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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
03 March 2022 (03.03.2022)

WIPO | PCT

(10) International Publication Number
WO 2022/047033 A1

(51) International Patent Classification:
C07K 16/10 (2006.01) *A61P 31/14* (2006.01)
A61K 39/395 (2006.01)

(21) International Application Number:
PCT/US2021/047757

(22) International Filing Date:
26 August 2021 (26.08.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/070,707 26 August 2020 (26.08.2020) US

(71) Applicant: **FLAGSHIP PIONEERING INNOVATIONS VI, LLC** [US/US]; 55 Cambridge Parkway, 8th Floor, Cambridge, MA 02142 (US).

(72) Inventors: **GRIGORYAN, Gevorg**; 55 Cambridge Parkway, 8th Floor, Cambridge, MA 02142 (US). **INGRAHAM, John**; 55 Cambridge Parkway, 8th Floor, Cambridge, MA 02142 (US).

(74) Agent: **BALICKY, Eric M.** et al.; Hamilton, Brook, Smith & Reynolds, P.C., 530 Virginia Rd, P.O. Box 9133, Concord, MA 01742-9133 (US).

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

Published:
— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

WO 2022/047033 A1

(54) Title: ANTIGEN BINDING MOLECULES TARGETING SARS-COV-2

(57) Abstract: The invention provides, in various embodiments, polypeptides (e.g., camelid antibodies and antigen binding fragments thereof) that specifically bind to Spike glycoprotein of severe acute respiratory syndrome coronavirus (e.g., SARS-CoV-2-Spike). The invention also provides, in various embodiments, fusion proteins comprising one or more of the polypeptides, polynucleotides encoding the polypeptides, vectors and host cells suitable for expressing the polypeptides, and methods for treating viral infections (e.g., COVID-19).

Antigen Binding Molecules Targeting SARS-CoV-2

RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 63/070,707, filed on August 26, 2020. The entire teachings of the above application are incorporated herein by reference.

INCORPORATION BY REFERENCE OF MATERIAL IN ASCII TEXT FILE

[0002] This application incorporates by reference the Sequence Listing contained in the following ASCII text file being submitted concurrently herewith:

a) File name: 5708_1029001_Sequence_Listing.txt; created August 26, 2021, 33,014 Bytes in size.

BACKGROUND

[0003] The SARS-Coronavirus-2 (SARS-CoV-2), a novel coronavirus, first caused a cluster of pneumonia cases (COVID-19) in Wuhan, China. As of March 1, 2020, 79,968 patients in China had tested positive for COVID-19, 2,873 deaths had occurred, equivalent to a mortality rate of 3.6% (95% CI 3.5-3.7) (Baud D. et al., *Lancet Infect Dis.* (2020)). This figure, however, may be an underestimate of the potential threat of COVID-19 in symptomatic patients (*Id.*).

[0004] COVID-19 has been spreading rapidly throughout the world, resulting in a pandemic. The Coronavirus disease (COVID-2019) situation report released from the World Health Organization on April 21, 2020 reported 2,397,216 confirmed infections and 162,956 deaths. Among them, 83,006 new cases and 5,109 deaths were added within the previous 24 hours. Quarantine, isolation, and infection-control measures have been relied on to prevent disease spread, and supportive care for those who become ill (Baden and Rubin, *N Engl J Med.*, (2020)).

SUMMARY

[0005] There is a critical need to develop specific antiviral therapeutic agents for preventing transmission of COVID-19 as well as treating COVID-19 patients.

[0006] The invention disclosed herein is based, in part, on the discovery that polypeptides of the present invention specifically bind to the Spike glycoprotein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2-Spike). Accordingly, the invention generally relates to

compositions (e.g., polypeptides, pharmaceutical compositions) and methods that are useful for reducing Spike (e.g., SARS-CoV-2-Spike) mediated viral entry into a cell.

[0007] Provided herein are polypeptides that specifically bind SARS-CoV-2-Spike. In one aspect, the invention provides polypeptides that specifically bind SARS-CoV-2-Spike, wherein the polypeptide comprises a paratope that is identical to the paratope of an antibody comprising an amino acid sequence selected from:

a) SEQ ID NO: 4; or

b) SEQ ID NO: 5.

[0008] In some embodiments, the polypeptide comprises an immunoglobulin heavy chain variable domain (V_H).

[0009] In another aspect, the invention provides a polypeptide that specifically binds SARS-CoV-2-Spike, wherein the polypeptide comprises an immunoglobulin heavy chain variable domain (V_H) amino acid sequence comprising a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2) and a heavy chain complementarity determining region 3 (HCDR3) that are identical to the HCDR1, HCDR2 and HCDR3, respectively, of the amino acid sequence of SEQ ID NO: 4 or SEQ ID NO: 5.

[0010] In some embodiments, the polypeptide further comprises a paratope that is identical to the paratope of a single domain antibody comprising:

a) SEQ ID NO: 4; or

b) SEQ ID NO: 5.

[0011] In another aspect, the invention provides a polypeptide that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 2, wherein:

a) X_1 is not T;

b) X_2 is not V; or

c) X_3 is not V,

or any combination of the foregoing.

[0012] In some embodiments, the polypeptide is a single-domain antibody or an antigen-binding fragment thereof.

[0013] In some embodiments, the polypeptide is linked to a second polypeptide. The term "linked" means attached, via a covalent or noncovalent interaction. Conjugation can employ a suitable linking agent. Non-limiting examples include peptide linkers, compound linkers, and chemical cross-linking agents. In certain embodiments, the linker is a disulfide bond.

[0014] In some embodiments, the polypeptides of the invention are fusion proteins.

[0015] In other aspects, the invention provides a polynucleotide encoding a polypeptide disclosed herein, a vector comprising such polynucleotide, and a host cell comprising such polynucleotide or vector.

[0016] In another aspect, the invention provides a method of treating a subject in need thereof (e.g., a subject having a SARS-CoV infection, such as COVID-19), comprising administering to the subject an effective amount of a polypeptide disclosed herein or a composition (e.g., pharmaceutical composition) comprising a polypeptide disclosed herein.

[0017] In another aspect, the invention provides a method of inhibiting fusion between SARS-CoV-2-Spike and a cell (e.g., a cell in a subject), comprising contacting the cell with an effective amount of a composition comprising a polypeptide disclosed herein or a composition (e.g., pharmaceutical composition) comprising a polypeptide disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0019] The foregoing will be apparent from the following more particular description of example embodiments, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating embodiments.

[0020] FIG. 1 depicts the amino acid sequence of the receptor binding domain (RBD of SARS-Cov-1-Spike (SEQ ID NO: 21). The epitope residues bound by the Reference antibody disclosed herein are indicated by asterisks.

[0021] FIG. 2 depicts an alignment of non-limiting examples of heavy chain variable (V_H) sequences that are useful in polypeptides of the invention. The heavy chain complementarity determining region (HCDR) sequences are indicated using bold letters and underlining. Paratope residues are indicated with black "*." Additional possible paratope residues are indicated with red "*." The position of X_n, where n is a number from 1-3, is indicated by the corresponding numbers at the bottom of the alignment. Also see V_H consensus (SEQ ID NO: 2) in Table 1. The antibody sequences were computationally generated using information from the sequence and structure of a reference polypeptide (Reference). Paratope positions were defined as antibody residues within 5 angstroms of the antigen in the parent structures.

[0022] FIG. 3 depicts the affinities of a reference VHH molecule (Reference), and two variant VHH molecules of the invention, AB-1 and AB-2, to COV-2 RBD, as measured using yeast surface display.

[0023] FIG. 4 illustrates the ability of a polypeptide of the invention (AB-1) to neutralize mutant SARS-COV-2 pseudovirus. The bars represent fold-difference (WT- Mutant)/(WT) in IC_{50} values for antibody neutralization of pseudoviruses bearing emerging mutations or deletions relative to the parent D614G-parent virus. Values between -2.5 and 2.5 are considered as not significant in this assay.

DETAILED DESCRIPTION

[0024] A description of example embodiments follows.

[0025] Several aspects of the invention are described below, with reference to examples for illustrative purposes only. It should be understood that numerous specific details, relationships, and methods are set forth to provide a full understanding of the invention. One having ordinary skill in the relevant art, however, will readily recognize that the invention can be practiced without one or more of the specific details or practiced with other methods, protocols, reagents, cell lines and animals. The invention is not limited by the illustrated ordering of acts or events, as some acts may occur in different orders and/or concurrently with other acts or events. Furthermore, not all illustrated acts, steps or events are required to implement a methodology in accordance with the invention. Many of the techniques and procedures described, or referenced herein, are well understood and commonly employed using conventional methodology by those skilled in the art.

Polypeptides Binding SARS-CoV-2 Spike Protein

[0026] SARS-CoV-2 is the causative agent of COVID-19. SARS-CoV-2-Spike, or S protein of the SARS-CoV-2, a protein that facilitates entry of the SARS-CoV-2 virus into a host cell, such as a human host cell. Without being bound by theory, it is believed SARS-CoV-2-Spike facilitates infection by binding to an entry receptor on the surface of a host cell (e.g., angiotensin converting enzyme 2 (ACE2) or Transmembrane protease, serine 2 (TMPRSS2)). A non-limiting example of a wildtype SARS-CoV-2-Spike sequence is NCBI RefSeq YP_009724390 (SEQ ID NO: 1).

MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFS
NVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIV
NNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMD

LEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQT
LLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSET
KCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISN
CVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIA
DYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGST
PCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKN
KCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVS
VITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEH
VNNSYECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLGAENSVAYSNNSIAIPT
NFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDK
NTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQY
GDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIP
FAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQN
AQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAA
EIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKN
FTTAPAICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVN
NTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLN
ESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCCSCGSCC
KFDEDDSEPVLKGVKLHYT (SEQ ID NO: 1).

[0027] As used herein, SARS-CoV-2 Spike includes wild-type SARS-CoV-2 Spike proteins (e.g., SEQ ID NO: 1), variants of wild-type SARS-CoV-2 Spike proteins, and modified forms of wild-type and variant SARS-CoV-2 Spike proteins.

[0028] In one aspect, the invention provides a polypeptide that binds to a SARS-CoV-2 Spike protein comprising SEQ ID NO:1.

[0029] In some embodiments, the polypeptide binds to a variant of SARS-CoV-2-Spike. In some embodiments, the variant comprises an amino acid sequence that has at least about 90% sequence identity to the wildtype SARS-CoV-2-Spike sequence (e.g., SEQ ID NO: 1), for example, having at least about: 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% sequence identity to the wildtype SARS-CoV-2-Spike sequence. In some embodiments, the sequence identity is about: 90-99.9%, 90-99.8%, 92-99.8%, 92-99.6%, 94-99.6%, 94-99.5%, 95-99.5%, 95-99.4%, 96-99.4%, 96-99.2%, 97-99.2% or 97-99%.

[0030] In some embodiments, the variant SARS-CoV-2-Spike comprises, relative to SEQ ID NO:1, one or more mutations selected from: L5F, S13I, T19R, A67V, 69del, 70del, D80G, T95I,

G142D, 144del, W152C, E154K, F157S, A222V, D253G, G261D, V367F, K417N, N439K, L452R, Y453F, S477N, E484K, F486L, S494P, E484Q, N501T, N501Y, F565L, A570D, D614G, Q677H, P681H, P681R, A701V, T716I, T859N, F888L, S982A, D950N, Q957R, Q1071H, V1176F, D1118H, K1191N, or a combination thereof, 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, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, or all 45.

[0031] In some embodiments, the variant SARS-CoV-2-Spike comprises, relative to SEQ ID NO:1, one or more mutations selected from: 69del, 70del, 144del, E484K, S494P, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H or K1191N, or a combination thereof. In some embodiments, the variant SARS-CoV-2-Spike comprises 69del, 70del, 144del, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H. In some embodiments, the variant SARS-CoV-2-Spike further comprises E484K, S494P or K1191N, or a combination thereof.

[0032] In some embodiments, the variant SARS-CoV-2-Spike comprises, relative to SEQ ID NO:1, one or more mutations selected from: D80A, D215G, 241del, 242del, 243del, K417N, E484K, N501Y, D614G or A701V, or a combination thereof. In some embodiments, the variant SARS-CoV-2-Spike comprises D80A, D215G, 241del, 242del, 243del, K417N, E484K, N501Y, D614G, and A701V.

[0033] In some embodiments, the variant SARS-CoV-2-Spike comprises, relative to SEQ ID NO:1, one or more mutations selected from: T19R, G142D, 156del, 157del, R158G, L452R, T478K, D614G, P681R or D950N, or a combination thereof. In some embodiments, the variant SARS-CoV-2-Spike comprises T19R, 156del, 157del, R158G, L452R, T478K, D614G, P681R, and D950N. In some embodiments, the variant SARS-CoV-2-Spike further comprises G142D.

[0034] In some embodiments, the variant SARS-CoV-2-Spike comprises, relative to SEQ ID NO:1, one or more mutations selected from: 69del, 70del, 144del, A222V, G261D, V367F, K417N, N439K, Y453F, S477N, E484K, F486L, N501T, N501Y, A570D or D614G, or a combination thereof.

[0035] In some embodiments, the variant SARS-CoV-2-Spike comprises, relative to SEQ ID NO:1, one or more mutations selected from: E484K, N501Y or D614G, or a combination thereof.

[0036] In some embodiments, the modified SARS-CoV-2 Spike protein comprises, relative to SEQ ID NO:1, one or more mutations selected from: F817P, A892P, A899P, A942P, K986P, V987P.

[0037] In some embodiments, the polypeptide binds the SARS-CoV-2 Spike protein receptor-binding domain (RBD) (SEQ ID NO: 17), or an epitope within RBD (e.g., recognized by RBD Community 7).

[0038] RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNS ASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTG CVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYF PLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGP (SEQ ID NO: 17).

[0039] In some embodiments, the polypeptide binds to a variant of the SARS-CoV-2-Spike RBD or an epitope within the SARS-CoV-2-Spike RBD. In some embodiments, the variant comprises an amino acid sequence that has at least about 90% sequence identity to the wildtype SARS-CoV-2-Spike RBD (e.g., SEQ ID NO: 17), for example, having at least about: 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% sequence identity. In some embodiments, the sequence identity is about: 90-99.9%, 90-99.8%, 92-99.8%, 92-99.6%, 94-99.6%, 94-99.5%, 95-99.5%, 95-99.4%, 96-99.4%, 96-99.2%, 97-99.2% or 97-99%.

[0040] In some embodiments, the polypeptide that binds to a SARS-CoV-2 Spike protein (*e.g.*, SEQ ID NO: 1, SEQ ID NO: 17) and comprises an immunoglobulin heavy chain variable region, wherein the polypeptide does not comprise all three of SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 9. In some embodiments, the polypeptide comprises 1 or 2 CDRs selected from SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 9.

[0041] In another aspect, the invention provides a polypeptide that specifically binds a SARS-CoV-2 Spike protein, wherein the polypeptide comprises an immunoglobulin heavy chain variable region (V_H) comprising an amino acid sequence that is at least 55% (*e.g.*, at least 60, 65, 70, 75, 80, 85, 90, 95, 98, or 99%) identical to SEQ ID NO: 3, wherein the polypeptide does not comprise all three of SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 9. In some embodiments, the polypeptide comprises 1 or 2 CDRs selected from SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 9.

[0042] In some embodiments, the polypeptide binds to a wildtype SARS-CoV-2 Spike protein (*e.g.*, SEQ ID NO:1). In some embodiments, the polypeptide binds to one or more epitope residues of a wildtype SARS-CoV-2 Spike protein (*e.g.*, one or more epitope residues in the SARS-CoV-2 Spike RBD).

[0043] As used herein, the term "reference" or "reference polypeptide" refers to a polypeptide (*e.g.*, immunoglobulin molecule) that specifically binds to SARS-CoV-1, and is not a polypeptide of the invention. The sequence of a reference polypeptide and a polypeptide of the

invention may be compared to illustrate structural differences between them (*e.g.*, differences at one or more amino acid positions, such as amino acid substitutions). In some embodiments, a polypeptide of the invention will have more than insubstantial differences (*e.g.*, one or more substantial differences) in comparison to a reference polypeptide, such that, generally, polypeptides of the invention will, under controlled conditions, exhibit one or more (*i.e.*, one, two, or all three) of: a different function, in a different way, to achieve a different result, in comparison to a reference polypeptide. Reference polypeptides will vary by one or more amino acids from a polypeptide of the invention, *e.g.*, in some embodiments by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more amino acids. In certain embodiments, a reference polypeptide diverges from a polypeptide provided by the invention by at least about: 0.4, 0.8, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55% or more amino acid identity.

[0044] In some embodiments, the "reference polypeptide" is an antibody, referred to herein as "the Reference Antibody," which comprises a V_H region comprising the amino acid sequence of SEQ ID NO: 3. The Reference Antibody is a single-domain camelid antibody that binds SARS-CoV-2 spike RBD and block interaction with ACE2. For additional information about the Reference Antibody, *see*, *e.g.*, Protein Data Bank (PDB) accession 6WAQ, Huo *et al.*, *Neutralizing nanobodies bind SARS-CoV-2 spike RBD and block interaction with ACE2*, Nat Struct Mol Biol. 27(9):846-54 (2020), and Wrapp *et al.*, *Structural Basis for Potent Neutralization of Betacoronaviruses by Single-Domain Camelid Antibodies*, Cell 181(5):1004-15 (2020)).

[0045] As used herein, the term "sequence identity," refers to the extent to which two nucleotide sequences, or two amino acid sequences, have the same residues at the same positions when the sequences are aligned to achieve a maximal level of identity, expressed as a percentage. For sequence alignment and comparison, typically one sequence is designated as a reference sequence, to which a test sequences are compared. The sequence identity between reference and test sequences is expressed as the percentage of positions across the entire length of the reference sequence where the reference and test sequences share the same nucleotide or amino acid upon alignment of the reference and test sequences to achieve a maximal level of identity. As an example, two sequences are considered to have 70% sequence identity when, upon alignment to achieve a maximal level of identity, the test sequence has the same nucleotide or amino acid residue at 70% of the same positions over the entire length of the reference sequence.

[0046] Alignment of sequences for comparison to achieve maximal levels of identity can be readily performed by a person of ordinary skill in the art using an appropriate alignment method or algorithm. In some instances, the alignment can include introduced gaps to provide for the maximal level of identity. Examples include the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), and visual inspection (see generally Ausubel *et al., Current Protocols in Molecular Biology*).

[0047] When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequent coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters. A commonly used tool for determining percent sequence identity is Protein Basic Local Alignment Search Tool (BLASTP) available through National Center for Biotechnology Information, National Library of Medicine, of the United States National Institutes of Health. (Altschul et al., 1990).

[0048] The term "polypeptide" "peptide" or "protein" denotes a polymer of at least two amino acids covalently linked by an amide bond, regardless of length or post-translational modification (*e.g.*, glycosylation or phosphorylation). A protein, peptide or polypeptide can comprise any suitable L-and/or D-amino acid, for example, common α-amino acids (*e.g.*, alanine, glycine, valine), non-α-amino acids (*e.g.*, β-alanine, 4-aminobutyric acid, 6-aminocaproic acid, sarcosine, statine), and unusual amino acids (*e.g.*, citrulline, homocitruline, homoserine, norleucine, norvaline, ornithine). The amino, carboxyl and/or other functional groups on a peptide can be free (*e.g.*, unmodified) or protected with a suitable protecting group. Suitable protecting groups for amino and carboxyl groups, and methods for adding or removing protecting groups are known in the art and are disclosed in, for example, Green and Wuts, *"Protecting Groups in Organic Synthesis,"* John Wiley and Sons, 1991. The functional groups of a protein, peptide or polypeptide can also be derivatized (*e.g.*, alkylated) or labeled (*e.g.*, with a detectable label, such as a fluorogen or a hapten) using methods known in the art. A protein, peptide or polypeptide can comprise one or more modifications (*e.g.*, amino acid linkers, acylation, acetylation, amidation, methylation, terminal modifiers (*e.g.*, cyclizing modifications),

N-methyl-α-amino group substitution), if desired. In addition, a protein, peptide or polypeptide can be an analog of a known and/or naturally-occurring peptide, for example, a peptide analog having conservative amino acid residue substitution(s).

[0049] In one aspect, the invention provides a polypeptide that specifically binds SARS-CoV-2-Spike, wherein the polypeptide comprises an immunoglobulin heavy chain variable domain (V_H) comprising a paratope that is identical to the paratope of an antibody comprising an amino acid sequence selected from:

a) SEQ ID NO: 4; or

b) SEQ ID NO: 5.

[0050] *See* Table 1 for SEQ ID NOs: 4 and 5 and FIG. 2 for the paratope residues of antibodies comprising SEQ ID NOs: 4 and 5. In some embodiments, the paratope comprises amino acid residues corresponding to S52, W53 or Y53, S54, G56, S57, T58, Y59, G102, T103 or V103, V104 or H104, V105 or L105, S106, E107, W108, Y110 and D111 of SEQ ID NO: 2. In some embodiments, said paratope further comprises amino acid residues corresponding to G55 and D62 of SEQ ID NO: 2.

[0051] The term "paratope" refers to a set of amino acid residues in an antibody or an antigen-binding fragment thereof that contribute to a binding interaction with an epitope of a target protein. The binding interaction can be a hydrogen bond, a salt bridge, a van der Waal interaction, an ionic bond or a combination thereof. A binding interaction may be direct, or indirect, e.g., via a coordinated intermediate molecule, such as an ion or water. The residues of a paratope, in some embodiments, comprise only residues that are part of a defined CDR. In other embodiments, the residues of a paratope further comprise one or more residues that are not part of a defined CDR.

[0052] In some embodiments, the paratope is oriented less than about 5.0 angstroms from an epitope on a target antigen when the polypeptide is bound to the target antigen, e.g., less than about: 4.5, 4.0, 3.5, 3.0, 2.5, 2.4, 2.3, 2.2, 2.1, 2.0, 1.9, 1.8, 1.7, 1.6, 1.5, 1.4, 1.3, 1.2, 1.1, 1.0 or 0.9 angstroms, or about: 0.9-5.0, 0.9-4.8. 1.0-5, 1.0-4.5, 1.0-4.0, 1.0-3.5, 1.1-3.5, 1.1-3.0, 1.2-3.0, 1.2-2.5, 1.3-2.5, 1.3-2.4, 1.4-2.4, 1.4-2.3, 1.5-2.3, 1.5-2.2, 1.6-2.2, 1.6-2.1, 1.7-2.1, 1.7-2.0 or 1.8-2.0 angstroms, from the epitope. In some embodiments, all of the amino acid residues constituting the paratope are oriented less than about 5.0 angstroms from an epitope on a target antigen when the polypeptide is bound to the target antigen. In other embodiments, less than all of the amino acid residues constituting the paratope (e.g., about 40%, 50%, 60%, 70%, 80%, 90% of the amino acid residues) in the paratope are oriented less than about 5.0 angstroms from an epitope on a target antigen when the polypeptide is bound to the target antigen.

[0053] In some embodiments, the polypeptide comprising a paratope disclosed herein further comprises an immunoglobulin light chain variable domain (V_L).

[0054] In another aspect, the invention provides a polypeptide that specifically binds SARS-CoV-2-Spike, wherein the polypeptide comprises a V_H amino acid sequence comprising a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2) and a heavy chain complementarity determining region 3 (HCDR3) that are identical in amino acid sequence to the HCDR1, HCDR2 and HCDR3, respectively, of SEQ ID NO: 4 or SEQ ID NO: 5.

[0055] The CDR (e.g., HCDR1, HCDR2 and/or HCDR3) can be a CDR defined by any art-recognized method for identifying CDR residues of an antibody, as described further herein (e.g., a CDR as defined by Kabat, a CDR as defined by Chothia).

[0056] In some embodiments, the polypeptide further comprises a paratope that is identical to the paratope of a single domain antibody comprising the amino acid sequence of:

a) SEQ ID NO: 4; or

b) SEQ ID NO: 5.

[0057] In another aspect, the invention provides a polypeptide that specifically binds SARS-CoV-2-Spike, comprising a V_H comprising the amino acid sequence of SEQ ID NO: 2, wherein:

a) X_1 is not T;

b) X_2 is not V; or

c) X_3 is not V,

or any combination of the foregoing.

[0058] The sequence identified as SEQ ID NO: 2 is shown below, which is a consensus V_H sequence for SEQ ID Nos: 3-5 herein.

QVQLQESGGGLVQAGGSLRLSCAASGRTFSEYAMGWFRQAPGKEREFVATISWSGGST YYTDSVKGRFTISRDNAKNTVYLQMNSLKPDDTAVYYCAAAGLGX$_1$X$_2$X$_3$SEWDYDYD YWGQGTQVTVSSGS (SEQ ID NO: 2).

[0059] In some embodiments:

a) X_1 is T or V;

b) X_2 is V or H; or

c) X_3 is V or L,

or any combination of the foregoing.

[0060] In some embodiments:

a) X_1 is V;

b) X_2 is H; or

c) X_3 is L,

or any combination of the foregoing.

[0061] In some embodiments, X_1 is not T. In some embodiments, X_1 is T or V. In some embodiments, X_1 is T. In some embodiments, X_1 is V. In some embodiments, X_2 is not V. In some embodiments, X_2 is V or H. In some embodiments, X_2 is V. In some embodiments, X_2 is H. In some embodiments, X_3 is not V. In some embodiments, X_3 is V or L. In some embodiments, X_3 is V. In some embodiments, X_3 is L.

[0062] In some embodiments, the polypeptide comprises a V_H that comprises an HCDR1, an HCDR2 and an HCDR3 that are identical in amino acid sequence to the HCDR1, HCDR2 and HCDR3, respectively, of SEQ ID NO: 4 or SEQ ID NO: 5 (*see* Table 1 for SEQ ID NOs: 4 and 5 and FIG. 2 for corresponding HCDR1, HCDR2 and HCDR3 sequences).

[0063] In some embodiments, a polypeptide disclosed herein comprises a V_H that is at least about 70% identical to the amino acid sequence of SEQ ID NO: 3. For example, the V_H can be at least about: 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% or 99% identical to the amino acid sequence of SEQ ID NO: 3. In some embodiments, the V_H is at least about 85% or at least about 90% identical to the amino acid sequence of SEQ ID NO: 3. The sequence identified as SEQ ID NO: 3 is shown below, which corresponds to a human V_H domain:

QVQLQESGGGLVQAGGSLRLSCAASGRTFSEYAMGWFRQAPGKEREFVATISWSGGST YYTDSVKGRFTISRDNAKNTVYLQMNSLKPDDTAVYYCAAAGLGTVVSEWDYDYDY WGQGTQVTVSSGS (SEQ ID NO: 3)

[0064] In some embodiments, a polypeptide disclosed herein comprises a V_H that is at least about 70% identical to the amino acid sequence of SEQ ID NO: 4 or SEQ ID NO: 5, or a combination of the foregoing. For example, the V_H can be at least about: 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% or 99% identical to the amino acid sequence of SEQ ID NO: 4 or SEQ ID NO: 5, or a combination of the foregoing. In some embodiments, the V_H is at least about 85% or at least about 90% identical to the amino acid sequence of SEQ ID NO: 4 or SEQ ID NO: 5, or a combination of the foregoing.

[0065] In some embodiments, a polypeptide disclosed herein comprises an amino acid sequence that is at least about 70% identical to the amino acid sequence of SEQ ID NO: 15 or SEQ ID NO: 16, or a combination of the foregoing. For example, the amino acid sequence can be at least about: 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% or 99% identical to the amino acid sequence of SEQ ID NO: 15 or SEQ ID NO: 16, or a combination of the foregoing. In some embodiments, the amino acid sequence is at least about 85% or at least about 90% identical to the amino acid sequence of SEQ ID NO: 15 or SEQ ID NO: 16, or a combination of the foregoing.

[0066] In some embodiments, a polypeptide disclosed herein comprises a V_H that comprises at least 1 amino acid substitution relative to the amino acid sequence of SEQ ID NO: 3. For example, the number of amino acid substitutions can be at least about: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20, or about: 1-20, 1-19, 2-19, 2-18, 2-17, 3-17, 3-16, 4-16, 4-15, 5-15, 5-14, 6-14, 6-13, 7-13, 7-12, 8-12, 8-11 or 9-11. In some embodiments, the V_H comprises about 1-10 amino acid substitutions, relative to the amino acid sequence of SEQ ID NO: 3.

[0067] In some embodiments, a polypeptide disclosed herein comprises a V_H that comprises at least 1 amino acid substitution relative to the amino acid sequence of SEQ ID NO: 4 or SEQ ID NO: 5, or a combination of the foregoing. For example, the number of amino acid substitutions can be at least about: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20, or about: 1-20, 1-19, 2-19, 2-18, 2-17, 3-17, 3-16, 4-16, 4-15, 5-15, 5-14, 6-14, 6-13, 7-13, 7-12, 8-12, 8-11 or 9-11. In some embodiments, the V_H comprises about 1-10 amino acid substitutions, relative to the amino acid sequence of SEQ ID NO: 4 or SEQ ID NO: 5, or a combination of the foregoing.

[0068] In some embodiments, a polypeptide disclosed herein comprises an amino acid sequence that comprises at least 1 amino acid substitution relative to the amino acid sequence of SEQ ID NO: 15 or SEQ ID NO: 16, or a combination of the foregoing. For example, the number of amino acid substitutions can be at least about: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20, or about: 1-20, 1-19, 2-19, 2-18, 2-17, 3-17, 3-16, 4-16, 4-15, 5-15, 5-14, 6-14, 6-13, 7-13, 7-12, 8-12, 8-11 or 9-11. In some embodiments, the amino acid sequence comprises about 1-10 amino acid substitutions, relative to the amino acid sequence of SEQ ID NO: 15 or SEQ ID NO: 16, or a combination of the foregoing.

[0069] In some embodiments, the amino acid substitutions are conservative substitutions. The term “conservative amino acid substitution(s)” or “conservative substitution(s)” refers to an amino acid substitution having a value of 0 or greater in BLOSUM62.

[0070] In some embodiments, the amino acid substitutions are highly conservative substitutions. The term “highly conservative amino acid substitution(s)” or “highly conservative

substitution(s)" refers to an amino acid substitution having a value of at least 1 (e.g., at least 2) in BLOSUM62.

[0071] In some embodiments, a polypeptide disclosed herein comprises a V_H that comprises the amino acid sequence of SEQ ID NO: 4 or SEQ ID NO: 5. In some embodiments, a polypeptide disclosed herein comprises a V_H that comprises the amino acid sequence of SEQ ID NO: 4. In some embodiments, a polypeptide disclosed herein comprises a V_H that comprises the amino acid sequence of SEQ ID NO: 5.

[0072] In some embodiments, a polypeptide disclosed herein comprises the amino acid sequence of SEQ ID NO: 15 or SEQ ID NO: 16. In some embodiments, a polypeptide disclosed herein comprises the amino acid sequence of SEQ ID NO: 15. In some embodiments, a polypeptide disclosed herein comprises the amino acid sequence of SEQ ID NO: 16.

[0073] In some embodiments, a polypeptide disclosed herein comprises a V_H that contains human framework regions.

[0074] In some embodiments, a polypeptide disclosed herein is an immunoglobulin molecule, such as an antibody (e.g., a whole antibody, an intact antibody) or an antigen-binding fragment of an antibody. As used herein, the term "antibody" refers to an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term "antibody" refers to a full-length antibody.

[0075] In some embodiments, a polypeptide disclosed herein is a single-domain antibody or an antigen-binding fragment thereof. As used herein, the term "single-domain antibody (sdAb)" or "nanobody" refers to an immunoglobulin molecule consisting of a single monomeric variable antibody domain and capable of specific binding to a target. The single-domain antibody can be of any species, such as a murine antibody, a human antibody or a humanized single-domain antibody.

[0076] In some embodiments, a polypeptide disclosed herein is a heavy-chain antibody comprising two or more heavy chains, but lacking light chains, or an antigen-binding fragment thereof. Non-limiting examples of heavy chain antibodies include camelid Vhh (also referred to as VHH or V_HH) antibodies. Camelid antibodies are antibodies from the *Camelidae* family of mammals that include llamas, camels, and alpacas.

[0077] In some embodiments, a polypeptide disclosed herein is an antibody comprising two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds or multimers thereof (for example, IgM). Each heavy chain comprises a heavy chain variable region (V_H) and

a heavy chain constant region (comprising domains CH1, hinge CH2 and CH3). Each light chain comprises a light chain variable region (V_L) and a light chain constant region (CL). The V_H and the V_L regions may be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed within framework regions (FR). V_H and V_L each comprises three CDRs and four FR segments, arranged from the amino-terminus to the carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. The antibody can be of any species, such as a murine antibody, a human antibody or a humanized antibody.

[0078] The extent of the framework region and the CDRs of an antibody can be identified using one of several suitable methodologies that are well known in the art, for example, by the Kabat definition, the Chothia definition, the AbM definition, and/or the contact definition. Publicly and/or commercially available tools for identifying framework and/or CDR regions include, IgBlast (accessible at www.ncbi.nlm.nih.gov/igblast/), Scaligner (available from drugdesigntech at www.scaligner.com/), IMGT rules and/or tools (see, for example, www.imgt.org/IMGTScientificChart/Nomenclature/IMGT-FRCDRdefinition.html, also accessible at www.imgt.org/), Chothia Canonical Assignment (accessible at www.bioinf.org.uk/abs/chothia.html), Antigen receptor Numbering And Receptor CalssificatiIon (ANARCI, accessible at opig.stats.ox.ac.uk/webapps/newsabdab/sabpred/anarci/), or the Paratome web server (accessible at www.ofranlab.org/paratome/, see Vered Kunik, et al, Nucleic Acids Research, Volume 40, Issue W1, 1 July 2012, Pages W521–W524).

[0079] As used herein, a "CDR" encompasses any CDR defined by an art-recognized method for identifying the CDR residues on an antibody. See, *e.g.*, Kabat, E.A., *et al.*, (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, Chothia *et al.*, (1989) Nature 342:877; Chothia, C. *et al.*, (1987) J. Mol. Biol. 196:901-917; Al-lazikani *et al.*, (1997) J. Molec. Biol. 273:927-948; and Almagro, J. Mol. Recognit. 17:132-143 (2004). See also hgmp.mrc.ac.uk and bioinf.org.uk/abs. Two antibodies are determined to have the same CDR as one another with respect to a HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and/or LCDR3, when the identity of that CDR is determined for both antibodies using the same method.

[0080] In some embodiments, a polypeptide disclosed herein is an antigen-binding fragment of an antibody. The term "antigen-binding fragment" refers to a portion of an immunoglobulin molecule (e.g., a single-domain antibody) that retains the antigen binding properties of the parental full-length single-domain antibody. Non-limiting examples of antigen-binding fragments include a V_H region, a V_L region, an Fab fragment, an $F(ab')_2$ fragment, an Fd

fragment, an Fv fragment, and a domain antibody (dAb) consisting of one V_H domain or one V_L domain, etc. V_H and V_L domains may be linked together via a synthetic linker to form various types of single-chain antibody designs in which the V_H/V_L domains pair intramolecularly, or intermolecularly in those cases when the V_H and V_L domains are expressed by separate chains, to form a monovalent antigen binding site, such as single chain Fv (scFv) or diabody. In some embodiments, a polypeptide disclosed herein is an antigen binding fragment selected from Fab, $F(ab')_2$, Fab', scFv, or Fv. In some embodiments, the polypeptide is a scFv.

[0081] In some embodiments, a polypeptide disclosed herein (e.g., an antibody or antigen-binding fragment) is incorporated into a cell-based therapy. In some embodiments, the polypeptide is an engineered T cell receptor. In some embodiments, the polypeptide is a chimeric antigen receptor (CAR) (e.g., expressed on a T (CAR-T) cell, natural killer (CAR-NK) cell, or macrophage (CAR-M) cell). In some embodiments, the CAR comprises a transmembrane domain and an antigen-recognition moiety, wherein the antigen-recognition moiety binds SARS-CoV-2 (for example, an epitope within RBD, e.g., recognized by RBD Community 7).

[0082] In some embodiments, the polypeptide is an antibody mimetic. The term "antibody mimetic" refers to polypeptides capable of mimicking an antibody's ability to bind an antigen, but structurally differ from native single-chain antibody structures. Non-limiting examples of antibody mimetics include Adnectins, Affibodies, Affilins, Affimers, Affitins, Alphabodies, Anticalins, Avimers, DARPins, Fynomers, Kunitz domain peptides, monobodies, nanobodies, nanoCLAMPs, and Versabodies.

[0083] In some embodiments, a polypeptide disclosed herein further comprises an antibody heavy chain constant domain sequence. In some embodiments, the antibody heavy chain constant region is selected from the group consisting of an IgA constant region, an IgD constant region, an IgE constant region, an IgG constant region and an IgM constant region. In some embodiments, the IgG constant region is an IgG1 constant region, an IgG2 constant region, an IgG3 constant region or an IgG4 constant region. In some embodiments, the IgG2 constant region is an IgG2a, an IgG2b constant region or an IgG2c constant region. In some embodiments, the IgA constant region is an IgA1 constant region or an IgA2 constant region. In some embodiments, the antibody heavy chain constant region is an IgG1 constant region (e.g., IGHV5-51).

[0084] In some embodiments, a polypeptide disclosed herein further comprises an immunoglobulin light chain variable domain (V_L). The V_H and V_L domains may be linked together via a linker (e.g., a synthetic linker) to form various types of single-chain antibody designs in which the V_H/V_L domains pair intramolecularly, or intermolecularly in those cases

when the V_H and V_L domains are expressed by separate chains, to form a monovalent antigen binding site.

[0085] In some embodiments, the polypeptide further comprises an antibody light chain constant domain sequence. In some embodiments, the antibody light chain constant domain is selected from the group consisting of a κ constant domain and a λ constant domain. In some embodiments, the antibody heavy chain constant region is an IgG1 constant region, and the antibody light chain constant region is a κ constant region.

[0086] In some embodiments, the antibody heavy chain constant region sequence is at least about 60% identical to the amino acid sequence of SEQ ID NO: 12. For example, the antibody heavy chain constant region sequence can be at least about: 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% or 99% identical to the amino acid sequence of SEQ ID NO: 12. In some embodiments, the antibody heavy chain constant region sequence is at least about 70% or at least about 80% identical to the amino acid sequence of SEQ ID NO: 12. The sequence identified as SEQ ID NO: 12 is shown below:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 12).

[0087] In some embodiments, the antibody light chain constant region sequence is at least about 60% identical to the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 14. For example, the antibody light chain constant region sequence can be at least about: 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% or 99% identical to the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 14. In some embodiments, the antibody light chain constant region sequence is at least about 70% or at least about 80% identical to the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 14. The sequences identified as SEQ ID NO: 13 or SEQ ID NO: 14 are shown below:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 13).

GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPS KQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS (SEQ ID NO: 14).

[0088] In some embodiments, the antibody heavy chain constant region sequence comprises at least 1 amino acid substitution relative to the amino acid sequence of SEQ ID NO: 12. For example, the number of amino acid substitutions can be at least about: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20, or about: 1-20, 1-19, 2-19, 2-18, 2-17, 3-17, 3-16, 4-16, 4-15, 5-15, 5-14, 6-14, 6-13, 7-13, 7-12, 8-12, 8-11 or 9-11. In some embodiments, the antibody heavy chain constant region sequence comprises about 1-10 amino acid substitutions, relative to the amino acid sequence of SEQ ID NO: 12.

[0089] In some embodiments, the antibody light chain constant region sequence comprises at least 1 amino acid substitution relative to the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 14. For example, the number of amino acid substitutions can be at least about: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20, or about: 1-20, 1-19, 2-19, 2-18, 2-17, 3-17, 3-16, 4-16, 4-15, 5-15, 5-14, 6-14, 6-13, 7-13, 7-12, 8-12, 8-11 or 9-11. In some embodiments, the antibody light chain constant region sequence comprises about 1-10 amino acid substitutions, relative to the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 14.

[0090] In some embodiments, the amino acid substitutions are conservative substitutions. In some embodiments, the amino acid substitutions are highly conservative substitutions.

[0091] In some embodiments, a polypeptide disclosed herein is linked to a second polypeptide.

[0092] In some embodiments, the polypeptide and the second polypeptide are linked to each other via a linker. In some embodiments, the linker is a disulfide bond.

[0093] In some embodiments, the second polypeptide comprises a polypeptide described herein. In some embodiments, the second polypeptide comprises:

a) a paratope that is identical to the paratope of a single-domain antibody comprising the amino acid sequence of SEQ ID NO: 3;

b) a V_H comprising a HCDR1, a HCDR2 and a HCDR3 that are identical to the HCDR1, HCDR2 and HCDR3, respectively, of a single-domain antibody comprising the amino acid sequence of SEQ ID NO: 3;

c) a V_H that is at least 85% identical to the amino acid sequence of SEQ ID NO: 3; or

d) a V_H comprising about 1-10 amino acid substitutions, relative to the amino acid sequence of SEQ ID NO: 3,

or any combination of the foregoing.

[0094] In some embodiments:

a) the polypeptide comprises the amino acid sequence of SEQ ID NO: 4 and the second polypeptide comprises the amino acid sequence of SEQ ID NO: 3, 4 or 5; or

b) the polypeptide comprises the amino acid sequence of SEQ ID NO: 5 and the second polypeptide comprises the amino acid sequence of SEQ ID NO: 3, 4 or 5.

[0095] In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 4 and the second polypeptide comprises the amino acid sequence of SEQ ID NO: 3. In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 4 and the second polypeptide comprises the amino acid sequence of SEQ ID NO: 4. In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 4 and the second polypeptide comprises the amino acid sequence of SEQ ID NO: 5. In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 5 and the second polypeptide comprises the amino acid sequence of SEQ ID NO: 3. In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 5 and the second polypeptide comprises the amino acid sequence of SEQ ID NO: 4. In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 5 and the second polypeptide comprises the amino acid sequence of SEQ ID NO: 5.

[0096] In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 15 and the second polypeptide comprises the amino acid sequence of SEQ ID NO: 3. In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 15 and the second polypeptide comprises the amino acid sequence of SEQ ID NO: 4. In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 15 and the second polypeptide comprises the amino acid sequence of SEQ ID NO: 5. In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 15 and the second polypeptide comprises the amino acid sequence of SEQ ID NO: 15. In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 15 and the second polypeptide comprises the amino acid sequence of SEQ ID NO: 16. In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 16 and the second polypeptide comprises the amino acid sequence of SEQ ID NO: 3. In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 16 and the second polypeptide comprises the amino acid sequence of SEQ ID NO: 4. In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 16 and the second polypeptide comprises the amino acid sequence of SEQ ID NO: 5. In some embodiments, the polypeptide comprises the amino acid sequence of

SEQ ID NO: 16 and the second polypeptide comprises the amino acid sequence of SEQ ID NO: 15. In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 16 and the second polypeptide comprises the amino acid sequence of SEQ ID NO: 16.

[0097] In some embodiments, the polypeptide, the second polypeptide, or both, are linked to a third polypeptide comprising a V_L.

[0098] In some embodiments, the third polypeptide further comprises an antibody light chain constant domain sequence. In some embodiments, the antibody light chain constant domain is selected from the group consisting of a κ constant domain and a λ constant domain.

[0099] In some embodiments, the antibody light chain constant region sequence is at least about 60% identical to the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 14. For example, the antibody light chain constant region sequence can be at least about: 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% or 99% identical to the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 14. In some embodiments, the antibody light chain constant region sequence is at least about 70% or at least about 80% identical to the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 14.

[00100] In some embodiments, the antibody light chain constant region sequence comprises at least 1 amino acid substitution relative to the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 14. For example, the number of amino acid substitutions can be at least about: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20, or about: 1-20, 1-19, 2-19, 2-18, 2-17, 3-17, 3-16, 4-16, 4-15, 5-15, 5-14, 6-14, 6-13, 7-13, 7-12, 8-12, 8-11 or 9-11. In some embodiments, the antibody light chain constant region sequence comprises about 1-10 amino acid substitutions, relative to the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 14. In some embodiments, the amino acid substitutions are conservative substitutions. In some embodiments, the amino acid substitutions are highly conservative substitutions.

[00101] In some embodiments, a polypeptide of the invention competes with a reference antibody for binding to the wildtype SARS-CoV-1-Spike, a SARS-CoV-1-Spike variant, or a combination thereof, wherein the reference antibody specifically binds the wildtype SARS-CoV-1-Spike. The term "specifically binding" or "specifically binds" refers to preferential interaction, i.e., significantly higher binding affinity, between an antibody, or an antigen-binding fragment thereof, and its epitope relative to other antigens or amino acid sequences.

[00102] In some embodiments, the reference antibody comprises a V_H amino acid sequence of SEQ ID NO: 3. In some embodiments, the reference antibody comprises HCDR1, HCDR2 and

HCDR3 amino acid sequences of SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 9, respectively.

[00103] In some embodiments, a polypeptide of the invention is an isolated polypeptide. In some embodiments, the isolated polypeptide is recombinantly produced. In some embodiments, the isolated polypeptide is synthetically produced.

[00104] In some embodiments, the polypeptide is conjugated to a heterologous moiety. The term "conjugated" refers to attached, via a covalent or noncovalent interaction. Conjugation can employ any of suitable linking agents. Non-limiting examples include peptide linkers, compound linkers, and chemical cross-linking agents.

[00105] In some embodiments, the heterologous moiety is a therapeutic agent, a diagnostic agent or a combination thereof. In some embodiments, the heterologous moiety is polyethylene glycol (PEG), hexadecanoic acid, hydrogels, nanoparticles, multimerization domains and carrier peptides.

[00106] In some embodiments, the nanoparticle is a lipid nanoparticle. In some embodiments, the nanoparticle is a polymer nanoparticle. In some embodiments, the polymer is an amphiphilic polymer. In other embodiments, the polymer is a hydrophobic or hydrophilic polymer. Non-limiting examples of polymers include poly(lactic acid)-poly(ethylene glycol), poly(lactic-co-glycolic acid)-poly(ethylene glycol), poly(lactic-co-glycolic) acid (PLGA), poly(lactic-co-glycolic acid)-d-α-tocopheryl polyethylene glycol succinate, poly(lactic-co-glycolic acid)-ethylene oxide fumarate, poly(glycolic acid)-poly(ethylene glycol), polycaprolactone-poly(ethylene glycol), or any salts thereof. In some embodiments, the polymer nanoparticle comprises poly(lactic-co-glycolic) acid (PLGA).

[00107] In some embodiments, the carrier polypeptide is albumin or an Fc polypeptide.

[00108] In some embodiments, a polypeptide of the invention:

a) is capable of binding to an epitope of SARS-CoV-2 RBD comprising amino acid residues corresponding to amino acid residues Y36, N37, S38, T39, F40, F41, S42, T43, F44, K45, C46, A51, T52, G71, D72, V74, R75, I169, G170 and Y174 of SARS-CoV-1 RBD;

b) binds SARS-CoV-2 with an affinity of 10 μM or less;

c) reduces binding of SARS-CoV-2 to angiotensin-converting enzyme 2 (ACE2); or

d) reduces infectivity of SARS-CoV-2 in human cells,

or a combination of any of the foregoing.

[00109] A non-limiting example of a wildtype SARS-CoV-1-Spike sequence is SEQ ID NO: 21.

[00110] NLCPFGEVFNATKFPSVYAWERKKISNCVADYSVLYNSTFFSTFKCYGVSAT KLNDLCFSNVYADSFVVKGDDVRQIAPGQTGVIADYNYKLPDDFMGCVLAWNTRNID ATSTGNYNYKYRYLRHGKLRPFERDISNVPFSPDGKPCTPPALNCYWPLNDYGFYTTTG IGYQPYRVVVLSFEGSLEVLFQ (SEQ ID NO: 21)

[00111] In some embodiments, a polypeptide of the invention is capable of binding to an epitope of SARS-CoV-2 RBD comprising amino acid residues corresponding to amino acid residues Y36, N37, S38, T39, F40, F41, S42, T43, F44, K45, C46, A51, T52, G71, D72, V74, R75, I169, G170 and Y174 of SARS-CoV-1 RBD. In certain embodiments, a polypeptide of the invention can also bind SARS-CoV1 RBD, e.g., amino acid residues Y36, N37, S38, T39, F40, F41, S42, T43, F44, K45, C46, A51, T52, G71, D72, V74, R75, I169, G170 and Y174 of SARS-CoV-1 RBD.

[00112] In some embodiments, the polypeptide binds SARS-CoV-2-Spike with a binding constant (K_D) of about 10 μM or less. As used herein the term "K_D," also referred to as "binding constant," "equilibrium dissociation constant" or "affinity constant," is a measure of the extent of a reversible association between two molecular species (e.g., antibody and target protein) and includes both the actual binding affinity as well as the apparent binding affinity. Binding affinity can be determined using methods known in the art including, for example, by measurement of surface plasmon resonance, e.g., using a Biolayer interferometry (Octet, ForteBio) or a surface plasmon resonance (Biacore) system and assay. A reference that compares various surface technologies for measuring binding affinity and kinetics is Yang, D., Singh, A., Wu, H., & Kroe-Barrett, R., *Comparison of biosensor platforms in the evaluation of high affinity antibody-antigen binding kinetics*, Analytical Biochemistry 508: 78-96 (2016), the contents of which are incorporated herein by reference in their entirety.

[00113] In some embodiments, the polypeptide binds SARS-CoV-2-Spike with a K_D of about: 5 μM, 2 μM, 1 μM, 500 nM, 200 nM, 100 nM, 50 nM, 20 nM, 10 nM, 5 nM, 2 nM, 1 nM, 0.5 nM, 0.2 nM or 0.1 nM or less. In some embodiments, the polypeptide binds SARS-CoV-2 with a K_D of 100 nM or less.

[00114] In some embodiments, the polypeptide binds SARS-CoV-2-Spike with a K_D of about: 10^{-10}-10^{-5} M, 10^{-10}-$5x10^{-6}$ M, $2x10^{-10}$-$5x10^{-6}$ M, $2x10^{-10}$-$2x10^{-6}$ M, $5x10^{-10}$-$2x10^{-6}$ M, $5x10^{-10}$-10^{-7} M, 10^{-9}-10^{-7} M, 10^{-9}-$5x10^{-8}$ M, $2x10^{-9}$-$5x10^{-8}$ M, $2x10^{-9}$-$2x10^{-8}$ M, $5x10^{-9}$-$2x10^{-8}$ M or $5x10^{-9}$-10^{-8} M.

[00115] In some embodiments, the polypeptide (e.g., camelid antibody) binds SARS-CoV-2-Spike or a fragment thereof (for example, an epitope within RBD, e.g., recognized by RBD Community 7) or a variant thereof with a K_D of about 10^{-6} M or less, e.g., about: 500 nM, 200

nM, 100 nM, 50 nM, 20 nM, 10 nM, 5 nM, 2 nM, 1 nM, 0.5 nM, 0.2 nM or 0.1 nM or less; or about: 10^{-10}-10^{-6} M, 10^{-10}-$5x10^{-7}$ M, $2x10^{-10}$-$5x10^{-7}$ M, $2x10^{-10}$-$2x10^{-7}$ M, $5x10^{-10}$-$2x10^{-7}$ M, $5x10^{-10}$-10^{-7} M, 10^{-9}-10^{-7} M, 10^{-9}-$5x10^{-8}$ M, $2x10^{-9}$-$5x10^{-8}$ M, $2x10^{-9}$-$2x10^{-8}$ M, $5x10^{-9}$-$2x10^{-8}$ M or $5x10^{-9}$-10^{-8} M.

[00116] In some embodiments, the polypeptide binds SARS-CoV-1-Spike with a K_D of about 10 μM or less. In some embodiments, the K_D is about: 5 μM, 2 μM, 1 μM, 500 nM, 200 nM, 100 nM, 50 nM, 20 nM, 10 nM, 5 nM, 2 nM, 1 nM, 0.5 nM, 0.2 nM or 0.1 nM or less. In some embodiments, the polypeptide binds SARS-CoV-1-Spike with a K_D of about: 10^{-10}-10^{-5} M, 10^{-10}-$5x10^{-6}$ M, $2x10^{-10}$-$5x10^{-6}$ M, $2x10^{-10}$-$2x10^{-6}$ M, $5x10^{-10}$-$2x10^{-6}$ M, $5x10^{-10}$-10^{-7} M, 10^{-9}-10^{-7} M, 10^{-9}-$5x10^{-8}$ M, $2x10^{-9}$-$5x10^{-8}$ M, $2x10^{-9}$-$2x10^{-8}$ M, $5x10^{-9}$-$2x10^{-8}$ M or $5x10^{-9}$-10^{-8} M.

[00117] In some embodiments, the polypeptide (e.g., camelid antibody) binds SARS-CoV-2-Spike or a fragment thereof (for example, an epitope within RBD, e.g., recognized by RBD Community 7) or a variant thereof with an association constant of about 10^6/Ms or less, e.g., about: $5x10^5$/Ms, $2x10^5$/Ms, 10^5/Ms, $5x10^4$/Ms, $2x10^4$/Ms, 10^4/Ms or less; or about: 10^4-10^6/Ms, 10^4-$5x10^5$/Ms, $2x10^4$-$5x10^5$/Ms, $2x10^4$-$2x10^5$/Ms, $5x10^4$-$2x10^5$/Ms or $5x10^4$-10^5/Ms.

[00118] In some embodiments, the polypeptide (e.g., camelid antibody) binds SARS-CoV-2-Spike or a fragment thereof (for example, an epitope within RBD, e.g., recognized by RBD Community 7) or a variant thereof with a dissociation constant of about 10^{-2}/s or less, e.g., about: $8x10^{-3}$/s, $6x10^{-3}$/s, $4x10^{-3}$/s, $2x10^{-3}$/s, 10^{-3}/s, $8x10^{-4}$/s, $6x10^{-4}$/s, $4x10^{-4}$/s, $2x10^{-4}$/s, 10^{-4}/s, $8x10^{-5}$/s, $6x10^{-5}$/s, $4x10^{-5}$/s, $2x10^{-5}$/s, 10^{-5}/s, $8x10^{-6}$/s, $6x10^{-6}$/s, $4x10^{-6}$/s, $2x10^{-6}$/s or 10^{-6}/s, or less; or about: 10^{-6}-10^{-2}/s, $2x10^{-6}$-10^{-2}/s, $2x10^{-6}$-$5x10^{-3}$/s, $5x10^{-6}$-$5x10^{-3}$/s, $5x10^{-6}$-$2x10^{-3}$/s, 10^{-5}-$2x10^{-3}$/s, 10^{-5}-10^{-3}/s, $2x10^{-5}$-10^{-3}/s, $2x10^{-5}$-$5x10^{-4}$/s, $5x10^{-5}$-$5x10^{-4}$/s, $5x10^{-5}$-$2x10^{-4}$/s or 10^{-4}-$2x10^{-4}$/s.

[00119] In some embodiments, the polypeptide (e.g., camelid antibody) binds SARS-CoV-2-Spike or a fragment thereof (for example, an epitope within RBD, e.g., recognized by RBD Community 7) or a variant thereof with a R_{max} of about 0.05 or more, e.g., about: 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0 or more; or about: 0.1-2.0, 0.15-2.0, 0.15-1.8, 0.2-1.8, 0.2-1.6, 0.25-1.6, 0.25-1.4, 0.3-1.4, 0.3-1.2, 0.35-1.2, 0.35-1.0, 0.4-1.0, 0.4-0.95, 0.45-0.95, 0.45-0.9, 0.5-0.9, 0.5-0.85, 0.55-0.85, 0.55-0.8, 0.6-0.8, 0.6-0.75, 0.65-0.75 or 0.65-0.7.

[00120] In some embodiments, the polypeptide reduces binding of SARS-CoV-2-Spike to an entry receptor (e.g., ACE2). In some embodiments, the polypeptide competes with an entry receptor (e.g., ACE2) for binding to the S1 subunit receptor binding domain of SARS-Cov-2-Spike (RBD).

[00121] In some embodiments, the entry receptor is ACE2. In some embodiments, the ACE2 is a mammalian ACE2. In some embodiments, the ACE2 is a primate ACE2. In some embodiments, the primate ACE2 is human ACE2 (GeneID 59272; exemplary protein sequence NP_001358344.1). In some embodiments, the primate ACE2 is a non-human primate ACE2 selected from the group consisting of chimpanzee ACE2 (e.g., A0A2J8KU96, XP_016798468.1, XP_016798469.1, PNI38578.1), macaque ACE2 (e.g., B6DUF2, NP_001129168.1, XP_024647450.1), cynomolgus ACE2 (e.g., A0A2K5X283, XP_005593094.1) and combinations thereof.

[00122] In some embodiments, the polypeptide reduces binding of SARS-CoV-2-Spike to its cellular target receptor (e.g., ACE2) by at least about 10%. For example, by at least about: 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%. In some embodiments, the polypeptide reduces binding of SARS-CoV-2-Spike to ACE2 by at least about 30%.

[00123] In some embodiments, the level of binding between SARS-CoV-2-Spike and its cellular target receptor (e.g., ACE2) in the presence of the polypeptide is less than about 90% relative to the level of binding in the absence of the polypeptide, for example, less than about: 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1%.

[00124] In some embodiments, the level of binding between SARS-CoV-2-Spike and its cellular target receptor (e.g., ACE2) in the presence of the polypeptide is about 1-90% relative to the level of binding in the absence of the polypeptide, for example, about: 2-90%, 2-85%, 3-85%, 3-80%, 4-80%, 4-75%, 5-75%, 5-70%, 6-70%, 6-65%, 7-65%, 7-60%, 8-60%, 8-55%, 9-55%, 9-50%, 10-50%, 10-45%, 15-45%, 15-40%, 20-40%, 20-35%, 25-35% or 25-30%, relative to the level of binding in the absence of the polypeptide.

[00125] In some embodiments, the polypeptide reduces binding of SARS-CoV-1-Spike to its cellular target receptor by at least about 10%. For example, by at least about: 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%.

[00126] In some embodiments, the polypeptide (e.g., camelid antibody) neutralizes the SARS-CoV-2 infectivity of human host cells with an IC_{50} of about 25,000 ng/mL or less, e.g., about: 20,000 ng/mL, 15,000 ng/mL, 10,000 ng/mL, 5,000 ng/mL, 2,500 ng/mL, 1,000 ng/mL, 750 ng/mL, 500 ng/mL, 250 ng/mL, 100 ng/mL, 75 ng/mL, 50 ng/mL, 25 ng/mL or 10 ng/mL or less; e.g., about: 10-25,000 ng/mL, 10-20,000 ng/mL, 25-20,000 ng/mL, 25-15,000 ng/mL, 50-

15,000 ng/mL, 50-10,000 ng/mL, 75-10,000 ng/mL, 75-5,000 ng/mL, 100-5,000 ng/mL, 100-2,500 ng/mL, 250-2,500 ng/mL, 250-1,000 ng/mL, 500-1,000 ng/mL or 500-750 ng/mL.

[00127] In some embodiments, the polypeptide (e.g., camelid antibody) neutralizes the SARS-CoV-2 infectivity of human host cells with an IC_{80} of about 50,000 ng/mL or less, e.g., about: 25,000 ng/mL, 15,000 ng/mL, 10,000 ng/mL, 5,000 ng/mL, 2,500 ng/mL, 1,000 ng/mL, 750 ng/mL, 500 ng/mL, 250 ng/mL, 100 ng/mL, 75 ng/mL, 50 ng/mL, 25 ng/mL or 10 ng/mL or less; e.g., about: 10-50,000 ng/mL, 10-25,000 ng/mL, 25-25,000 ng/mL, 25-15,000 ng/mL, 50-15,000 ng/mL, 50-10,000 ng/mL, 75-10,000 ng/mL, 75-5,000 ng/mL, 100-5,000 ng/mL, 100-2,500 ng/mL, 250-2,500 ng/mL, 250-1,000 ng/mL, 500-1,000 ng/mL or 500-750 ng/mL.

[00128] In some embodiments, the polypeptide reduces binding of SARS-CoV-2 to a host protease (e.g., the Type II transmembrane serine protease (TMPRSS2)). Without being bound to theory, the main host protease that mediates SARS-CoV-2-Spike activation on primary target cells and initial viral entry is TMPRSS2.

[00129] In some embodiments, the host protease is TMPRSS2. In some embodiments, the TMPRSS2 is a mammalian TMPRSS2. In some embodiments, the TMPRSS2 is a primate TMPRSS2, e.g., human TMPRSS2 or a non-human primate TMPRSS2 (e.g., chimpanzee TMPRSS2, macaque TMPRSS2 or cynomolgus TMPRSS2).

[00130] In some embodiments, the polypeptide reduces binding of SARS-CoV-2 to the host protease (e.g., TMPRSS2) by at least about 10%, e.g., by at least about: 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%.

[00131] In some embodiments, the level of binding between SARS-CoV-2 and the host protease (e.g., TMPRSS2) in the presence of the polypeptide is less than about 90% relative to the level of binding in the absence of the polypeptide, for example, less than about: 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1%.

[00132] In some embodiments, the level of binding between SARS-CoV-2 and the host protease (e.g., TMPRSS2) in the presence of the polypeptide is about 1-90% relative to the level of binding in the absence of the polypeptide, for example, about: 2-90%, 2-85%, 3-85%, 3-80%, 4-80%, 4-75%, 5-75%, 5-70%, 6-70%, 6-65%, 7-65%, 7-60%, 8-60%, 8-55%, 9-55%, 9-50%, 10-50%, 10-45%, 15-45%, 15-40%, 20-40%, 20-35%, 25-35% or 25-30%, relative to the level of binding in the absence of the polypeptide.

[00133] In some embodiments, the polypeptide reduces entry of SARS-CoV-2 into host cells (e.g., human host cells). In some embodiments, the polypeptide reduces entry of SARS-CoV-2

into host cells by at least about 10%, e.g., by at least about: 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%.

[00134] In some embodiments, the polypeptide reduces infectivity of SARS-CoV-2 in host cells (e.g., human host cells). In some embodiments, the polypeptide reduces infectivity of SARS-CoV-2 in host cells (e.g., human host cells) by at least about 10%, e.g., by at least about: 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%. In some embodiments, the polypeptide reduces infectivity of SARS-CoV-2 in human cells by at least about 30%.

[00135] In some embodiments, the polypeptide reduces re-infection of SARS-CoV-2 in host cells (e.g., human host cells). In some embodiments, the polypeptide reduces re-infection of SARS-CoV-2 in host cells (e.g., human host cells) by at least about 10%, e.g., by at least about: 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%. In some embodiments, the polypeptide reduces re-infection of SARS-CoV-2 in human cells by at least about 30%.

[00136] Infectivity or re-infection can be measured using techniques such as a pseudovirus neutralization assay or a live virus neutralization assay (see, e.g., Pinto et al., *Cross-neutralization of SARS-CoV-2 by a human monoclonal SARS-CoV antibody*, Nature 583: 290-95 (2020), the contents of which are incorporated herein by reference). A kit, for example, the GenScript cPass™ SARS-CoV-2 neutralization antibody detection kit (Genscript Biotech, Piscataway, NJ), can be used according to manufacturer's protocol.

[00137] In some embodiments, the host cells are selected from the group consisting of lung type II pneumocytes, ileal absorptive enterocytes, nasal goblet secretory cells, and combinations thereof.

Fusion Proteins

[00138] In another aspect, the invention provides a fusion protein comprising one or more of the polypeptides described herein.

[00139] The term “fusion protein” refers to a synthetic, semi-synthetic or recombinant single protein molecule. A fusion protein can comprise all or a portion of two or more different proteins and/or polypeptides that are attached by covalent bonds (e.g., peptide bonds). Fusion proteins of the invention can be produced recombinantly or synthetically, using routine methods and reagents that are well known in the art. For example, a fusion protein of the invention can be produced recombinantly in a suitable host cell (*e.g.,* bacteria) according to methods known in the

art. *See, e.g.*, *Current Protocols in Molecular Biology*, Second Edition, Ausubel *et al.* eds., John Wiley & Sons, 1992; and *Molecular Cloning: a Laboratory Manual*, 2nd edition, Sambrook *et al.*, 1989, Cold Spring Harbor Laboratory Press. For example, a nucleic acid molecule comprising a nucleotide sequence encoding a fusion protein described herein can be introduced and expressed in suitable host cell (*e.g.*, E. coli), and the expressed fusion protein can be isolated/purified from the host cell (*e.g.*, in inclusion bodies) using routine methods and readily available reagents. For example, DNA fragments coding for different protein sequences (*e.g.*, a light-responsive domain, a heterologous peptide component) can be ligated together in-frame in accordance with conventional techniques. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of nucleic acid fragments can be carried out using anchor primers that give rise to complementary overhangs between two consecutive nucleic acid fragments that can subsequently be annealed and re-amplified to generate a chimeric nucleic acid sequence (see Ausubel *et al.*, Current Protocols in Molecular Biology, 1992).

Nucleic Acids, Expression Vectors, Expression Host Cells

[00140] In another aspect, the invention provides one or more polynucleotides encoding any one of the polypeptides or fusion proteins described herein. In some embodiments, the polypeptide or fusion protein of the invention is encoded by a single polynucleotide. In some embodiments, the polypeptide or fusion protein of the invention is encoded by multiple polynucleotides.

[00141] In some embodiments, the polynucleotide comprises a nucleotide sequence that is codon-optimized for a chosen host cell.

[00142] In another aspect, the invention provides an expression vector comprising any one or more of the polynucleotides described herein.

[00143] The term “expression vector” refers to a replicable nucleic acid from which one or more proteins can be expressed when the expression vector is transformed into a suitable expression host cell.

[00144] In some embodiments, the expression vector further comprises an expression control polynucleotide sequence operably linked to the polynucleotide, a polynucleotide sequence encoding a selectable marker, or both. In some embodiments, the expression control polynucleotide sequence comprises a promoter sequence, an enhancer sequence, or both. In some embodiments, the expression control polynucleotide sequence comprises an inducible promoter sequence. The term “promoter” refers to a region of DNA to which RNA polymerase binds and

initiates the transcription of a gene. The term "operably linked" means that the nucleic acid is positioned in the recombinant polynucleotide, *e.g.*, vector, in such a way that enables expression of the nucleic acid under control of the element (*e.g.*, promoter) to which it is linked. The term "selectable marker element" is an element that confers a trait suitable for artificial selection. Selectable marker elements can be negative or positive selection markers.

[00145] In another aspect, the invention provides an expression host cell comprising any one or more of the polynucleotides or expression vectors described herein.

[00146] The term "expression host cell" refers to a cell useful for receiving, maintaining, reproducing and amplifying a vector.

[00147] Non-limiting examples of expression host cells include mammalian cells such as hybridoma cells, Chinese hamster ovary (CHO) cells, COS cells, human embryonic kidney (HEK), yeast cells such as Pichia pastoris cells, or bacterial cells such as DH5α, etc.

Compositions

[00148] In another aspect, the invention provides a composition comprising any one of the polypeptides or fusion proteins described herein. In some embodiments, the composition is a pharmaceutical composition.

[00149] In some embodiments, the composition (e.g., pharmaceutical composition) further comprises pharmaceutically acceptable carriers, excipients, stabilizers, diluents or tonifiers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)). Suitable pharmaceutically acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed. Non-limiting examples of pharmaceutically acceptable carriers, excipients, stabilizers, diluents or tonifiers include buffers (e.g., phosphate, citrate, histidine), antioxidants (e.g., ascorbic acid or methionine), preservatives, proteins (e.g., serum albumin, gelatin or immunoglobulins); hydrophilic polymers, amino acids, carbohydrates (e.g., monosaccharides, disaccharides, glucose, mannose or dextrins); chelating agents (e.g., EDTA), sugars (e.g., sucrose, mannitol, trehalose or sorbitol), salt-forming counter-ions (e.g., sodium), metal complexes (e.g., Zn-protein complexes); non-ionic surfactants (e.g., Tween), PLURONICS™ and polyethylene glycol (PEG).

[00150] In some embodiments, the composition (e.g., pharmaceutical composition) of the invention is formulated for a suitable administration schedule and route. Non-limiting examples of administration routes include oral, rectal, mucosal, intravenous, intramuscular, subcutaneous and topical, etc. In some embodiments, the composition (e.g., pharmaceutical composition) of

the invention is stored in the form of an aqueous solution or a dried formulation (e.g., lyophilized).

[00151] In some embodiments, the composition is formulated to be administered by infusion (e.g., intravenous infusion).

[00152] In some embodiments, the composition is formulated to be administered with a additional therapeutic agent(s) as a combination therapy.

Methods of Use

[00153] In another aspect, the invention provides a method of reducing the likelihood of a SARS-CoV-2 infection in a subject, comprising administering to the subject an effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable carrier and, wherein as an active ingredient, any one of the polypeptides or fusion proteins described herein.

[00154] In some embodiments, the likelihood of SARS-CoV-2 infection in the subject is reduced by at least about 10%, e.g., by at least about: 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%.

[00155] In some embodiments, the likelihood of SARS-CoV-2 infection in the subject in the presence of the polypeptide is about 1-90% relative to the likelihood in the absence of the polypeptide, for example, about: 2-90%, 2-85%, 3-85%, 3-80%, 4-80%, 4-75%, 5-75%, 5-70%, 6-70%, 6-65%, 7-65%, 7-60%, 8-60%, 8-55%, 9-55%, 9-50%, 10-50%, 10-45%, 15-45%, 15-40%, 20-40%, 20-35%, 25-35% or 25-30%.

[00156] The term "subject" or "patient" refers to an animal (e.g., a mammal). A subject to be treated according to the methods described herein may be one who has been diagnosed with a particular condition (e.g., COVID-19), or one at risk of developing such conditions. Diagnosis may be performed by any method or technique known in the art. One skilled in the art will understand that a subject to be treated according to the present disclosure may have been subjected to standard tests or may have been identified, without examination, as one at risk due to the presence of one or more risk factors associated with the disease or condition.

[00157] In some embodiments, the subject has COVID-19. In some embodiments, the subject has been diagnosed with COVID-19. In other embodiments, the subject is at risk of developing COVID-19.

[00158] In some embodiments, the subject is a mammal. In some embodiments, the subject is a mammal selected from the group consisting of a dog, a cat, a mouse, a rat, a hamster, a guinea

pig, a horse, a pig, a sheep, a cow, a chimpanzee, a macaque, a cynomolgus, and a human. In some embodiments, the subject is a primate. In some embodiments, the subject is a human.

[00159] In some embodiments, the subject has a heart disease. In some embodiments, the subject has a heart disease selected from the group consisting of a congenital heart disease, a coronary artery disease, a hypertensive heart disease, an inflammatory heart disease, a pulmonary heart disease, a rheumatic heart disease, a valvular heart disease, a cardiomyopathy, heart failure, and combinations thereof. In some embodiments, the subject has a congestive heart failure. In some embodiments, the subject has an inflammatory heart disease selected from the group consisting of endocarditis, cardiomegaly, myocarditis, and combinations thereof.

[00160] In some embodiments, the subject has diabetes.

[00161] In some embodiments, the subject has a lung disease. Non-limiting examples of lung diseases include acute respiratory distress syndromes, asthma, bronchitis, COPD, emphysema, lung tumors, pleural cavity diseases (e.g., pleural mesothelioma or tension pneumothorax), pulmonary vascular diseases (e.g., embolisms, edema, arterial hypertension or hemorrhage), respiratory tract infections (e.g., pneumonia or other upper or lower respiratory tract infections).

[00162] In some embodiments, the subject is a tobacco smoker.

[00163] In some embodiments, the subject is immune-compromised (e.g., has an underlying disorder or is on immunosuppressive therapy).

[00164] In some embodiments, the subject is 40 years or older, e.g., at least: 45, 50, 55, 60, 65, 70, 75, 80, 85, 90 years old.

[00165] "A therapeutically effective amount," "an effective amount" or "an effective dosage" is an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result (*e.g.*, treatment, healing, inhibition or amelioration of physiological response or condition, *etc.*). The full therapeutic effect does not necessarily occur by administration of one dose, and may occur only after administration of a series of doses. Thus, a therapeutically effective amount may be administered in one or more administrations. A therapeutically effective amount may vary according to factors such as disease state, age, sex, and weight of a mammal, mode of administration and the ability of a therapeutic, or combination of therapeutics, to elicit a desired response in an individual.

[00166] An effective amount of an agent to be administered can be determined by a clinician of ordinary skill using the guidance provided herein and other methods known in the art. Relevant factors include the given agent, the pharmaceutical formulation, the route of administration, the type of disease or disorder, the identity of the subject (e.g., age, sex, weight) or host being treated, and the like. For example, suitable dosages can be from about 0.001 mg/kg

to about 100 mg/kg, from about 0.01 mg/kg to about 100 mg/kg, from about 0.01 mg/kg to about 10 mg/kg, from about 0.01 mg/kg to about 1 mg/kg body weight per treatment. Determining the dosage for a particular agent, subject and disease is well within the abilities of one of skill in the art. Preferably, the dosage does not cause or produces minimal adverse side effects.

[00167] Desired response or desired results include effects at the cellular level, tissue level, or clinical results. As such, "a therapeutically effective amount" or synonym thereto depends upon the context in which it is being applied. For example, in some embodiments it is an amount of the composition sufficient to achieve a treatment response as compared to the response obtained without administration of the composition. In other embodiments, it is an amount that results in a beneficial or desired result in a subject as compared to a control. As defined herein, a therapeutically effective amount of a composition of the present disclosure may be readily determined by one of ordinary skill by routine methods known in the art. Dosage regimen and route of administration may be adjusted to provide the optimum therapeutic response.

[00168] In some embodiments, the method is used for prophylactic therapy. In some embodiments, the effective dosage is sufficient to prevent the subject of being infected by SARS-CoV-2.

[00169] In some embodiments, the method is used for treating SARS-CoV-2 infection. The term "treating" or "treatment" refers to the medical management of a subject with the intent to improve, ameliorate, stabilize (i.e., not worsen), prevent or cure a disease, pathological condition, or disorder—such as the particular indications exemplified herein. This term includes active treatment (treatment directed to improve the disease, pathological condition, or disorder), causal treatment (treatment directed to the cause of the associated disease, pathological condition, or disorder), palliative treatment (treatment designed for the relief of symptoms), preventative treatment (treatment directed to minimizing or partially or completely inhibiting the development of the associated disease, pathological condition, or disorder); and supportive treatment (treatment employed to supplement another therapy). Treatment also includes diminishment of the extent of the disease or condition; preventing spread of the disease or condition; delay or slowing the progress of the disease or condition; amelioration or palliation of the disease or condition; and remission (whether partial or total), whether detectable or undetectable. "Ameliorating" or "palliating" a disease or condition means that the extent and/or undesirable clinical manifestations of the disease, disorder, or condition are lessened and/or time course of the progression is slowed or lengthened, as compared to the extent or time course in the absence of treatment. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already

with the condition or disorder, as well as those prone to have the condition or disorder or those in which the condition or disorder is to be prevented.

[00170] In some embodiments, the effective dosage is sufficient to inhibit viral load in the subject. In some embodiments, the reduction in viral load is by at least about 10%, e.g., by at least about: 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%. In some embodiments, the reduction in viral load is about 10-99%, e.g., about: 10-98%, 15-98%, 15-97%, 20-97%, 20-96%, 25-96%, 25-95%, 30-95%, 30-94%, 35-94%, 35-93%, 40-93%, 40-92%, 45-92%, 45-91%, 50-91%, 50-90%, 55-90%, 55-85%, 60-85%, 60-80%, 65-80%, 65-75%, or 70-75%.

[00171] In some embodiments, the effective dosage is sufficient to reduce binding of the virus to its target proteins, target cells, or both. In some embodiments, the reduction in binding is by at least about 10%, e.g., by at least about: 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%. In some embodiments, the reduction in binding is about 10-99%, e.g., about: 10-98%, 15-98%, 15-97%, 20-97%, 20-96%, 25-96%, 25-95%, 30-95%, 30-94%, 35-94%, 35-93%, 40-93%, 40-92%, 45-92%, 45-91%, 50-91%, 50-90%, 55-90%, 55-85%, 60-85%, 60-80%, 65-80%, 65-75%, or 70-75%.

[00172] In some embodiments, the effective dosage is sufficient to inhibit virus mediated fusion with a target cell. In some embodiments, the reduction in fusion is by at least about 10%, e.g., by at least about: 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%. In some embodiments, the reduction in fusion is about 10-99%, e.g., about: 10-98%, 15-98%, 15-97%, 20-97%, 20-96%, 25-96%, 25-95%, 30-95%, 30-94%, 35-94%, 35-93%, 40-93%, 40-92%, 45-92%, 45-91%, 50-91%, 50-90%, 55-90%, 55-85%, 60-85%, 60-80%, 65-80%, 65-75%, or 70-75%.

[00173] In some embodiments, the effective dosage is sufficient to interfere with conformational changes in the viral envelope proteins necessary for cell infectivity.

[00174] A therapeutic agent described herein can be administered via a variety of routes of administration, including, for example, oral, dietary, topical, transdermal, rectal, parenteral (*e.g.*, intra-arterial, intravenous, intramuscular, subcutaneous injection, intradermal injection), intravenous infusion and inhalation (*e.g.*, intrabronchial, intranasal or oral inhalation, intranasal drops) routes of administration, depending on the compound and the particular disease to be treated. Administration can be local or systemic as indicated. The preferred mode of administration can vary depending on the particular compound chosen.

[00175] In some embodiments, a polypeptide, composition, or pharmaceutical composition disclosed herein is administered to a subject in combination with one or more additional therapeutic agents (*e.g.*, concurrently or sequentially with one or more additional therapeutic agents). In some embodiments, a subject has been previously treated with one or more therapeutic agents prior to being administered a polypeptide, composition, or pharmaceutical composition disclosed herein. In some embodiments, the method further comprises administering a therapeutically effective amount of one or more additional therapeutic agents to the subject at the same time as, or following administration of a polypeptide, composition, or pharmaceutical composition disclosed herein.

[00176] Non-limiting examples of additional therapeutic agents include antibiotics (e.g., azithromycin), antibodies or antigen-binding fragments thereof (e.g., other SARS-CoV-2-Spike-binding peptides), antimalarial agents (e.g., chloroquine or hydroxychloroquine), antiviral agents (e.g., favipiravir, lopinavir and/or ritonavir), cytokines (e.g., type 1 interferons such as interferon beta-1a), nucleotide analogs (e.g., remdesivir), protease inhibitors (e.g., danoprevir), Renin-Angiotensin-Aldosterone System Inhibitors (e.g., ACE2 inhibitors or angiotensin-receptor blockers (ARBs)).

[00177] In some embodiments, the antiviral agent is selected from the group consisting of amantadine, favipiravir, lopinavir, oseltamivir (Tamiflu), pleconaril, rimantadine, ritonavir, an anti-sense RNA to SARS-CoV-2, a siRNA to SARS-CoV-2, an additional anti-SARS-CoV-2 monoclonal antibody, and combinations thereof.

[00178] In some embodiments, the additional anti-SARS-CoV-2 monoclonal antibody targets the RBD of the S protein of SARS-CoV-2. In some embodiments, the additional anti-SARS-CoV-2 monoclonal antibody is a neutralizing monoclonal antibody. Non-limiting examples of anti-SARS-CoV-2 monoclonal antibodies include bamlanivimab (LY-CoV555 or LY3819253), etesevimab (LY-CoV016 or LY3832479), casirivimab (REGN10933), and imdevimab (REGN10987). *See*, e.g., www.covid19treatmentguidelines.nih.gov/therapies/anti-sars-cov-2-antibody-products/anti-sars-cov-2-monoclonal-antibodies.

[00179] In some embodiments, the ACE2 inhibitor is selected from the group consisting of an RNAi to ACE2, a siRNA to ACE2, CRISPR-based inhibitor of ACE2, a soluble ACE2, a soluble ACE2 variant, an anti-ACE2 antibody, a vaccine, and combinations thereof. In some embodiments, the antibiotic is azithromycin. In some embodiments, the antimalarial agent comprises chloroquine or hydroxychloroquine. In some embodiments, the vaccine is a nucleic acid vaccine or an inactivated virus vaccine. In some embodiments, the vaccine is mrna-1273,

BNT162, INO-4800, AZD1222, Ad5-nCoV, PiCoVacc, NVX-CoV2373, or a combination thereof.

[00180] Administration of the two or more therapeutic agents encompasses co-administration of the therapeutic agents in a substantially simultaneous manner, such as in a pharmaceutical combination. Alternatively, such administration encompasses co-administration in multiple containers, or separate containers (*e.g.*, capsules, powders, and liquids) for each therapeutic agent. Such administration also encompasses use of each type of therapeutic agent in a sequential manner, either at approximately the same time or at different times. The composition described herein and the additional therapeutic agent(s) can be administered via the same administration route or via different administration routes.

[00181] In another aspect, the invention provides a method of preventing a SARS-CoV-2 infection in a subject, comprising administering to the subject an effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable carrier and, wherein as an active ingredient, any one of the polypeptides or fusion proteins described herein.

[00182] In another aspect, the invention provides a method of treating a SARS-CoV-2 infection in a subject, comprising administering to the subject an effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable carrier and, wherein as an active ingredient, any one of the polypeptides or fusion proteins described herein.

[00183] In another aspect, the invention provides a method of reducing viral load of SARS-CoV-2 in a subject, comprising administering to the subject an effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable carrier and, wherein as an active ingredient, any one of the polypeptides or fusion proteins described herein.

[00184] In another aspect, the invention provides a method of inhibiting binding of SARS-CoV-2 to a target cell, comprising contacting the target cell an effective amount of any one of the polypeptides or fusion proteins described herein.

[00185] In another aspect, the invention provides a method of inhibiting binding of SARS-CoV-2 to a target protein on a target cell, comprising contacting the target cell an effective amount of any one of the polypeptides or fusion proteins described herein.

[00186] In another aspect, the invention provides a method of inhibiting virus mediated fusion with a target cell, comprising contacting the target cell an effective amount of any one of the polypeptides or fusion proteins described herein.

[00187] Unless otherwise defined, all terms of art, notations and other scientific terms or terminology used herein are intended to have the meanings commonly understood by those of

skill in the art to which this invention pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art. It will be further understood that terms, such as those defined in commonly-used dictionaries, should be interpreted as having a meaning that is consistent with their meaning in the context of the relevant art and/or as otherwise defined herein.

[00188] The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

[00189] As used herein, the indefinite articles "a," "an" and "the" should be understood to include plural reference unless the context clearly indicates otherwise.

[00190] Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise," and variations such as "comprises" and "comprising", will be understood to imply the inclusion of, *e.g.*, a stated integer or step or group of integers or steps, but not the exclusion of any other integer or step or group of integer or step. When used herein, the term "comprising" can be substituted with the term "containing" or "including."

[00191] As used herein, "consisting of" excludes any element, step, or ingredient not specified in the claim element. When used herein, "consisting essentially of" does not exclude materials or steps that do not materially affect the basic and novel characteristics of the claim. Any of the terms "comprising," "containing," "including," and "having," whenever used herein in the context of an aspect or embodiment of the invention, can in some embodiments, be replaced with the term "consisting of," or "consisting essentially of" to vary scopes of the disclosure.

[00192] As used herein, the conjunctive term "and/or" between multiple recited elements is understood as encompassing both individual and combined options. For instance, where two elements are conjoined by "and/or," a first option refers to the applicability of the first element without the second. A second option refers to the applicability of the second element without the first. A third option refers to the applicability of the first and second elements together. Any one of these options is understood to fall within the meaning, and, therefore, satisfy the requirement of the term "and/or" as used herein. Concurrent applicability of more than one of the options is also understood to fall within the meaning, and, therefore, satisfy the requirement of the term "and/or."

[00193] When a list is presented, unless stated otherwise, it is to be understood that each individual element of that list, and every combination of that list, is a separate embodiment. For

example, a list of embodiments presented as "A, B, or C" is to be interpreted as including the embodiments, "A," "B," "C," "A or B," "A or C," "B or C," or "A, B, or C."

Exemplification

[00194] Example 1. Direct Binding between the Antigen-Binding Molecule and the S1 Subunit Receptor Binding Domain of SARS-Cov-2-Spike (RBD)

[00195] The molecule of interest is recombinantly expressed, purified, and quantified. An ELISA plate is coated with 1 µg/mL in PBS of commercial SARS-Cov-2 RBD overnight. The plate is then washed 3x with 200 µL of PBS-T on an automated plate washer and then blocked with 5% BSA in PBS for 1 hour at 37 °C to block non-specific binding. The plate is then washed 3x with 200 µL of PBS-T on an automated plate washer and the molecule of interest is titrated in a dilution series in the ELISA plate and incubated for 1 hour at 37 °C. The plate is then washed 3x with 200 µL of PBS-T on an automated plate washer and a secondary antibody (conjugated with HRP) specific to the target molecule is added a fixed dilution to the plate. The plate is incubated for 1 hour at 37 °C. The plate is then washed 3x with 200 µL of PBS-T on an automated plate washer and binding is probed with by adding TMB substrate to the wells of interest. After 5-10 minutes, the reaction is stopped with an acidic stop solution and the signal is quantified by measuring absorbance at 450 nm on a plate reader. A positive signal corresponds to an absorbance reading about background for a negative control (typically an antibody isotope control). A semi-quantitative understanding of the binding can be inferred by assessing strength of binding at greater dilutions of the target molecule. In theory, candidate molecules with greater affinity to the SARS-Cov-2 RBD should show greater signal at greater dilutions compared to weaker binders.

[00196] Example 2. The Antigen-Binding Molecule Competes with Human ACE2 (hACE2) for Binding to RBD *In Vitro.*

[00197] The molecule of interest is recombinantly expressed, purified, and quantified. An ELISA plate is coated with 1 µg/mL in PBS of commercial SARS-Cov-2 RBD overnight. The plate is then washed 3x with 200 µL of PBS-T on an automated plate washer and then blocked with 5% BSA in PBS for 1 hour at 37 °C to block non-specific binding. The plate is then washed 3x with 200 µL of PBS-T on an automated plate washer and the molecule of interest is titrated in a dilution series in the ELISA plate. At the same time, a fixed concentration of commercial hACE2 (biotinylated) is co-incubated with the candidate molecule. The fixed concentration of hACE2 corresponds to the concentration at which 50% of maximal binding to SARS-Cov-2 RBD determined empirically in this assay format. The plate is incubated for 1 hour at 37 °C. The

plate is then washed 3x with 200 µL of PBS-T on an automated plate washer and a secondary antibody (streptavidin conjugated with HRP) specific to the biotinylated hACE2 is added a fixed dilution to the plate. The plate is incubated for 1 hour at 37 °C. The plate is then washed 3x with 200 µL of PBS-T on an automated plate washer and binding is probed with by adding TMB substrate to the wells of interest. After 5-10 minutes, the reaction is stopped with an acidic stop solution and the signal is quantified by measuring absorbance at 450 nm on a plate reader. A positive signal corresponds to a decrease in absorbance for wells with the candidate molecule compared to wells without the candidate molecule above background for a negative control (typically an antibody isotope control). A semi-quantitative understanding of the target molecule's ability to compete with hACE2 can be inferred by assessing total decrease of hACE2 binding at greater dilutions of the target molecule. In theory, candidate molecules with greater ability to compete with hACE2 for SARS-Cov-2 RBD should show greater signal at greater dilutions compared to molecules with weaker ability.

[00198] Example 3. The Antigen-Binding Molecule Competes with hACE2 for Binding to RBD on Cell Surface.

[00199] The molecule of interest is recombinantly expressed, purified, and quantified. A cell line expressing hACE2 at wild-type levels is cultured, harvested without trypsinization (to prevent surface protein destruction), and counted. A fixed number of cells are added to a 96 well plate and incubated with the molecule of interest titrated in a dilution series. At the same time, a fixed concentration of commercial SARS-Cov-2 (biotinylated) is co-incubated with the candidate molecule. The fixed concentration of SARS-Cov-2 corresponds to the concentration at which 50% of maximal binding to hACE2 on the cell determined empirically in this assay format. The plate of cells is incubated for 1 hour at 4 ℃ in the dark. The plate is centrifuged at 400xg for 5 minutes, the supernatant is aspirated, and the cells washed with 0.2% BSA in PBS. This process is repeated 2 more times for a total of 3 washes. A secondary antibody (streptavidin conjugated with Alexa Fluor 488) specific to the biotinylated SARS-Cov-2 is added a fixed dilution to the plate. The plate of cells is incubated for 1 hour at 4 ℃ in the dark. The plate is centrifuged at 400xg for 5 minutes, the supernatant is aspirated, and the cells washed with 0.2% BSA in PBS. The cells are then resuspended in a fixed volume of 0.2% BSA in PBS and subjected to flow cytometry. Total binding of the SARS-Cov-2 RBD is analyzed by median fluorescence intensity (MFI) of the FIT-C channel. A positive signal corresponds to a decrease in MFI for wells with the candidate molecule compared to wells without the candidate molecule above background for a negative control (typically an antibody isotope control). A semi-

quantitative understanding of the ability to compete with SARS-Cov-2 RBD can be inferred by assessing total decrease of SARS-Cov-2 RBD MFI at greater dilutions of the target molecule. In theory, candidate molecules with greater ability to compete with the SARS-Cov-2 RBD for hACE2 (which can also be considered the affinity for SARS-Cov-2 RBD) should show greater signal at greater dilutions molecules with weaker ability.

[00200] Example 4. Yeast display characterization of antibody designs

[00201] Antibodies were formatted as a single VH domain fused to a c-Myc epitope tag and tested for binding using yeast display. VH-c-Myc fusion constructs were synthesized as DNA fragments (TWIST Biosciences), with overhangs for cloning into a yeast display vector. DNA insert and digested vector were transformed into yeast, and the full plasmid was generated *in vivo* by homologous recombination. Cells were induced for VH-c-Myc expression and display on the yeast cell surface. Induced cells were stained for binding to biotinylated SARS-CoV-2 receptor binding domain (RBD) antigen (ACROBiosystems, Newark, DE) or SARS-CoV RBD (Genscript) and anti-c-myc (Exalpha) for VHc-Myc expression. For clones that showed binding, the equilibrium dissociation constant (K_D) was evaluated by performing a titration curve with various concentrations of SARS-CoV-2 RBD or SARS-CoV antigen. All clones that bound SAR-CoV-2 RBD also demonstrated cross-reactive binding to SARS-CoV RBD. Binding specificity and K_D values are summarized in Table 2.

[00202] Example 5. Production of VH-Fc fusions

[00203] VH-cMyc fusion constructs were reformatted into VH-Fc fusions using conventional methods. Homodimeric VHH antibodies were generated by using an expression plasmid containing a CMV promoter to drive expression and a signal peptide to promote secretion of the fully folded VH-Fc fusion into the supernatant. Mammalian expression host systems (such as the Chinese hamster ovary - CHO S cell line) were employed for protein expression using cationic lipid-based transient transfection methodologies with fed-batch production procedures. The expressed, soluble VH-Fc fusions were purified using an affinity capture-based purification method using Protein A affinity resin (such as the MabSelect Sure resin, GE Healthcare) and aqueous buffers. Elution was performed under acidic conditions with low pH (such as pH 3.5) and then neutralized to pH7.5 using 2M Tris base. The purified VHH antibodies were further stabilized in aqueous buffers (such as Histidine-based buffers containing NaCl) for long-term storage/freezing.

[00204] Example 6. Binding kinetics and affinity of VHH antibodies

[00205] Binding kinetics and affinity were measured with Bio-layer interferometry (BLI) using a ForteBio Octet Red96e instrument. Antibodies were captured onto biosensors.

Biosensors were then incubated with a serial dilution of SARS-CoV-2 RBD (Acro Biosystems) for association and dissociation.

[00206] Example 7. Material and Methods

[00207] Expression of Full-Length IgG

[00208] Back-translated DNA coding for the VHH variable domain (scFc) sequences of designed antibodies were PCR amplified and cloned into a human hG1 Fc vector containing a CMV promoter sequence, signal peptide, and corresponding constant regions using NEBuilder® HiFi DNA Assembly (New England Biolabs, Ipswich, MA). VHH proteins were expressed in ExpiCHO cells following manufacturer's methods (Thermo Fisher Scientific, Inc., Waltham, MA) and purified by affinity chromatography using MabSelect Sure Protein A affinity resin (Cytiva, Marlborough, MA) per manufacturer's methods.

[00209] Neutralization Assay

[00210] Pre-titrated amounts of rVSV-SARS-CoV-2 were incubated with serially diluted monoclonal antibodies at 37°C for 1 hour before addition to confluent Vero (ATCC CCL-81) monolayers in 96-well plates. Infection proceeded for 16-18 hours at 37°C in 5% CO_2 before cells were fixed in 4% paraformaldehyde and stained with 10 μg/mL Hoechst. Cells were imaged using a CellInsight CX5 imager and infection was quantified by automated enumeration of total cells and those expressing green fluorescent protein (GFP). Infection was normalized to the average number of cells infected with rVSV-SARS-CoV-2 incubated with human IgG isotype control.

[00211] Generation of WT and Mutant SARS-CoV-2 Spike Proteins

[00212] Spike proteins were generated for epitope binning studies and structural biology using the HexaPro background (containing residues 14-1208 (Genbank: MN908947) of the ectodomain, six proline substitutions (F817P, A892P, A899P, A942P, K986P, V987P), as well as the D614G mutation established in all/most variants associated with spillover/spillback in Northern Europe, and replacement of cleavage site residues 682-685 ("RRAR" (SEQ ID NO: 18) to "GSAS" (SEQ ID NO: 19)). The resulting spike variants were cloned into a phCMV mammalian expression vector containing an N-terminal Gaussia luciferase signal sequence (MGVKVLFALICIAVAEA (SEQ ID NO: 20)) and a C-terminal foldon trimerization domain, followed by an HRV-3C cleavage site and a Twin-Strep-Tag. Plasmids were transformed into Stellar competent cells and isolated using a Plasmid Plus Midi kit (Qiagen, Hilden, Germany).

[00213] SARS-CoV-2 HexaPro spike was transiently transfected into Freestyle 293-F or ExpiCHO-S cells (Thermo Fisher Scientific, Inc., Waltham, MA). Both cell lines were maintained and transfected according to manufacturer's protocols. Briefly, 293-F cells were

grown to a density of $2.0x10^6$ cells/mL and diluted to $1.0x10^6$ cell/mL on the day of transfection (day 0). Plasmid DNA and polyethyleneimine were mixed in Opti-MEM (Thermo Fisher Scientific, Inc., Waltham, MA), incubated for 25 minutes, and then added to the cells. Cell cultures were incubated at 37°C, 8% CO_2, and 120 RPM, and harvested on day 5. For ExpiCHO cultures, manufacturer's "High Titer" protocol was used. Briefly, cells were grown to a density of $1x10^7$ cells/mL and diluted to $6x10^6$ cells/mL on the day of transfection (day 0). Plasmid DNA and Expifectamine were mixed in Opti-PRO SFM (Thermo Fisher Scientific, Inc., Waltham, MA) according to manufacturer's instructions, and added to the cells. On day 1, cells were fed with manufacturer-supplied feed and enhancer according to the suggested protocol, and cultures were then incubated at 32°C, 5% CO_2 and 115 RPM. ExpiCHO cultures were harvested on day 7. All cultures were clarified by centrifugation, followed by addition of BioLock (IBA Lifesciences, Göttingen, Germany), and supernatants were flowed through a 0.22 μM sterile filter and purified on an Ä KTA GO (Cytiva, Marlborough, MA) using a 5mL StrepTrap-HP column equilibrated with TBS buffer (25mM Tris pH 7.6, 200mM NaCl, 0.02% NaN_3), and eluted in TBS buffer supplemented with 5mM d-desthiobiotin (Sigma-Aldrich, St. Louis, MO). The strep-tags were cleaved using HRV-3C protease, and the proteins were further purified by size-exclusion-chromatography (SEC) on a Superdex 6 increase 10/300 column (GE Healthcare, Chicago, IL) in TBS.

[00214] High-throughput Surface Plasmon Resonance (HT-SPR) Epitope Binning

[00215] Epitope binning was performed with a sandwich assay format on a Carterra LSA SPR instrument equipped with a CMDP sensor chip at 25°C and in a HBSTE-BSA running buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.05% Tween-20, supplemented with 0.5 mg/ml BSA). Two microfluidic modules, a 96-channel print-head (96PH) and a single flow cell (SFC), were used to deliver samples onto the sensor chip. Surface preparation was performed with 25 mM MES (pH 5.5) with 0.05% Tween-20 as a running buffer. The chip was activated with a freshly prepared solution of 130 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) + 33 mM N-hydroxysulfosuccinimide (Sulfo-NHS) in 0.1 M MES (pH 5.5) using the SFC. Antibodies were immobilized using the 96PH for 10 minutes at 10 μg/mL diluted into 10 mM sodium acetate (pH 4.25). Unreactive esters were quenched with a 7-minute injection of 1 M ethanolamine-HCl (pH 8.5) using the SFC. The binning analysis was performed over this array with the HBSTE-BSA buffer as the running buffer and sample diluent. The RBD antigen was injected in each cycle for 4 minutes at 50 nM (1.3 μg/mL) and followed immediately by a 4-minute injection of the analyte antibody at 30 μg/mL (200 nM for IgG constructs). The surface was regenerated each cycle with double pulses (17 seconds per pulse) of 10 mM Glycine (pH

2.0). The data was processed and analyzed with Epitope Tool software (Carterra, Salt Lake City, UT).

[00216] Example 8. Epitope and Affinities

[00217] The affinities of monoclonal antibodies against the soluble G614 HexaPro spike ectodomain and monomeric RBD were examined. Using a high-throughput surface plasmon resonance analysis, the antibodies that react with the RBD were sorted into 7 different "communities" (Coronavirus Immunotherapy Consortium (COVIC)). RBD communities were defined as the inability between two members of the same community to make SPR sandwich pairs, indicating a shared epitope for binding. It was found that Reference, AB-1 and AB-2 cluster into "RBD Community 7," a distinct "RBD community" epitope bin (COVIC, data not shown). Affinities to G614 HexaPro spike ectodomain and to monomeric RBD are described in Table 2.

[00218] Example 9. VHH Molecules Bind to SARS-COV-2 RBD by YSD

[00219] ScFv constructs fused to a c-myc epitope tag were synthesized as DNA fragments (Twist Biosciences) with overhangs for cloning into a yeast display vector. DNA inserts and digested vectors were transformed into yeast, and the full plasmid was generated *in vivo* by homologous recombination. Cells were induced for scFv expression and display on the yeast cell surface. Induced cells were stained for binding to biotinylated SARS-CoV-2 receptor binding domain (RBD) antigen (ACROBiosystems, Newark, DE) or SARS-CoV RBD (Genscript Biotech, Piscataway, NJ) and anti-c-myc (Exalpha Biologicals Inc, Shirley, MA) for scFv Expression. Affinity was determined by staining constructs at titrating concentrations of antigen and calculating K_D at equilibrium by plotting median fluorescence intensity at each concentration (Table 2, FIG. 3).

[00220] Example 10. VHH Molecules Neutralize SARS-COV-2 *In Vitro*

[00221] AB-1 and AB-2 were submitted to the Coronavirus Immunotherapy Consortium (COVIC) for neutralization studies. Both molecules show the ability to block infection of Vero monolayers of both pseudo and authentic WT virus (Table 2).

[00222] AB-1 was chosen to further investigate susceptibility of emerging mutations in COV-2 RBD. Pseudovirus neutralization studies were performed with pseudovirus bearing point mutations found in multiple human and mink variants. Data are presented in FIG. 4. AB-1 was resistant to most mutations as indicated by IC_{50} fold-change between -2.5 and 2.5.

Table 1. Amino Acid Sequences

Name	SEQ ID	Amino Acid Sequence
V_H Consensus	NO: 2	QVQLQESGGGLVQAGGSLRLSCAASGRTFSEYAMGWFRQAPGK EREFVATISWSGGSTYYTDSVKGRFTISRDNAKNTVYLQMNSLK PDDTAVYYCAAAGLGX$_1$X$_2$X$_3$SEWDYDYDYWGQGTQVTVSSGS
V_H of Reference	NO: 3	QVQLQESGGGLVQAGGSLRLSCAASGRTFSEYAMGWFRQAPGK EREFVATISWSGGSTYYTDSVKGRFTISRDNAKNTVYLQMNSLK PDDTAVYYCAAAGLGTVVSEWDYDYDYWGQGTQVTVSSGS
V_H of AB-1	NO: 4	QVQLQESGGGLVQAGGSLRLSCAASGRTFSEYAMGWFRQAPGK EREFVATISWSGGSTYYTDSVKGRFTISRDNAKNTVYLQMNSLK PDDTAVYYCAAAGLGVVLSEWDYDYDYWGQGTQVTVSSGS
V_H of AB-2	NO: 5	QVQLQESGGGLVQAGGSLRLSCAASGRTFSEYAMGWFRQAPGK EREFVATISYSGGSTYYTDSVKGRFTISRDNAKNTVYLQMNSLKP DDTAVYYCAAAGLGVHVSEWDYDYDYWGQGTQVTVSSGS
HCDR1 of Reference, AB-1, 2	NO: 6	GRTFSEYA
HCDR2 of Reference, AB-1, 2	NO: 7	ISWSGGST
HCDR3 Consensus	NO: 8	AAAGLGX$_1$X$_2$X$_3$SEWDYDYDY
HCDR3 of Reference	NO: 9	AAAGLGTVVSEWDYDYDY
HCDR3 of AB-1	NO: 10	AAAGLGVVLSEWDYDYDY
HCDR3 of AB-2	NO: 11	AAAGLGVHVSEWDYDYDY
Heavy Chain IgG1	NO: 12	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS NTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
Light Chain κ	NO: 13	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE VTHQGLSSPVTKSFNRGEC
Light Chain λ	NO: 14	GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKA DGSPVKAGVETTKPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQ VTHEGSTVEKTVAPTECS

Name	SEQ ID	Amino Acid Sequence
AB-1	NO: 15	QVQLQESGGGLVQAGGSLRLSCAASGRTFSEYAMGWFRQAPGK EREFVATISWSGGSTYYTDSVKGRFTISRDNAKNTVYLQMNSLK PDDTAVYYCAAAGLGVVLSEWDYDYDYWGQGTQVTVSSDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GK
AB-2	NO: 16	QVQLQESGGGLVQAGGSLRLSCAASGRTFSEYAMGWFRQAPGK EREFVATISYSGGSTYYTDSVKGRFTISRDNAKNTVYLQMNSLKP DDTAVYYCAAAGLGVHVSEWDYDYDYWGQGTQVTVSSDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GK

Heavy Chain IgG1, Light Chain κ, Light Chain λ sequences are non-limiting example sequences.

Table 2. Yeast display affinity measurements

Clone	YSD K_D (nM)		Octet K_D (nM)
	SARS-CoV-2 RBD	SARS-CoV RBD	
Reference	6.0	+	+
AB-1	9.3	+	+
AB-2	6.6	+	+

REFERENCES

1. Baud D, et al. (2020) Real estimates of mortality following COVID-19 infection. *Lancet Infect Dis.*: S1473-3099(20)30195-X. doi: 10.1016/S1473-3099(20)30195-X. [Epub ahead of print].

2. Lindsey R. Baden and Eric J. Rubin (2020) Covid-19 - The Search for Effective Therapy, *N Engl J Med.*, doi: 10.1056/NEJMe2005477.

3. Ziegler et al. (2020) SARS-CoV-2 receptor ACE2 is an interferon-stimulated gene in human airway epithelial cells and is detected in specific cell subsets across tissues, *Cell*, DOI: 10.1016/j.cell.2020.04.035 [Journal pre-proof].

[00223] The teachings of all patents, published applications and references cited herein are incorporated by reference in their entirety.

[00224] While example embodiments have been particularly shown and described, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the embodiments encompassed by the appended claims.

CLAIMS

What is claimed is:

1. A polypeptide that specifically binds a severe acute respiratory syndrome coronavirus 2 Spike glycoprotein (SARS-CoV-2-Spike), comprising a paratope that is identical to the paratope of an antibody comprising an amino acid sequence selected from:
   a) SEQ ID NO: 4; or
   b) SEQ ID NO: 5.

2. The polypeptide of claim 1, comprising an immunoglobulin heavy chain variable region (V_H).

3. A polypeptide that specifically binds a severe acute respiratory syndrome coronavirus 2 Spike glycoprotein (SARS-CoV-2-Spike), comprising an immunoglobulin heavy chain variable domain (V_H) amino acid sequence comprising a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2) and a heavy chain complementarity determining region 3 (HCDR3) that are identical to the HCDR1, HCDR2 and HCDR3, respectively, of the amino acid sequence of SEQ ID NO: 4 or SEQ ID NO: 5

4. The polypeptide of claim 3, further comprising a paratope that is identical to the paratope of a single-domain antibody comprising:
   a) SEQ ID NO: 4; or
   b) SEQ ID NO: 5.

5. A polypeptide that specifically binds a severe acute respiratory syndrome coronavirus 2 Spike glycoprotein (SARS-CoV-2-Spike), comprising an immunoglobulin heavy chain variable domain (V_H) comprising the amino acid sequence of SEQ ID NO: 2, wherein:
   a) X_1 is not T;
   b) X_2 is not V; or
   c) X_3 is not V,
   or any combination of the foregoing.

6. The polypeptide of claim 5, wherein:
   a) X_1 is T or V;

b) X_2 is V or H; or

a) X_3 is V or L,

or any combination of the foregoing.

7. The polypeptide of claim 6, wherein:

a) X_1 is V;

b) X_2 is H; or

c) X_3 is L,

or any combination of the foregoing.

8. The polypeptide of any one of claims 5-7, wherein the V_H comprises a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2) and a heavy chain complementarity determining region 3 (HCDR3) that are identical in amino acid sequence to the HCDR1, HCDR2 and HCDR3, respectively, of SEQ ID NO: 4 or SEQ ID NO: 5.

9. The polypeptide of any one of claims 1-8, wherein the V_H is at least 85% identical to the amino acid sequence of SEQ ID NO: 4 or SEQ ID NO: 5, or a combination of the foregoing.

10. The polypeptide of any one of claims 1-9, wherein the V_H comprises about 1-10 amino acid substitutions, relative to the amino acid sequence of SEQ ID NO: 4 or SEQ ID NO: 5, or a combination of the foregoing.

11. The polypeptide of claim 10, wherein the amino acid substitutions are conservative substitutions.

12. The polypeptide of claim 11, wherein the amino acid substitutions are highly conservative substitutions.

13. The polypeptide of any one of claims 1-12, wherein the V_H contains a human framework region.

14. The polypeptide of any one of claims 1-13, wherein the polypeptide is a single-domain antibody or an antigen-binding fragment thereof.

15. The polypeptide of any one of claims 1-13, further comprising an antibody heavy chain constant domain sequence.

16. The polypeptide of claim 15, wherein the antibody heavy chain constant domain is selected from the group consisting of an IgA constant domain, an IgD constant domain, an IgE constant domain, an IgG constant domain and an IgM constant domain.

17. The polypeptide of claim 16, wherein the antibody heavy chain constant domain is an IgG1 heavy chain constant domain.

18. The polypeptide of any one of claims 1-17, wherein the polypeptide is linked to a second polypeptide.

19. The polypeptide of claim 18, wherein the second polypeptide comprises a polypeptide of any one of claims 1-17.

20. The polypeptide of claim 18, wherein the second polypeptide comprises:

a) a paratope that is identical to the paratope of a single-domain antibody comprising the amino acid sequence of SEQ ID NO: 3;

b) a V_H comprising a HCDR1, a HCDR2 and a HCDR3 that are identical to the HCDR1, HCDR2 and HCDR3, respectively, of a single-domain antibody comprising the amino acid sequence of SEQ ID NO: 3;

c) a V_H that is at least 85% identical to the amino acid sequence of SEQ ID NO: 3; or

d) a V_H comprising about 1-10 amino acid substitutions, relative to the amino acid sequence of SEQ ID NO: 3,

or any combination of the foregoing.

21. The polypeptide of claim 18, wherein:

a) the polypeptide comprises the amino acid sequence of SEQ ID NO: 4 and the second polypeptide comprises the amino acid sequence of SEQ ID NO: 3, 4 or 5; or

b) the polypeptide comprises the amino acid sequence of SEQ ID NO: 5 and the second polypeptide comprises the amino acid sequence of SEQ ID NO: 3, 4 or 5.

22. The polypeptide of any one of claims 18-21 wherein the polypeptide and the second polypeptide are linked to each other via a linker.

23. The polypeptide of claim 22, wherein the linker is a disulfide bond.

24. The polypeptide of any one of claims 1-17, wherein the polypeptide is linked to an immunoglobulin light chain variable domain (V_L).

25. The polypeptide of any one of claims 18-23, wherein the polypeptide, the second polypeptide, or both, are linked to a third polypeptide comprising an immunoglobulin light chain variable domain (V_L).

26. The polypeptide of claim 24 or 25, wherein the third polypeptide further comprises an antibody light chain constant domain sequence.

27. The polypeptide of claim 26, wherein the antibody light chain constant domain is selected from the group consisting of a κ constant domain or a λ constant domain.

28. The polypeptide of any one of claims 1-27, wherein the polypeptide is conjugated to a heterologous moiety.

29. The polypeptide of claim 28, wherein the heterologous moiety is a therapeutic agent, a diagnostic agent or a combination thereof.

30. The polypeptide of claim 28, wherein the heterologous moiety is selected from the group consisting of polyethylene glycol (PEG), hexadecanoic acid, a hydrogel, a lipid nanoparticle, a polymer nanoparticle, and a heterologous polypeptide sequence, or a combination thereof.

31. The polypeptide of claim 30, wherein the polymer nanoparticle comprises poly(lactic-co-glycolic) acid (PLGA).

32. The polypeptide of claim 28, wherein the heterologous polypeptide sequence comprises a carrier polypeptide.

33. The polypeptide of claim 32, wherein the carrier polypeptide is albumin or an Fc polypeptide.

34. The polypeptide of any one of claims 1-33, wherein the polypeptide:

a) binds SARS-CoV-2 with a K_D of 10 μM or less;

b) reduces binding of SARS-CoV-2 to angiotensin-converting enzyme 2 (ACE2); or

c) reduces infectivity of SARS-CoV-2 in human cells,

or any combination of the foregoing.

35. The polypeptide of claim 34, wherein the SARS-CoV-2 is a variant comprising T19R, 156del, 157del, R158G, L452R, T478K, D614G, P681R and D950N, and optionally further comprising G142D.

36. The polypeptide of claim 34 or 35, wherein the polypeptide binds SARS-CoV-2 with a K_D of 100 nM or less.

37. The polypeptide of any one of claims 34-36, wherein the polypeptide reduces binding of SARS-CoV-2 to ACE2 by at least about 30%.

38. The polypeptide of any one of claims 34-37, wherein the ACE2 is a primate ACE2.

39. The polypeptide of claim 38, wherein the primate ACE2 is human ACE2 (GeneID 59272; exemplary protein sequence NP_001358344.1).

40. The polypeptide of claim 38, wherein the primate ACE2 is a non-human primate ACE2 selected from the group consisting of chimpanzee ACE2, macaque ACE2, cynomolgus ACE2 and combinations thereof.

41. The polypeptide of claim 34 or 35, wherein the polypeptide reduces infectivity of SARS-CoV-2 in human cells by at least about 30%.

42. A fusion protein comprising the polypeptide of any one of claims 1-41.

43. A polynucleotide comprising a sequence encoding the polypeptide of any one of claims 1-41 or the fusion protein of claim 42.

44. An expression vector comprising the polynucleotide of claim 43.

45. A host cell comprising the polynucleotide of claim 43 or the expression vector of claim 44.

46. A composition comprising the polypeptide of any one of claims 1-41 or the fusion protein of claim 42.

47. The composition of claim 46, further comprising one or more pharmaceutical excipients, diluents, or carriers.

48. A method of treating a subject in need thereof, comprising administering an effective amount of the composition of claim 46 or 47 to the subject.

49. A method of inhibiting fusion between SARS-CoV-2-Spike and a cell in a subject, comprising contacting the cell with an effective amount of the composition of claim 46 or 47.

50. The method of claim 48 or 49, wherein the subject has COVID-19.

51. The method of claim 48 or 49, wherein the subject is at risk of developing COVID-19.

52. The method of claim any one of claims 48-51, wherein the subject is a human.

53. The method of any one of claims 48-52, wherein the subject has a heart disease.

54. The method of claim 53, wherein the heart disease is selected from the group consisting of a congestive heart disease, a coronary artery disease, a hypertensive heart disease, an inflammatory heart disease, a pulmonary heart disease, a rheumatic heart disease, a valvular heart disease, a cardiomyopathy, heart failure, and combinations thereof.

55. The method of claim 54, wherein the heart failure is a congestive heart failure.

56. The method of claim 54, wherein the inflammatory heart disease is selected from the group consisting of endocarditis, cardiomegaly, myocarditis, and combinations thereof.

57. The method of any one of claims 48-56, wherein the subject has diabetes.

58. The method of any one of claims 48-57, wherein the subject has a lung disease.

59. The method of claim 58, wherein the lung disease is selected from the group consisting of acute respiratory distress syndrome, asthma, bronchitis, COPD, emphysema, a lung tumor, a pleural cavity disease, a pulmonary vascular disease, a respiratory tract infection, and combinations thereof.

60. The method of claim 59, wherein:

a) the respiratory tract infection is an upper respiratory tract infection, a lower respiratory tract infection, or pneumonia;

b) the pleural cavity disease is pleural mesothelioma or tension pneumothorax;

c) the pulmonary vascular disease is embolisms, edema, arterial hypertension or hemorrhages; or

d) a combination thereof.

61. The method of any one of claims 48-60, wherein the subject is a tobacco smoker.

62. The method of any one of claims 48-61, wherein the subject is immune-compromised.

63. The method of claim 62, wherein the subject is on immunosuppressive therapy.

64. The method of any one of claims 48-63, wherein the subject is 40 years or older.

65. The method of any one of claims 48-64, further comprising administering a therapeutically effective amount of an additional therapeutic agent to the subject.

66. The method of claim 65, wherein the additional therapeutic agent is selected from the group consisting of an antiviral agent, an ACE2 inhibitors, an additional SARS-CoV-2-Spike-binding antibody, an antibiotic, an antimalarial agent, a vaccine, and combinations thereof.

67. The method of claim 66, wherein:

a) the additional SARS-CoV-2-Spike-binding antibody is selected from the group consisting of bamlanivimab, etesevimab, casirivimab, imdevimab, and combinations thereof;

b) the antiviral agent is selected from the group consisting of oseltamivir (Tamiflu), favipiravir, amantadine, remdesivir, rimantadine, pleconaril, an anti-sense RNA to SARS-CoV-2, a siRNA to SARS-CoV-2, and combinations thereof;

c) the ACE2 inhibitor is selected from the group consisting of an RNAi to ACE2, a siRNA to ACE2, CRISPR-based inhibitor of ACE2, a soluble ACE2, a soluble ACE2 variant, an anti-ACE2 antibody, and combinations thereof;

d) the antibiotic comprises azithromycin;

e) the antimalarial agent comprises a chloroquine;

f) the vaccine is a nucleic acid vaccine or an inactivated virus vaccine; or

g) a combination thereof.

68. The method of claim 67, wherein the vaccine is mrna-1273, BNT162, INO-4800, AZD1222, Ad5-nCoV, PiCoVacc, NVX-CoV2373, or a combination thereof.

```
                                  **********      **                ** **
NLCPFGEVFNATKFPSVYAWERKKISNCVADYSVLYNSTFFSTFKCYGVSATKLNDLCFSNVYADSFVVKGDDVRQIAPGQTGVIA

                                 * *                                  **
DYNYKLPDDFMGCVLAWNTRNIDATSTGNYNYKYRYLRHGKLRPFERDISNVPFSPDGKPCTPPALNCYWPLNDYGFYTTTGIGYQ

    *
PYRVVVLSFEGSLEVLFQ  (SEQ ID NO: 21)
```

FIG. 1

HCDR1 HCDR2

Reference QVQLQESGGGLVQAGGSLRLSCAASGRTFSEYAMGWFRQAPGKEREFVATISWSGGSTYYTDSVKGRFTI

AB-1 QVQLQESGGGLVQAGGSLRLSCAAS**GRTFSEYA**MGWFRQAPGKEREFVAT**ISWSGGST**YYTDSVKGRFTI

AB-2 QVQLQESGGGLVQAGGSLRLSCAAS**GRTFSEYA**MGWFRQAPGKEREFVAT**ISYSGGST**YYTDSVKGRFTI

HCDR3

Reference SRDNAKNTVYLQMNSLKPDDTAVYYCAAAGLGTVVSEWDYDYWGQGTQVTVSSGS (SEQ ID NO: 3)

AB-1 SRDNAKNTVYLQMNSLKPDDTAVYYC**AAAGLG*VVL*SEWDYDY**WGQGTQVTVSSGS (SEQ ID NO: 4)

AB-2 SRDNAKNTVYLQMNSLKPDDTAVYYC**AAAGLG*VH*VSEWDYDY**WGQGTQVTVSSGS (SEQ ID NO: 5)

123

FIG. 2

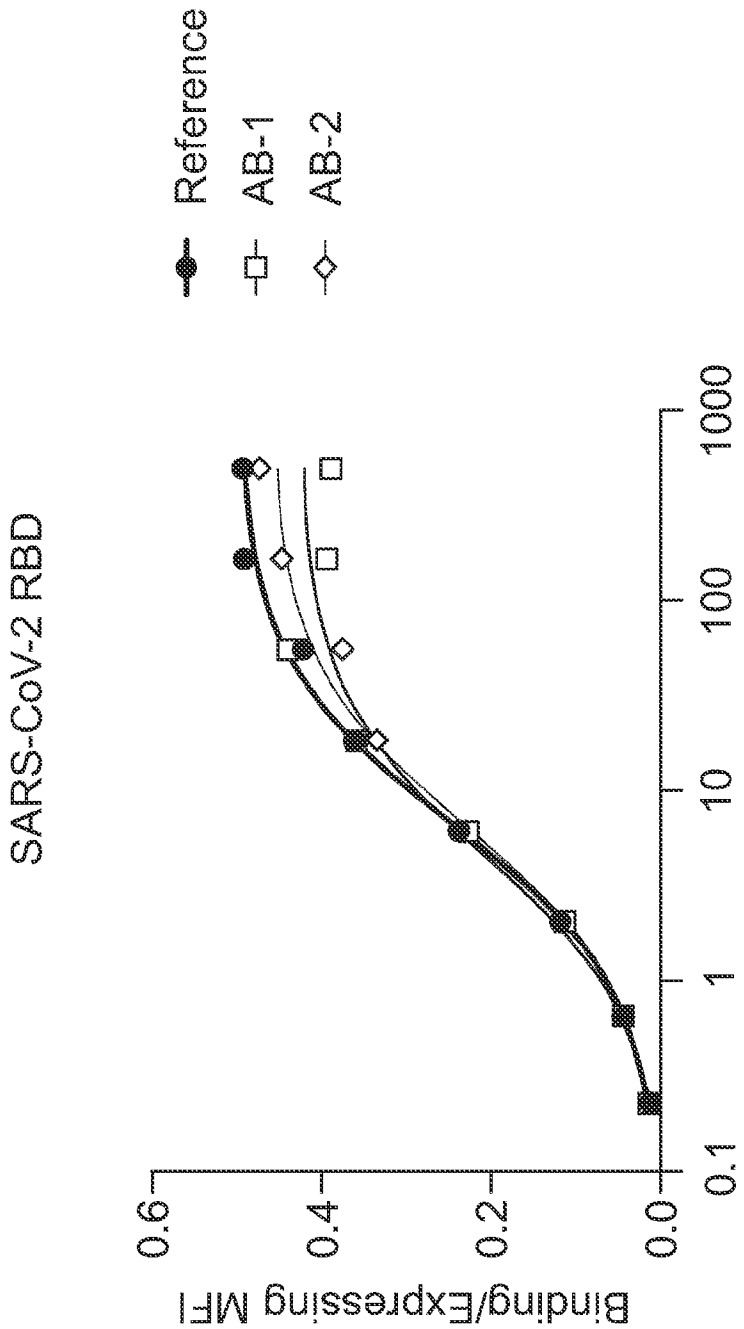

SARS-CoV-2 RBD
Reference
AB-1
AB-2
Binding/Expressing MFI
0.0
0.2
0.4
0.6
0.1
1
10
100
1000
[Antigen] in nM

FIG. 3

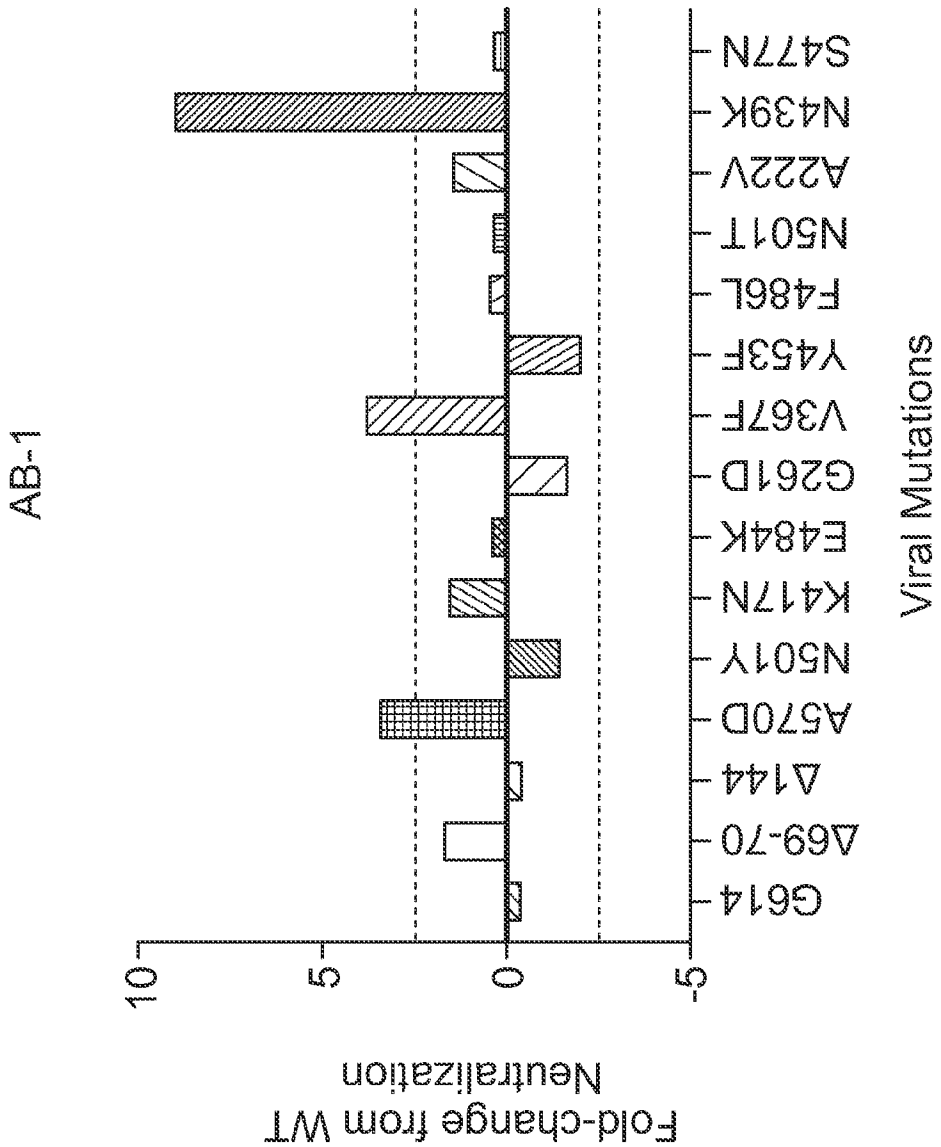

AB-1
Fold-change from WT
Neutralization
10
5
0
-5
G614
Δ69-70
Δ144
A570D
N501Y
K417N
E484K
G261D
V367F
Y453F
F486L
N501T
A222V
N439K
S477N
Viral Mutations

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2021/047757

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/10 A61K39/395 A61P31/14
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61P

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DANIEL WRAPP ET AL: "Structural Basis for Potent Neutralization of Betacoronaviruses by Single-Domain Camelid Antibodies", CELL, vol. 181, no. 5, 28 May 2020 (2020-05-28), pages 1004-1015.e15, XP055764639, Amsterdam NL ISSN: 0092-8674, DOI: 10.1016/j.cell.2020.04.031 cited in the application figures 4, 6 ----- -/--	1-68

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 November 2021	02/12/2021

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Brouns, Gaby

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE GenBank [Online] 4 May 2020 (2020-05-04), Wrapp D ET AL: "Lama glama immunoglobulin heavy chain variable region mRNA, partial cds", XP055864500, Database accession no. MT350284 abstract -----	1-68
X	WU YANLING ET AL: "Identification of Human Single-Domain Antibodies against SARS-CoV-2", CELL HOST & MICROBE, ELSEVIER, NL, vol. 27, no. 6, 14 May 2020 (2020-05-14), page 891, XP086178478, ISSN: 1931-3128, DOI: 10.1016/J.CHOM.2020.04.023 [retrieved on 2020-05-14] figures 2, 3 -----	1-68
X	Schoof Michael ET AL: "An ultra-potent synthetic nanobody neutralizes SARS-CoV-2 by locking Spike into an inactive conformation", bioRxiv, 17 August 2020 (2020-08-17), XP055865529, DOI: 10.1101/2020.08.08.238469 Retrieved from the Internet: URL:https://www.biorxiv.org/content/10.1101/2020.08.08.238469v2.full.pdf [retrieved on 2021-11-24] figures 3, 4 -----	1-68
X	Xiang Yufei ET AL: "Versatile, Multivalent Nanobody Cocktails Efficiently Neutralize SARS-CoV-2", bioRxiv, 25 August 2020 (2020-08-25), XP055852802, United States DOI: 10.1101/2020.08.24.264333 Retrieved from the Internet: URL:https://www.biorxiv.org/content/10.1101/2020.08.24.264333v3.full.pdf [retrieved on 2021-10-19] figures 1, 5 ----- -/--	1-68

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HUO JIANGDONG ET AL: "Neutralizing nanobodies bind SARS-CoV-2 spike RBD and block interaction with ACE2", NAT. STRUCT. MOL. BIOL, NATURE PUBLISHING GROUP US, NEW YORK, vol. 27, no. 9, 13 July 2020 (2020-07-13), pages 846-854, XP037271196, ISSN: 1545-9993, DOI: 10.1038/S41594-020-0469-6 [retrieved on 2020-07-13] cited in the application figures 2, 3	1-68
X	Thomas J. Esparza ET AL: "High Affinity Nanobodies Block SARS-CoV-2 Spike Receptor Binding Domain Interaction with Human Angiotensin Converting Enzyme", bioRxiv, 24 July 2020 (2020-07-24), XP055763653, DOI: 10.1101/2020.07.24.219857 Retrieved from the Internet: URL:https://www.biorxiv.org/content/10.1101/2020.07.24.219857v1.full.pdf [retrieved on 2021-01-11] figures 6, 7	1-68
X,P	WO 2021/156490 A2 (VIB VZW [BE]; UNIV GENT [BE] ET AL.) 12 August 2021 (2021-08-12) sequences 47, 49 figures 19, 21, 23	5-7, 9-12, 14-17, 28, 32-34, 36-68
A,P	ZUPANCIC JENNIFER M. ET AL: "Engineered Multivalent Nanobodies Potently and Broadly Neutralize SARS-CoV-2 Variants", ADVANCED THERAPEUTICS, vol. 4, no. 8, 2 August 2021 (2021-08-02), page 2100099, XP055865509, ISSN: 2366-3987, DOI: 10.1002/adtp.202100099 Retrieved from the Internet: URL:https://onlinelibrary.wiley.com/doi/full-xml/10.1002/adtp.202100099> figure 1	18-23

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/047757

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2021/047757

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2021156490 A2	12-08-2021	NONE	