliver a nucleic acid expressing an immunogen (e.g., an immunogen with the amino acid sequence of one of more of SEQ ID NOs: 2-4, 40-43, 65-68, and/or 75-80 or variants thereof having at least 85-99% sequence identity thereto, for example at least greater than 90% sequence identity thereto) include a

retrovirus, adenovirus (e.g., Ad2, Ad5, Ad11, Ad12, Ad24, Ad26, Ad34, Ad35, Ad40, Ad48, Ad49, Ad50, Ad52 (e.g., a RhAd52), Ad59 (e.g., a RhAd59), and Pan9 (also known as AdC68)), parvovirus (e.g., adeno-associated viruses), coronavirus, negative strand RNA viruses such as orthomyxovirus (e.g., influenza virus), rhabdovirus (e.g., rabies and vesicular stomatitis virus), paramyxovirus (e.g. measles and Sendai), positive strand RNA viruses, such as picornavirus and alphavirus, and double stranded DNA viruses including adenovirus, herpesvirus (e.g., Herpes Simplex virus types 1 and 2, Epstein-Barr virus, cytomegalovirus), and poxvirus (e.g., vaccinia, modified vaccinia Ankara (MVA), fowlpox and canarypox). Other viruses useful for delivering polynucleotides encoding immunogens (e.g., polypeptides) include Norwalk virus, togavirus, coronavirus, reoviruses, papovavirus, hepadnavirus, and hepatitis virus, for example. Examples of retroviruses include: avian leukosis-sarcoma, mammalian C-type, B-type viruses, D-type viruses, HTLV-BLV group, lentivirus, spumavirus (Coffin, J. M., *Retroviridae: The viruses and their replication*, *In Fundamental Virology*, Third Edition, B. N. Fields, et al., Eds., Lippincott-Raven Publishers, Philadelphia, 1996). For example, the vector can be Ad26. These adenovirus vectors can be derived from, for example, human, chimpanzee, or rhesus adenoviruses. Other examples include murine leukemia viruses, murine sarcoma viruses, mouse mammary tumor virus, bovine leukemia virus, feline leukemia virus, feline sarcoma virus, avian leukemia virus, human T-cell leukemia virus, baboon endogenous virus, Gibbon ape leukemia virus, Mason Pfizer monkey virus, simian immunodeficiency virus, simian sarcoma virus, Rous sarcoma virus and lentiviruses. Other examples of vectors are described, for example, in McVey et al., (U.S. Patent. No. 5,801,030); incorporated herein in its entirety by reference. The nucleic acid material (e.g., including a nucleic acid molecule) of the viral vector may be encapsulated, e.g., in a lipid membrane or by structural proteins (e.g., capsid proteins), that may include one or more viral polypeptides (e.g., a glycoprotein). The viral vector can be used to infect cells of a subject, which, in turn, promotes the translation of the heterologous gene(s) of the viral vector into the immunogens. For example, a viral vector can be genetically modified to contain one or more nucleic acid sequences set forth in SEQ ID NOs: 44-51 and 61-64, or variants thereof having at least 85-99% sequence identity thereto, for example at least greater than 90% sequence identity thereto, and complements thereof.

Adenoviral vectors disclosed in International Patent Application Publications WO 2006/040330 and WO 2007/104792, each incorporated by reference herein, are particularly useful as vectors. These adenoviral vectors can encode and/or deliver one or more of the immunogens described herein (e.g., one or more of the SET1, SET2, and SET3 immunogens, one or more of the EG1, EG2, EG3, and/or EG4 immunogens, one or more of the MEM1, MEM2, and/or MEM3 immunogens, and/or one or more of the NUL1, NUL2, and/or NUL3 immunogens, or any combination of the SET1, SET2, SET3, EG1, EG2, EG3, EG4, MEM1, MEM2, MEM3, NUL1, NUL2, and/or NUL3 immunogens, or variants thereof with up to 85% sequence identity thereto) to treat a subject having a pathological condition associated with a viral infection (e.g., a SARS-CoV-2 infection or a lineage variant thereof). In some embodiments, one or more recombinant adenovirus vectors can be administered to the subject in order to express more than one type of immunogen (e.g., one or more of the SET1, SET2, and SET3 immunogens, one or more of the EG1, EG2, EG3, and/or EG4 immunogens, or combinations of the SET1, SET2, SET3, EG1, EG2, EG3, and/or EG4 immunogens, or variants thereof with up to 85% sequence identity thereto). In some embodiments, a recombinant adenovirus vector can be modified to change the hexon HVR domains (e.g.,

replace one or more HVRs with those of a different serotype). Besides adenoviral vectors, other viral vectors and techniques are known in the art that can be used to facilitate delivery and/or expression of one or more of the immunogens in a subject (e.g., a human). These viruses include poxviruses (e.g., vaccinia virus and modified vaccinia virus Ankara (MVA); see, e.g., U.S. Patent Nos. 4,603,112 and 5,762,938, each incorporated by reference herein), herpesviruses, togaviruses (e.g., Venezuelan Equine Encephalitis virus; see, e.g., U.S. Patent No. 5,643,576, incorporated by reference herein), picornaviruses (e.g., poliovirus; see, e.g., U.S. Patent No. 5,639,649, incorporated by reference herein), baculoviruses, and others described by Wattanapitayakul and Bauer (*Biomed. Pharmacother.* 54:487 (2000), incorporated by reference herein).

Gene transfer techniques using these viruses are known to those skilled in the art. Retrovirus vectors for example may be used to stably integrate the polynucleotide into the host genome, although such recombination is not preferred. Replication-defective adenovirus vectors by contrast remain episomal and therefore allow transient expression. The replication-defective adenoviral vector may contain a deletion in or of one or more of the E1, E3, and/or E4 regions. Alternatively, the adenoviral vector may contain one or more of the E1, E3, and/or E4 regions and may be replication-competent.

Vectors capable of driving expression in insect cells (for example baculovirus vectors), in human cells, in yeast or in bacteria may be employed in order to produce quantities of the SET1, SET2, SET3, EG1, EG2, EG3, EG4, MEM1, MEM2, MEM3, NUL1, NUL2, and/or NUL3 immunogens, or variants thereof with up to 85% sequence identity thereto, encoded by the polynucleotides of the disclosure, for example, for use as a subunit vaccine or in an immunoassay.

In some embodiments, the vector is an expression vector. In some embodiments, the viral vector is a virus selected from the group consisting of a retrovirus, adenovirus, adeno-associated virus, parvovirus, coronavirus, negative strand RNA viruses, orthomyxovirus, rhabdovirus, paramyxovirus, positive strand RNA viruses, picornavirus, alphavirus, double stranded DNA viruses, herpesvirus, Epstein-Barr virus, cytomegalovirus, fowlpox, and canarypox. In some embodiments, the vector is an adenovirus. In some embodiments, the adenovirus is selected from the group consisting of Ad26, Ad52, Ad59, Ad2, Ad5, Ad11, Ad12, Ad24, Ad34, Ad35, Ad40, Ad48, Ad49, Ad50, and Pan9. Preferably, the adenovirus is Ad26. In some embodiments, the Ad52 is a rhesus Ad52 or the Ad59 is a rhesus Ad59. In some embodiments, the vector is a replication-defective vector. In some embodiments, the replication-defective vector is a viral vector (e.g., an adenoviral vector) that contains a deletion in or of one or more of the E1, E3, and/or E4 regions. In other embodiments, the viral vector (e.g., an adenoviral vector) includes one or more of the E1, E3, and/or E4 regions and is replication-competent.

Antibodies

Anti-SARS-CoV-2 antibodies of the disclosure are capable of specifically binding to a S protein of SARS-CoV-2 or a variant thereof or a Spike polypeptide of a lineage variant thereof (such as the SET1, SET2, SET3, EG1, EG2, EG3, and/or EG4 immunogens described herein), and are capable of inhibiting a SARS-CoV-2-mediated activity (e.g., viral spread, infection, and or cell fusion) in a subject (e.g., a human). The result of such binding may be, for example, a reduction in viral titer (e.g., viral load), by about 1% (e.g., 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%) or more, after administration of an antibody to a subject infected with SARS-CoV-2 or a variant

thereof. The anti-SARS-CoV-2 antibodies may selectively bind to an epitope comprising all, or a portion of, the NTD or RBD region of a S protein of SARS-CoV-2 or a variant thereof.

In particular, the anti-SARS-CoV-2 antibodies may selectively bind to an epitope containing all, or a portion of, any one of SEQ ID NOs: 2-4. In some embodiments, the anti-SARS-CoV-2 antibodies may bind to an epitope of a S protein of SARS-CoV-2 or a variant thereof that contains one or more of the mutations S13I, L18F, T20N, P26S, Δ69-70, D80A, D80Y, L141F, Δ144, W152C, M153T, M153I, F157L, Δ242-244, D253G, S255F, A262S, V367F, K417N, K417T, N439K, L452R, Y453F, S477N, S477R, E484K, S494P, N501T, N501Y, Q613H, D614G, and P681R relative to the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 35.

For example, an antibody of the disclosure may neutralize one or more of the B.1.1.7, B.1.429, B.1.1.28, B.1.351, or A23.1 lineages of SARS-CoV-2. In particular, an antibody may bind to an epitope containing all, or a portion of, SEQ ID NOs: 2-4, or a variant thereof with up to 85% sequence identity thereto, such as an epitope within the NTD or RBD region of the Spike protein of SEQ ID NOs: 2-4. For example, antibodies may bind to an epitope of a coronavirus spike protein containing one or more of the mutations S13I, L18F, T20N, P26S, Δ69-70, D80A, D80Y, L141F, Δ144, W152C, M153T, M153I, F157L, Δ242-244, D253G, S255F, A262S, V367F, K417N, K417T, N439K, L452R, Y453F, S477N, S477R, E484K, S494P, N501T, N501Y, Q613H, D614G, and P681R relative to the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 35. The antibodies may neutralize one coronavirus, such as a coronavirus of lineage B.1.1.7. The antibodies may also neutralize more than one coronavirus, such as coronaviruses of lineages B.1.1.7 and B.1.429. The antibodies can therefore be used to inhibit or treat a coronavirus infection (e.g., infection by SARS-CoV-2 or a variant thereof).

The antibodies may be specifically generated by using one or more of the SET1, SET2, and SET3 immunogens of SEQ ID NOs: 2-4, or variants thereof with up to 85% sequence identity thereto. Furthermore, the anti-SARS-CoV-2 antibodies may selectively bind to an epitope containing all, or a portion of, any one of SEQ ID NOs: 40-43 or any one of SEQ ID NOs: 65-68. In some embodiments, the anti-SARS-CoV-2 antibodies may bind to an epitope of a S protein of SARS-CoV-2 or a variant thereof that contains one or more of the mutations: V3G, L5F, P9L, S13I, L18F, T19R, T20N, P26S, A27S, T33I, V36F, V36I, S45F, H49Y, Q52R, L54F, W64R, A67V, Δ69-70, G75V, T76I, D80A, D80G, P85S, S94F, T95I, S98F, I105V, D111N, S112L, L118F, V120L, V126A, V127F, E132Q, D138Y, G142D, Δ144, N148T, W152R, Δ156-157, F157S, R158G, S162I, T167S, S172A, L176F, D178H, G181V, L189F, R190S, V193L, D198Y, I203V, Y204H, I210T, D215G, L216F, A222V, V227A, D228H, I233V, R237K, Δ242-244, H245Y, Δ246-252, D253G, D253N, W258L, A262S, A263P, V267L, P272L, R273S, E281Q, T284I, V289L, A292S, T299I, K300M, T307I, V308L, E309Q, F318S, V320F, T323I, V227I, P330S, L335F, P337S, G339S, R346S, R346K, A348S, K356R, R357K, V362F, V367F, V367L, S371T, T376I, V382L, P384L, T385N, N394S, N394H, V395I, R403K, E406Q, R408I, Q414K, K417T, K417N, D427N, D427Y, T430I, N439K, N440S, N440K, L452M, L452R, L452Q, L461F, K462T, I468V, T470I, E471Q, T478K, E484K, F490S, S494P, N501T, N501Y, Y505H, V510L, L513F, A520S, A522S, A522V, T531S, V534I, V534F, N540S, F543L, T547I, T549I, E554D, K558N, P561S, F565L, A570D, T572I, A575S, E583Q, E583D, L585F, G594S, V595I, I598V, T604A, T604I, Q607K, Q613H, D614G, N616S, V622F, A623S, D627G, P631S, T632S, T638I, S640F, N641S, A647S, A653V, H655Y, Y660F, I670V, A672V, Q677H, P681H, P681R, A688V, S691P, A694V, M697I, A701V, S704L, A706V, T716I, T716V, T719I,

M731I, T732I, T732A, M740V, T747I, T747N, N751D, S758G, T761I, T761R, G769V, A771S, V772I, Q779K, E780D, V785I, T791I, K795R, D796H, S803A, P809S, P812S, P812L, L822F, V826L, T827I, A831V, K835R, D843N, A845S, A845V, K854N, T859N, T859I, M869I, I870V, A871V, A879S, A879V, T883I, F888L, A890V, A892V, A899S, M902I, A903S, I909V, V911I, V915I, L922F, A924S, S929I, D936Y, S939F, S940F, G946R, D950N, D950H, Q957R, T961M, V963A, S967N, V976F, S982A, K986N, K986R, R995G, I997V, T998I, Q1005H, T1006I, T1009I, R1014K, A1016V, A1020S, A1025G, T1027I, V1033A, K1038Q, V1040F, K1045N, L1049I, M1050I, A1056V, H1058Y, L1063F, T1066N, Q1071H, K1073N, A1078S, D1084Y, D1084E, A1086S, V1094F, H1101D, H1101Y, V1104L, E1111K, D1118H, D1118Y, G1124V, D1127G, I1130V, V1133F, D1139H, L1141W, D1146Y, E1150D, D1153Y, P1162L, P1162S, D1163Y, N1173S, A1174V, V1176F, E1182D, R1185H, K1191N, N1192S, E1195Q, E1202Q, K1205N, Q1208H, I1216T, G1219V, G1219C, V1228L, M1229I, V1230L, M1237I, T1238I, C1243F, C1247F, S1252F, S1252P, D1260N, P1263L, V1264L, H1271Y, T1273I, and T1273A, relative to the amino acid sequence of SEQ ID NO: 1 or 35 (e.g., the antibodies may bind an epitope of a S protein that includes one or more of these mutations).

For example, an antibody of the disclosure may neutralize one or more of the B.1.1.7, B.1.429, B.1.1.28, B.1.351, A23.1, B.1.617.1, B.1.617.2, B.1.427, B.1.525, B.1.526, P.1, P.2, P.3, C.36, C.37, B.1.1.519, B.1.526.1, B.1.526.2, R.1, B.1.258.17, B.1.575, B.1.214.2, A.2.5.2, AT.1, B.1.1.523, and B.1.620. lineages of SARS-CoV-2. In particular, an antibody may bind to an epitope containing all, or a portion of, the polypeptide sequence set forth in any one of SEQ ID NOs: 40-43 and 65-68, or a variant thereof with up to 85% sequence identity thereto, such as an epitope within the NTD or RBD region of the Spike protein of SEQ ID NOs: 40-43 or SEQ ID NOs: 65-68. For example, antibodies may bind to an epitope of a coronavirus spike protein that is or that contains one or more of the mutations: V3G, L5F, P9L, S13I, L18F, T19R, T20N, P26S, A27S, T33I, V36F, V36I, S45F, H49Y, Q52R, L54F, W64R, A67V, Δ69-70, G75V, T76I, D80A, D80G, P85S, S94F, T95I, S98F, I105V, D111N, S112L, L118F, V120L, V126A, V127F, E132Q, D138Y, G142D, Δ144, N148T, W152R, Δ156-157, F157S, R158G, S162I, T167S, S172A, L176F, D178H, G181V, L189F, R190S, V193L, D198Y, I203V, Y204H, I210T, D215G, L216F, A222V, V227A, D228H, I233V, R237K, Δ242-244, H245Y, Δ246-252, D253G, D253N, W258L, A262S, A263P, V267L, P272L, R273S, E281Q, T284I, V289L, A292S, T299I, K300M, T307I, V308L, E309Q, F318S, V320F, T323I, V227I, P330S, L335F, P337S, G339S, R346S, R346K, A348S, K356R, R357K, V362F, V367F, V367L, S371T, T376I, V382L, P384L, T385N, N394S, N394H, V395I, R403K, E406Q, R408I, Q414K, K417T, K417N, D427N, D427Y, T430I, N439K, N440S, N440K, L452M, L452R, L452Q, L461F, K462T, I468V, T470I, E471Q, T478K, E484K, F490S, S494P, N501T, N501Y, Y505H, V510L, L513F, A520S, A522S, A522V, T531S, V534I, V534F, N540S, F543L, T547I, T549I, E554D, K558N, P561S, F565L, A570D, T572I, A575S, E583Q, E583D, L585F, G594S, V595I, I598V, T604A, T604I, Q607K, Q613H, D614G, N616S, V622F, A623S, D627G, P631S, T632S, T638I, S640F, N641S, A647S, A653V, H655Y, Y660F, I670V, A672V, Q677H, P681H, P681R, A688V, S691P, A694V, M697I, A701V, S704L, A706V, T716I, T716V, T719I, M731I, T732I, T732A, M740V, T747I, T747N, N751D, S758G, T761I, T761R, G769V, A771S, V772I, Q779K, E780D, V785I, T791I, K795R, D796H, S803A, P809S, P812S, P812L, L822F, V826L, T827I, A831V, K835R, D843N, A845S, A845V, K854N, T859N, T859I, M869I, I870V, A871V, A879S, A879V, T883I, F888L, A890V, A892V, A899S, M902I, A903S, I909V, V911I, V915I, L922F, A924S, S929I, D936Y, S939F, S940F, G946R, D950N, D950H, Q957R,

T961M, V963A, S967N, V976F, S982A, K986N, K986R, R995G, I997V, T998I, Q1005H, T1006I, T1009I, R1014K, A1016V, A1020S, A1025G, T1027I, V1033A, K1038Q, V1040F, K1045N, L1049I, M1050I, A1056V, H1058Y, L1063F, T1066N, Q1071H, K1073N, A1078S, D1084Y, D1084E, A1086S, V1094F, H1101D, H1101Y, V1104L, E1111K, D1118H, D1118Y, G1124V, D1127G, I1130V, V1133F, D1139H, L1141W, D1146Y, E1150D, D1153Y, P1162L, P1162S, D1163Y, N1173S, A1174V, V1176F, E1182D, R1185H, K1191N, N1192S, E1195Q, E1202Q, K1205N, Q1208H, I1216T, G1219V, G1219C, V1228L, M1229I, V1230L, M1237I, T1238I, C1243F, C1247F, S1252F, S1252P, D1260N, P1263L, V1264L, H1271Y, T1273I, and T1273A, relative to the amino acid sequence of SEQ ID NO: 1 or 35 (e.g., the antibodies may bind an epitope of a S protein that includes one or more of these mutations). The antibodies may neutralize one coronavirus, such as a coronavirus of lineage B.1.1.7. The antibodies may also neutralize more than one coronavirus, such as coronaviruses of lineages B.1.1.7 and B.1.429 (or one or more of the lineages listed above). The antibodies can therefore be used to inhibit or treat a coronavirus infection (e.g., infection by SARS-CoV-2 or a variant thereof).

The antibodies may be specifically generated by using one or more of the SET1, SET2, SET3, EG1, EG2, EG3, and EG4 immunogens of SEQ ID NOs: 40-43 or SEQ ID NOs: 65-68, or variants thereof with up to 85% sequence identity thereto. The specific binding of an antibody or antigen-binding fragment thereof to S protein of SARS-CoV-2 or a variant thereof can be determined by any of a variety of established methods. The affinity can be represented quantitatively by various measurements, including the concentration of antibody needed to achieve half-maximal inhibition of viral spread (e.g., viral titer) *in vitro* (IC₅₀) and the equilibrium constant (K_D) of the antibody-SARS-CoV-2 polyprotein complex dissociation. The equilibrium constant, K_D, that describes the interaction of SARS-CoV-2 polyprotein with an antibody is the chemical equilibrium constant for the dissociation reaction of a SARS-CoV-2 polyprotein-antibody complex into solvent-separated SARS-CoV-2 polyprotein and antibody molecules that do not interact with one another.

In some embodiments, anti-SARS-CoV-2 antibodies of the disclosure may specifically binding to a MEM and/or NUL protein of SARS-CoV-2 or a variant thereof or polypeptide of a lineage variant thereof (such as the MEM1, MEM2, MEM3, NUL1, NUL2, and NUL3 immunogens described herein), and are capable of inhibiting a SARS-CoV-2-mediated activity (e.g., viral spread, infection, and or cell fusion) in a subject (e.g., a human). The result of such binding may be, for example, a reduction in viral titer (e.g., viral load), by about 1% (e.g., 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%) or more, after administration of an antibody to a subject infected with SARS-CoV-2 or a variant thereof. The MEM antibodies may be specifically generated by using one or more of the MEM1, MEM2, and MEM3 immunogens of SEQ ID NOs: 75-77, or variants thereof with up to 85% sequence identity thereto. The NUL antibodies may be specifically generated by using one or more of the NUL1, NUL2, and NUL3 immunogens of SEQ ID NOs: 78-80, or variants thereof with up to 85% sequence identity thereto. The anti-SARS-CoV-2 MEM antibodies may selectively bind to an epitope comprising all, or a portion of, any one of SEQ ID NOs: 75-77. The anti-SARS-CoV-2 NUL antibodies may selectively bind to an epitope comprising all, or a portion of, any one of SEQ ID NOs: 78-80. The MEM and NUL antibodies may neutralize one coronavirus, such as a coronavirus of lineage B.1.1.7. The antibodies can therefore be used to inhibit or treat a coronavirus infection (e.g., infection by SARS-CoV-2 or a variant thereof).

Antibodies are those that specifically bind to a SARS-CoV-2 or a variant thereof polyprotein (e.g., the S, MEM, or NUL region of SARS-CoV-2 or a variant thereof) with a K_D value of less than 1 μ M (e.g., 900 nM, 800 nM, 700 nM, 600 nM, 500 nM, 400 nM, 300 nM, 200 nM, 100 nM, 95 nM, 90 nM, 85 nM, 80 nM, 75 nM, 70 nM, 65 nM, 60 nM, 55 nM, 50 nM, 45 nM, 40 nM, 35 nM, 30 nM, 25 nM, 20 nM, 15 nM, 10 nM, 5 nM, 4 nM, 3 nM, 2 nM, or 1 nM). In certain cases, antibodies are those that specifically bind to a SARS-CoV-2 or a variant thereof polyprotein with a K_D value of less than 1 nM (e.g., 990 pM, 980 pM, 970 pM, 960 pM, 950 pM, 940 pM, 930 pM, 920 pM, 910 pM, 900 pM, 890 pM, 880 pM, 870 pM, 860 pM, 850 pM, 840 pM, 830 pM, 820 pM, 810 pM, 800 pM, 790 pM, 780 pM, 770 pM, 760 pM, 750 pM, 740 pM, 730 pM, 720 pM, 710 pM, 700 pM, 690 pM, 680 pM, 670 pM, 660 pM, 650 pM, 640 pM, 630 pM, 620 pM, 610 pM, 600 pM, 590 pM, 580 pM, 570 pM, 560 pM, 550 pM, 540 pM, 530 pM, 520 pM, 510 pM, 500 pM, 490 pM, 480 pM, 470 pM, 460 pM, 450 pM, 440 pM, 430 pM, 420 pM, 410 pM, 400 pM, 390 pM, 380 pM, 370 pM, 360 pM, 350 pM, 340 pM, 330 pM, 320 pM, 310 pM, 300 pM, 290 pM, 280 pM, 270 pM, 260 pM, 250 pM, 240 pM, 230 pM, 220 pM, 210 pM, 200 pM, 190 pM, 180 pM, 170 pM, 160 pM, 150 pM, 140 pM, 130 pM, 120 pM, 110 pM, 100 pM, 90 pM, 80 pM, 70 pM, 60 pM, 50 pM, 40 pM, 30 pM, 20 pM, 10 pM, 5 pM, or 1 pM).

Antibodies of the disclosure can also be characterized by a variety of *in vitro* binding assays. Examples of experiments that can be used to determine the K_D or IC_{50} of a SARS-CoV-2 or a variant thereof antibody include, e.g., surface plasmon resonance, isothermal titration calorimetry, fluorescence anisotropy, and ELISA-based assays, among others. ELISA represents a particularly useful method for analyzing antibody activity, as such assays typically require minimal concentrations of antibodies. A common signal that is analyzed in a typical ELISA assay is luminescence, which is typically the result of the activity of a peroxidase conjugated to a secondary antibody that specifically binds a primary antibody (e.g., a SARS-CoV-2 or a variant thereof antibody). Antibodies are capable of binding SARS-CoV-2 or a variant thereof and epitopes derived thereof, such as epitopes containing one or more residues of any one of SEQ ID NOs: 2-4, 40-43, 65-68 and/or 75-80, as well as isolated peptides derived from SARS-CoV-2 or a variant thereof that structurally pre-organize various residues in a manner that may simulate the conformation of these amino acids in the native protein. For instance, antibodies may bind peptides containing the amino acid sequence of any one of SEQ ID NOs: 2-4, 40-43, 65-68, and/or 75-80, or a peptide containing between about 10 and about 30 continuous or discontinuous amino acids of any one of SEQ ID NOs: 2-4, 40-43, 65-68, and/or 75-80. In a direct ELISA experiment, this binding can be quantified, e.g., by analyzing the luminescence that occurs upon incubation of an HRP substrate (e.g., 2,2'-azino-di-3-ethylbenzthiazoline sulfonate) with an antigen-antibody complex bound to an HRP-conjugated secondary antibody.

Antibodies include those that are generated by immunizing a host (e.g., a mammalian host, such as a human) with the polypeptides of SEQ ID NOs: 2-4, 40-43, 65-68, 75-80. The antibodies can be prepared recombinantly and, if necessary, humanized, for subsequent administration to a human recipient if the host in which the antibodies against a modified S protein of SARS-CoV-2 or a variant thereof are generated is not a human.

Compositions

Compositions of the disclosure may include DNA or RNA vectors containing a heterologous nucleic acid molecule encoding an antigenic or therapeutic gene product, or fragment thereof, corresponding to all or a fragment of one or more of the SET1, SET2, and SET3 immunogens of SEQ ID NOs: 2-4, or a variant thereof with up to 85% sequence identity thereto, as described herein. The DNA or RNA vector may include all or a fragment of the nucleic acid molecule of any one of SEQ ID NOs: 7-12, or a variant thereof having at least 85% (e.g., 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%) sequence identity to any one of SEQ ID NOs: 7-12, or a complement thereof.

Compositions of the disclosure may further include DNA or RNA vectors containing a heterologous nucleic acid molecule encoding an antigenic or therapeutic gene product, or fragment thereof, corresponding to all or a fragment of one or more of the EG1, EG2, EG3, and EG4 immunogens of SEQ ID NOs: 40-43 (or SEQ ID NOs: 65-68), respectively, or a variant thereof with up to 85% sequence identity thereto, as described herein. The DNA or RNA vector may include all or a fragment of the nucleic acid molecule of any one of SEQ ID NOs: 44-51 and 61-64, or a variant thereof having at least 85% (e.g., 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%) sequence identity to any one of SEQ ID NOs: 44-51 and 61-64, or a complement thereof. Compositions of the disclosure may further include DNA or RNA vectors containing a heterologous nucleic acid molecule encoding an antigenic or therapeutic gene product, or fragment thereof, corresponding to all or a fragment of one or more of the MEM1, MEM2, and MEM3 immunogens of SEQ ID NOs: 75-77, respectively, or a variant thereof with up to 85% sequence identity thereto, as described herein. The DNA or RNA vector may include all or a fragment of the nucleic acid molecule of any one of SEQ ID NOs: 69-71, or a variant thereof having at least 85% (e.g., 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%) sequence identity to any one of SEQ ID NOs: 69-71, or a complement thereof.

Compositions of the disclosure may further include DNA or RNA vectors containing a heterologous nucleic acid molecule encoding an antigenic or therapeutic gene product, or fragment thereof, corresponding to all or a fragment of one or more of the NUL1, NUL2, and NUL3 immunogens of SEQ ID NOs: 78-80, respectively, or a variant thereof with up to 85% sequence identity thereto, as described herein. The DNA or RNA vector may include all or a fragment of the nucleic acid molecule of any one of SEQ ID NOs: 72-74, or a variant thereof having at least 85% (e.g., 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%) sequence identity to any one of SEQ ID NOs: 72-74, or a complement thereof.

The DNA or RNA vector of the disclosure may also contain a nucleic acid molecule with all or a fragment of the nucleic acid sequence of SEQ ID NO: 5, 6, 29, or 39, or a variant thereof with up to 85% sequence identity thereto.

Alternatively, a composition of the disclosure can include an immunogenic polypeptide with the amino acid sequence of all or a fragment of any one or more of SEQ ID NOs: 2-4, or a variant thereof with up to 85% sequence identity thereto. Furthermore, a composition of the disclosure can include an immunogenic polypeptide with the amino acid sequence of all or a fragment of any one or more of SEQ ID NOs: 40-43 and 65-68, or a variant thereof with up to 85% sequence identity thereto. The

immunogenic polypeptide composition of the disclosure may further include an immunogenic polypeptide with the amino acid sequence of all or a fragment of any one or more of SEQ ID NOs: 75-80, or a variant thereof with up to 85% sequence identity thereto. The composition may be an immunogenic composition, which is capable of eliciting the production of anti-coronavirus antisera (e.g., neutralizing antisera).

5 A composition of the disclosure may also contain an anti-coronavirus antibody (e.g., an anti-Spike antibody or a broadly neutralizing anti-Spike antibody) capable of binding SARS-CoV-2 or a variant thereof and epitopes derived thereof, such as epitopes containing one or more of residues of any one of SEQ ID NOs: 2-4. For example, a composition can include an antibody capable of binding epitopes within the NTD or RBD region of the polypeptide of any one or more of SEQ ID NOs: 2-4. The antibody
10 may be generated by immunization of a host (e.g., a mammal) with a polypeptide of any one of SEQ ID NOs: 2-4. For example, an antibody may be generated by immunization of a host with two or more polypeptides having the amino acid sequence of SEQ ID NOs: 2-4, or a variant thereof with up to 85% sequence identity thereto, or one or more nucleic acid molecules encoding the polypeptides. For example, the antibodies may be generated by administering a vector containing a nucleic acid molecule
15 encoding combinations of different immunogens (e.g., the vector may contain a nucleic acid molecule with the nucleotide sequence of SEQ ID NOs: 7 and 8, SEQ ID NOs: 7 and 9, SEQ ID NOs: 8 and 9, or SEQ ID NOs: 7-9, or variants thereof with up to 85% sequence identity thereto). The nucleic acid molecule of the vector may encode two or more immunogens, such as immunogens with the amino acid sequences of SEQ ID NOs: 10 and 11, SEQ ID NOs: 10 and 12, SEQ ID NOs: 11 and 12, or SEQ ID
20 NOs: 10-12, or variants thereof with up to 85% sequence identity thereto. The vector may also include a nucleic acid molecule containing the nucleotide sequence of SEQ ID NO: 6, or a variant thereof with up to 85% sequence identity thereto, that encodes an immunogen with the amino acid sequence of SEQ ID NO: 1, or a variant thereof with up to 85% sequence identity thereto. Antibodies generated by administering the vector to a mammal (to elicit the production of the antibodies) can be used to purified or
25 otherwise prepared and used to manufacture a composition of the disclosure.

A composition of the disclosure may contain, or further contain, an anti-coronavirus antibody (e.g., an anti-Spike antibody or a broadly neutralizing anti-Spike antibody) capable of binding SARS-CoV-2 or a variant thereof and epitopes derived thereof, such as epitopes containing one or more of residues of any one of SEQ ID NOs: 40-43 and 65-68. For example, a composition can include an antibody capable
30 of binding epitopes within the NTD or RBD region of the polypeptide of any one or more of SEQ ID NOs: 40-43 and 65-68. The antibody may be generated by immunization of a host (e.g., a mammal) with a polypeptide of any one of SEQ ID NOs: 40-43 and 65-68. For example, an antibody may be generated by immunization of a host with two or more polypeptides having the amino acid sequence of SEQ ID NOs: 40-43, or a variant thereof with up to 85% sequence identity thereto, or one or more nucleic acid
35 molecules encoding the polypeptides. For example, an antibody may be generated by immunization of a host with two or more polypeptides having the amino acid sequence of SEQ ID NOs: 65-68, or a variant thereof with up to 85% sequence identity thereto, or one or more nucleic acid molecules encoding the polypeptides. For example, the antibodies may be generated by administering a vector containing a nucleic acid molecule encoding combinations of different immunogens (e.g., the vector may contain a
40 nucleic acid molecule with the nucleotide sequence of SEQ ID NOs: 44 and 45, SEQ ID NOs: 44 and 46, SEQ ID NOs: 44 and 47, SEQ ID NOs: 45 and 47, SEQ ID NOs: 46 and 47, or

each of SEQ ID NOs: 44-51, or variants thereof with up to 85% sequence identity thereto). For example, the antibodies may be generated by administering a vector containing a nucleic acid molecule encoding combinations of different immunogens (e.g., the vector may contain a nucleic acid molecule with the nucleotide sequence of SEQ ID NOs: 61 and 62, SEQ ID NOs: 61 and 63, SEQ ID NOs: 61 and 64, SEQ ID NOs: 61 and 63, SEQ ID NOs: 62 and 64, SEQ ID NOs: 63 and 64, or each of SEQ ID NOs: 61-64, or variants thereof with up to 85% sequence identity thereto). The nucleic acid molecule of the vector may encode two or more immunogens, such as immunogens with the amino acid sequences of SEQ ID NOs: 40 and 41, SEQ ID NOs: 40 and 42, SEQ ID NOs: 40 and 43, SEQ ID NOs: 41 and 42, SEQ ID NOs: 41 and 43, SEQ ID NOs: 42 and 43, or SEQ ID NOs: 40-43, or variants thereof with up to 85% sequence identity thereto. The nucleic acid molecule of the vector may encode two or more immunogens, such as immunogens with the amino acid sequences of SEQ ID NOs: 65 and 66, SEQ ID NOs: 65 and 67, SEQ ID NOs: 65 and 68, SEQ ID NOs: 66 and 67, SEQ ID NOs: 66 and 68, SEQ ID NOs: 67 and 68, or SEQ ID NOs: 65-68, or variants thereof with up to 85% sequence identity thereto. The vector may also include a nucleic acid molecule containing the nucleotide sequence of SEQ ID NO: 29 or 39, or a variant thereof with up to 85% sequence identity thereto, that encodes an immunogen with the amino acid sequence of SEQ ID NO: 35, or a variant thereof with up to 85% sequence identity thereto. Antibodies generated by administering the vector to a mammal (to elicit the production of the antibodies) can be used to purified or otherwise prepared and used to manufacture a composition of the disclosure.

A composition of the disclosure may contain, or further contain, an anti-MEM antibody and/or anti-NUL antibody capable of binding SARS-CoV-2 or a variant thereof and epitopes derived thereof, such as epitopes containing one or more of residues of any one of SEQ ID NOs: 75-80. The antibody may be generated by immunization of a host (e.g., a mammal) with a polypeptide of any one of SEQ ID NOs: 75-80. For example, an antibody may be generated by immunization of a host with two or more polypeptides having the amino acid sequence of SEQ ID NOs: 75-80, or a variant thereof with up to 85% sequence identity thereto, or one or more nucleic acid molecules encoding the polypeptides. For example, the antibodies may be generated by administering a vector containing a nucleic acid molecule encoding combinations of different immunogens. The vector may include a nucleic acid molecule containing one or more of the nucleotide sequences of SEQ ID NO: 69-74, or a variant thereof with up to 85% sequence identity thereto, that encodes one or more immunogens with the amino acid sequence of SEQ ID NO: 75-80, or a variant thereof with up to 85% sequence identity thereto. Antibodies generated by administering the vector to a mammal (to elicit the production of the antibodies) can be used to purified or otherwise prepared and used to manufacture a composition of the disclosure.

A composition of the disclosure may contain a viral vector (e.g., an adenovirus vector or a poxvirus vector) containing a nucleic acid molecule(s) of the disclosure. Recombinant adenoviruses offer several significant advantages for use as vectors for the expression of, for example, one or more of the immunogens (e.g., SARS-CoV-2 or a variant thereof polypeptides). The viruses can be prepared to high titer, can infect non-replicating cells, and can confer high-efficiency transduction of target cells *ex vivo* following contact with a target cell population. Furthermore, adenoviruses do not integrate their DNA into the host genome. Thus, their use as expression vectors has a reduced risk of inducing spontaneous proliferative disorders. In animal models, adenoviral vectors have generally been found to mediate high-level expression for approximately one week. The duration of transgene expression (expression of a

nucleic acid molecule) can be prolonged by using cell or tissue-specific promoters. Other improvements in the molecular engineering of the adenovirus vector itself have produced more sustained transgene expression and less inflammation. This is seen with so-called "second generation" vectors harboring specific mutations in additional early adenoviral genes and "gutless" vectors in which virtually all the viral genes are deleted utilizing a Cre-Lox strategy (Engelhardt et al., *Proc. Natl. Acad. Sci. USA* 91:6196 (1994) and Kochanek et al., *Proc. Natl. Acad. Sci. USA* 93:5731 (1996), each herein incorporated by reference).

Therapeutic formulations of the compositions are prepared for administration to a subject (e.g., a human) using standard methods known in the art by mixing the active ingredient having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences (20th edition), ed. A. Gennaro, 2000, Lippincott, Williams & Wilkins, Philadelphia, PA). Therapeutic formulations of the compositions are prepared using standard methods known in the art by mixing the active ingredient having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences (20th edition), ed. A. Gennaro, 2000, Lippincott, Williams & Wilkins, Philadelphia, PA). Acceptable carriers, include saline, or buffers such as phosphate, citrate and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone, amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN[™], PLURONICS[™], or PEG.

Optionally, but preferably, the formulation contains a pharmaceutically acceptable salt, preferably sodium chloride, and preferably at about physiological concentrations. Optionally, the formulations can contain a pharmaceutically acceptable preservative. The preservative concentration may range from about 0.1 to about 2.0%, typically v/v. Suitable preservatives include those known in the pharmaceutical arts, such as benzyl alcohol, phenol, m-cresol, methylparaben, and propylparaben. Optionally, the formulations can include a pharmaceutically acceptable surfactant at a concentration of about 0.005 to about 0.02%.

Optionally, the compositions may be formulated to include for co-administration, or sequential administration with, an adjuvant and/or an immunostimulatory agent, (e.g., a protein), such as receptor molecules, nucleic acids, immunogenic proteins, pharmaceuticals, chemotherapy agents, and accessory cytokines. For example, interleukin-3 (IL-3), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-7 (IL-7), interleukin-8 (IL-8), interleukin-10 (IL-10), interleukin-11 (IL-11), interleukin-12 (IL-12), interleukin-13 (IL-13), lipid A, phospholipase A2, endotoxins, staphylococcal enterotoxin B, Type I interferon, Type II interferon, transforming growth factor- β (TGF- β), lymphotoxin migration inhibition factor, granulocyte-macrophage colony-stimulating factor (CSF), monocyte-macrophage CSF, granulocyte CSF, vascular epithelial growth factor (VEGF), angiogenin, transforming growth factor (TGF- α), heat shock proteins (HSPs), carbohydrate moieties of blood groups, Rh factors, fibroblast growth factors, nucleotides, DNA, RNA, mRNA, MART, MAGE, BAGE, mutant p53, tyrosinase, AZT, angiostatin, endostatin, or a combination thereof, may be included in formulations of, or for co-administration with, the compositions.

The pharmaceutical compositions can be administered in a therapeutically effective amount that provides an immunogenic and/or protective effect against an infective agent (e.g., a SARS-CoV-2 or a variant thereof). In some embodiments, a composition containing a nucleic acid molecule, polypeptide, vector, and/or antibodies may be formulated for administration at a dose of at least 1-1,000 µg (e.g., at least 15, 16, 17, 18, 19, 20, 30, 40, 50, 60, 70, 80, 90, 100, 125, 150, 175, 200, 225, 250, 275, or 300 µg or more). In some embodiments, the subject is administered two or more (e.g., two, three, four, five, six, seven, eight, nine, or ten) doses of the composition. The dose may be in a volume of 0.2 mL to 1.0 mL or up to 1 L (e.g., if prepared as an infusion). In some embodiments, a composition containing a nucleic acid molecule, vector, polypeptide, and/or antibodies is administered at a dose of 50 µg.

In some embodiments, the subject is administered a single type of a pharmaceutical composition described herein (e.g., a pharmaceutical composition containing either a SET1 immunogen (e.g., SEQ ID NO: 2), or a nucleic acid molecule (e.g., a vector) encoding a SET1 immunogen (e.g., SEQ ID NO: 10), a pharmaceutical composition containing a SET2 immunogen (e.g., SEQ ID NO: 3), or a nucleic acid molecule (e.g., a vector) encoding a SET2 immunogen (e.g., SEQ ID NO: 11), or a pharmaceutical composition containing a SET3 immunogen (e.g., SEQ ID NO: 4), or a nucleic acid molecule (e.g., a vector) encoding a SET3 immunogen (e.g., SEQ ID NO: 12), a pharmaceutical composition containing either an EG1 immunogen (e.g., SEQ ID NO: 40 or 65), or a nucleic acid molecule (e.g., a vector) encoding an EG1 immunogen (e.g., SEQ ID NO: 44, 48, or 61), a pharmaceutical composition containing an EG2 immunogen (e.g., SEQ ID NO: 41 or 66), or a nucleic acid molecule (e.g., a vector) encoding an EG2 immunogen (e.g., SEQ ID NO: 45, 49, or 62), a pharmaceutical composition containing an EG3 immunogen (e.g., SEQ ID NO: 42 or 67), or a nucleic acid molecule (e.g., a vector) encoding an EG3 immunogen (e.g., SEQ ID NO: 46, 50, or 63), or a pharmaceutical composition containing an EG4 immunogen (e.g., SEQ ID NO: 43 or 68), or a nucleic acid molecule (e.g., a vector) encoding an EG4 immunogen (e.g., SEQ ID NO: 47, 51, or 64)).

In some embodiments, the subject is administered two different types of the pharmaceutical compositions described herein (e.g., a pharmaceutical composition containing an EG1 immunogen (e.g., SEQ ID NO: 40 or 65), or a nucleic acid molecule encoding an EG1 immunogen (e.g., SEQ ID NO: 44, 48, or 61)), and a pharmaceutical composition containing an EG2 immunogen (e.g., SEQ ID NO: 41 or 66), or a nucleic acid molecule (e.g., a vector) encoding an EG2 immunogen (e.g., SEQ ID NO: 45, 49, or 62)).

In some embodiments, the subject is administered three different types of the pharmaceutical compositions described herein (e.g., a pharmaceutical composition containing an EG1 immunogen (e.g., SEQ ID NO: 40 or 65), or a nucleic acid molecule (e.g., a vector) encoding an EG1 immunogen (e.g., SEQ ID NO: 44, 48, or 61), a pharmaceutical composition containing an EG2 immunogen (e.g., SEQ ID NO: 41 or 66), or a nucleic acid molecule (e.g., a vector) encoding an EG2 immunogen (e.g., SEQ ID NO: 45, 49, or 62), and a pharmaceutical composition containing an EG3 immunogen (e.g., SEQ ID NO: 42 or 67), or a nucleic acid molecule (e.g., a vector) encoding an EG3 immunogen (e.g., SEQ ID NO: 46, 50, or 63)).

In some embodiments, the subject is administered four different types of the pharmaceutical compositions described herein (e.g., a pharmaceutical composition containing an EG1 immunogen (e.g., SEQ ID NO: 40 or 65), or a nucleic acid molecule (e.g., a vector) encoding an EG1 immunogen (e.g.,

SEQ ID NO: 44, 48, or 61), a pharmaceutical composition containing EG2 immunogen (e.g., SEQ ID NO: 41 or 66), or a nucleic acid molecule (e.g., a vector) encoding an EG2 immunogen (e.g., SEQ ID NO: 45, 49, or 62), a pharmaceutical composition containing an EG3 immunogen (e.g., SEQ ID NO: 42 or 67), or a nucleic acid molecule (e.g., a vector) encoding an EG3 immunogen (e.g., SEQ ID NO: 46, 50, or 63),
 5 and a pharmaceutical composition containing an EG4 immunogen (e.g., SEQ ID NO: 43 or 68), or a nucleic acid molecule (e.g., a vector) encoding an EG4 immunogen (e.g., SEQ ID NO: 47, 51, or 64).

In any of these examples, the pharmaceutical compositions described may further include one or more of the following: a pharmaceutical composition containing a MEM1 immunogen (e.g., SEQ ID NO: 75), or a nucleic acid molecule (e.g., a vector) encoding an MEM immunogen (e.g., SEQ ID NO: 69), a
 10 pharmaceutical composition containing a MEM2 immunogen (e.g., SEQ ID NO: 76), or a nucleic acid molecule (e.g., a vector) encoding a MEM2 immunogen (e.g., SEQ ID NO: 70), a pharmaceutical composition containing a MEM3 immunogen (e.g., SEQ ID NO: 77), a nucleic acid molecule (e.g., a vector) encoding an MEM3 immunogen (e.g., SEQ ID NO: 71), a pharmaceutical composition containing a NUL1 immunogen (e.g., SEQ ID NO: 78), or a nucleic acid molecule (e.g., a vector) encoding an NUL
 15 immunogen (e.g., SEQ ID NO: 72), a pharmaceutical composition containing a NUL2 immunogen (e.g., SEQ ID NO: 79), or a nucleic acid molecule (e.g., a vector) encoding a NUL2 immunogen (e.g., SEQ ID NO: 73), a pharmaceutical composition containing a NUL3 immunogen (e.g., SEQ ID NO: 80), and a nucleic acid molecule (e.g., a vector) encoding an NUL3 immunogen (e.g., SEQ ID NO: 74).

In any of these examples, the subject may also be administered an amount of a pharmaceutical
 20 composition containing a nucleic acid molecule containing the nucleotide sequence of SEQ ID NO: 5, nucleotides 19-3837 of SEQ ID NO: 6, the nucleotide sequence of SEQ ID NO: 6, the nucleotide of SEQ ID NO: 29, or the nucleotide sequence of SEQ ID NO: 39, a polypeptide containing the amino acid sequence of SEQ ID NO: 1, a polypeptide having at least 85% sequence identity to amino acids 18-1208 of SEQ ID NO: 1, or a polypeptide containing the amino acid sequence of SEQ ID NO: 35, or at least 85%
 25 sequence identity thereof.

The compositions utilized in the methods described herein can be formulated, for example, for administration intramuscularly, intravenously, intradermally, percutaneously, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intranasally, intravitreally, intravaginally, intrarectally, topically, intratumorally, peritoneally,
 30 subcutaneously, subconjunctivally, intravesicularly, mucosally, intrapericardially, intraumbilically, intraocularly, orally, topically, locally, by inhalation, by injection, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, by catheter, by lavage, by gavage, in cremes, or in lipid compositions.

Pharmaceutical compositions according to the disclosure described herein may be formulated to
 35 release the composition immediately upon administration (e.g., targeted delivery) or at any predetermined time period after administration using controlled or extended release formulations. Administration of the pharmaceutical composition in controlled or extended release formulations is useful where the composition, either alone or in combination, has (i) a narrow therapeutic index (e.g., the difference between the plasma concentration leading to harmful side effects or toxic reactions and the plasma
 40 concentration leading to a therapeutic effect is small; generally, the therapeutic index, TI, is defined as the ratio of median lethal dose (LD₅₀) to median effective dose (ED₅₀)); (ii) a narrow absorption window at the

site of release (e.g., the gastro-intestinal tract); or (iii) a short biological half-life, so that frequent dosing during a day is required in order to sustain a therapeutic level.

Many strategies can be pursued to obtain controlled or extended release in which the rate of release outweighs the rate of metabolism of the pharmaceutical composition. For example, controlled release can be obtained by the appropriate selection of formulation parameters and ingredients, including, e.g., appropriate controlled release compositions and coatings. Suitable formulations are known to those of skill in the art. Examples include single or multiple unit tablet or capsule compositions, oil solutions, suspensions, emulsions, microcapsules, microspheres, nanoparticles, patches, and liposomes.

The compositions may be sterilized by conventional sterilization techniques or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation may be administered in powder form or combined with a sterile aqueous carrier prior to administration. The pH of the preparations typically will be between 3 and 11, more preferably between 5 and 9 or between 6 and 8, and most preferably between 7 and 8, such as 7 to 7.5. The resulting compositions in solid form may be packaged in multiple single dose units, each containing a fixed amount of an immunogenic composition (e.g., a vaccine as described herein or an anti-SARS-CoV-2 or a variant thereof antibody described herein) and, if desired, one or more immunomodulatory agents, such as in a sealed package of tablets or capsules, or in a suitable dry powder inhaler (DPI) capable of administering one or more doses.

Methods of Treatment Using a Composition of the Disclosure

One or more of the pharmaceutical compositions described herein (e.g., an immunogenic composition and/or an anti-SARS-CoV-2 or a variant thereof antibody-containing composition) can be used to treat a subject (e.g., a human) at risk of exposure to a coronavirus (e.g., SARS-CoV-2 or a variant thereof), a subject susceptible to a coronavirus (e.g., SARS-CoV-2 or a variant thereof), or to treat a subject infected with a coronavirus (e.g., SARS-CoV-2 or a variant thereof). In particular, the compositions can be used to treat (pre- or post-exposure) infection by a SARS-CoV-2 or a variant thereof. In some embodiments, the treatment can induce a protective level of anti-coronavirus antibodies (e.g., antibodies against a modified S, MEM, and/or NUL protein of SARS-CoV-2 or a variant thereof (e.g., one or more of the SET1, SET2, SET3, EG1, EG2, EG3, EG4, MEM1, MEM2, MEM3, NUL1, NUL2, and NUL3 immunogens), e.g., anti-Spike antibodies, e.g., anti-Spike neutralizing antibodies, e.g., broadly neutralizing anti-Spike antibodies, e.g., anti-membrane antibodies, e.g., anti-membrane neutralizing antibodies, e.g., broadly neutralizing anti-membrane antibodies, e.g., anti-nucleocapsid antibodies, e.g., anti-nucleocapsid neutralizing antibodies, e.g., broadly neutralizing anti-nucleocapsid antibodies). In some embodiments, the protective level is a titer of at least about 70 as measured using the pseudovirus neutralization assay described herein, a titer of at least about 25 as measured using the live virus neutralization assay described herein, or is above a level of at least about 80% of a median or mean level of a cohort of convalescent humans as determined by a pseudovirus neutralization assay or live virus neutralization assay as described herein. In some embodiments, treatment with a composition may reduce a SARS-CoV-2 or a variant thereof-mediated activity in a subject, such as viral titer, viral spread, infection, and or cell fusion. In some embodiments, SARS-CoV-2 or a variant thereof-mediated activity is

viral load in the respiratory tract (e.g., the upper respiratory tract and/or the lower respiratory tract). In some embodiments, SARS-CoV-2 or a variant thereof-mediated activity is viral load in the lung, nares, and/or trachea. In some embodiments, the SARS-CoV-2 or a variant thereof viral load is decreased by about 1% or more (e.g., 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, 99.9%, 99.99%, or more). In some embodiments, SARS-CoV-2 or a variant thereof titer in a treated subject infected with SARS-CoV-2 or a variant thereof is decreased by at least about 1% or more (e.g., 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%, 95%, 99%, 99.9%, 99.99%, or more) after administration of a composition (e.g., vaccine) to the subject.

The compositions (e.g., any of the compositions described herein) can be used to induce an immune response (e.g., a humoral and/or cellular immune response) in a subject (e.g., a human subject). The immune response induced may be different (e.g., different in the specificity, robustness, or durability) depending on the composition or combination of compositions administered. For example, a composition can induce an antibody response with different antibody types (e.g., different proportions of IgM, IgA, IgG1, IgG2, IgG3, or FcγR2A.1) or different functional characteristics (e.g., ability to induce antibody-dependent neutrophil phagocytosis (ADNP), antibody-dependent complement deposition (ADCD), antibody-dependent monocyte cellular phagocytosis (ADCP), or antibody-dependent NK cell activation (IFN-γ secretion, CD107a degranulation, and MIP-1β expression)). Compositions described herein (e.g., SS-Spike and SS-SdCT) may induce an ADCD response that can be monitored (e.g., to assess therapeutic efficacy). Compositions described herein (e.g., SS-RBD-foldon and SS-S.Ecto-dF-PP-foldon) may induce an antibody-dependent NK cell activation response that can be monitored (e.g., to assess therapeutic efficacy). Compositions may also induce cellular responses with different characteristics (e.g., Th1, Th2, or Th17 responses). Compositions described herein (e.g., SS-Spike, SS-SdCT, and SS-S.Ecto-dF-PP-foldon) may induce an S-specific CD4⁺ or CD8⁺ T cell response that can be monitored (e.g., to assess therapeutic efficacy).

The vectors (e.g., mammalian, bacterial, or viral (e.g., Ad26) derived expression vectors) can be used to deliver a nucleic acid expressing an immunogen (e.g., one of more of SEQ ID NOs: 2-4 or variants thereof, having at least 85-99% sequence identity thereto, for example at least greater than 90% sequence identity thereto) to a subject in a method of inhibiting and/or treating a SARS-CoV-2 or a variant thereof infection or infection by a lineage variant thereof. For example, a vector can be used to deliver a nucleic acid (e.g., a nucleic acid containing the nucleotide sequence of one or more of SEQ ID NOs: 7-12, or a variant thereof with up to 85% sequence identity thereto) encoding an immunogen with the amino acid sequence of one or more SEQ ID NOs: 2-4. For example, a vector can be used to deliver a nucleic acid (e.g., a nucleic acid containing the nucleotide sequence of one or more of SEQ ID NOs: 7-12, or a variant thereof with up to 85% sequence identity thereto) that encodes an immunogen with the amino acid sequence of all or a fragment of any one of SEQ ID NOs: 2-4, or a variant thereof with up to 85% sequence identity thereto. The vectors (e.g., mammalian, bacterial, or viral derived expression vectors) can be genetically modified to contain one or more nucleic acid sequences set forth in SEQ ID NOs: 7-12 or variants thereof having at least 85-99% sequence identity thereto, for example at least greater than 90% sequence identity thereto, and complements thereof.

The vectors (e.g., mammalian, bacterial, or viral (e.g., Ad26) derived expression vectors) can be used to deliver a nucleic acid expressing an immunogen (e.g., one of more of SEQ ID NOs: 40-43 and 65-68 (e.g., any combination of SEQ ID NOs: 40-42 or any combination of SEQ ID NOs: 65-68)) or variants thereof, having at least 85-99% sequence identity thereto, for example at least greater than 90% sequence identity thereto) to a subject in a method of inhibiting and/or treating a SARS-CoV-2 or a variant thereof infection or infection by a lineage variant thereof. For example, a vector can be used to deliver a nucleic acid (e.g., a nucleic acid containing the nucleotide sequence of one or more of SEQ ID NOs: 44-51 and 61-64, or a variant thereof with up to 85% sequence identity thereto) encoding an immunogen with the amino acid sequence of one or more SEQ ID NOs: 40-43 and 65-68. For example, a vector can be used to deliver a nucleic acid (e.g., a nucleic acid containing the nucleotide sequence of one or more of SEQ ID NOs: 44-51 and 61-64, or a variant thereof with up to 85% sequence identity thereto) that encodes an immunogen with the amino acid sequence of all or a fragment of any one of SEQ ID NOs: 40-43 and 65-68, or a variant thereof with up to 85% sequence identity thereto. The vectors (e.g., mammalian, bacterial, or viral derived expression vectors) can be genetically modified to contain one or more nucleic acid sequences set forth in SEQ ID NOs: 44-51 and 65-68 or variants thereof having at least 85-99% sequence identity thereto, for example at least greater than 90% sequence identity thereto, and complements thereof.

The vectors (e.g., mammalian, bacterial, or viral (e.g., Ad26) derived expression vectors) can be used to deliver a nucleic acid expressing an immunogen (e.g., one of more of SEQ ID NOs: 40-43 and 65-68 (e.g., any combination of SEQ ID NOs: 75-80)) or variants thereof, having at least 85-99% sequence identity thereto, for example at least greater than 90% sequence identity thereto) to a subject in a method of inhibiting and/or treating a SARS-CoV-2 or a variant thereof infection or infection by a lineage variant thereof. For example, a vector can be used to deliver a nucleic acid (e.g., a nucleic acid containing the nucleotide sequence of one or more of SEQ ID NOs: 69-74, or a variant thereof with up to 85% sequence identity thereto) encoding an immunogen with the amino acid sequence of one or more SEQ ID NOs: 75-80. For example, a vector can be used to deliver a nucleic acid (e.g., a nucleic acid containing the nucleotide sequence of one or more of SEQ ID NOs: 75-80, or a variant thereof with up to 85% sequence identity thereto) that encodes an immunogen with the amino acid sequence of all or a fragment of any one of SEQ ID NOs: 75-80, or a variant thereof with up to 85% sequence identity thereto. The vectors (e.g., mammalian, bacterial, or viral derived expression vectors) can be genetically modified to contain one or more nucleic acid sequences set forth in SEQ ID NOs: 69-74 or variants thereof having at least 85-99% sequence identity thereto, for example at least greater than 90% sequence identity thereto, and complements thereof.

In particular, adenoviral vectors (e.g., vectors derived from Ad2, Ad5, Ad11, Ad12, Ad24, Ad26, Ad34, Ad35, Ad40, Ad48, Ad49, Ad50, Ad52 (RhAd52), Ad59 (RhAd59), and Pan9 (also known as AdC68)) disclosed in International Patent Application Publications WO 2006/040330 and WO 2007/104792, each incorporated by reference herein, are particularly useful as vectors in methods of delivering an immunogen to a subject. For example, the vector can be Ad26. Other examples of vectors are described, for example, in McVey et al., (U.S. Patent. No. 5,801,030); incorporated herein, in its entirety, by reference.

Useful gene therapy methods for the delivery of immunogens to a subject in need thereof include those described in PCT publication no. WO 2006/060641, U.S. Patent No. US 7,179,903, and PCT publication no. WO 2001/036620, which described the use of, for example, an adenovirus vector (e.g., vectors derived from Ad2, Ad5, Ad11, Ad12, Ad24, Ad26, Ad34, Ad35, Ad40, Ad48, Ad49, Ad50, Ad52 (RhAd52), Ad59 (RhAd59), and Pan9 (also known as AdC68)) for therapeutic protein delivery.

One or more of the compositions (e.g., pharmaceutical compositions (e.g., immunogenic compositions and antibodies against a modified S protein of SARS-CoV-2 or a variant thereof)) described herein can be used in the treatment of a subject with or at risk of exposure to a coronavirus (SARS-CoV-2 or a lineage variant thereof). The treatment can include administration of one or more of the compositions described herein, either alone or with one or more additional therapeutic agents (e.g., proinflammatory (e.g., interferons) or anti-inflammatory agents (e.g., corticosteroids, e.g., dexamethasone)) and/or one or more therapeutic interventions (e.g., surgery and prone positioning). The therapeutic agents and/or interventions can be administered sequentially (e.g., administration of one or more of any of the compositions described herein before disease or at an early stage of disease (e.g., within a week of symptom onset), then administration of an additional therapeutic agent (e.g., an anti-inflammatory agent (e.g., a corticosteroid, e.g., dexamethasone) at a later stage of disease (e.g., after a week of symptom onset))) or simultaneously (e.g., administration of one or more of any of the compositions described herein and/or one or more additional therapeutic agents). Additional therapeutic agents can include corticosteroids (e.g., glucocorticoids (e.g., dexamethasone, prednisone, and hydrocortisone)), interferons (e.g., interferon beta), deoxycholic acid, colony stimulating factors (e.g., G-CSF and GM-CSF), and non-steroidal anti-inflammatory drugs (e.g., aspirin, propionic acid derivatives such as ibuprofen, fenoprofen, ketoprofen, flurbiprofen, oxaprozin and naproxen, acetic acid derivatives such as sulindac, indomethacin, etodolac, diclofenac, enolic acid derivatives such as piroxicam, meloxicam, tenoxicam, droxicam, lornoxicam and isoxicam, fenamic acid derivatives such as mefenamic acid, meclofenamic acid, flufenamic acid, tolfenamic acid, and COX-2 inhibitors such as celecoxib, etoricoxib, lumiracoxib, parecoxib, rofecoxib, rofecoxib, and valdecoxib). Other agents that can be administered in combination with the compositions described herein include remdesivir, chloroquine, hydroxychloroquine, baricitinib, lopinavir/ritonavir, umifenovir, favipiravir, tocilizumab, and ribavirin.

Administration

The pharmaceutical compositions can be administered to a subject (e.g., a human) pre- or post-exposure to an infective agent (e.g., a coronavirus, such as SARS-CoV-2 or a variant thereof) to treat, prevent, ameliorate, inhibit the progression of, or reduce the severity of one or more symptoms of infection (e.g., a coronavirus infection, such as a SARS-CoV-2 or a variant thereof infection). For example, the compositions can be administered to a subject having a SARS-CoV-2 or a variant thereof infection. Examples of symptoms of diseases caused by a viral infection, such as SARS-CoV-2 or a variant thereof, that can be treated using the compositions include, for example, fever, pneumonia, respiratory failure, weight loss, joint pain, rash, conjunctivitis, muscle pain, headache, retro-orbital pain, edema, lymphadenopathy, malaise, asthenia, sore throat, cough, nausea, vomiting, diarrhea, and hematospermia. These symptoms, and their resolution during treatment, may be measured by, for example, a physician during a physical examination or by other tests and methods known in the art. A

pharmaceutical composition described herein can be administered to a subject (e.g., a human) pre- or post-exposure to an infective agent (e.g., a coronavirus, such as SARS-CoV-2 or a variant thereof) to reduce or prevent the risk of mortality caused by the infective agent.

The method of administration can vary depending on various factors (e.g., the components of the composition being administered and the severity of the condition being treated). Formulations suitable for oral or nasal administration may consist of liquid solutions, such as an effective amount of the composition dissolved in a diluent (e.g., water, saline, or PEG-400), capsules, sachets, tablets, or gels, each containing a predetermined amount of the chimeric Ad5 vector composition. The pharmaceutical composition may also be an aerosol formulation for inhalation, for example, to the bronchial passageways. Aerosol formulations may be mixed with pressurized, pharmaceutically acceptable propellants (e.g., dichlorodifluoromethane, propane, or nitrogen). In particular, administration by inhalation can be accomplished by using, for example, an aerosol containing sorbitan trioleate or oleic acid, for example, together with trichlorofluoromethane, dichlorofluoromethane, dichlorotetrafluoroethane, or any other biologically compatible propellant gas.

Immunogenicity of the composition may be significantly improved if it is co-administered with an immunostimulatory agent and/or adjuvant. Suitable adjuvants well-known to those skilled in the art include, for example, aluminum phosphate, aluminum hydroxide, QS21, Quil A (and derivatives and components thereof), calcium phosphate, calcium hydroxide, zinc hydroxide, glycolipid analogs, octadecyl esters of an amino acid, muramyl dipeptides, polyphosphazene, lipoproteins, ISCOM matrix, DC-Chol, DDA, cytokines, and other adjuvants and derivatives thereof.

The compositions may be administered to provide pre-exposure prophylaxis or after a subject has been diagnosed as having a viral infection (e.g., SARS-CoV-2 or a variant thereof infection) or a subject exposed to an infective agent, such as a virus (e.g., a coronavirus infection, such as a SARS-CoV-2 or a variant thereof). The composition may be administered, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 35, 40, 45, 50, 55, or 60 minutes, 2, 4, 6, 10, 15, or 24 hours, 2, 3, 5, or 7 days, 2, 4, 6 or 8 weeks, or even 3, 4, or 6 months pre-exposure to a SARS-CoV-2 or a variant thereof, or may be administered to the subject 15-30 minutes or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 20, 24, 48, or 72 hours, 2, 3, 5, or 7 days, 2, 4, 6 or 8 weeks, 3, 4, 6, or 9 months, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20 years or post-exposure to a coronavirus (e.g., SARS-CoV-2 or a variant thereof).

When treating viral infection (e.g., a SARS-CoV-2 or a variant thereof infection), the compositions may be administered to the subject either before the occurrence of symptoms or a definitive diagnosis or after diagnosis or symptoms become evident. For example, the composition may be administered, for example, immediately after diagnosis or the clinical recognition of symptoms or 2, 4, 6, 10, 15, or 24 hours, 2, 3, 5, or 7 days after diagnosis or detection of symptoms.

One or more doses (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 doses) of an immunogenic composition or anti-SARS-CoV-2 or a variant thereof antibody-containing composition may be administered to a subject in need thereof. In some embodiments, a subject is administered at least one dose. In some embodiments, a subject is administered at least two doses. In some embodiments, doses are administered on the same day. In some embodiments, doses are administered on different days. In some embodiments, an immunogenic composition is administered to a subject in need thereof as a prime, a boost, or as a prime-boost. In some embodiments, the boost is administered 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, or 28 days, 5, 6, 7, 8, 9, 10, 11, or 12 weeks, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, or 23 months, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20 years after the prime of a prime-boost regimen. In other embodiments, multiple boost doses are administered, in which each boost dose is administered at least 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, or 28 days, 5, 6, 7, 8, 9, 10, 11, or 12 weeks, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, or 23 months, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20 years apart.

One or more doses of any of the compositions described herein (e.g., any of the immunogenic compositions described herein) may be administered with one or more additional therapeutic agents either sequentially or simultaneously.

Dosages

The dose of the compositions or the number of treatments using the compositions may be increased or decreased based on the severity of, occurrence of, or progression of, the disease in the subject (e.g., based on the severity of one or more symptoms of, e.g., viral infection).

The pharmaceutical compositions can be administered in a therapeutically effective amount that provides an immunogenic and/or protective effect against an infective agent (e.g., a SARS-CoV-2 or a variant thereof). In some embodiments, a composition containing a nucleic acid molecule, polypeptide, vector, and/or antibodies may be administered in a dose of at least 1 µg to 100 mg (e.g., at least 10 µg, 20 µg, 30 µg, 40 µg, 50 µg, 60 µg, 70 µg, 80 µg, 90 µg, 100 µg, 125 µg, 150 µg, 175 µg, 200 µg, 225 µg, 250 µg, 275 µg, 300 µg, 325 µg, 350 µg, 375 µg, 400 µg, 425 µg, 450 µg, 475 µg, 500 µg, 525 µg, 550 µg, 575 µg, 600 µg, 625 µg, 650 µg, 675 µg, 700 µg, 725 µg, 750 µg, 775 µg, 800 µg, 825 µg, 850 µg, 875 µg, 900 µg, 925 µg, 950 µg, 975 µg, 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg, 20 mg, 25 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg or more). In some embodiments, a composition containing a nucleic acid molecule, vector, and/or antibody is administered at a dose of about 50 µg (e.g., a dose between about 25 µg and about 75 µg). In some embodiments, a composition containing a nucleic acid molecule, vector, and/or antibody is administered at a dose of about 5 mg (e.g., a dose of about 1 mg to about 10 mg).

In some instances, administration of an effective amount of a composition of the disclosure (e.g., an immunogen, such as a protein having all or a fragment of the amino acid sequence of one or more of SEQ ID NOs: 2-4, 40-44, 65-68, and 75-80 or a nucleic acid molecule (e.g., a vector, such as a viral vector) encoding the immunogen, which can be administered alone or in combination with a protein having the amino acid sequence of SEQ ID NO: 1 or 35) induces a protective level (e.g., above a titer of at least about 70 as measured using the pseudovirus neutralization assay described herein, above a titer of at least about 25 as measured using the live virus neutralization assay described herein, or is above a level of at least about 80% of a median or mean level of a cohort of convalescent humans as determined by a pseudovirus neutralization assay or live virus neutralization assay as described herein) of anti-coronavirus antibodies (e.g., antibodies against a modified S, MEM, and/or NUL protein of SARS-CoV-2 or a variant thereof (e.g., one or more of the SET1, SET2, SET3, EG1, EG2, EG3, EG4, MEM1, MEM2, MEM3, NUL1, NUL2, and NUL3 immunogens), e.g., anti-Spike antibodies, e.g., anti-Spike neutralizing antibodies, e.g., broadly neutralizing anti-Spike antibodies, e.g., anti-membrane antibodies, e.g., anti-

membrane neutralizing antibodies, e.g., broadly neutralizing anti-membrane antibodies, e.g., anti-nucleocapsid antibodies, e.g., anti-nucleocapsid neutralizing antibodies, e.g., broadly neutralizing anti-nucleocapsid antibodies). In some instances, the protective level is a titer of at least about 70 (e.g., at least about 80, at least about 100, or at least about 120) as measured using the pseudovirus

neutralization assay described herein. In some instances, the protective level is a titer of at least about 100, as measured using the pseudovirus neutralization assay described herein. In some instances, administration of an effective amount of a composition results in a protective level of anti-coronavirus antibodies (e.g., antibodies against a modified S protein of SARS-CoV-2 or a variant thereof, e.g., anti-Spike antibodies, e.g., anti-Spike neutralizing antibodies, e.g., broadly neutralizing anti-Spike antibodies) that are maintained for at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 years or more.

In some instances, administration of an effective amount of a composition (e.g., an immunogen, such as a protein having all or a fragment of the amino acid sequence of one or more of SEQ ID NOs: 2-4, 40-44, 65-68, and 75-80 or a nucleic acid molecule (e.g., a vector, such as a viral vector) encoding the immunogen, which can be administered alone or in combination with a protein having the amino acid sequence of SEQ ID NO: 1 or 35) reduces SARS-CoV-2 or a variant thereof serum viral loads determined from a subject having a SARS-CoV-2 or a variant thereof infection by at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more compared to viral loads determined from the patient prior to administration of an effective amount of a composition. In some instances, administration of an effective amount of a composition reduces serum viral loads to an undetectable level compared to viral loads determined from the patient prior to administration of an effective amount of a composition. In some instances, administration of an effective amount of a composition results in a reduced and/or undetectable serum viral load that may be maintained for at least about 1, 2, 3, 4, 5, 6, 7 days; 1, 2, 3, 4, weeks; 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months; or 1 year or more.

The dosage administered depends on the subject to be treated (e.g., the age, body weight, capacity of the immune system, and general health of the subject being treated), the form of administration (e.g., as a solid or liquid), the manner of administration (e.g., by injection, inhalation, or dry powder propellant), and the cells targeted (e.g., epithelial cells, such as blood vessel epithelial cells, nasal epithelial cells, or pulmonary epithelial cells). The composition is preferably administered in an amount that provides a sufficient level of the antigenic or therapeutic gene product, or fragment thereof (e.g., a level of an antigenic gene product that elicits an immune response without undue adverse physiological effects in the host caused by the antigenic gene product).

The method of delivery, for example a DNA or RNA vaccine, may also determine the dose amount. In some cases, dosage administered by injections by intravenous (i.v.) or intramuscular (i.m.) route may require variable amounts of a DNA or RNA vaccine, for example from 10 µg-1 mg. However, administration using a gene gun may require a dose of a DNA or RNA vaccine between 0.2 µg and 20 µg (e.g., 0.2, 0.1, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 µg). In some instances, the use of a gene gun to deliver a dose of a DNA or RNA vaccine may require only ng quantities of DNA or RNA, for example between 10 ng and 200 ng (e.g., 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, 60, 70, 80, 90, 100, 125, 150, 175, or 200 ng).

In other embodiments, the delivery vector is a virus (e.g., an Ad26 virus) and the subject can be administered at least about 1×10^3 viral particles (VP)/dose or between 1×10^1 and 1×10^{20} VP/dose (e.g., 1×10^1 , 1×10^2 , 1×10^3 , 1×10^4 , 1×10^5 , 1×10^6 , 1×10^7 , 1×10^8 , 1×10^9 , 1×10^{10} , 1×10^{11} , 1×10^{12} , 1×10^{13} , 1×10^{14} , 1×10^{15} , 1×10^{16} , 1×10^{17} , 1×10^{18} , 1×10^{19} , or 1×10^{20} VP/dose). For example, the subject can be administered about 1×10^6 to about 1×10^{14} VP/dose (e.g., about 1×10^7 , about 1×10^8 , about 1×10^9 , about 1×10^{10} , about 1×10^{11} , about 1×10^{12} , about 1×10^{13} , about 1×10^{14} , or about 1×10^{15} VP/dose). For example, the subject can be administered about 1×10^{11} , about 1×10^{12} , about 1×10^{13} , or about 1×10^{14} VP/dose.

In addition, single or multiple administrations of the compositions of the disclosure may be given (pre- or post-exposure and/or pre- or post-diagnosis) to a subject (e.g., one administration or administration two or more times). For example, subjects who are particularly susceptible to, for example, viral infection (e.g., a SARS-CoV-2 or a variant thereof infection) may require multiple treatments to establish and/or maintain protection against the virus. Levels of induced immunity provided by the pharmaceutical compositions described herein can be monitored by, for example, measuring amounts of neutralizing secretory and serum antibodies. The dosages may then be adjusted or repeated as necessary to trigger the desired level of immune response. For example, the immune response triggered by a single administration (prime) of a composition may not sufficiently potent and/or persistent to provide effective protection. Accordingly, in some embodiments, repeated administration (boost), such that a prime boost regimen is established, can significantly enhance humoral and cellular responses to the antigen of the composition.

Alternatively, the efficacy of treatment can be determined by monitoring the level of the antigenic or therapeutic gene product, or fragment thereof, expressed in a subject (e.g., a human) following administration of the compositions. For example, the blood or lymph of a subject can be tested for antigenic or therapeutic gene product, or fragment thereof, using, for example, standard assays known in the art.

In some instances, efficacy of treatment can be determined by monitoring a change in the serum viral load from a sample from the subject obtained prior to and after administration of an effective amount of a composition (e.g., an immunogen, such as a protein having all or a fragment of the amino acid sequence of one or more of SEQ ID NOs: 2-4, or a nucleic acid molecule (e.g., a vector, such as a viral vector) encoding the immunogen, which can be administered alone or in combination with a protein having the amino acid sequence of SEQ ID NO: 1). A reduction in serum viral load of at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more compared to viral load determined from the subject prior to administration of an effective amount of a composition may indicate that the subject is receiving benefit from the treatment. If a viral load does not decrease by at least about 10%, 20%, 30%, or more after administration of a composition, the dosage of the composition to be administered may be increased. For example, by increasing the μ g or mg amount of a DNA vaccine (e.g., a DNA vaccine containing a nucleic acid molecule with a nucleic acid sequence of one or more of SEQ ID NOs: 7-12, which can be administered alone or in combination with any one of the nucleic acid molecules with the sequence of SEQ ID NO: 6, 44-51, 61-64, and 69-74) administered to the subject or by increasing the number of viral particles (VP) of an adenovirus vector-based vaccine (e.g., an adenovirus vector-based vaccine containing the nucleic acid sequence of one or more of SEQ ID NOs: 7-12, which can be

administered alone or in combination with a nucleic acid molecule with the sequence of SEQ ID NO: 6, 44-51, 61-64, and 69-74).

In some instances, efficacy of treatment can be determined by monitoring a change in the serum viral load from a sample from the subject obtained prior to and after administration of an effective amount of a composition (e.g., an immunogen, such as a protein having all or a fragment of the amino acid sequence of one or more of SEQ ID NOs: 40-43 and 65-68, or a nucleic acid molecule (e.g., a vector, such as a viral vector) encoding the immunogen, which can be administered alone or in combination with a protein having the amino acid sequence of SEQ ID NO: 1 or 35). A reduction in serum viral load of at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more compared to viral load determined from the subject prior to administration of an effective amount of a composition may indicate that the subject is receiving benefit from the treatment. If a viral load does not decrease by at least about 10%, 20%, 30%, or more after administration of a composition, the dosage of the composition to be administered may be increased. For example, by increasing the μ g or mg amount of a DNA vaccine (e.g., a DNA vaccine containing a nucleic acid molecule with a nucleic acid sequence of one or more of SEQ ID NOs: 44-51 and 61-64, which can be administered alone or in combination with any one of the nucleic acid molecules with the sequence of SEQ ID NOs: 29, 39, and 69-74) administered to the subject or by increasing the number of viral particles (VP) of an adenovirus vector-based vaccine (e.g., an adenovirus vector-based vaccine containing the nucleic acid sequence of one or more of SEQ ID NOs: 44-51 and 61-64, which can be administered alone or in combination with a nucleic acid molecule with the sequence of SEQ ID NOs: 29, 39, and 69-74).

A single dose of a composition may achieve protection, pre-exposure or pre-diagnosis. In addition, a single dose administered post-exposure or post-diagnosis can function as a treatment according to the disclosure.

A single dose of a composition can also be used to achieve therapy in subjects being treated for an infection (e.g., a coronavirus infection, such as a SARS-CoV-2 or a variant thereof infection). Multiple doses (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or more doses) can also be administered, in necessary, to these subjects.

Methods of Diagnosing and Predicting Susceptibility to Coronavirus Infection

Diagnostic Methods

Provided herein are methods for identifying, diagnosing, and/or predicting the susceptibility of a subject to a coronavirus infection. The method includes measuring the level or amount of a broadly neutralizing anti-coronavirus antibody (bNAb) against two or more lineages of coronavirus (such as an anti-Spike antibody) in a sample (e.g., a whole blood sample, e.g., a serum or plasma sample) from the subject. In some embodiments, the two or more lineages of coronavirus are selected from the group consisting of B.1.1.7, B.1.429, B.1.1.28, B.1.351, A23.1, B.1.617.1, B.1.617.2, B.1.427, B.1.525, B.1.526, P.1, P.2, P.3, C.36, C.37, B.1.1.519, B.1.526.1, B.1.526.2, R.1, B.1.258.17, B.1.575, B.1.214.2, A.2.5.2, AT.1, B.1.1.523, and B.1.620. In some embodiments, the coronavirus is SARS-CoV-2 or a variant thereof. In some embodiments, the subject is determined to be susceptible to the coronavirus infection if the broadly neutralizing anti-coronavirus antibody (e.g., a broadly neutralizing anti-Spike, anti-membrane, or anti-nucleocapsid antibody) amount or level is below a protective level (e.g., below a titer of at least

about 70 as measured using the pseudovirus neutralization assay described herein, below a titer of at least about 25 as measured using the live virus neutralization assay described herein, or below 80% of a median level of a cohort of convalescent humans (e.g., a group of humans who have recovered or are recovering from a coronavirus infection (e.g., SARS-CoV-2 or a variant thereof)) as determined by a pseudovirus neutralization assay or live virus neutralization assay) and determined to not be susceptible to the coronavirus infection if the broadly neutralizing anti-coronavirus antibody (e.g., a broadly neutralizing anti-Spike, anti-membrane, or anti-nucleocapsid antibody) level is above a protective level. In some embodiments, the protective level is a broadly neutralizing anti-coronavirus antibody titer (e.g., a broadly neutralizing anti-Spike, anti-membrane, or anti-nucleocapsid antibody titer) of at least about 70 (e.g., about 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 110, 115, 120, 125, 130, 140, 150, 175, 200, 225, 250, 275, 300, 325, 350 or more) as determined in a pseudovirus neutralization assay. In some embodiments, the protective level is a broadly neutralizing anti-coronavirus antibody titer (e.g., a broadly neutralizing anti-Spike, anti-membrane, or anti-nucleocapsid antibody titer) of at least about 83 as determined in a pseudovirus neutralization assay. In some embodiments, the protective level is a broadly neutralizing anti-coronavirus antibody titer (e.g., a broadly neutralizing anti-Spike, anti-membrane, or anti-nucleocapsid antibody titer) of at least about 25 (e.g., about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 110, 115, 120, 125, 130, 140, 150, 175, 200 or more) as determined in a live virus neutralization assay. In some embodiments, the protective level is a broadly neutralizing anti-coronavirus antibody titer (e.g., a broadly neutralizing anti-Spike, anti-membrane, or anti-nucleocapsid antibody titer) of at least about 35 as determined in a live virus neutralization assay. In some embodiments, the protective level is a broadly neutralizing anti-coronavirus antibody titer (e.g., a broadly neutralizing anti-Spike, anti-membrane, or anti-nucleocapsid antibody titer) that is at least about 60% (e.g., about 61%, about 62%, about 63%, about 64%, about 65%, about 66%, about 67%, about 68%, about 69%, about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 100%, about 110%, about 120%) of a median or mean level of a cohort of convalescent humans as determined by a pseudovirus neutralization assay or live virus neutralization assay as described herein. In some embodiments, the protective level is a broadly neutralizing anti-coronavirus antibody titer (e.g., a broadly neutralizing anti-Spike, anti-membrane, or anti-nucleocapsid antibody titer) that is at least about 80% of a median or mean level of a cohort of convalescent humans as determined by a pseudovirus neutralization assay or live virus neutralization assay as described herein. A subject determined to be susceptible to the coronavirus infection (a subject with a broadly neutralizing anti-coronavirus antibody (e.g., a broadly neutralizing anti-Spike, anti-membrane, or anti-nucleocapsid antibody) amount or level is below a protective level (e.g., below a titer of at least about 70 as measured using the pseudovirus neutralization assay described herein, below a titer of at least about 25 as measured using the live virus neutralization assay described herein, or below 80% of a median level of a cohort of convalescent humans (e.g., a group of humans

who have recovered or are recovering from a coronavirus infection (e.g., SARS-CoV-2 or a variant thereof)) as determined by a pseudovirus neutralization assay or live virus neutralization assay)) can be administered a therapy (e.g., administered any of the compositions described herein), such as an effective amount of one or more of the pharmaceutical compositions (e.g., immunogenic compositions and antibodies against a modified S, MEM, and/or NUL protein of SARS-CoV-2 or a variant thereof) described herein. A subject may be re-administered a therapy until the subject is determined to not be susceptible to the coronavirus infection (e.g., until the subject has a level of a broadly neutralizing anti-coronavirus antibody (e.g., a broadly neutralizing anti-Spike antibody, such as an antibody that specifically binds to one or more of the SET1, SET2, SET3, EG1, EG2, EG3, EG4, MEM1, MEM2, MEM3, NUL1, NUL2, and NUL3 immunogens) that is above a protective level (e.g., a level above a titer of at least about 70 as measured using the pseudovirus neutralization assay described herein, above a titer of at least about 25 as measured using the live virus neutralization assay described herein, or is at a level that is at least 80% of a median level (and preferably at or above a median level) of a broadly neutralizing anti-coronavirus antibody of a cohort of convalescent humans (e.g., a group of humans who have recovered or are recovering from a coronavirus infection (e.g., SARS-CoV-2 or a variant thereof)) as determined by a pseudovirus neutralization assay or live virus neutralization assay)). The method may also involve determining whether the anti-Spike antibody is an RBD-specific antibody. The method may also involve determining whether the anti-Spike antibody is an S1-specific antibody. The method may also involve determining whether the anti-Spike antibody is an S2-specific antibody. The method may also involve identifying the subclass (e.g., IgM, IgA, IgG1, IgG2, IgG3, or FcγR2A.1) and/or effector function (e.g., antibody-dependent neutrophil phagocytosis (ADNP), antibody-dependent complement deposition (ADCD), antibody-dependent monocyte cellular phagocytosis (ADCP), or antibody-dependent NK cell activation (IFN- γ secretion, CD107a degranulation, and MIP-1 β expression)) of the anti-coronavirus antibody. The method may further include administering one or more of the pharmaceutical compositions (e.g., one of the immunogenic compositions and/or a composition containing an antibody against a modified S (e.g., one or more of the SET1, SET2, SET3, EG1, EG2, EG3, and EG4 immunogens), MEM (e.g., one or more of the MEM1, MEM2, and MEM3 immunogens) and/or NUL (e.g., NUL1, NUL2, and NUL3 immunogen) protein of SARS-CoV-2 or a variant thereof) described herein to a subject determined to be in need of further therapy.

The method may include measuring the coronavirus (e.g., SARS-CoV-2 or a variant thereof) viral load in a sample from the subject. In some embodiments, the sample is a bronchoalveolar lavage (BAL) or a nasal swab (NS). In some embodiments, the sample is a bodily fluid (e.g., blood, e.g., whole blood or plasma) from the subject. In some embodiments, the sample is a tissue sample (e.g., a respiratory tract tissue sample) from the subject. In some embodiments, viral load is a detectable nucleic acid (e.g., subgenomic mRNA) level or a detectable protein (e.g., nucleocapsid protein (NUL)) level. In some embodiments, the detectable nucleic acid (e.g., subgenomic mRNA) is determined by RNA-seq, RT-qPCR, qPCR, multiplex qPCR or RT-qPCR, LAMP, microarray analysis, or hybridization (e.g., ISH (e.g., FISH)). In some embodiments, the detectable protein (e.g., nucleocapsid protein (NUL)) is determined by an immunoassay (e.g., an immunohistochemical (IHC) assay or a lateral flow immunoassay). In some embodiments, a detectable viral load indicates that the subject is susceptible to disease (e.g., a SARS-CoV-2 or a variant thereof-mediated disease, e.g., COVID-19, e.g., severe COVID-19 disease). In some

embodiments, a viral load of greater than at least about 3.5 log₁₀ sgRNA copies/mL (e.g., about 3.75 log₁₀ sgRNA copies/mL, about 3.8 log₁₀ sgRNA copies/mL, about 3.9 log₁₀ sgRNA copies/mL, about 4.0 log₁₀ sgRNA copies/mL, about 4.25 log₁₀ sgRNA copies/mL, about 4.5 log₁₀ sgRNA copies/mL, about 4.75 log₁₀ sgRNA copies/mL, about 5.0 log₁₀ sgRNA copies/mL, about 5.5 log₁₀ sgRNA copies/mL, about 6.0 log₁₀ sgRNA copies/mL, about 6.5 log₁₀ sgRNA copies/mL, about 7.0 log₁₀ sgRNA copies/mL, about 7.5 log₁₀ sgRNA copies/mL, about 8.0 log₁₀ sgRNA copies/mL, about 8.5 log₁₀ sgRNA copies/mL, about 9 log₁₀ sgRNA copies/mL, about 10 log₁₀ sgRNA copies/mL, about 11 log₁₀ sgRNA copies/mL, about 12 log₁₀ sgRNA copies/mL, about 13 log₁₀ sgRNA copies/mL or more). In some embodiments, a viral load of greater than 3.85 log₁₀ sgRNA copies/mL in BAL or 3.78 log₁₀ sgRNA copies/mL in NS indicates that the subject is susceptible to disease (e.g., a SARS-CoV-2 or a variant thereof-mediated disease, e.g., COVID-19, e.g., severe COVID-19 disease). In some embodiments, a viral load of greater than 3.85 log₁₀ sgRNA copies/mL in BAL or 3.78 log₁₀ sgRNA copies/mL in NS indicates that the subject is susceptible to severe COVID-19 disease. In some embodiments, a viral load of greater than about 2.0 log₁₀ sgRNA copies/g (e.g., about 2.0 log₁₀ sgRNA copies/g, about 2.5 log₁₀ sgRNA copies/g, about 3.0 log₁₀ sgRNA copies/g, about 3.5 log₁₀ sgRNA copies/g, about 4.0 log₁₀ sgRNA copies/g, about 4.25 log₁₀ sgRNA copies/g, about 4.5 log₁₀ sgRNA copies/g, about 4.75 log₁₀ sgRNA copies/g, about 5.0 log₁₀ sgRNA copies/g, about 5.5 log₁₀ sgRNA copies/g, about 6.0 log₁₀ sgRNA copies/g, about 6.5 log₁₀ sgRNA copies/g, about 7.0 log₁₀ sgRNA copies/g, about 7.5 log₁₀ sgRNA copies/g, about 8.0 log₁₀ sgRNA copies/g, about 8.5 log₁₀ sgRNA copies/g, about 9 log₁₀ sgRNA copies/g, about 10 log₁₀ sgRNA copies/g, about 11 log₁₀ sgRNA copies/g, about 12 log₁₀ sgRNA copies/g, about 13 log₁₀ sgRNA copies/g or more) of tissue indicates that the subject is susceptible to severe COVID-19 disease. In some embodiments, a viral load of greater than about 8.0 log₁₀ sgRNA copies/g in lung tissue, about 7.0 log₁₀ sgRNA copies/g in nares tissue, about 6.0 log₁₀ sgRNA copies/g in trachea tissue, about 5.5 log₁₀ sgRNA copies/g in heart tissue, or about 2.0 log₁₀ sgRNA copies/g in GI, spleen, liver, kidney, or brain tissue indicates that the subject is susceptible to severe COVID-19 disease. In some embodiments, a viral load of greater than about 3% (e.g., about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%) SARS-CoV-2 vRNA staining by ISH indicates that the subject is susceptible to disease (e.g., a SARS-CoV-2 or a variant thereof-mediated disease, e.g., COVID-19, e.g., severe COVID-19 disease). In some embodiments, a viral load of greater than about 5% (e.g., about 5%, about 6%, about 7%, about 8%, about 9%, about 10%) SARS-CoV-2 vRNA staining by ISH indicates that the subject is susceptible to severe COVID-19 disease. In some embodiments, a viral load of greater than about 5% (e.g., about 5%, about 6%, about 7%, about 8%, about 9%, about 10%) SARS-CoV-2 vRNA staining by ISH indicates that the subject is susceptible to severe COVID-19 disease. In some embodiments, coronavirus (e.g., SARS-CoV-2 or a variant thereof) viral load is measured one or more times over about 1, 2, 3, 4, 5, or 6 days or 1, 2, 3, 4, 5, 6, or 7 weeks post-infection.

Monitoring Responsiveness

Provided herein are methods for monitoring an anti-coronavirus immune response of a subject to a therapeutic composition (e.g., any of the compositions or immunogenic compositions described herein) for treating or inhibiting or reducing the risk of a coronavirus infection. The method includes measuring

the level or amount of an anti-coronavirus antibody (e.g., an anti-Spike, anti-membrane, or anti-nucleocapsid antibody or a broadly neutralizing anti-Spike, anti-membrane, or anti-nucleocapsid antibody) in the subject. In some embodiments, the coronavirus is SARS-CoV-2 or a variant thereof. In some embodiments, the anti-coronavirus antibody (e.g., an anti-Spike, anti-membrane, or anti-nucleocapsid antibody) is a neutralizing antibody. The anti-coronavirus antibody (e.g., an anti-Spike, anti-membrane, or anti-nucleocapsid antibody, e.g., an anti-Spike, anti-membrane, or anti-nucleocapsid neutralizing antibody, e.g., a broadly neutralizing anti-Spike, anti-membrane, or anti-nucleocapsid antibody) may be measured in a short timeframe (e.g., in order to measure the robustness of the antibody response) or a longer timeframe (e.g., in order to measure the durability of the antibody response) after administration of a therapeutic composition (e.g., any of the compositions or immunogenic compositions described herein). In some embodiments, the anti-coronavirus antibody (e.g., an anti-Spike, anti-membrane, or anti-nucleocapsid antibody or a broadly neutralizing anti-Spike, anti-membrane, or anti-nucleocapsid antibody) is measured about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 days, about 5, 6, 7, 8, 9, 10, 11, or 12 weeks, about 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, or 23 months, or about 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20 years after the subject is administered the therapeutic composition (e.g., any of the compositions or immunogenic compositions described herein).

The subject is determined to be responsive to the therapeutic composition if the anti-coronavirus antibody (e.g., an anti-Spike, anti-membrane, or anti-nucleocapsid antibody or a broadly neutralizing anti-Spike, anti-membrane, or anti-nucleocapsid antibody) detected in the subject (e.g., in the subject's blood) is above a protective level (e.g., above a titer of at least about 70 as measured using the pseudovirus neutralization assay described herein, above a titer of at least about 25 as measured using the live virus neutralization assay described herein, or is at a level that is at least 80% of a median level (and preferably at or above a median level) of an anti-coronavirus antibody of a cohort of convalescent humans (e.g., a group of humans who have recovered or are recovering from a coronavirus infection (e.g., SARS-CoV-2 or a variant thereof)) as determined by a pseudovirus neutralization assay or live virus neutralization assay). Alternatively, the subject is determined to be non-responsive to the therapeutic composition if the anti-coronavirus antibody (e.g., an anti-Spike, anti-membrane, or anti-nucleocapsid antibody or a broadly neutralizing anti-Spike, anti-membrane, or anti-nucleocapsid antibody) detected in the subject is below a protective level (e.g., below a titer of at least about 70 as measured using the pseudovirus neutralization assay described herein, below a titer of at least about 25 as measured using the live virus neutralization assay described herein, or is at a level that is below 80% of a median level of a cohort of convalescent humans as determined by a pseudovirus neutralization assay or live virus neutralization assay). A protective level of an anti-coronavirus antibody (e.g., an anti-Spike, anti-membrane, or anti-nucleocapsid neutralizing antibody) corresponds to a titer of at least about 70 (e.g., about 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 110, 115, 120, 125, 130, 140, 150, 175, 200, 225, 250, 275, 300, 325, 350 or more) as determined in a pseudovirus neutralization assay (e.g., the pseudovirus neutralization assay described herein). In some embodiments, the protective level is an anti-coronavirus antibody titer (e.g., an anti-Spike, anti-membrane, or anti-nucleocapsid neutralizing antibody titer) of at least about 25 (e.g., about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45,

50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 110, 115, 120, 125, 130, 140, 150, 175, 200 or more) as determined in a live virus neutralization assay (e.g., the pseudovirus neutralization assay described herein). In some
 5 embodiments, the protective level is an anti-coronavirus antibody titer (e.g., an anti-Spike, anti-membrane, or anti-nucleocapsid neutralizing antibody titer) that is at least about 60% (e.g., about 61%, about 62%, about 63%, about 64%, about 65%, about 66%, about 67%, about 68%, about 69%, about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about
 10 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 100%, about 110%, about 120%) of a median or mean level of a cohort of convalescent humans as determined by a pseudovirus neutralization assay or live virus neutralization assay as described herein.

If, over time, an anti-coronavirus antibody (e.g., an anti-Spike, anti-membrane, or anti-
 15 nucleocapsid antibody or a broadly neutralizing anti-Spike, anti-membrane, or anti-nucleocapsid antibody) titer in the subject (e.g., in the blood of a subject) falls below or fails to reach a protective level (e.g., below a titer of at least about 70 as measured using the pseudovirus neutralization assay described herein, below a titer of at least about 25 as measured using the live virus neutralization assay described herein, or below 80% of a median level of a cohort of convalescent humans as determined by a
 20 pseudovirus neutralization assay or live virus neutralization assay described herein), the subject may be administered or may be re-administered a coronavirus vaccine composition (e.g., one or more of the therapeutic or immunogenic compositions described herein) alone or in combination with an additional therapeutic agent, such as one or more of the additional therapeutic agents described herein.
 Administration of a composition of the disclosure to a subject in need thereof can be performed one or
 25 more times (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more times) over one or more days (e.g., 1, 2, 3, 4, 5, 6, or 7 days), weeks (e.g., 1, 2, 3, 4, 5, 6, 7, or 8 weeks), months (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months), or years (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more years), or over the life of the subject, as needed to maintain a protective level of an anti-coronavirus antibody in the subject, thereby protecting the subject against coronavirus infection (e.g., infection by SARS-CoV-2 or a variant thereof).

30 The method may include measuring the coronavirus (e.g., SARS-CoV-2 or a variant thereof) viral load in a sample from the subject. In some embodiments, the coronavirus is SARS-CoV-2 or a variant thereof. In some embodiments, the viral load is measured about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 30, 36, 42, or 48 hours or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 days post-infection. In some
 35 embodiments, the sample is a bronchoalveolar lavage (BAL) or a nasal swab (NS). In some embodiments, the sample is a bodily fluid (e.g., blood, e.g., whole blood or plasma) from the subject. In some embodiments, the sample is a tissue sample (e.g., a respiratory tract tissue sample) from the subject. In some embodiments, viral load is a detectable nucleic acid (e.g., subgenomic mRNA) level or a detectable protein (e.g., nucleocapsid protein (NUL)) level. In some embodiments, the detectable nucleic
 40 acid (e.g., subgenomic mRNA) is determined by RNA-seq, RT-qPCR, qPCR, multiplex qPCR or RT-qPCR, LAMP, microarray analysis, or hybridization (e.g., ISH (e.g., FISH)). In some embodiments, the

detectable protein (e.g., nucleocapsid protein (NUL)) is determined by an immunoassay (e.g., an immunohistochemical (IHC) assay or a lateral flow immunoassay). The subject is determined to be responsive to the therapeutic composition if the viral load is below a pre-assigned level. In some embodiments, the pre-assigned level is less than about $3.5 \log_{10}$ sgRNA copies/mL BAL or NS or less than about $5.0 \log_{10}$ sgRNA copies/g of tissue (e.g., lung, nares, trachea, heart, GI, spleen, liver, kidney, or brain tissue). In some embodiments, the subject is determined to be responsive to the therapeutic composition if the viral load decreases in the subject.

If the subject is not determined to be responsive as determined by viral load, then the subject may be administered or may be re-administered a coronavirus vaccine composition (e.g., one or more of the therapeutic or immunogenic compositions described herein) alone or in combination with an additional therapeutic agent, such as one or more of the additional therapeutic agents described herein.

Administration of a composition of the disclosure to a subject in need thereof can be performed one or more times (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more times) over one or more days (e.g., 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, or 28 days) as needed to reduce the viral load.

EXAMPLES

The following examples are to illustrate the invention. They are not meant to limit the invention in any way.

Example 1. Signature-based Epitope Targeted (SET) Vaccine Concept Applied To SARS-Cov-2 Background

SET design

The underlying strategy for this design came from Signature-based Epitope Targeted (SET) vaccines that were designed to treat HIV (Bricault et al., 2019). The strategy is used to design vaccines against variable pathogens based on the premise that presenting the immune system with the relevant natural diversity, including resistance mutations, in a commonly targeted epitope region, will elicit antibodies that will be selected during affinity maturation that are able to interact with and neutralize the critical variants (see, e.g., International Patent Application No. PCT/US2017/057045, incorporated herein by reference).

The idea was inspired by the natural evolution of bNAbs in chronic HIV-1 infections. The natural emergence of such antibodies requires years of antibody lineage coevolution with the evolving viral quasispecies within an infected host (Bhiman et al., 2015; Bonsignori et al., 2017; Bonsignori et al., 2016; Gao et al., 2014). The autologous viruses within a host escapes contemporaneous antibodies (Abs), and B-cells are in turn selected that can recognize the escaped viruses by acquiring somatic hypermutations. Iterative cycles of viral escape and B-cell maturation result in substantial accumulation of mutations in the B-cell lineage over time; all bNAbs show high levels of somatic hypermutation, and acquire heterologous neutralization breadth only as the lineage matures. During the course of the evolution of the virus during an HIV infection, the escape mutations that are sampled are the ones that are fit and enable continuing viral replication while persisting the presence of the host's immune response.

As key epitope regions are repeatedly targeted in different human hosts, mutations that confer antibody escape are often recapitulated across hosts, thus across the population of infected people particular escape mutations are often sampled recurrently, and toggle back and forth within the evolutionary landscape of the virus according dynamic balance of immune escape and viral fitness (Bricault et al., 2019; Korber et al., 2017; Stephenson et al., 2020). Given this, the repeated cycles of immune escape and selection for antibody tolerance of resistance mutations in a single host can eventually give rise to antibodies with heterologous breadth across the circulating population, as the same patterns of resistance mutations that arise in a given host over will often sample the same resistance forms that commonly found in other individuals, and that can limit heterologous breadth. Thus, it was hypothesized that a vaccine that presented an optimal, immunologically sensitive form of a key target epitope that was commonly recognized in HIV infected people, coupled with two complementary forms of HIV-1 ENV that carried commonly circulating immune resistance mutations might elicit antibodies with neutralization breadth, with the intent to trigger an antibody response to the optimal sensitive form, and accelerate the development of breadth by exposing maturing antibody lineage to the variant forms that it critically need to recognize to achieve neutralization breadth at the population level.

To inform SET vaccine design, a phylogenetically-corrected signature strategy similar to one previously used for the development of HIV-1 broadly neutralizing antibodies (Bricault et al., 2019) was used to define recurrent common patterns of resistance at the population level. The initial HIV-1 Env SET vaccine design focused on the V2 epitope and included an Env that was designed to be optimally sensitive to V2 bNAbs, and two complementary variants to cover the most relevant diversity for conferring resistance to HIV V2 antibodies. This vaccination strategy elicited sera that had very good heterologous neutralization breadth profiles in guinea pigs (Bricault et al., 2019). The best ranking for breadth and potency involved delivering all three SET variants simultaneously, not in series, and different forms of Env and different adjuvants yielded different levels of bNAb response, but the SET vaccines improved heterologous responses over natural strains every scenario tested (see Bricault et al., 2019). The strategy used to produce the HIV-1 SET vaccine cocktails were used as a guide in the production of a COVID-19 Spike SET cocktail that could be used to develop a COVID-19 Spike vaccine.

Translating SET design strategy to a SARS-CoV-2

SARS-CoV-2 is not a chronic virus, although some immunocompromised individuals have prolonged infections, and in such cases, antibodies are often given therapeutically. This is one scenario where the virus can evolve under immune pressure in vivo; in one such case resistance mutations in both the RDB and NTD supersite evolved over a long disease course (Choi et al., 2020). In more typical SARS CoV-2 infections, antibodies have been shown to continue to evolve over months, and to acquire greater breadth over time (Gaebler et al., 2020). Persistent low levels virus was found in the small bowel of 7/14 asymptomatic individuals, which was proposed to be stimulating the continuing antibody evolution, although no viral sequences were obtained to track antibody/viral co-evolution in these cases (Gaebler et al., 2020). The B.1.1.7 viral lineages which have been rapidly expanding carry multiple mutations in the Spike protein. No clear stepwise evolution of this virus was evident in the sampling across the population, but a long branch in the tree leading to the ancestor of the B.1.1.7 lineages lead to the speculation that the evolution and accumulation of multiple mutations may have occurred in a chronic

infection. Mutations in B.1.1.7 lineages may have resulted in a favorable phenotype for the virus, which began to spread rapidly.

It is known from HIV work that presenting epitope diversity and including sensitive and resistant forms in a cocktail can give rise to antibodies with improved heterologous breadth (Bradley et al., 2017; Bricault et al., 2019). We used this concept in a SARS CoV-2 setting to identify viral variants that could be combined in a vaccine cocktail with the goal of producing a vaccine with enhanced breadth of antibody response to the RDB in order to treat diverse coronaviruses. The immunogens described herein can be used to produce a second generation vaccine that could neutralize existing SARS-CoV-2 lineage variants, as well as newly arising vaccine resistant variants.

In the original design of SET vaccines for HIV-1, a phylogenetically corrected signature strategy was used to identify critical sites of resistance that were both frequent in the circulating population. This information was used to develop immunogens that would avoid the development of resistance. To be statistically informed, the signature strategy depended upon having data from very large panels of HIV-1 Env variants (preferably many hundreds) tested against panels of many neutralizing antibodies with known targets (dozens of monoclonal antibodies with multiple examples per antibody class). Such variant data is not yet available for SARS CoV-2. Furthermore, the evolutionary trajectory of pandemic variation in SARS CoV-2 is different than HIV-1. While mutations have accumulated at a slow pace during the COVID-19, if a favorable variant evolves, it can rapidly expand in a population. In the case of the G clade carrying the D614G mutation, it often became the most prevalent variant over a period of 3-6 weeks after it had first been sampled in a geographic region and was observed to be 3-6 fold more infectious in pseudotype assays (Korber et al., 2020). In the case of the B.1.1.7 variant, which has rapidly expanded in local regions within the United Kingdom and has now been detected and found with increasing in frequency in many countries all over the world (Davies et al., 2020; Rambaut et al., 2020; Volz et al., 2021), it was often observed to have become the dominant form in a local region with 2-3 months of initial detection (Shen et al., 2021), and the D69/70 mutation it carried was found to be ~2-fold more infectious in a pseudotype assay (Kemp et al., 2021). Thus, there is a need to define key antibody resistance mutations in a low diversity setting, where relatively rapid transitions over time of the dominant forms of the virus could be accounted for, and hopefully even anticipated, to create a vaccine design that may be able to stay ahead of viral diversification.

In this vaccine design, it was apparent that a relatively small and manageable set of mutations in the key epitope regions were either recurrent in different lineages or currently expanding in lineages of concern, and so a SET design would be feasible. It was hypothesized that the resistant forms that were currently evident and frequently sampled or resampled could re-emerge in new Spike backbones as the virus continues to evolve during the pandemic; such that a vaccine that covered much of the spectrum of contemporary resistance mutations may be able to continue to be effective against newly emergent lineages. The previously described computational tools that were designed to define the most relevant HIV resistance mutations for SET vaccines would not be appropriate tool for the purpose of defining SARS CoV-2 resistance signature mutations in such a low diversity setting. Thus, a different computational approach to define the most common circulating resistance mutations, their covariation patterns, and their levels of recurrence to design a SARS CoV-2 SET vaccine cocktail was used.

Over the course of the pandemic, two critical epitope regions in the SARS CoV-2 Spike protein have emerged as the key targets of potent neutralizing antibodies: the supersite in the N-terminal domain (NTD) (Cerutti et al., 2021; Chi et al., 2020; McCallum et al., 2021) and the Receptor Binding Domain (RBD) (Barnes et al., 2020a; Barnes et al., 2020b). We focused on these two regions simultaneously in the SET cocktail design. The NTD supersite was defined as including Spike positions 14-20, 140-158, 242-264, whereas the RBD region was defined as positions 330-521. Current evidence points to neutralizing antibodies (NAbs) directed against the Spike protein as a correlate of vaccine elicited protective immunity against SARS-CoV-2, emphasizing the importance of NAbs targeting these regions (Tostanoski et al., 2020; Yu et al., 2020). However, non-neutralizing antibodies with other functionalities and CD8+ T cell responses may also be beneficial (McMahan et al., 2020), and by presenting the common diversity in these the RBD and NTD domains, it is also likely to expand the breadth of these other beneficial natural responses as a side benefit.

To define the signatures of resistance in the case of SARS-CoV-2, a summary of known resistance mutations and infectivity enhancing mutations in the Spike protein that has been gathering from the literature over the past year was created. The resistance mutations shown in **FIGs. 3-14** were identified in the COVID-19 literature because convalescent sera or neutralizing antibodies isolated from convalescent sera were tested against commonly circulating variants in pseudotype viral assay, or because variants were selected in vitro after exposure to antibodies or sera. Simultaneously, computational tools to track the emergence of Spike variants globally, with Spike diversity at each position in the protein summarized daily at cov.lanl.gov from in GISAID updates were developed (Korber et al., 2020).

Here, two new computational tools to specifically track covariation and common patterns of linked mutations in the pandemic by geographic region (xSpike, for “explore Spike”, and Shiver, for “SARS-CoV-2: Historically Variants in Epitope Regions”) are described. The output of xSpike was overlaid with NAb resistance mutation summaries for vaccine design purposes, and results cross-checked using the output of xSpike.

Implementation

To create a SARS-CoV-2 vaccine that would be practically useful in the context of a vector-based vaccine delivery, there was a need to constrain the inclusion of the ancestral form of the Spike plus two or three additional strains that would best cover relevant contemporary variants; a relatively small vaccine cocktail would also be simpler and more cost effective in the context of an RNA delivery strategy. The Spike protein of the ancestral strain Wuhan variant (e.g., SEQ ID NO: 34) would be the analog of an “optimal” HIV variant; it turns out that it is still the most common form of the two epitope regions (spanning the RBD + NTD supersite) sampled globally, making up 54% between December 1, 2020, and February 20, 2021, based on GISAID sampling, although as newer variants increase in frequency this may be a transient scenario. Furthermore, it is known that this ancestral form of the Spike first isolated in Wuhan is highly immunogenic and provides extremely good vaccine protection in clinical trials (Mercado et al., 2020; Polack et al., 2020), and the ancestral Spike has already been developed for human use and global deployment in both Adenovirus and RNA delivery platforms, which were the two delivery strategies intended to be used to test the SARS CoV-2 SET concept. To design the complementary SET Spikes for

a polyvalent vaccine SARS CoV-2 emerging diversity and antibody resistance, the following was performed:

Key resistance and common mutations within the RDB and NTD supersite that were sampled and those that were observed to be transmitted at a significant level were identified. Meeting this criterion included capturing mutations that were both relevant to NAb breadth, and mutations that would be well tolerated at a structural and functional level.

GISAID global SARS CoV-2 Spike protein sequence data was analyzed geographically, in order to not over emphasize sampling variants from the United Kingdom (UK), which is by far the most frequently sampled nation in the world, and also to weight more sparsely sampled regions (the UK makes up over half of the global sample (**FIG. 1**)). This weighting is important as the diversity patterns in Spike are known to be highly regionally dependent (**FIG. 2**), and newly emerging variants may originate in any part of the world and disperse. Thus, there was a need for a strategy that could balance sampling bias and utilize the full global data in a simple and strategic way. Given the reality of the current sampling scenario, treating the important and rich data set from the United Kingdom as its own sample seemed the best course. To have enough data from other regions in the world to be informative in geographically regional data subsets, the global data down by continents (Europe excluding the United Kingdom (Europe without UK), Africa, Asia, North America, and South America were broken down.

Next, we used the code xSpike to map regional covariation patterns in Spike to identify covariation patterns among circulating Spikes. We applied our analysis globally, in each of the major geographic regions described above, and sampled in GISAID. Five variants of concern and of related form, each of which have strong lineage-based covariation patterns, were identified by looking at different continents, as depicted in Table 2:

Table 2: SARS-CoV-2 Spike Protein Covariation Patterns By Continent

Variant/Synonym	Point of First Detection	Percent sampled January 26, 2020 – February 23, 2021	Reference
B.1.1.7 GR/501Y.V1	United Kingdom	93%	Rambuat et al., 2020
B.1.429+B.1427 GH/452R	California, United States	20%	Zhang et al., 2021
B.1.1.28 GR/484.V2	South Africa	97%	Wibmer et al., 2021
B.1.351 GH/501Y.V2	Brazil	54%	Gröhs Ferrareze et al., 2021
A23.1 367F.V1	Uganda	No recent samples, 100% in December, 2020	Bugembe et al., 2021

There is an additional variant of concern: North American lineages that contain Q677P (Hodcroft et al., 2021). These mutations were found in various Spike backbones in North America (**FIG. 6**), but so far do not accompany any changes in the NTD supersite or RBD, so were not of concern for the vaccines (**FIGs. 15-17**). These changes were thought to be just some of many changes in recurrent globally that add positive charge near the site at 681, and it was hypothesized that these were all likely to impact

proteolytic furin cleavage. Also, Brazil carries several forms of the emerging E484K variant (Gröhs Ferrareze et al., 2021; Naveca et al., 2021), but only the B.1.351 variant carries multiple mutations in epitope regions (**FIG. 7**).

Samples were collected in the time window between October 1, 2020, and February 10, 2021, to balance an emphasis on newly emerging variants with the value of having more extensive geographically varied sampling within each continent. By using a collection window of 100 days, we were able to observe a large enough sample size to detect newly emerging variants. As a control, we revisited the data between December 1, 2020, and a ten day later sampling of GISAID on February 20, 2021, using the SHIVER code. Only very rare variants shifted, thus the portrait of variation revealed was robust to different sampling time choices, and need not alter the SET design choices. It was hypothesized, that the diversity represented in the vaccine may be able to anticipate likely future variants in the key epitope regions. This is because recurrent viable variants that have emerged through the course of the epidemic were represented. Most of the mutations of interest have been identified in the virus samples recure in the context of many different Spike backbones, thus they are fit variants that also may contribute to resistance. Epitope variants within all five of the key variants of concern currently circulating were represented in the SET design. But even if the evolutionary trajectory takes SARS-CoV-2 away from the current SET design, the approach we used would still be expected to be effective against currently co-circulating variants and others that later arise.

Using the output of xSpike, we designed the SET Spike vaccine cocktails to display common and recurrent NAb resistance mutations, favoring the context of commonly found co-variation patterns in each of the individual SET proteins. By tapping into covariation patterns for the design, and limiting mutations outside of the key epitope regions, it was hypothesized that in order to increase the likelihood of creating a well-folded Spike immunogen that may serve as a vaccine, multiple mutations covering the spectrum of combinations of variants will need to be incorporated.

Output of xSpike

To highlight important covariation patterns, first, mutual information (Korber et al., 1993), a classic strategy for identifying covariations patterns of mutation within the full Spike protein, was used. This strategy was not particularly revealing, and only captured already well-known variants of concern. It was evident from simply looking by eye at the global SARS co-variation tables created daily at cov.lanl.gov that there were other less frequent but still common patterns of co-variation being missed by a mutual information approach, so other statistics, including Cramer's V, were explored and found to be a much more sensitive indicator of patterns of covariation (**FIG. 3**). These patterns were not phylogenetically corrected, they simply reflect shared patterns of mutations among commonly circulating forms of Spike. As these are the variant forms that second generation vaccine sera will need to be effective against, to the extent that currently available circulating forms can be captured, it seems an appropriate view of the data.

Covariation patterns among the 50 most variable sites were then tracked globally and regionally, and alignments of natural variants as defined by the 50 most variable regions are summarized in **FIGs. 3-12**. In this way, rare mutations were excluded from consideration. The subset of variable positions that are in the RBD are highlighted in pink text, the subset found in the NTD supersite were highlighted in

green text, and recurrent mutations that are potentially linked to increased infectivity but may not be related to NAb resistance were highlighted in blue text.

The covariation patterns in **FIGs. 3-12** were reduced to two pages that covered all common distinct forms of the virus *within* the RBD and NTD supersite (**FIGs. 13-14**) that were co-circulating globally. Variants that did perturb the NTD supersite or RBD were not included. This covariation data was superimposed upon additional information regarding the natural frequencies of each mutation and patterns of resistance that were documented in the literature, and reduced the variants found among over 250,000 globally circulating Spike sequences sampled between October 1st, 2020, and February 10th, 2021, to a set of the most common variation patterns that could be interleaved and superimposed on the RBD and NTD regions of three Spike backbones (**FIGs. 15-17**).

Based on our analysis, we designed three SET immunogens:

SET1 – containing the following recurrent mutations: L18F, T20N, P26S, D80A, M153T, M153I, Δ242-244, K417N, Y453F, E484K, N501Y, and D614G (relative to SEQ ID NO: 1);

SET2 – containing the following recurrent mutations: S13I, Δ69-70, Δ144, W152C, D253G, A262S, L452R, S477N, and D614G (relative to SEQ ID NO: 1); and

SET3 – containing the following recurrent mutations: D80Y, L141F, F157L, S255F, V367F, K417T, N439K, S477R, S494P, N501T, Q613H, and P681R (relative to SEQ ID NO: 1).

We also analyzed these mutations in the context of each SET immunogen using *in silico* computational approaches to confirm that the combination of the mutations would not disrupt the function and/or structure of the Spike protein. During our analysis of the M153T mutation in SET1, we found that M153I or other conservative substitutions may be equally effective.

Changes outside of the epitope regions were minimized to avoid joining unintended conflicting mutational patterns, although two highly recurrent sites that were common covariation partners outside of the RBD and NTD were included in SET1: P26S and D80A. P681R, D69/70, and variation in 613/614, which may impact both neutralization potency and infectivity were also included. P681H is far more common than P681R globally, but the R form is associated with the variant of concern that is currently expanding in central Africa, and there is limited space for variant coverage in the 3 SET cocktail, so the R form with strong covariation connections to other sites was chosen for inclusion in SET3 to represent positive charge at this site. The Spike mutations included in each SET protein interspersed patterns of resistance in the local protein sequence and structural space; while trying to keep this as disperse as possible (**FIGs. 18-20**). One intent of this is to avoid so severely disrupting antibody contact regions that complete resistance is conferred, and the variant can no longer act to select more cross-reactive antibodies during via somatic mutation if antibody recognition of an epitope is completely lost. The other intent is to avoid local combinations of mutations that would inhibit proper protein folding. The final designs capture the most common known resistance mutations, in covariation patterns within SET proteins that are consistent natural co-variation patterns based on the output of xSpike. The potential for the introduction of incompatible mutations in the SET proteins was reviewed by using molecular dynamic modeling of Spike (Mansbach et al., 2020), and the mutations introduced into each SET design were observed to be compatible with, and to yield, stable proteins that can adopt native-like conformations.

In summary, the SET designs were intended for use in conjunction with the ancestral Wuhan strain (e.g., SEQ ID NO: 1). Together these spike variants capture the most critical patterns of resistance

mutations that enable escape from sera and subsets of monoclonal antibodies. SET1 covers related mutations found in the variant of concern, B.1.351, that is expanding in S. Africa, and B.1.1.28 that is common in Brazil. SET2 captures mutations in the B.1.1.7 variant that was first detected in the United Kingdom, and has rapidly come to dominate regional epidemics, and is predicted by some modelers to become the globally dominant form this spring. Three key mutations that were not anticipated to be conflicting with B.1.1.7 are found in B.1.429, the variant that originated in California, and it is rapidly spreading in North America; these were also integrated into the SET2 design. SET3 contains epitope region mutations in a variant that is rapidly spreading in Central Africa, and also found in Cambodia the UK, and North America, A23.1 (**FIGs. 21-22**). Each of the three SET design includes several additional common resistance mutations as well in the epitope regions and covariation patterns with other mutations found within the particular SET design that includes them. The individual mutations that in combination make up the each of the five variants of concern are also very often found as single mutations in a G614 backbone, or in conjunction with other covariation partners (**FIGs. 5-14**), and so were important recurring mutations in their own right, even outside the context of the variant of concern. Additions of resistance mutations can be introduced into backbones of different variant of concerns, as illustrated in **FIG. 22**.

Conclusion

In the spring of 2020, the coronavirus was advancing through an immunologically naïve population; a mutation arose that increased infectivity and was rapidly selected globally, as the virus was adapting to its new host. Although antibody resistance mutations remained very rare through the summer of 2020 (Korber et al., 2020), resistance mutations were beginning to arise in the summer/fall of 2020, in particular as the virus began to propagate through a population with convalescent sera present. In these subjects, resistance has greater potential to be favored, and variants of concern that carried multiple resistance mutations began to be sampled more frequently and to expand. By summer 2021, the virus will likely be moving through vaccinated populations. It was hypothesized that including commonly emergent resistance mutations in a SET vaccine will stimulate antibody responses that can tolerate those changes, and that the resistance forms sampled by the virus already can be used to predict common escape paths the virus will continue to use in the future.

Taken together, this example demonstrates the rational design of three different coronavirus spike protein variants (e.g., SET1 (**FIG. 15**), SET2 (**FIG. 16**), and SET3 (**FIG. 17**)) that can be used singly (e.g., SET1, SET2, or SET3), in combination (e.g., SET1 and SET2; SET1 and SET3; SET2 and SET3; or SET1, SET2, and SET3), and/or with the Wuhan coronavirus spike variant (e.g., SEQ ID NO:1; e.g., see U.S. Application number 63/066,147), e.g., as a monovalent or polyvalent vaccine, respectively, in the treatment of coronavirus infection.

Example 2. The Epigraph (EG) Vaccine Concept Applied To New and Emerging SARS-Cov-2 Variants

Implementation

To create a SARS-CoV-2 vaccine that would be practically useful in the context of a vector-based vaccine delivery, the ancestral form of the Spike plus three or four additional strains that would best cover

relevant contemporary variants were considered; a relatively small vaccine cocktail would also be simpler and more cost effective in the context of an RNA delivery strategy. By June 2021 it became apparent the ancestral form of the virus from Wuhan was being replaced by novel variants, and the dominant forms at that time were the Alpha B.1.1.7 variant, which was in the process of transitioning to the Delta variant, B.1.617.2. Thus four Epigraph (EG) immunogens (SEQ ID NOs: 40-43) and four Spike-modified EG immunogens (SEQ ID NOs: 65-68) were designed based on applying the Epigraph algorithm (Theiler *et al.*, *Statistics in Medicine* 37:181 (2018); Theiler *et al.*, *Scientific Reports* 6:33987 (2016)) to the full set of 300,000 sequences sampled in the 60 day period April 9 – June 9 2021, based on the June 18th GISAID data available sample. At the timeframe of this design, the B.1.1.7 lineage was most commonly sampled, and corresponds to Epigraph 1 (EG1), but many other variants were co-circulating globally, and all were well-covered. For example, the common Delta mutations are distributed between EG2 and EG3, and Gamma mutations between EG1 and EG2. The Epigraphs are serially designed to complementary artificial Spike sequences that best cover combinations in local regions which as considered surrogates for T cell epitopes and linear sections of B cell epitopes, optimized using a using a graph theory approach (Theiler *et al.*, *Statistics in Medicine* 37:181 (2018); Theiler *et al.*, *Scientific Reports* 6:33987 (2016)). They integrate local mutation patterns (preserving the juxtaposition of neighboring mutations) found in many different common variants into a small and manageable number of Spike backbones. EG2 best complements EG1, in terms of potential epitope coverage of the global population of viruses; EG3 is the best complement of (EG1 + EG2); EG4 is the best complement of (EG1 + EG2 + EG3). The intent of these immunogens is to compress the natural sequence diversity observed into a small number of vaccine antigens without pairing local combinations of amino acids that are structurally incompatible or rare in nature. Many mutations were co-circulating and there was clear convergence between mutational patterns in different lineages (**FIG. 24**); such recurrent mutations in past lineages, that are very well covered in the Epigraph designs, may have attributes that confer selective advantage and enable them to recur in lineages that will emerge in the future. Unlike the Spike SET design, which focus only on key epitope regions, the Epigraphs cover the diversity throughout the entire Spike protein (**FIG. 25** and **FIG. 26**), and so may facilitate both T cell and B cell response cross-reactivity with variants. Thus there are dozens of mutations spanning each of the full Epigraph sequences, as shown if **FIG. 26**. The full combination of mutations for each EG design (e.g., SEQ ID NOs: 40-43 and 65-68) are as follows:

SEQ ID NO: 40 and SEQ ID NO: 65 (both referred herein as EG1) contains $\Delta 69-70$, $\Delta 144$, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H, relative to SEQ ID NO: 35 and 1, respectively.

SEQ ID NO: 41 and SEQ ID NO: 66 (both referred herein as EG2) contains L5F, L18F, T20N, P26S, V36F, Q52R, D80G, T95I, I105V, L118F, V127F, D138Y, $\Delta 156-157$, R158G, T167S, D178H, R190S, I203V, D215G, A222V, I233V, $\Delta 242-244$, D253G, A262S, P272L, T284I, T299I, V308L, F318S, V227I, P337S, R346S, K356R, V367L, P384L, N394S, R408I, K417T, D427N, N439K, L452R, I468V, T478K, E484K, L513F, A522S, T531S, N540S, T549I, K558N, E583D, G594S, T604A, Q613H, D614G, V622F, P631S, S640F, H655Y, I670V, S691P, A701V, T732A, T747I, S758G, G769V, Q779K, D796H, P809S, L822F, A831V, A845S, T859N, I870V, A879S, F888L, A899S, I909V, L922F, S939F, D950N, T961M, I997V, T1006I, A1016V, T1027I, V1040F, L1049I, H1058Y, Q1071H, D1084Y, V1094F, V1104L,

I1130V, D1139H, D1153Y, P1162S, V1176F, K1191N, Q1208H, G1219V, V1228L, M1237I, S1252F, V1264L, and T1273I, relative to SEQ ID NO: 35 and 1, respectively.

SEQ ID NO: 42 and SEQ ID NO: 67 (both referred herein as EG3) contains P9L, T19R, T33I, H49Y, A67V, Δ69-70, D80A, S98F, S112L, V126A, G142D, W152R, S162I, L176F, L189F, D198Y, I210T, A222V, D228H, H245Y, W258L, V267L, E281Q, A292S, T307I, T323I, L335F, R346K, R357K, V367F, T376I, T385N, V395I, E406Q, K417N, D427Y, N440K, L452Q, K462T, E471Q, E484K, F490S, N501T, V510L, A520S, V534I, T547I, P561S, A570D, T572I, E583Q, V595I, T604I, A623S, T632S, N641S, A653V, A672V, P681R, A694V, S704L, T716V, M731I, M740V, N751D, T761I, A771S, E780D, T791I, S803A, P812S, T827I, D843N, K854N, A871V, T883I, A892V, M902I, V911I, A924S, D936Y, G946R, Q957R, S967N, V976F, K986N, T998I, T1009I, A1020S, V1033A, K1045N, A1056V, T1066N, A1078S, A1086S, H1101D, E1111K, G1124V, V1133F, D1146Y, P1162L, A1174V, R1185H, E1195Q, K1205N, G1219C, M1229I, T1238I, C1247F, D1260N, and H1271Y, relative to SEQ ID NO: 35 and 1, respectively.

SEQ ID NO: 43 and SEQ ID NO: 68 (both referred herein as EG4) contains V3G, S13I, L18F, A27S, V36I, S45F, L54F, W64R, G75V, T76I, P85S, S94F, D111N, V120L, E132Q, N148T, F157S, S172A, G181V, V193L, Y204H, L216F, V227A, R237K, Δ246-252, D253N, A263P, R273S, V289L, K300M, E309Q, V320F, P330S, G339S, A348S, V362F, S371T, V382L, N394H, R403K, Q414K, T430I, N440S, L452M, L461F, T470I, T478K, S494P, N501Y, Y505H, A522V, V534F, F543L, E554D, F565L, A570D, A575S, L585F, I598V, Q607K, D614G, N616S, D627G, T638I, A647S, Y660F, Q677H, A688V, M697I, A706V, T716I, T719I, T732I, T747N, T761R, V772I, V785I, K795R, P812L, V826L, K835R, A845V, T859I, M869I, A879V, A890V, A903S, V915I, S929I, S940F, D950H, V963A, V976F, S982A, K986R, R995G, Q1005H, R1014K, A1025G, K1038Q, M1050I, L1063F, K1073N, D1084E, H1101Y, D1118Y, D1127G, L1141W, E1150D, D1163, N1173S, E1182D, N1192S, E1202Q, I1216T, V1230L, C1243F, S1252P, P1263L, and T1273A, relative to SEQ ID NO: 35 and 1, respectively.

Conclusion

Taken together, this example demonstrates the rational design of four different coronavirus spike protein variants (e.g., EG1, EG2, EG3, and EG4) that can be used singly (e.g., EG1, EG2, EG3, or EG4), in combination (e.g., EG1 and EG2; EG1 and EG3; EG1 and EG4; EG2 and EG3, EG2 and EG4; EG3 and EG4; or EG1, EG2, and EG3; EG1, EG2, and EG4; EG1, EG3, and EG4; or EG1, EG2, EG3, and EG4; or any of the aforementioned combinations of EG1, EG2, EG3, and/or EG4 in combination with SET1, SET2, and/or SET3), and/or with the Wuhan coronavirus spike variant, or optimized variant thereof (e.g., SEQ ID NO: 1 or 35; e.g., see U.S. Application number 63/066,147), e.g., as a monovalent or polyvalent vaccine, respectively, in the treatment of coronavirus infection.

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Example 3. Administration of SET1-3 nucleic acid vaccines to a human subject

A human subject can be administered a nucleic acid vaccine (e.g., a DNA vaccine or an RNA vaccine) composition of this disclosure pre- or post-exposure to SARS-CoV-2 or a variant thereof according to the methods described herein. The human subject may be identified as being at high risk for infection, such as an individual who has or will be traveling to a region where infection of SARS-CoV-2 or a variant thereof is prevalent, or may be identified as presenting with symptoms consistent with an infection of SARS-CoV-2 or a variant thereof. The nucleic acid vaccine administered may include the nucleic acid sequence of SET1 (e.g., SEQ ID NOs: 7 or 10 or a variant thereof with 85% sequence identity thereto, or a complementary sequence thereof), SET2 (e.g., SEQ ID NOs: 8 or 11 or a variant thereof with 85% sequence identity thereto, or a complementary sequence thereof), and/or SET3, (e.g., SEQ ID NOs: 9 or 12 or a variant thereof with 85% sequence identity thereto, or a complementary sequence thereof), or any combination of SET1-3.

For example, a human identified as having a risk of infection of SARS-CoV-2 or a variant thereof and may be administered a nucleic acid vaccine (e.g., a DNA vaccine or an RNA vaccine) containing a nucleic acid molecule encoding a modified Spike (S) protein of SARS-CoV-2 or a variant thereof (e.g., a nucleic acid molecule of any one or more of SEQ ID NOs: 7-12, or a variant thereof with 85% sequence identity thereto, or a complementary sequence thereof), e.g., in an adenoviral vector (e.g., Ad26) at a dose of between 10 µg and 10 mg. The nucleic acid vaccine (e.g., a DNA vaccine or an RNA vaccine) may contain the nucleic acid sequence of SEQ ID NOs: 7, 8, 9, 10, 11, and/or 12.

The subject may also be administered a nucleic acid vaccine (e.g., a DNA vaccine or an RNA vaccine) containing a nucleic acid molecule encoding a 2019-nCoV (Wuhan/WIV04/2019) nucleic acid

(e.g., a nucleic acid molecule with the nucleic acid sequence of one of SEQ ID NOs: 5-6). The subject may also be administered a nucleic acid vaccine (e.g., a DNA vaccine or an RNA vaccine) containing a nucleic acid molecule encoding a modified membrane (MEM) and/or nucleocapsid (NUL) protein of SARS-CoV-2 or a variant thereof (e.g., a nucleic acid molecule of any one or more of SEQ ID NOs: 69-74, or a variant thereof with 85% sequence identity thereto, or a complementary sequence thereof), e.g., in an adenoviral vector (e.g., Ad26) at a dose of between 10 µg and 10 mg.

The subject can then be monitored for presentation of symptoms of 2019-nCoV infection, the resolution of symptoms, and/or the production of antibodies against the modified S, MEM, or NUL protein of SARS-CoV-2 or a variant thereof. If necessary, a second dose or additional doses of the nucleic acid vaccine(s) can be administered.

Example 4. Administration of EG1-4 nucleic acid vaccines to a human subject

A human subject can be administered a nucleic acid vaccine (e.g., a DNA vaccine or an RNA vaccine) composition of this disclosure pre- or post-exposure to SARS-CoV-2 or a variant thereof according to the methods described herein. The human subject may be identified as being at high risk for infection, such as an individual who has or will be traveling to a region where infection of SARS-CoV-2 or a variant thereof is prevalent, or may be identified as presenting with symptoms consistent with an infection of SARS-CoV-2 or a variant thereof. The nucleic acid vaccine administered may include, e.g., the nucleic acid sequence of EG1 (e.g., SEQ ID NOs: 44, 48, or 61, or a variant thereof with 85% sequence identity thereto, or a complementary sequence thereof), EG2 (e.g., SEQ ID NOs: 45, 49, or 62, or a variant thereof with 85% sequence identity thereto, or a complementary sequence thereof), EG3, (e.g., SEQ ID NOs: 46, 50, or 63, or a variant thereof with 85% sequence identity thereto, or a complementary sequence thereof), and/or EG4 (e.g., SEQ ID NOs: 47, 51, OR 64, or a variant thereof with 85% sequence identity thereto, or a complementary sequence thereof), or any combination of EG1-4.

For example, a human identified as having a risk of infection of SARS-CoV-2 or a variant thereof and may be administered a nucleic acid vaccine (e.g., a DNA vaccine or an RNA vaccine) containing a nucleic acid molecule encoding a modified Spike (S) protein of SARS-CoV-2 or a variant thereof (e.g., a nucleic acid molecule of any one or more of SEQ ID NOs: 44-51 AND 61-64, or a variant thereof with 85% sequence identity thereto, or a complementary sequence thereof), e.g., in an adenoviral vector (e.g., Ad26) at a dose of between 10 µg and 10 mg. The nucleic acid vaccine (e.g., a DNA vaccine or an RNA vaccine) may contain the nucleic acid sequence of one or more of SEQ ID NOs: 44-51 and 61-64.

The subject may also be administered a nucleic acid vaccine (e.g., a DNA vaccine or an RNA vaccine) containing a nucleic acid molecule encoding a 2019-nCoV (Wuhan/WIV04/2019) nucleic acid (e.g., a nucleic acid molecule with the nucleic acid sequence of one of SEQ ID NOs: 29 or 39). The subject may also be administered a nucleic acid vaccine (e.g., a DNA vaccine or an RNA vaccine) containing a nucleic acid molecule encoding a modified membrane (MEM) and/or nucleocapsid (NUL) protein of SARS-CoV-2 or a variant thereof (e.g., a nucleic acid molecule of any one or more of SEQ ID NOs: 69-74, or a variant thereof with 85% sequence identity thereto, or a complementary sequence thereof), e.g., in an adenoviral vector (e.g., Ad26) at a dose of between 10 µg and 10 mg.

The subject can then be monitored for presentation of symptoms of 2019-nCoV infection, the resolution of symptoms, and/or the production of antibodies against the modified S, MEM, or NUL protein of SARS-CoV-2 or a variant thereof. If necessary, a second dose or additional doses of the nucleic acid vaccine(s) can be administered.

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Example 5. Combinatorial administration of SET1-3 and EG1-4 nucleic acid vaccines to a human subject

A human subject can be administered a nucleic acid vaccine (e.g., a DNA vaccine or an RNA vaccine) composition of this disclosure pre- or post-exposure to SARS-CoV-2 or a variant thereof according to the methods described herein. The human subject may be identified as being at high risk for infection, such as an individual who has or will be traveling to a region where infection of SARS-CoV-2 or a variant thereof is prevalent, or may be identified as presenting with symptoms consistent with an infection of SARS-CoV-2 or a variant thereof. The nucleic acid vaccine administered may include the nucleic acid sequence of SET1 (e.g., SEQ ID NOs: 7 or 10 or a variant thereof with 85% sequence identity thereto, or a complementary sequence thereof), SET2 (e.g., SEQ ID NOs: 8 or 11 or a variant thereof with 85% sequence identity thereto, or a complementary sequence thereof), and/or SET3, (e.g., SEQ ID NOs: 9 or 12 or a variant thereof with 85% sequence identity thereto, or a complementary sequence thereof) being administered in combination with EG1 (e.g., SEQ ID NOs: 44, 48, or 61, or a variant thereof with 85% sequence identity thereto, or a complementary sequence thereof), EG2 (e.g., SEQ ID NOs: 45, 49, or 62, or a variant thereof with 85% sequence identity thereto, or a complementary sequence thereof), EG3, (e.g., SEQ ID NOs: 46, 50, or 63, or a variant thereof with 85% sequence identity thereto, or a complementary sequence thereof), and/or EG4 (e.g., SEQ ID NOs: 47, 51, or 64, or a variant thereof with 85% sequence identity thereto, or a complementary sequence thereof).

For example, a human identified as having a risk of infection of SARS-CoV-2 or a variant thereof may be administered a nucleic acid vaccine (e.g., a DNA vaccine or an RNA vaccine) containing a nucleic acid molecule encoding more than one modified Spike (S) proteins of SARS-CoV-2 or a variant thereof (e.g., a nucleic acid molecule of one or more of SEQ ID NOs: 7-12 as well as a nucleic acid molecule of one or more of SEQ ID NOs: 44-51 and 61-64, or any variants thereof with 85% sequence identity thereto, or a complementary sequence thereof), e.g., in an adenoviral vector (e.g., Ad26) at a dose of between 10 µg and 10 mg. The nucleic acid vaccine (e.g., a DNA vaccine or an RNA vaccine) may further contain the nucleic acid sequence of one or more of SEQ ID NOs: 7-12 as well as a nucleic acid sequence of one or more of SEQ ID NOs: 44-51 and 61-64.

The subject may also be administered a nucleic acid vaccine (e.g., a DNA vaccine or an RNA vaccine) containing a nucleic acid molecule encoding a 2019-nCoV (Wuhan/WIV04/2019) nucleic acid (e.g., a nucleic acid molecule with the nucleic acid sequence of one or more of SEQ ID NOs: 5-6 and 29-39). The subject may also be administered a nucleic acid vaccine (e.g., a DNA vaccine or an RNA vaccine) containing a nucleic acid molecule encoding a modified membrane (MEM) and/or nucleocapsid (NUL) protein of SARS-CoV-2 or a variant thereof (e.g., a nucleic acid molecule of any one or more of SEQ ID NOs: 69-74, or a variant thereof with 85% sequence identity thereto, or a complementary sequence thereof), e.g., in an adenoviral vector (e.g., Ad26) at a dose of between 10 µg and 10 mg.

The subject can then be monitored for presentation of symptoms of 2019-nCoV infection, the resolution of symptoms, and/or the production of antibodies against the modified S, MEM, or NUL protein of SARS-CoV-2 or a variant thereof. If necessary, a second dose or additional doses of the nucleic acid vaccine(s) can be administered.

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Example 6. Administration of SET1-3 immunogenic S proteins of SARS-CoV-2 polypeptide to a human subject

A human subject can be administered an immunogenic composition (e.g., containing a modified S protein of a coronavirus) of this disclosure pre- or post-exposure to SARS-CoV-2 or a variant thereof according to the methods described herein. The human subject may be identified as being at high risk for infection, such as an individual who has or will be traveling to a region where infection of SARS-CoV-2 or a variant thereof is prevalent, or may be identified as presenting with symptoms consistent with an infection of SARS-CoV-2 or a variant thereof.

For example, a human with an underlying health condition (e.g., one or more of hypertension, diabetes, and cardiovascular disease) may be identified as having a risk of infection of SARS-CoV-2 or a variant thereof and may be administered a modified S protein of SARS-CoV-2 or a variant thereof as an immunogen (e.g., a Spike protein of any one of SEQ ID NOs: 2-4, or a variant thereof with at least 85% sequence identity thereto), e.g., at a dose of between 10 µg and 10 mg. The immunogen is one or more polypeptides encoded by SEQ ID NOs: 2, 3, or 4.

The subject may also be administered a 2019-nCoV Spike protein as an immunogen (e.g., a Spike protein having the sequence of SEQ ID NO: 1, SEQ ID NO: 38, or SEQ ID NO: 35, or a variant thereof with at least 85% sequence identity thereto), e.g., at a dose of between 10 µg and 10 mg. The subject may also be administered an immunogen containing a modified membrane (MEM) and/or nucleocapsid (NUL) protein of SARS-CoV-2 or a variant thereof (e.g., a polypeptide of any one or more of SEQ ID NOs: 75-80, or a variant thereof with 85% sequence identity thereto), e.g., in an adenoviral vector (e.g., Ad26) at a dose of between 10 µg and 10 mg.

The subject can then be monitored for presentation of symptoms of infection of SARS-CoV-2 or a variant thereof, the resolution of symptoms, and/or the production of antibodies against the S, MEM, or NUL protein of SARS-CoV-2 or a variant thereof. If necessary, a second dose or additional doses of the immunogen(s) can be administered.

Example 7. Administration of EG1-4 immunogenic S proteins of SARS-CoV-2 polypeptide to a human subject

A human subject can be administered an immunogenic composition (e.g., containing a modified S protein of a coronavirus) of this disclosure pre- or post-exposure to SARS-CoV-2 or a variant thereof according to the methods described herein. The human subject may be identified as being at high risk for infection, such as an individual who has or will be traveling to a region where infection of SARS-CoV-2 or a variant thereof is prevalent, or may be identified as presenting with symptoms consistent with an infection of SARS-CoV-2 or a variant thereof.

For example, a human with an underlying health condition (e.g., one or more of hypertension, diabetes, and cardiovascular disease) may be identified as having a risk of infection of SARS-CoV-2 or a

variant thereof and may be administered a modified S protein of SARS-CoV-2 or a variant thereof as an immunogen (e.g., a Spike protein of any one of SEQ ID NOs: 40-43 and 65-68, or a variant thereof with at least 85% sequence identity thereto), e.g., at a dose of between 10 µg and 10 mg. The immunogen is one or more polypeptides encoded by SEQ ID NOs: 40-43 and 65-68. The subject may also be administered a 2019-nCoV Spike protein as an immunogen (e.g., a Spike protein having the sequence of SEQ ID NO: 35, or a variant thereof with at least 85% sequence identity thereto), e.g., at a dose of between 10 µg and 10 mg. The subject may also be administered an immunogen containing a modified membrane (MEM) and/or nucleocapsid (NUL) protein of SARS-CoV-2 or a variant thereof (e.g., a polypeptide of any one or more of SEQ ID NOs: 75-80, or a variant thereof with 85% sequence identity thereto), e.g., in an adenoviral vector (e.g., Ad26) at a dose of between 10 µg and 10 mg.

The subject can then be monitored for presentation of symptoms of infection of SARS-CoV-2 or a variant thereof, the resolution of symptoms, and/or the production of antibodies against the S, MEM, or NUL protein of SARS-CoV-2 or a variant thereof. If necessary, a second dose or additional doses of the immunogen(s) can be administered.

Example 8. Combinatorial administration of SET1-3 and EG1-4 immunogenic S proteins of SARS-CoV-2 polypeptides to a human subject

A human subject can be administered an immunogenic composition (e.g., containing a modified S protein of a coronavirus) of this disclosure pre- or post-exposure to SARS-CoV-2 or a variant thereof according to the methods described herein. The human subject may be identified as being at high risk for infection, such as an individual who has or will be traveling to a region where infection of SARS-CoV-2 or a variant thereof is prevalent, or may be identified as presenting with symptoms consistent with an infection of SARS-CoV-2 or a variant thereof.

For example, a human with an underlying health condition (e.g., one or more of hypertension, diabetes, and cardiovascular disease) may be identified as having a risk of infection of SARS-CoV-2 or a variant thereof and may be administered more than one modified S proteins of SARS-CoV-2 or a variant thereof as an immunogen (e.g., a Spike protein with the polypeptide sequence of one or more of SEQ ID NOs: 2-4 as well as a Spike protein with the polypeptide sequence of one or more of SEQ ID NOs: 40-43, or any variants thereof with at least 85% sequence identity thereto), e.g., at a dose of between 10 µg and 10 mg. The immunogens may be one or more polypeptides encoded by SEQ ID NOs: 7-12, 44-51, and/or 61-64.

The subject may also be administered a 2019-nCoV Spike protein as an immunogen (e.g., a Spike protein having the sequence of SEQ ID NO: 35, or a variant thereof with at least 85% sequence identity thereto), e.g., at a dose of between 10 µg and 10 mg. The subject may also be administered an immunogen containing a modified membrane (MEM) and/or nucleocapsid (NUL) protein of SARS-CoV-2 or a variant thereof (e.g., a polypeptide of any one or more of SEQ ID NOs: 75-80, or a variant thereof with 85% sequence identity thereto), e.g., in an adenoviral vector (e.g., Ad26) at a dose of between 10 µg and 10 mg.

The subject can then be monitored for presentation of symptoms of infection of SARS-CoV-2 or a variant thereof, the resolution of symptoms, and/or the production of antibodies against the S, MEM, or

NUL protein of SARS-CoV-2 or a variant thereof. If necessary, a second dose or additional doses of the immunogen(s) can be administered.

Example 9. Administration of antibodies against SET1-3 S proteins of SARS-CoV-2 or a variant thereof to a human subject at risk of, or presenting symptoms of, infection by SARS-CoV-2 or a variant thereof

A human subject infected with SARS-CoV-2 or a variant thereof or identified as having a risk of infection by SARS-CoV-2 or a variant thereof (e.g., a subject that has traveled to a region where infection of SARS-CoV-2 or a variant thereof is prevalent) can be administered an antibody against a modified S protein of SARS-CoV-2 or a variant thereof that binds an epitope within the amino acid sequence of any one of SEQ ID NOs: 2-4, such as the NTD and/or RBD region of SEQ ID NOs: 2-4, and, in particular, an epitope containing one or more of the SET1, SET2, and SET3 mutations. For example, the antibody may have been generated against one or more of the polypeptides of SEQ ID NOs: 2-4. The antibody composition can be administered to the subject at a dose of the antibody of between 1-1,000 mg. In addition, administration can optionally include 1-1000 mg of an antibody against a modified membrane (MEM) and/or nucleocapsid (NUL) protein of SARS-CoV-2 or a variant thereof that binds to an epitope with the amino acid sequence of any one of SEQ ID NOs: 75-80, or a variant thereof with 85% sequence identity thereto. The antibody composition may be administered to the subject as a prophylactic therapy, e.g., prior to or post-exposure to a SARS-CoV-2 or a variant thereof. The subject can then be monitored for presentation of symptoms of infection of SARS-CoV-2 or a variant thereof or the resolution of symptoms. If necessary, a second dose or subsequent doses of the antibody composition can be administered to the subject.

Example 10. Administration of antibodies against EG1-4 S proteins of SARS-CoV-2 or a variant thereof to a human subject at risk of, or presenting symptoms of, infection by SARS-CoV-2 or a variant thereof

A human subject infected with SARS-CoV-2 or a variant thereof or identified as having a risk of infection by SARS-CoV-2 or a variant thereof (e.g., a subject that has traveled to a region where infection of SARS-CoV-2 or a variant thereof is prevalent) can be administered an antibody against a modified S protein of SARS-CoV-2 or a variant thereof that binds an epitope within the amino acid sequence of any one of SEQ ID NOs: 40-43 and/or 65-68, such as the NTD and/or RBD region of SEQ ID NOs: 40-43, and, in particular, an epitope containing one or more of the EG1, EG2, EG3 and EG4 mutations. For example, the antibody may have been generated against one or more of the polypeptides of SEQ ID NOs: 40-43 and 65-68. The antibody composition can be administered to the subject at a dose of the antibody of between 1-1,000 mg. In addition, administration can optionally include 1-1000 mg of an antibody against a modified membrane (MEM) and/or nucleocapsid (NUL) protein of SARS-CoV-2 or a variant thereof that binds to an epitope with the amino acid sequence of any one of SEQ ID NOs: 75-80, or a variant thereof with 85% sequence identity thereto. The antibody composition may be administered to the subject as a prophylactic therapy, e.g., prior to or post-exposure to a SARS-CoV-2 or a variant thereof. The subject can then be monitored for presentation of symptoms of infection of SARS-CoV-2 or

a variant thereof or the resolution of symptoms. If necessary, a second dose or subsequent doses of the antibody composition can be administered to the subject.

Example 11. Combinatorial administration of antibodies against SET1-3 and EG1-4 S proteins of SARS-CoV-2 or a variant thereof to a human subject at risk of, or presenting symptoms of, infection by SARS-CoV-2 or a variant thereof

A human subject infected with SARS-CoV-2 or a variant thereof or identified as having a risk of infection by SARS-CoV-2 or a variant thereof (e.g., a subject that has traveled to a region where infection of SARS-CoV-2 or a variant thereof is prevalent) can be administered an antibody against a modified S protein of SARS-CoV-2 or a variant thereof that binds an epitope within the amino acid sequence of any one of SEQ ID NOs: 2-4, 40-43, and/or 65-68, such as the NTD and/or RBD region of SEQ ID NOs: 2-4, 40-43, and/or 65-68, and, in particular, an epitope containing one or more of the SET1, SET2, SET3, EG1, EG2, EG3 and EG4 mutations. For example, the antibody may have been generated against one or more of the polypeptides of SEQ ID NOs: 2-4, 40-43, and/or 65-68. The antibody composition can be administered to the subject at a dose of the antibody of between 1-1,000 mg. In addition, administration can optionally include 1-1000 mg of an antibody against a modified membrane (MEM) and/or nucleocapsid (NUL) protein of SARS-CoV-2 or a variant thereof that binds to an epitope with the amino acid sequence of any one of SEQ ID NOs: 75-80, or a variant thereof with 85% sequence identity thereto. The antibody composition may be administered to the subject as a prophylactic therapy, e.g., prior to or post-exposure to a SARS-CoV-2 or a variant thereof. The subject can then be monitored for presentation of symptoms of infection of SARS-CoV-2 or a variant thereof or the resolution of symptoms. If necessary, a second dose or subsequent doses of the antibody composition can be administered to the subject.

Other Embodiments

All publications, patents, and patent applications mentioned in this specification are incorporated herein by reference to the same extent as if each independent publication or patent application was specifically and individually indicated to be incorporated by reference.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations following, in general, the principles and including such departures from the invention that come within known or customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth, and follows in the scope of the claims.

Other embodiments are within the claims.

CLAIMS

1. An isolated nucleic acid molecule comprising a nucleotide sequence that encodes a polypeptide having at least 85% sequence identity to amino acids 18-1208 of any one of SEQ ID NOs: 1 to 4 or a complementary sequence thereof, wherein the polypeptide has at least one of the following mutations: S13I, L18F, T20N, P26S, Δ69-70, D80A, D80Y, L141F, Δ144, W152C, M153T, M153I, F157L, Δ242-244, D253G, S255F, A262S, V367F, K417N, K417T, N439K, L452R, Y453F, S477N, S477R, E484K, S494P, N501T, N501Y, Q613H, D614G, and P681R relative to the amino acid sequence of SEQ ID NO: 1 or 35.
2. The nucleic acid molecule of claim 1, wherein:
 - a) the polypeptide is capable of eliciting an immune response in a subject; or
 - b) the polypeptide has at least 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity to, or the polypeptide sequence of, any one of SEQ ID NOs: 1 to 4.
3. The nucleic acid molecule of claim 1 or 2, wherein the polypeptide comprises two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, twenty-four, twenty-five, twenty-six, or twenty-seven of the mutations, wherein preferably the polypeptide comprises eight to twelve of the mutations.
4. The nucleic acid molecule any one of claims 1-3, wherein the polypeptide has:
 - (a) one or more of the mutations: L18F, T20N, P26S, D80A, M153T, M153I, Δ242-244, K417N, Y453F, E484K, N501Y, and D614G relative to the amino acid sequence of SEQ ID NO: 1 or 35;
 - (b) one or more of the mutations: S13I, Δ69-70, Δ144, W152C, D253G, A262S, L452R, S477N, and D614G relative to the amino acid sequence of SEQ ID NO: 1 or 35; or
 - (c) one or more of the mutations: D80Y, L141F, F157L, S255F, V367F, K417T, N439K, S477R, S494P, N501T, Q613H, D614G, and P681R relative to the amino acid sequence of SEQ ID NO: 1 or 35.
5. The nucleic acid molecule of claim 4, wherein:
 - (i) the polypeptide of (a) comprises two, three, four, five, six, seven, eight, nine, ten, or eleven of the mutations;
 - (ii) the polypeptide of (b) comprises two, three, four, five, six, seven, eight, or nine of the mutations; or
 - (iii) the polypeptide of (c) comprises two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, or thirteen of the mutations.
6. The nucleic acid molecule of claim 5, wherein the polypeptide of (a) comprises each of the mutations: L18F, T20N, P26S, D80A, M153T, Δ242-244, K417N, Y453F, E484K, N501Y, and D614G relative to the amino acid sequence of SEQ ID NO: 1 or 35.

7. The nucleic acid molecule of claim 5, wherein the polypeptide of (b) comprises each of the mutations: S13I, Δ69-70, Δ144, W152C, D253G, A262S, L452R, S477N, and D614G relative to the amino acid sequence of SEQ ID NO: 1 or 35.
8. The nucleic acid molecule of claim 5, wherein the polypeptide of (c) comprises each of mutations: D80Y, L141F, F157L, S255F, V367F, K417T, N439K, S477R, S494P, N501T, Q613H, D614G, and P681R relative to the amino acid sequence of SEQ ID NO: 1 or 35.
9. The nucleic acid molecule of any one of claims 1-6, wherein the polypeptide has the amino acid sequence of SEQ ID NO: 2.
10. The nucleic acid molecule of any one of claims 1-5 or 7, wherein the polypeptide has the amino acid sequence of SEQ ID NO: 3.
11. The nucleic acid molecule of any one of claims 1-5 or 8, wherein the polypeptide has the amino acid sequence of SEQ ID NO: 4.
12. The nucleic acid molecule of any one of claims 1-5, wherein the nucleotide sequence has at least 85% sequence identity to all or a portion of any one of SEQ ID NOs: 7 to 9, or a complementary sequence thereof.
13. The nucleic acid molecule of claim 12, wherein the nucleic acid sequence has at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the nucleic acid sequence of any one of SEQ ID NOs: 7 to 9, or a complementary sequence thereof.
14. The isolated nucleic acid molecule of claim 12 or 13, wherein the nucleic acid molecule, or a portion thereof, is capable of eliciting an immune response in a subject.
15. The nucleic acid molecule of any one of claims 12-14, wherein the nucleic acid molecule has the nucleic acid sequence of SEQ ID NO: 7.
16. The nucleic acid molecule of any one of claims 12-14, wherein the nucleic acid molecule has the nucleic acid sequence of SEQ ID NO: 8.
17. The nucleic acid molecule of any one of claims 12-14, wherein the nucleic acid molecule has the nucleic acid sequence of SEQ ID NO: 9.
18. An isolated polypeptide encoded by the nucleic acid molecule of any one of claims 1-17.

19. The polypeptide of claim 18, wherein said polypeptide has at least 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity to, or the amino acid sequence of, any one of SEQ ID NOs: 2 to 4 and at least one of the mutations.
20. The polypeptide of claim 18 or 19, wherein the polypeptide, or a portion thereof, is capable of eliciting an immune response in a subject.
21. The polypeptide of any one of claims 18-20, wherein the polypeptide has the amino acid sequence of SEQ ID NO: 2.
22. The polypeptide of any one of claims 18-20, wherein the polypeptide has the amino acid sequence of SEQ ID NO: 3.
23. The polypeptide of any one of claims 18-20, wherein the polypeptide has the amino acid sequence of SEQ ID NO: 4.
24. An isolated vector comprising one or more of the nucleic acid molecules of any one of claims 1-17.
25. The vector of claim 24, wherein the vector is replication-defective.
26. The vector of claim 24 or 25, wherein the vector is a mammalian, bacterial, or viral vector.
27. The vector of claim 26, wherein the vector is an expression vector.
28. The vector of claim 26, wherein the viral vector is a virus selected from the group consisting of a retrovirus, adenovirus, adeno-associated virus, parvovirus, coronavirus, negative strand RNA viruses, orthomyxovirus, rhabdovirus, paramyxovirus, positive strand RNA viruses, picornavirus, alphavirus, double stranded DNA viruses, herpesvirus, Epstein-Barr virus, cytomegalovirus, fowlpox, and canarypox.
29. The vector of claim 28, wherein the vector is an adenovirus.
30. The vector of claim 28, wherein the adenovirus is selected from the group consisting of Ad26, Ad52, Ad59, Ad2, Ad5, Ad11, Ad12, Ad24, Ad34, Ad35, Ad40, Ad48, Ad49, Ad50, and Pan9, wherein preferably the adenovirus is Ad26.
31. The vector of claim 30, wherein the Ad52 is a rhesus Ad52 or the Ad59 is a rhesus Ad59.
32. An isolated antibody that specifically binds to the polypeptide of any one of claims 18-23.

33. The antibody of claim 32, wherein the antibody is generated by administering the nucleic acid molecule of any one of claims 1-17, the polypeptide of any one of claims 18-23, or the vector of any one of claims 24-31 to a mammal.
34. The antibody of claim 33, wherein the nucleic acid molecule comprises a nucleic acid sequence of any one of SEQ ID NOs: 7 to 9 or a variant thereof with at least 85% sequence identity thereto, or a complementary sequence thereof, the polypeptide comprises the amino acid sequence of any one of SEQ ID NOs: 2 to 4, or a variant thereof with at least 85% sequence identity thereto, or the vector is an Ad26 vector comprising a nucleic acid sequence of any one of SEQ ID NOs: 7 to 9 or a variant thereof with at least 85% sequence identity thereto, or a complementary sequence thereof.
35. The antibody of claim 33 or 34, wherein the mammal is a human, cow, goat, mouse, or rabbit.
36. The antibody of any one of claims 32-35, wherein the antibody is humanized.
37. The antibody of any one of claims 32-36, wherein the antibody is an IgG.
38. The antibody of any one of claims 32-37, wherein the antibody is a bis-Fab, Fv, Fab, Fab'-SH, F(ab')₂, a diabody, a linear antibody, or a scFV.
39. A method of producing an antibody comprising administering one or more of the nucleic acid molecules of any one of claims 1-17, one or more of the polypeptides of any one of claims 18-23, and/or one or more of the vectors of any one of claims 24-31 to a subject to elicit production of neutralizing antisera in said subject.
40. The method of claim 39, wherein the one or more nucleic acid molecules comprises a nucleic acid sequence of any one of SEQ ID NOs: 7 to 9 or a variant thereof with at least 85% sequence identity thereto, or a complementary sequence thereof, the one or more polypeptides comprise the amino acid sequence of any one of SEQ ID NOs: 2 to 4, or a variant thereof with at least 85% sequence identity thereto, or the one or more vectors comprise an Ad26 vector comprising a nucleic acid sequence of any one of SEQ ID NOs: 7 to 9 or a variant thereof with at least 85% sequence identity thereto, or a complementary sequence thereof, wherein preferably the method elicits the production of neutralizing anti-2019-nCoV antisera after administration to said subject.
41. An antibody produced by the method of claim 39.
42. The antibody of any one of claims 32-38 and 41, wherein the antibody binds to an epitope within a coronavirus spike protein, such as a coronavirus spike protein that comprises the amino acid sequence of any one of SEQ ID NOs: 1-4, wherein preferably the antibody binds to an epitope within the N-terminal domain (NTD) or the receptor binding domain (RBD) of the coronavirus spike protein, for example, the antibody specifically binds to a coronavirus spike protein that contains one or more of the mutations set

forth in claim 1 and/or the antibody neutralizes one or more of the B.1.1.7, B.1.429, B.1.1.28, B.1.351, or A23.1 lineages of 2019-nCoV .

43. A composition comprising the nucleic acid molecule of any one of claims 1-17, the polypeptide of any one of claims 18-23, the vector of any one of claims 24-31, or the antibody of any one of claims 32-38, 41 and 42.

44. The composition of claim 43, further comprising a pharmaceutically acceptable carrier, excipient, or diluent.

45. The composition of claim 43 or 44, further comprising an adjuvant or an immunostimulatory agent.

46. An immunogenic composition comprising the nucleic acid molecule of any one of claims 1-17, the polypeptide of any one of claims 18-23, the vector of any one of claims 24-31, or the antibody of any one of claims 32-38, 41, and 42.

47. The immunogenic composition of claim 46, wherein the immunogenic composition is a vaccine.

48. The immunogenic composition of claim 47, wherein the vaccine is a monovalent or a polyvalent vaccine.

49. The immunogenic composition of claim 47 or 48, wherein the immunogenic composition is capable of treating or reducing the risk of a coronavirus infection, such as, for example, infection by a 2019-nCoV virus or a variant thereof, in a subject in need thereof.

50. The immunogenic composition of any one of claims 46-49, wherein said immunogenic composition elicits production of neutralizing anti-2019-nCoV antisera in said subject.

51. The immunogenic composition of any one of claims 46-50, wherein the subject is a mammal.

52. The immunogenic composition of claim 51, wherein the mammal is a human.

53. The immunogenic composition of claim 52, wherein the human has an underlying health condition.

54. The immunogenic composition of claim 53, wherein the underlying health condition is hypertension, diabetes, or cardiovascular disease.

55. A method of identifying, diagnosing, and/or predicting the susceptibility of a subject to a coronavirus infection comprising determining whether the subject has a protective level of a broadly neutralizing anti-coronavirus antibody (bNAb) against two or more lineages of coronavirus (such as an anti-Spike antibody) in a sample from the subject, wherein preferably the protective level is:

- (i) a level that is at or above a titer of at least about 70, as determined using a pseudovirus neutralization assay; or
- (ii) a level that is at or above a titer of at least about 25, as determined using a live virus neutralization assay; or
- (iii) a level that is at least 80% of a median level of an anti-coronavirus antibody in a cohort of convalescent humans, as determined by a pseudovirus neutralization assay or live virus neutralization assay.

56. The method of claim 55, wherein the method further comprises administering an effective amount of one or more of the compositions of any one of claims 43-45 or one or more of the immunogenic compositions of any one of claims 46-54 to the subject having less than a protective level of the bNAb.

57. The method of claim 55 or 56, wherein the method further comprises identifying a subclass and/or an effector function of the bNAb (e.g., the broadly neutralizing anti-Spike antibody).

58. The method of claim 57, wherein:

- (a) the subclass is IgM, IgA, IgG1, IgG2, IgG3, or FcγR2A; and/or
- (b) the effector function is antibody-dependent neutrophil phagocytosis (ADNP), antibody-dependent complement deposition (ADCD), antibody-dependent monocyte cellular phagocytosis (ADCP), or antibody-dependent NK cell activation.

59. The method of any one of claims 55-58, wherein the sample is a bodily fluid from the subject, wherein preferably the bodily fluid is blood.

60. The method of any one of claims 55-59, wherein the coronavirus is 2019-nCoV.

61. The method of any one of claims 55-60, wherein the two or more lineages of coronavirus are selected from the group consisting of B.1.1.7, B.1.429, B.1.1.28, B.1.351, and A23.1.

62. A method of treating or reducing the risk of a coronavirus infection in a subject in need thereof, comprising administering a therapeutically effective amount of the composition of any one of claims 43-45 or the immunogenic composition of any one of claims 44-52 to said subject.

63. The method of claim 62, comprising administering a therapeutically effective amount of more than one said composition of any one of claims 43-45 or more than one said immunogenic composition of any one of claims 44-52 to said subject.

64. The method of claim 63, comprising administering a therapeutically effective amount of three different types of the composition of any one of claims 43-45 or three different types of the immunogenic composition of any one of claims 44-52 to said subject.

65. The method of any one of claims 62-64, further comprising administering:

- a) an amount of a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 5, nucleotides 19-3837 of SEQ ID NO: 6, or the nucleotide sequence of SEQ ID NO: 6, and/or
- b) a polypeptide comprising the amino acid sequence of SEQ ID NO: 1 or a polypeptide having at least 85% sequence identity to amino acids 18-1208 of SEQ ID NO: 1.

66. The method of claim 65, comprising administering:

- i) an Ad26 vector comprising the nucleic acid molecule; and/or
- ii) an Ad26 vector comprising a nucleic acid molecule that encodes the polypeptide.

67. The method of any one of claims 62-66, further comprising measuring an anti-coronavirus antibody (e.g., an anti-Spike antibody) level in the subject.

68. The method of claim 67, wherein the anti-coronavirus antibody level in the subject is measured before and/or after administration of the composition or the immunogenic composition.

69. The method of claim 68, wherein the anti-coronavirus antibody level in the subject is measured one or more times over about 1, 2, 3, 4, 5, or 6 days, 1, 2, 3, 4, 5, 6, or 7 weeks, 2, 3, 4, 5, or 6 months, 1, 2, 3, 4, or 5 years after administration.

70. The method of any one of claims 62-69, wherein the anti-coronavirus antibody level of the subject is below a protective level and wherein the method further comprises re-administering the composition of any one of claims 43-45 or the immunogenic composition of any one of claims 46-54 to said subject or administering a different anti-coronavirus composition to the subject.

71. The method of claim 70, wherein the protective level is a level sufficient to reduce symptoms or duration of a coronavirus-mediated disease.

72. The method of claim 70 or 71, wherein the protective level is:

- (i) a level that is at or above a titer of at least about 70, as determined using a pseudovirus neutralization assay; or
- (ii) a level that is at or above a titer of at least about 25, as determined using a live virus neutralization assay; or
- (iii) a level that is at least 80% of a median level of an anti-coronavirus antibody in a cohort of convalescent humans, as determined by a pseudovirus neutralization assay or live virus neutralization assay.

73. The method of any one of claims 62-72, wherein the coronavirus infection is infection by 2019-nCoV, wherein preferably said 2019-nCoV is of the lineage B.1.1.7, B.1.429, B.1.1.28, B.1.351, or A23.1.

74. A method of reducing a coronavirus-mediated activity in a subject infected with a 2019-nCoV or a variant thereof, comprising administering a therapeutically effective amount of the composition of any one of claims 43-45 or the immunogenic composition of any one of claims 46-54 to said subject.

75. The method of claim 74, comprising administering a therapeutically effective amount of two or more types of the composition of any one of claims 43-45 or two or more types of the immunogenic composition of any one of claims 46-54 to said subject.

76. The method of claim 75, comprising administering a therapeutically effective amount of three different types of the composition of any one of claims 43-45 or three different types of the immunogenic composition of any one of claims 46-54 to said subject.

77. The method of any one of claims 74-76, further comprising administering:

- a) an amount of a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 5, nucleotides 19-3837 of SEQ ID NO: 6, or the nucleotide sequence of SEQ ID NO: 6, and/or
- b) a polypeptide comprising the amino acid sequence of SEQ ID NO: 1 or a polypeptide having at least 85% sequence identity to amino acids 18-1208 of SEQ ID NO: 1.

78. The method of claim 77, comprising administering:

- i) an Ad26 vector comprising the nucleic acid molecule; and/or
 - ii) an Ad26 vector comprising a nucleic acid molecule that encodes the polypeptide,
- wherein preferably the therapeutically effective amount of the composition or the immunogenic composition is sufficient to produce a log serum anti-Spike antibody titer greater than 2 in a subject, as measured by an ELISA assay.

79. The method of any one of claims 74-78, wherein the therapeutically effective amount is between 15 µg and 300 µg of the one or more of compositions of any one of claims 43-45 or the one or more immunogenic compositions of any one of claims 46-54.

80. The method of any one of claims 74-79, wherein said activity is viral titer, viral spread, infection, or cell fusion.

81. The method of claim 80, wherein said viral titer is decreased after administration of the one or more compositions of any one of claims 43-45 or the one or more immunogenic compositions of any one of claims 46-54.

82. The method of claim 81, wherein the viral titer is decreased by 25% or more.

83. The method of claim 82, wherein the viral titer is decreased by 50% or more.

84. The method of claim 83, wherein the viral titer is decreased by 75% or more.

85. The method of claim 84, wherein the coronavirus is undetectable after said administration.
86. The method of any one of claims 74-85, wherein said administering occurs prior to exposure to the coronavirus.
87. The method of claim 86, wherein said administering occurs at least 1 hour prior to exposure to said coronavirus.
88. The method of claim 87, wherein said administering occurs at least 1 week, 1 month, or a year prior to exposure to said coronavirus.
89. The method of any one of claims 74-85, wherein said administering occurs post-exposure to the coronavirus.
90. The method of claim 89, wherein said administering occurs at least 15 minutes post-exposure to said coronavirus.
91. The method of claim 90, wherein said administering occurs at least 1 hour, 1 day, 1 week, post-exposure to said coronavirus.
92. The method of any one of claims 74-91, wherein said subject is administered at least one dose of the one or more compositions or the one or more immunogenic compositions.
93. The method of claim 92, wherein said subject is administered at least two doses of the one or more compositions or the one or more immunogenic compositions.
94. The method of claim 93, wherein the composition or the immunogenic composition is administered to said subject as a prime, a boost, or as a prime-boost.
95. The method of any one of claims 74-94, wherein the composition or the immunogenic composition is administered intramuscularly, intravenously, intradermally, percutaneously, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intranasally, intravitreally, intravaginally, intrarectally, topically, intratumorally, peritoneally, subcutaneously, subconjunctively, intravesicularly, mucosally, intrapericardially, intraumbilically, intraocularly, orally, topically, locally, by inhalation, by injection, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, by catheter, by lavage, by gavage, in creams, or in lipid compositions.
96. The method of any one of claims 74-95 wherein the subject is a mammal.

97. The method of claim 96, wherein the mammal is a human.
98. The method of claim 97, wherein the human has an underlying health condition.
99. The method of claim 98, wherein the underlying health condition is hypertension, diabetes, or cardiovascular disease.
100. The method of any one of claims 74-99, wherein the method promotes an immune response in said subject.
101. The method of claim 100, wherein the immune response is a humoral immune response.
102. The method of claim 101, wherein the humoral immune response is an IgG response.
103. A composition for use in treating or reducing the risk of a coronavirus infection, such as a 2019-nCoV infection, in a subject in need thereof, comprising a therapeutically effective amount of one or more the compositions of any one of claims 43-45 or one or more the immunogenic compositions of any one of claims 46-54.
104. A composition for use in reducing a coronavirus-mediated activity in a subject infected with a 2019-nCoV or a variant thereof, comprising a therapeutically effective amount of the composition of any one of claims 43-45 or the immunogenic composition of any one of claims 46-54.
105. The composition for use according to claim 104, comprising a therapeutically effective amount of two or more types of the composition of any one of claims 43-45 or two or more types of the immunogenic composition of any one of claims 46-54.
106. The composition for use according to claim 105, comprising a therapeutically effective amount of three different types of the composition of any one of claims 43-45 or three different types of the immunogenic composition of any one of claims 46-54.
107. The composition for use according to any one of claims 104-106, for administration with
- a) an amount of a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 5, nucleotides 19-3837 of SEQ ID NO: 6, or the nucleotide sequence of SEQ ID NO: 6, and/or
 - b) a polypeptide comprising the amino acid sequence of SEQ ID NO: 1 or a polypeptide having at least 85% sequence identity to amino acids 18-1208 of SEQ ID NO: 1.
108. The composition for use according to claim 107, wherein said composition comprises:
- i) an Ad26 vector comprising the nucleic acid molecule of the composition or the immunogenic composition; and/or

ii) an Ad26 vector comprising a nucleic acid molecule that encodes the polypeptide of the composition or the immunogenic composition.

109. A method of manufacturing an immunogenic composition for treating or reducing the risk of a coronavirus infection in a subject in need thereof, said method comprising the steps of:

(a) admixing at least one of the nucleic acid molecules of any one of claims 1-17, at least one of the polypeptides of any one of claims 18-23, at least one of the vectors of any one of claims 24-31, at least one of the compositions of any one of claims 43-45, and at least one of the antibodies of any one of claims 32-38, 41, and 42 with a pharmaceutically acceptable carrier, excipient, or diluent to form the immunogenic composition; and

(b) placing the immunogenic composition in a container.

110. A kit comprising:

(a) a first container comprising at least one said nucleic acid molecule of any one of claims 1-17, at least one said polypeptide of any one of claims 18-23, at least one said vector of any one of claims 24-31, at least one said composition of any one of claims 43-45, at least one said immunogenic composition of any one of claims 46-54, and/or at least one said antibody of any one of claims 32-38 or 41-42;

(b) instructions for use thereof; and optionally

(c) a second container comprising a pharmaceutically acceptable carrier, excipient, or diluent.

111. The kit of claim 110, wherein the first container further comprises a pharmaceutically acceptable carrier, excipient, or diluent.

112. A kit of claim 110 or 111, wherein the kit optionally includes an adjuvant and/or an immunostimulatory agent.

113. A composition comprising:

(a) at least two different nucleic acid molecules, wherein each of the nucleic acid molecules encodes a polypeptide having at least 85% sequence identity to amino acids 18-1208 of any one of SEQ ID NOs: 40 to 43 and 65 to 68, or a complementary sequence thereof, wherein the polypeptides do not comprise amino acids 18-1208 of SEQ ID NO: 35; or

(b) at least two different polypeptides having at least 85% sequence identity to amino acids 18-1208 of any one of SEQ ID NOs: 40 to 43 and 65 to 68, wherein the polypeptides do not comprise amino acids 18-1208 of SEQ ID NO: 35.

114. The composition of claim 113, wherein the at least two different nucleic acid molecules of the composition are selected from the group consisting of:

(a) a nucleic acid molecule having the nucleic acid sequence of any one of SEQ ID NOs: 44-47, or a variant thereof with at least 85% sequence identity thereto;

(b) a nucleic acid molecule having the nucleic acid sequence of any one of SEQ ID NOs: 48-51, or a variant thereof with at least 85% sequence identity thereto, and/or

(c) a nucleic acid molecule having the nucleic acid sequence of any one of SEQ ID NOs: 61-64, or a variant thereof with at least 85% sequence identity thereto.

115. The composition of claim 114, wherein the composition comprises three or more different nucleic acid molecules of (a), (b), and/or (c).

116. The composition of any one of claims 113 to 115, wherein the nucleic acid molecules are inserted into one or more nucleic acid vectors.

117. The composition of claim 116, wherein the nucleic acid molecules are tandemly inserted into a single said nucleic acid vector.

118. The composition of any one of claims 113 to 115, wherein one or more of the nucleic acid molecules are inserted into different nucleic acid vectors.

119. The composition of claim 116 or 117, wherein the nucleic acid vector comprises:

- (a) each of the nucleic acid molecules of SEQ ID NOs: 44-46, and optionally SEQ ID NO: 47;
- (b) each of the nucleic acid molecules of SEQ ID NOs: 48-50, and optionally SEQ ID NO: 51; or
- (c) each of the nucleic acid molecules of SEQ ID NOs: 61-63, and optionally SEQ ID NO: 64.

120. The composition of any one of claims 116 to 119, wherein the one or more vectors are replication defective.

121. The composition of claim 120, wherein the one or more vectors are a mammalian, bacterial, or viral vector.

122. The composition of claim 121, wherein the one or more vectors are an expression vector.

123. The composition of claim 121, wherein the viral vector is a virus selected from the group consisting of a retrovirus, adenovirus, adeno-associated virus, parvovirus, coronavirus, negative strand RNA viruses, orthomyxovirus, rhabdovirus, paramyxovirus, positive strand RNA viruses, picornavirus, alphavirus, double stranded DNA viruses, herpesvirus, Epstein-Barr virus, cytomegalovirus, fowlpox, and canarypox.

124. The composition of claim 123, wherein the vector is an adenovirus.

125. The composition of claim 124, wherein the adenovirus is selected from the group consisting of Ad26, Ad52, Ad59, Ad2, Ad5, Ad11, Ad12, Ad24, Ad34, Ad35, Ad40, Ad48, Ad49, Ad50, and Pan9, wherein preferably the adenovirus is Ad26.

126. The composition of claim 125, wherein the Ad52 is a rhesus Ad52 or the Ad59 is a rhesus Ad59.

127. The composition of any one of claims 113-126, wherein the composition is an immunogenic composition.
128. The composition of claim 127, wherein the immunogenic composition is a vaccine.
129. The composition of claim 128, wherein the vaccine is a monovalent or a polyvalent vaccine.
130. The composition of claim 128 or 129, wherein the immunogenic composition is capable of treating or reducing the risk of a coronavirus infection, such as, for example, infection by a 2019-nCoV virus or a variant thereof, in a subject in need thereof.
131. The composition of any one of claims 127-130, wherein the immunogenic composition elicits production of neutralizing anti-2019-nCoV antisera in said subject.
132. The composition of claim 130 or 131, wherein the subject is a mammal.
133. The composition of claim 132, wherein the mammal is a human.
134. The composition of claim 133, wherein the human has an underlying health condition.
135. The composition of claim 134, wherein the underlying health condition is hypertension, diabetes, or cardiovascular disease.
136. A composition comprising two or more different antibodies that specifically binds to the polypeptide of any one of SEQ ID NOs: 40-43 and 65-68.
137. The composition of claim 136, wherein the antibodies are generated in a mammal by administering:
- (a) a nucleic acid molecule comprising the sequence of two or more of SEQ ID NOs: 44 to 51 and 61 to 64, or a variant thereof with at least 85% sequence identity thereto, or a complementary sequence thereof;
 - (b) a polypeptide comprising the sequence of two or more of SEQ ID NOs: 40 to 43 and 65 to 68, or a variant thereof with at least 85% sequence identity thereto; or
 - (c) the composition of any one of claims 113-129.
138. The composition of claim 137, wherein the mammal is a human, cow, goat, mouse, or rabbit.
139. The composition of any one of claims 136-138, wherein the antibodies are humanized.
140. The composition of any one of claims 136-139, wherein the antibodies are IgG antibodies.

141. The composition of any one of claims 136-140, wherein the antibodies are a bis-Fab, Fv, Fab, Fab'-SH, F(ab')₂, a diabody, a linear antibody, or a scFv.

142. A method of producing antibodies in a subject, comprising administering to the subject:

(a) two or more different nucleic acid molecules comprising the sequence of any one of SEQ ID NOs: 44 to 51 and 61 to 64, or a variant thereof with at least 85% sequence identity thereto, or a complementary sequence thereof;

(b) two or more different polypeptides comprising the sequence of any one of SEQ ID NOs: 40 to 43 and 65 to 68, or a variant thereof with at least 85% sequence identity thereto; or

(c) the composition of any one of claims 113-129,

wherein administration of (a), (b), or (c) elicits production of neutralizing anti-2019-nCoV antisera in the subject.

143. The method of claim 142, wherein the composition comprises an Ad26 vector comprising the two or more different nucleic acid molecules.

144. A composition comprising antisera comprising the antibodies produced by the method of claim 142 or 143.

145. The composition of any one of claims 136 to 141 and 144, wherein the antibodies bind to an epitope within a coronavirus spike protein, such as a coronavirus spike protein that comprises the amino acid sequence of any one of SEQ ID NOs: 40-43 and 65-68, wherein preferably the antibody binds to an epitope within the N-terminal domain (NTD) or the receptor binding domain (RBD) of the coronavirus spike protein, for example, the antibodies:

(a) neutralize one or more of the B.1.1.7, B.1.429, B.1.1.28, B.1.351, A23.1, B.1.617.1, B.1.617.2, B.1.427, B.1.525, B.1.526, P.1, P.2, P.3, C.36, C.37, B.1.1.519, B.1.526.1, B.1.526.2, R.1, B.1.258.17, B.1.575, B.1.214.2, A.2.5.2, AT.1, B.1.1.523, and B.1.620 lineages of 2019-nCoV; and/or

(b) bind to a coronavirus spike protein that contains one or more of the following mutations: V3G, L5F, P9L, S13I, L18F, T19R, T20N, P26S, A27S, T33I, V36F, V36I, S45F, H49Y, Q52R, L54F, W64R, A67V, Δ 69-70, G75V, T76I, D80A, D80G, P85S, S94F, T95I, S98F, I105V, D111N, S112L, L118F, V120L, V126A, V127F, E132Q, D138Y, G142D, Δ 144, N148T, W152R, Δ 156-157, F157S, R158G, S162I, T167S, S172A, L176F, D178H, G181V, L189F, R190S, V193L, D198Y, I203V, Y204H, I210T, D215G, L216F, A222V, V227A, D228H, I233V, R237K, Δ 242-244, H245Y, Δ 246-252, D253G, D253N, W258L, A262S, A263P, V267L, P272L, R273S, E281Q, T284I, V289L, A292S, T299I, K300M, T307I, V308L, E309Q, F318S, V320F, T323I, V227I, P330S, L335F, P337S, G339S, R346S, R346K, A348S, K356R, R357K, V362F, V367F, V367L, S371T, T376I, V382L, P384L, T385N, N394S, N394H, V395I, R403K, E406Q, R408I, Q414K, K417T, K417N, D427N, D427Y, T430I, N439K, N440S, N440K, L452M, L452R, L452Q, L461F, K462T, I468V, T470I, E471Q, T478K, E484K, F490S, S494P, N501T, N501Y, Y505H, V510L, L513F, A520S, A522S, A522V, T531S, V534I, V534F, N540S, F543L, T547I, T549I, E554D, K558N, P561S, F565L, A570D, T572I, A575S, E583Q, E583D, L585F, G594S, V595I, I598V, T604A, T604I, Q607K, Q613H, D614G, N616S, V622F, A623S, D627G, P631S, T632S, T638I,

S640F, N641S, A647S, A653V, H655Y, Y660F, I670V, A672V, Q677H, P681H, P681R, A688V, S691P, A694V, M697I, A701V, S704L, A706V, T716I, T716V, T719I, M731I, T732I, T732A, M740V, T747I, T747N, N751D, S758G, T761I, T761R, G769V, A771S, V772I, Q779K, E780D, V785I, T791I, K795R, D796H, S803A, P809S, P812S, P812L, L822F, V826L, T827I, A831V, K835R, D843N, A845S, A845V, K854N, T859N, T859I, M869I, I870V, A871V, A879S, A879V, T883I, F888L, A890V, A892V, A899S, M902I, A903S, I909V, V911I, V915I, L922F, A924S, S929I, D936Y, S939F, S940F, G946R, D950N, D950H, Q957R, T961M, V963A, S967N, V976F, S982A, K986N, K986R, R995G, I997V, T998I, Q1005H, T1006I, T1009I, R1014K, A1016V, A1020S, A1025G, T1027I, V1033A, K1038Q, V1040F, K1045N, L1049I, M1050I, A1056V, H1058Y, L1063F, T1066N, Q1071H, K1073N, A1078S, D1084Y, D1084E, A1086S, V1094F, H1101D, H1101Y, V1104L, E1111K, D1118H, D1118Y, G1124V, D1127G, I1130V, V1133F, D1139H, L1141W, D1146Y, E1150D, D1153Y, P1162L, P1162S, D1163Y, N1173S, A1174V, V1176F, E1182D, R1185H, K1191N, N1192S, E1195Q, E1202Q, K1205N, Q1208H, I1216T, G1219V, G1219C, V1228L, M1229I, V1230L, M1237I, T1238I, C1243F, C1247F, S1252F, S1252P, D1260N, P1263L, V1264L, H1271Y, T1273I, and T1273A, relative to the amino acid sequence of SEQ ID NO: 1 or 35.

146. The composition of any one of claims 136-141, 144, and 145 comprising at least three or more different said antibodies.

147. The composition of claim 146, wherein the composition comprises:

- (a) at least three or more antibodies that bind coronavirus spike proteins comprising the amino acid sequence of SEQ ID NOs: 40-42, and optionally SEQ ID NO: 43; or
- (b) at least three or more antibodies that bind coronavirus spike proteins comprising the amino acid sequence of SEQ ID NOs: 65-67, and optionally SEQ ID NO: 68.

148. The composition of claim 146 and 147, further comprising a pharmaceutically acceptable carrier, excipient, or diluent.

149. The composition of any one of claims 146-148, further comprising an adjuvant or an immunostimulatory agent.

150. The composition of any one of claims 113-141 and 144-149, further comprising the composition of any one of claims 43-54 and 103-108.

151. The composition of any one of claims 43-54, 103-108, 113-141, and 144-150, further comprising:

- (a) a nucleic acid molecule that encodes a polypeptide with the amino acid sequence of SEQ ID NOs: 75-77, or a variant thereof with at least 85% sequence identity thereto; and/or
- (b) a nucleic acid molecule that encodes a polypeptide with the amino acid sequence of any one of SEQ ID NOs: 78-80, or a variant thereof with at least 85% sequence identity thereto.

152. The composition of claim 151, wherein the nucleic acid molecule of (a) has at least 85% identity to any one of SEQ ID NOs: 69-71, and/or the nucleic acid molecule of (b) has at least 85% identity to any one of SEQ ID NOs: 72-74.

153. The method of claim 55, wherein the method further comprises administering an amount of one or more of the compositions of any one of claims 113-141 and 144-152.

154. The method of claim 55 or 153, wherein the method further comprises identifying a subclass and/or an effector function of the bNAb, wherein preferably the bNAb is an anti-Spike antibody.

155. The method of claim 154, wherein:

(a) the subclass is IgM, IgA, IgG1, IgG2, IgG3, or FcγR2A; and/or

(b) the effector function is antibody-dependent neutrophil phagocytosis (ADNP), antibody-dependent complement deposition (ADCD), antibody-dependent monocyte cellular phagocytosis (ADCP), or antibody-dependent NK cell activation.

156. The method of any one of claims 55 or 153-155, wherein the sample is a bodily fluid from the subject, wherein preferably the bodily fluid is blood.

157. The method of any one of claims 55 or 153-156, wherein the coronavirus is 2019-nCoV.

158. The method of any one of claims 55 or 153-157, wherein the two or more lineages of coronavirus are selected from the group consisting of B.1.1.7, B.1.429, B.1.1.28, B.1.351, A23.1, B.1.617.1, B.1.617.2, B.1.427, B.1.525, B.1.526, P.1, P.2, P.3, C.36, C.37, B.1.1.519, B.1.526.1, B.1.526.2, R.1, B.1.258.17, B.1.575, B.1.214.2, A.2.5.2, AT.1, B.1.1.523, and B.1.620.

159. A method of treating or reducing the risk of a coronavirus infection in a subject in need thereof, comprising administering a therapeutically effective amount of the composition of any one of claims 113-141 and 144-152.

160. The method of claim 159, comprising administering two or more different types of said composition.

161. The method of claim 160, comprising administering three or more different types of said composition.

162. The method of any one of claims 159-161, further comprising administering:

(a) an amount of a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 29, nucleotides 19-3837 of SEQ ID NO: 39, or the nucleotide sequence of SEQ ID NO: 39, and/or

(b) a polypeptide comprising amino acids 18-1208 of SEQ ID NO: 35 or a polypeptide having at least 85% sequence identity to amino acids 18-1208 of SEQ ID NO: 35.

163. The method of claim 162, wherein the composition comprises:

- (a) an Ad26 vector comprising the nucleic acid molecule; and/or
- (b) an Ad26 vector comprising a nucleic acid molecule that encodes the polypeptide.

164. The method of any one of claims 159-163, further comprising measuring an anti-coronavirus antibody level, such as an anti-Spike antibody level, in the subject.

165. The method of claim 164, wherein the anti-coronavirus antibody level in the subject is measured before and/or after administration of the composition or the immunogenic composition.

166. The method of claim 165, wherein the anti-coronavirus antibody level in the subject is measured one or more times over about 1, 2, 3, 4, 5, or 6 days, 1, 2, 3, 4, 5, 6, or 7 weeks, 2, 3, 4, 5, or 6 months, 1, 2, 3, 4, or 5 years after administration.

167. The method of any one of claims 159-166, wherein the anti-coronavirus antibody level of the subject is below a protective level and wherein the method further comprises re-administering the composition of any one of claims 113-141 and 144-152 to said subject or administering a different anti-coronavirus composition to the subject.

168. The method of claim 167, wherein the protective level is a level sufficient to reduce symptoms or duration of a coronavirus-mediated disease.

169. The method of claim 167 or 168, wherein the protective level is:

- (a) a level that is at or above a titer of at least about 70, as determined using a pseudovirus neutralization assay; or
- (b) a level that is at or above a titer of at least about 25, as determined using a live virus neutralization assay; or
- (c) a level that is at least 80% of a median level of an anti-coronavirus antibody in a cohort of convalescent humans, as determined by a pseudovirus neutralization assay or live virus neutralization assay.

170. The method of any one of claims 159-169, wherein the coronavirus infection is infection by 2019-nCoV, wherein preferably said 2019-nCoV is of the lineage B.1.1.7, B.1.429, B.1.1.28, B.1.351, A23.1, B.1.617.1, B.1.617.2, B.1.427, B.1.525, B.1.526, P.1, P.2, P.3, C.36, C.37, B.1.1.519, B.1.526.1, B.1.526.2, R.1, B.1.258.17, B.1.575, B.1.214.2, A.2.5.2, AT.1, B.1.1.523, or B.1.620.

171. A method of reducing a coronavirus-mediated activity in a subject infected with a 2019-nCoV or a variant thereof, comprising administering a therapeutically effective amount of the composition of any one of claims 113-141 and 144-152 to said subject.

172. The method of claim 171, comprising administering two or more different types of the composition to said subject.

173. The method of claim 172, comprising administering three or more different types of the composition to said subject.

174. The method of any one of claims 171-173, further comprising administering:

(a) an amount of a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 29, nucleotides 19-3837 of SEQ ID NO: 39, or the nucleotide sequence of SEQ ID NO: 39, and/or

(b) a polypeptide comprising amino acids 18-1208 of SEQ ID NO: 35 or a polypeptide having at least 85% sequence identity to amino acids 18-1208 of SEQ ID NO: 35.

175. The method of claim 174, wherein the composition comprises:

(a) an Ad26 vector comprising the nucleic acid molecule; and/or

(b) an Ad26 vector comprising a nucleic acid molecule that encodes the polypeptide, wherein preferably the method produces a log serum anti-Spike antibody titer greater than 2 in the subject, as measured by an ELISA assay.

176. The method of any one of claims 171-175, comprising administering an amount of the composition of about 15 µg to about 300 µg.

177. The method of any one of claims 171-176, wherein said coronavirus-mediated activity is viral titer, viral spread, infection, or cell fusion.

178. The method of claim 177, wherein said viral titer is decreased after administration of the composition of any one of claims 113-1141 and 144-152.

179. The method of claim 178, wherein the viral titer is decreased by 25% or more.

180. The method of claim 179, wherein the viral titer is decreased by 50% or more.

181. The method of claim 180, wherein the viral titer is decreased by 75% or more.

182. The method of claim 181, wherein the coronavirus is undetectable after administration of said composition.

183. The method of any one of claims 171-182, wherein said administering occurs prior to exposure to the coronavirus.

184. The method of claim 183, wherein said administering occurs at least 1 hour prior to exposure to said coronavirus.

185. The method of claim 184, wherein said administering occurs at least 1 week, 1 month, or a year prior to exposure to said coronavirus.

186. The method of any one of claims 171-182, wherein said administering occurs post-exposure to the coronavirus.

187. The method of claim 186, wherein said administering occurs at least 15 minutes post-exposure to said coronavirus.

188. The method of claim 187, wherein said administering occurs at least 1 hour, 1 day, 1 week, post-exposure to said coronavirus.

189. The method of any one of claims 171-188, wherein said subject is administered at least one dose of the composition.

190. The method of claim 189, wherein said subject is administered at least two doses of the compositions.

191. The method of claim 190, wherein the composition is administered to said subject as a prime, a boost, or as a prime-boost.

192. The method of any one of claims 171-191, wherein the composition is administered intramuscularly, intravenously, intradermally, percutaneously, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intranasally, intravitreally, intravaginally, intrarectally, topically, intratumorally, peritoneally, subcutaneously, subconjunctively, intravesicularly, mucosally, intrapericardially, intraumbilically, intraocularly, orally, topically, locally, by inhalation, by injection, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, by catheter, by lavage, by gavage, in creams, or in lipid compositions.

193. The method of any one of claims 171-192, wherein the subject is a mammal.

194. The method of claim 193, wherein the mammal is a human.

195. The method of claim 194, wherein the human has an underlying health condition.

196. The method of claim 195, wherein the underlying health condition is hypertension, diabetes, or cardiovascular disease.

197. The method of any one of claims 171-196, wherein the method promotes an immune response in said subject.

198. The method of claim 197, wherein the immune response is a humoral immune response.

199. The method of claim 198, wherein the humoral immune response is an IgG response.

200. A composition for use in treating or reducing the risk of a coronavirus infection, such as a 2019-nCoV infection, in a subject in need thereof, comprising of one or more the compositions of any one of claims 113-141 and 144-152.

201. A composition for use in reducing a coronavirus-mediated activity in a subject infected with a 2019-nCoV or a variant thereof, comprising the composition of any one of claims 113-141 and 144-152.

202. The composition for use according to claim 201, comprising two or more different types of the composition.

203. The composition for use according to claim 202, comprising three or more different types of the composition.

204. The composition for use according to claim 202 or 203, wherein the composition comprises:

- (a) the nucleic acid molecules of each of SEQ ID NOs: 44-46 and optionally SEQ ID NO: 47;
- (b) the nucleic acid molecules of each of SEQ ID NOs: 48-50, and optionally SEQ ID NO: 51;
- (c) the nucleic acid molecules of each of SEQ ID NOs: 61-63, and optionally SEQ ID NO: 64;
- (d) the polypeptides of each of SEQ ID NOs: 40-42, and optionally SEQ ID NO: 43;
- (e) the polypeptides of each of SEQ ID NOs: 65-67, and optionally SEQ ID NO: 68;
- (f) one or more vectors comprising the nucleic acid sequences of each of SEQ ID NOs: 44-46, and optionally SEQ ID NO: 47, or comprising a nucleic acid sequence encoding the polypeptide of each of SEQ ID NOs: 40-42, and optionally SEQ ID NO: 43;
- (g) one or more vectors comprising the nucleic acid sequences of each of SEQ ID NOs: 48-50, and optionally SEQ ID NO: 51, or comprising a nucleic acid sequence encoding the polypeptide of each of SEQ ID NOs: 40-42, and optionally SEQ ID NO: 43;
- (h) one or more vectors comprising the nucleic acid sequences of each of SEQ ID NOs: 61-63, and optionally SEQ ID NO: 64, or comprising a nucleic acid sequence encoding the polypeptide of each of SEQ ID NOs: 65-67, and optionally SEQ ID NO: 68;
- (i) at least two different antibodies that specifically bind a coronavirus spike proteins comprising the amino acid sequence of SEQ ID NOs: 40-42, and optionally SEQ ID NO: 43; or
- (j) at least two different antibodies that specifically bind coronavirus spike proteins comprising the amino acid sequence of SEQ ID NOs: 65-67, and optionally SEQ ID NO: 68.

205. The composition for use according to any one of claims 201-204, wherein the composition is to be administered with:

(a) an amount of a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 29, nucleotides 19-3837 of SEQ ID NO: 39, or the nucleotide sequence of SEQ ID NO: 39, and/or

(b) a polypeptide comprising amino acids 18-1208 of SEQ ID NO: 35 or a polypeptide having at least 85% sequence identity to amino acids 18-1208 of SEQ ID NO: 35.

206. The composition for use according to claim 205, wherein said composition comprises:

(a) an Ad26 vector comprising the nucleic acid molecules; and/or

(b) an Ad26 vector comprising a nucleic acid molecule that encodes the polypeptides.

207. A method of manufacturing an immunogenic composition for treating or reducing the risk of a coronavirus infection in a subject in need thereof, said method comprising the steps of:

(a) admixing:

- (i) at least two different nucleic acid molecules, wherein each of the nucleic acid molecules encodes a polypeptide having at least 85% sequence identity to amino acids 18-1208 of any one of SEQ ID NOs: 40 to 43 and 65 to 68, or a complementary sequence thereof, wherein the polypeptide does not comprise amino acids 18-1208 of SEQ ID NO: 35; or
- (ii) at least two different polypeptides having at least 85% sequence identity to amino acids 18-1208 of any one of SEQ ID NOs: 40 to 43 and 65 to 68, wherein the polypeptides do not comprise amino acids 18-1208 of SEQ ID NO: 35; or
- (iii) one or more vectors comprising at least two different nucleic acid molecules, wherein each of the nucleic acid molecules encodes a polypeptides having at least 85% sequence identity to amino acids 18-1208 of SEQ ID NOs: 40 to 43 and 65 to 68, wherein the polypeptides do not comprise amino acids 18-1208 of SEQ ID NO: 35; or
- (iv) two or more of the antibodies that specifically bind to the polypeptide of any one of SEQ ID NOs: 40-43 and 65-68,

with a pharmaceutically acceptable carrier, excipient, or diluent to form the immunogenic composition; and

(b) placing the immunogenic composition in a container.

208. A kit comprising:

(a) a first container comprising:

- (i) at least two different nucleic acid molecules, wherein each of the nucleic acid molecules encodes a polypeptide having at least 85% sequence identity to amino acids 18-1208 of any one of SEQ ID NOs: 40 to 43 and 65 to 68, or a complementary sequence thereof, wherein the polypeptide does not comprise amino acids 18-1208 of SEQ ID NO: 35; or
- (ii) at least two different polypeptides having at least 85% sequence identity to amino acids 18-1208 of any one of SEQ ID NOs: 40 to 43 and 65 to 68, wherein the polypeptides do not comprise amino acids 18-1208 of SEQ ID NO: 35; or
- (iii) one or more vectors comprising at least two different nucleic acid molecules, wherein each of the nucleic acid molecules encodes a polypeptides having at least 85%

- sequence identity to amino acids 18-1208 of SEQ ID NOs: 40 to 43 and 65 to 68, wherein the polypeptides do not comprise amino acids 18-1208 of SEQ ID NO: 35; or
- (iv) two or more of the antibodies that specifically bind to the polypeptide of any one of SEQ ID NOs: 40-43 and 65-68, or
 - (v) at least one of the compositions of any one of claims 113-141 and 143-152; and
- (b) instructions for use thereof;
- wherein, optionally, the kit further comprises:
- (c) a second container comprising a pharmaceutically acceptable carrier, excipient, or diluent.

209. The kit of claim 208, wherein the first container further comprises a pharmaceutically acceptable carrier, excipient, or diluent.

210. The kit of claim 208 or 209, wherein the first or second container further comprises an adjuvant and/or an immunostimulatory agent.

211. The composition of claim 113, wherein the composition comprises:

- (a) each of the polypeptides of SEQ ID NOs: 40-42, and optionally SEQ ID NO: 43; or
- (b) each of the polypeptides of SEQ ID NOs: 65-67, and optionally SEQ ID NO: 68.

FIG. 1

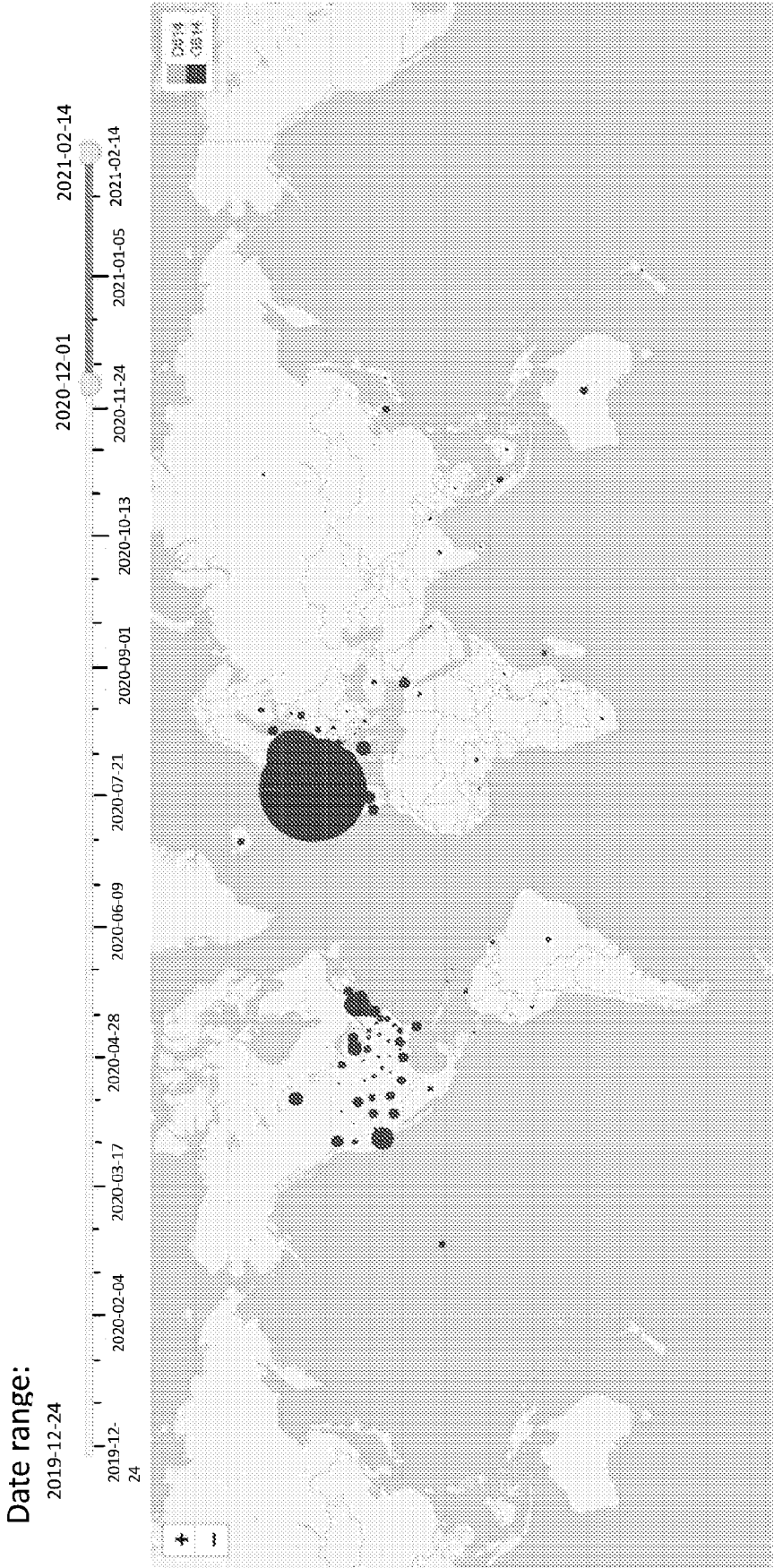


FIG. 2

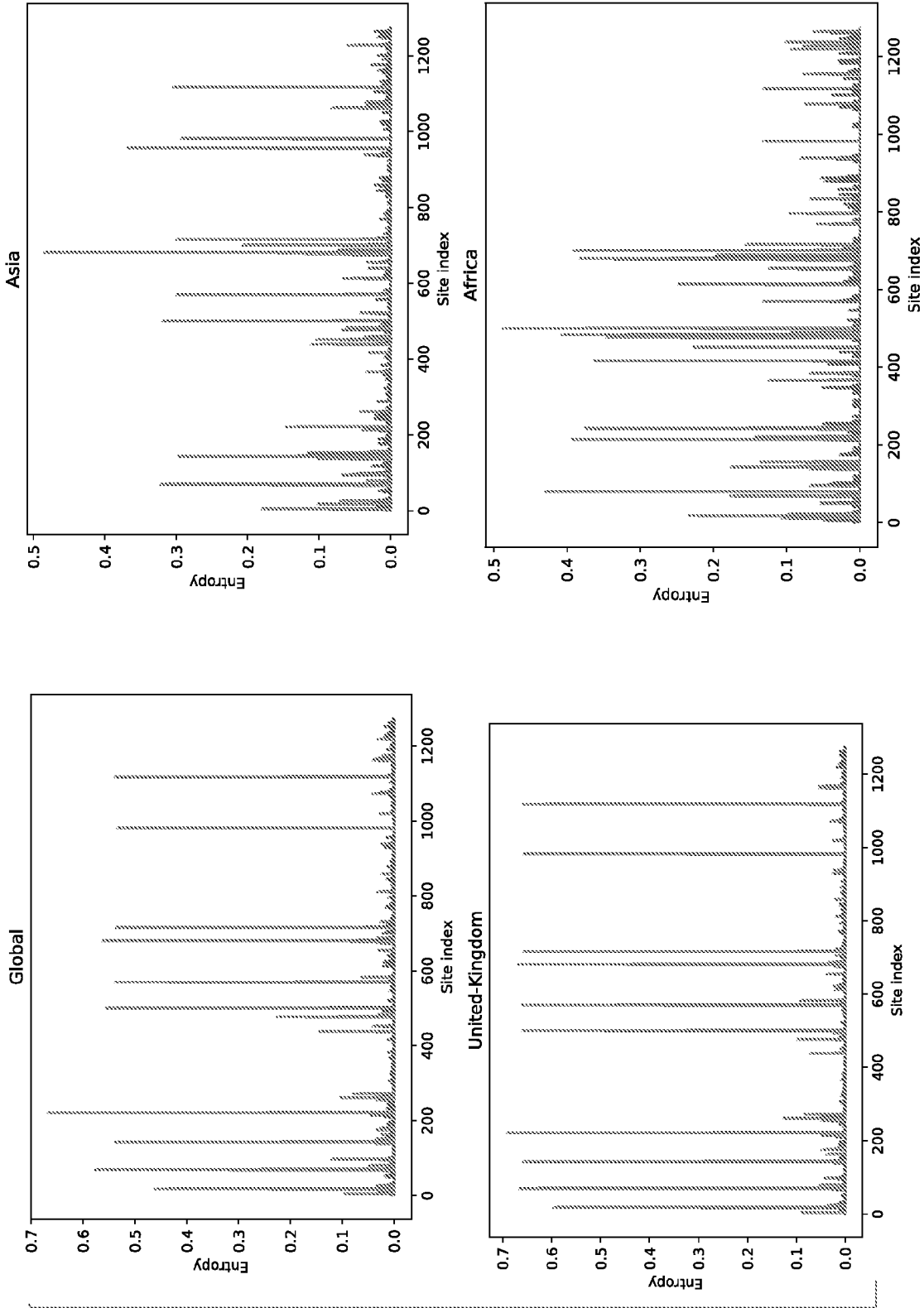


FIG. 2 CONT.

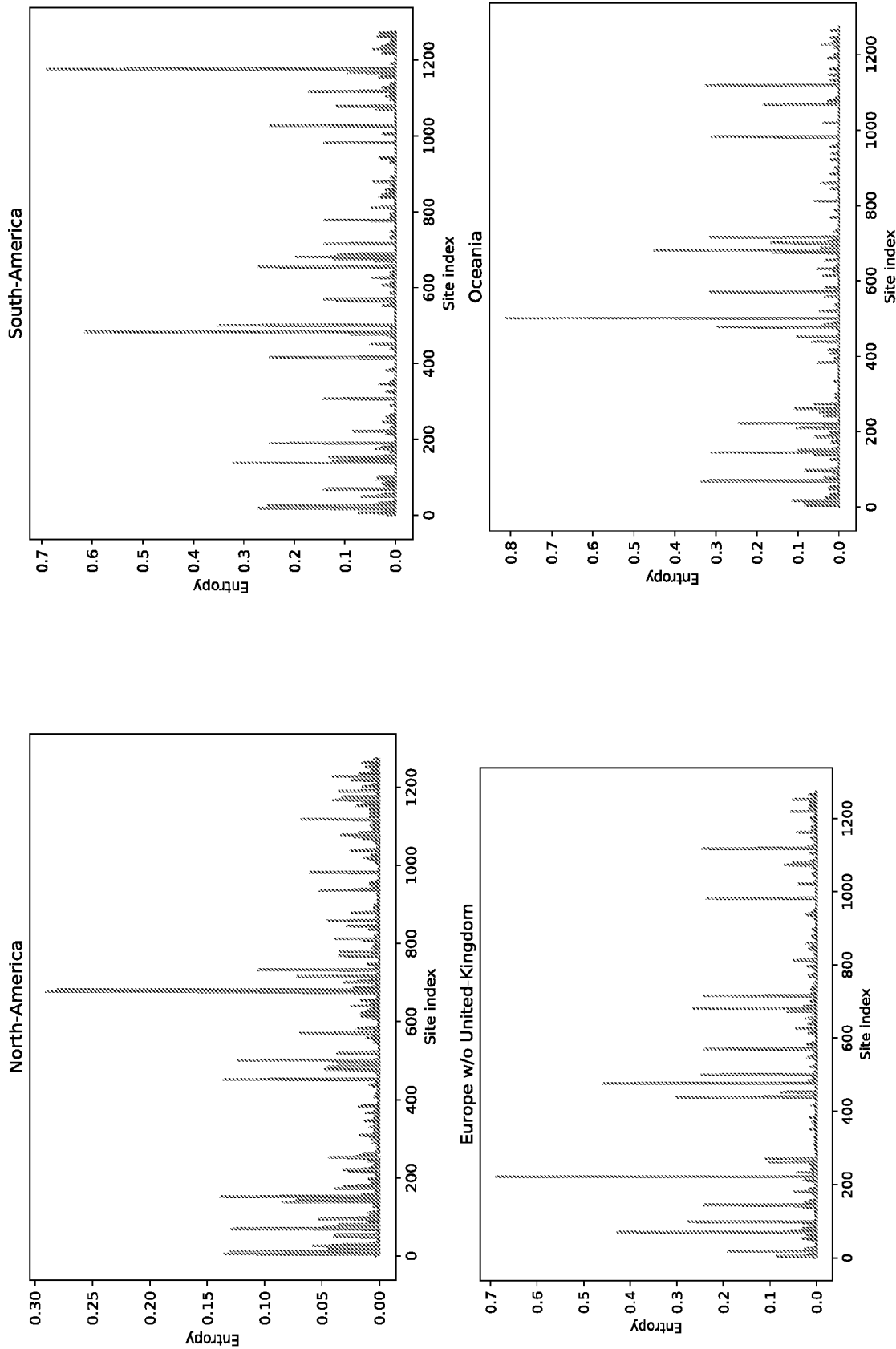


FIG. 3A

Cramer's V

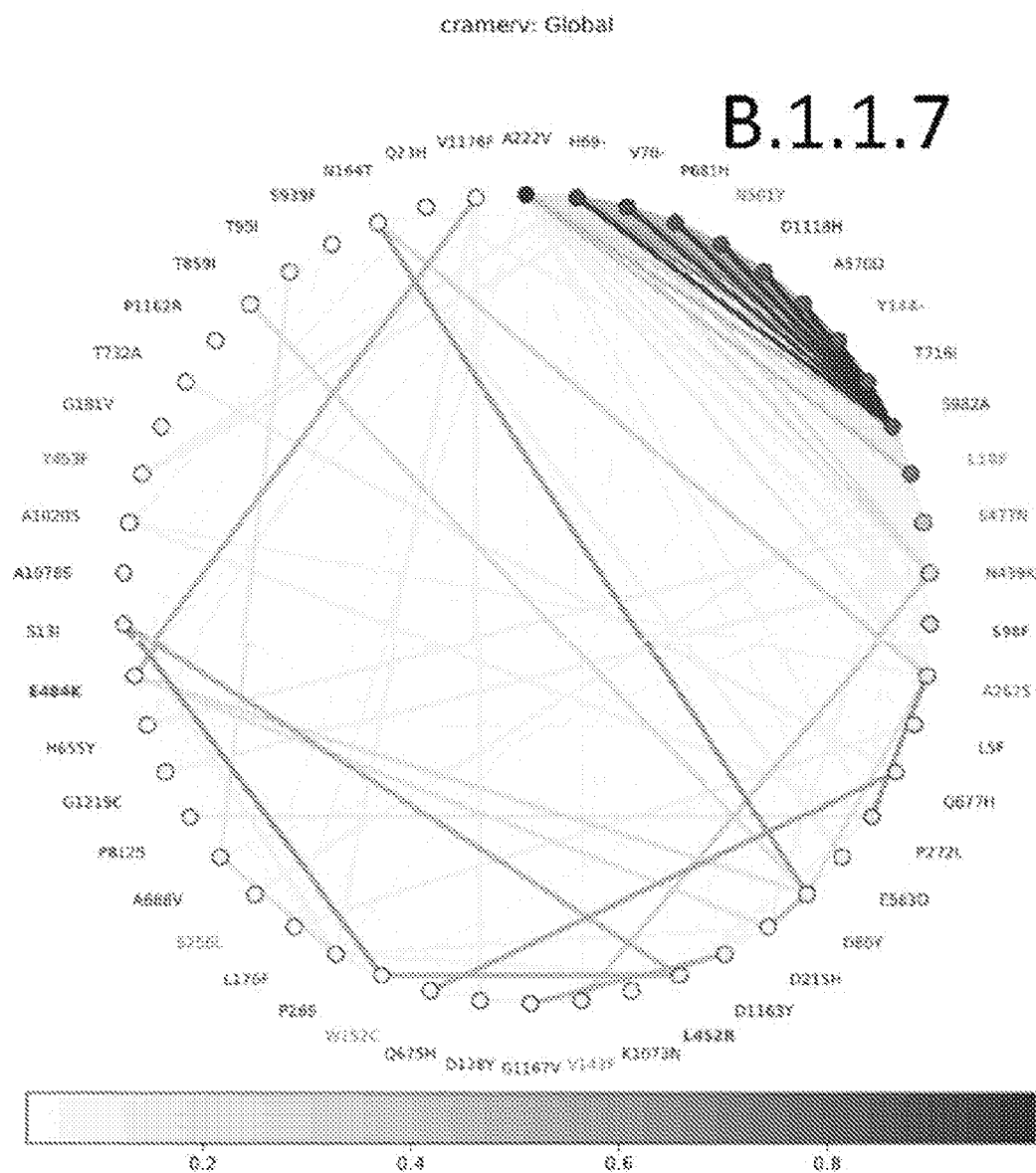


FIG. 3B

Cramer's V

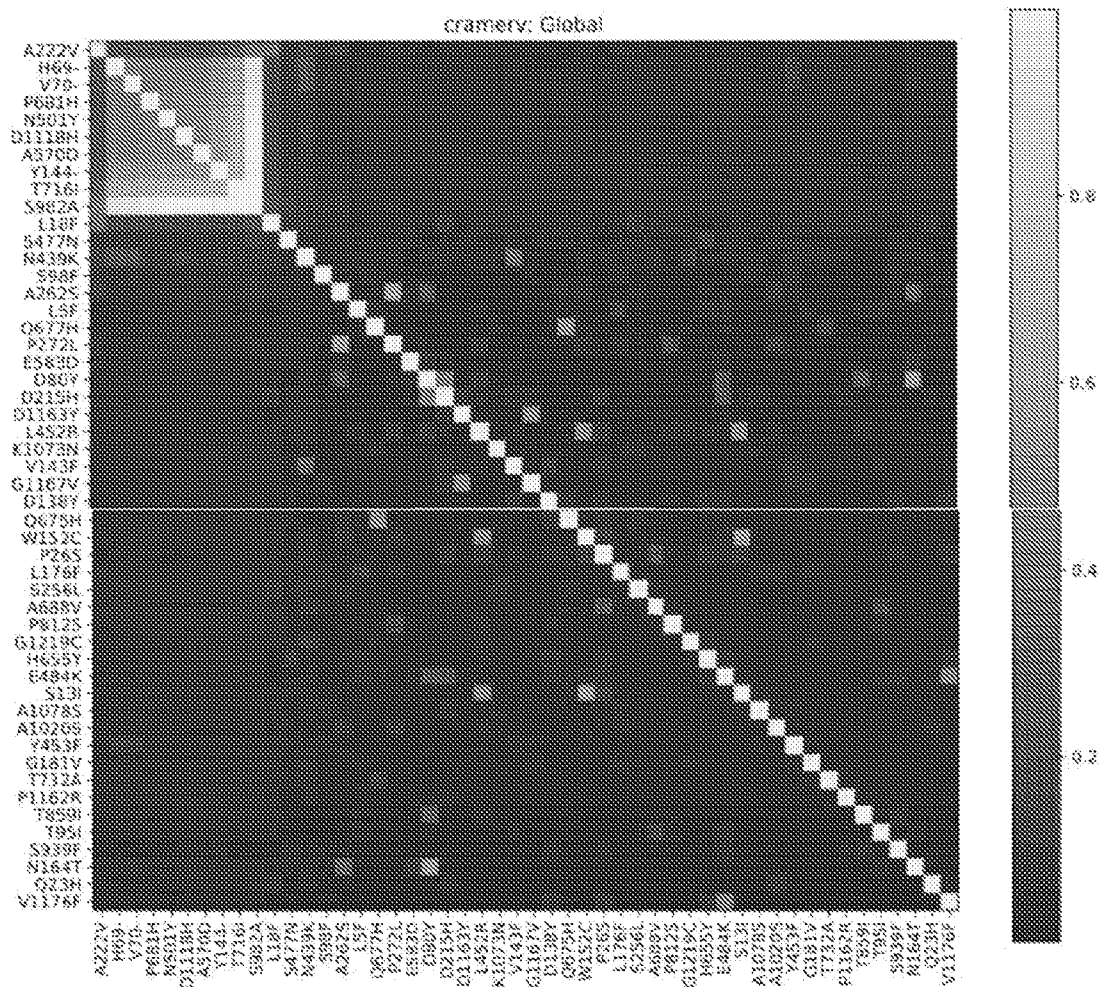


FIG. 3C

Mutual information

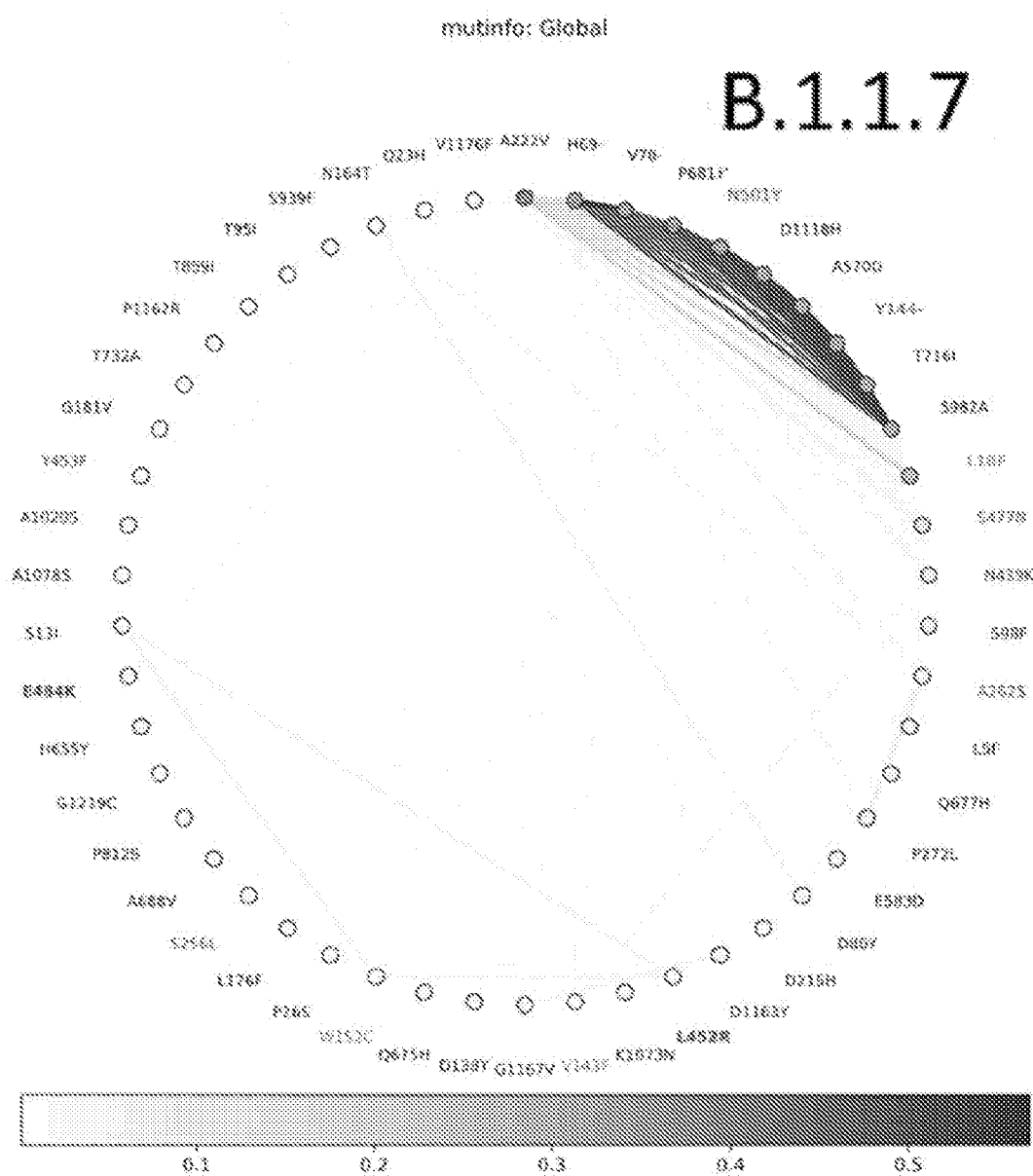


FIG. 3D

Mutual information

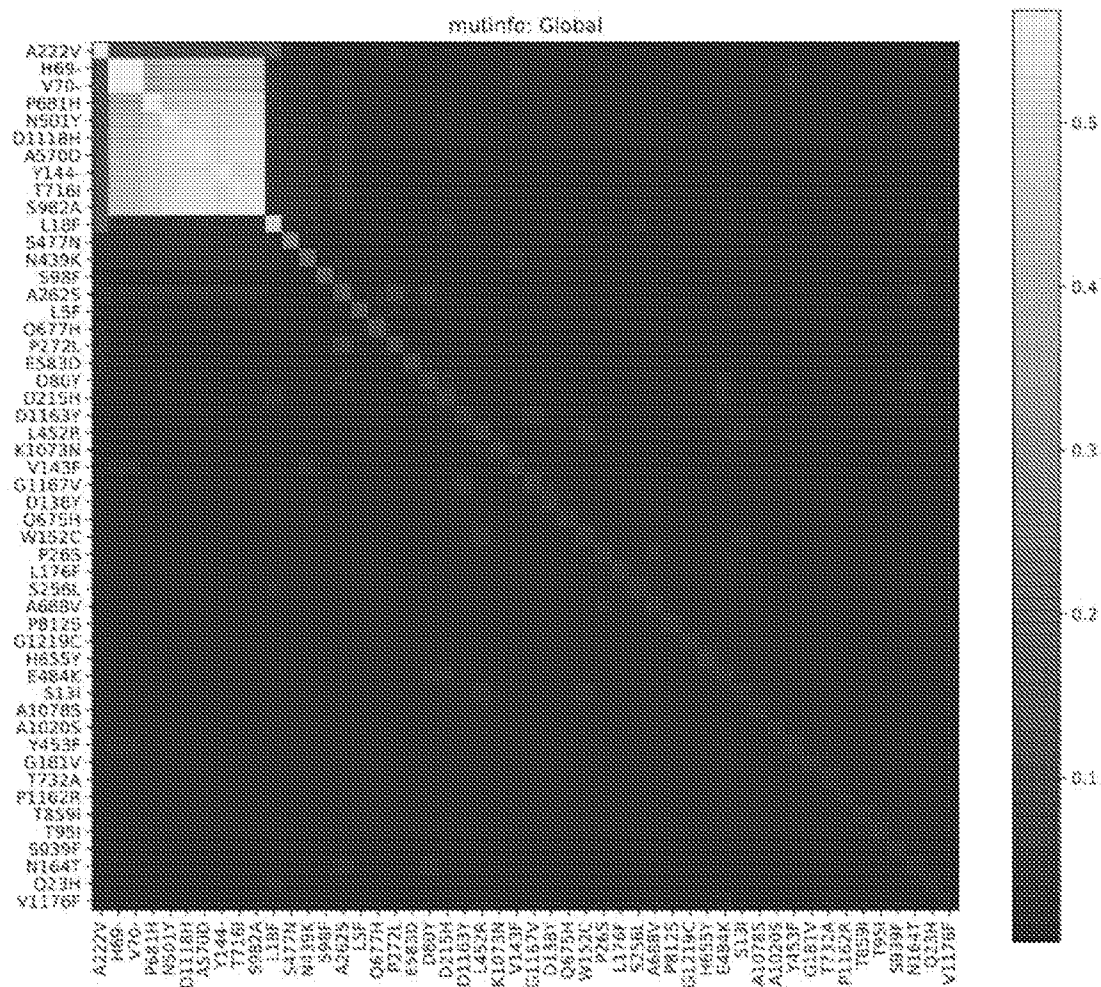


FIG. 4A

Cramer's V

cramerv: North-America

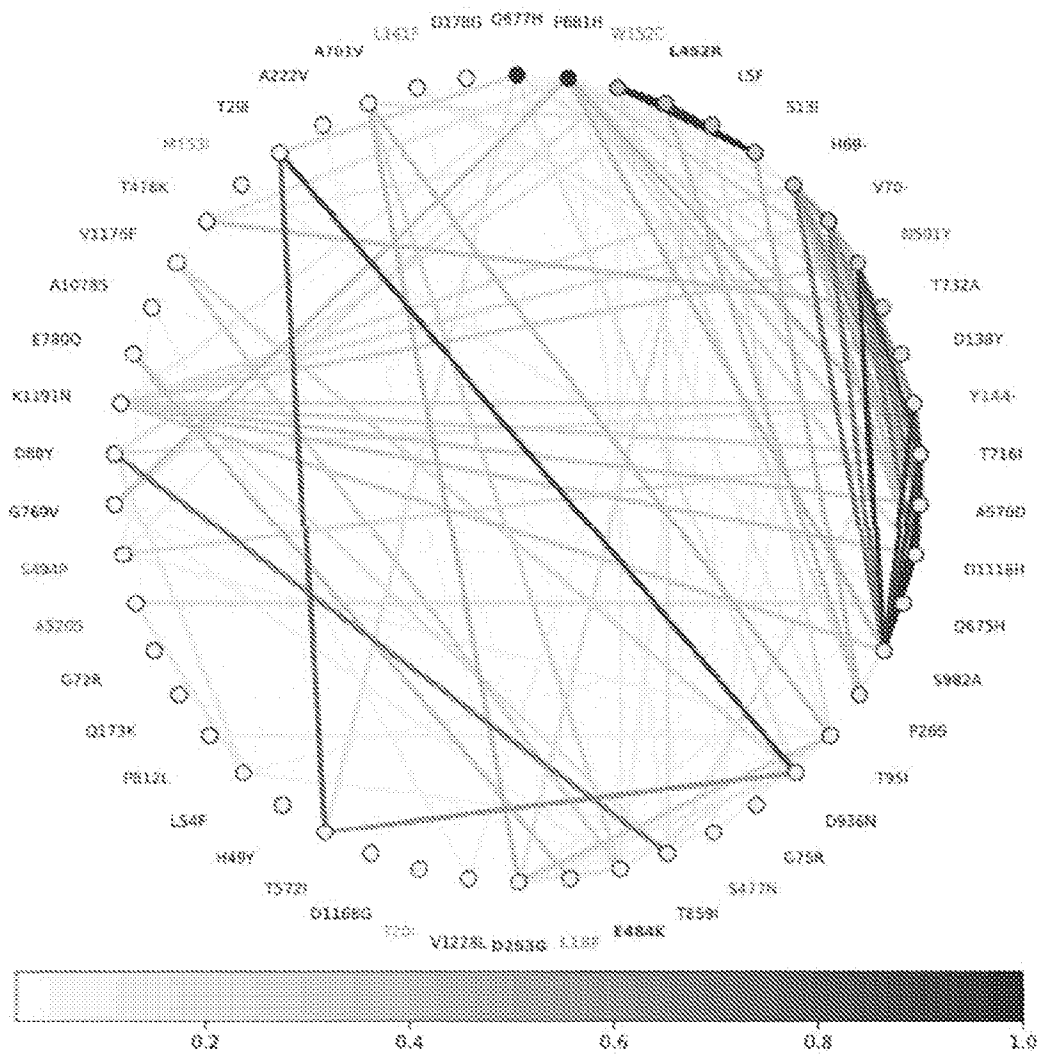


FIG. 4B

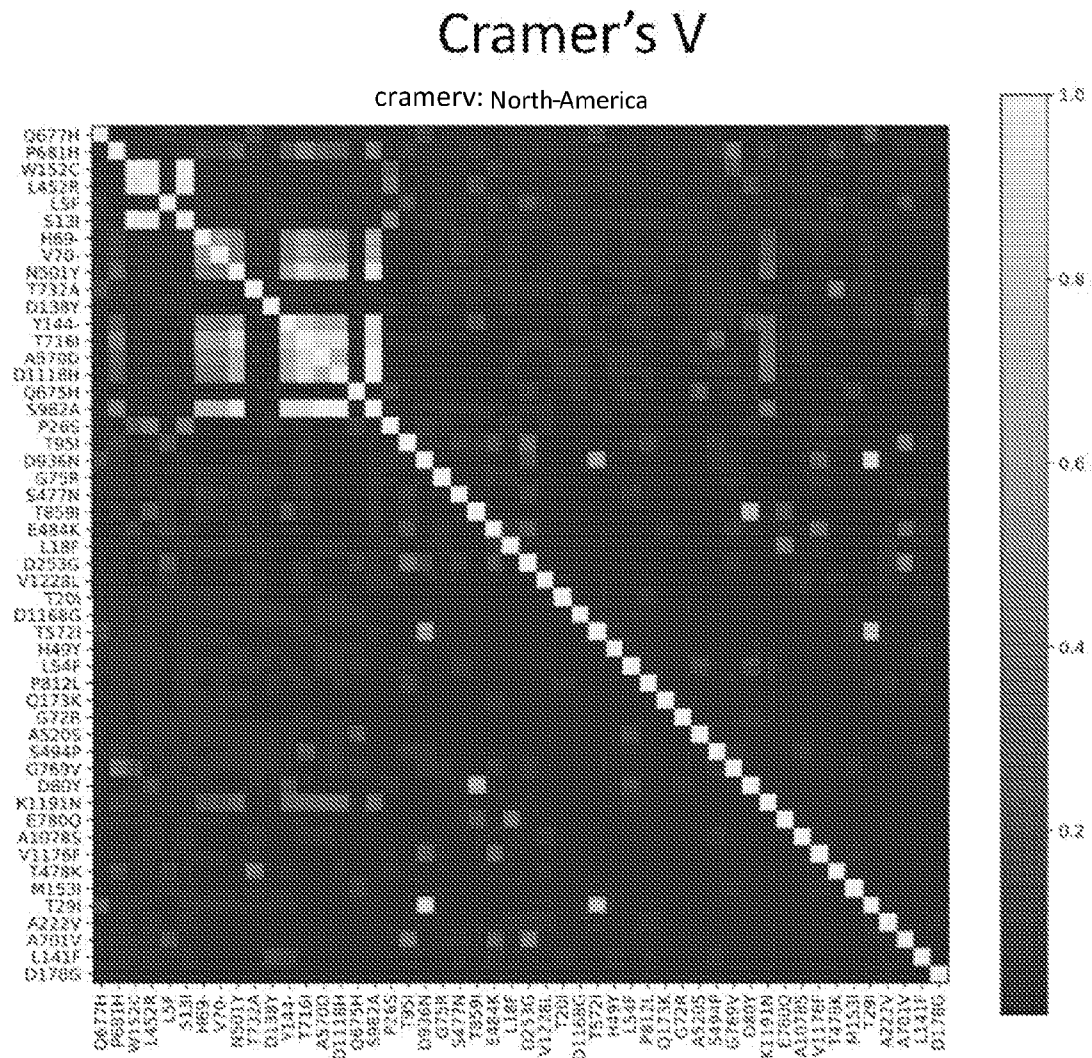


FIG. 4C

Mutual information

mutinfo: North-America

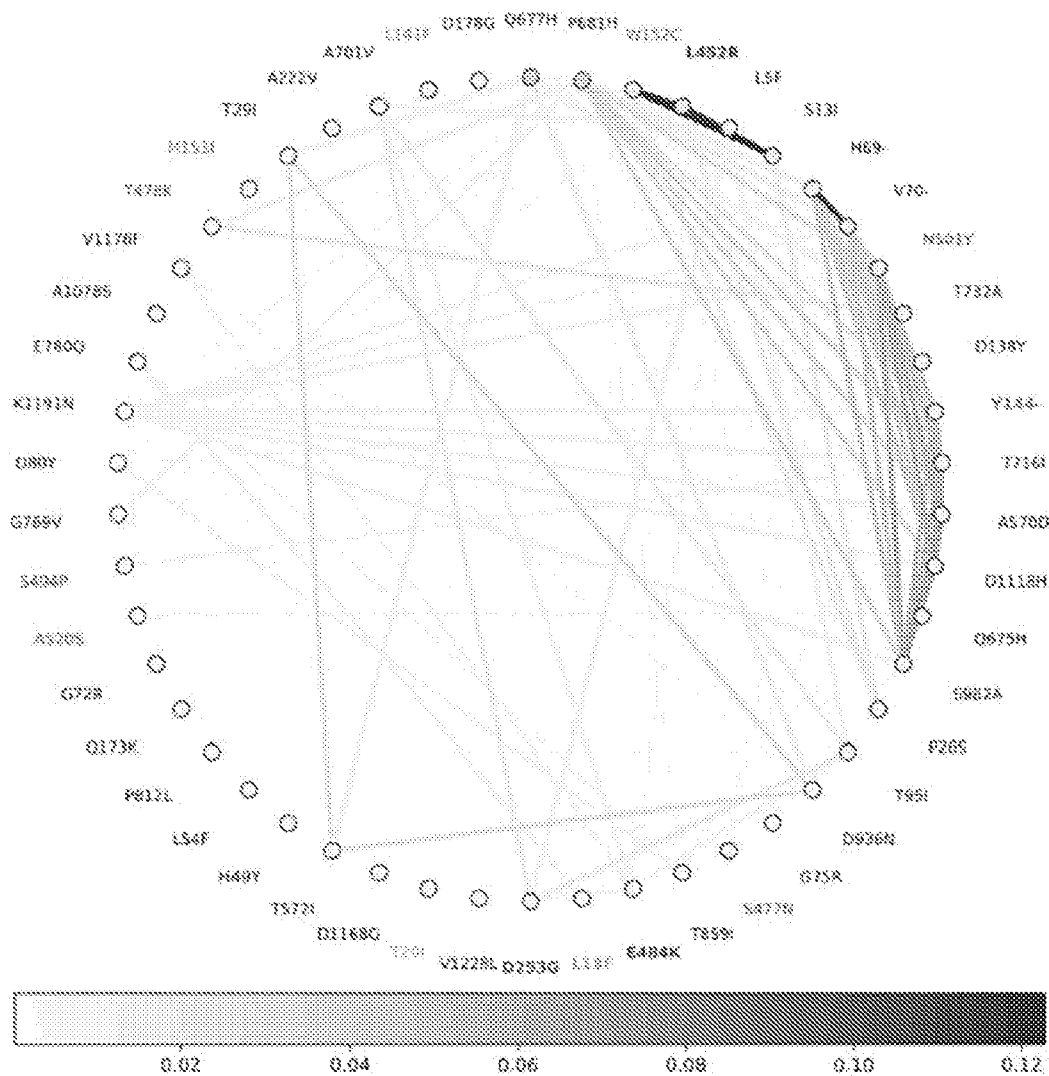


FIG. 4D

Mutual information

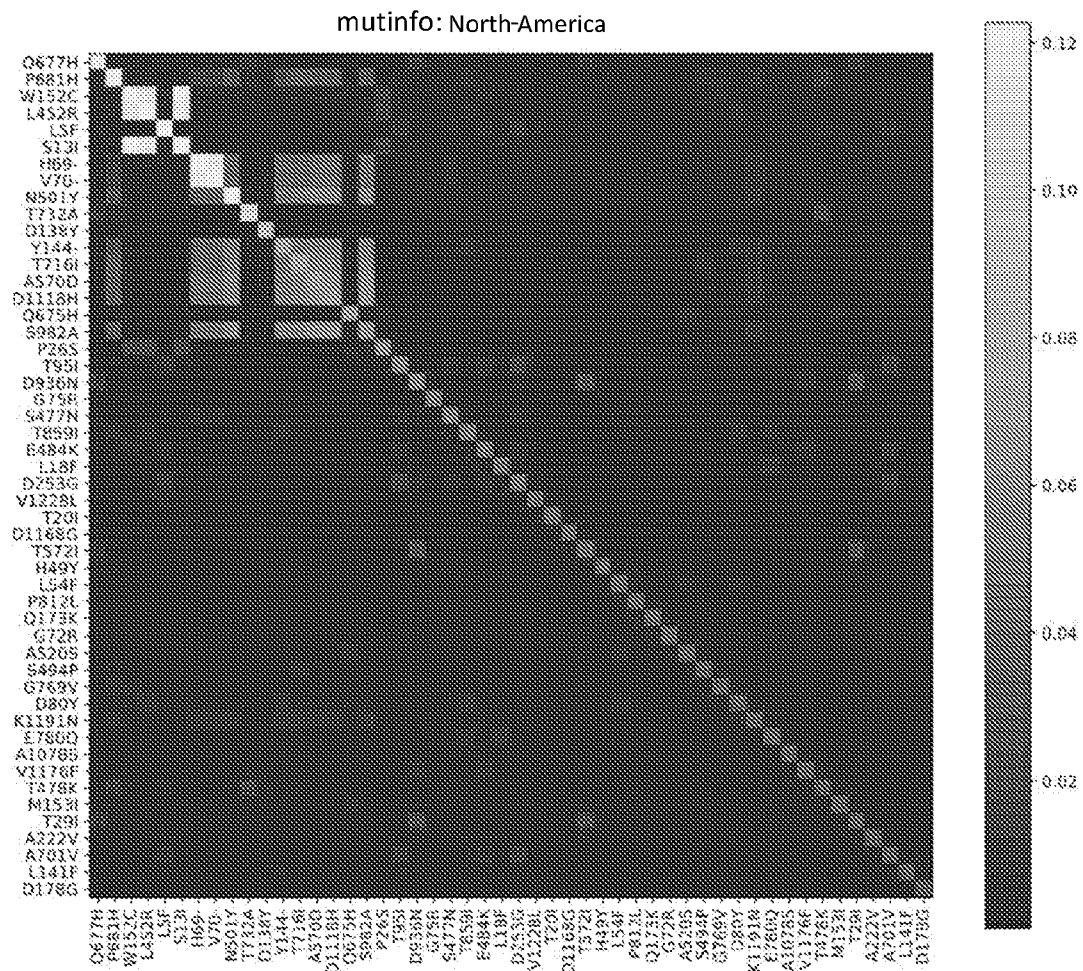


FIG. 5A

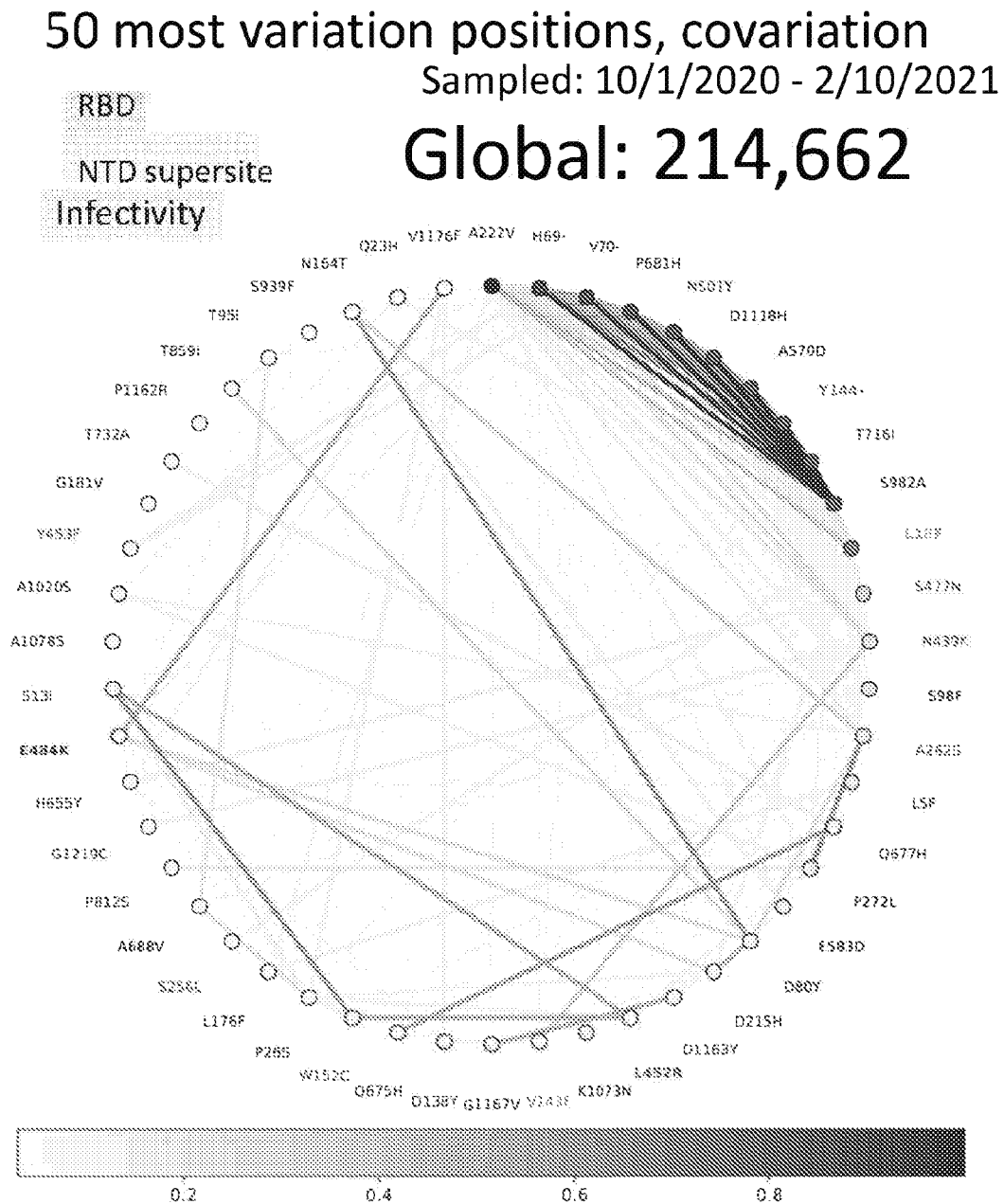


FIG. 5B

B.1.1.7 related	B.1.1.248 related	
B.1.429 related	B.1.351 related	
Most common patterns for: Global		
	1111111111	
11111111222224444455556666778899000111112		
1122678993445678125673557807857788131538277166671		
53836900588342461526229237410355718622992038823769		
LSLQPHVDTSDVWNLGDASAPNLYSENAEHQDPATTPTSSAKADPDGVG		
.....YD.....H.I....A...H.....	44269	Spike mutations
.....	41691	
.....V.....	33596	
..F.....V.....	30398	
.....N.....	9643	
.....F.....	4601	
.....K.....	3941	
.....V.SL.....	2468	
.....H.....	2014	
.....H.....	1763	
.....V.....D.....	1712	
.....K.....	1220	
..Fxx--.....YD.....H.I....A...H.....	1004	
.....V.....YV.....	1004	
F.....	937	
.....F.....K.....	926	
..F.....VL.....	897	
..F.....HV.....	880	
.....F.....	781	
.....F.V.....	660	
..I.....C.....R.....	641	
.....N.....	628	
F.F.....V.....	607	
..FH.....V.....	602	
.....V.S.....	597	
F.....YD.....H.I....A...H.....	579	
.....N..Y.....	530	
.....Y.....T..V.S.....	506	
F.....V.....	498	
.....V.....T.....	487	
.....Y.....	483	
.....P.....	470	
.....V.....C.....	457	
.....T.....	448	
.....	409	
.....Y.....	385	

FIG. 5B CONT.

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F..... 381
.....R..... 351
.....H..... 339
.....A..... 315
.....V.....S..... 304
.....S.....V.....V..... 301
.....Y..... 300
.....I..... 292
.....V.....R..... 289
.....V.....H..... 283
.....R..... 277
.....V.....S.....H..... 270
.....F.....YD.....H.I.....A.....H..... 270
.....V.....S..... 267
.....R..... 265
.....K.....F..... 250
.....F..... 248
.....V..... 244
F.....F.V..... 232
F.....V.V..... 223
.....YD.....H.I.....FA.....H..... 221
.....I..... 217
F.....H..... 199
.....V..... 196
.....YD.....H.I.....A.....H..... 190
.....H.A..... 184
F.....V.....Y..... 184
.....N..... 174
.....V.SL.....S..... 172
.....H..... 169
.....S..... 167
.....Y.....T.....V.S.....D..... 163
.....H.....YD.....H.I.....A.....H..... 157
.....L..... 156
.....K.....V..... 151
I.S.....C.....R..... 150
F.....V.....Y..... 149
.....F..... 144
F.....V.....F..... 141
.....K.....YD.....H.I.....A.....H.....V 139
.....F.....YD.....H.I.....A.....H..... 130
.....N..... 129
F.....V.....L..... 127
.....R..... 117
.....S..... 117
.....V.L..... 113
.....V.....S..... 107
.....I..... 105
.....A.....G.....KY..... 104

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FIG. 5B CONT.

.....V.....V..... 98
.....R.V..... 97
.....C..... 96
.....H.S..... 95
.....ID.....H.I.S.A.N..... 94
.....V.SL..... 93
.....Y..... 92
.....I..... 91
.....Y..... 90
.....D..... 89
.....A.....G.....KY..... 88
..... 87
.....N.....S..... 86
.....S.....N..... 85
.....S.....Y.....KY.Y.....F..... 84
.....V.....Q..... 83
.....Y.....N..... 82
.....T..... 81
.....H.I..... 80
.....I.....K..... 79
.....S..... 78
.....N..... 77
.....V.....I..... 76
.....I.....S..... 75
..... 74
.....S..... 73

FIG. 6A

North America, 30794

cramerv: North-America

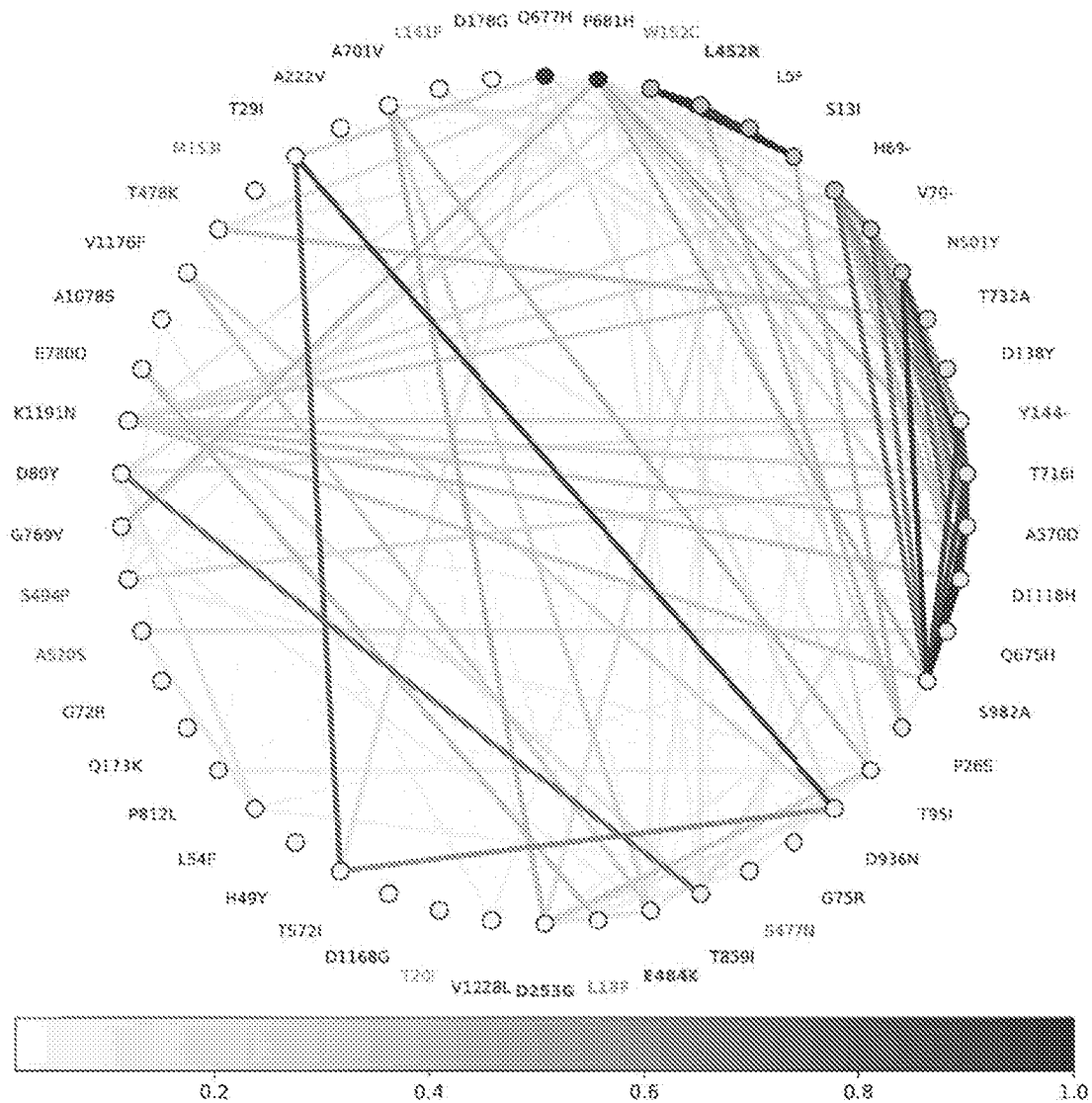


FIG. 6B

North America, 30794

8.1.1.7 related

B.1.429 related

Most common patterns for: North-America

```

                                     1111111
      1111111224444455556667777788990111112
112224567778993445577255778992777776013681536716792
52806994902505814233823278441002571162902962888618
LSLTPTNLMVGGDTOLYHQPADLSTESNAATKQFATTGPTDSADGVKV
***** 18720
*****.H. 1466
*****.H. 1067
.I.....C.....R. 617
*****.P. 468
F..... 447
*****~ 385
*****.A. 316
*****.T. 279
*****.Y. 256
*****~.....Y.D...H.I.....A.H... 250
*****.R. 214
*****.G. 194
...I..... 181
*****.Y. 172
*****.K. 158
*****.H. 157
F.....H. 149
.I.S.....C.....R. 147
*****.H. 146
*****.L 144
*****.R. 141
*****.L 139
*****.N. 138
...I.....I.H.....N. 116
*****.S. 114
*****.K.....H.A. 113
*****F. 112
...F..... 102
*****.H...S. 86
*****.G. 83
*****.P. 82
*****.I. 82
*****.V. 80
*****.Y. 80
```

North America, 30794

.....F..... 74
.....Y.D.....H.I.....A.H.N. 71
.....V..... 69
.....F..... 66
.....G..... 64
.....Q..... 64
.....N..... 62
.....I..... 61
.....T..... 61
.....I..... 60
.....I.....G.....K.....V..... 58
.....R..... 53
.....Q..... 52
.....P.....H.I..... 50
.....S..... 46
.....H.....I..... 46
.....R..... 45
.....K.....F..... 45
.....Q.I..... 44
.....I..... 42
.....S..... 42
.....K..... 41
.....R..... 41
.....R.....V..... 40
.....V..... 39
.....Y..... 39
.....R.....S.....H..... 35
.....S.....H..... 35
.....Y..... 34
.....K.....H..... 32
.....V..... 30
.....V..... 30
.....N..... 28
.....H.....I..... 27
.....P.....H.....Y..... 26
.....Y..... 25
.....Y.....F..... 25
.....T..... 24
.....G.....T.....R.....N..... 22
.....I.....G.N..... 21
.....E..... 20
.....F.....V..... 20
.....I.....G.....V..... 19
.....N.....R..... 19
.....R..... 18
.....L.....H..... 18
.....I..... 17
.....L..... 17
.....I.....H..... 17
.....F..... 16
.....H..... 15
.....I.....C.....R.....N..... 15
.....P..... 15
.....L..... 14
.....L.....K.....V..... 14
.....Y.....R..... 14
.....V..... 13
.....I..... 13
.....F.....S..... 12
.....I.....C.....V.R..... 12
.....F.....N..... 12
.....P.....S.....H..... 12
.....L..... 11
.....S..... 11
.....P..... 11
.....I..... 11
.....I.....G.N.....V..... 10
.....H..... 10
.....R..... 10
.....Q..... 10
.....H..... 10
.....F.....A..... 10
.....R..... 9
.....I.....A..... 9
.....V..... 9
.....V..... 8
.....H..... 8

FIG. 7A

South America

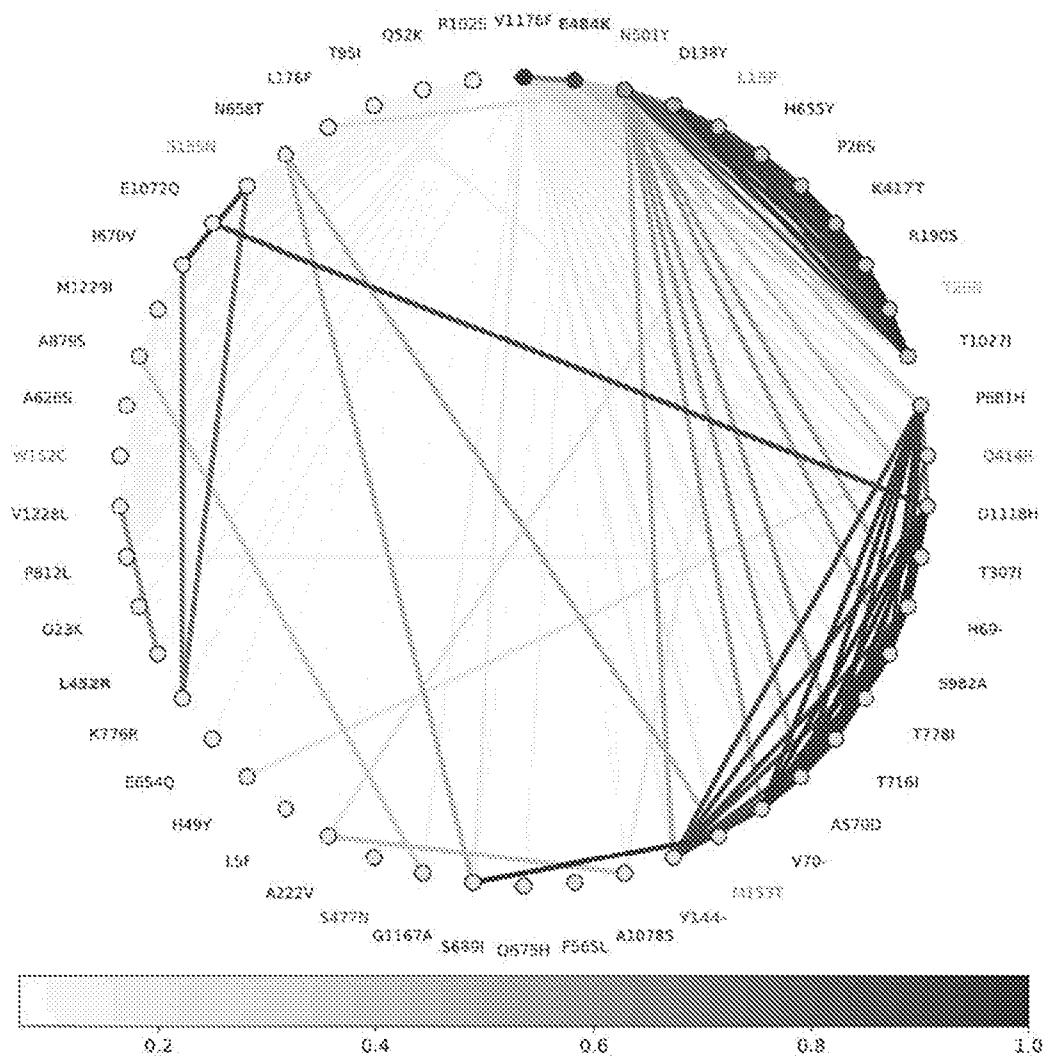


FIG. 8A

Asia

cramerv; Asia

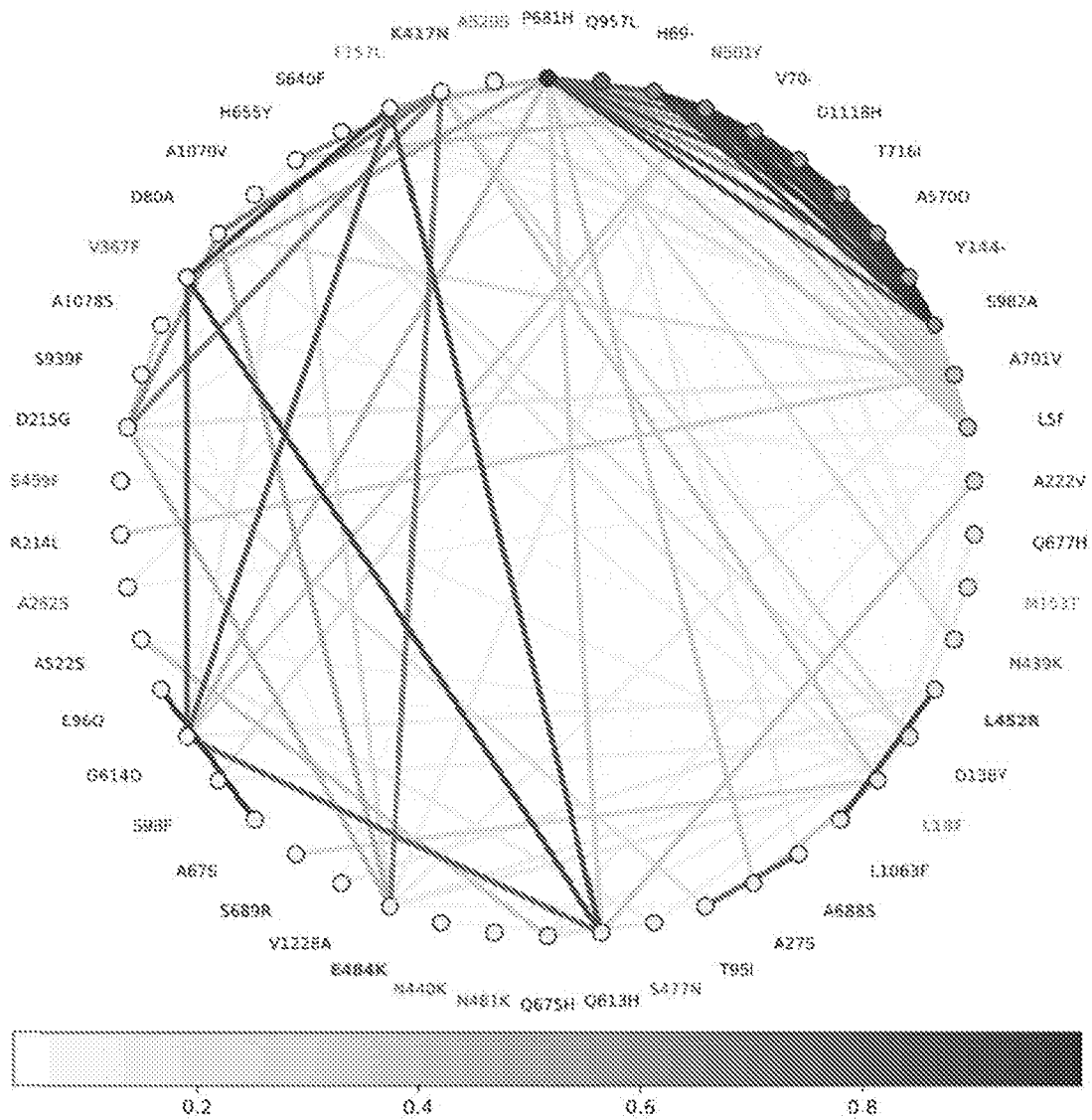


FIG. 9B

Common B.1.1.7 derivatives

Common xxx derivatives

Most common patterns for: Oceania

	11111	
11111122222234444555556666667788900112		
1112678993445581124567835780257813577880115826112		
52383900588142360522114492741080342457181629208788		
L55LQHVDTSDLYWAFIDALPGTPNLSENAKAEDTEQQPAATPTSAVTOV		
.....G.....		197
.....T.....G.....		152
.....Y.....D.....G.....H.....I.....A.....H.....		0
.....V.....G.....		30
.....N.....G.....		28
.....N.....G.....F.....		17
.....R.....G.....V.....		17
.....G.....		16
.....I.....C.....R.....G.....		13
.....F.....V.....G.....		12
.....G.....H.....		12
.....F.....G.....		9
F.....G.....		8
.....L.....N.....G.....F.....		8
.....I.....N.....G.....F.....		8
.....GI.....R.....		7
.....L.....G.....H.....		7
.....G.....V.....		7
.....G.....I.....		6
.....G.....H.....		6
.....G.....R.....		5
.....G.....R.....		5
.....S.....G.....		5
.....G.....L.....		5
.....I.....G.....		4
.....V.....DG.....		4
.....N.....G.....		4
.....R.....G.....		4
.....G.....H.....		4
.....F.....G.....		4
.....G.....K.....		4
.....Y.....G.....		3
.....K.....G.....		3
.....F.....Y.....D.....G.....H.....I.....A.....H.....		0
F.....G.....		3
.....I.....G.....		3
.....V.....G.....H.....		3
.....G.....S.....		3

FIG. 10A

Africa

cramerv: Africa

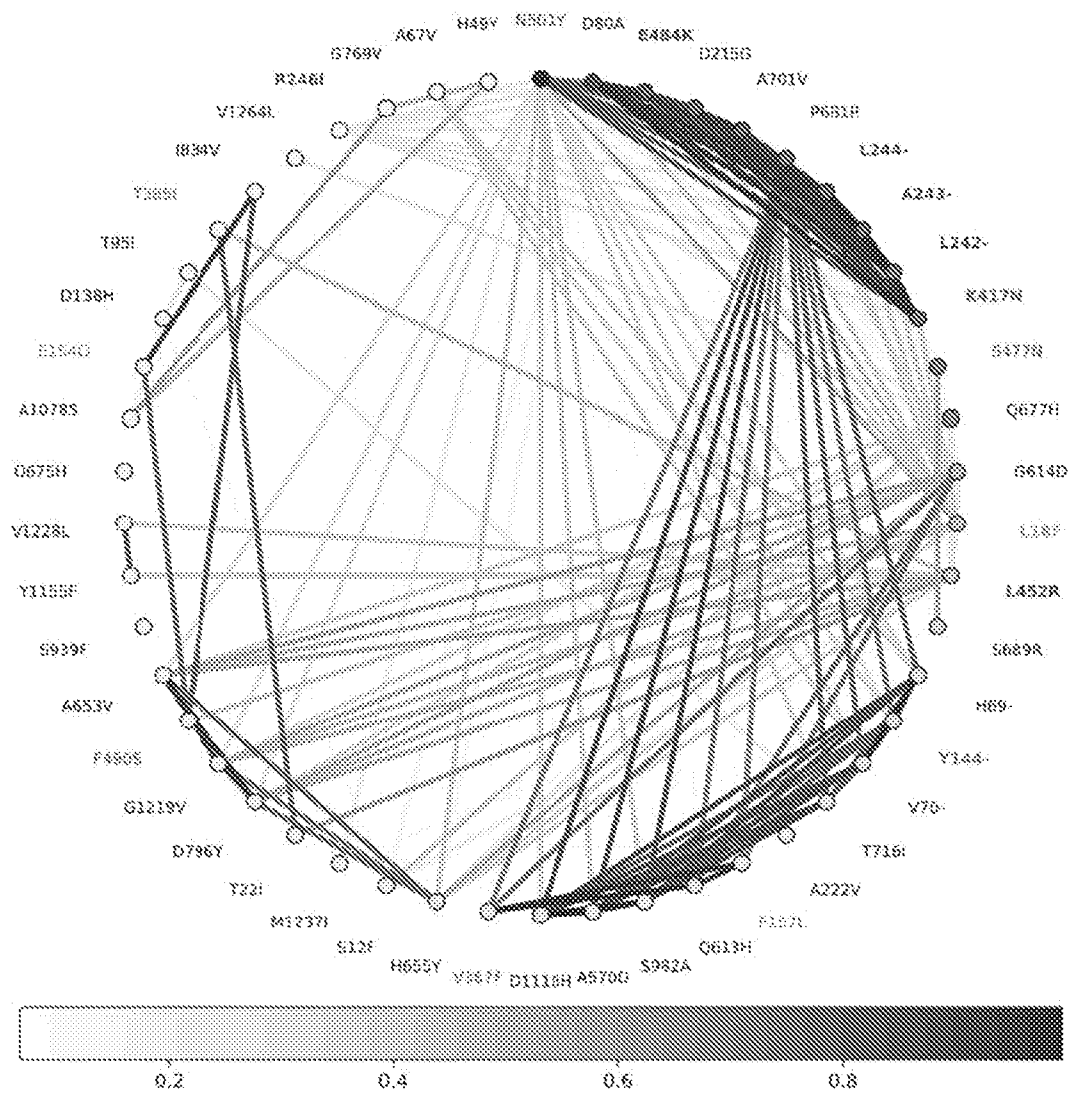


FIG. 10B

Common 8.1.1.7 related

Common 8.1.351 related

Most common patterns for: Africa

```

11111111
11112222223344444556666666677778990112222
11246678934551244446815789071155778801693387151236
28297900584475223467572740103435571916964928859874
SLTHAHVDTDYEFDALALRVTKLSEFNAQDAHQOPSATGDISSADYGMV
.....G..... 352
.....N.....G..... 63
.....A.....G.~~~.N..K.Y..G.....V..... 61
.F.....A.....G.~~~.N..K.Y..G.....V..... 34
.....N.....G.....R..... 28
.....~.~.....YD.G.....H..I...A.H..... 27
.....G.....H..... 21
.....V.....G..... 21
.....L.....F.....H.....R..... 19
.F.....R...Y..VY.H.....Y.....V... 16
.....G.....R..... 14
.....G.....F..... 13
.....Q.....S...G.....V..... 12
..I.....I.R.....G..... 12
.....G.....H..... 10
.....R.....G.....F.L.. 10
.....G.....I.. 9
.....G.....I..... 8
.....G.....H.....L 8
.....A.....~I..N..K.Y..G.....V..... 7
..V~.~.....K...G.....H..... 7
F.....G.....H..... 7
F.....R.....G.....H..... 7
.....G.....H..... 6
..I.....R.....G..... 6
.....G.....HR..... 6
.....I.....G.....I..... 5
.....N.....G.....H..... 5
.....Y.....G..... 5
..Y.....G.....V...S..... 5
.....G.....V..... 5
.....S...N..... 4
.F.....G..... 4
.....V.....G.....V..... 4
.....G.Y..... 3
.....~.~..... 3
.....N.....G.....R.....I.. 3
.....V.....G.....I.. 3

```


FIG. 11B

B.1.1.7 related B.1.351 related

Most common patterns for: Europe w/o United-Kingdom

```

1111111111
11111222224444556666666788899000011122
1256667899344581236735570725777788111538277716615
58644790058834315232292371064357918623992013882392
LLPLNAHVDTSDVYMGDAIAPNLYSNAAESQONPATPSTSSAQKADPDGS
.....V.....18220
.....N.....7888
.....F.....4795
.....F.....3746
.....--.....YD.....H.I.....A.....H.....
.....--.....K.....2824
.F.....V.....2042
.....V.SL.....1030
.....--.....F.....K.....882
.....--.....K.....823
.....--.....F.....798
.....V.....T.....487
.....V.....C.....453
.....H.....424
.....H.....369
.....V.....362
.....R.....T.K.....337
.....F.....F.....325
.....I.....321
.....V.....S.....297
.....V.....S.....260
.....V.....S.....260
.....V.....H.....252
F.....V.....251
.....V.....233
.....S.....208
F.....203
.....T.....192
.....V.....L.....183
.....V.....R.....175
.F.....V.....Y.....172
.....SQ.....156
.....I.....137
.....N.....127
.....V--.....K.....118
.....K.....V.....118

```

FIG. 11B CONT.

```

.....C.....V.....115
.....N.....111
.....V.....L.....110
.....R.....108
.....Y.....101
.....S.....99
.....V.....S.....98
.....R.V.....94
.....T.....93
.....I.....87
.....F.....87
.....HV.....72
.....S.....N.....70
.....S.....67
.....F.F.....V.....61
.....N.....S.....60
FF.....V.....59
.....F.....Y.....53
F.....N.....50
.....F.....N.....F.....47
.....F.....46
FF.....C.....V.....46
.....K.....Y.....45
.....H.....45
.....F.....K.....G.....43
.....Y.....43
F.....V.....L.....42
.....F.....Y.F.....41
.....F.....S.....F.....39
.....F.....37
F.....K.....37
.....V.T.....37
.....I.....S.....37
.....V.....N.....35
.....V.....Y.....34
.....H.....V.....34
.....V.....I.....34
.....F.....V.....32
.....F.F.....30
.....F.....V.....30
.....V.S.....F.....30
.....Y.....29
.....F.....A.....G.....Y.....29
.....F.....N.....29
.....F.....S.....29
F.....F.....29
.....Y.....N.....R.....28
.....F.....V.....28
.....A.....G.....M.....27
.....A.....G.....Y.....26
.....Q.....26
.....N.....I.....25
.....V.S.L.R.....25
.....N.....V.....24
.....F.....H.....24
.....K.....F.....23
F.....T.....22
.....Y.....V.....22
.....Y.....R.I.....A.....R.....21
.....K.....H.....S.....21
.....V.S.....21
.....N.....F.....20
.....F.....H.....F.....20
.....V.V.....Y.....20
.....K.....H.....18
.....F.....V.....18
.....Y.....V.....18
.....K.....K.....16
.....V.....H.....16
.....V.....I.....16
.....V.V.....16
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FIG. 12B CONT.

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FIG. 13 CONT.

RBD
NTD supersite
Infectivity

B.1.1.7 B.1.429 B.1.1.28 B.1.351 A231
GR/501Y.V1 GH/452R GR/484.V2 GH/501Y.V2 367F.V1

Asia, relevant patterns not captured globally

```

11111
11112222344444445555666666667799900012
1266789993455112661345578802271145778880135867712
58779005668437452277902971410203405571891697230558
LLAAMDTESDYMFDAIVKNNLSSAENAAAQDSHQPPASATSQSLAAOV
.....L....F.....H....R..... 13
.....T.....Y..... 3
.....S....G..... 10
.....K....G..... 31
.....F....G..... 12
F.....F....G..... 5
.....R....G.....L 4
.....R....G..... 4
.....G..... 25
.....T.....G..... 40
.....Y.T.....G..... 11
.....I.....G..... 3
.....G.R..... 2

```

FIG. 13 CONT.

RBD
NTD supersite
Infectivity

B.1.1.7 B.1.429 B.1.1.28 B.1.351 A231
GR/501Y.V1 GH/452R GR/484.V2 GH/501Y.V2 367F.V1

North America, relevant patterns not captured globally

```

1111111
1111111122444445555666777778899011112
11224567778934455772557789027778013681538716792
53806994902505814233829278441002571162902962888618
LSLYPTHLNAGGDYDLYNNQADLSTE SNAATX PATTGEPTDSADGVKV
.....S..... 114
.....S..H..... 35
.....F.....S..... 13
.....F.....S..H..... 12
.....H.....I..... 50
.....K.....H..... 32
F.....I.....K.....V..... 58
.....L.....K.....V..... 14
.....H..A..... 113
F.....I..... 21
F.....I.....V..... 10
..... 18
.....R..... 35
.....R.....N..... 22
.....V..... 64
F.....I.....V..... 19
.....I..... 60
.....I.....H..... 17
.....I.....A..... 9
.....T..... 24
.....L..... 80
.....L..... 14
.....R..... 10
..... 66
..... 30
.....I..... 181
.....Y..... 25
.....F..... 16
.....Q..... 10
```

South America, relevant patterns not captured globally

```

111111111
111111112344444555666666677788900011122
1222456790345557920115780672555778817717827716722
58036929052842316027472741506458051966829272887689
LLTQPHQWTRDYNNLRATQKLSENF AAEHNICPSTKTPASTEADGVAM
..N.S.....Y.....S...Y..K.....Y.....I....F.. 54
.....R.....L.. 4
.....R.....I..... 23
.....R..... 8
.....Y.....R..... 4
.....N.....V....R.....F.. 5
.....T.....I.....F.. 14
.....T.....T...I.....F.. 5
```

FIG. 13 CONT.

RBD
NTD supersite
Infectivity

B.1.1.7 B.1.429 B.1.1.28 B.1.351 A231
GR/501Y.V1 GH/452R GR/484.V2 GH/501Y.V2 367F.V1

Africa, relevant patterns not captured globally

```

11111111
111122222233444455666666677778990112222
1124687893455 1244446815789071155778801693387151236
28297900584475223467572740103435571916964928859874
SLTHAMDTDYEFDAALRVTKLSEFNAQDAHQPSATGDISSAOYGMV
.....L.....F.....H.....R..... 19
.F.....R...Y...VY.H.....Y.....V... 16
..... 3
.....Q.....S...G.....V..... 12
.....S...N..... 4
.....A.....G.---...K...G.....V..... 61
.F.....A.....G.---...K...G.....V..... 34
.....A.....---...K...G.....V..... 7
F.....R.....G...H..... 7
.I.....R.....G..... 12
.....R.....G.....F.L.. 10
F.....R.....G...H..... 7
.I.....R.....G..... 6

```

FIG. 14

B.1.1.7 B.1.429 B.1.1.28 B.1.351 A231
GR/501Y.V1 GH/452R GR/484.V2 GH/501Y.V2 367F.V1

United-Kingdom, relevant patterns not captured globally

```

111111
1111112222222244455566666677788999001111
1222667893446781125566737907812578801215238271666
58136790088464610525612297410332571866329992038237
LLRQPAHVDS DYHNLGIDASSGAPNSSNAEQVHQPAATTPTSSSAKDPDG
.....D.....H..I.....A..H... 352
.....V..... 148
.....V..S..... 76
.....F.....N.... 321
.....V..... 35
.....V..... 29
.....V..... 168
.....V..... 112

```

Oceania, relevant patterns not captured globally

```

111111
111111222222234444555566666677889900112
1112678993445581124567835780257813577880115826112
52383900588142360522114492741080342457181629208788
LSSLQHVDTSDLYMMFIDALPGTPNLSENAKAEDTEQQAATPTSAVTDV
..... 2
.....R..... 2
.....G..... 152
.....R.....G.....V..... 17
.....G.....H..... 7
.....S.....G..... 5
.....G..... 4
.....I.....G..... 3

```

Europe w/o United-Kingdom,
relevant patterns not captured globally

```

1111111111
11111122222244445566666666788899000011122
1256667899344581236735570725777788111538277716615
58644790058834315232292371064357918623992013882392
LLPLWAHVDTSDVYMGDAIAPNLYSNAAESQNPATPSTSSAQKADPDGS
.....F.....K..... 882
.....F.....K.....G..... 43
.....F..F..... 30
.....T.....T..K..... 337
.....T..... 93
.....Y..T..... 37
.....V..... 32
..... 27
.....V..SL..R..... 25
..... 22
.....H..... 21
.....Y.....V..... 18
.....K.....K..... 16

```

FIG. 15

SET Vaccine Design

Variants of concern mutations are color coded.

UK B.1.1.7 US B.1.429 BR B.1.1.28 ZA B.1.351 UG A23.1
GR/501Y.V1 GH/452R.V1 GR/484.V2 GH/501Y.V2 D/367F.V1

Orange amino acids are sites that are selected in vivo as resistant or resistant to at least some antibodies.
Red amino acids are known to be very serious resistance mutations for sera and multiple antibodies.

SET1	Where is it found?		Count
L18P	Recurrent in many lineages	Global	40644
T20N	GR/484.V2	S. America	59
P26S	GR/484.V2	Global, many back bones	821
D80A	GH/501Y.V2	Africa, increasing	196
M153T	Recurrent	Asia, S & N America, Europe	3195
Δ242-244	GH/501Y.V2	Africa, increasing	243
K417N	GH/501Y.V2	Africa, increasing	188
Y453P	Common	UK, Europe	1449
E484K	GH/501Y.V2 GR/484.V2	Africa US, Brazil	799
N501Y	GH/501Y.V2 GR/484.V2	Global	45067
	GR/501Y.V1		
D614G		Global	377214

FIG. 16

SET Vaccine Design

Variants of concern mutations are color coded.

UK B.1.1.7 US B.1.429 BR B.1.1.28 ZA B.1.351 UG A23.1
GR/501Y.V1 GH/452R.V1 GR/484.V2 GR/501Y.V2 Q/367F.V1

Orange amino acids are sites that are selected in vivo as resistant or resistant to at least some antibodies.
Red amino acids are known to be very serious resistance mutations for sera and multiple antibodies.

SET2		Where is it found?	Count
S13I	GH/452R	North America, increasing	951
A69V	GR/501Y.V1	Global, multiple contexts	51840
A188V	GR/501Y.V1	Global, multiple contexts	44535
W152C	GH/452R	North America, increasing	887
D253G	low level	North America	1061
A262S	recurrent	Europe, Asia	5323
L452R	GH/452R + others	Global	1298
S477N	common, transient	Oceania, Global	20936
D614G		Global	377214

FIG. 17

SET Vaccine Design

Varicants of concern mutations are color coded.

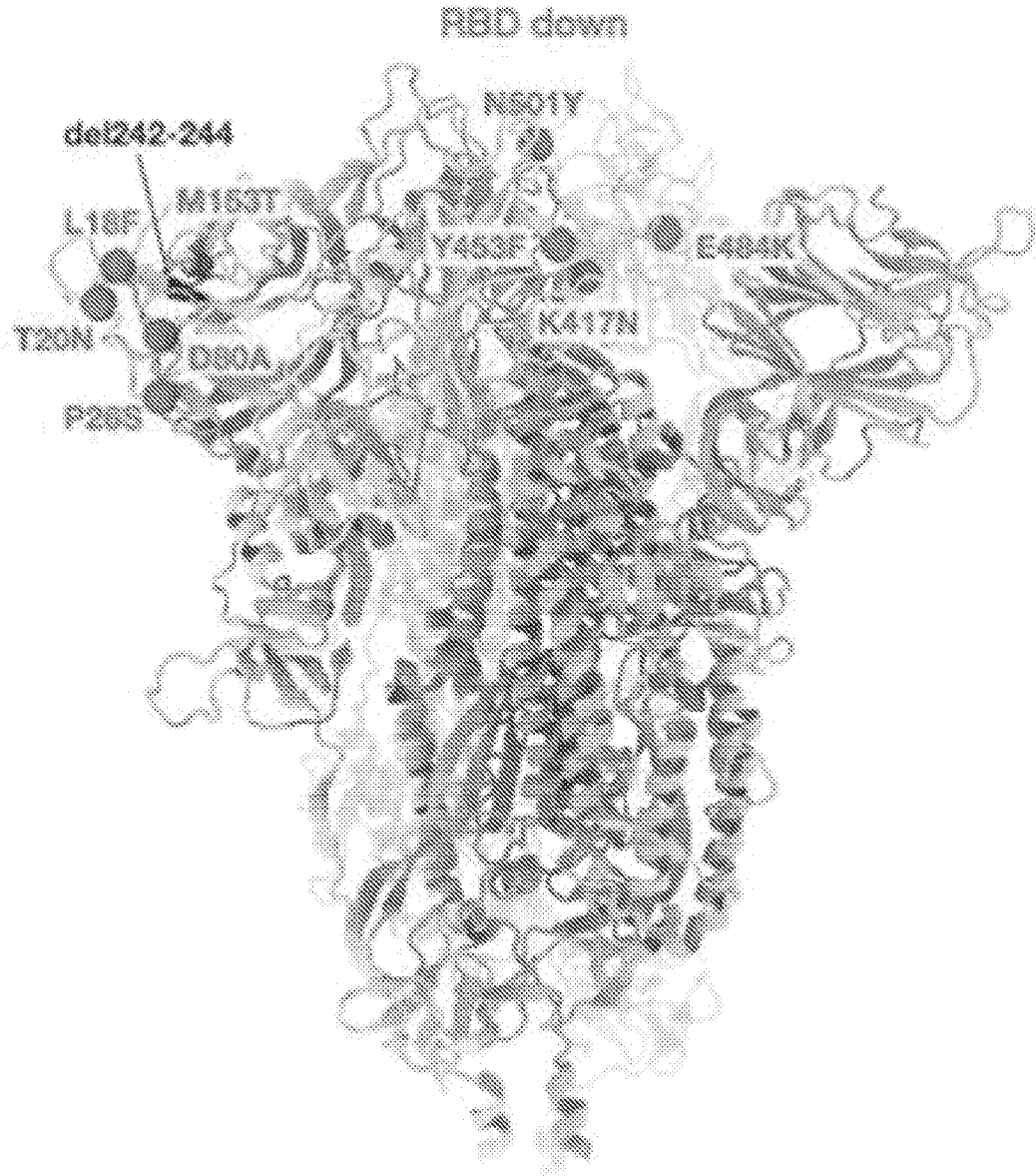
UK B.1.1.7 US B.1.429 BR B.1.1.28 ZA B.1.351 UG A23.1
 GR/501Y.V1 GH/452R.V1 GR/484.V2 GH/501Y.V2 Q/367F.V1

Orange amino acids are sites that are selected in vivo as resistant or resistant to at least some antibodies.
 Red amino acids are known to be very serious resistance mutations for sera and multiple antibodies.

SET3		Where is it found?	Count
D80Y		UK, US	2041
L141F	A23.1	N. America/N America	157
F157L	A23.1	Africa/Asia/N America	281
S255F	low level	UK	822
V367F	A23.1	UG/UK/Cambodia/Canada	347
K417T	GR/484.V2	Brazil	57
N439K	Common	UK, Europe, Asia, Oceania	7907
S477R		Oceania, Europe	20936
S494P	Recent	US/UK	645
N501Y		US/UK	569
Q613H	A23.1	Africa/Asia/N America	722
D614	A23.1	Global, mostly early 2020	19323
P681R	A23.1	Africa/Asia/N America	404

FIG. 18

SET 1



SET 1

FIG. 18 CONT.

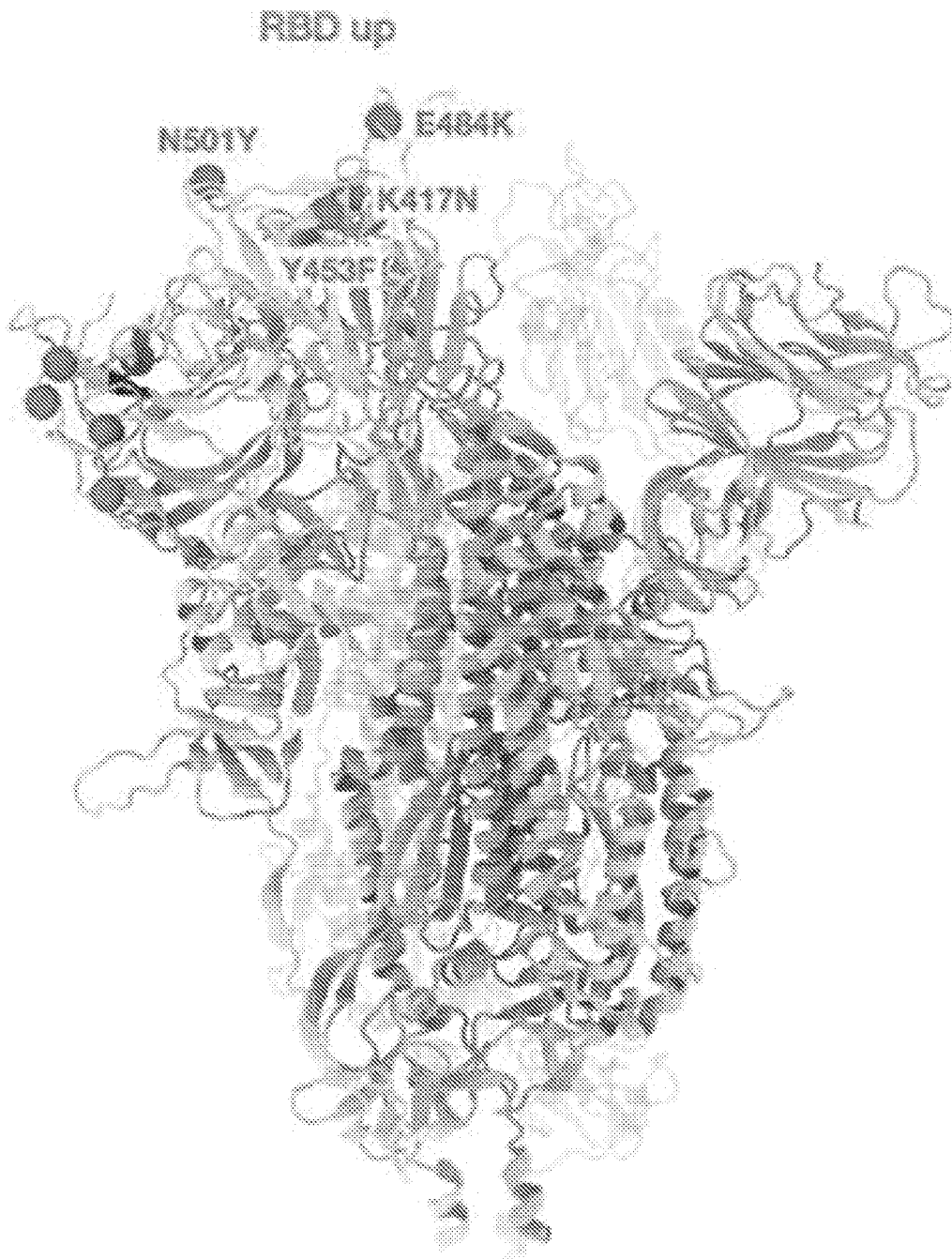


FIG. 19

SET 2

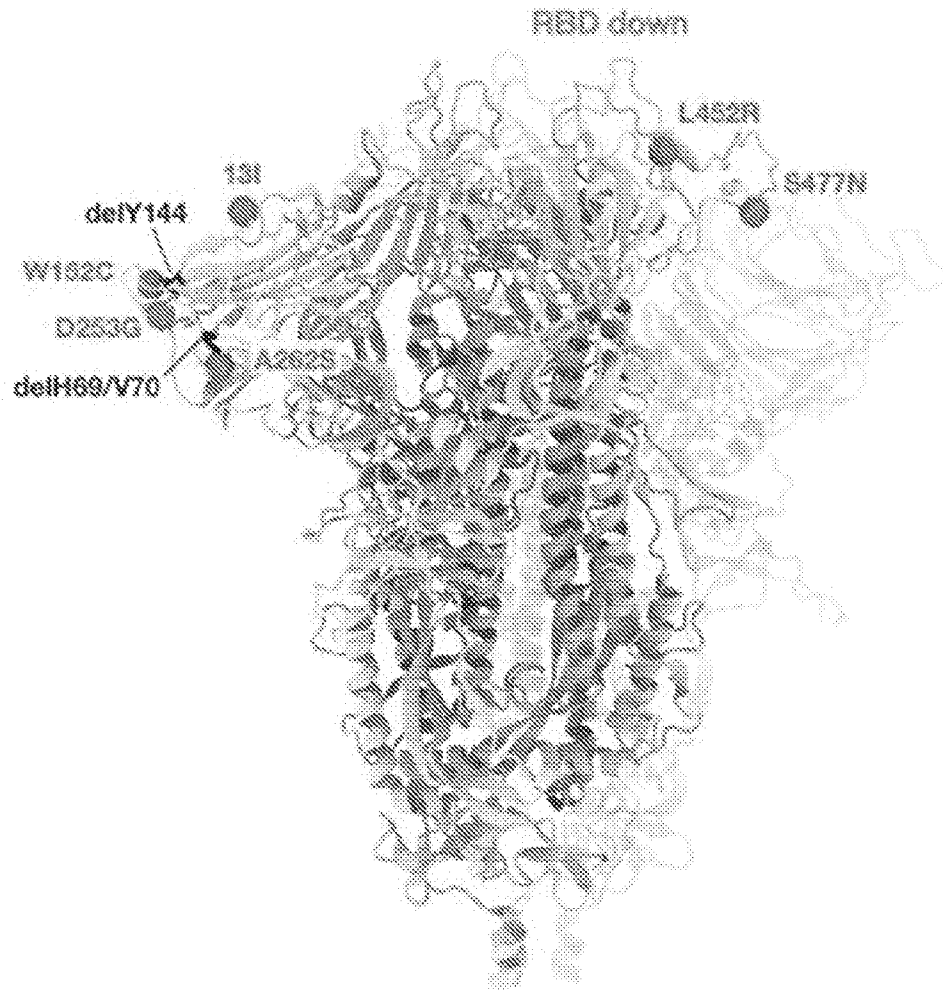


FIG. 19 CONT.

SET 2



FIG. 20

SET 3

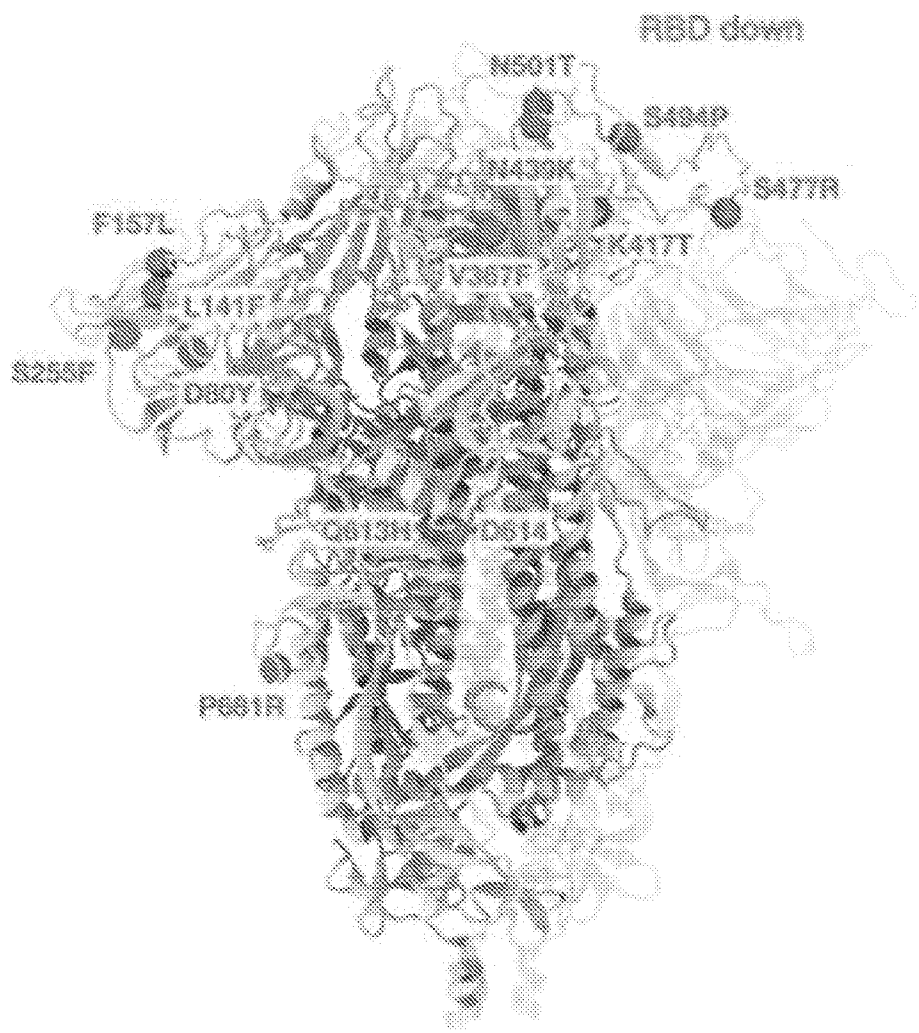


FIG. 20 CONT.

SET 3

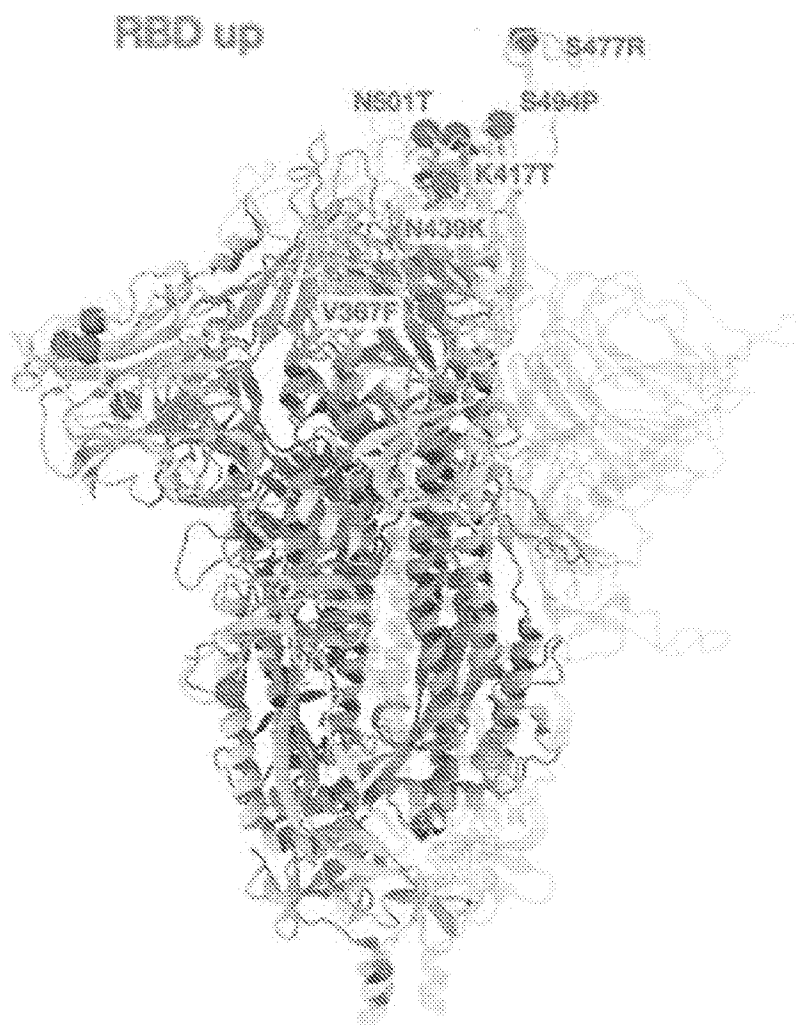


FIG. 21

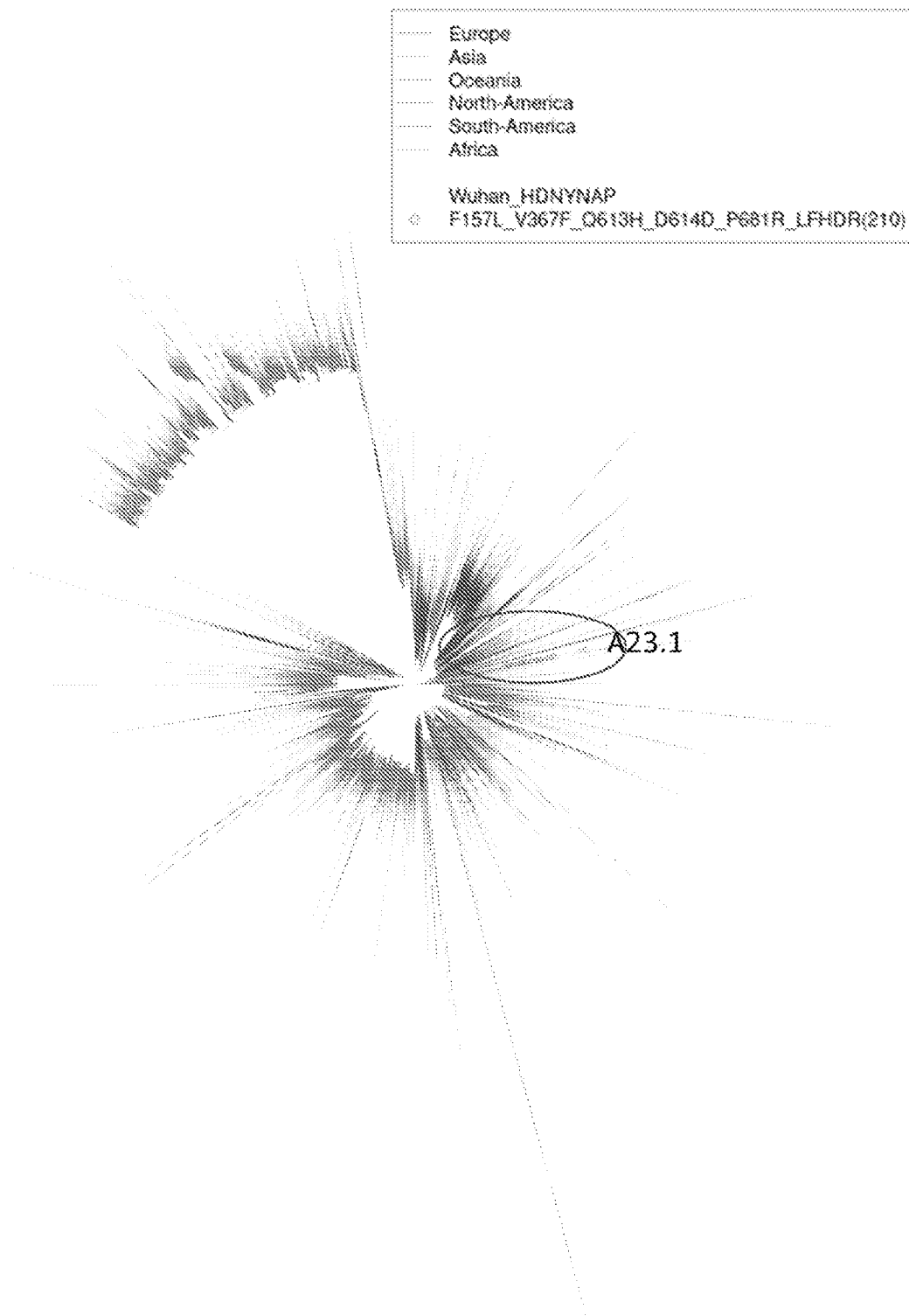
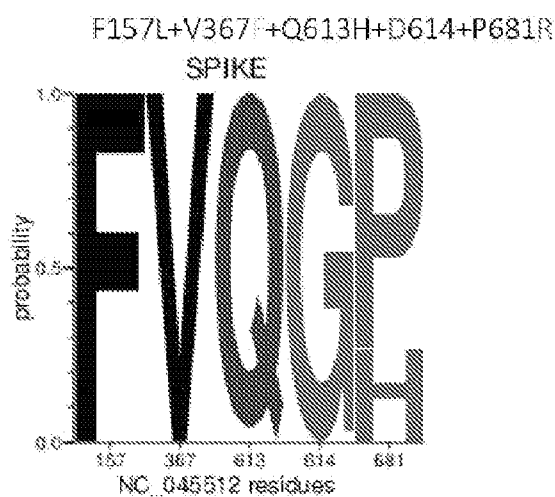


FIG. 22A



Belgium 6
Cambodia 12
Canada 30 (New-Brunswick 28)
Denmark 4 (Hovedstaden 3)
Ghana 1
India 2 (Karnataka 2)
Netherlands 1
Rwanda 24 (Kigali 24)
Switzerland 3
USA 5 (Arizona 2, New-Mexico 1, New-York 1, Oklahoma 1)
Uganda 48 (No local data 48)
United-Arab-Emirates 1
United-Kingdom 127

FIG. 22B

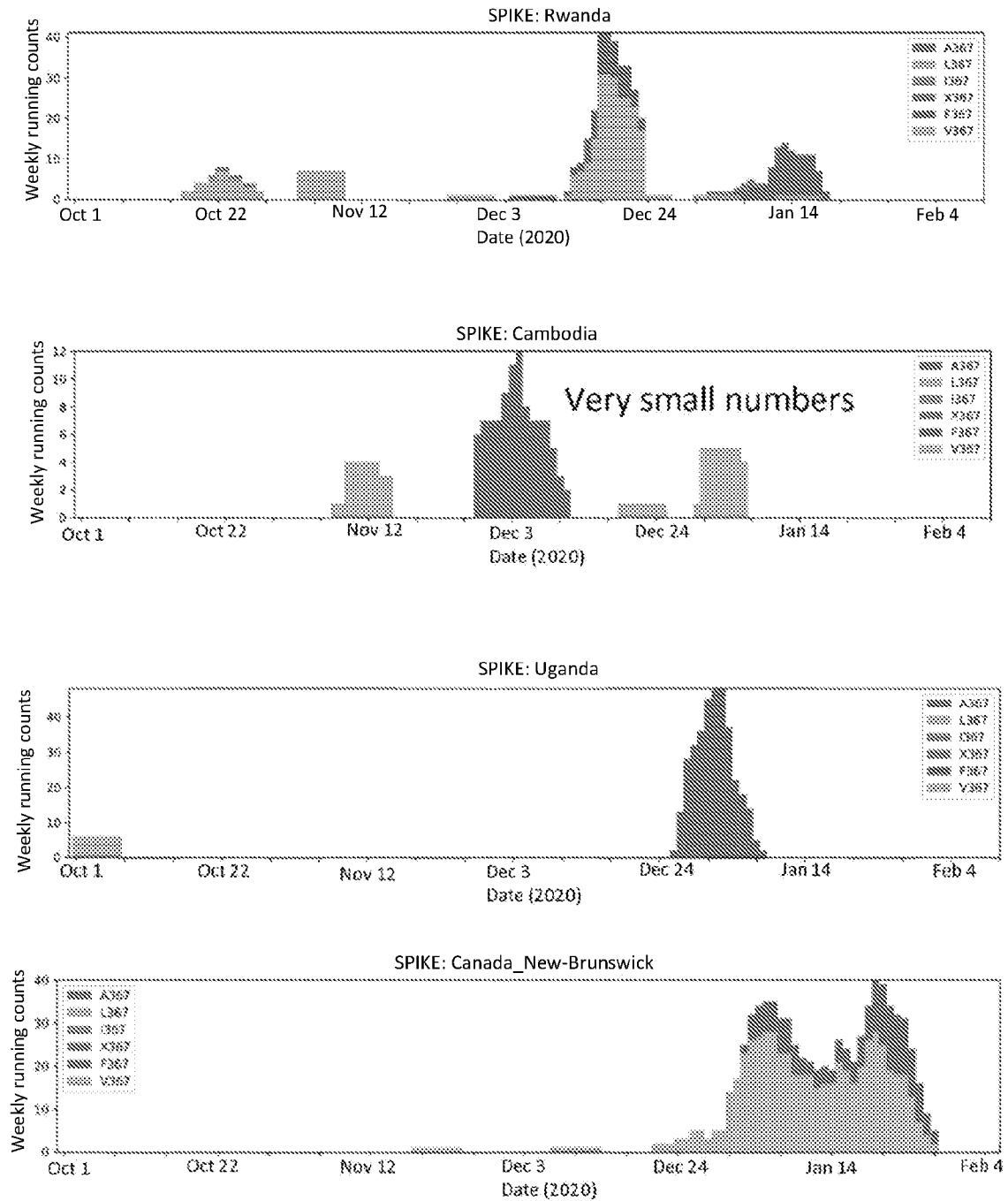


FIG. 22C

Form.	Count	
RLFVEQGP	241081	Most common
-----H	85835	Recurrent global
----K---	1554	Recurrent, global
-----H--	530	UK/DE/US +
-----D-	260	scattered
-----R	390	US/Bangladesh +
----K--H	143	US/UK +
--LF-HDR	124	CA/UG/UK/Cambodia+
---F----	98	Switzerland/US +
I-LFKHDR	49	UK (ALDP)
I-LF-HDR	48	UK/UG
-FLF-HDR	42	UK/US
---F-HDR	3	Cambodia

FIG. 22D

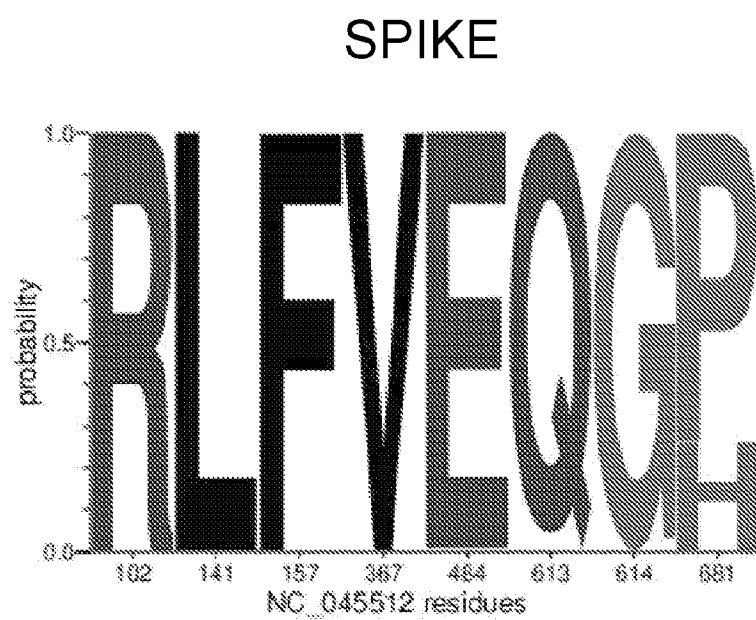


FIG. 23 CONT.

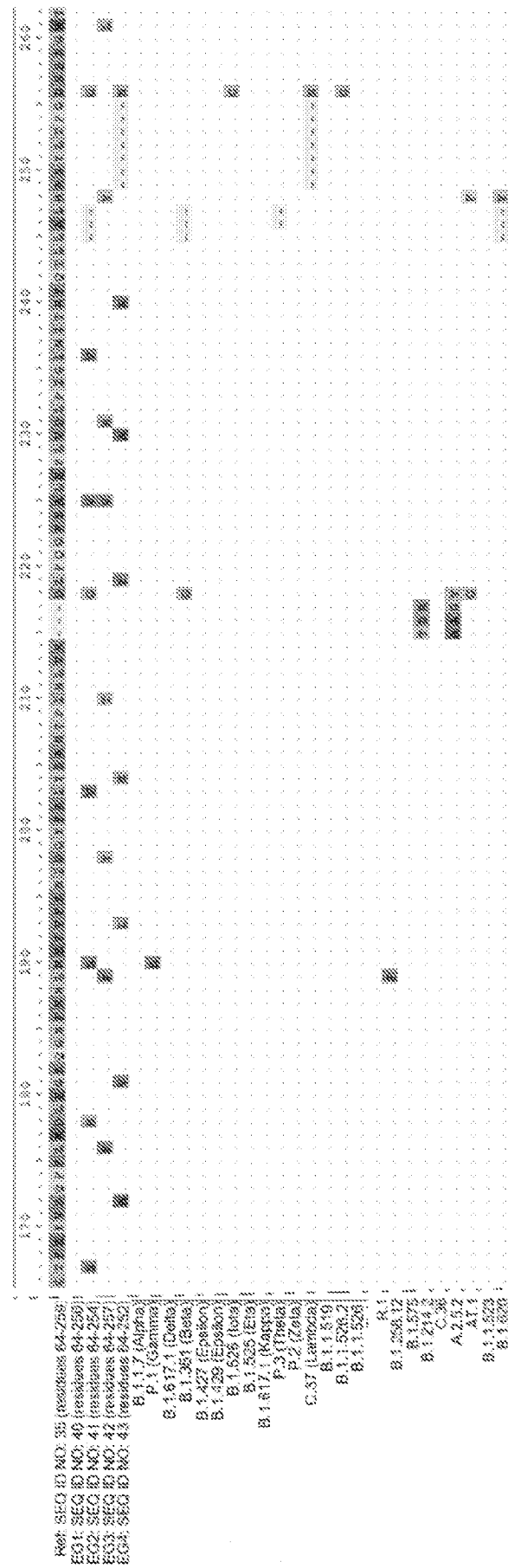


FIG. 24

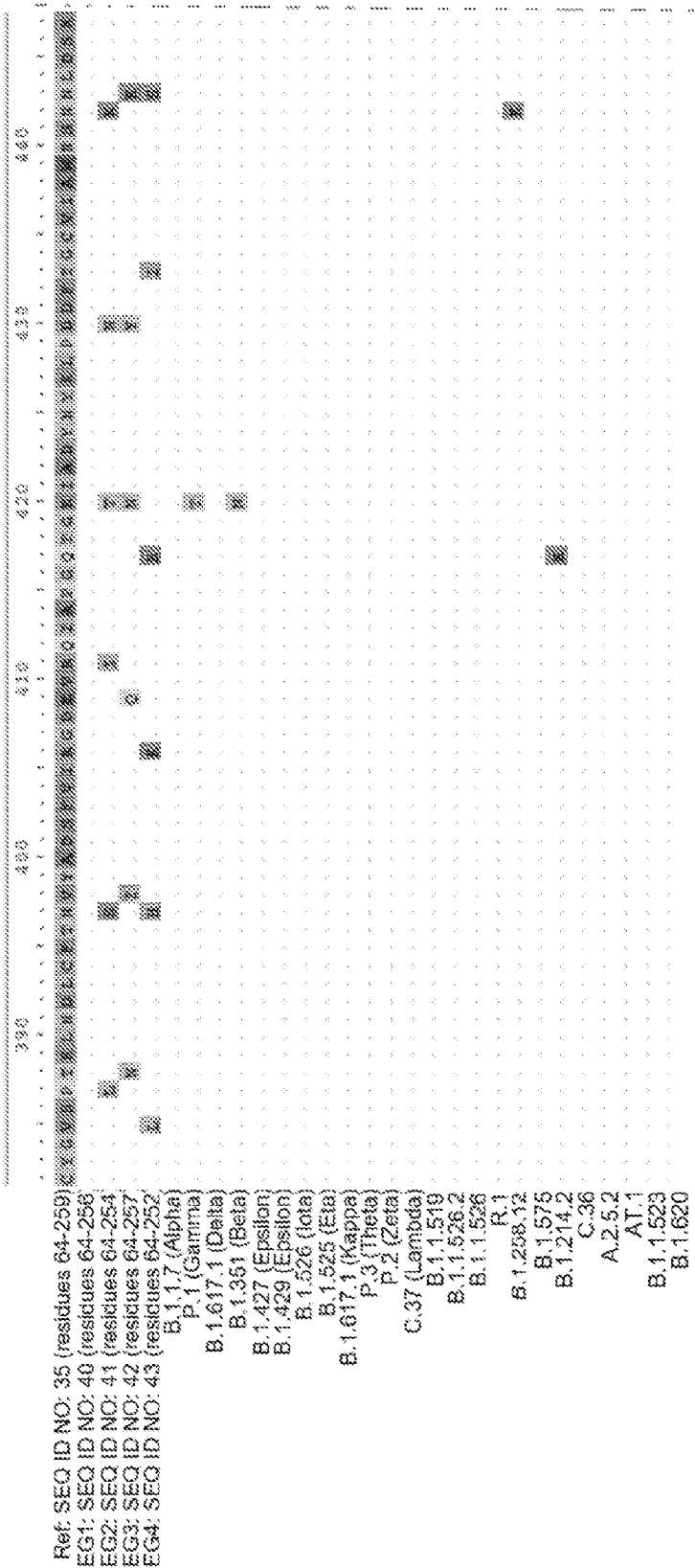


FIG. 24 CONT.

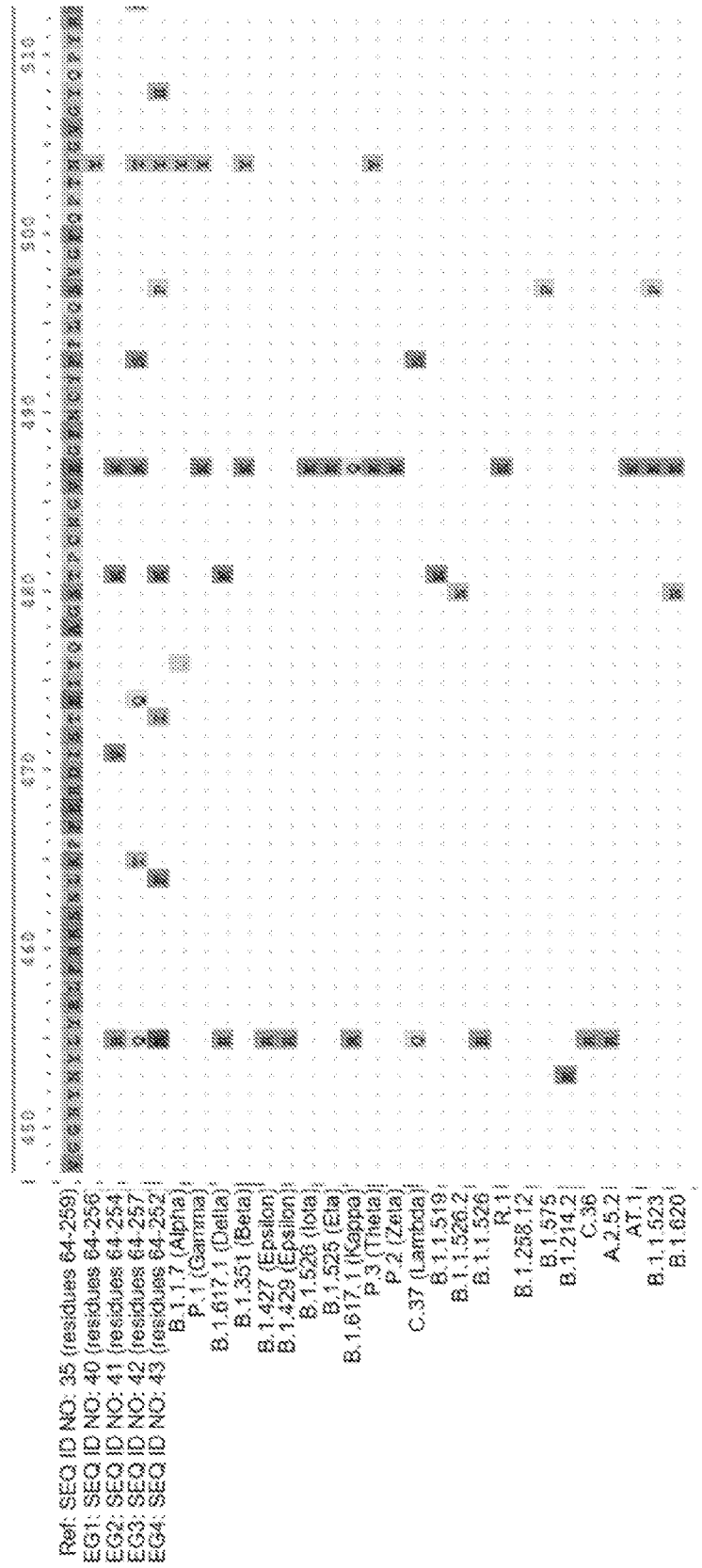


FIG. 25A

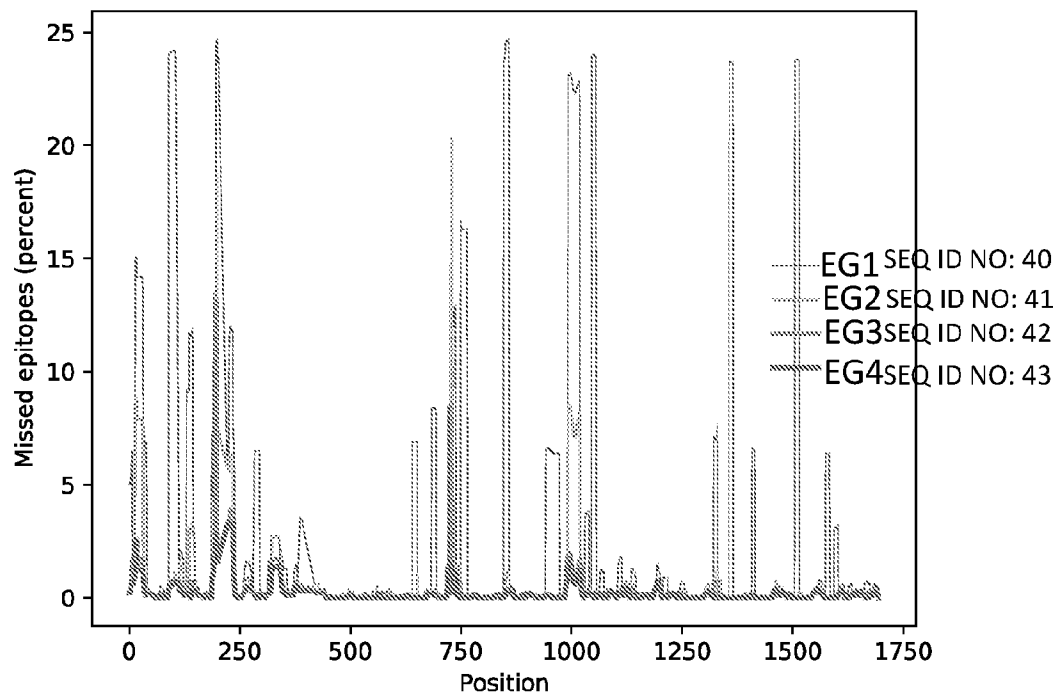


FIG. 25B

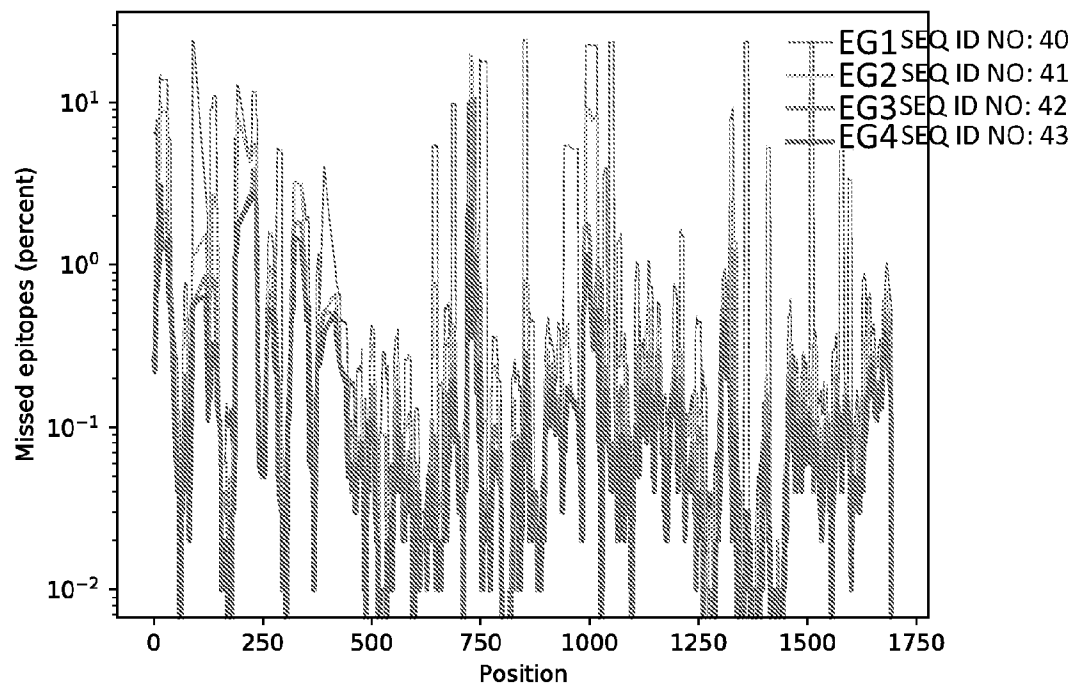


FIG. 26

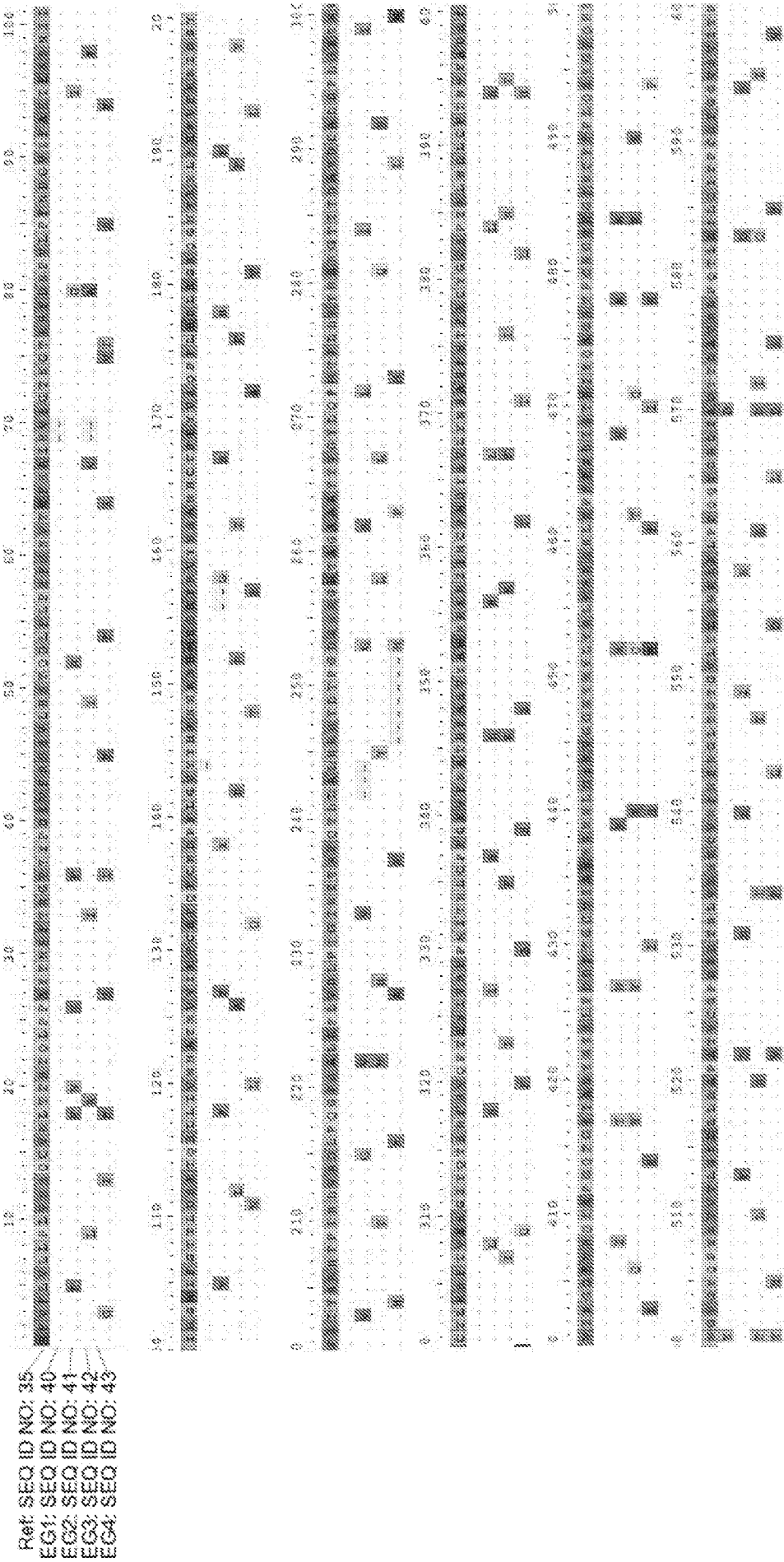


FIG. 26 CONT.

