

Polypeptides, Compositions, and their Use to Treat or Limit Development of an
5 **Infection**

Cross Reference

This application claims priority to U.S. Provisional Application Serial Nos.
62/977,036 filed February 14, 2020; 63/046,159, filed: June 30, 2020, and 63/064,235, filed
10 August 11, 2020; each incorporated by reference herein in their entirety.

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15 The government has certain rights in the invention.

Sequence Listing Statement:

A computer readable form of the Sequence Listing is filed with this application by
electronic submission and is incorporated into this application by reference in its entirety. The
20 Sequence Listing is contained in the file created on February 11, 2021, having the file name
"20-1008-PCT_SeqList_ST25.txt" and is 1077 kb in size.

Background

The recent emergence of a previously unknown virus in Wuhan, China has resulted in
25 the ongoing COVID-19 pandemic that has caused more than 18,700,000 infections and
700,000 fatalities as of August 6, 2020 (WHO). Rapid viral isolation and sequencing revealed
by January 2020 that the newly emerged zoonotic pathogen was a coronavirus closely related
to SARS-CoV and was therefore named SARS-CoV-2. SARS-CoV-2 is believed to have
originated in bats based on the isolation of the closely related RaTG13 virus from
30 *Rhinolophus affinis* and the identification of the RmYN02 genome sequence in
metagenomics analyses of *Rhinolophus malayanus*, both from Yunnan, China.

Summary

In one aspect, the disclosure provides polypeptides comprising an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS: 1-84, 138-146, and 167-184, wherein X1 is absent or is an amino acid linker, and wherein residues in parentheses are optional and may be present or some or all of the optional residues may be absent. In various specific embodiments, the polypeptides comprise the amino acid sequence selected from the group consisting of SEQ ID NOS: 1-12 and 142-151, comprise the amino acid sequence selected from the group consisting of SEQ ID NOS: 1-8, or comprise the amino acid sequence selected from the group consisting of SEQ ID NOS: 1 or 5. In another embodiment, the disclosure provides nanoparticles comprising a plurality of such polypeptides.

In another aspect, the disclosure provides nanoparticles, comprising:

(a) a plurality of first assemblies, each first assembly comprising a plurality of identical first proteins; and,

(b) a plurality of second assemblies, each second assembly comprising a plurality of second proteins;

wherein the amino acid sequence of the first protein differs from the sequence of the second protein; wherein the plurality of first assemblies non-covalently interact with the plurality of second assemblies to form the nanoparticle; and wherein the nanoparticle displays on its surface an immunogenic portion of a SARS-CoV-2 antigen or a variant or homolog thereof, present in the at least one second protein. In one embodiment, the second proteins comprises an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS: 85-124 or 185-193, or consisting of SEQ ID NOS: 85-88, wherein X1 for at least one second protein comprises an immunogenic portion of a SARS-CoV-2 antigen or a variant or homolog thereof, X2 is absent or an amino acid linker, and residues in parentheses are optional. In another embodiment, X1 in at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the second proteins comprises an amino acid sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid sequence identity to a Spike (S) protein extracellular domain (ECD) amino acid sequence, an S1 subunit amino acid sequence, an S2 subunit amino acid sequence, an S1 receptor binding domain (RBD) amino acid sequence, and/or an N-terminal

domain (NTD) amino acid sequence, from SARS-CoV-2, or a variant or homolog thereof. In a further embodiment, X1 in at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the second proteins comprises an amino acid sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid sequence identity to the amino acid sequence selected from the group consisting of SEQ ID NO:125-137. In a further embodiment, the first protein comprises an amino acid sequence at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected the group consisting of SEQ ID NOS:152-159, wherein residues in parentheses are optional and may be present or some or all of the optional residues may be absent.

In various other aspects, the disclosure provides compositions comprising a plurality of nanoparticles disclosed herein, nucleic acid molecules, such as mRNA, encoding the polypeptide disclosed herein, expression vectors comprising the nucleic acid molecules disclosed herein operatively linked to a suitable control sequence, cells comprising the polypeptide, the nanoparticle, the composition, the nucleic acid, and/or the expression vector disclosed herein, and pharmaceutical compositions, kits, and vaccines comprising the polypeptide, the nanoparticle, the composition, the nucleic acid, the expression vector, and/or the cell disclosed herein.

In another aspect, the disclosure provides methods to treat or limit development of a SARS-CoV-2 infection, comprising administering to a subject in need thereof an amount effective to treat or limit development of the infection the polypeptide, nanoparticle, composition, nucleic acid, pharmaceutical composition, or vaccine disclosed herein.

Description of the Figures

Figure 1 (A-H). Design, *In Vitro* Assembly, and Characterization of SARS-CoV-2 RBD Nanoparticle Immunogens (A) Molecular surface representation of the SARS-CoV-2 S-2P trimer in the prefusion conformation (PDB 6VYB). Each protomer is colored distinctly, and N-linked glycans are rendered dark blue (the glycan at position N343 was modeled based on PDB 6WPS and the receptor-binding motif (RBM) was modeled from PDB 6M0J). The single open RBD is boxed. (B) Molecular surface representation of the SARS-CoV-2 S RBD, including the N-linked glycans at positions 331 and 343. The ACE2 receptor-binding site or RBM is indicated with a black outline. (C) Structural models of the trimeric RBD-I53-50A (RBD in light blue and I53-50A in light gray) and pentameric I53-50B (orange) components.

Upon mixing *in vitro*, 20 trimeric and 12 pentameric components assemble to form nanoparticle immunogens with icosahedral symmetry. Each nanoparticle displays 60 copies of the RBD. (D) Structural model of the RBD-12GS-I53-50 nanoparticle immunogen. Although a single orientation of the displayed RBD antigen and 12-residue linker are shown for simplicity, these regions are expected to be flexible relative to the I53-50 nanoparticle scaffold. (E) Dynamic light scattering (DLS) of the RBD-8GS-, RBD-12GS-, and RBD-16GS-I53-50 nanoparticles compared to unmodified I53-50 nanoparticles. (F) Representative electron micrographs of negatively stained RBD-8GS-, RBD-12GS-, and RBD-16GS-I53-50 nanoparticles. The samples were imaged after one freeze/thaw cycle. Scale bars, 100 nm. (G) Hydrogen/Deuterium-exchange mass spectrometry of monomeric RBD versus trimeric RBD-8GS-I53-50A component, represented here as a butterfly plot, confirms preservation of the RBD conformation, including at epitopes recognized by known neutralizing Abs. In the plot, each point along the horizontal sequence axis represents a peptide where deuterium uptake was monitored from 3 seconds to 20 hours. Error bars shown on the butterfly plot indicate standard deviations from two experimental replicates. The difference plot below demonstrates that monomeric RBD and RBD-8GS-I53-50A are virtually identical in local structural ordering across the RBD. (H) Pie charts summarizing the glycan populations present at the N-linked glycosylation sites N331 and N343 in five protein samples: monomeric RBD, S-2P trimer, and RBD-8GS-, RBD-12GS-, and RBD-16GS-I53-50A trimeric components. The majority of the complex glycans at both sites were fucosylated; minor populations of afucosylated glycans are set off by dashed lines. Oligo, oligomannose.

Figure 2 (A-B). Antigenic Characterization of SARS-CoV-2 RBD-I53-50

Nanoparticle Immunogens (A) Bio-layer interferometry of immobilized mACE2-Fc, CR3022 mAb, and S309 mAb binding to RBD-8GS-, RBD-12GS-, and RBD-16GS-I53-50 nanoparticles displaying the RBD antigen at 50% or 100% valency. The monomeric SARS-CoV-2 RBD was included in each experiment as a reference. (B) The binding signal at 880 s, near the end of the association phase, is plotted for each experiment in panel (A) to enable comparison of the binding signal obtained from each nanoparticle.

Figure 3 (A-E). Physical and Antigenic Stability of RBD Nanoparticle

Immunogens and S-2P Trimer (A) Chemical denaturation by guanidine hydrochloride. The ratio of intrinsic tryptophan fluorescence emission at 350/320 nm was used to monitor protein tertiary structure. Major transitions are indicated by shaded regions. Representative data from one of three independent experiments are shown. (B) Summary of SDS-PAGE and nsEM

stability data over four weeks. SDS-PAGE showed no detectable degradation in any sample. nsEM revealed substantial unfolding of the S-2P trimer at 2-8°C after three days incubation, and at 22-27°C after four weeks. N/A, not assessed. (C) Summary of antigenicity data over four weeks. The antigens were analyzed for mACE2-Fc (solid lines) and CR3022 mAb (dashed lines) binding by bio-layer interferometry after storage at the various temperatures. The plotted value represents the amplitude of the signal near the end of the association phase normalized to the corresponding <-70°C sample at each time point. (D) Summary of UV/vis stability data over four weeks. The ratio of absorbance at 320/280 nm is plotted as a measure of particulate scattering. Only the S-2P trimer and the RBD-12GS-I53-50 nanoparticle showed any increase in scattering, and only at ambient temperature. (E) DLS of the RBD-12GS-I53-50 nanoparticle indicated a monodisperse species with no detectable aggregate at all temperatures and time points. The data in panels B-E is from a four-week real-time stability study that was performed once.

Figure 4 (A-D). RBD-I53-50 Nanoparticle Immunogens Elicit Potent Antibody Responses in BALB/c and Human Immune Repertoire Mice (A-B) Post-prime (week 2) (A) and post-boost (week 5) (B) anti-S binding titers in BALB/c mice, measured by ELISA. Each symbol represents an individual animal, and the geometric mean from each group is indicated by a horizontal line. The dotted line represents the lower limit of detection of the assay. 8GS, RBD-8GS-I53-50; 12GS, RBD-12GS-I53-50; 16GS, RBD-16GS-I53-50; HCS, human convalescent sera. The inset depicts the study timeline. The immunization experiment was repeated twice and representative data are shown. (C-D) Post-prime (week 2) (C) and post-boost (week 5) (D) anti-S binding titers in Kymab Darwin™ mice, which are transgenic for the non-rearranged human antibody variable and constant region germline repertoire, measured by ELISA and plotted as in (A). The inset depicts the study timeline. The immunization experiment was performed once.

Figure 5 (A-H). RBD-I53-50 Nanoparticle Immunogens Elicit Potent and Protective Neutralizing Antibody Responses (A-B) Serum pseudovirus neutralizing titers post-prime (A) or post-boost (B) from mice immunized with monomeric RBD, S-2P trimer, or RBD-I53-50 nanoparticles. Each circle represents the reciprocal IC₅₀ of an individual animal. The geometric mean from each group is indicated by a horizontal line. Limit of detection shown as a gray dotted line. The animal experiment was performed twice, and representative data from duplicate measurements are shown. (C-D) Serum live virus neutralizing titers post-prime (C) or post-boost (D) from mice immunized as described in (A).

(E–F) Serum pseudovirus neutralizing titers from Kymab DarwinTM mice post-prime (E) and post-boost (F), immunized as described in (A). The animal experiment was performed once, and the neutralization assays were performed at least in duplicate. (G–H) Seven weeks post-boost, eight BALB/c mice per group were challenged with SARS-CoV-2 MA. Two days post-challenge, viral titers in lung tissue (G) and nasal turbinates (H) were assessed. Limit of detection depicted as a gray dotted line.

Figure 6 (A–J). RBD Nanoparticle Vaccines Elicit Robust B Cell Responses and Antibodies Targeting Multiple Epitopes in Mice and a Nonhuman Primate (A–B)

Number of (A) RBD+ B cells (B220+CD3–CD138–) and (B) RBD+ GC precursors and B cells (CD38+/-GL7+) detected across each immunization group. (C–D) Frequency of (C) RBD+ GC precursors and B cells (CD38+/-GL7+) and (D) IgD+, IgM+, or class-switched (IgM–IgD–; swIg+) RBD+ GC precursors and B cells. (A–D) N=6 across two experiments for each group. Statistical significance was determined by one-way ANOVA, and Tukey's multiple comparisons tests were performed for any group with a p-value less than 0.05. Significance is indicated with stars: * p < 0.05, **** p < 0.0001. (E) Ratio post-boost (week 5) of S-2P ELISA binding titer (Figure 4D) to pseudovirus neutralization titers (Figure 5F) in Kymab DarwinTM mice. The ratio is the [GMT (EC50) of five mice]:[the GMT (IC50) of five mice] or the EC50:IC50 of all HCS tested. A lower value signifies a higher quality response. (F) Ratio post-boost (week 5) of S-2P ELISA binding titer (Figure 4B) to either pseudovirus (Figure 5B) or live virus (Figure 5D) neutralization titers in BALB/c mice. The ratio is the [GMT (EC50) of ten mice]:[the GMT (IC50) of ten mice] or the EC50:IC50 of all HCS tested. (G) SARS-CoV-2 RBD with monomeric ACE2, CR3022 Fab, and S309 Fab bound. (H–J) Determination of vaccine-elicited Ab epitope specificity by competition BLI. A dilution series of polyclonal NHP Fabs was pre-incubated with RBD on the BLI tip. The polyclonal Fab concentration was maintained with the addition of competitor to each dilution point. The 1:3 dilution series of polyclonal Fabs is represented from dark to light, with a dark gray line representing competitor loaded to apo-RBD (no competition). Competition with (H) 200 nM ACE2, (I) 400 nM CR3022, or (J) 20 nM S309.

Figure 7 (A–E). Additional characterization of RBD Nanoparticle Immunogens.

(A) Size exclusion chromatography of RBD-I53-50 nanoparticles, unmodified I53-50 nanoparticle, and trimeric RBD-I53-50A components on a SuperoseTM 6 Increase 10/300 GL. (B) SDS-PAGE of SEC-purified RBD-I53-50 nanoparticles under reducing and non-reducing conditions before and after one freeze/thaw cycle. (C) Dynamic light scattering of RBD-I53-

50 nanoparticles before and after one freeze/thaw cycle indicates monodisperse nanoparticles with a lack of detectable aggregates in each sample. (D) Hydrogen/Deuterium-exchange mass spectrometry, represented here as heatmaps, reveals the structural accessibility and dynamics on RBD (PDB 6W41). Color codes indicate deuterium uptake levels. Monomeric RBD and RBD-8GS-I53-50A have indistinguishable uptake patterns, and are presented in a single heatmap at each time point. (E) Top, bar graphs reveal similar glycan profiles at the N-linked glycosylation sites N331 and N343 in five protein samples: monomeric RBD, S-2P trimer, and RBD-8GS-, RBD-12GS-, and RBD-16GS-I53-50A trimeric components. Bottom, comprehensive glycan profiling on other N-linked glycosylation sites besides N331 and N343 that are found in the S-2P trimer. The axis of each bar graph is scaled to 0-80%. M9 to M5, oligomannose with 9 to 5 mannose residues, are colored dark gray. Hybrid and FHybrid, hybrid types with or without fucosylation are gray. Subtypes in complex type, shown in light gray, are classified based on antennae number and fucosylation.

Figure 8 (A-B). Determination of hACE2 and CR3022 Fab Affinities by Bio-layer Interferometry. (A) Analysis of monomeric hACE2 binding to immobilized monomeric RBD and trimeric RBD-8GS-, RBD-12GS-, and RBD-16GS-I53-50A components. (B) Analysis of CR3022 Fab binding to immobilized monomeric RBD and trimeric RBD-8GS-, RBD-12GS-, and RBD-16GS-I53-50A components. Affinity constants (Table 5) were determined by global fitting of the kinetic data from six analyte concentrations to a 1:1 binding model.

Figure 9 (A-D). Characterization of Partial Valency RBD Nanoparticles (A) Representative electron micrographs of negatively stained RBD-8GS-, RBD-12GS-, and RBD-16GS-I53-50 nanoparticles displaying the RBD at 50% valency. The samples were imaged after one freeze/thaw cycle. Scale bars, 100 nm. (B) SDS-PAGE of purified RBD-8GS-, RBD-12GS-, and RBD-16GS-I53-50 nanoparticles displaying the RBD at 50% valency. Both RBD-bearing and unmodified I53-50A subunits are visible on the gels. (C) Dynamic light scattering (DLS) of 50% valency RBD-8GS-, RBD-12GS-, and RBD-16GS-I53-50 nanoparticles both before and after freeze/thaw. No aggregates or unassembled components were observed. (D) UV/vis absorption spectra of 50% valency RBD-8GS-, RBD-12GS-, and RBD-16GS-I53-50 nanoparticles. Turbidity in the samples is low, as indicated by the low absorbance at 320 nm.

Figure 10 (A-E). Day 28 Stability Data. (A) SDS-PAGE of purified monomeric RBD, S-2P trimer, RBD-I53-50A components and RBD-12GS-I53-50 nanoparticle in

reducing and non-reducing conditions. No degradation of any immunogen was observed after a four-week incubation at any temperature analyzed. (B) Analysis of mACE2-Fc and CR3022 IgG binding to monomeric RBD, RBD-I53-50A trimeric components, and RBD-12GS-I53-50 nanoparticle by BLI after a four-week incubation at three temperatures. Monomeric RBD was used as a reference standard in nanoparticle component and nanoparticle BLI experiments. The RBD-12GS-I53-50 nanoparticle lost minimal binding at the higher temperatures after four weeks; the remaining antigens did not lose any mACE2-Fc or CR3022 IgG binding over the course of the study. (C) UV/vis spectroscopy showed minimal absorbance in the near-UV, suggesting a lack of aggregation/particulates after a four week-incubation at three temperatures, with the exception of S-2P trimer, which gained significant absorbance around 320 nm at ambient temperature. RBD-12GS-I53-50 nanoparticle samples at 22-27°C at several earlier time points exhibited similar peaks near 320 nm (see Supplementary Item 2). (D) nsEM of RBD-12GS-I53-50 nanoparticle (top) and S-2P trimer (bottom) after a four-week incubation at three temperatures. Intact monodisperse nanoparticles were observed at all temperatures, with no observed degradation or aggregation. The S-2P trimer remained well folded in the <-70 and 22-27°C samples, but was unfolded in samples incubated at 2-8°C. Scale bars: RBD-12GS-I53-50, 100 nm; S-2P, 50 nm. (E) DLS of the RBD-12GS-I53-50 nanoparticle after a four-week incubation at three temperatures. No aggregation was observed at any temperature.

Figure 11. Subclasses of vaccine-elicited Abs and anti-scaffold antibody titers. Levels of vaccine-elicited IgG specific to the (top) trimeric I53-50A component, (middle) pentameric I53-50B component, and (bottom) assembled I53-50 nanoparticle two weeks post-prime (left) and post-boost (right) in BALB/c mice.

Figure 12 (A-D). B Cell Gating Strategy and Durability of the Vaccine-Elicited Immune Response. (A) Representative gating strategy for evaluating RBD-specific B cells, germinal center (GC) precursors and B cells (CD38+/-GL7+), and B cell isotypes. Top row, gating strategy for measuring numbers of live, non-doublet B cells. These cells were further analyzed as depicted in the middle and bottom rows. Middle row, representative data from a mouse immunized with the monomeric RBD formulated with AddaVax™, RBD+CD38+/-GL7+ cells that did not bind decoys were counted as antigen-specific GC precursors and B cells. Bottom row, representative data from a mouse immunized with the RBD-12GS-I53-50 nanoparticle formulated with AddaVax™. GC precursors and B cells were further analyzed to characterize B cell receptor isotypes. (B-C) Levels of (B) S-specific IgG and (C)

pseudovirus neutralization in sera collected 20 (RBD-16GS-I53-50) or 24 (monomeric RBD, S-2P, RBD-8GS-I53-50, and RBD-12GS-I53-50) weeks post-boost. Sera were collected from the two animals from each group that were not challenged with MA-SARS-CoV-2. (D)

Numbers of S-2P-specific Ab secreting cells in the bone marrow of BALB/c mice

- 5 immunized with either S-2P trimer or RBD-16GS-I53-50 nanoparticle, measured by ELISpot. Cells were harvested 17 weeks post-boost (see panel B inset). The animal experiment was performed once. Statistical significance was determined by two-tailed unpaired t test. *, p = 0.02.

10 Detailed Description

All references cited are herein incorporated by reference in their entirety. Within this application, unless otherwise stated, the techniques utilized may be found in any of several well-known references such as: Molecular Cloning: A Laboratory Manual (Sambrook, et al., 1989, Cold Spring Harbor Laboratory Press), Gene Expression Technology (Methods in

- 15 Enzymology, Vol. 185, edited by D. Goeddel, 1991. Academic Press, San Diego, CA), "Guide to Protein Purification" in Methods in Enzymology (M.P. Deutscher, ed., (1990) Academic Press, Inc.); PCR Protocols: A Guide to Methods and Applications (Innis, et al. 1990. Academic Press, San Diego, CA), Culture of Animal Cells: A Manual of Basic Technique, 2nd Ed. (R.I. Freshney. 1987. Liss, Inc. New York, NY), Gene Transfer and
- 20 Expression Protocols, pp. 109-128, ed. E.J. Murray, The Humana Press Inc., Clifton, N.J.), and the Ambion 1998 Catalog (Ambion, Austin, TX).

As used herein, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise.

As used herein, "about" means +/- 5% of the recited parameter.

- 25 As used herein, the amino acid residues are abbreviated as follows: alanine (Ala; A), asparagine (Asn; N), aspartic acid (Asp; D), arginine (Arg; R), cysteine (Cys; C), glutamic acid (Glu; E), glutamine (Gln; Q), glycine (Gly; G), histidine (His; H), isoleucine (Ile; I), leucine (Leu; L), lysine (Lys; K), methionine (Met; M), phenylalanine (Phe; F), proline (Pro; P), serine (Ser; S), threonine (Thr; T), tryptophan (Trp; W), tyrosine (Tyr; Y), and valine
- 30 (Val; V).

All embodiments of any aspect of the disclosure can be used in combination, unless the context clearly dictates otherwise.

Unless the context clearly requires otherwise, throughout the description and the claims, the words ‘comprise’, ‘comprising’, and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of “including, but not limited to”. Words using the singular or plural number also include the plural and singular number, respectively. Additionally, the words “herein,” “above,” and “below” and words of similar import, when used in this application, shall refer to this application as a whole and not to any particular portions of the application.

In a first aspect, the disclosure provides polypeptides comprising an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS: 1-84, 138-146, and 167-184, wherein X1 is absent or is an amino acid linker, and wherein residues in parentheses are optional and may be present or some or all of the optional residues may be absent.

As shown in the examples that follow, the polypeptides of this aspect can be used to generated self-assembling protein nanoparticle immunogens that elicit potent and protective antibody responses against SARS-CoV-2. The nanoparticle vaccines induce neutralizing antibody titers roughly ten-fold higher than the prefusion-stabilized S ectodomain trimer despite a more than five-fold lower dose. Antibodies elicited by the nanoparticle immunogens target multiple distinct epitopes, suggesting that they may not be easily susceptible to escape mutations, and exhibit a significantly lower binding:neutralizing ratio than convalescent human sera, which may minimize the risk of vaccine-associated enhanced respiratory disease.

The amino acid sequence of exemplary polypeptides of this aspect of the disclosure are provided below.

Table 1

Protein name	Protein type	Expressed sequence (optional residues in parentheses; X1 is optional linker)
SARS-CoV-2 RBD-153-50A-16GS-he-Ris	SARS-CoV-2-153-50A fusion protein	RFPNITNLCFFGSEVFNATRFASVYAWNRRKRIISNCVADYSVLYNSASFSTFKCYGVSPTEK LNDLCFTNVIYADSFVIRGDEVQRQIAPGQTGKIADYNKLPDDFTGCVIAWNSNNLDSKV GGNINYLRLFRHSNLSKPFERDISTETIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVG YQPYRVVVLSEFELLHAPATVCSFKKSTGGSGGSGSGSGSGSGSEKAAKAEAAARKMEEL FKKKHIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIRKLSVLKEKGAI GAGTVTSVEQARKAVESGAEFIVSEHLDEEISQFAKEKGVFYMPGVMTFTLVKAMKLG HTILKLPFGSEVVGPEFVKAMEGPFPPNVKFPVPTGGVNLDNVAENFKAGVLAVGVGSALVK GTPDEVREKAKAFVEKIRGATE (SEQ ID NO:1) (mgilpspgmpallsivslslvllmgcva)RFPNITNLCFFGSEVFNATRFASVYAWNRRK RIISNCVADYSVLYNSASFSTFKCYGVSPTEKLNLCFTNVIYADSFVIRGDEVQRQIAPGQT GKIADYNKLPDDFTGCVIAWNSNNLDSKVGGNINYLRLFRHSNLSKPFERDISTETIYQ

	AGSTPCNGVEGFNCYFPLQSYGFGQPTNGVGYQPYRVVLSFELLHAPATVCGPKKST (G GSGGSGSGGSGSGSGSEKAAKAEAAAR) KMEELFKKKHIVAVLRANSVEEAIEKAVAVFA GGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTSTSVEQARKAVESGAEFIVSPHL DEEISQFAKEKGVFYMFGVMTPTLVKAMKLGHTILKLFPGGEVVGPPQFVKAMKGFFPNV KFVPTGGVNLNDVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATE (SEQ ID NO:2) RFPNITNLCPPGGEVFNATRFASVYAWNRKRISNCVADYSVLVNSASFSTFKCYGVSPTK LNDLCFTNVIADSFVIRGDEVQRQIAPGQTGKIADYNYKLDDFTGCVIAWNSNNLDSKV GGNYNYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCYFPLQSYGFGQPTNGV QPYRVVLSFELLHAPATVCGPKKST (X1) KMEELFKKKHIVAVLRANSVEEAIEKA VAVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTSTSVEQARKAVESGAEFI VSPHLDEEISQFAKEKGVFYMFGVMTPTLVKAMKLGHTILKLFPGGEVVGPPQFVKAMKG FFPNVKFVPTGGVNLNDVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATE (SEQ ID NO:3) (mgilpspgmpallslvsllelimgcva) RFPNITNLCPPGGEVFNATRFASVYAWNRK RISNCVADYSVLVNSASFSTFKCYGVSPTKLNLCFTNVIADSFVIRGDEVQRQIAPGQT GKIADYNYKLDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFFERDISTEIQ AGSTPCNGVEGFNCYFPLQSYGFGQPTNGVGYQPYRVVLSFELLHAPATVCGPKKST (X 1) KMEELFKKKHIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSVL KEKGAIIGAGTSTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMFGVMTPTLV KAMKLGHTILKLFPGGEVVGPPQFVKAMKGFFPNVKFVPTGGVNLNDVAEWFKAGVLAVG VGSALVKGTPDEVREKAKAFVEKIRGATE (SEQ ID NO:4) ETGTRFPNITNLCPPGGEVFNATRFASVYAWNRKRISNCVADYSVLVNSASFSTFKCYGV SPTKLNLCFTNVIADSFVIRGDEVQRQIAPGQTGKIADYNYKLDDFTGCVIAWNSNNL DSKVGGNYNYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCYFPLQSYGFGQPT NGVGYQPYRVVLSFELLHAPATVCGPKKST (GSGGSGSGGSGSGSGSEKAAKAEAAAR) KMEELFKKKHIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSVL KEKGAIIGAGTSTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMFGVMTPTLV KAMKLGHTILKLFPGGEVVGPPQFVKAMKGFFPNVKFVPTGGVNLNDVAEWFKAGVLAVG VGSALVKGTPDEVREKAKAFVEKIRGATE (SEQ ID NO:5) (mgilpspgmpallslvsllelimgcvaetgt) RFPNITNLCPPGGEVFNATRFASVYA WNRKRISNCVADYSVLVNSASFSTFKCYGVSPTKLNLCFTNVIADSFVIRGDEVQRQIA PGQTGKIADYNYKLDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFFERDIST EIQAGSTPCNGVEGFNCYFPLQSYGFGQPTNGVGYQPYRVVLSFELLHAPATVCGPKK ST (GSGGSGSGGSGSGSGSEKAAKAEAAAR) KMEELFKKKHIVAVLRANSVEEAIEKAV AVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTSTSVEQARKAVESGAEFIV SPHLDEEISQFAKEKGVFYMFGVMTPTLVKAMKLGHTILKLFPGGEVVGPPQFVKAMKG FFPNVKFVPTGGVNLNDVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATE (SEQ ID NO:6) ETGTRFPNITNLCPPGGEVFNATRFASVYAWNRKRISNCVADYSVLVNSASFSTFKCYGV SPTKLNLCFTNVIADSFVIRGDEVQRQIAPGQTGKIADYNYKLDDFTGCVIAWNSNNL DSKVGGNYNYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCYFPLQSYGFGQPT NGVGYQPYRVVLSFELLHAPATVCGPKKST (X1) KMEELFKKKHIVAVLRANSVEEAIE KAVAVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTSTSVEQARKAVESGA EFIVSPHLDEEISQFAKEKGVFYMFGVMTPTLVKAMKLGHTILKLFPGGEVVGPPQFVK AMKGFFPNVKFVPTGGVNLNDVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRG ATE (SEQ ID NO:7) mgilpspgmpallslvsllelimgcvaetgt) RFPNITNLCPPGGEVFNATRFASVYAW NRKRISNCVADYSVLVNSASFSTFKCYGVSPTKLNLCFTNVIADSFVIRGDEVQRQIA PGQTGKIADYNYKLDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFFERDISTE IQAGSTPCNGVEGFNCYFPLQSYGFGQPTNGVGYQPYRVVLSFELLHAPATVCGPKKS T (GSGGSGSGGSGSGSGSEKAAKAEAAAR) KMEELFKKKHIVAVLRANSVEEAIEKAVA VFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTSTSVEQARKAVESGAEFIVS
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		PHLDEEISQFAKEKGVFYMFGVMTPTTELVKAMKLGHTILKLFPGGEVVGPFQFVKAMKGF FNVKFPVPTGGVNLNDNAEWFKAGVLAVGVGSALVKGTFDEVREKAKAFVEKIRGATE ((SEQ ID NO:8) (mgilpspgmpallslvslsllimgcvaetgt)RFPNITNLCPPGEVFNATRFASVYA WNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIA PGQTGKIADYNKLPDDFTGCVIAWNSNNLDSKVGGNYNLYRLFRKSNLKPFERDIST EIIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKK ST (GGSGGSGSGSGSGSGSEKAKEAAR) KMEELFKKKHIVAVLRANSVEEAIEKAV AVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGACTVTSVEQARKAVESGAEFIV SPHLDEEISQFAKEKGVFYMFGVMTPTTELVKAMKLGHTILKLFPGGEVVGPFQFVKAMKGF FNVKFPVPTGGVNLNDNAEWFKAGVLAVGVGSALVKGTFDEVREKAKAFVEKIRGATE ((GGSHHHHHHH) (SEQ ID NO:9) ETGTRFPNITNLCPPGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGV SPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNKLPDDFTGCVIAWNSNNL DSKVGGNYNLYRLFRKSNLKPFERDISTEIIYQAGSTPCNGVEGFNCYFPLQSYGFQPT NGVGYQPYRVVLSFELLHAPATVCGPKKST (GGSGGSGSGSGSGSGSEKAKEAAR) KMEELFKKKHIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSVL KEKGAIIGACTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMFGVMTPTTEL VKAMKLGHTILKLFPGGEVVGPFQFVKAMKGF FNVKFPVPTGGVNLNDNAEWFKAGVLAVGV GSALVKGTFDEVREKAKAFVEKIRGATE (GGSHHHHHHH) (SEQ ID NO:10) (mgilpspgmpallslvslsllimgcvaetgt)RFPNITNLCPPGEVFNATRFASVYA WNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIA PGQTGKIADYNKLPDDFTGCVIAWNSNNLDSKVGGNYNLYRLFRKSNLKPFERDIST EIIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKK ST (X1) KMEELFKKKHIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKAL SVLKEKGAIIGACTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMFGVMT PTTELVKAMKLGHTILKLFPGGEVVGPFQFVKAMKGF FNVKFPVPTGGVNLNDNAEWFKAGV LAVGVGSALVKGTFDEVREKAKAFVEKIRGATE (GGSHHHHHHH) (SEQ ID NO:11---- ETGTRFPNITNLCPPGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGV SPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNKLPDDFTGCVIAWNSNNL DSKVGGNYNLYRLFRKSNLKPFERDISTEIIYQAGSTPCNGVEGFNCYFPLQSYGFQPT NGVGYQPYRVVLSFELLHAPATVCGPKKST (X1) KMEELFKKKHIVAVLRANSVEEAIE KAVAVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGACTVTSVEQARKAVESGA EFIVSPHLDEEISQFAKEKGVFYMFGVMTPTTELVKAMKLGHTILKLFPGGEVVGPFQFVK AMKGF FNVKFPVPTGGVNLNDNAEWFKAGVLAVGVGSALVKGTFDEVREKAKAFVEKIRG ATE (GGSHHHHHHH) (SEQ ID NO:12---
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SARS- CoV-2 RBD~ 153~ 50A*~ 8GS- he~ Nls	SARS- CoV-2- 153-50A fusion protein	RFPNITNLCPPGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTK LNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNKLPDDFTGCVIAWNSNNLDSK VGNNYNLYRLFRKSNLKPFERDISTEIIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGV GYQPYRVVLSFELLHAPATVCGPKKSTGGSGGSGSGSEKAKEAAR KMEELFKKKHIVA VLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGACTVTSV EQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMFGVMTPTTELVKAMKLGHTILKLF GEVVGPFQFVKAMKGF FNVKFPVPTGGVNLNDNAEWFKAGVLAVGVGSALVKGTFDEVRE KAKAFVEKIRGATE SEQ ID NO:13) (mgilpspgmpallslvslsllimgcvaetgt)RFPNITNLCPPGEVFNATRFASVYA WNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIA PGQTGKIADYNKLPDDFTGCVIAWNSNNLDSKVGNNYNLYRLFRKSNLKPFERDIST EIIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKK STGGSGGSGSGSEKAKEAAR KMEELFKKKHIVAVLRANSVEEAIEKAVAVFAGGVHLI EITFTVPDADTVIKALSVLKEKGAIIGACTVTSVEQARKAVESGAEFIVSPHLDEEISQ FAKEKGVFYMFGVMTPTTELVKAMKLGHTILKLFPGGEVVGPFQFVKAMKGF FNVKFPVPTG
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		GVNLDNVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATE (GGSNNHHHHHHH 8) (SEQ ID NO:14) ETGTRFFNITNLCFFGEVFNATRFASVYAWNRRKRISNCVADYSVLYNSASFSTFKCYGV SPTKLNLCFTNVIYADSFVIRGDEVQRQIAPGQTGKIADYNKLPDDFTGCVIWNNSNNL DSKVGGNYYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQPT NGVGYQPYRVVLSFELLHAPATVCGPKKSTGGSGGSGSEKAAKAEAAKMEELFKKH KIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGT VTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMFGVMTPTTELVKAMKLGHITIL KLFPGEVVVGPFVKAMKGPFPNVKRVFTGGVNLNDNVAEWFKAGVLAVGVGSALVKGTPD EVREKAKAFVEKIRGATE (SEQ ID NO:15) ETGTRFFNITNLCFFGEVFNATRFASVYAWNRRKRISNCVADYSVLYNSASFSTFKCYGV SPTKLNLCFTNVIYADSFVIRGDEVQRQIAPGQTGKIADYNKLPDDFTGCVIWNNSNNL DSKVGGNYYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQPT NGVGYQPYRVVLSFELLHAPATVCGPKKSTGGSGGSGSEKAAKAEAAKMEELFKKH KIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGT VTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMFGVMTPTTELVKAMKLGHITIL KLFPGEVVVGPFVKAMKGPFPNVKRVFTGGVNLNDNVAEWFKAGVLAVGVGSALVKGTPD EVREKAKAFVEKIRGATE (GGSNNHHHHHHH) (SEQ ID NO:16) RFPNITNLCFFGEVFNATRFASVYAWNRRKRISNCVADYSVLYNSASFSTFKCYGVSPTEK LNDLCFTNVIYADSFVIRGDEVQRQIAPGQTGKIADYNKLPDDFTGCVIWNNSNNLDSKV GGNYYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVG YQPYRVVLSFELLHAPATVCGPKKST () KMEELFKKHKIVAVLRANSVEEAIEKAVAV FAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSGP HLDEEISQFAKEKGVFYMFGVMTPTTELVKAMKLGHITILKLFPGEVVVGPFVKAMKGPFP NVKRVFTGGVNLNDNVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATE (SEQ ID NO:17) (mgilpspgmpallsllsvllmgsaetgt)RFPNITNLCFFGEVFNATRFASVYA WNRKRISNCVADYSVLYNSASFSTFKCYGVSPTEKLNLCFTNVIYADSFVIRGDEVQRQI A PGQTGKIADYNKLPDDFTGCVIWNNSNNLDSKVGGNYYLYRLFRKSNLKPFFERDIST EIQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKK ST (X1) KMEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKA LSVLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMFGVMT PTELVKAMKLGHITILKLFPGEVVVGPFVKAMKGPFPNVKRVFTGGVNLNDNVAEWFKAGV LAVGVGSALVKGTPDEVREKAKAFVEKIRGATE (GGSNNHHHHHHH) (SEQ ID NO:18) ETGTRFFNITNLCFFGEVFNATRFASVYAWNRRKRISNCVADYSVLYNSASFSTFKCYGV SPTKLNLCFTNVIYADSFVIRGDEVQRQIAPGQTGKIADYNKLPDDFTGCVIWNNSNNL DSKVGGNYYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQPT NGVGYQPYRVVLSFELLHAPATVCGPKKST (X1) KMEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSV LKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMFGVMTPTTE LVKAMKLGHITILKLFPGEVVVGPFVKAMKGPFPNVKRVFTGGVNLNDNVAEWFKAGVLAV GVGSAALVKGTPDEVREKAKAFVEKIRGATE (SEQ ID NO:19) ETGTRFFNITNLCFFGEVFNATRFASVYAWNRRKRISNCVADYSVLYNSASFSTFKCYGV SPTKLNLCFTNVIYADSFVIRGDEVQRQIAPGQTGKIADYNKLPDDFTGCVIWNNSNNL DSKVGGNYYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQPT NGVGYQPYRVVLSFELLHAPATVCGPKKST (X1) KMEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSV LKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMFGVMTPTTE LVKAMKLGHITILKLFPGEVVVGPFVKAMKGPFPNVKRVFTGGVNLNDNVAEWFKAGVLAV GVGSAALVKGTPDEVREKAKAFVEKIRGATE (GGSNNHHHHHHH) (SEQ ID NO:20)
SARS-CoV-2	SARS-CoV-2-	RFPNITNLCFFGEVFNATRFASVYAWNRRKRISNCVADYSVLYNSASFSTFKCYGVSPTEK LNDLCFTNVIYADSFVIRGDEVQRQIAPGQTGKIADYNKLPDDFTGCVIWNNSNNLDSKV

RBD- 153- 50A*- 12GS- he- His	153-50A fusion protein	GGNYNYLYRLFRKSNLKPFERDISTEIQAGSTPCNGVEGFNCYFFLQSYGFGPTNGVG YQPYRVVVLSPFELLHAPATVCGPKKSTGSGSGSGSGSGSEKAAKAEAAARKMEELFKKH KIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGT VTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMPGVMTPTTELVKAMKLGHTIL KLFPGEVVGQFVKAMKGPFNVKFFVPTGGVNLNDNVAEWFKAGVLAVGVGSALVKGTPE EVREKAKAFVEKIRGATE (SEQ ID NO:21) (mgilpspgmpallsylsvllsllmgcvaetgt)RFPNITNLCFFGEVFNATRFASVYA WNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVIYADSFVIRGDEVVRQIA PGQTGKIADYNYKLPPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDIST EIQAGSTPCNGVEGFNCYFFLQSYGFGPTNGVGYPYRVVVLSPFELLHAPATVCGPKK STGSGSGSGSGSGSEKAAKAEAAARKMEELFKKHKIVAVLRANSVEEAIEKAVAVFAGG VHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDE EISQFAKEKGVFYMPGVMTPTTELVKAMKLGHTILKLFPGEVVGQFVKAMKGPFNVKFF VPTGGVNLNDNVAEWFKAGVLAVGVGSALVKGTPEDEVREKAKAFVEKIRGATE (GGSHHH HHHH) (SEQ ID NO:22) ETGTRFPNITNLCFFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGV SPTKLNDLCFTNVIYADSFVIRGDEVVRQIAPGQTGKIADYNYKLPPDDFTGCVIAWNSNNL DSKVGGNYNYLYRLFRKSNLKPFERDISTEIQAGSTPCNGVEGFNCYFFLQSYGFGPT NGVGYPYRVVVLSPFELLHAPATVCGPKKSTGSGSGSGSGSGSEKAAKAEAAARKMEEL FKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIG AGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMPGVMTPTTELVKAMKLG HTILKLFPGEVVGQFVKAMKGPFNVKFFVPTGGVNLNDNVAEWFKAGVLAVGVGSALVK GTPDEVREKAKAFVEKIRGATE (SEQ ID NO:23) ETGTRFPNITNLCFFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGV SPTKLNDLCFTNVIYADSFVIRGDEVVRQIAPGQTGKIADYNYKLPPDDFTGCVIAWNSNNL DSKVGGNYNYLYRLFRKSNLKPFERDISTEIQAGSTPCNGVEGFNCYFFLQSYGFGPT NGVGYPYRVVVLSPFELLHAPATVCGPKKSTGSGSGSGSGSGSEKAAKAEAAARKMEEL FKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIG AGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMPGVMTPTTELVKAMKLG HTILKLFPGEVVGQFVKAMKGPFNVKFFVPTGGVNLNDNVAEWFKAGVLAVGVGSALVK GTPDEVREKAKAFVEKIRGATE (GGSHHHHHHHH) (SEQ ID NO:24) RFPNITNLCFFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTK LNDLCFTNVIYADSFVIRGDEVVRQIAPGQTGKIADYNYKLPPDDFTGCVIAWNSNNLDSKV GGNYNYLYRLFRKSNLKPFERDISTEIQAGSTPCNGVEGFNCYFFLQSYGFGPTNGVG YQPYRVVVLSPFELLHAPATVCGPKKST (X1) KMEELFKKHKIVAVLRANSVEEAIEKAV AVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQARKAVESGAEFIV SPHLDEEISQFAKEKGVFYMPGVMTPTTELVKAMKLGHTILKLFPGEVVGQFVKAMKG PFNVKFFVPTGGVNLNDNVAEWFKAGVLAVGVGSALVKGTPEDEVREKAKAFVEKIRGATE (SEQ ID NO:25) (mgilpspgmpallsylsvllsllmgcvaetgt)RFPNITNLCFFGEVFNATRFASVYA WNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVIYADSFVIRGDEVVRQIA PGQTGKIADYNYKLPPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDIST EIQAGSTPCNGVEGFNCYFFLQSYGFGPTNGVGYPYRVVVLSPFELLHAPATVCGPKK ST (X1) KMEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKA LSVLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMPGVMT PTTELVKAMKLGHTILKLFPGEVVGQFVKAMKGPFNVKFFVPTGGVNLNDNVAEWFKAGV LAVGVGSALVKGTPEDEVREKAKAFVEKIRGATE (GGSHHHHHHHH) (SEQ ID NO:26) ETGTRFPNITNLCFFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGV SPTKLNDLCFTNVIYADSFVIRGDEVVRQIAPGQTGKIADYNYKLPPDDFTGCVIAWNSNNL DSKVGGNYNYLYRLFRKSNLKPFERDISTEIQAGSTPCNGVEGFNCYFFLQSYGFGPT NGVGYPYRVVVLSPFELLHAPATVCGPKKST (X1) KMEELFKKHKIVAVLRANSVEEAIEKAV AVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMPGVMTPTTE
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		LVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFFNVKFFVPTGGVNLDNVAEWFKAGVLAV GVGSALVKGTPDEVREKAKAFVEKIRGATE (SEQ ID NO:27) ETGTRFPNITNLCPFGSEVFNATRFASVYAWNKKRISNCVADYSVLYNSASFSTFKCYGV SPTKLNLDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNKLPDDFTGCVIAWNSNNL DSKVGGNYYNYLRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCFYFELQSYGFPQT NGVGYQFYRVVLSFELLHAPATVCGPKKST (X1) KMERLFPKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTTVPDADTVIKALSV LKEKGAIIGACTVTSVRQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMFGVMTPTTE LVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFFNVKFFVPTGGVNLDNVAEWFKAGVLAV GVGSALVKGTPDEVREKAKAFVEKIRGATE (GGSHHHHHHHH) (SEQ ID NO:28)
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SARS- CoV-2 2PSEA G-S- TEV- FO- I53- 50A*- 12GS- he- His	SARS- CoV-2- I53-50A fusion protein	QCVNLTTRTQLPPAYTNSFTRGVVYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSG TNGTKRFDFNPVLPFNDGVYFASTEKSNIRGWIFGTTLDSTQSLIIVNNATNVVIVKVC EFQFCNDFFLGVYYHKNNESWMESEFRVYSSANNCTFEYVSQPFLLMDLEGKQGNFKNLR EFVFKNIDGYFKIYKSKHTPINLVRDLPGQFSALEPLVDLPICINITRFQTLALHRSYL TPGDSSSGWTAGAAAAYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLRSTFV EKGIYQTSNFRVQPTESIVRFPNITNLCPFGSEVFNATRFASVYAWNKKRISNCVADYSV LYNSASFSTFKCYGVSPKLNLDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNKLP DDFTGCVIAWNSNNLDSKVGGNYYNYLRLFRKSNLKPFFERDISTEIQAGSTPCNGVEG FNCYFELQSYGFPQTNGVGYQFYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNMFNFN GLTGTGVLTESNKKFLPFQFGRDIADTTDAVRDPQTLRIIDITPCSFGGVSVITPGTN TSNQVAVLYQDVNCTEVPVAIHADQLPTWRVYSTGNSVFTQTRAGCLIGAETHVNSYEC DIPICAGICASYQTQTNPSGAGSVASQSI IAYTMSLGAENSVAYSNNNSIAIPNFTTIS VTTEILPVSMTKTSVDCTMYICGDS TECSNLLQYGSFCTQLNRLTGI AVEQDKNTQE VFAQVKQIYKTPFIKDFGGFNFPSQILPDPSPKSKRSFIEDLLFNKVTLADAGFIKQYGD CLGDIAARDLICAKRFNGLTVLPPLLTDEMIACQYTSALLAGTITSGWTFGAGAALQIPF AMQMAYRFNGIGVTVQNVLYENQKLIANQFNSAIGKIQDSLSTASALGKLQDVVNQNAQ ALNTLVKQLSSNFGAISSVLNDILSRLDPPAEVQIDRLITGRLQSLQTYVTQQLIRAA EIRASANLAATKMSSECVLGQSKRVDFCGKGYHLMSPQSAFPGVFLHVTVYVPAQEKNN TTAPAICHGDKAHFPRFEGVFSNGTHWFTQRFNYEPQITTDNTFVSGNCDVVIQIYV NTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDISGINASVNIQKELDRLNEVAKNL NESLIDLQELGKYEQYIKsggrenlyfqqgggsgyipeaprdggayvrkdgewllstf lgGSGSGSGSGSGSEKAAKAEAAARKMEELFKKKHKIVAVLRANSVEEAIEKAVAVFAGG VHLIEITFTTVPDADTVIKALSVLKEKGAIIGACTVTSVRQARKAVESGAEFIVSPHLDE EISQFAKEKGVFYMFGVMTPTTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFFNVKFF VPTGGVNLDNVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATE SEQ ID NO:29) (mgilpspgmpallslvslslsvllmgcvaetgt) QCVNLTTRTQLPPAYTNSFTRGVVY PDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDFNPVLPFNDGVYFASTEK SNIRGWIFGTTLDSTQSLIIVNNATNVVIVKVC EFQFCNDFFLGVYYHKNNESWMESE FRVYSSANNCTFEYVSQPFLLMDLEGKQGNFKNLREFVFKNIDGYFKIYKSKHTPINLVRD LPQGFSALEPLVDLPICINITRFQTLALHRSYLTPGDSSSGWTAGAAAAYVGYLQPRTF FLKYNENGTITDAVDCALDPLSETKCTLRSTFVEKGIYQTSNFRVQPTESIVRFPNIT NLCPFGSEVFNATRFASVYAWNKKRISNCVADYSVLYNSASFSTFKCYGVSPKLNLDLCF TNVYADSFVIRGDEVQRQIAPGQTGKIADYNKLPDDFTGCVIAWNSNNLDSKVGGNYY LYLRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCFYFELQSYGFPQTPNGVGYQFYRV VLSFELLHAPATVCGPKKSTNLVKNKCVNMFNFNGLTGTGVLTESNKKFLPFQFGRDI ADTTDAVRDPQTLRIIDITPCSFGGVSVITPGTN TSNQVAVLYQDVNCTEVPVAIHADQ LPTWRVYSTGNSVFTQTRAGCLIGAETHVNSYEC DIPICAGICASYQTQTNPSGAGSV ASQSI IAYTMSLGAENSVAYSNNNSIAIPNFTTISVTTEILPVSMTKTSVDCTMYICGDS TECSNLLQYGSFCTQLNRLTGI AVEQDKNTQE VFAQVKQIYKTPFIKDFGGFNFPSQI LPDPSPKSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLICAKRFNGLTVLPPL LTDEMIACQYTSALLAGTITSGWTFGAGAALQIPFAMQMAYRFNGIGVTVQNVLYENQKLI ANQFNSAIGKIQDSLSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILS RLDPPAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSSECVLGQSKRVDF FCGKGYHLMSPQSAFPGVFLHVTVYVPAQEKNNFTTAPAICHGDKAHFPRFEGVFSNGT
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	HWFVTRQNFYEPQIITTDNTFVSGNCDVVGIVNNTVYDPLQPELDSFKEELDHYFKNH TSPDVLGDISGINASVNNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKsgrenl yfqqgggsgyipeaprdgqayvrkdgeawl1stflgSGSGSGSGSGSGSEKAAKAEAAAR KMEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSVLKE KGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMFGVMTPTLVK AMKLGHTILKLPFGVVGQFVKAMKGPPNVKVFVFTGGVNLDNVAENFFKAGVLAVGVG SALVKGTFDEVREKAKAFVEKIRGATE(GGSRHHHHHHH) (SEQ ID NO:30) ETGTQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAI HVSGTNGTKRFDNVLFPFNDGVYFPASTEKSNIRGWIFGTTLDSTKQSLILVNNATNVV IKVCEFGFCNDPFLGVYHKNNKSWMESEFRVYSSANNCTFEYVSQPFMLDLEGKQGNF KNLREPFVKNIIDGYFKIYSKHTPINLVRDLPGQFSALRPLVDLPIGINITRFQTLALH RSYLTFGDSSSGWTAGAAAYVGYLQPTFLLKYNENGTITDAVDCALDPLSTFKCTLK SFTVERGIYQTSNFRVQPTESIVRFPNITNLCPPFGEVFNATRFASVYAWNRKRISNCVA DYSVLVNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQAFGQTGKIADYN YKLDDFTGCVIANNMNLDSKVGNYLYLPLFRKSNLKFPERDISTEIIYQAGSTPCN GVEGFNOCYFPLQSYGQFTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVN FWFNGLTGTGVLTESNKKFLPQQFGRIADTTDAVRDPQTLEILDITPCSFQGVSVIT EGTNTSNQVAVLYQDVNCTEVFVAIHADQLTFTWRVYSTGSNVFQTRAGCLIGAEHVNN SYECDIPIGAGICASYQTQTNSEFSGAGSVASQSI IAYTMSLGAENSVAYSNNSIAIPEN FTISVTEILPVSMTKESVDCTMYICGDESTECNLLQYGSFCTQLNRALTGIAVEQDK NTQEVFAQVKQIYKTFPIKDFGGFNFSQILPDPSKESKRSFIEDLLFNKVTLLADAGFIK QYGDCLGDI AARDLICAQKFNGLTVLFPLLTDEMIAQYTSALLAGTITSGWTFGAGAAL QIPFAMQMAYRFNGIGVTVQNVLYENQKLIANQFNSAIGKIQDSLSTASALGKLQDVVN QNAQALNTLVKQLSSNFGAIESVNLNLSRLDPPEAEVQIDRLITGRQLSLQTYVTQQL IRAAEIPRASANLAATKMESECVLGQSKRVDFCGRGYHLSMFPQSAPHGVSFLHVTYVPAQ EKNFTTAPAICHGDKARFPREGVFSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVI GIVNNTVYDPLQPELDSFKEELDHYFKNHTSPDVLGDISGINASVNNIQKEIDRLNEV AKNLNESLIDLQELGKYEQYIKsgrenlyfqqgggsgyipeaprdgqayvrkdgeawl 1stflgSGSGSGSGSGSGSEKAAKAEAAARKMEELFKKHKIVAVLRANSVEEAIEKAVAV FAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSP HLDEEISQFAKEKGVFYMFGVMTPTLVKAMKLGHTILKLPFGVVGQFVKAMKGPP NVKVFVFTGGVNLDNVAENFFKAGVLAVGVGSALVKGTFDEVREKAKAFVEKIRGATE (SEQ ID NO:31) ETGTQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAI HVSGTNGTKRFDNVLFPFNDGVYFPASTEKSNIRGWIFGTTLDSTKQSLILVNNATNVV IKVCEFGFCNDPFLGVYHKNNKSWMESEFRVYSSANNCTFEYVSQPFMLDLEGKQGNF KNLREPFVKNIIDGYFKIYSKHTPINLVRDLPGQFSALRPLVDLPIGINITRFQTLALH RSYLTFGDSSSGWTAGAAAYVGYLQPTFLLKYNENGTITDAVDCALDPLSTFKCTLK SFTVERGIYQTSNFRVQPTESIVRFPNITNLCPPFGEVFNATRFASVYAWNRKRISNCVA DYSVLVNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQAFGQTGKIADYN YKLDDFTGCVIANNMNLDSKVGNYLYLPLFRKSNLKFPERDISTEIIYQAGSTPCN GVEGFNOCYFPLQSYGQFTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVN FWFNGLTGTGVLTESNKKFLPQQFGRIADTTDAVRDPQTLEILDITPCSFQGVSVIT EGTNTSNQVAVLYQDVNCTEVFVAIHADQLTFTWRVYSTGSNVFQTRAGCLIGAEHVNN SYECDIPIGAGICASYQTQTNSEFSGAGSVASQSI IAYTMSLGAENSVAYSNNSIAIPEN FTISVTEILPVSMTKESVDCTMYICGDESTECNLLQYGSFCTQLNRALTGIAVEQDK NTQEVFAQVKQIYKTFPIKDFGGFNFSQILPDPSKESKRSFIEDLLFNKVTLLADAGFIK QYGDCLGDI AARDLICAQKFNGLTVLFPLLTDEMIAQYTSALLAGTITSGWTFGAGAAL QIPFAMQMAYRFNGIGVTVQNVLYENQKLIANQFNSAIGKIQDSLSTASALGKLQDVVN QNAQALNTLVKQLSSNFGAIESVNLNLSRLDPPEAEVQIDRLITGRQLSLQTYVTQQL IRAAEIPRASANLAATKMESECVLGQSKRVDFCGRGYHLSMFPQSAPHGVSFLHVTYVPAQ EKNFTTAPAICHGDKARFPREGVFSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVI GIVNNTVYDPLQPELDSFKEELDHYFKNHTSPDVLGDISGINASVNNIQKEIDRLNEV AKNLNESLIDLQELGKYEQYIKsgrenlyfqqgggsgyipeaprdgqayvrkdgeawl 1stflgSGSGSGSGSGSGSEKAAKAEAAARKMEELFKKHKIVAVLRANSVEEAIEKAVAV FAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSP HLDEEISQFAKEKGVFYMFGVMTPTLVKAMKLGHTILKLPFGVVGQFVKAMKGPP
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	NVHFVPTGGVNLNDVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATE (GG SHHHHHHHH) (SEQ ID NO:32) QCVNLTRRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTFWFAIHVSG TNGTKRFDNFVLPFNDGVYFASTKSNIRGWIFGTTLDSKTQSLLIVNNATNVVIVKVC EFQFCNDFFLGVIYHKNKSNWMESEFRVYSSANNCTFEYVSQPFMLDLEGKQGNFNKLR RFVFKNIDGYFKIYSKHTPINLVRDLPGQFSALEPLVDLPIGINITRFPQTLLALHRSYL TFGDBSSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLKSFYV RKGIIYQTSNFRVQPTESIVRFNNITNLCFFGEVFNATRFASVYAWNRRKRSNCVADYSV LYNSASFSTFKCYGVSPTKLNLDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNKILP DDFTGCVIAWNSNNLDSKVGNNYLYLRLFRKSNLKPFERDISTEYQAGSTPCNGVEG FNCYFPLQSYGFPQPTNGVGYQFYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFN GLTGTGVLTESNKKFLPFQFGRDIADTTDAVRDPQTLEILDITPCSPGGVSVITPGTN TSNQVAVLYQDVNCTEVPVVAIHADQLTFTWRVYSTGSNVFTQTRAGCLIGAHEVNNSEYEC DIPIGAGICASYQTQTNPSGAGSVASQSI IAYTMSLGAENSVAYSNNNSIAIPTNFTIS VTTEILPVSMTKTSVDCTMYICGDSFTECSNLLQYGSFCTQLNRALTGLAVEQDKNTQE VFAQVKEQIYKTPPIKDFGCFNFSQILPDESKFSPERSFIEDLLFNKVTLADAGFIKQYGD CLGDIAARDLICAQKFNGLTVLPPLLTDEMIAYTSALLAGTITSGWTFGAGAALQIPF AMQMA YRFNGIGVTVQNVLYENQKLIANQFNSAIGKIQDLSSTASALGKLQDVVNQNAQ ALNTLVKQLSSNFGAISSVLNDILSLDPPFAEVQIDRLITGRLOSLQTYVTQQLIRAA EIRASANLAATKMSSECVLGQSKRVDFCGKGYHMSFPQSAPHGVVFLHVTYVPAQEKNF TTAPAI CHDQKARFFREGVFGNGTHWFTQGRNFYEPQIITDNTFVSGNCDVIGIVN NTVYDPLQPELDSFKEELDXYFKNHTSPDVLGDISGINASVVNIQKEIDRLNEVAKNL NESLIDLQELGKYEYIKsgrenlyfqqggggsgyipeaprdggayvrkdgevwllstf lg (X1) KMEELFKKHKIIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKA LSVLKEKGAIIAGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMPGVMT PTELNVKAMKLGHTILKLPFGEVVGVPQFVKAMKGFPPNVKVFVPTGGVNLNDVAEWFKAGV LAVGVGSALVKGTPDEVREKAKAFVEKIRGATE (SEQ ID NO:33) (mgilpspgmpallslyslsllmvgcvaetgt) QCVNLTRRTQLPPAYTNSFTRGVYYP FDKVFSSSVLHSTQDLFLPFTSNVTFWFAIHVSGTNGTKRFDNFVLPFNDGVYFASTK SNIRGWIFGTTLDSKTQSLLIVNNATNVVIVKVCPEFQFCNDFFLGVIYHKNKSNWMESE FRVYSSANNCTFEYVSQPFMLDLEGKQGNFNKLRREFVFKNIDGYFKIYSKHTPINLVRD LPQGFSALEPLVDLPIGINITRFPQTLLALHRSYLTPGDBSSSGWTAGAAAYVGYLQPR TFLLKYNENGTITDAVDCALDPLSETKCTLKSFYVVEKGIYQTSNFRVQPTESIVRFNNIT NLCFFGEVFNATRFASVYAWNRRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNLDLCF ENVYADSFVIRGDEVQRQIAPGQTGKIADYNKILPDDFTGCVIAWNSNNLDSKVGNNY LYRLFRKSNLKPFERDISTEYQAGSTPCNGVEGFNCYFPLQSYGFPQPTNGVGYQFYRV VVLSPFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQFGRDI ADTTDAVRDPQTLEILDITPCSPGGVSVITPGTNSTNQVAVLYQDVNCTEVPVVAIHADQ LTPTWRVYSTGSNVFTQTRAGCLIGAHEVNNSEYECDIPIGAGICASYQTQTNPSGAGSV ASQSI IAYTMSLGAENSVAYSNNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDS TECSNLLQYGSFCTQLNEALTGIAVEQDKNTQEVFAQVVKIYKTPPIKDFGCFNFSQI LPDPSKSPKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLICAQKFNGLTVLPPL LTDEMIAYTSALLAGTITSGWTFGAGAALQIPFAMQMA YRFNGIGVTVQNVLYENQKLI ANQFNSAIGKIQDLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILS RLDPPFAEVQIDRLITGRLOSLQTYVTQQLIRAAEIRASANLAATKMSSECVLGQSKRV DFCGKGYHMSFPQSAPHGVVFLHVTYVPAQEKNF TTAPAI CHDQKARFFREGVFGNGT HWFVTQGRNFYEPQIITDNTFVSGNCDVIGIVNNTVYDPLQPELDSFKEELDXYFKNH TSPDVLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEYIKsgrenl yfqqggggsgyipeaprdggayvrkdgevwllstflg (X1) KMEELFKKHKIIVAVLRANS VEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIAGAGTVTSVEQARRA VESGAEFIVSPHLDEEISQFAKEKGVFYMPGVMT PTELNVKAMKLGHTILKLPFGEVVG VPQFVKAMKGFPPNVKVFVPTGGVNLNDVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFV EKIRGATE (GGSHHHHHHHH) (SEQ ID NO:34) ETGTQCVNLTRRTQLPEAYTNSFTRGVYYPDKVFRSSVLAHQDLFLPFFSNVTFWFAI HVSGTNGTKRFDNFVLPFNDGVYFASTKSNIRGWIFGTTLDSKTQSLLIVNNATNVV IEVCEPQFCNDFFLGVIYHKNKSNWMESEFRVYSSANNCTFEYVSQPFMLDLEGKQGNF KNLREFVFKNIDGYFKIYSKHTPINLVRDLPGQFSALEPLVDLPIGINITRFPQTLLALH
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		RSYLTSGDSSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLK SFTVEKGIYQTSNFRVQPTESIVRFPNITNLCFFGEVFNATRFASVYAWNRRKISNCVA DYSVLNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYN YKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRFRKSNLKPFEEDISTEIIYQAGSTPCN GVEGFNCYFFLQSYGFPQTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVN FNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFQGVSVIT PGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFTQTRAGCLIGAEHVNN SYECDIPIGAGICASYQTQTSRSPSGAGSVASQSI IAYTMSLGAENSVAYSNNSIAIFTN FTISVTTEILPVSMTKTSVDCTMYICGDSSTECSNLLLLQYGSFCTQLNRALTGIAVEQDK NTQEVFAQVKQIYKTPPIKDFGGFNFSQLPDPSPKSKRSFIEDLLFNKVTLADAGFIK QYGDCLGDI AARDLICAQKFNGLTVLPELLTDEMIAQYTSALLAGTITSGWTFGAGAAL QIPFAMQMAXRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDLSSTASALGKIQDVVN QNAQALNTLVKQLSSNFGAIISSVLNDILSRLOPPEAEVQIDRLITGRQLSQTIVTQQL IRAAEIRASANLAATKMSCEVLGQSKRVDFCGKGYHLSMFFQSAFNGVVFVFLHVTYVFAQ EKNFTTAPAI CHDCKANFPREGVFSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVI GIVNNTVYDPLQPELDSFKELDKYFKNHTSPDVLDGDISGINASVNNIQKEIDRLNEV AKNLLRESLIDLQELGKYEQYIKgsagrenlyfsggggsgyipeaprdggayvrkdgeawl letflg(X1)KMEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADT VIKALSVLKEKGAIIIGAGTIVTSVEQARKAVESGAEFIVSPHLDEEISQFAKERGVFYMP GYMTPTTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGFPPNVKFPVPTGGVNLNDVAEWF KAGVLAVGVGSALVKGTFPDEVREKAKAFVEKIRGATE (SEQ ID NO:35) ETGTQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAI HVSNGTNGTKRFDNPLFPNDGVYFASTEKSNIRGWIFGTTLDSTQSLIIVNNATNVV IKVCFEQFCNDFFLGVYHKNKSNWMESEFRVYSSANNCTFEYVSQPFMLDLEGKQGNF KNLREFVFKNIDGYFKIYSKHTPINLVRDLPGGFSALEPLVDLPIGINITRFQTLALH RSYLTSGDSSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLK SFTVEKGIYQTSNFRVQPTESIVRFPNITNLCFFGEVFNATRFASVYAWNRRKISNCVA DYSVLNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYN YKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRFRKSNLKPFEEDISTEIIYQAGSTPCN GVEGFNCYFFLQSYGFPQTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVN FNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFQGVSVIT PGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFTQTRAGCLIGAEHVNN SYECDIPIGAGICASYQTQTSRSPSGAGSVASQSI IAYTMSLGAENSVAYSNNSIAIFTN FTISVTTEILPVSMTKTSVDCTMYICGDSSTECSNLLLLQYGSFCTQLNRALTGIAVEQDK NTQEVFAQVKQIYKTPPIKDFGGFNFSQLPDPSPKSKRSFIEDLLFNKVTLADAGFIK QYGDCLGDI AARDLICAQKFNGLTVLPELLTDEMIAQYTSALLAGTITSGWTFGAGAAL QIPFAMQMAXRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDLSSTASALGKIQDVVN QNAQALNTLVKQLSSNFGAIISSVLNDILSRLOPPEAEVQIDRLITGRQLSQTIVTQQL IRAAEIRASANLAATKMSCEVLGQSKRVDFCGKGYHLSMFFQSAFNGVVFVFLHVTYVFAQ EKNFTTAPAI CHDCKANFPREGVFSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVI GIVNNTVYDPLQPELDSFKELDKYFKNHTSPDVLDGDISGINASVNNIQKEIDRLNEV AKNLLRESLIDLQELGKYEQYIKgsagrenlyfsggggsgyipeaprdggayvrkdgeawl letflg(X1)KMEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADT VIKALSVLKEKGAIIIGAGTIVTSVEQARKAVESGAEFIVSPHLDEEISQFAKERGVFYMP GYMTPTTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGFPPNVKFPVPTGGVNLNDVAEWF KAGVLAVGVGSALVKGTFPDEVREKAKAFVEKIRGATE (GGSHHHHHHH) (SEQ ID NO:36)
SARS- CoV-2 2PSEA G-3- 153- 50A*- 12GS-	SARS- CoV-2- 153-50A fusion protein	QCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVS TNGTKRFDNPLFPNDGVYFASTEKSNIRGWIFGTTLDSTQSLIIVNNATNVV IKVCFEQFCNDFFLGVYHKNKSNWMESEFRVYSSANNCTFEYVSQPFMLDLEGKQGNF KNLREFVFKNIDGYFKIYSKHTPINLVRDLPGGFSALEPLVDLPIGINITRFQTLALH RSYLTSGDSSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLK SFTVEKGIYQTSNFRVQPTESIVRFPNITNLCFFGEVFNATRFASVYAWNRRKISNCVA DYSVLNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYN YKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRFRKSNLKPFEEDISTEIIYQAGSTPCN GVEGFNCYFFLQSYGFPQTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVN FNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFQGVSVIT PGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFTQTRAGCLIGAEHVNN SYECDIPIGAGICASYQTQTSRSPSGAGSVASQSI IAYTMSLGAENSVAYSNNSIAIFTN FTISVTTEILPVSMTKTSVDCTMYICGDSSTECSNLLLLQYGSFCTQLNRALTGIAVEQDK NTQEVFAQVKQIYKTPPIKDFGGFNFSQLPDPSPKSKRSFIEDLLFNKVTLADAGFIK QYGDCLGDI AARDLICAQKFNGLTVLPELLTDEMIAQYTSALLAGTITSGWTFGAGAAL QIPFAMQMAXRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDLSSTASALGKIQDVVN QNAQALNTLVKQLSSNFGAIISSVLNDILSRLOPPEAEVQIDRLITGRQLSQTIVTQQL IRAAEIRASANLAATKMSCEVLGQSKRVDFCGKGYHLSMFFQSAFNGVVFVFLHVTYVFAQ EKNFTTAPAI CHDCKANFPREGVFSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVI GIVNNTVYDPLQPELDSFKELDKYFKNHTSPDVLDGDISGINASVNNIQKEIDRLNEV AKNLLRESLIDLQELGKYEQYIKgsagrenlyfsggggsgyipeaprdggayvrkdgeawl letflg(X1)KMEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADT VIKALSVLKEKGAIIIGAGTIVTSVEQARKAVESGAEFIVSPHLDEEISQFAKERGVFYMP GYMTPTTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGFPPNVKFPVPTGGVNLNDVAEWF KAGVLAVGVGSALVKGTFPDEVREKAKAFVEKIRGATE (GGSHHHHHHH) (SEQ ID NO:36)

he- His	FNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFN GLTGTGVLTESSNEKFLPFQGFQPDIAADTTDAVRDPQTEILIDITPCSPGGVSVITPGTN TSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFPQTRAGCLIGAEHVNNSEYEC DIPIGAGICASYQTQTNPSGAGSVASQSI IAYTMSLGAENSVAYSNNISIAIPTNFTIS VTEILPVSMTKTSVDCTMYICGDSSTECNLLQYGSFCTQLNRALTGIAVEQDKNTQE VFAQVKQIYKTPPIKDFGGFNFQSQILPDPSPKSKRSFIEDLLFNKVTLADAGFIKQYGD CLGDI AARDLICAQKFNGLTVLPPLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPF AMQMAYRFNGIGVTQNVLYENQKLIANQFNNSAIGKIQDLSSTASALGKLQDVVNQNAQ ALNTLVKQLSSNFGAISSVLNDILSRDPPFAEVQIDRLITGRQLSLQTYVTQQLIRAA EIRASANLAATKMSCEVLGQSKRVDFCGKGYHLMSPQSAPHGVVFLHVTYVPAQEKNF TTAPAICHGDKAHFPREGVFEVSNGETHWFVTQRNFYEPQIITDNTFVSGNCDVVGIVN NTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDISGINASVVNIQKEIDRLNEVAKNL NESLIDLQELGKYEQYIKGSGSGSGSGSGSEKAAKAEKMEELFKKHKIVAVLRANS SVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIIGAGTVTSVEQARK AVESGAEFIVSPHLDEEISQFAKEKGVFYMFGVMTPTTELVRAMKLGHTIILKLPFGVVG PQFVKAMKGPFPNVKFPVPTGGVNLNVAEWFKAGVLAAGVGSALVKGTFDEVREKAKAF VEKIRGATE (SEQ ID NO:37) (mgilpspgmpallsllvallsvllmgcvaetgt)QCVNLTTRTQLPPAYTNSFTRGVVY PDKVFRSSVLHSTQDLFLPFFFSNVTFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEK SNIIRGWIFGTTLDSEKTSLLIVNNATNVVIVKCEFPQFCNDPFLGVYHKNKSWMESE FRVYSSANNCTFEYVSQPFLLMDLEGKQGNFRNLREFVFNKIDGYFKIYSKHTPINLVRD LPQGFSALEFLVDLPICINITRFQTLALHRSYLTGDSGSGWTAGAAAYVGYLQPRF FLKYNENGTITDAVDCALDPLSETEKTLRSEFTVEKGIYQTSNFRVQPTESIVRFPNIT NLCPFGVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTRLNDLCF TNVYADSFVIRGDEVRCIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNINY LYRLFRKSNLKPFFERDISTEIIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRV VVLGSELLRAAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESSNEKFLPFQGFGRDI ADTTDAVRDPQTEILIDITPCSPGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQ LTPTWRVYSTGSNVFPQTRAGCLIGAEHVNNSEYEC DIPIGAGICASYQTQTNPSGAGSV ASQSI IAYTMSLGAENSVAYSNNISIAIPTNFTISVTEILPVSMTKTSVDCTMYICGDS TECSNLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFQSQI LPDPSPKSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDI AARDLICAQKFNGLTVLPPL LTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLI ANQFNNSAIGKIQDLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILS RLDPPFAEVQIDRLITGRQLSLQTYVTQQLIRAAEIRASANLAATKMSCEVLGQSKRV FCGKGYHLMSPQSAPHGVVFLHVTYVPAQEKNF TTAPAICHGDKAHFPREGVFEVSNGT HWFVTQRNFYEPQIITDNTFVSGNCDVVGIVNNTVYDPLQPELDSFKEELDKYFKNH TSPDVLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKGSGSGS GGSGSEKAAKAEKMEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFT VPDADTVIKALSVLKEKGAIIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEK GVFYMFGVMTPTTELVRAMKLGHTIILKLPFGVVG PQFVKAMKGPFPNVKFPVPTGGVNL NVAEWFKAGVLAAGVGSALVKGTFDEVREKAKAFVEKIRGATE (GGSHHHHHHHH) (SEQ ID NO:38) ETGTQCVNLTTRTQLPEAYTNSFTRGVVYPDKVFRSSVLHSTQDLFLPFFFSNVTFHAI HVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIRGWIFGTTLDSEKTSLLIVNNATNVV IVKCEFPQFCNDPFLGVYHKNKSWMESEFRVYSSANNCTFEYVSQPFLLMDLEGKQGNF RNLREFVFNKIDGYFKIYSKHTPINLVRDLPQGFSALEFLVDLPICINITRFQTLALH RSYLTGDSGSGWTAGAAAYVGYLQPRFLLKYNENGTITDAVDCALDPLSETEKTLK SEFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGVFNATRFASVYAWNRKRISNCV ADYSVLYNSASFSTFKCYGVSPTRLNDLCFTNVYADSFVIRGDEVRCIAPGQTGKIADYN YKLPDDFTGCVIAWNSNNLDSKVGGNINYLYRLFRKSNLKPFFERDISTEIIYQAGSTPCN GVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVN FNFNGLTGTGVLTESSNEKFLPFQGFGRDIAADTTDAVRDPQTEILIDITPCSPGGVSVIT PGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFPQTRAGCLIGAEHVNN SEYEC DIPIGAGICASYQTQTNPSGAGSVASQSI IAYTMSLGAENSVAYSNNISIAIPTN FTISVTEILPVSMTKTSVDCTMYICGDSSTECNLLQYGSFCTQLNRALTGIAVEQDK NTQEVFAQVKQIYKTPPIKDFGGFNFQSQILPDPSPKSKRSFIEDLLFNKVTLADAGFIK QYGDCLGDI AARDLICAQKFNGLTVLPPLTDEMIAQYTSALLAGTITSGWTFGAGAAL
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	QIPFAMQMAYRFNGIGVTVQNVLYENQKLIANQFNBSAIGKIQDSLSSTASALGKLQDVVN QNAQALNTLVKQLSSNFGAISSVLNDILSRLOPPEAEVQIDRLITGRLOSLQTYVTQQL IRAAEIRASANLAATKMESECVLGQSKRVDFCGKGYHLMSPFQSAFHGVVFLHVTYVFAQ EKNFTTAPAICHGDKAHFPREGVFSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVI GIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDISGINASVUNIQKEIDRLNEV AKNLNESLIDLQELGKYEQYIKSGSGSGSGSGSEKAAKAEAAARKMEELFKKHKIVAV LRANSVEEAIEKAVAVPAGGVHLIEITFTVPDADTVIKALSVLKEKGAIGAGTVTSVE QARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMFGVMTPTTELKAMKLGHTILKLFPG EVVGFQFVKAMKGPFPNVKVFVPTGGVNLDNVAEMFKAGVLAVGVGSALVKGTFDEVREK AKAFVEKIRGATE (SEQ ID NO:39) ETGTQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWPHAI HVSGETNGTKRFDNPVLPFNDGVYFASTEKSNIRGMIFGTTLDSEKTSQSLIIVNATNVV IKVCEPQFCNDPFLGVYHKNKNSWMESEFVYSSANNCTFEYVSQPFILMDLEGKQGNF KNLREFVFKNIDGYFKIYKHTPINALVRDLPGGFSALEPLVDLFIGINITRFQTLALH RSYLTPGDSSSGWTAGAAAYVGYLQPTFTLLKYNENGITDAVDCALDPLSEKTKLSFTV EKGIVQTSNFRVQPTESIVRFPNITNLCPFGGEVFNATRFASVYAWNRKRISSNCVA DYSVLNSASFSTFKCYGVSEPKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTKGIADYN YKLPDDFTGCVIANNNSNLDKVGGNYNLYRLFRKSNLAPFERDISTEIVQAGSTPCN GVEGECYFFLQSYGFQPTNGVGYQPYRVVLSFELLHAFATVCGPKKSTNLVKNKCVN FNFNGLTGTGVLTESNKKFLPQQFGKDIADTTDAVRDPQTLEILDTFCSPGGVSVIT PGTNTSNQVAVLYQDVNCTEVEVAIHADQLTPTWRVYSTGSRVFPQTRAGCLIGAHEVNN SYECDIPIGAGICASYQTQTSNPSGAGSVASQSI IAYTMSLGAENSVAYSNNISIAIPTN FTISVTTEILPVSMTKTSVDCMTMYICGDESTECNSNLLQYGSFCTQLNRALTGIAVEQDK NTQEVFAQVKQIYKTPPIKDPGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIK QYGDCLGDIAARDLICAKKFNGLTVLPELTTDEMIQYTSALLAGTITSGWTFGAGAAL QIPFAMQMAYRFNGIGVTVQNVLYENQKLIANQFNBSAIGKIQDSLSSTASALGKLQDVVN QNAQALNTLVKQLSSNFGAISSVLNDILSRLOPPEAEVQIDRLITGRLOSLQTYVTQQL IRAAEIRASANLAATKMESECVLGQSKRVDFCGKGYHLMSPFQSAFHGVVFLHVTYVFAQ EKNFTTAPAICHGDKAHFPREGVFSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVI GIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDISGINASVUNIQKEIDRLNEV AKNLNESLIDLQELGKYEQYIKSGSGSGSGSGSEKAAKAEAAARKMEELFKKHKIVAV LRANSVEEAIEKAVAVPAGGVHLIEITFTVPDADTVIKALSVLKEKGAIGAGTVTSVE QARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMFGVMTPTTELKAMKLGHTILKLFPG EVVGFQFVKAMKGPFPNVKVFVPTGGVNLDNVAEMFKAGVLAVGVGSALVKGTFDEVREK AKAFVEKIRGATE (GGSHHHHHHHH) (SEQ ID NO:40) QCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWPHAIHVS TNGTKRFDNPVLPFNDGVYFASTEKSNIRGMIFGTTLDSEKTSQSLIIVNATNVV IKVCEPQFCNDPFLGVYHKNKNSWMESEFVYSSANNCTFEYVSQPFILMDLEGKQGNFKNL REFVFKNIDGYFKIYKHTPINALVRDLPGGFSALEPLVDLFIGINITRFQTLALHRSYL TPGDSSSGWTAGAAAYVGYLQPTFTLLKYNENGITDAVDCALDPLSEKTKLSFTV EKGIVQTSNFRVQPTESIVRFPNITNLCPFGGEVFNATRFASVYAWNRKRISSNCVA DYSVLNSASFSTFKCYGVSEPKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTKGIADYN YKLPDDFTGCVIANNNSNLDKVGGNYNLYRLFRKSNLAPFERDISTEIVQAGSTPCN GVEGECYFFLQSYGFQPTNGVGYQPYRVVLSFELLHAFATVCGPKKSTNLVKNKCVN FNFNGLTGTGVLTESNKKFLPQQFGKDIADTTDAVRDPQTLEILDTFCSPGGVSVIT PGTNTSNQVAVLYQDVNCTEVEVAIHADQLTPTWRVYSTGSRVFPQTRAGCLIGAHEVNN SYECDIPIGAGICASYQTQTSNPSGAGSVASQSI IAYTMSLGAENSVAYSNNISIAIPTN FTISVTTEILPVSMTKTSVDCMTMYICGDESTECNSNLLQYGSFCTQLNRALTGIAVEQDK NTQEVFAQVKQIYKTPPIKDPGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIK QYGDCLGDIAARDLICAKKFNGLTVLPELTTDEMIQYTSALLAGTITSGWTFGAGAAL QIPFAMQMAYRFNGIGVTVQNVLYENQKLIANQFNBSAIGKIQDSLSSTASALGKLQDVVN QNAQALNTLVKQLSSNFGAISSVLNDILSRLOPPEAEVQIDRLITGRLOSLQTYVTQQL IRAAEIRASANLAATKMESECVLGQSKRVDFCGKGYHLMSPFQSAFHGVVFLHVTYVFAQ EKNFTTAPAICHGDKAHFPREGVFSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVI GIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDISGINASVUNIQKEIDRLNEV AKNLNESLIDLQELGKYEQYIKSGSGSGSGSGSEKAAKAEAAARKMEELFKKHKIVAV LRANSVEEAIEKAVAVPAGGVHLIEITFTVPDADTVIKALSVLKEKGAIGAGTVTSVE QARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMFGVMTPTTELKAMKLGHTILKLFPG EVVGFQFVKAMKGPFPNVKVFVPTGGVNLDNVAEMFKAGVLAVGVGSALVKGTFDEVREK AKAFVEKIRGATE (GGSHHHHHHHH) (SEQ ID NO:40)
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	GGVNLDNVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATE (SEQ ID NO:41) (mgilpspgmpallslvllsvllimgcvaetgt)QCVNLTTTQTQLPPAYTNSFTRGVYY PDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFLNPFVLPFNDGVYFASTEK SNIIRGWIFGTTLDSTQSLIVNNATNVVVKVCEFPQCNDFPLGVYYHKNNKSWMESE FRVYSSANNCTFEYVSQPFILMDLEGKQGNFKNLREFVFNIDGYFKIYSEHTPINLVRO LPQGFSALEPLVDLPIGINITRFQTLALHRSYLTFGDSSSGWTAGAAAYVGYLQPR FLLKYNENGTITDAVDCALDPLSETTECTLKSTVEKGIYQTSNFRVQPTESIVRKNIT NLCPPGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTEKLNLCF TNVYADSEVIRGDEVQRQIAPGQTGKIADYNKLPDDFTGCVIAWNSNNLDSKVGGNYNY LYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCYFFLQSYGFQPTNGVGYQFYRV VVLSEFELLHAPATVCGPKKSTNLVKNKCVNPNFNGLTGTGVLTESNKKRFLPFQFGRI ADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQ LTFTWRVYSTGSSNVFQTRAGCLIGAEHVNNSEYCDIPIGAGICASYQTQTSNPSGAGSV ASQSI IAYTMSLGAENSVAYSNNSIAIPTNFTISVTEILFVSMKTSTVDCTMYICGDS TECSNLLQYGSFCTQLNRALTGIAVEQDKNFQEVFAQVQYKYTEPKIDFGGFNFESQI LPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIARDLCAQKFNGLTVLPPL LTDEMIQYTSALLAGTITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLI ANQFNSAIGKIQDSLSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILS RLDPPEAEVQIDRLITGRQLSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVD PCGKGXHLMSFPQSAFHGVVFLHVTVYPAQEKNETTAPAI CHDGAHFFREGVFSNGT HWFVTQRNFYEPQIITDNTFVSGNCDVVGIVNNTVYDPLQPELDSFKEELDXYFKNH TSPDVLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIK (X1) KME ELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSVLKEKGA IIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFMPGVMTPELTVKAMK LGHTIILKLPGEVVGPPQFVKAMKGPFPNVKFPVPTGGVNLDNVAEWFKAGVLAVGVGSAL VEGTPDEVREKAKAFVEKIRGATE (GGSHHHHHHHH) (SEQ ID NO:42) ETGTQCVNLTTTQTQLPPAYTNSFTRGVYYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAI HVSGTNGTKRFLNPFVLPFNDGVYFASTEESNIIRGWIFGTTLDSETQSLIVNNATNVV IKVCEFPQCNDFPLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFILMDLEGKQGNF KNLREFVFNIDGYFKIYSEHTPINLVROLPQGFSALEPLVDLPIGINITRFQTLALH RSYLTFGDSSSGWTAGAAAYVGYLQPRFLLKYNENGTITDAVDCALDPLSETTECTLK SETVEKGIYQTSNFRVQPTESIVRFPNITNLCPPGEVFNATRFASVYAWNRKRISNOVA DYSVLYNSASFSTFKCYGVSPTEKLNLCFTNVYADSEVIRGDEVQRQIAPGQTGKIADYN XFLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFFERDISTEIQAGSTPCN GVEGFNCYFFLQSYGFQPTNGVGYQFYRVVVLSEFELLHAPATVCGPKKSTNLVKNKCVN PNFNGLTGTGVLTESNKKRFLPFQFGRIADTTDAVRDPQTLEILDITPCSFGGVSVIT PGTNTSNQVAVLYQDVNCTEVPVAIHADQLTFTWRVYSTGSSNVFQTRAGCLIGAEHVNN SYECDIPIGAGICASYQTQTSNPSGAGSVASQSI IAYTMSLGAENSVAYSNNSIAIPTN FTISVTEILFVSMKTSTVDCTMYICGDSSTECNLLQYGSFCTQLNRALTGIAVEQDK NTQEVFAQVQYKYTEPKIDFGGFNFESQILPDPSKPSKRSFIEDLLFNKVTLADAGFIK QYGDCLGDIARDLCAQKFNGLTVLPPLLTDEMIQYTSALLAGTITSGWTFGAGAAL QIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTASALGKLQDVVN QNAQALNTLVKQLSSNFGAISSVLNDILSRLDPEAEVQIDRLITGRQLSLQTYVTQQL IRAAEIRASANLAATKMSECVLGQSKRVDPCGKGXHLMSFPQSAFHGVVFLHVTVYPAQ SENFTTAPAI CHDGAHFFREGVFSNGTHWFVTQRNFYEPQIITDNTFVSGNCDVVG GIVNNTVYDPLQPELDSFKEELDXYFKNHTSPDVLGDISGINASVVNIQKEIDRLNEV AKNLNESLIDLQELGKYEQYIK () KMEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGV HLIEITFTVPDADTVIKALSVLKEKGAIIIGAGTVTSVEQARKAVESGAEFIVSEHLDEE ISQFAKEKGVFMPGVMTPELTVKAMKLGHTIILKLPGEVVGPPQFVKAMKGPFPNVKFP PTGGVNLDNVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATE (SEQ ID NO:43) ETGTQCVNLTTTQTQLPPAYTNSFTRGVYYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAI HVSGTNGTKRFLNPFVLPFNDGVYFASTEESNIIRGWIFGTTLDSETQSLIVNNATNVV IKVCEFPQCNDFPLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFILMDLEGKQGNF KNLREFVFNIDGYFKIYSEHTPINLVROLPQGFSALEPLVDLPIGINITRFQTLALH RSYLTFGDSSSGWTAGAAAYVGYLQPRFLLKYNENGTITDAVDCALDPLSETTECTLK
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		SFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPPFGEVFNATRFASVYAWNRRKRISNCVA DYSVLYNSASFSTPRCYGVSPTEKLNLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYN YKLPDDFTGCVIAWNSNNLDSKVGGNYYLYRLFRKSNLKPFERDISTEIIYQAGSTPCN GVEGFNCYFPLOSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVN FNFNGLTGTGVLTESNKKFLPFQCGRDIADTTDAVRDEQTLLEILDITPQSFQGVSVIT EGTNTISNQVAVLYQDVNCTEVFVAIHADQLTFTWRVISTGSNVFPQTRAGCLIGAEHVNN SYECDFIFIGAGICASYQTQTNSTPFGAGSVASQSI IAYTMSLGAENSVAYSNNSIAIPEN FTISVTTETILPVSMTKESVDCTMYICGDSSTECNHLILQYGSFCTQLNKAITGIAVEQDK NTQEVFAQVKQIYKTPPKIDFGGFNFBSQILLPDPSPKSKK3FIEDLLEKNVTLADAGFIK QYGDCLGDIARDLICAQKFNGLTVPFLLTDEMIQAQYTSALLAGTITSGWTFGAGAAL QIPFAMQMAYRFGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVN QNAQAINTLVKQLSSNFGAISSVLNDILSRLOPPEAEVQIDRLITGRLQSLQTYVTQQL IRAAEIRASANLAATKMSSECVLGQSKRVDFCGKGYHLSFPQSAHPGVVFLHVVYVPAQ EKNFTTAPAICHDGKAHFPREGVTVSNSTHWFVTQRNFYEFQIITTDHTFVSGNCDVVI GIVNNTVYDPLQPELDSFKEELDXYFKNHTSFEDVLDGDISGINASVVNIQKEIDRLNEV AKNLESLELDLQELGKVEQYIK () KMEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGV HLEITFTVPDADTVIKALSFLKEMGAIIGAGTVTSVEQAREAVESGAEFIVSPHLDEE ISQFAKEKGVFYMFGVMTPTTELVKAMKLGHTIILKLPFGEVVGPFQFVKAMKGPFVNVKVF PTGGVNLNDVAEWFEAGVQAVGVGEALNEGTFVEVAEKAKAFVEKIEGATE (GGSHRRH HHHH) (SEQ ID NO:44)
SARS- CoV-2 RBD- I3- Q1* secOp t- 3GS- he- His	SARS- CoV-2- I3-Q1 fusion protein	RFPNITNLCPPFGEVFNATRFASVYAWNRRKRISNCVADYSVLYNSASFSTPRCYGVSPTEK LNLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNKLPDDFTGCVIAWNSNNLDSKV GGNYYLYRLFRKSNLKPFERDISTEIIYQAGSTPCNGVEGFNCYFPLOSYGFQPTNGVG YQPYRVVLSFELLHAPATVCGPKKSTGGSGGSGSEKAAKAEAAARMEELFKENKIVAV LRANSVEEAKKKALAVFLGGVDLIEITFTVPDADTVIKELSFLEKMGAIIGAGTVTSVE QAREAVESGAEFIVSPHLDEEISQFAKEKGVFYMFGVMTPTTELVKAMKLGHTIILKLPFG EVVGFQFVEAMKGPFVNVKVFPTGGVNLNDVAEWFEAGVQAVGVGEALNEGTFVEVAEK AKAFVEKIEGATE (SEQ ID NO:45) (mgilpspgmpallsivslavllmgcvaetgt)RFPNITNLCPPFGEVFNATRFASVYA WNRKRISNCVADYSVLYNSASFSTPRCYGVSPTEKLNLCFTNVYADSFVIRGDEVQRQIA PGQTGKIADYNKLPDDFTGCVIAWNSNNLDSKVGGNYYLYRLFRKSNLKPFERDISTEII YQAGSTPCNGVEGFNCYFPLOSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKK STGGSGGSGSEKAAKAEAAARMEELFKENKIVAVLRANSVEEAKKKALAVFLGGVDLIE ITFTVPDADTVIKELSFLEKMGAIIGAGTVTSVEQAREAVESGAEFIVSPHLDEEISQF AKEEGVFYMFGVMTPTTELVKAMKLGHTIILKLPFGEVVGPFQFVEAMKGPFVNVKVFPTGG VNLNDVAEWFEAGVQAVGVGEALNEGTFVEVAEKAKAFVEKIEGATE (GGSHHHHHHHH) (SEQ ID NO:46) ETGTRFPNITNLCPPFGEVFNATRFASVYAWNRRKRISNCVADYSVLYNSASFSTPRCYGV SPTKLNLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNKLPDDFTGCVIAWNSNNL DSKVGGNYYLYRLFRKSNLKPFERDISTEIIYQAGSTPCNGVEGFNCYFPLOSYGFQPT NGVGYQPYRVVLSFELLHAPATVCGPKKSTGGSGGSGSEKAAKAEAAARMEELFKENK IVAVLRANSVEEAKKKALAVFLGGVDLIEITFTVPDADTVIKELSFLEKMGAIIGAGTV TSVEQAREAVESGAEFIVSPHLDEEISQFAKEKGVFYMFGVMTPTTELVKAMKLGHTIILK LPFGEVVGPFQFVEAMKGPFVNVKVFPTGGVNLNDVAEWFEAGVQAVGVGEALNEGTFVE VAEKAKAFVEKIEGATE (SEQ ID NO:47) ETGTRFPNITNLCPPFGEVFNATRFASVYAWNRRKRISNCVADYSVLYNSASFSTPRCYGV SPTKLNLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNKLPDDFTGCVIAWNSNNL DSKVGGNYYLYRLFRKSNLKPFERDISTEIIYQAGSTPCNGVEGFNCYFPLOSYGFQPT NGVGYQPYRVVLSFELLHAPATVCGPKKSTGGSGGSGSEKAAKAEAAARMEELFKENK IVAVLRANSVEEAKKKALAVFLGGVDLIEITFTVPDADTVIKELSFLEKMGAIIGAGTV TSVEQAREAVESGAEFIVSPHLDEEISQFAKEKGVFYMFGVMTPTTELVKAMKLGHTIILK LPFGEVVGPFQFVEAMKGPFVNVKVFPTGGVNLNDVAEWFEAGVQAVGVGEALNEGTFVE VAEKAKAFVEKIEGATE (GGSHHHHHHHH) (SEQ ID NO:48) RFPNITNLCPPFGEVFNATRFASVYAWNRRKRISNCVADYSVLYNSASFSTPRCYGVSPTEK LNLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNKLPDDFTGCVIAWNSNNLDSKV

		GGNYYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCYFFLQSYGFGQPTNGVG YQPYRVVVLSEFLLHAPATVCGPKKST (X1) MEELFKEHKIVAVLRANSVEEAKKKALA VFLGGVDLIEITFTVPDADTVIKELSFLEKMGAIIGAGTSTSVEQAREAVESGAEFIVS PHLDEEISQFAKEEGVFYMPGVMTPTLVKAMKLGHTILKLFPGEVVGPQFVEAMKGPFF PNVKFVPTGGVNLNDNVAEWFEAGVQAVGVGEALNEGTPVEVAEKAKAFVEKIEGATE (SEQ ID NO:49) (mgilpspgmpallslvslsvllimgcvaetgt)RFPNITNLCPPFGEVFNATRFASVYA WNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVIYADSFVIRGDEVVRQIA PGQTGKIADYNYKLPPDDFTGCVIANNNNLDSKVGGNYYLYRLFRKSNLKPFFERDIST EIQAGSTPCNGVEGFNCYFFLQSYGFGQPTNGVGYPYRVVVLSEFLLHAPATVCGPKK ST (X1) MEELFKEHKIVAVLRANSVEEAKKKALAVFLGGVDLIEITFTVPDADTVIKEL SFLEKMGAIIGAGTSTSVEQAREAVESGAEFIVS PHLDEEISQFAKEEGVFYMPGVMTPT TELVKAMKLGHTILKLFPGEVVGPQFVEAMKGPFFPNVKFVPTGGVNLNDNVAEWFEAGVQ AVGVGEALNEGTPVEVAEKAKAFVEKIEGATE (GGSHHHHHHHH) (SEQ ID NO:50) ETGTRFPNITNLCPPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGV SPTKLNDLCFTNVIYADSFVIRGDEVVRQIAPGQTGKIADYNYKLPPDDFTGCVIANNNNL DSKVGGNYYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCYFFLQSYGFGQPT NGVGYPYRVVVLSEFLLHAPATVCGPKKST (X1) MEELFKEHKIVAVLRANSVEEAKK KALAVFLGGVDLIEITFTVPDADTVIKELSFLEKMGAIIGAGTSTSVEQAREAVESGA FIVSPHLDEEISQFAKEEGVFYMPGVMTPTLVKAMKLGHTILKLFPGEVVGPQFVEAM KGPFFPNVKFVPTGGVNLNDNVAEWFEAGVQAVGVGEALNEGTPVEVAEKAKAFVEKIEGA TE (SEQ ID NO:51) ETGTRFPNITNLCPPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGV SPTKLNDLCFTNVIYADSFVIRGDEVVRQIAPGQTGKIADYNYKLPPDDFTGCVIANNNNL DSKVGGNYYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCYFFLQSYGFGQPT NGVGYPYRVVVLSEFLLHAPATVCGPKKST (X1) MEELFKEHKIVAVLRANSVEEAKK KALAVFLGGVDLIEITFTVPDADTVIKELSFLEKMGAIIGAGTSTSVEQAREAVESGA FIVSPHLDEEISQFAKEEGVFYMPGVMTPTLVKAMKLGHTILKLFPGEVVGPQFVEAM KGPFFPNVKFVPTGGVNLNDNVAEWFEAGVQAVGVGEALNEGTPVEVAEKAKAFVEKIEGA TE (GGSHHHHHHHH) (SEQ ID NO:52)
SARS- CoV-2 RBD- I3- G1*- secOp t- 12GS- he- His	SARS- CoV-2- I3-G1 fusion protein	RFPNITNLCPPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTK LNDLCFTNVIYADSFVIRGDEVVRQIAPGQTGKIADYNYKLPPDDFTGCVIANNNNLDSKV GGNYYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCYFFLQSYGFGQPTNGVG YQPYRVVVLSEFLLHAPATVCGPKKSTGSGSGGSGSGSGSEKAAKAEAAAPMEELFKEHK IVAVLRANSVEEAKKKALAVFLGGVDLIEITFTVPDADTVIKELSFLEKMGAIIGAGT TSVEQAREAVESGAEFIVS PHLDEEISQFAKEEGVFYMPGVMTPTLVKAMKLGHTILK LFPGEVVGPQFVEAMKGPFFPNVKFVPTGGVNLNDNVAEWFEAGVQAVGVGEALNEGTPVE VAEKAKAFVEKIEGATE (SEQ ID NO:53) (mgilpspgmpallslvslsvllimgcvaetgt)RFPNITNLCPPFGEVFNATRFASVYA WNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVIYADSFVIRGDEVVRQIA PGQTGKIADYNYKLPPDDFTGCVIANNNNLDSKVGGNYYLYRLFRKSNLKPFFERDIST EIQAGSTPCNGVEGFNCYFFLQSYGFGQPTNGVGYPYRVVVLSEFLLHAPATVCGPKK STGSGSGGSGSGSGSEKAAKAEAAAPMEELFKEHKIVAVLRANSVEEAKKKALAVFLGGV DLIEITFTVPDADTVIKELSFLEKMGAIIGAGTSTSVEQAREAVESGAEFIVS PHLDEE ISQFAKEEGVFYMPGVMTPTLVKAMKLGHTILKLFPGEVVGPQFVEAMKGPFFPNVKFV PTGGVNLNDNVAEWFEAGVQAVGVGEALNEGTPVEVAEKAKAFVEKIEGATE (GGSHHHH HHHH) (SEQ ID NO:54) ETGTRFPNITNLCPPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGV SPTKLNDLCFTNVIYADSFVIRGDEVVRQIAPGQTGKIADYNYKLPPDDFTGCVIANNNNL DSKVGGNYYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCYFFLQSYGFGQPT NGVGYPYRVVVLSEFLLHAPATVCGPKKSTGSGSGGSGSGSGSEKAAKAEAAAPMEELF KEHKIVAVLRANSVEEAKKKALAVFLGGVDLIEITFTVPDADTVIKELSFLEKMGAIIG AGTSTSVEQAREAVESGAEFIVS PHLDEEISQFAKEEGVFYMPGVMTPTLVKAMKLGHTILK

		TILKLPFGVVGPFQFVEAMKGFPPNVKFPVPTGGVNLNDVAEWFEAGVQAVGVGEALNEG TPVEVAEKAKAFVEKIEGATE (SEQ ID NO:55) ETGTRFPNITNLCPPGEVFNATRFASVYAWNKKRISNCVADYSVLVNSASFSTFKCYGV SPTKLNLDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNKLPDDFTGCVIAWNNSNNL DSKVGGNYYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCYFFLQSYGFGQPT NGVGYQPYRVVLSFELLHAPATVCGPKKSTGGSGGGGGSGSGSEKAAKAEAAKMEELF KEHKIVAVLRANSVEEAKKKALAVFLGGVDLIEITFTVPDADTVIKELSFLEKMGAIIG AGTVTSVEQAREAVESGAEFIVSPHLDEEISQFAKEEGVFYMPGVMTPTLVKAMKLGH TILKLPFGVVGPFQFVEAMKGFPPNVKFPVPTGGVNLNDVAEWFEAGVQAVGVGEALNEG TPVEVAEKAKAFVEKIEGATE (GGSHHHHHHHH) (SEQ ID NO:56) RFPNITNLCPPGEVFNATRFASVYAWNKKRISNCVADYSVLVNSASFSTFKCYGVSPTE LNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNKLPDDFTGCVIAWNNSNNLDSKV GGNYYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCYFFLQSYGFGQPTNGVG YQPYRVVLSFELLHAPATVCGPKKST(X1)MEELFKHEKIVAVLRANSVEEAKKKALA VFLGGVDLIEITFTVPDADTVIKELSFLEKMGAIIGAGTVTSVEQAREAVESGAEFIVS PHLDEEISQFAKEEGVFYMPGVMTPTLVKAMKLGHTILKLPFGVVGPFQFVEAMKGF PPNVKFPVPTGGVNLNDVAEWFEAGVQAVGVGEALNEGTPVEVAEKAKAFVEKIEGATE (SEQ ID NO:57) (mgilpspgmpallslvallsvlimgcvaetgt)RFPNITNLCPPGEVFNATRFASVYA WNRKRISNCVADYSVLVNSASFSTFKCYGVSPTEKLNLDLCFTNVYADSFVIRGDEVQRQI APGQTGKIADYNKLPDDFTGCVIAWNNSNNLDSKVGGNYYLYRLFRKSNLKPFFERDIST EIQAGSTPCNGVEGFNCYFFLQSYGFGQPTNGVGYQPYRVVLSFELLHAPATVCGPKK ST(X1)MEELFKHEKIVAVLRANSVEEAKKKALAVFLGGVDLIEITFTVPDADTVIKEL SFLEKMGAIIGAGTVTSVEQAREAVESGAEFIVSPHLDEEISQFAKEEGVFYMPGVMT PTLVKAMKLGHTILKLPFGVVGPFQFVEAMKGFPPNVKFPVPTGGVNLNDVAEWFEAGV QAVGVGEALNEGTPVEVAEKAKAFVEKIEGATE (GGSHHHHHHHH) (SEQ ID NO:58) ETGTRFPNITNLCPPGEVFNATRFASVYAWNKKRISNCVADYSVLVNSASFSTFKCYGV SPTKLNLDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNKLPDDFTGCVIAWNNSNNL DSKVGGNYYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCYFFLQSYGFGQPT NGVGYQPYRVVLSFELLHAPATVCGPKKST(X1)MEELFKHEKIVAVLRANSVEEAKK KALAVFLGGVDLIEITFTVPDADTVIKELSFLEKMGAIIGAGTVTSVEQAREAVESGA EFIVSPHLDEEISQFAKEEGVFYMPGVMTPTLVKAMKLGHTILELFPGEVVGPFQFVEAM KGFPPNVKFPVPTGGVNLNDVAEWFEAGVQAVGVGEALNEGTPVEVAEKAKAFVEKIEGA TE (SEQ ID NO:59) ETGTRFPNITNLCPPGEVFNATRFASVYAWNKKRISNCVADYSVLVNSASFSTFKCYGV SPTKLNLDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNKLPDDFTGCVIAWNNSNNL DSKVGGNYYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCYFFLQSYGFGQPT NGVGYQPYRVVLSFELLHAPATVCGPKKST(X1)MEELFKHEKIVAVLRANSVEEAKK KALAVFLGGVDLIEITFTVPDADTVIKELSFLEKMGAIIGAGTVTSVEQAREAVESGA EFIVSPHLDEEISQFAKEEGVFYMPGVMTPTLVKAMKLGHTILELFPGEVVGPFQFVEAM KGFPPNVKFPVPTGGVNLNDVAEWFEAGVQAVGVGEALNEGTPVEVAEKAKAFVEKIEGA TE (GGSHHHHHHHH) (SEQ ID NO:60)
SARS- CoV-2 RBD- I3- Q1*- secOp t- 16GS-	SARS- CoV-2- I3-Q1 fusion protein	RFPNITNLCPPGEVFNATRFASVYAWNKKRISNCVADYSVLVNSASFSTFKCYGVSPTE LNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNKLPDDFTGCVIAWNNSNNLDSKV GGNYYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCYFFLQSYGFGQPTNGVG YQPYRVVLSFELLHAPATVCGPKKSTGGSGGGGGSGSGSEKAAKAEAAKMEELF KEHKIVAVLRANSVEEAKKKALAVFLGGVDLIEITFTVPDADTVIKELSFLEKMGAIIG AGTVTSVEQAREAVESGAEFIVSPHLDEEISQFAKEEGVFYMPGVMTPTLVKAMKLGH TILKLPFGVVGPFQFVEAMKGFPPNVKFPVPTGGVNLNDVAEWFEAGVQAVGVGEALNEG TPVEVAEKAKAFVEKIEGATE (SEQ ID NO:61)

he- His	(mgilpspgmpallslyslsllavllimgcvaetgt) RFPNITNLCPFGEVFNATRFASVYA WNRKRISNCVADYSVLVNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIA PGQTGKIADYNYKLPPDDFTGCVIAWNSNNLDSKVGGNINYLRLFRKSNLKPFFERDIST EIQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKK STGGSGSGSGSGSGSGSGSEKAAKAEAAARMEEELFKHEHKIVAVLRANSVEEAKKKALAVF LGGVDLIEITFTVPDADTVIKELSLFKEMGAIIGAGTVTSVEQAREAVESGAEFIVSPH LDEEISQFAKEEGVFMFGVMTPTTELVKAMKLIGHTILKLFPGEVVGQPFVEAMKGFPPFN VFFVPTGCVNLDNVAEWFEAGVQAVGVGEALNEGTPVEVAEKAKAFVEKIEGATE (GGS HHHHHHH) (SEQ ID NO:62) ETGTRFFNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLVNSASFSTFKCYGV SPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPPDDFTGCVIAWNSNNL DSKVGGNINYLRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQPT NGVGYQPYRVVLSFELLHAPATVCGPKKSTGGSGSGSGSGSGSGSEKAAKAEAAARM EELFKHEHKIVAVLRANSVEEAKKKALAVFLGGVDLIEITFTVPDADTVIKELSLFKEMG AIIGAGTVTSVEQAREAVESGAEFIVSPHLDEEISQFAKEEGVFMFGVMTPTTELVKAM KLIGHTILKLFPGEVVGQPFVEAMKGFPPFNVKFVPTGCVNLDNVAEWFEAGVQAVGVGEA LNEGTPVEVAEKAKAFVEKIEGATE (SEQ ID NO:63) ETGTRFFNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLVNSASFSTFKCYGV SPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPPDDFTGCVIAWNSNNL DSKVGGNINYLRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQPT NGVGYQPYRVVLSFELLHAPATVCGPKKSTGGSGSGSGSGSGSGSEKAAKAEAAARM EELFKHEHKIVAVLRANSVEEAKKKALAVFLGGVDLIEITFTVPDADTVIKELSLFKEMG AIIGAGTVTSVEQAREAVESGAEFIVSPHLDEEISQFAKEEGVFMFGVMTPTTELVKAM KLIGHTILKLFPGEVVGQPFVEAMKGFPPFNVKFVPTGCVNLDNVAEWFEAGVQAVGVGEA LNEGTPVEVAEKAKAFVEKIEGATE (GGSHHHHHHH) (SEQ ID NO:64) RFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLVNSASFSTFKCYGVSPTEK LNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPPDDFTGCVIAWNSNNLDSKV GGNINYLRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGY QPYRVVLSFELLHAPATVCGPKKST (X1) MEELFKHEHKIVAVLRANSVEEAKKKALA VFLGGVDLIEITFTVPDADTVIKELSLFKEMGAIIGAGTVTSVEQAREAVESGAEFIVS PHLDEEISQFAKEEGVFMFGVMTPTTELVKAMKLIGHTILKLFPGEVVGQPFVEAMKGF PNVKFVPTGCVNLDNVAEWFEAGVQAVGVGEALNEGTPVEVAEKAKAFVEKIEGATE (SEQ ID NO:65) (mgilpspgmpallslyslsllavllimgcvaetgt) RFPNITNLCPFGEVFNATRFASVYA WNRKRISNCVADYSVLVNSASFSTFKCYGVSPTEKLNLCFTNVYADSFVIRGDEVQRQIA PGQTGKIADYNYKLPPDDFTGCVIAWNSNNLDSKVGGNINYLRLFRKSNLKPFFERDIST EIQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKK ST (X1) MEELFKHEHKIVAVLRANSVEEAKKKALAVFLGGVDLIEITFTVPDADTVIKEL SLFKEMGAIIGAGTVTSVEQAREAVESGAEFIVSPHLDEEISQFAKEEGVFMFGVMTPT TELVKAMKLIGHTILKLFPGEVVGQPFVEAMKGFPPFNVKFVPTGCVNLDNVAEWFEAGVQ AVGVGEALNEGTPVEVAEKAKAFVEKIEGATE (GGSNNNNNNHHH) (SEQ ID NO:66) ETGTRFFNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLVNSASFSTFKCYGV SPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPPDDFTGCVIAWNSNNL DSKVGGNINYLRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQPT NGVGYQPYRVVLSFELLHAPATVCGPKKST (X1) MEELFKHEHKIVAVLRANSVEEAKK KALAVFLGGVDLIEITFTVPDADTVIKELSLFKEMGAIIGAGTVTSVEQAREAVESGA EFIVSPHLDEEISQFAKEEGVFMFGVMTPTTELVKAMKLIGHTILKLFPGEVVGQPFVEAM KGFPPFNVKFVPTGCVNLDNVAEWFEAGVQAVGVGEALNEGTPVEVAEKAKAFVEKIEGA TE (SEQ ID NO:67) ETGTRFFNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLVNSASFSTFKCYGV SPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPPDDFTGCVIAWNSNNL DSKVGGNINYLRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQPT NGVGYQPYRVVLSFELLHAPATVCGPKKST (X1) MEELFKHEHKIVAVLRANSVEEAKK KALAVFLGGVDLIEITFTVPDADTVIKELSLFKEMGAIIGAGTVTSVEQAREAVESGA
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		FIVSPHLDEEISQFAKEEGVYMPGVMTPTTELVKAMKLGHTILKLFEPGEVVGPPQFVEAM KGPFFNVKFPVPTGGVNLGNVAEWFEAGVQAVGVGEALNEGTPVEVAEKAKAFVEKIEGA TE{GGSHHHHHHHH} (SEQ ID NO:68)
SARS- CoV-2 2FSGA G-S- TEV- EQ- I3- G1*- secOp t- I2GS- he- His	SARS- CoV-2- I3-01 fusion protein	QCVNLTTRTQLPFPAYTNSFTTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWTFHAIHVSG TNGTKRFDNVPVLPNDGVYFASTEKSNIIRGWIIFGTTLDSEKTSQSLIVNNATNVVIKVC EFQFCNDPFLGVYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLLMDLEGKQGNFKNLR EFVFKNIDGYFKIYSKHTPINLVRDLPPQGSALPLVDLPIGINITRFQTLALHRSYL TPGDSSSSGWTAGAAAYVGYLQFRTFLKYNENGTTITDAVDCALDPLSETKCTLKSFSTV EKGIVQTSNFRVQPTESIVRFPNITNLCPFGGEVFNATRFASVYAWNRKRISNCVADYSV LYNSASFSTFKCYGVSPTKLNDLCFTNVIADSFVIRGDEVQRQIAPGQTGKIADYNYKLP DDFTGCVIAWNSNNLDSKVGNNYNYLFRKSNLKPFRDITSTIYQAGSTPCNGVEG FNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLNAPATVCGPKKSTNLVKNKCVNFNFN GLTGTGVLTESNKKFLPFPQQGGRDIADTTDAVRDPQTLEILDITECSFGGVSVITPGTN TSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGNSVVFQTRAGCLIGAETHVNNNSYEC DIPIGAGICASYQTQTNSSPSCAGSVAQSIIAYTMSLGAENSVAYSNNNSIAIPTNFTIS VTTEILPVSMTKTSVDCTMYICGDSSTCSNLLQYGSFCTQLNRALTGIAVEQDKNTQE VFAQVKQIYKTPFIKDFGGFNFSQILPDPSPKPSKRSFIEDLLFNKVTADAGFIKQYGD CLGDIARDLCAQKFNGLTVPFLPDLTDEMIAQYTSALLAGTITSGWTFGAGAAALQIPF AMQMAYRFGNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQ ALNTLVKQLSSNFGAIISSVLNDILSRIDPPEAEVQIDRLITGRLQSLQTYVTQQLIRAA EIRASANLAATKMSCEVLGQSKRVDFCGEGYHLMSEFQSAPHGVVFLHVITYVPAQEKNF TTAPAICHGKAHFFREGVFSNGTHWFVTQRNFYEPQIITDNTFVSGNCDVVIIGIVN NTVYDPLQPELDSFKEELDKYFKNHTSPDVLQDISGINASVNIQKEIDRLNEVAKNL NESLIDLQELGKYEQYIKGsgrenlyfsgggggsgyipeaprdggayvrkdgewllstf lgGSGSGSGSGSGSEKAAKAEAAAMEELFKEHKIVAVLRANSVEEAKKKALAVFLGGV DLIEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQAREAVESGAEFIVSPHLDEE ISQFAKEEGVYMPGVMTPTTELVKAMKLGHTILKLFEPGEVVGPPQFVEAMKGPFFNVKFP FTGGVNLGNVAEWFEAGVQAVGVGEALNEGTPVEVAEKAKAFVEKIEGATE (SEQ ID NO:69)
		(mgilpspgmpallsalvsllelilmgcvaetgt)QCVNLTTRTQLPFPAYTNSFTTRGVY YYPDKVFRSSVLHSTQDLFLPFFSNVTWTFHAIHVSGTNGTKRFDNVPVLPNDGVYFASTEK SNIIRGWIIFGTTLDSEKTSQSLIVNNATNVVIKVCPEFQFCNDPFLGVYHKNNKSWMESE FRVYSSANNCTFEYVSQPFLLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRD LPQGSALPLVDLPIGINITRFQTLALHRSYLTPGDSSSSGWTAGAAAYVGYLQFRT FLKYNENGTTITDAVDCALDPLSETKCTLKSFSTVEKGIYQTSNFRVQPTESIVRFPNIT NLCPFGGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCF TNVIADSFVIRGDEVQRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGNNYNY LYRLEFRKSNLKPFRDITSTIYQAGSTPCNGVEGFGNCFYFPLQSYGNFQPTNGVGYQPYRV VLSFELLNAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFPQQGGRDI ADTTDAVRDPQTLEILDITPCSFSGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQ LTPTWRVYSTGNSVVFQTRAGCLIGAETHVNNNSYECDIPIGAGICASYQTQTNSSPSCAGS VAQSIIAYTMSLGAENSVAYSNNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDS TECSNLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPFIKDFGGFNFSQI LPDPSPKPSKRSFIEDLLFNKVTADAGFIKQYGDCLGDIARDLCAQKFNGLTVPFLP LTDDEMIAQYTSALLAGTITSGWTFGAGAAALQIPFAMQMAYRFGNGIGVTQNVLYENQKLI ANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAIISSVLNDILS RLDPEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSCEVLGQSKRVDF FCGEGYHLMSEFQSAPHGVVFLHVITYVPAQEKNFTTAPAICHGKAHFFREGVFSNGT HWFVTQRNFYEPQIITDNTFVSGNCDVVIIGIVNNTVYDPLQPELDSFKEELDKYFKNH TSPDVLQDISGINASVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKGsgrenl yfsgggggsgyipeaprdggayvrkdgewllstfllgGSGSGSGSGSGSEKAAKAEAAAR MEELFKEHKIVAVLRANSVEEAKKKALAVFLGGVDLIEITFTVPDADTVIKELSFLKEM GAIIGAGTVTSVEQAREAVESGAEFIVSPHLDEEISQFAKEEGVYMPGVMTPTTELVKA MKLGHTILKLFEPGEVVGPPQFVEAMKGPFFNVKFPVPTGGVNLGNVAEWFEAGVQAVGVGE ALNEGTPVEVAEKAKAFVEKIEGATE{GGSHHHHHHHH} (SEQ ID NO:70)
		ETGTQCVNLTTRTQLPFPAYTNSFTTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWTFHAI HVSGTNGTKRFDNVPVLPNDGVYFASTEKSNIIRGWIIFGTTLDSEKTSQSLIVNNATNVV

	IKVCEPQFCNDPFLGVYHHNNKSNWMESEFRVYSSANNCTFEYVSQPFMLDLEKQGNF KNLREFVEKNIDGYFKIYSKHTPINLVRLDLPQGFSALEPLVDLPFIGINITRFQTLALH RSYLTPEGSSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLK SETVEKGIYQTSNFRVQPTESIVRFPNITNLCFFGEVFNATRFASVYAWNRRKISNCVA DYSVLVNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTKGIADYN YKLPDDFTGCVIANNNNLDSKVGGNYNYLYRLFRKSNLKPFFERDISTEYIYQAGSTPCN GVEGFNCYFFLQSYGQPTNGVGYQPYRVVVLSPFELLHAPATVCGPKKSTNLVKNKCVN FNFNGLTGTGVLTESNKKFLPFQPGRDIAADTFDAVRDPQTLLEILDITPCSFQGVSVIT PGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSSNVQTRAGCLIGAEHVNN SYECDIPIGAGICASYQTQTSFSGAGSVASQSI IAYTMSLGAENSVAYSNNSIAIPTN FTISVTEILPVSMTKTSVDCTMYICGDSSTECNLLQYGSFCTQLNRALTGIAVEQDK NTQEVPAQVKQIYKTPPIKDPGGFNFSQLPDPSPKSKRSFIEDLLFNKVTLADAGFIK QYGDCLGDIARDLCAQKFNGLTVLPLLTDEMIAQYTSALLAGTITSGWTFGAGAAL QIPFAMQMAYRENGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTASALGKLQDVVN QNAQALNTLVKQLSSNFGAISSVLNDILSRDLPPEAEVQIDRLITGRQLSLQTYVTQQL IRAAEIRASANLAATKMECVLGQSKRVDFCGKGYHLSFPQSAFHGVVFLHVTYVFAQ EKNFTTAPAICHDCKAHFFREGVVFVSNQTHWFVTQRNFYEPQIITTDNTFVSGNCDVVI GIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDISGINASVYNIQKEIDRLNEV AKNLNESLIDLQELGKYEQYIKsgrenlyfgggggsgyipeaprdggayvrkdgeowl lsfllgSGSGSGSGSGSGSEKAAKAAEEAARMEELEKHKIVAVLANSVEEAKKKALAVF LGGVDLIEITFTVPDADTVIKELSLFKEMGAIIGACTVTSVEQAREAVESGAEFIVSPH LDEEISQFAKEEGVFYMPGVMTPTELVKAMKLGHTILKLPGEVVGPGFVEAMKGFPPN VKFVPTGGVNLNDNVAEWFAGVQAVGVGEALNEGTPVEVAEKAKAFVEKIEGATE (SEQ ID NO:71) ETGTQCVNLTTTRTQLPPAYTHSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAI RVSGTNGTKRFDNFVLPFNDGVYFASTEKSNIRGWIFGTTLSKTQSLLIVNNATNVV IKVCEPQFCNDPFLGVYHHNNKSNWMESEFRVYSSANNCTFEYVSQPFMLDLEKQGNF KNLREFVEKNIDGYFKIYSKHTPINLVRLDLPQGFSALEPLVDLPFIGINITRFQTLALH RSYLTPEGSSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLK SETVEKGIYQTSNFRVQPTESIVRFPNITNLCFFGEVFNATRFASVYAWNRRKISNCVA DYSVLVNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTKGIADYN YKLPDDFTGCVIANNNNLDSKVGGNYNYLYRLFRKSNLKPFFERDISTEYIYQAGSTPCN GVEGFNCYFFLQSYGQPTNGVGYQPYRVVVLSPFELLHAPATVCGPKKSTNLVKNKCVN FNFNGLTGTGVLTESNKKFLPFQPGRDIAADTFDAVRDPQTLLEILDITPCSFQGVSVIT PGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSSNVQTRAGCLIGAEHVNN SYECDIPIGAGICASYQTQTSFSGAGSVASQSI IAYTMSLGAENSVAYSNNSIAIPTN FTISVTEILPVSMTKTSVDCTMYICGDSSTECNLLQYGSFCTQLNRALTGIAVEQDK NTQEVPAQVKQIYKTPPIKDPGGFNFSQLPDPSPKSKRSFIEDLLFNKVTLADAGFIK QYGDCLGDIARDLCAQKFNGLTVLPLLTDEMIAQYTSALLAGTITSGWTFGAGAAL QIPFAMQMAYRENGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTASALGKLQDVVN QNAQALNTLVKQLSSNFGAISSVLNDILSRDLPPEAEVQIDRLITGRQLSLQTYVTQQL IRAAEIRASANLAATKMECVLGQSKRVDFCGKGYHLSFPQSAFHGVVFLHVTYVFAQ EKNFTTAPAICHDCKAHFFREGVVFVSNQTHWFVTQRNFYEPQIITTDNTFVSGNCDVVI GIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDISGINASVYNIQKEIDRLNEV AKNLNESLIDLQELGKYEQYIKsgrenlyfgggggsgyipeaprdggayvrkdgeowl lsfllgSGSGSGSGSGSGSEKAAKAAEEAARMEELEKHKIVAVLANSVEEAKKKALAVF LGGVDLIEITFTVPDADTVIKELSLFKEMGAIIGACTVTSVEQAREAVESGAEFIVSPH LDEEISQFAKEEGVFYMPGVMTPTELVKAMKLGHTILKLPGEVVGPGFVEAMKGFPPN VKFVPTGGVNLNDNVAEWFAGVQAVGVGEALNEGTPVEVAEKAKAFVEKIEGATE (GGS HHHHHHHH) (SEQ ID NO:72) QCVNLTTTRTQLPPAYTHSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSG TNGTKRFDNFVLPFNDGVYFASTEKSNIRGWIFGTTLSKTQSLLIVNNATNVV EFQFCNDPFLGVYHHNNKSNWMESEFRVYSSANNCTFEYVSQPFMLDLEKQGNFKNL EFVFKNIDGYFKIYSKHTPINLVRLDLPQGFSALEPLVDLPFIGINITRFQTLALHRSYL TFGSSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLKSTFTV RKGIYQTSNFRVQPTESIVRFPNITNLCFFGEVFNATRFASVYAWNRRKISNCVADYSV LYNASASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTKGIADYNKLP DDFTGCVIANNNNLDSKVGGNYNYLYRLFRKSNLKPFFERDISTEYIYQAGSTPCNGVEG
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	FNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNEN GLTGTGVLTESSNEKFLPFQQFGPDIAADTTDAVRDPQTEILIDITPCSPGGVSVITPGTEN TSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFPQTRAGCLIGAEHVNSYEC DIPIGAGICASYQTQTNPSGAGSVASQSI IAYTMSLGAENSVAYSNNNSIAIPTNFTIS VTEILPVSMTKTSVDCTMYICGDSSTECNLLQYGSFCTQLNRALTGIAVEQDKNTQE VFAQVKQIYKTPPIKDFGGFNFQSQILPDPFSKPSKRSFIEDLLFNKVTLADAGFIKQYGD CLGDI AARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPF AMQMAYRFNGIGVTQNVLYENQKLIANQFNNSAIGKIQDLSSTASALGKLQDVVNQNAQ ALNTLVKQLSSNFGAISSVLNDILSRDPPFAEVQIDRLITGRQLSLQTYVTQQLIRAA EIRASANLAATKMSCEVLGQSKRVDFCGKGYHLMSPQSAPHGVVFLHVTVYVPAQEKNF TTAPAICHGDKAHFPREGVFEVSNGTWTFVTQRNFYEPQIITDNTFVSGNCDVVGIVN NTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDISGINASVUNIQKEIDRLNEVAKNL NESLIDLQELGKYEQYIKsgrenlyfqqggsggyipeaprdggayvrkdgewllstf lg (X1) MEELFKEHKIIVAVLRANSVEEAKKALAVFLGGVDLIEITFTVPDADTVIKEL SFLKEMGAIIGAGTVTSVEQAREAVESGAEFIVSPHLDEEISQFAKEEGVFMFGVMT TELVKAMKLGHTILKLPFGEVVGPFVEAMRGFFPNVKFVFTGGVNLNVAEMFEAGVQ AVGVGEALNEGTFVEVAEAKAFVEKIEGATE (SEQ ID NO:73) (mgilpspgmpallsllsllsvllmgcvaetgt) QCVNLTTRTQLPPAYTNSFTRGVY PDKVFRSSVLHSTQDLFLPFFFSNVTFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEK SNIIRGWIFGTTLDSEKTSLLIVNNATNVVIVKCEFPQCNDFPLGVYHKNKNSWMESE FRVYSSANNCTFEYVSQPFLLMDLEGKQGNFRNLREFVFNKIDGYFKIYSKHTPINLVRD LPQGFSALEFLVDLPICINITRFQTLALHPSYLTGPDSSSGWTAGAAAYVGYLQPRF FLKYNENGTTITDAVDCALDPLSETKCTLRSEFTVEKGIYQTSNFRVQPTESIVRFPNIT NLCPFGEVFNATRFASVYANNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCF TNVYADSFVIRGDEVQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNINY LYRLFRKSNLKPFFERDISTEIIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRV VVLGSELLAAPATVCGPKKSTNLVKNKCVNFNENGLTGTGVLTESSNEKFLPFQQFGPD ADTTDAVRDPQTEILIDITPCSPGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQ LTPTWRVYSTGSNVFPQTRAGCLIGAEHVNSYECDIPIGAGICASYQTQTNPSGAGSV ASQSI IAYTMSLGAENSVAYSNNNSIAIPTNFTISVTEILPVSMTKTSVDCTMYICGDS TECSNLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFQSQI LPDPFSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDI AARDLICAQKFNGLTVLPPL LTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLI ANQFNNSAIGKIQDLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILS RLDPPFAEVQIDRLITGRQLSLQTYVTQQLIRAAEIRASANLAATKMSCEVLGQSKRV FCGKGYHLMSPQSAPHGVVFLHVTVYVPAQEKNFTTAPAICHGDKAHFPREGVFEVSNGT WTFVTQRNFYEPQIITDNTFVSGNCDVVGIVNNTVYDPLQPELDSFKEELDKYFKNH TSPDVLGDISGINASVUNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKsgrenl yfqqggsggyipeaprdggayvrkdgewllstflg (X1) MEELFKEHKIIVAVLRANSV EEAKKALAVFLGGVDLIEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQAREAV ESGAEFIVSPHLDEEISQFAKEEGVFMFGVMTPTTELVKAMKLGHTILKLPFGEVVGPF FVEAMRGFFPNVKFVFTGGVNLNVAEMFEAGVQAVGVGEALNEGTFVEVAEAKAFVE KIEGATE (GGSHHHHHHHH) (SEQ ID NO:74) ETGTQCVNLTTRTQLPEAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFFSNVTFHAI HVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIRGWIFGTTLDSEKTSLLIVNNATNVV IVKCEFPQCNDFPLGVYHKNKNSWMESEFRVYSSANNCTFEYVSQPFLLMDLEGKQGNF ANLREFVFNKIDGYFKIYSKHTPINLVRDLPQGFSALEFLVDLPICINITRFQTLALH PSYLTGPDSSSGWTAGAAAYVGYLQPRFLLKYNENGTTITDAVDCALDPLSETKCTLK SEFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYANNRKRISNCV ADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQIAPGQTGKIADY NYKLPDDFTGCVIAWNSNNLDSKVGGNINYLYRLFRKSNLKPFFERDISTEIIYQAGSTPCN GVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVN FNFENGLTGTGVLTESSNEKFLPFQQFGPDIAADTTDAVRDPQTEILIDITPCSPGGVSVIT PGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFPQTRAGCLIGAEHVNS SYECDIPIGAGICASYQTQTNPSGAGSVASQSI IAYTMSLGAENSVAYSNNNSIAIPTN FTISVTEILPVSMTKTSVDCTMYICGDSSTECNLLQYGSFCTQLNRALTGIAVEQDK NTQEVFAQVKQIYKTPPIKDFGGFNFQSQILPDPFSKPSKRSFIEDLLFNKVTLADAGFIK QYGDCLGDI AARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAAL
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		QIPFAMQMA YRFNGIGVTQNVLYENQKLIANQFN SAIGKIQDSLSTASALGKLQDVVN QNAQALNTLVKQLSSNFGA ISSV LNDILSR LDPPEAEVQIDRLITGR LQSLQTYVTQQL IRAAEIRASANLAATKMS ECVLGQSKRVDFCGKGYHLMSPFQSAPHGVVFLHVTYVPAQ EKNFTTAPAICHGDKASFPREGV FVSNGT HWFVTQRNFYEPQIITDNTFVSGNCDVVI GIVNNTVYDPLQPELDSFKEELDKYFKNHTSPD VDLGDISGINASV VNIQKEIDRLNEV AKNLNESLIDLQELGKYEQYIKgsgrenlyfqggggsgyipeaprdggayvrkdge wvl lstflg(XI)MEELFKRAKIVAVLRANSVEEAKKKALAVFLGGVDLIEITFTVPDADTV IKELSPFLKEMGAIIGAGTVTSVEQAREAVESGAEFIVSFLDDEIISQFAKEEGVFTYMPG VMTPTLVLKAMKLGHTILKLFPGEVVGPQFV EAMKGPFFENVKVFVTGGVNLNDVAEWFE AGVQAVGVGEALNEGTPVEVAEKAKAFVEKIEGATE (SEQ ID NO:75) ETGTQCVNLTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAI HVS GGTNGTKRFDNPFVLPFNDGVYFAST EKSNIRGWIFGTTLDSEKTSLLIIVNNATNVV IKVCEFPQFCNDPFLGVYHKNKNSWMESEFRVYSSANNCTFEYVSQPFMLDLEGKQGNF KNLRE FVFKNIDGYFKIY3KHTPINLV RDLPQGFSALEPLVDLPIGINITRFQTLALH RSYLT PGDSSSGWTAGAAAYVGYLQPTFLLKYNENG TITDAVDCALDPLSEKCTLK SFTV EKG IYQTSNFRVQPTESIVRFPNITNLCPFGGEVFNATRFASVYAMNRRISNCA DYSVL YNSASFSTFKCYGVSP TKLNDLCFTNVYADSFVIRGDEV RQIAPGQTGKIADYN YKLPDDFTGCVIAMNSNNLDSKVG GNYNYLYR LFRKSNLKPFERDISTE IYQAGSTPCN GVEG ENCYFPLQSYGFPQTNGVGYQPYRVV VLSFELLHAPATVCGPKKSTNLVKNKCVN FNFNGLTGTGVLTESNKKFLPFQ QFG RDIADTTDAVRDPQTLEILDITPCSF GGVSVIT PGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTG SNVFTQTRAGCLIGAEHVNN SYEC DIFIGAGICASYQTQTNSPFGAGSVASQSI IAYTMSLGAENSVAYSNNISIAIPTN FTISV TTEILPVSMTKTSVLDCTMY ICGDSTEC SNLLQYGSFCTQLNRALTGIAVEQDK NTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDSPSKPKRSFIEDLLFNKVT LADAGFIK QYGDCLGDIAARDLICAQKFNGLTVLFP LLTDEMIAQYTSALLAGTITSGWTFGAGAAL QIPFAMQMA YRFNGIGVTQNVLYENQKLIANQFN SAIGKIQDSLSTASALGKLQDVVN QNAQALNTLVKQLSSNFGA ISSV LNDILSR LDPPEAEVQIDRLITGR LQSLQTYVTQQL IRAAEIRASANLAATKMS ECVLGQSKRVDFCGKGYHLMSPFQSAPHGVVFLHVTYVPAQ EKNFTTAPAICHGDKASFPREGV FVSNGT HWFVTQRNFYEPQIITDNTFVSGNCDVVI GIVNNTVYDPLQPELDSFKEELDKYFKNHTSPD VDLGDISGINASV VNIQKEIDRLNEV AKNLNESLIDLQELGKYEQYIKgsgrenlyfqggggsgyipeaprdggayvrkdge wvl lstflg(XI)MEELFKRAKIVAVLRANSVEEAKKKALAVFLGGVDLIEITFTVPDADTV IKELSPFLKEMGAIIGAGTVTSVEQAREAVESGAEFIVSFLDDEIISQFAKEEGVFTYMPG VMTPTLVLKAMKLGHTILKLFPGEVVGPQFV EAMKGPFFENVKVFVTGGVNLNDVAEWFE AGVQAVGVGEALNEGTPVEVAEKAKAFVEKIEGATE (GGSNNHHHHHHH) (SEQ ID NO:76)
SARS- CoV-2 2PSGA G-S- I3- Q1*- secOp t- 12GS- he- His	SARS- CoV-2- I3-Q1 fusion protein	QCVNLTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVS TNGTKRFDNPFVLPFNDGVYFAST EKSNIRGWIFGTTLDSEKTSLLIIVNNATNVV IKVCEFPQFCNDPFLGVYHKNKNSWMESEFRVYSSANNCTFEYVSQPFMLDLEGKQGNF KNLRE FVFKNIDGYFKIY3KHTPINLV RDLPQGFSALEPLVDLPIGINITRFQTLALH RSYLT PGDSSSGWTAGAAAYVGYLQPTFLLKYNENG TITDAVDCALDPLSEKCTLK SFTV EKG IYQTSNFRVQPTESIVRFPNITNLCPFGGEVFNATRFASVYAMNRRISNCA DYSVL YNSASFSTFKCYGVSP TKLNDLCFTNVYADSFVIRGDEV RQIAPGQTGKIADYN YKLPDDFTGCVIAMNSNNLDSKVG GNYNYLYR LFRKSNLKPFERDISTE IYQAGSTPCN GVEG ENCYFPLQSYGFPQTNGVGYQPYRVV VLSFELLHAPATVCGPKKSTNLVKNKCVN FNFNGLTGTGVLTESNKKFLPFQ QFG RDIADTTDAVRDPQTLEILDITPCSF GGVSVIT PGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTG SNVFTQTRAGCLIGAEHVNN SYEC DIFIGAGICASYQTQTNSPFGAGSVASQSI IAYTMSLGAENSVAYSNNISIAIPTN FTISV TTEILPVSMTKTSVLDCTMY ICGDSTEC SNLLQYGSFCTQLNRALTGIAVEQDK NTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDSPSKPKRSFIEDLLFNKVT LADAGFIK QYGDCLGDIAARDLICAQKFNGLTVLFP LLTDEMIAQYTSALLAGTITSGWTFGAGAAL QIPFAMQMA YRFNGIGVTQNVLYENQKLIANQFN SAIGKIQDSLSTASALGKLQDVVN QNAQALNTLVKQLSSNFGA ISSV LNDILSR LDPPEAEVQIDRLITGR LQSLQTYVTQQL IRAAEIRASANLAATKMS ECVLGQSKRVDFCGKGYHLMSPFQSAPHGVVFLHVTYVPAQ EKNFTTAPAICHGDKASFPREGV FVSNGT HWFVTQRNFYEPQIITDNTFVSGNCDVVI GIVNNTVYDPLQPELDSFKEELDKYFKNHTSPD VDLGDISGINASV VNIQKEIDRLNEV AKNLNESLIDLQELGKYEQYIKgsgrenlyfqggggsgyipeaprdggayvrkdge wvl lstflg(XI)MEELFKRAKIVAVLRANSVEEAKKKALAVFLGGVDLIEITFTVPDADTV IKELSPFLKEMGAIIGAGTVTSVEQAREAVESGAEFIVSFLDDEIISQFAKEEGVFTYMPG VMTPTLVLKAMKLGHTILKLFPGEVVGPQFV EAMKGPFFENVKVFVTGGVNLNDVAEWFE AGVQAVGVGEALNEGTPVEVAEKAKAFVEKIEGATE (GGSNNHHHHHHH) (SEQ ID NO:76)

	AVESGAEFIVSPHLDEEISQFAKEEGVYFMPGVMTPTLVKAMKLGHTILKLFPGSEVVG PQFVEAMKGPFPNVKFPVFTGGVNLDNVAEWFEAGVQAVGVGEALNEGTPFEVEAEKAKAF VEKIEGATE (SEQ ID NO:77) (mgilpspgmpallsivslsvllimgcvaetgt)QCVNLTTTRTQLPPAYTNSFTRGVYY FDKVFSSSVLHSTQDLFLFFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEK SNIIRGWIFGTTLDSKTQSLIVNNATNVYIKVCEPQFCNDPFLGVYVHNNKSWMESE FRVYSSANNCTFEYVSQFFLMDLEGKQGNFKNLREFVFNIDGYFKIYSKHTPINLVRD LPQGFSALEPLVDLPICGINITRFQTLALHRSYLTFGDSSSGWTAGAAAYVGYLQFRT FLLKYNENGTITDAVDCALDPLSETKCTLKSTFVEKGIYQTSNFRVQPTESIVRFPNIT NLCFFGEVFNATRFASVYAWNKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCF TNVYADSFVIRGDEVQIAPGQTGKIADYNWYKLPDDFTGCVIAWNSNNLDSKVGSGNYY LYRLFRKSNLKPFRDISTEIQAGSTPCNGVEGFNCYFPLQSYGQPTNGVGYQPYRV VVLSPFELLHAFATVCGPKKSTNLVKNKCVNPNFNGLTGTGVLTESNKKFLPFQFGRDI ADTTDAVRDPQTLLEILDTFCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQ LPTWRVYSTGSNVFTQTRAGCLIGAEHVNNSEODIPIGAGICASYQTQTSNPSGAGSV ASQSI IAYTMSLGAENSVAYSNNSIAIPTNFISVTTTILPVSMKTSVDCMTYICGDS TECSNLLQYGSFCTQLNRLTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGSGNF3QI LPDPSKPSKRSFIEDLLENKVTILADAGFIKQYGDCLGDIAARDLICAGKFNGLTVLPFL LTDEMIAQYTSALLAGTITSGWTFGAGAAALQIPFAMQMAXRFNGIGVTONVLYENQKLI ANQFNSAIGKIQDLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAIBSVLNDILS RLDPPEAEVQIDRLITGRLOSLQTYVTQQLIRAAEIRASANLAATEMSECVLGQSKFVD FCGKGYHLSFFQSAPHGUVFLHVTYVPAQEKMTTAPAI CHDGKAHFPEGVFVSNGT HWFVTQRNFYEPQIIITDNTFVSGNCDVVI GIVNNTVYDPLQPELDSFKEELDNYFKNH TSPDVLGDISGINASVYVNIQKEIDRLNEVAKNLNESLIDLQELGKYEYIIGSGSGGS GGSGSEKAAKAEAAARKMEELFKHKIVAVLRANSVEEAKKALAVFLGGVDLIEITFT VPDADTVIKELSFLEMGAIIGAGTVTSVEQAREAVESGAEFIVSPHLDEEISQFAKEE GVYFMPGVMTPTLVKAMKLGHTILKLFPGSEVVG PQFVYAMKGPFPNVKFPVFTGGVNL DNVAEWFEAGVQAVGVGEALNEGTPFEVEAEKAKAFVEKIEGATE (GGSRRRRHHHH) (SEQ ID NO:78) ETGTQCVNLTTTRTQLPPAYTNSFTRGVYYFDKVFSSSVLHSTQDLFLFFFSNVTWFHAI HVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLIVNNATNVY IKVCEPQFCNDPFLGVYVHNNKSWMESEFRVYSSANNCTFEYVSQFFLMDLEGKQGNF KNLREFVFNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPICGINITRFQTLALH RSYLTFGDSSSGWTAGAAAYVGYLQFRTFLLKYNENGTITDAVDCALDPLSETKCTLK STFVEKGIYQTSNFRVQPTESIVRFPNITNLCFFGEVFNATRFASVYAWNKRISNCVA DYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQIAPGQTGKIADYN YKLPDDFTGCVIAWNSNNLDSKVGSGNYYLYRLFRKSNLKPFRDISTEIQAGSTPCN GVEGFNCYFPLQSYGQPTNGVGYQPYRVVVLSPFELLHAFATVCGPKKSTNLVKNKCVN PNFNGLTGTGVLTESNKKFLPFQFGRDIADTTDAVRDPQTLLEILDTFCSFGGVSVIT PGTNTSNQVAVLYQDVNCTEVPVAIHADQLPTWRVYSTGSNVFTQTRAGCLIGAEHVNN SYECDIPIGAGICASYQTQTSNPSGAGSVASQSI IAYTMSLGAENSVAYSNNSIAIPTN FTISVTTTILPVSMKTSVDCMTYICGDS TECSNLLQYGSFCTQLNRLTGIAVEQDK NTQEVFAQVKQIYKTPPIKDFGSGNF3QILPDPSKPSKRSFIEDLLENKVTILADAGFIK QYGDCLGDIAARDLICAGKFNGLTVLPFLLTDEMIAQYTSALLAGTITSGWTFGAGAAAL QIPFAMQMAXRFNGIGVTONVLYENQKLIANQFNSAIGKIQDLSSTASALGKLQDVVN QNAQALNTLVKQLSSNFGAIBSVLNDILSRLDPPEAEVQIDRLITGRLOSLQTYVTQQL IRAAEIRASANLAATEMSECVLGQSKFVD FCGKGYHLSFFQSAPHGUVFLHVTYVPAQ EKNFTTAPAI CHDGKAHFPEGVFVSNGTHWFVTQRNFYEPQIIITDNTFVSGNCDVVI GIVNNTVYDPLQPELDSFKEELDNYFKNH TSPDVLGDISGINASVYVNIQKEIDRLNEV AKNLNESLIDLQELGKYEYIIGSGSGSGSGSGSEKAAKAEAAARKMEELFKHKIVAV LRANSVEEAKKALAVFLGGVDLIEITFTVPDADTVIKELSFLEMGAIIGAGTVTSVE QAREAVESGAEFIVSPHLDEEISQFAKEEGVYFMPGVMTPTLVKAMKLGHTILKLFPG SEVVG PQFVYAMKGPFPNVKFPVFTGGVNLDNVAEWFEAGVQAVGVGEALNEGTPFEVEAEK AKAFVEKIEGATE (SEQ ID NO:79) ETGTQCVNLTTTRTQLPPAYTNSFTRGVYYFDKVFSSSVLHSTQDLFLFFFSNVTWFHAI HVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLIVNNATNVY IKVCEPQFCNDPFLGVYVHNNKSWMESEFRVYSSANNCTFEYVSQFFLMDLEGKQGNF
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	KNLREFVFKNIDGYFKIYSKHTPINLVRDLFQGFSALEFLVDLPIGINITRFQTLALH RSYLTFGDSSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLK SFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGGEVFNATRFASVYAWNRKRISNCVA DYSVLVNSASFSTFKCYGVSPFKLNDLCFTNVYADSFVIRGDEVQRQIAPGGTGKIADYN YKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKFPERDISTEIIYQAGSTFCN GVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVN FNFNGLTGTGVLTSNKKFLPFQGFGRDIADTTDAVRDPQTLEILDITPCSPGGVSVIT PGTNTSNQVAVLYQDVNCTEVFPVAIHADQLTPTWRVYSTGSGNVFQTRAGCLICAEHVNN SYECDIFIGAGICASYQTQTNSTSPGAGSVASQSI IAYTMSLGAENSVAYSNNSTAIPTN FTISVTEILPVSMTKTSVDCTMYICGDESTECNLLQYGSFCTQLNRALTGIAVEQDK NTQEVEFAQVKIYKTPFIKDFGGFNFSQILPDPSPKSKRSFIEDLLFNKVTLLADAGFIK QYGDCLGDI AARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAAL QIPFAMQMAIRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTASALGKLQDVVN QNAQALNTLVKQLSSNFGAIISSVLNDILSRDLPPEAEVQIDRLITGRLQSLQTYVTQQL IRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSPFQSAFHGVVFLHVTVYPAQ EKNFTTAPAICHHDGKAHFPREGVFSNGTHWFTQKNFYEPQIITTDNFTFVSGNCDVVI GIVNNTVYDPLQFELDSFKEELDKYFKNHTSPDVOLGDISGINASVUNIQKEIDRLNEV AKNLNESLIDLQELGKYEQYIKGSGSGSGSGSGSEKAAKAEAAAPKMEELFKKHIVAV LRANSVEEAKKKALAVFLGGVDLIEITFTVPDADTVIKELSFLEKMGAIIGACTVTSVE QAREAVESGAEFIVSPHLDEEISQFAKEEGVFYMPGVMTPTTELVKAMKLGHTILKLFPG EYVGPQFVEAMKGFPPNVKFFVPTGCVNLDNVAEWFEAGVQAVGVGEALNEGTPEVEAEK AKAEVEKIEGATE (GSGHHHHHHHH) (SEQ ID NO:80) QCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFAIHVSG TNGTKRFDNPFVLFNDGVYFASTEKSNIIIRGWIFGTTLDSKTQSLIIVNNATNVVIKVC EFQFONDFFLGVIYHKNKSNWSESEFRVYSSANNCTFEYVSQPFLLMDLEGKQGNFNKLR RNVFKNIDGYFKIYSKHTPINLVRDLFQGFSALEFLVDLPIGINITRFQTLALHRSYL TFGDSSSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETEKCTLKSTV RKGIYQTSNFRVQPTESIVRFPNITNLCPFGGEVFNATRFASVYAWNRKRISNCVADYSV LYNSASFSTFKCYGVSPFKLNDLCFTNVYADSFVIRGDEVQRQIAPGGTGKIADZNYKLP DDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKFPERDISTEIIYQAGSTFCNGVEG FNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFN GLTGTGVLTSNKKFLPFQGFGRDIADTTDAVRDPQTLEILDITPCSPGGVSVITPGTN TSNQVAVLYQDVNCTEVFPVAIHADQLTPTWRVYSTGSGNVFQTRAGCLICAEHVNNSEY CDIFIGAGICASYQTQTNSTSPGAGSVASQSI IAYTMSLGAENSVAYSNNSTAIPTNFTIS VTEILPVSMTKTSVDCTMYICGDESTECNLLQYGSFCTQLNRALTGIAVEQDKNTQE VFAQVKIYKTPFIKDFGGFNFSQILPDPSPKSKRSFIEDLLFNKVTLLADAGFIKQYGD CLGDI AARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPF AMQMAIRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTASALGKLQDVVNQNAQ ALNTLVKQLSSNFGAIISSVLNDILSRDLPPEAEVQIDRLITGRLQSLQTYVTQQLIRAA EIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSPFQSAFHGVVFLHVTVYPAQEKNF TTAPAICHHDGKAHFPREGVFSNGTHWFTQKNFYEPQIITTDNFTFVSGNCDVVI GIVNNTVYDPLQFELDSFKEELDKYFKNHTSPDVOLGDISGINASVUNIQKEIDRLNEVAKNL NESLIDLQELGKYEQYIK (X1) MEELFKKHIVAVLRANSVEEAKKKALAVFLGGVDLIE ITFTVPDADTVIKELSFLEKMGAIIGACTVTSVEQAREAVESGAEFIVSPHLDEEISQ FAKEEGVFYMPGVMTPTTELVKAMKLGHTILKLFPGEVVGPQFVEAMKGFPPNVKFFVPTG CVNLDNVAEWFEAGVQAVGVGEALNEGTPEVEAEKAKAEVEKIEGATE (SEQ ID NO:81) (mgilpspgmpallsivslsvllmgcvaetgt)QCVNLTTRTQLPPAYTNSFTRGVYYP DKVFRSSVLHSTQDLFLPFFSNVTWFAIHVSGTNGTKRFDNPFVLFNDGVYFASTEK SNIIIRGWIFGTTLDSKTQSLIIVNNATNVVIKVC EFQFONDFFLGVIYHKNKSNWSESE FRVYSSANNCTFEYVSQPFLLMDLEGKQGNFNKLRREFVFKNIDGYFKIYSKHTPINLVRD LPQGFSALEFLVDLPIGINITRFQTLALHRSYLTFGDSSSGWTAGAAAYVGYLQPRTF LLKYNENGTITDAVDCALDPLSETKCTLKSTFTVEKGIYQTSNFRVQPTESIVRFPNIT NLCPFGGEVFNATRFASVYAWNRKRISNCVADYSVLVNSASFSTFKCYGVSPFKLNDLCF TNVYADSFVIRGDEVQRQIAPGGTGKIADZNYKLPDDFTGCVIAWNSNNLDSKVGGNYNY LYRLFRKSNLKFPERDISTEIIYQAGSTFCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRV VLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTSNKKFLPFQGFGRDI ADTTDAVRDPQTLEILDITPCSPGGVSVITPGTNTSNQVAVLYQDVNCTEVFPVAIHADQ
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	LPTWRYVYSTGNSVFTQTRAGCLIGAEHVNNSEYECDIPIGAGICASYQTQTNPSGAGSV ASQSI IAYTMSLGAENSVAYSNNSIAIPTNFTISVTTTEILPVSMTESTVSDCTMYICGDS TECSNLLQYGSFCTQNLNALTGIAVEQDKNTQEVFAQVVKQIYKTPPIKDFGCGNFNSQI LPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIARDLICAQKFNGLTVLPPL LTDEMIQYTSALLAGTITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLI ANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISVNLNDILS RLDPPEAEVQIDRLITGRQLSLQTYVTQQLIRAAEIRASANLAATKMSCEVLGQSKRV FCGKGYHLMSPQSAFHGVVFLHVTYVFAQEKNTTAPAI CHDGKAHFFREGVVFVSNGT RWFVTQRNFYEPQIITTDNTFVSGNCDVVI GIVNNTVYDPLQPELDSFKEELDXYFKNH TSPDVLGDISGINASVNNIQKEIDRLNEVAKNLESIDLQELGKYEQYIK (X1) MEE LFKEHKIVAVLRANSVEEAKKALAVFLGGVDLIEITFTVPDADTVIKELSFLKEMGAI IGACTVTSVEQAREAVESGAEFIVSPHLDEEISQFAKEEGVFYMPGVMTPTLVKAMKL GHTILKLPGEVVGQPFVEAMKGPFPNVKFPVPTGGVNLDNVAEWFEAGVQAVGVGEALN EGTPEVAEAKAKAFVEKIEGATE (GGSHHHHHHHH) (SEQ ID NO:82) ETGTQCVNLTTRTQLFPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTFWFAI HVSCTNGTKRFDNVLFPNDGVYFASTEKSNIRGWIFGTTLSKTSQSLIIVNNATNVV IKVCEPQFCNDPFLGVYHKNKNSWMESEFVYSSANNCTFEYVSQPFILMDLEGKQGNF KNLREPVFNIDGYFKIYKNTPIINLVKDLPGQFSALEFLVDLPFIGINITRFQTLALH RSYLTFGDSSSGWTAGAAAYVGYLQPTFLLKYNENGTITDAVDCALDFLSFTKCTLK SFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGVEFNATRFASVYAWNRKRISNCVA DYSVLVNSASFSTFRCYGVSPTKLNDLCFTNVYADSFVINGDEVQRQIAPGQTKIADYN YKLPDDFTGCVIANNNNLDSKVGNNYLYLRLFRKSNLKPFERDISTEIIYQAGSTPCN GVEGFNCYFPLQSYGQFTNGVGYQPIRVVVLSEFLLHAPATVCGPKKSTNLVKNKCVN FNFENGLTGTGVLTESNKKFLPFQGFGRDIADTTDAVRDEQTLBILDITPCSFGGVSVIT PGTNTSNQVAVLYQDVNCTEVFVAIHADQLTPTWRYVYSTGNSVFTQTRAGCLIGAEHVNN SYECDIPIGAGICASYQTQTNPSGAGSVASQSI IAYTMSLGAENSVAYSNNSIAIPTN FTISVTTTEILPVSMTESTVSDCTMYICGDS TECSNLLQYGSFCTQNLNALTGIAVEQDK NTQEVFAQVVKQIYKTPPIKDFGCGNFNSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIK QYGDCLGDIARDLICAQKFNGLTVLPFLLTDEMIQYTSALLAGTITSGWTFGAGAAL QIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVN QNAQALNTLVKQLSSNFGAISVNLNDILSRLOPPEAEVQIDRLITGRQLSLQTYVTQQL IRAAEIRASANLAATKMSCEVLGQSKRVDFCGKGYHLMSPQSAFHGVVFLHVTYVFAQ EKNFTTAPAI CHDGKAHFFREGVVFVSNGTWFWVTQRNFYEPQIITTDNTFVSGNCDVVI GIVNNTVYDPLQPELDSFKEELDXYFKNNTSFDVLGDISGINASVNNIQKEIDRLNEV AKNLESIDLQELGKYEQYIK (X1) MEELFKEHKIVAVLRANSVEEAKKALAVFLGG VDLIEITFTVPDADTVIKELSFLKEMGAIIGACTVTSVEQAREAVESGAEFIVSPHLDE EISQFAKEEGVFYMPGVMTPTLVKAMKLGHTILKLPGEVVGQPFVEAMKGPFPNVKFP VPTGGVNLDNVAEWFEAGVQAVGVGEALNEGTPEVAEAKAKAFVEKIEGATE (SEQ ID NO:83) ETGTQCVNLTTRTQLFPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTFWFAI HVSCTNGTKRFDNVLFPNDGVYFASTEKSNIRGWIFGTTLSKTSQSLIIVNNATNVV IKVCEPQFCNDPFLGVYHKNKNSWMESEFVYSSANNCTFEYVSQPFILMDLEGKQGNF KNLREPVFNIDGYFKIYKNTPIINLVKDLPGQFSALEFLVDLPFIGINITRFQTLALH RSYLTFGDSSSGWTAGAAAYVGYLQPTFLLKYNENGTITDAVDCALDFLSFTKCTLK SFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGVEFNATRFASVYAWNRKRISNCVA DYSVLVNSASFSTFRCYGVSPTKLNDLCFTNVYADSFVINGDEVQRQIAPGQTKIADYN YKLPDDFTGCVIANNNNLDSKVGNNYLYLRLFRKSNLKPFERDISTEIIYQAGSTPCN GVEGFNCYFPLQSYGQFTNGVGYQPIRVVVLSEFLLHAPATVCGPKKSTNLVKNKCVN FNFENGLTGTGVLTESNKKFLPFQGFGRDIADTTDAVRDEQTLBILDITPCSFGGVSVIT PGTNTSNQVAVLYQDVNCTEVFVAIHADQLTPTWRYVYSTGNSVFTQTRAGCLIGAEHVNN SYECDIPIGAGICASYQTQTNPSGAGSVASQSI IAYTMSLGAENSVAYSNNSIAIPTN FTISVTTTEILPVSMTESTVSDCTMYICGDS TECSNLLQYGSFCTQNLNALTGIAVEQDK NTQEVFAQVVKQIYKTPPIKDFGCGNFNSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIK QYGDCLGDIARDLICAQKFNGLTVLPFLLTDEMIQYTSALLAGTITSGWTFGAGAAL QIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVN QNAQALNTLVKQLSSNFGAISVNLNDILSRLOPPEAEVQIDRLITGRQLSLQTYVTQQL IPAAEIRASANLAATKMSCEVLGQSKRVDFCGKGYHLMSPQSAFHGVVFLHVTYVFAQ EKNFTTAPAI CHDGKAHFFREGVVFVSNGTWFWVTQRNFYEPQIITTDNTFVSGNCDVVI
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		GIVNNTVYDPLQPELDSFKEELDKEYFNHTSPDVLGDISGINASVNNIQKEIDRLNEV AKNLNESLIDLQELGRYQYIK (X1) MEELFKKHKIVAVLRANSVEEAKKKALAVFLGG VDLIEITFTVPDADTVIKELSPLEKMGAIIGACTVTSVEQAREAVESGAEFIVSPHLDE EISQFAKEKGVFYMPGVMTPTTELKAMKLGHTILKLFPGEVVGPQFVKAMKGPFPNVK VPTGGVNLNDNVAEWFEAGVQAVGVGEALNEGTFVEVAEKAKAFVEKIRGATE (GGSHHH HHHHH) (SEQ ID NO:84)
SARS- CoV-2 RED- I53- 50A*- 12GS- he- His	SARS- CoV-2- I53-50A fusion protein	RFPNITNLCPPGEVFNATRFASVYAWNRKRISNCVADYSVLVNSASFSTFKCYGVSPSTK LNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLEDDFTGCVIANNNNLDSKV GGNYNYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCFYFPLQSYGFQPTNGVG VQPYRVVVLSPFELLHAPATVCGPKKST (X1) KMEELFKKHKIVAVLRANSVEEAEKAV AVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGACTVTSVEQARKAVESGAEFIV SPHLDEEISQFAKEKGVFYMPGVMTPTTELKAMKLGHTILKLFPGEVVGPQFVKAMKGP FPNVKVFVPTGGVNLNDNVAEWFRAGVLAVGVGSALVGTPTDEVREKAKAFVEKIRGATE SEQ ID NO:167 (mgilpspgmpallsllvslsllimgcvaetgt) RFPNITNLCPPGEVFNATRFASVYA WNRKRISNCVADYSVLVNSASFSTFKCYGVSPSTKLNDLCFTNVYADSFVIRGDEVQRQIA PGQTGKIADYNYKLEDDFTGCVIANNNNLDSKVGGNYNYLYRLFRKSNLKPFFERDIST EIQAGSTPCNGVEGFNCFYFPLQSYGFQPTNGVGYPYRVVVLSPFELLHAPATVCGPKK ST (GGSGGGSGGGSGGSEKAARAEAAAR) KMEELFKKHKIVAVLRANSVEEAEKAVAVFA GGVHLIEITFTVPDADTVIKALSVLKEKGAIIGACTVTSVEQARKAVESGAEFIVSPHL DEEISQFAKEKGVFYMPGVMTPTTELKAMKLGHTILKLFPGEVVGPQFVKAMKGPFPNV KVFPTGGVNLNDNVAEWFRAGVLAVGVGSALVKCTPDEVREKAKAFVEKIRGATE (GGSH HHHHHHH) SEQ ID NO:168
SARS- CoV-2 RED- I53- 50A*- 16GS- he- His	SARS- CoV-2- I53-50A fusion protein	RFPNITNLCPPGEVFNATRFASVYAWNRKRISNCVADYSVLVNSASFSTFKCYGVSPSTK LNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLEDDFTGCVIANNNNLDSKV GGNYNYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCFYFPLQSYGFQPTNGVG XQPYRVVVLSPFELLHAPATVCGPKKST (X1) KMEELFKKHKIVAVLRANSVEEAEKAV AVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGACTVTSVEQARKAVESGAEFIV SPHLDEEISQFAKEKGVFYMPGVMTPTTELKAMKLGHTILKLFPGEVVGPQFVKAMKGP FPNVKVFVPTGGVNLNDNVAEWFRAGVLAVGVGSALVKCTPDEVREKAKAFVEKIRGATE SEQ ID NO:169 (mgilpspgmpallsllvslsllimgcvaetgt) RFPNITNLCPPGEVFNATRFASVYA WNRKRISNCVADYSVLVNSASFSTFKCYGVSPSTKLNDLCFTNVYADSFVIRGDEVQRQIA PGQTGKIADYNYKLEDDFTGCVIANNNNLDSKVGGNYNYLYRLFRKSNLKPFFERDIST EIQAGSTPCNGVEGFNCFYFPLQSYGFQPTNGVGYPYRVVVLSPFELLHAPATVCGPKK ST (GGSGGGSGGGSGGSEKAARAEAAAR) KMEELFKKHKIVAVLRANSVEEAEKAV AVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGACTVTSVEQARKAVESGAEFIV SPHLDEEISQFAKEKGVFYMPGVMTPTTELKAMKLGHTILKLFPGEVVGPQFVKAMKGP FPNVKVFVPTGGVNLNDNVAEWFRAGVLAVGVGSALVKCTPDEVREKAKAFVEKIRGATE (G GGSHHHHHHHH) SEQ ID NO:170
SARS- CoV-2 2PSGA G-S- TEV- FO- I53- 50A*- 12GS- he- His	SARS- CoV-2- I53-50A fusion protein	QCVNLTTRTQLFPAYTNSFTRCVYYPDKVFRSSVLHSTQDLFLPFFSIVTFWFAIHVSG TWGTRFDFNVLPFNDGVYFASERKSNIRGNIFGTTLDSKTPQSLLIVNNATNVVIVKVC RFQPCNDFFLGYYHKNKSNMESEFRVYSSANNCTFEYV3QPFLMDLGRKQGNFKNLK EFVFRNIDGYFKIYKHTFIRLVRLPQGFSALEPLVDLPICINITRFQELLALHRSYL TFGDSSSGWTAGAAAYVGYLQFRTFLLKYNENGTITDAVDCALDPLSETKCTKLKSFYV EKGIIQTSNFRVQPTESIVRFPNITNLCPPGEVFNATRFASVYAWNRKRISNCVADYSV LVNSASFSTFKCYGVSPSTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLP DDFTGCVIANNNNLDSKVGGNYNYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEG FNCYFPLQSYGFQPTNGVGYPYRVVVLSPFELLHAPATVCGPKKSTNLVKNKCVNFNFN GLTGTGVLTESNKKFLPFQPGGRDIADTTDAVRDPQTLEILDTFCSPGGVSVITFGTN TSNQVAVLYQDVNCTEVEVAIHADQLTPTWRVYSTGSHVFQTRAGCLIGAENHVNNSYEC DIPIGAGICASYQTQTNPSGAG3VASQSIATYMSLGAENSVAYSNRSIAITPNTTIS VTTEILPVSMYKTSVDCMYICGDSSTECNLLQYGSFCTQLNRALTGIAVEQDKNTQE VFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNEVTLADAGFIKQYGD CLGDIAARDLICAKFNGLTVLFLPFLTDEMIQYTSALLACTITSGWTFGAGAAALQIFF

		AMQMA YRFNGIGVTQNVLYENQKLIANQFN SAIGKIQDLSSTASALGKLDQVNVQNAQ ALNTLVKQLSSNFGA ISSVLNDILSRLOPPEAEVQIDRLITGRLQSLQTYVTQQLIRAA EIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSPQSAFPGVVFVFLHVTYVPAQEKNF TTAPAICHGDKAHFFREGVFEVSNGTNHFVTQRNFYEPQIITDNTFVSGNCDVIGIVN NTVYDPLQPELDSFKEELDKYFKNHTSFDDVLDGDISGINASVUNIQKEIDRLNEVAKNL NESLIDLQELGKYEYIK (X1) KMEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHL YEITTFVFDADTVIKALSVLKEKGA I GAGTVTSVEQARKAVESGAEFTVSPHLDEEIS QFAKEKGVFYMFGVMTPTTEL VKAMKLGHTILKLFPGEVVGPQFVKAMKGPFPNVKFPVPT GGVNLDNVAEWFEAGVLA VGVGSALVKCTPDEVREKAKAFVEKIRGATE SEQ ID NO:171 (mgilpspgmpellisivellavllimgcvaetgt) QCVNLTRTQLPFPAYTNSFTRGVVY PDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPFVLPFNDGVYFASTEK SNIIRGWIFGTTLDSKTQSLLI VNNATNVVIKVCPEFQPCNDPFLGVVYHKNNKSWMESE FRVYSSANNCTFEYVSQFFLMDLEGKQGNFKNLREFVFNIDGYFKIYSKHTPINLVRL LPQGFSALEPLVDLPIGINITRFQTLALHRSVLTFGDSSSGWTAGAAAYVGLQFRT FLLKYNENGTITDAVDCALDPLSETKCTLKSFTEVENG IYQTSNFRVQPTESIVRFPNIT NLCPFGGEVFNATREFASVYANNKRKISNOCVADYSVLXNSASFSTFKCYGVSPTKLMDLCF ENVYADSFVIRGDEVQRQIAPGQGTGKIA DYNKLPDDFTGCVIANNSSNLDSEKGVGNVY LYRLFRKSNLKPFFERDISTEYIYQAGSTPCNGVEGFNCFYFPLQSYGFPQFTNGVGYQFYRV VVLSPFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPQQFGRDI ADTTDAVRDPQTLEILDTITPCSFPGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQ LTPTRVYSTGSENVFQTRAGCLIGAEHVNNSEYCDIPICAGICASYQTQTNSSPAGS ASQSI IAYTMSLGAENSVAYSNNISIAIPTNFTISVTTIELFVSMTKTSVDC TMYICGDS TECSNLLLQYGSFCTQLNRALTGI AVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQI LPLDESKPSKPSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLICAQKFNGLTVLFPPL LTDEMIACQYTSALLAGTITSGWTFGAGAALQIPFAMQMA YRFNGIGVTQNVLYENQKLI ANQFN SAIGKIQDLSSTASALGKLDQVNVQNAQALNTLVKQLSSNFGA ISSVLNDILS RLDPEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRV FCGKGYHLMSPQSAFPGVVFVFLHVTYVPAQEKNF TTAPAICHGDKAHFFREGVFEVSNGT NHFVTQRNFYEPQIITDNTFVSGNCDVIGIVNNTVYDPLQPELDSFKEELDKYFKNH TSPDDVLDGDISGINASVUNIQKEIDRLNEVAKMLNESLIDLQELGKYEYIK (gsgren lyfqqggggsgyipeaprdggayvrkdgewvllstflgSGSGSGSGSGSSEKAAKAEAA R) KMEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITTFVFPADTVIKALSVL KEKGA I GAGTVTSVEQARKAVESGAEFTVSPHLDEEISQFAKEKGVFYMFGVMTPTTEL VKAMKLGHTILKLFPGEVVGPQFVKAMKGPFPNVKFPVPTGGVNLDNVAEWFEAGVLA V GVGSALVKCTPDEVREKAKAFVEKIRGATE (GG3HHHHHHH) SEQ ID NO:172
SARS- CoV-2 2PSGA G-S- I53- 50A*- 12GS- he- His	SARS- CoV-2- 153-50A fusion protein	QCVNLTRTQLPFPAYTNSFTRGVVY PDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSG TNGTKRFDNPFVLPFNDGVYFASTEK SNIIRGWIFGTTLDSKTQSLLI VNNATNVVIKVC EFQPCNDPFLGVVYHKNNKSWMESEFRVYSSANNCTFEYVSQFFLMDLEGKQGNFKNL RFVFNKIDGYFKIYSKHTPINLVRLDPQGFSALEPLVDLPIGINITRFQTLALHRSYL TPGDSSSGWTAGAAAYVGLQFRTFLLKYNENGTITDAVDCALDPLSETKCTLKSFTEV EKGIYQTSNFRVQPTESIVRFPNITNLCPFGGEVFNATREFASVYANNKRKISNOCVADYSV LYNSASFSTFKCYGVSPTKLMDLCFTNVYADSFVIRGDEVQRQIAPGQGTGKIA DYNKLP DDFTGCVIANNSSNLDSEKGVGNVYLYRLFRKSNLKPFFERDISTEYIYQAGSTPCNGVEG FNCFYFPLQSYGFPQFTNGVGYQFYRVVVLSPFELLHAPATVCGPKKSTNLVKNKCVNFNFN GLTGTGVLTESNKKFLPQQFGRDIADTTDAVRDPQTLEILDTITPCSFPGVSVITPGTN TSNQVAVLYQDVNCTEVPVAIHADQLTPTRVYSTGSENVFQTRAGCLIGAEHVNNSEY DIPICAGICASYQTQTNSSPAGSVAASQSI IAYTMSLGAENSVAYSNNISIAIPTNFTIS VTTIELFVSMTKTSVDC TMYICGDS TECSNLLLQYGSFCTQLNRALTGI AVEQDKNTQEV FAQVKQIYKTPPIKDFGGFNFSQILPDESKPSKPSFIEDLLFNKVTLADAGFIKQYGD CLGDIAARDLICAQKFNGLTVLFPPLTDEMIACQYTSALLAGTITSGWTFGAGAALQIPF AMQMA YRFNGIGVTQNVLYENQKLIANQFN SAIGKIQDLSSTASALGKLDQVNVQNAQ ALNTLVKQLSSNFGA ISSVLNDILSRLOPPEAEVQIDRLITGRLQSLQTYVTQQLIRAA EIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSPQSAFPGVVFVFLHVTYVPAQEKNF TTAPAICHGDKAHFFREGVFEVSNGTNHFVTQRNFYEPQIITDNTFVSGNCDVIGIVN NTVYDPLQPELDSFKEELDKYFKNHTSFDDVLDGDISGINASVUNIQKEIDRLNEVAKNL NESLIDLQELGKYEYIK (X1) KMEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHL YEITTFVFDADTVIKALSVLKEKGA I GAGTVTSVEQARKAVESGAEFTVSPHLDEEIS

		QFAKEKGVFYMFGVMTPTTELVRAMKLGHTILKLFPGEVVGPQFVKAMKGFPPNVKVFVPT GGVNLDNVAEWFEAGVAVGVGSALVKGTPDEVREKAKAFVEKIRGATE SEQ ID NO:173 (mgilpspgmpallsivslsvllimgcvaetgt)QCVNLTTTRTQLFPAYTNSFTRGVYY EDKVFPSVSLHSTQDLFLFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEK SNIIRGWIFGTLDLSTQSLIVNNATNVYIKVCEPQPCNDPFLGVYVHNNKSWMESE FRVYSSANNCTFEYVSQPFLLMDLEGKQGNFKNLREFVFNIDGYFKIYSKHTPINLVRO LPQGFSALEPLVDLPICGNITRFQTLALHRSYLTFGDS33SGWTAGAAAYVGYLQFRT ELLKYNENGTITDAVDCALDPLSETKCTLKSTFVEKGIYQTSNFRVQPTESIVRFPNIT NLCPFGFVNATRFASVYAWNRRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCF TNVYADS FVIRGDEVQRAPGQTGKIADYNYKLDDFTGCVIAWNSNNLDSKVGSGNYY LYRLFRKSNLKPFFERDISTEYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRV VVLSPFELLHAPATVCGPKKSTNLVKNKCVNPNFNGLTGTGVLTESNKKFLPFQGFGRDI ADTTDAVRDPQTEILEITPESFGGVSVITPGTNTSNQVAVLYQDVNCTEVEVAIHADQ LTPTRVYVSTGSNVFPQTRAGCLIGAETHVMSVEODIPIGAGICASYQTQTNPSPSGAGSV ASQSIILAYTHSLGAENSVAYSNNSIAIPTNFTISVTTILPVGMTKTSVDCMTMYICGDS TECSNLLQYGSFCTQINRALGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGCGNFESQI LPDPSEPSKRSFIEDLEFNKVTILADAGFIKQYGDCLGDIARDLICAGKFNGLTYVLPFL LTDEMIAQYTSALLAGTITSGWTFGAGAAALQIPFAMQAYRFGNGICVTQNVLYENOKLI ANQFNSAIGKIQDLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAIISSVNLNDLS RLDPPEAEVQIDRLITGRQLQSLQTYVTQQLIRAAEIRASANLAATEMSECVLGQSKFVD FCGKGYHLSFPPQSAPHGVVFLHVTYVFAQEKMTTAPAI CHDGKAHFPREGVFSNGT HWFVTQRNFYEPQIITDNTFVSGNCDVIGIVNNTVIDPLQPELDSFKEELDNYFKNH TSPDVLGDSIGINAGVYVNIQKEIDRLNEVAKNLNESLIDQLBLGKYEQYIK (GSGSGG SGGSGSEKAAKAEERAR) KMEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEIT FTVPDADTVIKALSVLKEKGAIIAGSTVTSVQARRAVESGAEFIVSPALDEEISQFAK EFGVFMFGVMTPTTELVRAMKLGHTILKLFPGEVVGPQFVKAMKGFPPNVKVFVPTGGVN LDNVAEWFEAGVAVGVGSALVKGTPDEVREKAKAFVEKIRGATE (GSGHHHHHHHHH) SEQ ID NO:174
SARS- CoV-2 RBD- I3- G1*- secOp t- SGS- he- His	SARS- CoV-2- I3-01 fusion protein	RFPNITNLCPFGFVNATRFASVYAWNRRKRISNCVADYSVLYNSASFSTFKCYGVSPTK LNDLCFTNVYADS FVIRGDEVQRAPGQTGKIADYNYKLDDFTGCVIAWNSNNLDSKV GGNYYNLYRLFRKSNLKPFFERDISTEYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVG YQPYRVVVLSPFELLHAPATVCGPKKST (X1) MEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEIT FTVPDADTVIKALSVLKEKGAIIAGSTVTSVQARRAVESGAEFIVSPALDEEISQFAK EFGVFMFGVMTPTTELVRAMKLGHTILKLFPGEVVGPQFVKAMKGFPPNVKVFVPTGGVN LDNVAEWFEAGVAVGVGSALVKGTPDEVREKAKAFVEKIRGATE (GSGHHHHHHHHH) SEQ ID NO:175 (mgilpspgmpallsivslsvllimgcvaetgt)RFPNITNLCPFGFVNATRFASVYA WNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADS FVIRGDEVQRAPGQTGKIADYNYKLDDFTGCVIAWNSNNLDSKV GGNYYNLYRLFRKSNLKPFFERDISTEYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVG YQPYRVVVLSPFELLHAPATVCGPKKST (X1) MEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEIT FTVPDADTVIKALSVLKEKGAIIAGSTVTSVQARRAVESGAEFIVSPALDEEISQFAK EFGVFMFGVMTPTTELVRAMKLGHTILKLFPGEVVGPQFVKAMKGFPPNVKVFVPTGGVN LDNVAEWFEAGVAVGVGSALVKGTPDEVREKAKAFVEKIRGATE (GSGHHHHHHHHH) HH) SEQ ID NO:176
SARS- CoV-2 RBD- I3- G1*- secOp t- 12GS-	SARS- CoV-2- I3-01 fusion protein	RFPNITNLCPFGFVNATRFASVYAWNRRKRISNCVADYSVLYNSASFSTFKCYGVSPTK LNDLCFTNVYADS FVIRGDEVQRAPGQTGKIADYNYKLDDFTGCVIAWNSNNLDSKV GGNYYNLYRLFRKSNLKPFFERDISTEYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVG YQPYRVVVLSPFELLHAPATVCGPKKST (X1) MEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEIT FTVPDADTVIKALSVLKEKGAIIAGSTVTSVQARRAVESGAEFIVSPALDEEISQFAK EFGVFMFGVMTPTTELVRAMKLGHTILKLFPGEVVGPQFVKAMKGFPPNVKVFVPTGGVN LDNVAEWFEAGVAVGVGSALVKGTPDEVREKAKAFVEKIRGATE (GSGHHHHHHHHH) HH) SEQ ID NO:177

he- His		(mgilpspgmpallslyslsllsvllimgcvaetgt) RFPNITNLCFFGEVFNATRFASVYA WNRKRISNCVADYSVLYNSASFSTFKCYGVSPTEKLNLCFTNVIYADSFVIRGDEVPRQIA PGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNINYLRLFRKSNLKPFFERDIST EIIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKK ST (GSGSGSGSGSGSGSEKAAKAEAAAR) MEELFKHKIIVAVLRANSVEEAKKKALAVFLG GVDLIEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQAREAVESGAEFIVSPHLD REISQFAKEEGVFYMPGVMTPTLVKAMKLGHTILKLPFGGEVVGPFVPEAMKGPFFNVK FVPTGGVNLNDVAEWFEAGVQAVGVGEALNEGTFPEVAEKAKAFVEKIEGATE (GSGSRH AAAAHH) SEQ ID NO:178
SARS- CoV-2 RBD- I3- 01*- secOp t- 16GS- he- His	SARS- CoV-2- I3-01 fusion protein	RFPNITNLCFFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTEK LNLCFTNVIYADSFVIRGDEVPRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKV GGNINYLRLFRKSNLKPFFERDISTEIIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVG YQPYRVVLSFELLHAPATVCGPKKST (X1) MEELFKHKIIVAVLRANSVEEAKKKALA VFLGGVDLIEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQAREAVESGAEFIVS PHLDEEISQFAKEEGVFYMPGVMTPTLVKAMKLGHTILKLPFGGEVVGPFVPEAMKGPFF FNVKFPVPTGGVNLNDVAEWFEAGVQAVGVGEALNEGTFPEVAEKAKAFVEKIEGATE SEQ ID NO:179 (mgilpspgmpallslyslsllsvllimgcvaetgt) RFPNITNLCFFGEVFNATRFASVYA WNRKRISNCVADYSVLYNSASFSTFKCYGVSPTEKLNLCFTNVIYADSFVIRGDEVPRQIA PGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNINYLRLFRKSNLKPFFERDIST EIIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKK ST (GSGSGSGSGSGSGSEKAAKAEAAAR) MEELFKHKIIVAVLRANSVEEAKKKALA VFLGGVDLIEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQAREAVESGAEFIVS PHLDEEISQFAKEEGVFYMPGVMTPTLVKAMKLGHTILKLPFGGEVVGPFVPEAMKGPFF FNVKFPVPTGGVNLNDVAEWFEAGVQAVGVGEALNEGTFPEVAEKAKAFVEKIEGATE (G GSHHHHHHHH) SEQ ID NO:180
SARS- CoV-2 2PSGA G-S- TEV- FO- I3- 01*- secOp t- 12GS- he- His	SARS- CoV-2- I3-01 fusion protein	QCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSG TNGTKRFDNFVLPFNDGVYFASTEKSNIIIRGWIFGTTLDSKTSLLIVNNATNVVIVKVC EFQFCNDPFLGVYHKNMKSWMSEFRVYSSANNCTFEYVSQPFMLDLEGGKQGNFKNLR EFVFKNIDGYFKIYKHTPINLVRDLFQGFSALEPLVDLPFIGINITRFQTLALHRSYL TFPGDSSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETECTLKSTFV RKGIIYQTSNFRVQPTRESIVRFPNITNLCFFGEVFNATRFASVYAWNRKRISNCVADYSV LYNSASFSTFKCYGVSPTEKLNLCFTNVIYADSFVIRGDEVPRQIAPGQTGKIADYNYKLP DDFTGCVIAWNSNNLDSKVGGNINYLRLFRKSNLKPFFERDISTEIIYQAGSTPCNGVEG FNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFN GLTGTGVLTSNKKFLFPQGFQFDPDIADTTDAVRDPQTELEIDITPCSPGGVSVITPGTN TSNQVAVLYQDVNCTEVPVAIHADQLTPTWVYSTGSENVFQTRAGCLGAEHVNSYEC DIPICAGICASYQTQTNPSFGAGSVASQSI IAYTMSLGAENSVAYSNNIAIPNTFTTIS VTTEILPVSMTKTSVDCTMYICGSDSTBCSNLLQYGSFCTQLNRALTGIAVEQDKNTQE VFAQVNEQIVKTPPKDFGCGFNFSSQILPDESKEPSEKSFIEDILLFNKVTIADAGFIKQVGD CLGDIARNDLCAQKFNGLTVLPFLITDEMIAQYTSALLAGTITSQWTFGAGAAALQIPF AMQMAYRFNGIGVTVQVLYENQKLIANGQFNSAIGKIQDSLSTASALGKLQDVVRQNAQ ALNTLVKQLSSNFGAISSVLNDILSLDFFPEAEVQIDRLITGRLQSLQTYVTQQLIRAA EIPASANLAATKMSCEVLGQSKRVDFCGGYHLMSPFQSAPHGVVFLHVTVYVPAQEKNF TTAPAICHGDKARFFREGVFGNGTHWFVTQRNFYEPQIITDNTFVSGNCQVIGIVN NTVYDPLQFELDSFKEELDXYFKNHTSPDVLGDISGINASVVNIQKEIDRLNEVAKNL NESLIDLQELGKYEQYIK (X1) MEELFKHKIIVAVLRANSVEEAKKKALAVFLGGVDLI EITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQAREAVESGAEFIVSPHLDDEEISQ FAKEEGVFYMPGVMTPTLVKAMKLGHTILKLPFGGEVVGPFVPEAMKGPFFNVKFPVPTG GVNLNDVAEWFEAGVQAVGVGEALNEGTFPEVAEKAKAFVEKIEGATE SEQ ID NO:181 (mgilpspgmpallslyslsllsvllimgcvaetgt) QCVNLTTRTQLPPAYTNSFTRGVY YYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNFVLPFNDGVYFASTEK SNIIIRGWIFGTTLDSKTSLLIVNNATNVVIVKCEPQFCNDPFLGVYHKNMKSWMSE FRVYSSANNCTFEYVSQPFMLDLEGGKQGNFKNLRFEVFKNIDGYFKIYKHTPINLVRD LPQGFSALEPLVDLPFIGINITRFQTLALHRSYLTPGDSSSGWTAGAAAYVGYLQPRTF

37

		TECSNLLQYGSFCTQLNPALTGIAVEQDKNTQEVFAQVQKIYKTPPIKDFGGFNFSQI LPDPSPKPSKPSFIEDLLFNKVTADAGFIRQYGDCLGDIARDLCAQKFNGLTVLPPL LTDEMIAGYTSALLAGTITSGWTFGAGAAQIIPFAMQMAYRFNGIGVTVQNVLYENQKLI ANQFNSAIGKIQDSLSSTASALGKIQDVVNQNAQALNTLVKQLSSNFGAIISSVNDILS RLDPPEAEVQIDRLITGRLOSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVD FCGKGYHLMSPQSAHPGVVFLHVTVYPAQEKNFTTAPAICHDGKAHFFREGVTVSNGT RWFVTQRNFYEPQIITDNTFVSGNCDVYVIGIVNNTVYDPLQPELDSFKKEELDKYFKNH TSPDVLGDISGINASVVNIQKRIEKLNEVAKNLNESLIDLQELGKYEQYIN (GSGSGG SGSGSGSEKAAKAEAAAR) MEELFKKHKIVAVLRANSVEEAKKALAVFLGGVNLIEITF TVPDADTVIKELSLFKEMGAIIGAGTVTSVEQAPAEVESGAEFIVSPHLDEEISQFAKE EGVFYMPGVMTPTTELVKAMKLGHTILKLPFGEVVGQFVAMKGPFPNVKFEVPTGGVNL DNVAEWFEAGVQAVGVGEALNEGTFEVAAEKAKAFVERIEGATE (GGSNNNNNNNNH) SEQ ID NO:184
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>HexaPro-12GS-He-I5350A*-His:

(MEVFLVLLPLVSSQC)VNLTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFAHNVSGT
NGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIVKCEFPQCNDFPLGVYHKN
5 NKSWMSEFEFRVYSSANNCTFEYVSQPFMLDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTFNLNVRDLPGQFSAL
EPLVDLPIGINITRFQTLALHRSYLPFGDSSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDAVDCALDPLSE
TKCTLSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRRKISNCVADYSVLYNSAS
FSTFRCTYGVSPTEKNDLCFTNVYADSFVIRGDEVQRQIAPGQTKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGN
YNYLYRLFRKSNLKPFEKDISTEIIYQAGSTPCNGVEGFNCFYFLQSYGFQPTNGVGYQPYRVVLSFELLHAPAT
10 VCGPKHSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDFTLEILDITPCSFGGVSVI
TPGTNTSNQVAVLYQDVNCTEVFAIHADQLTPTWRVYSTGSNVFTQTRAGCLIGAHEVNNSECDIPFGAGICAS
YQTQTNSPGSASSVASQSIIAYTMSLGAENSVAYSNNISIAIPTNFITISVTTEILFVSMFKTSVDCTMYICGDSTE
CSNLLQYGSFCTQLNRLTGIAVEQDKNTQEVFAQVQKIYKTPPIKDFGGFNFSQILPDPSPKPSKPSFIEDLLF
15 NKVTADAGFIRQYGDCLGDIARDLCAQKFNGLTVLPPLLTDEMIAGYTSALLAGTITSGWTFGAGPALQIIPF
PMQMAYRFNGIGVTVQNVLYENQKLIANQFNSAIGKIQDSLSSTPSALGKIQDVVNQNAQALNTLVKQLSSNFGAI
SSVNDILSRLDPPEAEVQIDRLITGRLOSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYH
LMSFPQSAHPGVVFLHVTVYPAQEKNFTTAPAICHDGKAHFFREGVTVSNGTTHWFTQRNFYEPQIITDNTFVS
GNCDVYVIGIVNNTVYDPLQPELDSFKKEELDKYFKNHTSPDVLGDISGINASVVNIQKRIEKLNEVAKNLNESLI
DLQELGKYEQ (GSGSGSGSGSGSEKAAKAEAAAR) KMEELFKKHKIVAVLRANSVEEAEKAVAVFAGGVNLIEI
20 TFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQAPKAVESGAEFIVSPHLDEEISQFAKEKGVFYMFGVMTPTTE
LVKAMKLGHTILKLPFGEVVGQFVKAMKGPFPNVKFEVPTGGVNLDNVAEWFEAGVLAAGVGSALVKGTPDEVRE
KAKAFVEKIRGATE (GGSNNNNNNNNH) (SEQ ID NO:135)

>HexaPro-FO-12GS-He-I5350A*-His:

(MEVFLVLLPLVSSQC)VNLTRTQLPPAYTNSFTRGVYYPDKVFRSSVLASTQDLFLPFFSNVTWFAHNVSGT
NGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIVKCEFPQCNDFPLGVYHKN
25 NKSWMSEFEFRVYSSANNCTFEYVSQPFMLDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTFNLNVRDLPGQFSAL
EPLVDLPIGINITRFQTLALHRSYLPFGDSSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDAVDCALDPLSE
TKCTLSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRRKISNCVADYSVLYNSAS
FSTFRCTYGVSPTEKNDLCFTNVYADSFVIRGDEVQRQIAPGQTKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGN
30 YNYLYRLFRKSNLKPFEKDISTEIIYQAGSTPCNGVEGFNCFYFLQSYGFQPTNGVGYQPYRVVLSFELLHAPAT

VCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPQQFGRDIADTTDAVRDPQTLEILDITPCSFSGVSVI
 TPGTNTSNQVAVLYQDVNCTEVPAIHAHQLEPTWRYVSTGSNVFQTRAGCLIGAHEVMNSYECDDIPGAGICAS
 YQTQTNSPGSASSVASQSIIAYTMSLGAENSVAYSNNISIAIPTNFTISVTTEILFVSMTKTSVDCTMYICGDSTE
 CSNLLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKFSKRSPIEDLLF
 5 NKVTLADAGFIKQYGDCLGDIAARDLICAQKFNGLTVLPPLITDEMIAQYTSALLAGTITSGWTFGAGPALQIPF
 FPMQAYBFNGIGVGTQNVLYENQKLIANQFNISAIGKIQDSLSSTPSALGKLQDVVNQNAQALNTLVKQLSSNFGAI
 SSVLNDILSRDLPPEAEVQIDRLITGRQLQSLQTYVTQQILIRAAEIRASANLAATEMSECVLGQSKRVDFCGKGYH
 LMSFPQSAPHGCVVFLHVTVYPAQEKNFITAFAICRDGKAHFFREGVVFVSNGTWTFVTQRNFYEPQIITDNTFVS
 GNCDDVIGIVNNTVYDPLQPELDSFKEELDXYFKNHTSPDVLGDSSGINASVVNIQKEIDRLNEVAKNLNESLI
 10 DLQELGKEYEQ (GS) (GYIPEAPRDGQAYVRKIDGEWVLLSTFL) (GSGSGSGSGSGSEKAARAEAAAR) KMEELFK
 KKKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQARKAVESG
 AEFIVSPHLDEEISQFAKEKGVFYMFGVMTPTLVKAMKLGHTILKLPGEVVGPFVKAMKGPFPNVKFPVPTGG
 VNLDNVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATE (GSGHHHHHHHH) (SEQ ID
 NO:139)

15

>HexaPro-delHR2-12GS-He-I5350A*-His:
 (MFVFLVLLFLVSS)QC) VNLTRTQLFPAYTNSFTRCVYYFDKVFRRSSVLHSTQDLFLPFFSNVTWFHAIHVSG
 TNGTKRFDNPVLPFNDGVYFASTEKSNIIIRGNIFGTTLDSKTQSLLIVNNATNVVIKVCBFQPCNDFFLGVYYHK
 NNKSWMESEFRVYSSANNCTFEYVSQPFMLDLEGKQGNFKNLREFVFNIDGYFKIYKHTPINLVRDLPGQFSA
 20 LEPLVDLPIGINITRFQTLALHRSYLTFGDSSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDAVDCALDPLS
 ETKCTLKSFTEKGIYQTSNFRVQPTESIVRFPNITNLCPFGGEVFNATRFASVYANNPKRISRCVADYSVLYNSA
 3FSTFRCYGVSPKTLNLCFTNVYADSFVIRGDEVRIAPGQTKIADYNXKLPDDFTGCVIANNNNLDSKVG
 NNYLYRLFRKSNLKPFFERDISTEIIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYPYRVVLSFELLHAPA
 TVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPQQFGRDIADTTDAVRDPQTLEILDITPCSFSGVSV
 25 ITPGTNTSNQVAVLYQDVNCTEVPAIHAHQLEPTWRYVSTGSNVFQTRAGCLIGAHEVMNSYECDDIPGAGICAS
 SYQTQTNSPGSASSVASQSIIAYTMSLGAENSVAYSNNISIAIPTNFTISVTTEILFVSMTKTSVDCTMYICGDST
 ECSNLLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKFSKRSPIEDLL
 FNVTLADAGFIKQYGDCLGDIAARDLICAQKFNGLTVLPPLITDEMIAQYTSALLAGTITSGWTFGAGPALQIP
 FPMQAYBFNGIGVGTQNVLYENQKLIANQFNISAIGKIQDSLSSTPSALGKLQDVVNQNAQALNTLVKQLSSNFGA
 30 ISSVLNDILSRDLPPEAEVQIDRLITGRQLQSLQTYVTQQILIRAAEIRASANLAATEMSECVLGQSKRVDFCGKGY
 HLMSPQSAPHGCVVFLHVTVYPAQEKNFITAFAICRDGKAHFFREGVVFVSNGTWTFVTQRNFYEPQIITDNTFV
 SGNCDDVIGIVNNTVYDPLQPELDSFKEELDXYFKNHT (GSGSGSGSGSGSEKAARAEAAAR) KMEELFKKKIV
 AVLNRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQARKAVESGAEFIV
 3PHLDEEISQFAKEKGVFYMFGVMTPTLVKAMKLGHTILKLPGEVVGPFVKAMKGPFPNVKFPVPTGGVNLDN
 35 VAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATE (GSGHHHHHHHH) (SEQ ID NO:140)

>HexaPro-delHR2-FC-12GS-He-I5350A*-His:
 (MFVFLVLLFLVSS)QC) VNLTRTQLFPAYTNSFTRCVYYFDKVFRRSSVLHSTQDLFLPFFSNVTWFHAIHVSG
 TNGTKRFDNPVLPFNDGVYFASTEKSNIIIRGNIFGTTLDSKTQSLLIVNNATNVVIKVCBFQPCNDFFLGVYYHK
 40 NNKSWMESEFRVYSSANNCTFEYVSQPFMLDLEGKQGNFKNLREFVFNIDGYFKIYKHTPINLVRDLPGQFSA
 LEPLVDLPIGINITRFQTLALHRSYLTFGDSSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDAVDCALDPLS

ETKCTLKSPFTEKGIYQTSNFRVQFTESIVRFPNITHLCPPGGEVFNATRFASVYAWNRRKRISNCVADYSVLVNSA
 SFSTFKCYGVSPTEKINDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVG
 NNYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCYFPLQSYGFPQTNVGVGYQPYRVVLSFELLHAPA
 TVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLFFQGFGRDIADTTDAVRDPQTLEILLITEPCSFSGGVSV
 5 ITPGTNTSNQVAVLYQDVNCTEVEVAIHADQLTPTWRVYSTGSENVFQTRAGCLIGAEHVNNSECDIPGAGICA
 SYQTQTNSPGSASSVASQSI IAYTMSLGAENSVAYSNNNSIAIPTNFTISVTTTEILFVSMTKTSVDCTMYICGDS
 ECSNLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPFIKDFGGFNFSQILPDPSKPSKRSPIEDLL
 FNKYTLADAGFLQYGDCLGDIARDLICAQKFNGLTVLPPLTDEMLAQVTSALLAGTITSGWTFGAGPALQIF
 FPMQAMAYRFNGIGVTVQNVLYENQKLIANQFNSEAIGKIQDSLSSTPSALSKLQDVVNQNAQALNTLVKQLSSNFGA
 10 ISSVLNDILSRDPPEAEVQIDRLITGRQLSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGRGY
 AIMSEFPQSAPHGVVFLHVTYVPAQEKNTTAPAIOROKAHFPREGVTVSNGTHWFTVQRNFYEPQIITTDNTFV
 SGNCDDVVGIVNNTVYDPLQPELDSFKEELDKYFKNHT (GS) (GYLPEAFROGQAYVVKDGEWVLLSTFL) (GSG
 GSGSGSGSEKAAKAEAAAR) KMEELPKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKAL
 SVLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMFGVMTPTTELVKAMKLGHTILKL
 15 FPGEVVGPQFVKAMKGPFFPNVKFVPTGGVNLNDVAEWFKAGVLAVGVGSALVKSTPDEVREKAKAFVEKIRGATE
 (GGSHHHHHHH) (SEQ ID NO:141)

RBD-noRpk-50A Variants

20 >SARS-CoV-2 RBD_N501Y_16GS-he-153-50A*-His (UK):
 (MGILPSPGMPALLSLVSLLSVLLMGCVAETGT) RFPNITNLCPPGGEVFNATRFASVYAWNRRKRISNC
 VADYSVLVNSASFSTFKCYGVSPTEKINDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPDDF
 TGCVIAWNSNNLDSKVGNNYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCYFPLQSYGF
 QPTYGVGYQPYRVVLSFELLHAPATVCGPKKST (GGSGSGSGSGSGSGSEKAAKAEAAAR) KMEEL
 25 FKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVE
 QARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMFGVMTPTTELVKAMKLGHTILKLFPGEVVGPFVK
 AMKGPFFPNVKFVPTGGVNLNDVAEWFKAGVLAVGVGSALVKSTPDEVREKAKAFVEKIRGATE (GGSH
 HHHHHHH) (SEQ ID NO:142)

30 >SARS-CoV-2 RBD_K417N_E484K_N501Y_16GS-he-153-50A*-His (S.Africa)
 (MGILPSPGMPALLSLVSLLSVLLMGCVAETGT) RFPNITNLCPPGGEVFNATRFASVYAWNRRKRISNC
 VADYSVLVNSASFSTFKCYGVSPTEKINDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPDDF
 TGCVIAWNSNNLDSKVGNNYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVKGFNCFYFPLQSYGF
 QPTYGVGYQPYRVVLSFELLHAPATVCGPKKST (GGSGSGSGSGSGSGSEKAAKAEAAAR) KMEEL
 35 FKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVE
 QARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMFGVMTPTTELVKAMKLGHTILKLFPGEVVGPFVK
 AMKGPFFPNVKFVPTGGVNLNDVAEWFKAGVLAVGVGSALVKSTPDEVREKAKAFVEKIRGATE (GGSH

HHHHHHH) (SEQ ID NO:143)

>SARS-CoV-2_RBD-noRpk_16GS_I53-50A*_Brazil-ver_K417T_E484K_N501Y
(Brazil):

5 (MGILPSPGMPALLSLVSLLSVLLMGCVAETGT)RFPNITNLCPPGEVFNATRFASVYAWNRRKRISNC
VADYSVLVNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGTIADYNYKLPDDF
TGCVIAWNSNNLDSKVGNNYNYRLFRKSNLKPFFERDISTEIQAGSTPCNGVKGFNCYFPLQSYGF
QPTYGVGYQPYRVVVLSPFELLHAPATVCCGPKKST(GGSGGSGSGSGSGSGSEKAAKAEBAAR)KMEEL
FKKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVE
10 QARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMFGVMTPTLVKAMKLGHTILKLPFGEVVGPPQFVK
AMKGPFPNVKFPVPTGGVNLNDVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATE(GGSH
HHHHHHH) (SEQ ID NO:144)

>SARS-CoV-2_RBD-noRpk_16GS_I53-50A*_E484K:

15 (MGILPSPGMPALLSLVSLLSVLLMGCVAETGT)RFPNITNLCPPGEVFNATRFASVYAWNRRKRISNC
VADYSVLVNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGTIADYNYKLPDDF
TGCVIAWNSNNLDSKVGNNYNYRLFRKSNLKPFFERDISTEIQAGSTPCNGVKGFNCYFPLQSYGF
QPTNGVGYQPYRVVVLSPFELLHAPATVCCGPKKST(GGSGGSGSGSGSGSGSEKAAKAEBAAR)KMEEL
FKKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVE
20 QARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMFGVMTPTLVKAMKLGHTILKLPFGEVVGPPQFVK
AMKGPFPNVKFPVPTGGVNLNDVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATE(GGSH
HHHHHHH) (SEQ ID NO:145)

>SARS-CoV-2_RBD-noRpk_16GS_I53-50A*_I452R:

25 (MGILPSPGMPALLSLVSLLSVLLMGCVAETGT)RFPNITNLCPPGEVFNATRFASVYAWNRRKRISNC
VADYSVLVNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGTIADYNYKLPDDF
TGCVIAWNSNNLDSKVGNNYNYRLFRKSNLKPFFERDISTEIQAGSTPCNGVGFNCYFPLQSYGF
QPTNGVGYQPYRVVVLSPFELLHAPATVCCGPKKST(GGSGGSGSGSGSGSGSEKAAKAEBAAR)KMEEL
FKKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVE
30 QARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMFGVMTPTLVKAMKLGHTILKLPFGEVVGPPQFVK
AMKGPFPNVKFPVPTGGVNLNDVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATE(GGSH
HHHHHHH) (SEQ ID NO:146)

>SARS-CoV-2_RBD_N501Y_16GS-he-I53-50A*-His (UK):

35 (MGILPSPGMPALLSLVSLLSVLLMGCVA)RFPNITNLCPPGEVFNATRFASVYAWNRRKRISNCVADY

SVLYNSASESTFKCYGVSP TKLNDLCFTNVYADSFVIRGDEV RQIAPGQTGKIADYNYKLPDDFTGCV
IAWNSNNLDSKVGGN YNYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQPTY
GVGYQPYRVVLSFELLHAPATVCGPKKST (GGSGGSGSGSGSGSGSEKAAKAEAAAR) KMEELFKKH
KIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQARK
5 AVESGAEFIVSPHLDEEISQFAKEKGVFYMFGVMTPTLVKAMKLGHTILKLFPGEVVGPFVKAMKG
PPPNVKFVPTGGVNLNDNVAEWFKAGVLAVGVGSALVKCTPDEVREKAKAFVEKIRGATE (GGSHHHHH
HHH) (SEQ ID NO:147)

>SARS-CoV-2_RBD_K417N_E484K_N501Y_16GS-he-153-50A*-His (S.Africa)
10 (MGILSPGMPALLSLVSLLSVLLMGCVA) RFPNITNLCFFGEVFNATRFASVYAWNKRKISNCVADY
SVLYNSASESTFKCYGVSP TKLNDLCFTNVYADSFVIRGDEV RQIAPGQTGNIADYNYKLPDDFTGCV
IAWNSNNLDSKVGGN YNYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVKGFNCYFPLQSYGFQPTY
GVGYQPYRVVLSFELLHAPATVCGPKKST (GGSGGSGSGSGSGSGSEKAAKAEAAAR) KMEELFKKH
KIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQARK
15 AVESGAEFIVSPHLDEEISQFAKEKGVFYMFGVMTPTLVKAMKLGHTILKLFPGEVVGPFVKAMKG
PPPNVKFVPTGGVNLNDNVAEWFKAGVLAVGVGSALVKCTPDEVREKAKAFVEKIRGATE (GGSHHHHH
HHH) (SEQ ID NO:148)

>SARS-CoV-2_RBD-noRpk_16GS_153-50A*_Brazil-ver_K417T_E484K_N501Y
20 (Brazil):
(MGILSPGMPALLSLVSLLSVLLMGCVA) RFPNITNLCFFGEVFNATRFASVYAWNKRKISNCVADY
SVLYNSASESTFKCYGVSP TKLNDLCFTNVYADSFVIRGDEV RQIAPGQTGTIADYNYKLPDDFTGCV
IAWNSNNLDSKVGGN YNYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVKGFNCYFPLQSYGFQPTY
GVGYQPYRVVLSFELLHAPATVCGPKKST (GGSGGSGSGSGSGSGSEKAAKAEAAAR) KMEELFKKH
25 KIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQARK
AVESGAEFIVSPHLDEEISQFAKEKGVFYMFGVMTPTLVKAMKLGHTILKLFPGEVVGPFVKAMKG
PPPNVKFVPTGGVNLNDNVAEWFKAGVLAVGVGSALVKCTPDEVREKAKAFVEKIRGATE (GGSHHHHH
HHH) (SEQ ID NO:149)

30 >SARS-CoV-2_RBD-noRpk_16GS_153-50A*_E484K:
(MGILSPGMPALLSLVSLLSVLLMGCVA) RFPNITNLCFFGEVFNATRFASVYAWNKRKISNCVADY
SVLYNSASESTFKCYGVSP TKLNDLCFTNVYADSFVIRGDEV RQIAPGQTGKIADYNYKLPDDFTGCV
IAWNSNNLDSKVGGN YNYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVKGFNCYFPLQSYGFQPTN
GVGYQPYRVVLSFELLHAPATVCGPKKST (GGSGGSGSGSGSGSGSEKAAKAEAAAR) KMEELFKKH
35 KIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQARK

AVESGAEFIVSPHLDEEISQFAKEKGVFYMFGVMTPTLVKAMKLGHTILKLFPGEVVGPQFVKAMKG
 FFPNVKVFVFTGGVNLNDNVAEWFKAGVLAVGVGSALVKSTPDEVREKAKAFVEKIRGATE (GGSEHHHH
 HHH) (SEQ ID NO:150)

5 >SARS-CoV-2_RBD-noRpk_16GS_I53-50A*_I452R:
 (MGILPSPGMPALLSLVSLLSVLLMGCVA)RFPNITNLCPPGEVFNATRFASVYAWNRRKRISNCVADY
 SVLYNSASFSTFKCYGVSPFKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPDDFTGCV
 IAWNSNNLDSKVGGNYNRYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQFTN
 GVGYQPYRVVLSFELLHAPATVCGPKKST (GGSGSGSGSGSGSGSEKAAKAEAAAP)KMEELFKKH
 10 KIVAVLRANSVEEAIEKAVAVFAGGVHLEITFTVPDADTVIKALSVLKEGAILGAGTVTSVEQARK
 AVESGAEFIVSPHLDEEISQFAKEKGVFYMFGVMTPTLVKAMKLGHTILKLFPGEVVGPQFVKAMKG
 FFPNVKVFVFTGGVNLNDNVAEWFKAGVLAVGVGSALVKSTPDEVREKAKAFVEKIRGATE (GGSEHHHH
 HHH) (SEQ ID NO:151)

15 In various embodiments, the polypeptide comprises the amino acid sequence selected
 from the group consisting of SEQ ID NOS:1-12 and 142-151. In various other
 embodiments, the polypeptides comprises an amino acid sequence at least 95%, at least 96%,
 at least 97%, at least 98%, at least 99%, or at least 100% identical to the amino acid sequence
 selected from the group consisting of SEQ ID NOS: 1-8, or the group consisting of SEQ ID
 20 NOS: 1-4, SEQ ID NOS: 5-8, or the group consisting of SEQ ID NOS: 1 and 5, provided as
 exemplary embodiments in the examples that follow.

As used throughout the present application, the term “polypeptide” is used in its
 broadest sense to refer to a sequence of subunit D- or L-amino acids, including canonical and
 non-canonical amino acids. The polypeptides described herein may be chemically
 25 synthesized or recombinantly expressed. The polypeptides may be linked to other compounds
 to promote an increased half-life in vivo, such as by PEGylation, HESylation, PASylation,
 glycosylation, or may be produced as an Fc-fusion or in deimmunized variants. Such linkage
 can be covalent or non-covalent as is understood by those of skill in the art.

In a second aspect, the disclosure provides nanoparticles comprising a plurality of
 30 polypeptides according to any embodiment or combination of embodiments of the first aspect
 of the disclosure. In this aspect, a plurality (2, 3, 4, 5, 10, 20, 25, 50, 60, 100, or more)
 polypeptides of the first aspect of the disclosure are present in any suitable nanoparticle.

Nanoparticles of any embodiment or aspect of this disclosure can be of any suitable
 size for an intended use, including but not limited to about 10 nm to about 100 nm in
 35 diameter.

In a third aspect, the disclosure provides nanoparticles, comprising:

(a) a plurality of first assemblies, each first assembly comprising a plurality of identical first proteins; and,

(b) a plurality of second assemblies, each second assembly comprising a
5 plurality of second proteins;

wherein the amino acid sequence of the first protein differs from the sequence of the second protein;

wherein the plurality of first assemblies non-covalently interact with the plurality of second assemblies to form the nanoparticle; and,

10 wherein the nanoparticle displays on its surface an immunogenic portion of a SARS-CoV-2 antigen or a variant or homolog thereof, present in the at least one second protein.

In this aspect, the nanoparticle forms a three-dimensional structure formed by the non-covalent interaction of the first and second assemblies. A plurality (2, 3, 4, 5, 6, or more) of first polypeptides self-assemble to form a first assembly, and a plurality (2, 3, 4, 5,
15 6, or more) of second polypeptides self-assemble to form a second assembly. Non-covalent interaction of the individual self-assembling proteins results in self-assembly of the first protein into first assemblies, and self-assembly of the second proteins into second assemblies. A plurality of these first and second assemblies then self-assemble non-covalently via interfaces to produce the nanoparticles. The number of first polypeptides in the first
20 assemblies may be the same or different than the number of second polypeptides in the second assemblies. Nanoparticles of this disclosure can have any shape and/or symmetry suitable for an intended use, including, but not limited to, tetrahedral, octahedral, icosahedral, dodecahedral, and truncated forms thereof. In one exemplary embodiment, each first assembly is pentameric and each second assembly is trimeric.

25 Assembly of the first and second assemblies into nanoparticles is not random, but is dictated by non-covalent interactions (e.g., hydrogen bonds, electrostatic, Van der Waals, hydrophobic, etc.) between the various assemblies (i.e., the cumulative effect of interactions between first assemblies, interactions between second assemblies, and interactions between first and second assemblies). Consequently, nanoparticles of this disclosure comprise
30 symmetrically repeated, non-natural, non-covalent, protein-protein interfaces that orient the first and second assemblies into a nanoparticle having a highly ordered structure. While the formation of nanoparticles is due to non-covalent interactions of the first and second assemblies, in some embodiments, once formed, nanoparticles may be stabilized by covalent

linking between proteins in the first assemblies and the second assemblies. Any suitable covalent linkage may be used, including but not limited to disulfide bonds and isopeptide linkages.

First proteins and second proteins suitable for producing assemblies of this disclosure may be of any suitable length for a given nanoparticle. First proteins and second proteins may be between 30 and 250 amino acids in length.

In one embodiment, the second proteins comprise an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:85-124 or 185-193 (Table 2), wherein X1 for at least one second protein comprises an immunogenic portion of a SARS-CoV-2 antigen or a variant or homolog thereof, X2 is absent or an amino acid linker, and residues in parentheses are optional. The optional residues may be present, or some (i.e.: 1, 2, 3, 4, 5, 6, or more) or all of the optional residues may be absent.

Table 2

Protein name	Protein type	Expressed sequence (optional residues in parentheses)
SARS-CoV-2 RB D-153-50A*-16GS-he-His	SARS-CoV-2-153-50A fusion protein	X1- GGSGGSGSGSGSGSGSEKAAKAEAAARKMEELFKKHKIVAVLRANSVEEAIEKA VAVFAGGVHLEITFTVPDADTVIKALSVLKEKGALIGAGTVTSVEQARKAVES GAETIVSPHLDEEISQFAKEKGVFYMPGVMTPTTELVKAMKLGHITILKLFPGEVV GPQFVKAMKGPPFPNVKFPVPTGGVNLNVAEWFKAGVLAVGVGSALVKGTPDEV EKAKAFVEKIRGATE (SEQ ID NO:85) X1- (X2) KMEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLEITFTVPDADTV IKALSVLKEKGALIGAGTVTSVEQARKAVESGAETIVSPHLDEEISQFAKEKGV FYMPGVMTPTTELVKAMKLGHITILKLFPGEVVGPQFVKAMKGPPFPNVKFPVPTGGV NLNVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATE (SEQ ID NO:86) X1- GGSGGSGSGSGSGSGSEKAAKAEAAARKMEELFKKHKIVAVLRANSVEEAIEKA VAVFAGGVHLEITFTVPDADTVIKALSVLKEKGALIGAGTVTSVEQARKAVES GAETIVSPHLDEEISQFAKEKGVFYMPGVMTPTTELVKAMKLGHITILKLFPGEVV GPQFVKAMKGPPFPNVKFPVPTGGVNLNVAEWFKAGVLAVGVGSALVKGTPDEV EKAKAFVEKIRGATE(GGSHHHHHHH) (SEQ ID NO:87) X1- (X2) KMEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLEITFTVPDADTV IKALSVLKEKGALIGAGTVTSVEQARKAVESGAETIVSPHLDEEISQFAKEKGV FYMPGVMTPTTELVKAMKLGHITILKLFPGEVVGPQFVKAMKGPPFPNVKFPVPTGGV NLNVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATE (GGSHHH HHHH) (SEQ ID NO:88)

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SARS-CoV-2 RB D-153-50A*- 8GS-he- His	SARS-CoV-2-153-50A fusion protein	X1- GGSGGSGSGSEKAAKAEAAARKMEELFKKKHKIVAVLRANSVEEAIEKAVAVFAGGV HLIEITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSP HLDEEISQFAKEKGVFYMPGVMTPTTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFFNVKFFVPTGGV NLDNVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVE KIRGATE (SEQ ID NO:89) X1- (X2) KMEELFKKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTV IKALSVLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGV FYMPGVMTPTTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFFNVKFFVPTGGV NLDNVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATE (SEQ ID NO:90) X1- GGSGGSGSGSEKAAKAEAAARKMEELFKKKHKIVAVLRANSVEEAIEKAVAVFAGGV HLIEITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSP HLDEEISQFAKEKGVFYMPGVMTPTTELVKAMKLGHTILKLFPGEVVGPQFVKAM KGPFFNVKFFVPTGGV NLDNVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVE KIRGATE (GGSHHHHHHHH) (SEQ ID NO:91) X1- (X2) KMEELFKKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTV IKALSVLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGV FYMPGVMTPTTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFFNVKFFVPTGGV NLDNVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATE (GGSHHH HHHH) (SEQ ID NO:92)
SARS-CoV-2 RB D-153-50A*- 12GS-he- His	SARS-CoV-2-153-50A fusion protein	X1- GGSGGSGSGSGSEKAAKAEAAARKMEELFKKKHKIVAVLRANSVEEAIEKAVAVF AGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQARKAVESGAEF IVSPHLDEEISQFAKEKGVFYMPGVMTPTTELVKAMKLGHTILKLFPGEVVGPQF VKAMKGPFFNVKFFVPTGGV NLDNVAEWFKAGVLAVGVGSALVKGTPDEVREKAK AFVEKIRGATE (SEQ ID NO:93) X1- (X2) KMEELFKKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTV IKALSVLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGV FYMPGVMTPTTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFFNVKFFVPTGGV NLDNVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATE (SEQ ID NO:94) X1- GGSGGSGSGSGSEKAAKAEAAARKMEELFKKKHKIVAVLRANSVEEAIEKAVAVF AGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQARKAVESGAEF IVSPHLDEEISQFAKEKGVFYMPGVMTPTTELVKAMKLGHTILKLFPGEVVGPQF VKAMKGPFFNVKFFVPTGGV NLDNVAEWFKAGVLAVGVGSALVKGTPDEVREKAK AFVEKIRGATE (GGSHHHHHHHH) (SEQ ID NO:95) X1- (X2) KMEELFKKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTV IKALSVLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGV FYMPGVMTPTTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFFNVKFFVPTGGV NLDNVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATE (GGSHHH HHHH) (SEQ ID NO:96)

SARS-CoV-2 2PSSGAG- S-TEV- FO-I53- 50A*- 12GS-be- His (+ foldon)	SARS-CoV-2- I53-50A fusion protein	X1- gsgrenlyfsgggsgsyipeaprdggayvskdggewllstflgGSGSGSGSGSG SEKAARAEAAARKMEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFT TVPDADTVIKALSVLKEKGAIIGAGTIVTSVEQARKAVESGAEFIVSPHLDEEIS QFAKEKGVFYMPGVMTPTTELVKAMKLGHTILKLFPGGEVVGPPQFVKAMKGPFFNV KFVPTGGVNLNDVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATE (SEQ ID NO:97) X1- (X2) KMEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTTVPDADTV IKALSVLKEKGAIIGAGTIVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGV FYMPGVMTPTTELVKAMKLGHTILKLFPGGEVVGPPQFVKAMKGPFFNVKFVPTGGV NLNDVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATE (SEQ ID NO:98) X1- gsgrenlyfsgggsgsyipeaprdggayvskdggewllstflgGSGSGSGSGSGSG SEKAARAEAAARKMEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFT TVPDADTVIKALSVLKEKGAIIGAGTIVTSVEQARKAVESGAEFIVSPHLDEEIS QFAKEKGVFYMPGVMTPTTELVKAMKLGHTILKLFPGGEVVGPPQFVKAMKGPFFNV KFVPTGGVNLNDVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATE (GSGHHHHHHHH) (SEQ ID NO:99) X1- (X2) KMEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTTVPDADTV IKALSVLKEKGAIIGAGTIVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGV FYMPGVMTPTTELVKAMKLGHTILKLFPGGEVVGPPQFVKAMKGPFFNVKFVPTGGV NLNDVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATE (GSGHHHH HHHH) (SEQ ID NO:100)
SARS-CoV-2 2PSSGAG- S-I53- 50A*- 12GS-be- His (- foldon)	SARS-CoV-2- I53-50A fusion protein	X1- GSGSGSGSGSGSEKAARAEAAARKMEELFKKHKIVAVLRANSVEEAIEKAVAVF AGGVHLIEITFTTVPDADTVIKALSVLKEKGAIIGAGTIVTSVEQARKAVESGAEF IVSPHLDEEISQFAKEKGVFYMPGVMTPTTELVKAMKLGHTILKLFPGGEVVGPPQF VKAMKGPFFNVKFVPTGGVNLNDVAEWFKAGVLAVGVGSALVKGTPDEVREKAK AFVEKIRGATE (SEQ ID NO:101) X1- (X2) KMEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTTVPDADTV IKALSVLKEKGAIIGAGTIVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGV FYMPGVMTPTTELVKAMKLGHTILKLFPGGEVVGPPQFVKAMKGPFFNVKFVPTGGV NLNDVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATE (SEQ ID NO:102) X1- GSGSGSGSGSGSEKAARAEAAARKMEELFKKHKIVAVLRANSVEEAIEKAVAVF AGGVHLIEITFTTVPDADTVIKALSVLKEKGAIIGAGTIVTSVEQARKAVESGAEF IVSPHLDEEISQFAKEKGVFYMPGVMTPTTELVKAMKLGHTILKLFPGGEVVGPPQF VKAMKGPFFNVKFVPTGGVNLNDVAEWFKAGVLAVGVGSALVKGTPDEVREKAK AFVEKIRGATE (GSGHHHHHHHH) (SEQ ID NO:103) X1- (X2) RKMEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTTVPDADT VIKALSVLKEKGAIIGAGTIVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGV FYMPGVMTPTTELVKAMKLGHTILKLFPGGEVVGPPQFVKAMKGPFFNVKFVPTGGV NLNDVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATE (GSGHHH HHHH) (SEQ ID NO:104)
SARS-CoV-2	SARS-CoV-2- I2-01	X1- GSGSGSGSEKAARAEAAARKMEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTTVPDADT VIKALSVLKEKGAIIGAGTIVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGV FYMPGVMTPTTELVKAMKLGHTILKLFPGGEVVGPPQFVKAMKGPFFNVKFVPTGGV NLNDVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATE (GSGHHH HHHH) (SEQ ID NO:105)

RE D-13- 01*- secOpt- 8GS-he- His	fusion protein	LIEITFTVPDADTVIKELSFLEKMGAIIGAGTVTSVEQAREAVESGAEFIVSPH LDEEISQFAKEEGVFYMPGVMTPTLVKAMKLGHTILKLPGEVVGPPQFVEAMK GPPFNVRFPVPTGGVNLNDVAEWFEAGVQAVGVGEALNEGTFFVEVAEKAKAFVEK IEGATE (SEQ ID NO:105) X1- (X2)MEELFKENKIVAVLRANSVEEAKKKALAVFLGGVDLIEITFTVPDADTVI KELSFLEKMGAIIGAGTVTSVEQAREAVESGAEFIVSPHLDEEISQFAKEEGVF YMPGVMTPTLVKAMKLGHTILKLPGEVVGPPQFVEAMKGPPFNVRFPVPTGGVN LDNVAEWFEAGVQAVGVGEALNEGTFFVEVAEKAKAFVEKIEGATE (SEQ ID NO:106) X1- GGSGGGSGSGSEKAAKAEFAARMEEELFKENKIVAVLRANSVEEAKKKALAVFLGGVD LIEITFTVPDADTVIKELSFLEKMGAIIGAGTVTSVEQAREAVESGAEFIVSPH LDEEISQFAKEEGVFYMPGVMTPTLVKAMKLGHTILKLPGEVVGPPQFVEAMK GPPFNVRFPVPTGGVNLNDVAEWFEAGVQAVGVGEALNEGTFFVEVAEKAKAFVEK IEGATE (GGSHHHHHHH) (SEQ ID NO:107) X1- (X2)MEELFKENKIVAVLRANSVEEAKKKALAVFLGGVDLIEITFTVPDADTVI KELSFLEKMGAIIGAGTVTSVEQAREAVESGAEFIVSPHLDEEISQFAKEEGVF YMPGVMTPTLVKAMKLGHTILKLPGEVVGPPQFVEAMKGPPFNVRFPVPTGGVN LDNVAEWFEAGVQAVGVGEALNEGTFFVEVAEKAKAFVEKIEGATE (GGSHHHHH HHH) (SEQ ID NO:108)
SARS- CoV-2 RE D-13- 01*- secOpt- 12GS-he- His	SARS-CoV- 2-13-01 fusion protein	X1- GGSGGGSGSGSGSEKAAKAEFAARMEEELFKENKIVAVLRANSVEEAKKKALAVFL GGVDLIEITFTVPDADTVIKELSFLEKMGAIIGAGTVTSVEQAREAVESGAEFI VSPHLDEEISQFAKEEGVFYMPGVMTPTLVKAMKLGHTILKLPGEVVGPPQFV EAMKGPPFNVRFPVPTGGVNLNDVAEWFEAGVQAVGVGEALNEGTFFVEVAEKAKA FVEKIEGATE (SEQ ID NO:109) X1- (X2)MEELFKENKIVAVLRANSVEEAKKKALAVFLGGVDLIEITFTVPDADTVI KELSFLEKMGAIIGAGTVTSVEQAREAVESGAEFIVSPHLDEEISQFAKEEGVF YMPGVMTPTLVKAMKLGHTILKLPGEVVGPPQFVEAMKGPPFNVRFPVPTGGVN LDNVAEWFEAGVQAVGVGEALNEGTFFVEVAEKAKAFVEKIEGATE (SEQ ID NO:110) X1- GGSGGGSGSGSGSEKAAKAEFAARMEEELFKENKIVAVLRANSVEEAKKKALAVFL GGVDLIEITFTVPDADTVIKELSFLEKMGAIIGAGTVTSVEQAREAVESGAEFI VSPHLDEEISQFAKEEGVFYMPGVMTPTLVKAMKLGHTILKLPGEVVGPPQFV EAMKGPPFNVRFPVPTGGVNLNDVAEWFEAGVQAVGVGEALNEGTFFVEVAEKAKA FVEKIEGATE (GGSHHHHHHH) (SEQ ID NO:111) X1- (X2)MEELFKENKIVAVLRANSVEEAKKKALAVFLGGVDLIEITFTVPDADTVI KELSFLEKMGAIIGAGTVTSVEQAREAVESGAEFIVSPHLDEEISQFAKEEGVF YMPGVMTPTLVKAMKLGHTILKLPGEVVGPPQFVEAMKGPPFNVRFPVPTGGVN LDNVAEWFEAGVQAVGVGEALNEGTFFVEVAEKAKAFVEKIEGATE (GGSHHHHH HHH) (SEQ ID NO:112)
SARS- CoV-2 RB D-13- 01*- secOpt-	SARS-CoV- 2-13-01 fusion protein	X1- GGSGGGSGSGSGSGSEKAAKAEFAARMEEELFKENKIVAVLRANSVEEAKKKAL AVFLGGVDLIEITFTVPDADTVIKELSFLEKMGAIIGAGTVTSVEQAREAVESG AEFIVSPHLDEEISQFAKEEGVFYMPGVMTPTLVKAMKLGHTILKLPGEVVG PPQFVEAMKGPPFNVRFPVPTGGVNLNDVAEWFEAGVQAVGVGEALNEGTFFVEVAE KAKAFVEKIEGATE (SEQ ID NO:113)

16GS-he- His		X1- (X2)MEELFKEHKIVAVLRANSVEEAKKKALAVFLGGVDLIEITFTVPDADTVI KELSFLKEMGAIIGAGTVTSVEQAREAVESGAEFIVSPHLDEEISQFAKEEGVF YMPGVMTPTTELVKAMKLGHITILKLFPGEVVGPQFVEAMKGFFPNVKFVPTGGVN LDNVAEWFEAGVQAVGVGEALNEGTPVEVAEKAKAFVEKIEGATE (SEQ ID NO:114) X1- GGSGGGSGGGSGSGSEKAAKAEAAARMEELFKEHKIVAVLRANSVEEAKKKAL AVFLGGVDLIEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQAREAVESG AEFIVSPHLDEEISQFAKEEGVFYMPGVMTPTTELVKAMKLGHITILKLFPGEVVGP QFVEAMKGFFPNVKFVPTGGVNLDNVAEWFEAGVQAVGVGEALNEGTPVEVAE KAKAFVEKIEGATE (GGSHHHHHHH) (SEQ ID NO:115) X1- (X2)MEELFKEHKIVAVLRANSVEEAKKKALAVFLGGVDLIEITFTVPDADTVI KELSFLKEMGAIIGAGTVTSVEQAREAVESGAEFIVSPHLDEEISQFAKEEGVF YMPGVMTPTTELVKAMKLGHITILKLFPGEVVGPQFVEAMKGFFPNVKFVPTGGVN LDNVAEWFEAGVQAVGVGEALNEGTPVEVAEKAKAFVEKIEGATE (GGSHHHHH HHH) (SEQ ID NO:116)
SARS- CoV-2 2P SGAG-S- TEV-FO- I3-01*- secOpt- 12GS-he- His (* foldon)	SARS-CoV- 2-I3-01 fusion protein	X1- gsgrenlyfgggggsgyipeaprdggayvrkdgevvllstflgGSSES SEKAAKAEAAARMEELFKEHKIVAVLRANSVEEAKKKALAVFLGGVDLIEITFT VPDADTVIKELSFLKEMGAIIGAGTVTSVEQAREAVESGAEFIVSPHLDEEISQ FAKEEGVFYMPGVMTPTTELVKAMKLGHITILKLFPGEVVGPQFVEAMKGFFPNVK FVPTGGVNLDNVAEWFEAGVQAVGVGEALNEGTPVEVAEKAKAFVEKIEGATE (SEQ ID NO:117) X1- (X2)MEELFKEHKIVAVLRANSVEEAKKKALAVFLGGVDLIEITFTVPDADTVI KELSFLKEMGAIIGAGTVTSVEQAREAVESGAEFIVSPHLDEEISQFAKEEGVF YMPGVMTPTTELVKAMKLGHITILKLFPGEVVGPQFVEAMKGFFPNVKFVPTGGVN LDNVAEWFEAGVQAVGVGEALNEGTPVEVAEKAKAFVEKIEGATE (SEQ ID NO:118) X1- gsgrenlyfgggggsgyipeaprdggayvrkdgevvllstflgGSSES SEKAAKAEAAARMEELFKEHKIVAVLRANSVEEAKKKALAVFLGGVDLIEITFT VPDADTVIKELSFLKEMGAIIGAGTVTSVEQAREAVESGAEFIVSPHLDEEISQ FAKEEGVFYMPGVMTPTTELVKAMKLGHITILKLFPGEVVGPQFVEAMKGFFPNVK FVPTGGVNLDNVAEWFEAGVQAVGVGEALNEGTPVEVAEKAKAFVEKIEGATE (GGSHHHHHHHH) (SEQ ID NO:119) X1- (X2)MEELFKEHKIVAVLRANSVEEAKKKALAVFLGGVDLIEITFTVPDADTVI KELSFLKEMGAIIGAGTVTSVEQAREAVESGAEFIVSPHLDEEISQFAKEEGVF YMPGVMTPTTELVKAMKLGHITILKLFPGEVVGPQFVEAMKGFFPNVKFVPTGGVN LDNVAEWFEAGVQAVGVGEALNEGTPVEVAEKAKAFVEKIEGATE (GGSHHHHH HHH) (SEQ ID NO:120)
SARS- CoV-2 2PSSAG- S-I3- 01*- secOpt- 12GS-he- His	SARS-CoV- 2-I3-01 fusion protein	X1- GGSGGGSGGGSGSEKAFAEAAARMEELFKEHKIVAVLRANSVEEAKKKALAVFL GGVDLIEITFTVPDADTVIKELSFLKEMGAIIGAGTVTSVEQAREAVESGAEFIV VSPHLDEEISQFAKEEGVFYMPGVMTPTTELVKAMKLGHITILKLFPGEVVGPQFV EAMKGFFPNVKFVPTGGVNLDNVAEWFEAGVQAVGVGEALNEGTPVEVAEKAKA FVEKIEGATE (SEQ ID NO:121)

(- foldon)		X1- (X2) MEELFKENKIVAVLRANSVEEAKKKALAVFLGGVDLIEITFTVFPDADTVI KELSPFKRMGAIIGAGTVTSVEQAREAVESGAEFIVSPHLDEEISQFAKEEGVF YMPGVMTPTTELKAMKLGHTILKLPGEVVGPPQFVEAMKGPFPNVKFPVFTGGVN LDNVAEWFEAGVQAVGVGEALNEGTFVEVAEKAKAFVEKIEGATE (SEQ ID NO:122) X1- GGSGSGSGSGSGSSEKAAKAEFAARMEELFKENKIVAVLRANSVEEAKKKALAVFL GGVDLIEITFTVFPDADTVIKELSPFKRMGAIIGAGTVTSVEQAREAVESGAEFI VSPHLDEEISQFAKEEGVFYMPGVMTPTTELKAMKLGHTILKLPGEVVGPPQFV EAMKGPFPNVKFPVFTGGVLDNVAEWFEAGVQAVGVGEALNEGTFVEVAEKAKA FVEKIEGATE (GGSHHHHHHH) (SEQ ID NO:123) X1- (X2) MEELFKENKIVAVLRANSVEEAKKKALAVFLGGVDLIEITFTVFPDADTVI KELSPFKRMGAIIGAGTVTSVEQAREAVESGAEFIVSPHLDEEISQFAKEEGVF YMPGVMTPTTELKAMKLGHTILKLPGEVVGPPQFVEAMKGPFPNVKFPVFTGGVN LDNVAEWFEAGVQAVGVGEALNEGTFVEVAEKAKAFVEKIEGATE (GGSHHHHH HHH) (SEQ ID NO:124)
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I53_dn5A W16E	Degreased nanoparti cle protein	(METDTLLWVLLWVPGSTGDYKDE) KKYDGSKLRIIGILHARENAEIIALVL GALKRLQEFVGVERENIIETVPGSFELPYGSKLFVEKQKRLGKPLDAIIPIGVL IKGSTMHFEYICDSTTHQMLKLNFLGIPVIFGVLTCLTDEQAEARAGLIEGKM HNHGEDWGAAAVEMATKFN (LEGSEQKLI SEEDLNHHHHH) (SEQ ID NO:185)
I53_dn5A L29N	Degreased nanoparti cle protein	(METDTLLWVLLWVPGSTGDYKDE) KKYDGSKLRIIGILHARWNAEIIALVL GALKRLQEFVGVERENIIETVPGSFELPYGSKLFVEKQKRLGKPLDAIIPIGVL IKGSTMHFEYICDSTTHQMLKLNFLGIPVIFGVLTCLTDEQAEARAGLIEGKM HNHGEDWGAAAVEMATKFN (LEGSEQKLI SEEDLNHHHHH) (SEQ ID NO:186)
I53_dn5A 'mut101' [T116N / L118D / T119K]	Degreased nanoparti cle protein	(METDTLLWVLLWVPGSTGDYKDE) KKYDGSKLRIIGILHARWNAEIIALVL GALKRLQEFVGVERENIIETVPGSFELPYGSKLFVEKQKRLGKPLDAIIPIGVL IKGSTMHFEYICDSTTHQMLKLNFLGIPVIFGVLCDDKDEQAEARAGLIEGKM HNHGEDWGAAAVEMATKFN (LEGSEQKLI SEEDLNHHHHH) (SEQ ID NO:187)
I53_dn5A T116N	Degreased nanoparti cle protein	(METDTLLWVLLWVPGSTGDYKDE) KKYDGSKLRIIGILHARWNAEIIALVL GALKRLQEFVGVERENIIETVPGSFELPYGSKLFVEKQKRLGKPLDAIIPIGVL IKGSTMHFEYICDSTTHQMLKLNFLGIPVIFGVLCDDKDEQAEARAGLIEGKM HNHGEDWGAAAVEMATKFN (LEGSEQKLI SEEDLNHHHHH) (SEQ ID NO:188)
I53_dn5A L118D	Degreased nanoparti cle protein	(METDTLLWVLLWVPGSTGDYKDE) KKYDGSKLRIIGILHARWNAEIIALVL GALKRLQEFVGVERENIIETVPGSFELPYGSKLFVEKQKRLGKPLDAIIPIGVL IKGSTMHFEYICDSTTHQMLKLNFLGIPVIFGVLTCLTDEQAEARAGLIEGKM HNHGEDWGAAAVEMATKFN (LEGSEQKLI SEEDLNHHHHH) (SEQ ID NO:189)
I53_dn5A T119K	Degreased nanoparti cle protein	(METDTLLWVLLWVPGSTGDYKDE) KKYDGSKLRIIGILHARWNAEIIALVL GALKRLQEFVGVERENIIETVPGSFELPYGSKLFVEKQKRLGKPLDAIIPIGVL IKGSTMHFEYICDSTTHQMLKLNFLGIPVIFGVLTCLTDEQAEARAGLIEGKM HNHGEDWGAAAVEMATKFN (LEGSEQKLI SEEDLNHHHHH) (SEQ ID NO:190)

I53_dn5A .1	Degreased nanoparti cle protein	{METDTLLLVLLWVPGSTGDYKDEMG} KYDGSKLRIIGILHARGNAEIIILALV LGALKRLQEFQVGRRENIIETVPGSFELPYGSKLFVEKQKRLGKPLDAIIPIGV LIRGSTPHFDYIADSTTHQLMKLNFEIGIPVIFGVITADTDEQAEAPAGLIEGK MHNHGEDWGAAAVEMATKFN (LEGSEQKLISEEDLHHHHHH) (SEQ ID NO:191)
I53_dn5A .1 W16E	Degreased nanoparti cle protein	{METDTLLLVLLWVPGSTGDYKDEMG} KYDGSKLRIIGILHARENAEIIILALV LGALKRLQEFQVGRRENIIETVPGSFELPYGSKLFVEKQKRLGKPLDAIIPIGV LIRGSTPHFDYIADSTTHQLMKLNFEIGIPVIFGVITADTDEQAEAPAGLIEGK MHNHGEDWGAAAVEMATKFN (LEGSEQKLISEEDLHHHHHH) (SEQ ID NO:192)
I53_dn5A .2	Degreased nanoparti cle protein	{METDTLLLVLLWVPGSTGDYKDEMG} KYDGSKLRIIGILHARGNAEIIILELV LGALKRLQEFQVGRRENIIETVPGSFELPYGSKLFVEKQKRLGKPLDAIIPIGV LIRGSTPHFDYIADSTTHQLMKLNFEIGIPVIFGVITTESDEQAEAPAGTKAGN HGEDWGAAAVEMATKFN (LEGSEQKLISEEDLHHHHHH) (SEQ ID NO:193)

- In various embodiments of this third aspect, the second proteins comprise an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:85-88. In various other embodiments, the polypeptides comprise the amino acid sequence selected from the group consisting of SEQ ID NOS: 85-88, or the group consisting of SEQ ID NOS:85-86, or SEQ ID NOS: 85, provided as exemplary embodiments in the examples that follow.

- The nanoparticles of this third aspect display on their surface an immunogenic portion of a SARS-CoV-2 antigen or a variant or homolog thereof, present in the at least one second protein. In one embodiment, the immunogenic portion of a SARS-CoV-2 antigen or a variant or homolog thereof is present as fusion protein with at least one second protein; it can be present on a single second protein in the nanoparticle (present in a single copy on the nanoparticle), or present in a plurality of second proteins present in the nanoparticle. In various embodiments, the SARS-CoV-2 antigen or a variant or homolog thereof is present in at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the second proteins in the nanoparticle.

- In these fusion proteins, the second protein may be joined directly to the SARS-CoV-2 antigen or a variant or homolog thereof, or the second protein and the SARS-CoV-2 antigen or a variant or homolog thereof may be joined using a linker. As used throughout this disclosure, a linker is a short (e.g., 2-30) amino acid sequence used to covalently join two polypeptides. Any suitable linker sequence may be used, including but not limited to those disclosed herein.

Any suitable SARS-CoV-2 antigen or a variant or homolog thereof may be used. In one embodiment of this third aspect, X1 in at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the second proteins comprises an amino acid sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid sequence identity to a Spike (S) protein extracellular domain (ECD) amino acid sequence, an S1 subunit amino acid sequence, an S2 subunit amino acid sequence, an S1 receptor binding domain (RBD) amino acid sequence, and/or an N-terminal domain (NTD) amino acid sequence, from SARS-CoV-2, or a variant or homolog thereof.

In various further embodiments, X1 in at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the second proteins comprises an amino acid sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid sequence identity to the amino acid sequence selected from the group consisting of SEQ ID NO:125-137.

RFPNITNLCPPFGEVFNATRFASVYAWNRRKRISNCVADYSVLYNSASFSTFKCYGVSP TKLNDLCFTNV
YADSFVIRGDEVQRQIAPGQTGKIADYNYKL PDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLK
FERDISTEIIYQAGSTPCNGVEGFNCYFELQSYGFQPTNGVGYQPYRVVVL SFELLHAPATVCGPKKST
(RBD) SEQ ID NO:125

ETGTRFPNITNLCPPFGEVFNATRFASVYAWNRRKRISNCVADYSVLYNSASFSTFKCYGVSP TKLNDLC
FTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKL PDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRK
NLKPFERDISTEIIYQAGSTPCNGVEGFNCYFELQSYGFQPTNGVGYQPYRVVVL SFELLHAPATVCGP
KKST (RBD) SEQ ID NO:126

QCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAINVSGTNGTKRFDN
FVLPFNDGVYFASTEKSNIIPGWIFGTTLDSTQSLIIVNNATNVVVKVCEFPQCNDFPLGVYYHKNN
KSWMESEFRVYSSANNCTFEYVSQPFMDLEGKQGNFKNLREFVFNIDGYFKIYSKHTPINLVRLP
QGFSALEPIVDLPIGINITRFQTLALHRSYLT PGDSSSGWTAGAAAYVGYLQPRTFLLKYNENGTT
TDAVDCALDPLSETKCTLKSFTEKGIYQTSNFRVQPTESIVRFPNITNLCPPFGEVFNATRFASVYAW
NRKRISNCVADYSVLYNSASFSTFKCYGVSP TKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADY
NYKL PDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIIYQAGSTPCNGVEGFNCY
FELQSYGFQPTNGVGYQPYRVVVL SFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNK
KFLRFQQFGRDIADTTDAVRDPQTLEILDITPCFSGGVSVITRGNTSNQVAVLYQDVNCTEVRVAIH
ADQLTPTWRVYSTGSNVFQTRAGCLIGAETHVNNSECDIFIGAGICASYQTQTSNPSGAGSVASQSII
AYTMSLGAENSVAYSNNIAIPTNFTISVTTEILFVSMTKTSVDCTMYICGDSTECNNLLQYGSFCT

- QLNRALTGIABEQDENTQEVFAQVKQIYKTPFIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLA
DAGFIKQYGDCLGDIAARDLCAQKFNGLTVLPFLITDEMIAQYTSALLAGTITSGWTFGAGAAALQIP
FAMQMAYRFNGIGVTONVLYENQKLIANQFNSEAIGKIQDSLSTASALGKLQDVVNQNAQALNTLVKQ
LSSNFGAISSVLNDILSRLDPEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECV
5 LQQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFETAPACHDGKAHFPREGVFVSNQTH
WFTVQRNFYEPQIITTDNTFVSGNCDVVGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDI
SGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIK (Spike (S) protein extracellular
domain (ECD)) SEQ ID NO:127
- 10 (ETGT) QCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNG
TKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSTQSLLLIVNNATNVVLEKVEFCQFCNDPFLGV
YYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLLMDLEGKQGNFKNLREFVFNIDGYFKIYSKHTPIN
LVRDLPQGFSALEPLVDLPIGINITRFQTLALHRSYLTGDSSSGWTAGAAAAYVGYLQPRTFLLKY
NENGTITDAVDCALDPLSETKCTLKSTVEKGIYQTSNFRVQPTESIVREFNITNLCPPGEVFNATRF
15 ASVYAWNKRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQT
GKIADYNYKLPDDFTGCVIAWNSNNLDSKVGNNYLYALFRKSNLKPFERDISTEIIYQAGSTPCNGV
EGFNCYFPIQSYGFQPTNGVGYQPYRVVLSPELLHAPATVCGPKKSTNLVKNKCVNFNENGLTGTGV
LTESNKKFLFPQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQGVNCTE
VFVAIHADQLTPTWVYSTGSNVFQTRAGCLIGAHEVNNSYECDIPIGAGICASYQTQTNPSFGAGSV
20 ASQSIIAYTMSLGAENSVAYSNNISAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSSTECNLLQ
YGSFCTQLNRALTGIABEQDENTQEVFAQVKQIYKTPFIKDFGGFNFSQILPDPSKPSKRSFIEDLLF
NKVTLADAGFIKQYGDCLGDIAARDLCAQKFNGLTVLPFLITDEMIAQYTSALLAGTITSGWTFGAG
AALQIPFAMQMAYRFNGIGVTONVLYENQKLIANQFNSEAIGKIQDSLSTASALGKLQDVVNQNAQAL
NTLVKQLSSNFGAISSVLNDILSRLDPEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAAT
25 KMSECVLQQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFETAPACHDGKAHFPREGVF
VSNQTHWFTVQRNFYEPQIITTDNTFVSGNCDVVGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPD
VDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIK (Spike (S) protein
extracellular domain (ECD), including N-terminal linker related to signal peptide in
parentheses, which may be present or absent) SEQ ID NO:128
- 30 MGILPSPGMPALLSLVSLLSVLLMGCVAETGTQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLH
STQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSTQSL
LIVNNATNVVLEKVEFCQFCNDPFLGVYHKNKSWMESEFRVYSSANNCTFEYVSQPFLLMDLEGKQGN
FKNLREFVFNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLALHRSYLTGDS
35 SSSGWTAGAAAAYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLKSTVEKGIYQTSNFRVQ
PTESIVREFNITNLCPPGEVFNATRFASVYAWNKRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLND

LCFTNVYADSFVIRGDEVROIAPGQTGKIADYNYKLDDFTGCVIAWNSNNLDSKVGGNYYLYRLFR
 KSNLKPFFERDISTEIIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVC
 GEKKSTNLVKNKCVNFNFGLTGTGVLTESNKKFLPFQGFGRDIADTTDAVRDPQTLEILDITPCSF
 GVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSSNVFQTRAGCLIGAETHVNSYE
 5 CDIPIGAGICASYQTQTNSEPSGAGSVASQSI IAYTMSLGAENSVAYSNNISIAIPTNFTISVTTEILPV
 SMTKTSVDCTMYICGDSTECNNLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDF
 GGFNFSQILPDPSPKSKRSFIEDLLFNKVTADAGFIKQYGDCLGDIAARDLCAQKFNGLTVLPFL
 TDEMIAQYTSALLAGTITSGWTFGAGAAIQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFN
 SAIGKIQDLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRDPPEAEVQIDRLITGR
 10 QSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSPQSAFPGVFLHVTYVP
 AQEKNETTAPALCHDGAHFFREGVFSNGTHWFVTQRNFYEPQIITDNTFVSGNCDVIGIVNNTV
 YDPLQPELDSFKEELDKYFKNHTSPDVLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGK
 YEQYIK (SEQ ID NO:129) mu phosphatase signal peptide, and the ETGT is left over as a
 remnant after signal peptide cleavage

15 (MEVFLVLLPLVSSQC) VNLTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGT
 NGTERFDNPFVLPFNDGVYFASTEKSNIIIRGWIFGTTLDSKTQSLIVNNATNVVIVKCEFPQCNDFFLGVYHKN
 NKSWMSESEFRVYSSANNCTFEYVSQPFMLMLEGKQGNFKNLREFVFKNIDGYFKIYKNTFINLVRDLPGQFSAL
 EPLVDLPIGINITRPTLLALHRSYLPFGDSSSGWTAGAAAYVGYLQPRFTLLKYNEGTITDAVDCALDPLSE
 20 TKCTLKSFTEKGIYQTSNFRVQPTESIVRFPNITNLCPFGGVFNATRFASVYAMNRKRI SNQVADYSVLYNSAS
 FSTFKCYGVSPKLNLDLFTNVYADSFVIRGDEVROIAPGQTGKIADYNYKLDDFTGCVIAWNSNNLDSKVGGN
 YNYLYRLFRKSNLKPFFERDISTEIIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPAT
 VCGPKHSTNLVKNKCVNFNFGLTGTGVLTESNKKFLPFQGFGRDIADTTDAVRDPQTLEILDITPCSF
 GVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSSNVFQTRAGCLIGAETHVNSYEC
 25 DIPIGAGICASYQTQTNSEPSGAGSVASQSI IAYTMSLGAENSVAYSNNISIAIPTNFTISVTTEILFVSM
 TKTSDCTMYICGDSTECNNLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQIL
 PDPSPKSKRSFIEDLLFNKVTADAGFIKQYGDCLGDIAARDLCAQKFNGLTVLPFLTDEMIAQYTSALLAGTIT
 SGWTFGAGAAIQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFN
 30 SAIGKIQDLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRDPPEAEVQIDRLITGR
 QSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSPQSAFPGVFLHVTYVP
 AQEKNETTAPALCHDGAHFFREGVFSNGTHWFVTQRNFYEPQIITDNTFVSGNCDVIGIVNNTVYDPLQPELDS
 FKEELDKYFKNHTSPDVLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQ (SEQ ID NO:130)

35 (MEVFLVLLPLVSSQC) VNLTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGT
 NGTKRFDNPFVLPFNDGVYFASTEKSNIIIRGWIFGTTLDSKTQSLIVNNATNVVIVKCEFPQCNDFFLGVYHKN
 NKSWMSESEFRVYSSANNCTFEYVSQPFMLMLEGKQGNFKNLREFVFKNIDGYFKIYKNTFINLVRDLPGQFSAL
 EPLVDLPIGINITRPTLLALHRSYLPFGDSSSGWTAGAAAYVGYLQPRFTLLKYNEGTITDAVDCALDPLSE
 TKCTLKSFTEKGIYQTSNFRVQPTESIVRFPNITNLCPFGGVFNATRFASVYAMNRKRI SNQVADYSVLYNSAS
 FSTFKCYGVSPKLNLDLFTNVYADSFVIRGDEVROIAPGQTGKIADYNYKLDDFTGCVIAWNSNNLDSKVGGN

YNYLYRLFRKSNLKPFERDISTEIIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPAT
 VCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSGGVS
 TPGTNTSNQVAVLYQDVNCTEVEVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAHEVNNSECDIPGAGICAS
 YQTQTNSPGSASSVASQSIIAYTMSLGAENSVAYSNNNSIAIPTNFTISVTTEILPVSMTKTSVDCCTMYICGDS
 5 CSNLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGCGFNFSQILPDPSPKPSKRSPIEDLLF
 NKVTLADAGFIKQYGDCLGDI AARDLCAQKFNGLTVLFPLLTDEMIQYTSALLAGTITSGWTFGAGPALQIPF
 PMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTPSALGKLQDVVNQNAQALNTLVKQLSSNFGAI
 SSVLNDILSRLOPPEAEVQIDRLITGRQLSLQTYVTQQLIRAAEIRASANLAATKMSCEVLGQSKRVDFCGKGYH
 LMSFFQSAFHGVVFLHVTYVPAQEKNTTAPAI CHDGAHFFREGVFSNGTHWFTQNRNFEPIITDNTFVS
 10 GNCDVIGIVNNTVYDPLQPELDSFKEELDKYFKNHT (SEQ ID NO:131)

(QC) VNLTTTFQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSCTNGTKRFDNEVLFP
 NDGVYFASTEKSNIIRGWIFGTTLDSTQSLIVNNATNVVIXVCEFCNDPFLGVYHKNKSWMESEPRVYS
 SANNCTFEYVSQPFLLMDLGGKQGNFKNLREFVFKNIDGYFKIYSEHTPINLVRLDLPQGFSALEPLVDLPICINIT
 15 RFQTLALHRSYLTGDSSSGWTAGAAAYVGYLQFRTFLKYNENGTITDAVDCALDPLSETCTLKSFTVEKG
 IYQTSNFRVQPTESIVRFENITNLCPPGEVFNATRFASVYAWNRKRISNCVADYSVLVNSASFSTFKCYGVSPTK
 LNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPEDDFTGCVIANNSSNLDSEKVGNGYNYLYRLFRKSNL
 KPFERDISTEIIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKN
 KCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSGGVSVITPGTNTSNQVAVL
 20 YQDVNCTEVEVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAHEVNNSECDIPGAGICASYQTQTNSPGSASS
 VASQSIIAYTMSLGAENSVAYSNNNSIAIPTNFTISVTTEILPVSMTKTSVDCCTMYICGDSCTCSNLLQYGSFCT
 QLNRAITGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGCGFNFSQILPDPSPKPSKRSPIEDLLFNKVTADAGFIKQ
 YGDCLGDI AARDLCAQKFNGLTVLFPLLTDEMIQYTSALLAGTITSGWTFGAGPALQIPFPMQMAYRFNGIGV
 TQNVLYENQKLIANQFNSAIGKIQDSLSTPSALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLOP
 25 PEAEVQIDRLITGRQLSLQTYVTQQLIRAAEIRASANLAATKMSCEVLGQSKRVDFCGKGYHLMSSFFQSAFHGVV
 FLHVTYVPAQEKNTTAPAI CHDGAHFFREGVFSNGTHWFTQNRNFEPIITDNTFVS GNCDVIGIVNNT
 VYDPLQPELDSFKEELDKYFKNHTSPDVLGDISGINASVUNIQKEIDRLNEVAKNLNESLIDLQELGKYEQ
 (SEQ ID NO:132)

(QC) VNLTTTFQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSCTNGTKRFDNEVLFP
 NDGVYFASTEKSNIIRGWIFGTTLDSTQSLIVNNATNVVIXVCEFCNDPFLGVYHKNKSWMESEPRVYS
 SANNCTFEYVSQPFLLMDLGGKQGNFKNLREFVFKNIDGYFKIYSEHTPINLVRLDLPQGFSALEPLVDLPICINIT
 30 RFQTLALHRSYLTGDSSSGWTAGAAAYVGYLQFRTFLKYNENGTITDAVDCALDPLSETCTLKSFTVEKG
 IYQTSNFRVQPTESIVRFENITNLCPPGEVFNATRFASVYAWNRKRISNCVADYSVLVNSASFSTFKCYGVSPTK
 LNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPEDDFTGCVIANNSSNLDSEKVGNGYNYLYRLFRKSNL
 KPFERDISTEIIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKN
 KCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSGGVSVITPGTNTSNQVAVL
 35 YQDVNCTEVEVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAHEVNNSECDIPGAGICASYQTQTNSPGSASS
 VASQSIIAYTMSLGAENSVAYSNNNSIAIPTNFTISVTTEILPVSMTKTSVDCCTMYICGDSCTCSNLLQYGSFCT
 QLNRAITGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGCGFNFSQILPDPSPKPSKRSPIEDLLFNKVTADAGFIKQ
 40 YGDCLGDI AARDLCAQKFNGLTVLFPLLTDEMIQYTSALLAGTITSGWTFGAGPALQIPFPMQMAYRFNGIGV

TQNVLYENQKLIANQFNSAIGKIQDSLSSTPSALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRDP
PRAENVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSPQSAFHGVV
FLHVTYVPAQEKNFHTTAPAICHGDKAHFPREGVFSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVGIVNNT
VYDPLQPELDSFKEELDKYFKNHT (SEQ ID NO:133)

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VNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFAIHVSGTNGTKRFDNEVLPFNDGV
YFASTEKSNIIRGWIFGTTLDSKTQSLILIVNNATNVVIVKCEFPQFCNDPFLGVYHKNKNSWMESEFRVYSSANN
CTFEYVSQPFMLDLEGKQGNFKNLREFVFNIDGYFKIYSKHTFINLVRDLPGQFSALEPLVDLPIGINITRFQT
LLALHRSYLTFGDSSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDAVDCALDELSETKCTLSFTVEKGIYQF
SNFRVQPTESIVRFPNITNLCPFGVEFNATRFASVYAWNRRKISNCVADYSVLNYSASFSTFKCYGVSPTELNDL
CFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNKLPDDFTGCVIANNSSNNLDSKVGGNXYLYLFRKSNLKPFE
RDISTEIIYQAGSTPCNGVEGFNCYFPLQSYGFPQFTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVN
FNFNGLTGTGVLTESNKKFLPQQFGRIADTTDAVRDPQTLBILDITPCSGGCVSVITPCTNTSNQVAVLYQDV
NCTEVPVAIHADQLTPTWRVYSTGSSNVFQTRACCLIGAHEVNNSYECDIPIGAGICASYQTQTNSPGSASSVASQ
SIIAYTMSLGAENSVAYSNNISIAIPTNFTISVTEILFVSMKTSVDCTMYICGDSSTECNLLIQYGSFCTQLNR
ALTGIAVEQDKNTQEVFAQVHQIYKTPFIKDFGGFNFSQILPDPFSKPSKRSPIEDLLFNKVTLADAGFIKQYGD
LGDIAARDLICAQKFNGLTVLPLLLTDEMIAQYTSALLAGTITSGWTFGAGPALQIPFFMQMAYRPNIGIQTQNV
LYENQKLIANQFNSAIGKIQDSLSSTPSALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRDPPEAE
VQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSPQSAFHGVVFLHV
TYVPAQEKNFHTTAPAICHGDKAHFPREGVFSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVGIVNNTVYDE
LQPELDSFKEELDKYFKNHTSPDVLGDLSGINASVNNIQKEIDRLNEVAKNLNESLIDLQELGKYEQ (SEQ
ID NO:134)

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VNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFAIHVSGTNGTKRFDNEVLPFNDGV
YFASTEKSNIIRGWIFGTTLDSKTQSLILIVNNATNVVIVKCEFPQFCNDPFLGVYHKNKNSWMESEFRVYSSANN
CTFEYVSQPFMLDLEGKQGNFKNLREFVFNIDGYFKIYSKHTFINLVRDLPGQFSALEPLVDLPIGINITRFQT
LLALHRSYLTFGDSSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDAVDCALDELSETKCTLSFTVEKGIYQF
SNFRVQPTESIVRFPNITNLCPFGVEFNATRFASVYAWNRRKISNCVADYSVLNYSASFSTFKCYGVSPTELNDL
CFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNKLPDDFTGCVIANNSSNNLDSKVGGNXYLYLFRKSNLKPFE
RDISTEIIYQAGSTPCNGVEGFNCYFPLQSYGFPQFTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVN
FNFNGLTGTGVLTESNKKFLPQQFGRIADTTDAVRDPQTLBILDITPCSGGCVSVITPCTNTSNQVAVLYQDV
NCTEVPVAIHADQLTPTWRVYSTGSSNVFQTRACCLIGAHEVNNSYECDIPIGAGICASYQTQTNSPGSASSVASQ
SIIAYTMSLGAENSVAYSNNISIAIPTNFTISVTEILFVSMKTSVDCTMYICGDSSTECNLLIQYGSFCTQLNR
ALTGIAVEQDKNTQEVFAQVHQIYKTPFIKDFGGFNFSQILPDPFSKPSKRSPIEDLLFNKVTLADAGFIKQYGD
LGDIAARDLICAQKFNGLTVLPLLLTDEMIAQYTSALLAGTITSGWTFGAGPALQIPFFMQMAYRPNIGIQTQNV
LYENQKLIANQFNSAIGKIQDSLSSTPSALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRDPPEAE
VQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSPQSAFHGVVFLHV
TYVPAQEKNFHTTAPAICHGDKAHFPREGVFSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVGIVNNTVYDE
LQPELDSFKEELDKYFKNHT (SEQ ID NO:135)

ETCTQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWPHAIHVSGTNGTKRFDNFVL
 PFNDGVYFASFEESNIIRGWIFGTTLDSKTQSLIIVNNATNVVIVKCFQPCNDPFLGVVYHNNKSNMSESEFRV
 YSSANNCTFEYVSQPFMLDLEGKQGNFNLRREFVFNIDGYFKIYSKHTPINLVRDLPGQFSALEPLVNLFIGIN
 ITRFQTLALHRSYLTFGDSSSGWTAGAAAYVGYLQPRFTFLKYNENGTITDAVDCALDPLSETKCTLKSFTVE
 5 KGIYQTSNFRVQPTESIVRFPNITNLCEFFGEVFNATRFASVYAWNRRKRSNCVADYSVLYNSASFSTFKCYGVSP
 TKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGNGNYLYRLFRKS
 NLKPFERDISTEYIYQAGSTPCNGVEGFNCYFFLQSYGFGPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLV
 ANKCVNFNFNGLTGTGVLTESNKKFLPFQGFGRDIADTTDAVRDPQTLIILDITPCSFGGVSVITPGTNTSNQVA
 VLYQDVNCTEVPVAIHADQLTPTWRVYSTGNSVFPQTRAGCLIGAENVNNSYECDIPIGAGICASYQTQNTSPGSA
 10 SSVASQSIIAYTMSLGAENSVAYSNNSTAIPTNFTISVYTEILFVSMTKTSVDCTMYICGDSTECNSLLQYGSF
 CTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSPIEDLLFNKVTIADAGFI
 KQYGDCLGDIAARDLCAQKFNGLTVLPPLLTDEMIQYTSALLAGTITSGWTFGAGPALQIPFFMQMAYRENGI
 GVTQNVLYENQKLIANQFNSAIGKIQDSLSSTPSALGHLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRL
 DPPEAEVQIDRLITGRLOSLQTYVTQQLIRAAHIRASANLAATMSECVLGQSKRVDFCGKGYHLMSPQSAFPG
 15 VVFLHVTVYVPAQEKNFPTTAPAICHGDKAHFPREGVFSNGTHWVFTQRNFYEPQIITTDNTFVSGNCDVIGIVN
 NTVYDPLQPELDSFKEELDKYFNHTSPDVLGDISGINASVNIQKEIDRLNEVAKNLNESLIDLQELGEYEQ
 (SEQ ID NO:136)

ETCTQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWPHAIHVSGTNGTKRFDNFVL
 20 PFNDGVYFASFEESNIIRGWIFGTTLDSKTQSLIIVNNATNVVIVKCFQPCNDPFLGVVYHNNKSNMSESEFRV
 YSSANNCTFEYVSQPFMLDLEGKQGNFNLRREFVFNIDGYFKIYSKHTPINLVRDLPGQFSALEPLVNLFIGIN
 ITRFQTLALHRSYLTFGDSSSGWTAGAAAYVGYLQPRFTFLKYNENGTITDAVDCALDPLSETKCTLKSFTVE
 KGIYQTSNFRVQPTESIVRFPNITNLCEFFGEVFNATRFASVYAWNRRKRSNCVADYSVLYNSASFSTFKCYGVSP
 TKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGNGNYLYRLFRKS
 25 NLKPFERDISTEYIYQAGSTPCNGVEGFNCYFFLQSYGFGPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLV
 ANKCVNFNFNGLTGTGVLTESNKKFLPFQGFGRDIADTTDAVRDPQTLIILDITPCSFGGVSVITPGTNTSNQVA
 VLYQDVNCTEVPVAIHADQLTPTWRVYSTGNSVFPQTRAGCLIGAENVNNSYECDIPIGAGICASYQTQNTSPGSA
 SSVASQSIIAYTMSLGAENSVAYSNNSTAIPTNFTISVYTEILFVSMTKTSVDCTMYICGDSTECNSLLQYGSF
 CTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSPIEDLLFNKVTIADAGFI
 30 KQYGDCLGDIAARDLCAQKFNGLTVLPPLLTDEMIQYTSALLAGTITSGWTFGAGPALQIPFFMQMAYRENGI
 GVTQNVLYENQKLIANQFNSAIGKIQDSLSSTPSALGHLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRL
 DPPEAEVQIDRLITGRLOSLQTYVTQQLIRAAHIRASANLAATMSECVLGQSKRVDFCGKGYHLMSPQSAFPG
 VVFLHVTVYVPAQEKNFPTTAPAICHGDKAHFPREGVFSNGTHWVFTQRNFYEPQIITTDNTFVSGNCDVIGIVN
 NTVYDPLQPELDSFKEELDKYFNHT (SEQ ID NO:137)

35

In one specific embodiment, X1 in at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the second proteins comprises an amino acid sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid sequence identity to the amino acid sequence of SEQ ID NO:125, the SARS-CoV-2 RBD

provided as exemplary embodiments in the examples that follow. In various embodiments, X1 in at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the second proteins comprise mutations at 1, 2, 3, 4, 5, 6, 7, or all 8 positions relative to SEQ ID NO:125 selected from the group consisting of K90N, K90T, G119S, Y126F, T151I, E157K, E157A, S167P, N174Y, and L125R, including but not limited to mutations comprising one of the following naturally occurring mutations or combinations of mutations:

- N174Y (UK variant);
- K90N/E157K/N174Y (South African variant);
- K90N or T/E157K/N174Y (Brazil variant); or
- L125R (LA variant).

The amino acid residue numbering of these naturally occurring variants is based on their position within SEQ ID NO:125, while they are generally described based on their residue number in the Spike protein (i.e.: K417 in spike = K90 in RBD; G446 in spike = G119 in RBD; L452 in spike = L125 in RBD; Y453 in spike = Y126 in RBD; T478 in spike = T151 in RBD; E484 in spike = E157 in RBD; S494 in spike = S167 in RBD; N501 in spike = N174 in RBD).

In various further embodiments, X1 in at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the second proteins comprise mutations at 1, 2, 3, 4, 5, 6, 7, or all 8 positions relative to SEQ ID NO:130 selected from the group consisting of L18F, T20N, P26S, deletion of residues 69-70, D80A, D138Y, R190S, D215G, K417N, K417T, G446S, L452R, Y453F, T478I, E484K, S494P, N501Y, A570D, D614G, H655Y, P681H, A701V, T716L including but not limited to mutations comprising one of the following naturally occurring mutations or combinations of mutations:

- N501Y, optionally further including 1, 2, 3, 4, or 5 of deletion of one or both of residues 69-70, A570D, D614G, P681H, and/or T716L (UK variant);
- K417N/E484K/N501Y, optionally further including 1, 2, 3, 4, or 5 of L18F, D80A, D215G, D614G, and/or A701V (South African variant);
- K417N or T/E484K/N501Y, optionally further including 1, 2, 3, 4, or 5 of L18F, T20N, P26S, D138Y, R190S, D614G, and/or H655Y (Brazil variant); or
- L452R (LA variant).

As will be understood by those of skill in the art, when X1 comprises an amino acid sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid sequence identity to the amino acid sequence of SEQ ID

NO:125 (or any other disclosed antigen), it may include additional amino acids at the amino- or carboxy-terminus. Thus, for example, when X1 comprises an amino acid sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid sequence identity to the amino acid sequence of SEQ ID NO:125, X1 may
 5 comprise the amino acid sequence of SEQ ID NO:126, which includes additional amino acids at its N-terminus relative to SEQ ID NO:125.

In a further embodiment, X1 in at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the second proteins comprise 1, 2, 3, or all 4 mutations relative to SEQ ID NO:125 selected from the group consisting of K90N, K90T, E157K, and N174Y.

10 The plurality of second assemblies may in total comprise a single SARS-CoV-2 antigen, or may comprise 2 or more different SARS-CoV-2 antigen. In one embodiment, the plurality of second assemblies in total comprises 2, 3, 4, 5, 6, 7, 8, or more different SARS-CoV-2 antigens. In one exemplary such embodiment, the plurality of second assemblies in total comprise 2, 3, 4, 5, 6, 7, 8, or more polypeptides comprising the amino acid sequence of
 15 any one of SEQ ID NOS: 1-84.

In one embodiment, X1 in at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the second proteins comprises the amino acid sequence of SEQ ID NO:125. In another embodiment, X1 in 100% of the second proteins comprises the amino acid sequence of SEQ ID NO:125, and all second proteins are identical.

20 In a further embodiment, all second assemblies comprise at least one second protein comprising the amino acid sequence of any one of SEQ ID NOS: 1-84. In another embodiment, all second proteins comprise the amino acid sequence of any one of SEQ ID NOS: 1-84.

The nanoparticles comprise a plurality of identical first proteins. In one embodiment,
 25 the first protein comprises an amino acid sequence at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected the group consisting of SEQ ID NOS:152-159, wherein residues in parentheses are optional and may be present or some (i.e.: 1, 2, 3, 4, 5, 6, or more) or all of the optional residues may be absent. In a specific embodiment, the first protein comprises an amino acid
 30 sequence at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:155.

Name	Amino Acid Sequence	Identified interface residues	Surface residues not near interface
I53-50B SEQ ID NO:152	(M)NQSHKDYETVRIAVVEAPW HAEIVDACVSFAEAMRDIGGDR FAVDVFDVPGAYEIPLHARTLAE TGRYGAVLGTAFFVVGGIYRHEF VASAVIDGMMNVQLDTGVPVLSA VLTPHRYRDSDAHTLLFLALFAV KGMEARACVEILAAREKIAA	I53-50B: 24,28,36,124,125,127,128,129, 131,132,133,135,139	I53-50B: 6,7,8,9,10,11,13,18,20, 21,34,38,39,40,43,44,48, 51,63,67,70,87,101,105, 118,143,147,152,153,154
I53-50B.1 SEQ ID NO:153	(M)NQSHKDHETVRIAVVEAPW HAEIVDACVSFAEAMRDIGGDR FAVDVFDVPGAYEIPLHARTLAE TGRYGAVLGTAFFVVGGIYRHEF VASAVIDGMMNVQLDTGVPVLSA VLTPHRYRDSDAHTLLFLALFAV KGMEARACVEILAAREKIAA	I53-50B: 24,28,36,124,125,127,128,129, 131,132,133,135,139	I53-50B: 6,7,8,9,10,11,13,18,20, 21,34,38,39,40,43,44,48, 51,63,67,70,87,101,105, 118,143,147,152,153,154
I53-50B.1NegT2 SEQ ID NO:154	(M)NQSHKDHETVRIAVVEAPW HAEIVDACVSFAEAMRDIGGDR FAVDVFDVPGAYEIPLHARTLAE TGRYGAVLGTAFFVVGGIYRHEF VASAVIDGMMNVQLDTGVPVLSA VLTPHRYRDSDAHTLLFLALFAV KGMEARACVEILAAREKIAA	I53-50B: 24,28,36,124,125,127,128,129, 131,132,133,135,139	I53-50B: 6,7,8,9,10,11,13,18,20, 21,34,38,39,40,43,44,48, 51,63,67,70,87,101,105, 118,143,147,152,153,154
I53-50B.4PosT1 SEQ ID NO:155	(M)NQSHKDHETVRIAVVEAPW HAEIVDACVSFAEAMRDIGGDR FAVDVFDVPGAYEIPLHARTLAE TGRYGAVLGTAFFVVGGIYRHEF VASAVIDGMMNVQLDTGVPVLSA VLTPHRYRDSDAHTLLFLALFAV KGMEARACVEILAAREKIAA	I53-50B: 24,28,36,124,125,127,128,129, 131,132,133,135,139	I53-50B: 6,7,8,9,10,11,13,18,20, 21,34,38,39,40,43,44,48, 51,63,67,70,87,101,105, 118,143,147,152,153,154

I53-50-v4 pentameric component
(MGSSHHHHHSSGLVPRGSEQKLISEEDLGS)NQHSQKDQETVRIAVVRARWHAFIVDACV
SAFEAAMRKIGGERFAVDVFDVPGAYEIPLHARTLAKTGRYGAVLGTAFFVVGGIYRHEFVA
5 SAVIDGMMNVQLDTGVPVLSAVLTPHNYDKSNAKTLLFLALFAVKGMEARACVEILAAREK
IAA(GSLEGS) (SEQ ID NO:156)

I53-50-v1 pentameric component B
(M)NQSHKDHETVRIAVVRARWHAEIVDACVSFAEAMRDIGGDRFAVDVFDVPGAYEIPL
10 HARTLAETGRYGAVLGTAFFVVGGIYRHEFVASAVIDGMMNVQLDTGVPVLSAVLTPHNYDK
SKAHTLLFLALFAVKGMEARACVEILAAREKIAA(GS) (SEQ ID NO:157)

I53-50-v2 pentameric component B
(M)NQSHKDHETVRIAVVRARWHAFIVDACVSFAEAMRDIGGDRFAVDVFDVPGAYEIPL
15 HARTLAETGRYGAVLGTAFFVVGGIYRHEFVASAVIDGMMNVQLDTGVPVLSAVLTPHNYDK
SNAKTLLFLALFAVKGMEARACVEILAAREKIAA(GS) (SEQ ID NO:158)

I53-50-v3 pentameric component B
(M)NQSHKDHETVRIAVVRARWHAFIVDACVSFAEAMRDIGGDRFAVDVFDVPGAYEIPL
20 HARTLAETGRYGAVLGTAFFVVGGIYRHEFVASAVIDGMMNVQLDTGVPVLSAVLTPHNYDK
SNAKTLLFLALFAVKGMEARACVEILAAREKIAA(GS) (SEQ ID NO:159)

In an exemplary embodiment, the first protein comprises the amino acid sequence of SEQ ID NO:155. In various further such embodiments, the at least one or a plurality (20%, 33%, 40%, 50%, 75%, etc.) of the second assemblies comprises at least one second protein comprising the amino acid sequence selected from the group consisting of SEQ ID NO:85-88, or all second assemblies comprise at least one second protein comprising the amino acid sequence selected from the group consisting of SEQ ID NO:85-88.

In one specific embodiment,

- (a) the first protein comprises the amino acid sequence of SEQ ID NO:155;
 - (b) all second proteins comprise the amino acid sequence of SEQ ID NO:85,
- wherein X1 in at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the second proteins comprise an amino acid sequence at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid sequence identity to the amino acid sequence of SEQ ID NO:125.

In another specific embodiment,

- (a) the first protein comprises the amino acid sequence of SEQ ID NO:155;
 - (b) all second proteins comprise the amino acid sequence of SEQ ID NO:85,
- wherein X1 in at least 50%, 60%, 70%, 80%, 90%, or 100% of the second proteins comprise an amino acid sequence at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid sequence identity to the amino acid sequence of SEQ ID NO:125.

In a further specific embodiment:

- (a) the first protein comprises the amino acid sequence of SEQ ID NO:155;
- (b) all second proteins comprise the amino acid sequence selected from the group consisting of SEQ ID NO:1-8.

In one specific embodiment:

- (a) the first protein comprises the amino acid sequence of SEQ ID NO:155;
- (b) all second proteins comprise the amino acid sequence of SEQ ID NO:1 or 5.

The disclosure further provides compositions, comprising a plurality of nanoparticles of any embodiment or combination of embodiments of the disclosure. In one embodiment, the compositions comprise a plurality of nanoparticles of the specific embodiments disclosed above.

In a fourth aspect, the disclosure provides nucleic acids encoding a polypeptide or fusion protein of the disclosure. The nucleic acid sequence may comprise RNA (such as mRNA) or DNA. Such nucleic acid sequences may comprise additional sequences useful for promoting expression and/or purification of the encoded protein, including but not limited to polyA sequences, modified Kozak sequences, and sequences encoding epitope tags, export signals, and secretory signals, nuclear localization signals, and plasma membrane localization signals. It will be apparent to those of skill in the art, based on the teachings herein, what nucleic acid sequences will encode the proteins of the invention.

In a fifth aspect, disclosure provides expression vectors comprising the isolated nucleic acid of any embodiment or combination of embodiments of the disclosure operatively linked to a suitable control sequence. "Expression vector" includes vectors that operatively link a nucleic acid coding region or gene to any control sequences capable of effecting expression of the gene product. "Control sequences" operably linked to the nucleic acid sequences of the disclosure are nucleic acid sequences capable of effecting the expression of the nucleic acid molecules. The control sequences need not be contiguous with the nucleic acid sequences, so long as they function to direct the expression thereof. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the nucleic acid sequences and the promoter sequence can still be considered "operably linked" to the coding sequence. Other such control sequences include, but are not limited to, polyadenylation signals, termination signals, and ribosome binding sites. Such expression vectors can be of any type known in the art, including but not limited to plasmid and viral-based expression vectors. The control sequence used to drive expression of the disclosed nucleic acid sequences in a mammalian system may be constitutive (driven by any of a variety of promoters, including but not limited to, CMV, SV40, RSV, actin, EF) or inducible (driven by any of a number of inducible promoters including, but not limited to, tetracycline, ecdysone, steroid-responsive).

In a sixth aspect, the present disclosure provides cells comprising the polypeptide, the nanoparticle, the composition, the nucleic acid, and/or the expression vector of any embodiment or combination of embodiments of the disclosure, wherein the cells can be either prokaryotic or eukaryotic, such as mammalian cells. In one embodiment the cells may be transiently or stably transfected with the nucleic acids or expression vectors of the disclosure. Such transfection of expression vectors into prokaryotic and eukaryotic cells can be accomplished via any technique known in the art. A method of producing a polypeptide

according to the invention is an additional part of the invention. The method comprises the steps of (a) culturing a host according to this aspect of the invention under conditions conducive to the expression of the polypeptide, and (b) optionally, recovering the expressed polypeptide.

- 5 In a seventh aspect, the disclosure provides pharmaceutical compositions/vaccines comprising
- (a) the polypeptide, the nanoparticle, the composition, the nucleic acid, the expression vector, and/or the cell of embodiment or combination of embodiments herein; and
 - (b) a pharmaceutically acceptable carrier.

- 10 As shown in the examples that follow, the nanoparticle immunogens elicit potent and protective antibody responses against SARS-CoV-2. The nanoparticle vaccines of the disclosure induce neutralizing antibody titers roughly ten-fold higher than the prefusion-stabilized S ectodomain trimer despite a more than five-fold lower dose. Antibodies elicited by the nanoparticle immunogens target multiple distinct epitopes, suggesting that they may
- 15 not be easily susceptible to escape mutations, and exhibit a significantly lower binding:neutralizing ratio than convalescent human sera, which may minimize the risk of vaccine-associated enhanced respiratory disease.

- The compositions/vaccines may further comprise (a) a lyoprotectant; (b) a surfactant; (c) a bulking agent; (d) a tonicity adjusting agent; (e) a stabilizer; (f) a preservative and/or (g)
- 20 a buffer. In some embodiments, the buffer in the pharmaceutical composition is a Tris buffer, a histidine buffer, a phosphate buffer, a citrate buffer or an acetate buffer. The composition may also include a lyoprotectant, e.g. sucrose, sorbitol or trehalose. In certain embodiments, the composition includes a preservative e.g. benzalkonium chloride, benzethonium, chlorohexidine, phenol, m-cresol, benzyl alcohol, methylparaben, propylparaben,
- 25 chlorobutanol, o-cresol, p-cresol, chlorocresol, phenylmercuric nitrate, thimerosal, benzoic acid, and various mixtures thereof. In other embodiments, the composition includes a bulking agent, like glycine. In yet other embodiments, the composition includes a surfactant e.g., polysorbate-20, polysorbate-40, polysorbate- 60, polysorbate-65, polysorbate-80 polysorbate-85, poloxamer-188, sorbitan monolaurate, sorbitan monopalmitate, sorbitan monostearate,
- 30 sorbitan monooleate, sorbitan trilaurate, sorbitan tristearate, sorbitan trioleate, or a combination thereof. The composition may also include a tonicity adjusting agent, e.g., a compound that renders the formulation substantially isotonic or isoosmotic with human blood. Exemplary tonicity adjusting agents include sucrose, sorbitol, glycine, methionine,

mannitol, dextrose, inositol, sodium chloride, arginine and arginine hydrochloride. In other embodiments, the composition additionally includes a stabilizer, e.g., a molecule which substantially prevents or reduces chemical and/or physical instability of the nanostructure, in lyophilized or liquid form. Exemplary stabilizers include sucrose, sorbitol, glycine, inositol, sodium chloride, methionine, arginine, and arginine hydrochloride.

The nanoparticles may be the sole active agent in the composition, or the composition may further comprise one or more other agents suitable for an intended use, including but not limited to adjuvants to stimulate the immune system generally and improve immune responses overall. Any suitable adjuvant can be used. The term "adjuvant" refers to a compound or mixture that enhances the immune response to an antigen. Exemplary adjuvants include, but are not limited to, Adju-PhosTM, AdjuvaxTM, albumin-heparin microparticles, Algal Glucan, Algammulin, Alum, Antigen Formulation, AS-2 adjuvant, autologous dendritic cells, autologous PBMC, AvridineTM, B7-2, BAK, BAY R1005, Bupivacaine, Bupivacaine-HCl, BWZL, Calcitriol, Calcium Phosphate Gel, CCR5 peptides, CFA, Cholera holotoxin (CT) and Cholera toxin B subunit (CTB), Cholera toxin A1-subunit-Protein A D-fragment fusion protein, CpG, CRL1005, Cytokine-containing Liposomes, D-Murapalmitine, DDA, DHEA, Diphtheria toxoid, DL-PGL, DMPC, DMPG, DOC/Alum Complex, Fowlpox, Freund's Complete Adjuvant, Gamma Inulin, Gerbu Adjuvant, GM-CSF, GMDP, hGM-CSF, hIL-12 (N222L), hTNF-alpha, IFA, IFN-gamma in pcDNA3, IL-12 DNA, IL-12 plasmid, IL-12/GMCSF plasmid (Sykes), IL-2 in pcDNA3, IL-2/Ig plasmid, IL-2/Ig protein, IL-4, IL-4 in pcDNA3, ImiquimodTM, ImmTherTM, Immunoliposomes Containing Antibodies to Costimulatory Molecules, Interferon-gamma, Interleukin-1 beta, Interleukin-12, Interleukin-2, Interleukin-7, ISCOM(s)TM, Iscoprep 7.0.3TM, Keyhole Limpet Hemocyanin, Lipid-based Adjuvant, Liposomes, Loxoribine, LT(R192G), LT-OA or LT Oral Adjuvant, LT-R192G, LTK63, LTK72, MF59, MONTANIDE ISA 51, MONTANIDE ISA 720, MPLTM, MPL-SE, MTP-PE, MTP-PE Liposomes, Murametide, Murapalmitine, NAGO, nCT native Cholera Toxin, Non-Ionic Surfactant Vesicles, non-toxic mutant E112K of Cholera Toxin mCT-E112K, p-Hydroxybenzoic acid methyl ester, pCIL-10, pCIL12, pCMVmCAT1, pCMVN, Peptomer-NP, Pleuran, PLG, PLGA, PGA, and PLA, Phuronic L121, PMMA, PODDSTM, Poly rA, Poly rU, Polysorbate 80, Protein Cochleates, QS-21, Quadri A saponin, Quil-A, Rehydralgel HPA, Rehydralgel LV, RIBI, Ribilike adjuvant system (MPL, TMD, CWS), S-28463, SAF-1, Sclavo peptide, Sendai Proteoliposomes, Sendai-containing Lipid Matrices, Span 85, Specol, Squalane 1, Squalene 2, Stearyl Tyrosine, Tetanus toxoid (TT),

Theramide™, Threonyl muramyl dipeptide (TMDP), Ty Particles, and Walter Reed Liposomes. Selection of an adjuvant depends on the subject to be treated. Preferably, a pharmaceutically acceptable adjuvant is used.

5 In an eighth aspect, the disclosure provides methods to treat or limit development of a SARS-CoV-2 infection, comprising administering to a subject in need thereof an amount effective to treat or limit development of the infection of the polypeptide, nanoparticle, composition, nucleic acid, pharmaceutical composition, or vaccine of any embodiment herein (referred to as the "immunogenic composition"). The subject may be any suitable mammalian subject, including but not limited to a human subject.

10 When the method comprises limiting a SARS-CoV-2 infection, the immunogenic composition is administered prophylactically to a subject that is not known to be infected, but may be at risk of exposure to SARS-CoV-2. As used herein, "limiting development" includes, but is not limited to accomplishing one or more of the following: (a) generating an immune response (antibody and/or cell-based) to of SARS-CoV-2 in the subject; (b)
15 generating neutralizing antibodies against SARS-CoV-2 in the subject (b) limiting build-up of SARS-CoV-2 titer in the subject after exposure to SARS-CoV-2; and/or (c) limiting or preventing development of SARS-CoV-2 symptoms after infection. Exemplary symptoms of SARS-CoV-2 infection include, but are not limited to, fever, fatigue, cough, shortness of breath, chest pressure and/or pain, loss or diminution of the sense of smell, loss or diminution
20 of the sense of taste, and respiratory issues including but not limited to pneumonia, bronchitis, severe acute respiratory syndrome (SARS), and upper and lower respiratory tract infections.

In one embodiment, the methods generate an immune response in a subject in the subject not known to be infected with SARS-CoV-2, wherein the immune response serves to
25 limit development of infection and symptoms of a SARS-CoV-2 infection. In one embodiment, the immune response comprises generation of neutralizing antibodies against SARS-CoV-2. In an exemplary such embodiment, the immune response comprises generation of SARS-CoV-2 spike protein antibody-specific responses with a mean geometric titer of at least 1×10^5 . In a further embodiment, the immune response comprises generation
30 of antibodies against multiple antigenic epitopes.

As used herein, an "effective amount" refers to an amount of the immunogenic composition that is effective for treating and/or limiting SARS-CoV-2 infection. The polypeptide, nanoparticle, composition, nucleic acid, pharmaceutical composition, or vaccine

of any embodiment herein are typically formulated as a pharmaceutical composition, such as those disclosed above, and can be administered via any suitable route, including orally, parentally, by inhalation spray, rectally, or topically in dosage unit formulations containing conventional pharmaceutically acceptable carriers, adjuvants, and vehicles. The term

- 5 parenteral as used herein includes, subcutaneous, intravenous, intra-arterial, intramuscular, intrasternal, intratendinous, intraspinal, intracranial, intrathoracic, infusion techniques or intraperitoneally. Polypeptide compositions may also be administered via microspheres, liposomes, immune-stimulating complexes (ISCOMs), or other microparticulate delivery systems or sustained release formulations introduced into suitable tissues (such as blood).
- 10 Dosage regimens can be adjusted to provide the optimum desired response (e.g., a therapeutic or prophylactic response). A suitable dosage range may, for instance, be 0.1 µg/kg-100 mg/kg body weight of the polypeptide or nanoparticle thereof. The composition can be delivered in a single bolus, or may be administered more than once (e.g., 2, 3, 4, 5, or more times) as determined by attending medical personnel.

- 15 In one embodiment, the administering comprises administering a first dose and a second dose of the immunogenic composition, wherein the second dose is administered about 2 weeks to about 12 weeks, or about 4 weeks to about 12 weeks after the first dose is administered. In various further embodiments, the second dose is administered about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks after the first dose. In another embodiment, three doses may
- 20 be administered, with a second dose administered about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks after the first dose, and the third dose administered about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks after the second dose.

In various other embodiments of prime-boost dosing, the administering comprises

- (a) administering a prime dose to the subject of a DNA, mRNA, or adenoviral
- 25 vector vaccine, wherein the DNA, mRNA, or adenoviral vector vaccine encodes an amino acid sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid sequence identity to the amino acid sequence of SEQ ID NO:125-137; and

- (b) administering a boost dose to the subject of the polypeptide, nanoparticle,
- 30 composition, nucleic acid, pharmaceutical composition, or vaccine of any embodiment or combination disclosed herein.

In an alternative embodiment, the administering comprises

(a) administering a prime dose to the subject of any embodiment or combination disclosed herein; and

(b) administering a boost dose to the subject of a DNA, mRNA, or adenoviral vector vaccine, wherein the DNA, mRNA, or adenoviral vector vaccine encodes an amino acid sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid sequence identity to the amino acid sequence of SEQ ID NO:125-137.

In either of these embodiments, any suitable DNA, mRNA, or adenoviral vector vaccine may be used in conjunction with the immunogenic compositions of the present disclosure, including but not limited to vaccines to be developed as well as those available from Moderna, Pfizer/BioNTech, Johnson & Johnson, etc.

In another embodiment of the methods, the subject is infected with a severe acute respiratory (SARS) virus, including but not limited to SARS-CoV-2, wherein the administering elicits an immune response against the SARS virus in the subject that treats a SARS virus infection in the subject. When the method comprises treating a SARS-CoV-2 infection, the immunogenic compositions are administered to a subject that has already been infected with SARS-CoV-2, and/or who is suffering from symptoms (as described above) indicating that the subject is likely to have been infected with SARS-CoV-2.

As used herein, "treat" or "treating" includes, but is not limited to accomplishing one or more of the following: (a) reducing SARS-CoV-2 titer in the subject; (b) limiting any increase of SARS-CoV-2 titer in the subject; (c) reducing the severity of SARS-CoV-2 symptoms; (d) limiting or preventing development of SARS-CoV-2 symptoms after infection; (e) inhibiting worsening of SARS-CoV-2 symptoms; (f) limiting or preventing recurrence of SARS-CoV-2 symptoms in subjects that were previously symptomatic for SARS-CoV-2 infection; and/or (e) survival.

The disclosure further provides kits, which may be used to prepare the nanoparticles and compositions of the disclosure. In one embodiment, the kits comprise:

(a) the polypeptide of any embodiment or combination of embodiments disclosed herein, such as in the first aspect; and

(b) a first protein comprising an amino acid sequence at least at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected the group consisting of SEQ ID NOS:152-159, wherein residues in parentheses are optional and may be present or absent.

In one embodiment, the polypeptide comprises the amino acid sequence of SEQ ID NO:1 or 5, and the first protein comprises the amino acid sequence of SEQ ID NO:155.

In another embodiment, the kits comprise:

(a) a nucleic acid encoding the polypeptide of any embodiment or combination of
5 embodiments disclosed herein, such as in the first aspect; and

(b) a nucleic acid encoding first protein comprising an amino acid sequence at least at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected the group consisting of SEQ ID NOS:152-159, wherein residues in parentheses are optional and may be present or absent.

10 In one embodiment, the polypeptide comprises the amino acid sequence of SEQ ID NO:1 or 5, and the first protein comprises the amino acid sequence of SEQ ID NO:155.

In a further embodiment, the kits comprise:

(a) an expression vector comprising a nucleic acid encoding the polypeptide any
embodiment or combination of embodiments disclosed herein, such as in the first aspect,
15 operatively linked to a suitable control sequence; and

(b) an expression vector comprising a nucleic acid encoding first protein
comprising an amino acid sequence at least at least 75%, 80%, 85%, 90%, 91%, 92%, 93%,
94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected the
group consisting of SEQ ID NOS:152-159, wherein residues in parentheses are optional and
20 may be present or absent, wherein the nucleic acid is operatively linked to a suitable control
sequence.

In one embodiment, the polypeptide comprises the amino acid sequence of SEQ ID NO:1 or 5, and the first protein comprises the amino acid sequence of SEQ ID NO:155.

In another embodiment, the kits comprise:

25 (a) a cell comprising an expression vector, wherein the expression vector
comprises a nucleic acid encoding the polypeptide any embodiment or combination of
embodiments disclosed herein, such as in the first aspect, operatively linked to a suitable
control sequence; and

(b) a cell comprising an expression vector, wherein the expression vector
30 comprises a nucleic acid encoding first protein comprising an amino acid sequence at least at
least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%
identical to the amino acid sequence selected the group consisting of SEQ ID NOS:152-159,

wherein residues in parentheses are optional and may be present or absent, wherein the nucleic acid is operatively linked to a suitable control sequence.

In one embodiment, the polypeptide comprises the amino acid sequence of SEQ ID NO:1 or 5, and the first protein comprises the amino acid sequence of SEQ ID NO:155.

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Examples

Elicitation of potent neutralizing antibody responses by designed protein nanoparticle vaccines for SARS-CoV-2

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Summary

A safe, effective, and scalable vaccine is urgently needed to halt the ongoing SARS-CoV-2 pandemic. Here, we describe the structure-based design of self-assembling protein nanoparticle immunogens that elicit potent and protective antibody responses against SARS-CoV-2 in mice. The nanoparticle vaccines display 60 copies of the SARS-CoV-2 spike (S) glycoprotein receptor-binding domain (RBD) in a highly immunogenic array and induce neutralizing antibody titers roughly ten-fold higher than the prefusion-stabilized S ectodomain trimer despite a more than five-fold lower dose. Antibodies elicited by the nanoparticle immunogens target multiple distinct epitopes on the RBD, suggesting that they may not be easily susceptible to escape mutations, and exhibit a significantly lower binding:neutralizing ratio than convalescent human sera, which may minimize the risk of vaccine-associated enhanced respiratory disease. The high yield and stability of the protein components and assembled nanoparticles, especially compared to the SARS-CoV-2 prefusion-stabilized S trimer, indicate that manufacture of the nanoparticle vaccines will be highly scalable.

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Design, *In Vitro* Assembly, and Characterization of SARS-CoV-2 RBD Nanoparticle Immunogens

To design vaccine candidates that induce potent neutralizing Ab responses, we focused on the RBD of the SARS-CoV-2 S glycoprotein (Figure 1A–B). To overcome the limited immunogenicity of this small, monomeric antigen, we multivalently displayed the RBD on the exterior surface of the two-component protein nanoparticle I53-50. I53-50 is a computationally designed, 28 nm, 120-subunit complex with icosahedral symmetry

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constructed from trimeric (I53-50A) and pentameric (I53-50B) components (all amino acid sequences provided in Table 3). The nanoparticle can be assembled *in vitro* by simply mixing independently expressed and purified I53-50A and I53-50B. The RBD (residues 328–531) was genetically fused to I53-50A using linkers comprising 8, 12, or 16 glycine and serine residues (hereafter referred to as RBD-8GS-, RBD-12GS-, or RBD-16GS-I53-50A) to enable flexible presentation of the antigen extending from the nanoparticle surface (Figure 1C). All RBD-I53-50A constructs were recombinantly expressed using mammalian (Expi293F) cells to ensure proper folding and glycosylation of the viral antigen. Initial yields of purified RBD-I53-50A proteins (~30 mg purified protein per liter Expi293F cells) were ~20-fold higher than for the prefusion-stabilized S-2P trimer (Kirchdoerfer et al., 2018; Pallesen et al., 2017; Walls et al., 2020; Wrapp et al., 2020) (~1.5 mg/L), and increased to ~60 mg/L following promoter optimization. The RBD-I53-50A proteins were mixed with pentameric I53-50B purified from *E. coli* in a ~1:1 molar ratio (subunit:subunit) to initiate nanoparticle assembly (Figure 1D).

Table 3. Amino acid sequences of proteins used in this work (See figures 1-6)

>RBD-8GS-I53-50A
MGILPSPGMPALLSLVSLLSVLLMGCVAETGTRFFPNITNLCPFGVEVFNATRFASVYAWNRRKISNCVADYSVL YNSASFSTFKCYGVSPKTLNDLCFTNVYADSFVIRGDEVQRQIAPGQGTGKIADYNKYLPDDFTGCVIWNNSNNL DSKVGGNYNLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCFYFPLQSYGFQPTNGVGYPYRVVLS FELLHAPATVCGPKKSTGGSGGGSGGSEKAAKAEEAARKMEELFKKKHIVAVLRANSVEEAIEKAVAVFAGGVHL IEITFTVPDADTVIKALSVLKEKGAIIGAGTSTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMFGV MTPTLVLKAMKLGHTILKLPFGVEVVGQFVKAMKGPFPNVKFPVPTGGVNLNDNVAEWFKAGVLAVGVGSALVRG TFDEVREKAKAFVEKIRGATEGGSHHHHHHHH (SEQ ID NO: 14)
>RBD-12GS-I53-50A
MGILPSPGMPALLSLVSLLSVLLMGCVAETGTRFFPNITNLCPFGVEVFNATRFASVYAWNRRKISNCVADYSVL YNSASFSTFKCYGVSPKTLNDLCFTNVYADSFVIRGDEVQRQIAPGQGTGKIADYNKYLPDDFTGCVIWNNSNNL DSKVGGNYNLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCFYFPLQSYGFQPTNGVGYPYRVVLS FELLHAPATVCGPKKSTGGSGGGSGGSGGSEKAAKAEEAARKMEELFKKKHIVAVLRANSVEEAIEKAVAVFAG GVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTSTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFY MPGVMTPTLVLKAMKLGHTILKLPFGVEVVGQFVKAMKGPFPNVKFPVPTGGVNLNDNVAEWFKAGVLAVGVGS LVKSTPDEVREKAKAFVEKIRGATEGGSHHHHHHHH (SEQ ID NO: 22)
>RBD-16GS-I53-50A
MGILPSPGMPALLSLVSLLSVLLMGCVAETGTRFFPNITNLCPFGVEVFNATRFASVYAWNRRKISNCVADYSVL YNSASFSTFKCYGVSPKTLNDLCFTNVYADSFVIRGDEVQRQIAPGQGTGKIADYNKYLPDDFTGCVIWNNSNNL DSKVGGNYNLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCFYFPLQSYGFQPTNGVGYPYRVVLS FELLHAPATVCGPKKSTGGSGGGSGGSGGSEKAAKAEEAARKMEELFKKKHIVAVLRANSVEEAIEKAVAVFAG GVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTSTSVEQARKAVESGAEFIVSPHLDEEISQFAKEK GVFYMPGVMTPTLVLKAMKLGHTILKLPFGVEVVGQFVKAMKGPFPNVKFPVPTGGVNLNDNVAEWFKAGVLAVG VGSALVKGTPDEVREKAKAFVEKIRGATEGGSHHHHHHHH (SEQ ID NO: 2)
>Monomeric SARS-CoV-2 RBD
MGILPSPGMPALLSLVSLLSVLLMGCVAETGTRFFPNITNLCPFGVEVFNATRFASVYAWNRRKISNCVADYSVL YNSASFSTFKCYGVSPKTLNDLCFTNVYADSFVIRGDEVQRQIAPGQGTGKIADYNKYLPDDFTGCVIWNNSNNL DSKVGGNYNLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCFYFPLQSYGFQPTNGVGYPYRVVLS

FELLHAFATVCGPKKSTHHHHHHH (SEQ ID NO: 160)
>SARS-CoV-2 S-2P Trimer
MGILPSPGMPALLSLVSLLSVLLMGCVAETGTCVNLTRTQLPPAYTNSFTRGVYYPDKVFKSSVLHSTQDL FLPFFSNVTFHAIHVSSTNGTKRFDNFVLPFNDGVYFASSTKSNIRGWIFGTTLLDSKTQSLIVNNATNVV LKYCPQPCNDPFLGVYHKKNSWMESEFRVYSSANNCTFEYVSQFFLMDLEGKQGNFKNLAEFVFNIDGY FKIYXKHTPINLVRDLPGQPSALEPLVDLPIGINITRFQTLALHRSYLTFGDS3SGWTAGAAAYVGYLQPR TFLIKYNNGTITDAVDCALDPLSETKCTLKSTFVREKGIYQTSNFRVQPTESIVRFPNITNLCPFGSEVFNATR FASVYAWNRRIRISNCVADYSVLVNSASSTFKCYGVSPTEKLNLCFTNVYADSFVIRGDEVQIAPGQTGKIA DYNKLPDDFTGCVIAWNSNNLDSKVGNNYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCFYFPL QSYGFPQPTNGVGYQPYRVVLSFELLHAFATVCGPKKSTNLVKNKCVNENFNGLTGTGVLTESNKKFLPQQF GRDIADSTDAVRDPQTLEILDITPCSPGGVSITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYST GSNVFQTRAGCLIGASHVNSYECDIPIGAGICASYQTQTSNPSGAGSVASQSI IAYTMSLGAENSVAYSNNNS IAIPTNFTISVTTELIPVSMTKTSVDCTMYIOGDSSTECNLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAF VKQIYKTPPIKDFGGFNFSQILPDPKPSKRSFIEDLLFNKVLADAGFIKQYGDCLGDIAARDLICAKQKFN LTVLPFLLTDEMIAQYTSALLAGTITSQWTFGAGAAALQIPFAMQMAFRENGIGVTQNVLYRNQKLIANQFN IGKIQDSSLSSTASALGKLQPVVQNAQALNTLVKQLSSNFGAISSVLNDILSRLDPPRAEVQIORLITGRLOQ LQTYVTQQLIPAAIRKASANLAATKMSSECVLQQSKRVDFCGKGYHMSFPQSAFHGVVFLHVTYVPAQEKNF TAPAICHGDKAHFFREGVFSNGTHWFTQRNFYEPQIITTDNTFVSGNCDVVIGIVMNTVYDPLQPELDSFK EELDKYFNKNTSPDVLGDISGINASVNNIQKEIDRLNEVAHNLNESLIDLQELGKVEQYIKASGRENLYFQG GGGGYIPEAPRDGQAYVRKDGWVLLSTFLGHHHHHHH (SEQ ID NO: 161)
>hACE2 (R518G) -Fc
MARAWIFFLLCLAGRALASTIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNI TEENVQNMNAGDKNSAFL EQQSTLAQMYFLQEIQNLTIVKLQALQALQNGSSVLSSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECLEP GLNEIMANSLDYNERLWAWESWRSEVGEKQLRPLYEYVVLKNEMARANHYEDYGDYWRGDYEVNGVDYDYSR GQLIESVEHTEFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDMWGRFWTNLYSLVFPFGQKFNID VTDAMVDQAWDAQRIKFAEKFFVSVGLPNMTQGFWENSMLTDPGNVQKAVCHPTAWDLGRGDFRILMCTKVT MDDFLTANHEMCHIQYDMAYAAQPFLLRNGANEGFHEAVGEIMSLSAATPKHLKSLIGLLSPDFQEDNETEINF LLKQALTIVGTLPFTYMLEKWRWVFKGEIFKDLQWMMKWWEMKREIVGVVEPVPHDETCDPASLFHVSNDYS FIRXYTGTLYQFQFQALQQAANHEGFLHKCDISNSTEAGQNLNMLRLGKSEPTWLALENVVGAKNMNVRL LNYFEPLFTWLKQDNKNSFVGWSTDWSPYADPLVPRGSGGGGDPPEKSCDKTHTCPPCPAPELLGGF3VFLFP PKPKDFTLMSPTPEYTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPAEIKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPEVLDSDGSPFLYSKLTVDKSPWQQGNVVFSCVMHEALNNHYTQKSLSLSPGK (SEQ ID NO: 162)
>mACE2 -Fc
MPWGLQPLATLYLLGMLVASVLAQSTIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNI TEENVQNMNAG EKWSAFLKQSTLAQMYFLQEIQNLTIVKLQALQALQNGSSVLSSEDKSKRLNTILNTMSTIYSTGKVCNPNPQ ECLLLDPLGNEIMEKSLDYNERLWAWEGWRSEVGEKQLRPLYEYVVLKNEMARANHYKDYGDYWRGNIYEVNGV DGYDYNRDQILIEDVERTFEEIKPLYEHLHAYVRAKLMNAYPSYISPTGCLPAHLLGDMWGRFWTNLYSLTVFP GQKFNIDVTDAMVNCANNAQRIKFAEKFFVSVGLPNMTQGFWENSMLTDPGNVQKAVCHPTAWDLGRGDFRI IMCTKVTMDFLTANHEMCHIQYDMAYAAQPFLLRNGANEGFHEAVGEIMSLSAATPKHLKSLIGLLSPDFQED NETEINFLLKQALTIVGTLPFTYMLEKWRWVFKGEIFKDLQWMMKWWEMKREIVGVVEPVPHDETCDPASLF HVSNDYSFIRIYVTRTLYQFQFQALQQAANHEGFLHKCDISNSTEAGQKLNLMLKLGKSEPTWLALENVVGAK NMNVRELLNLYFEPLFTWLKQDNKNSFVGWSTDWSPYADQSIKVRISLKSALGDKAYEWNNDNEMYLFRSSVAYA MRTYFLEIKHQITLFGEDVRVADLKPRISFNFYVTAPKNVSDITPTEVERAIRISRSRINDAFRLNDNSLE FLGIQFTLAPPYQSPVTDPLVPRGSGGGGDPPEKSCDKTHTCPPCPAPELLGGF3VFLFPFKPKDTLMSRFP EVTCCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP AEIKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPEVLDSDGS FFLYSKLTIVKSRWQQGNVVFSCVMHEALNNHYTQKSLSLSPGK (SEQ ID NO: 163)
>EBD-8G5-F53-50A
MGILPSPGMPALLSLVSLLSVLLMGCVAETGTRFPNITNLCPFGSEVFNATRFASVYAWNRRIRISNCVADYSVL YNSASFSTFKCYGVSPTEKLNLCFTNVYADSFVIRGDEVQIAPGQFGKIADYNKLPDDFTGCVIAWNSNNL DSKVGNNYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCFYFPLQSYGFPQPTNGVGYQPYRVVLS FELLHAFATVCGPKKSTGGSGSGSGSEKAAKAEAAARKMEELFKKKHIVAVLRANSVEEAIEKAVAVFAGGVHL IEITFTVPDATTIVKALSVLKEKGAIFGATVTSVEQARKAVSSGAEITVSPHLDEISQFAKEHGVPTMPGV MTPTTELVAHMLGRTILKLPFGGVVGFQFVRAMKGFFFNVEFVPTGGVNLDNVAEWFRAGVLAVGVSSALVKG TPDEVREKARAFVEXIRGATEGGSHHHHHHHH

>RBD-12GS-I53-50A
MGILPSPGMPALLSLVSLLSVLLMGCAETGTRFPNITNLCFFGEVFNATRFASVYAWNRRKRISNCVADYSVL YNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNKLPDDFTGCVIAWNSNNL DSKVGGNYNLYRLFRKSNLKPFERDISTEIIYQAGSTFCNGVEGFNCYFPLQSYGFGPTNGVGQPYRVVVLS FELLHAPATVCGPKKSTGSGSGSGSGSGSEKAAKAEAAARKMEELFKKHKIIVAVLRANSVEEAIEKAVAVFAG GVHLIEITFTVPDADTVIKALSVLKEGAILGAGTVTSVEQARKAVESGAEFIVSPHLDERISQFAKEKGVFY MPGVMTPTTELVKAMKLGHTILKLPGEVVGQFVKAMKGFPPNVKFPVPTGGVNLNDVNAEWFKAGVLAQVGS LVKGTFFDEVREKAKAFVEKIRGATEGGSHHHHHHH
>RBD-16GS-I53-50A
MGILPSPGMPALLSLVSLLSVLLMGCAETGTRFPNITNLCFFGEVFNATRFASVYAWNRRKRISNCVADYSVL YNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNKLPDDFTGCVIAWNSNNL DSKVGGNYNLYRLFRKSNLKPFERDISTEIIYQAGSTFCNGVEGFNCYFPLQSYGFGPTNGVGQPYRVVVLS FELLHAPATVCGPKKSTGSGSGSGSGSGSGSEKAAKAEAAARKMEELFKKHKIIVAVLRANSVEEAIEKAVA VFAGGVHLIEITFTVPDADTVIKALSVLKEGAILGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEK GVFYMPGVMTPTTELVKAMKLGHTILKLPGEVVGQFVKAMKGFPPNVKFPVPTGGVNLNDVNAEWFKAGVLAQV VGSALVKGTFFDEVREKAKAFVEKIRGATEGGSHHHHHHH
>Monomeric SARS-CoV-2 RBD
MGILPSPGMPALLSLVSLLSVLLMGCAETGTRFPNITNLCFFGEVFNATRFASVYAWNRRKRISNCVADYSVL YNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNKLPDDFTGCVIAWNSNNL DSKVGGNYNLYRLFRKSNLKPFERDISTEIIYQAGSTFCNGVEGFNCYFPLQSYGFGPTNGVGQPYRVVVLS FELLHAPATVCGPKKSTHHHHHHHH
>SARS-CoV-2 S-2F Trimer
MGILPSPGMPALLSLVSLLSVLLMGCAETGTRFPNITNLCFFGEVFNATRFASVYAWNRRKRISNCVADYSVL FLPFFSNVTWPHAINVSGTNGTKRFDNPFVLPFNDGVYFASTEKSNIRGWIFGTTLDSTQSLIIVNNATNVV IKVCEFPQCNDFFLGVYYHKNKSWMESEFRVYSSANMCTFEYVSQFFLMDLEGKQGNFENLREFVFNIDGY FKIYSKHTPINLVRLPQGPSALEPLVDLPICINITRFQTLALHRSYLTGDSSESGWTAGAAAYVGYLQPR TFLKYNEWGTTITDAVDCALDPLSETKCTLSFTVERKGIYQTSNFRVQPTESIVRFPNITNLCFFGEVFNATR FASVYAWNRRKRISNCVADYSVLNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKI ADYNKLPDDFTGCVIAWNSNNLDSKVGGNYNLYRLFRKSNLKPFERDISTEIIYQAGSTFCNGVEGFNCYFPL QSYGFGPTNGVGQPYRVVVLSFELLHAPATVCGPKKSTNLVNNKCVNENFNGLTGTGVLTRSNKKFLPFQGF GEDIADTTDAVRDPQTLRLILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRYST GSNVFQTRAGCLIGAETHVNSYECDIPIGAGICASYQTQTSFSGAGSVASQSI IAYTMSLGAENSVAYSNN IAIPTNFTISVTTTELPSMTKTSVDCTMYICGDSSTECNSNLLQYGSFCTQLNRLTGIAVEQDENTQEVFAQ VKQIYKTPPKDFGGFNFSQILPDPSEKPSKRSFIEDLLFNKVTLAGAGFIKQYGDCLGDIAARDLICAQKFNG LTVLPLLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQMAFRFNGICVTVQNVLYENQKLIANQFNSA IGKIQDSSLSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISVNDILSPLDPPRAEVQIDRLITGRLOS LQTVVTQQLIPAAEIRASANLAATKMSCEVLGQSKRVDFCGKGYHLSMFPQSAFNGVFLHVTVYPAQEKNT TAPAICHDKAHFFREGVVSNGTHWFVTQRNFYEPQIITDNTFVSGNCDVVIGIVNNTVYDFLQPELDSFK EELDKYFKNHTSPDVLGDISGINASVVNIQKEIDRLNEVANNLNRSLIDLQELGKYRQYIRGSGRENLYFQG GGSGGYIPEAPRDGCAYVPRDGEWVLLSTFLGHHHHHHHH
>HACE2 (R518G) -Fc
MARAWIFFLLCLAGPALASTIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQNMNAGDKNSAFL KEQSTLAQMYPLQEIQNLTVKLQIQALQQNGSSVLSRDKSKKLNITILTMSTIYSTGKVCNPNQPCCLLLLP GLNEIMANSIDYNERLWAWESWRSEVGKQLRFLYEYVVLNEMARANHYEDYGDYWRGDIYVNGVDYDYSR GQLIEDVEHTFEETKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLGDMWGRFNTNLYSLFVFPQKPNID VTDMVDAQWDAQRIFKEAEKFFVSVGLPNNMTQGEWENSMLTDFGNVQKAVCHPTAWDLGKGDIFRLMCTKVT MDDFLTANHEMCHIQYDMAYAAQPFLLRNGANEGFNEAVGEIMSLSAATPEHLKLSIGLLSPDFQEDNETEINF LLKQALTIIVGTLPTTYMLEKNPMMVFKGEIPKQDQWKKWEMEREIVGVVEPVPHDETTCDFASLFVSNDS FIRYTTGTLYQFQFQALCQAAKHEGFLHKCDISNSTEAGQKLFNMLALGKSEFWTLALENVVGAKNMNVRF LNYFEPLFTWLRLDQKNNSFVGSSTWSPYADPLVPRGSGGGDPEPKSCDKTHTCPPCPAPELLGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVFKNWYVDGVEVHNAKTKFREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYVTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPEVLSDDSGSPFLYSKLTVDKSRWQQGNVVFSCVMHREALRNHYTQKSLSLSPGE
>mACE2 -Fc
MPWGSLLQPLATLYLLGMLVASVLAQSTIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQNMNAG

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EKWSAFLEQSTLAQMYFLGEIQNLTVKLQIQALQONGSSVLSEDKSKRLNTILNTMSTLYSTGKVCNPNNEQ
RCLLLDPLGNEIMEKSLDYNERLWAEGRSEVGEQLRPLYEYVVLNEMARANHXYKDYGDYWRGNYEVNGV
DGYDYNRQQLIEDVERTFEEIKPLYEHLHAYVRAKLMNAYPSYISPTGCLPAHLGDMWGRFWTNLYSLTVPF
GQHPNIDVTAMVNAQAWNAQRIFKEAEKFFSVGLPMTQGFWENSMLTDPGNVQKVVCHEPTAWDLGEGDFRI
INCTKVMTDDFLTAHHENGHIQYDMAYAAQPFLLRNGANEGFHEAVGEIMSLSAATPKHLKSIQLLSPDFQED
NETEINFLKQALFIVGTLFFTYMLEKWRPMWFFKGEIFKQDMKKWEMKREIVGVVSEFVPHDETVCDFASLF
HVSNDYSFIRYTYRTRYQFQFQZALQQAARHREGPLHKCDISRSTEAGQKLLNMLKLGKSEFWTLAENNVGAK
NMNVRPLLNYFEPLFTWLKDQNKNSFVGNSTWSPYADQS IKVRI SLKSA LGDKAYEWNNDNEMYLFRRSEVAYA
MRTYFLEIKHQITILFGEEDVRVADLKPRISFNFYVTAPKNVSDIIFKTEVEEAIRISRSRINDAFRLNDNSLE
FLGIQTTLAPFYQSPVTDPLVFRGSGGGGDFEPKSCDKTHTCPFCAPPELLGGFVSFLFPKPKDTLMISRTP
EVTGVVVDVSHEDPEVVFNNVVDGVVHNKTKFPRERQYNSTYRVVSVLTVLHQDWLNGKEYNCKEVSNAKALPA
PIEKTISKANGQPRPQVYTLPPSRDELTKNQVSLTCLVGFYPSPDIYAVEWESNGQPENNYKTPPVLDSDGS
FFLYSKLTVDKSRWQQGNVFSQSVNHAEALHNHYTQKSLSLSPGK

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Size-exclusion chromatography (SEC) of the SARS-CoV-2 RBD-I53-50 nanoparticles revealed predominant peaks corresponding to the target icosahedral assemblies and smaller peaks comprising residual unassembled RBD-I53-50A components (Figures 7A and 7B). Dynamic light scattering (DLS) and negative stain electron microscopy (nsEM) confirmed the homogeneity and monodispersity of the various RBD-I53-50 nanoparticles, both before and after freeze/thaw (Figures 1E, 1F, and 7C). The average hydrodynamic diameter and percent polydispersity measured by DLS for RBD-8GS-, RBD-12GS-, and RBD-16GS-I53-50 before freeze/thaw were 38.5 (27%), 37 (21%), and 41 (27%) nm, respectively, compared to 30 (22%) nm for unmodified I53-50 nanoparticles. Hydrogen/Deuterium-exchange mass spectrometry confirmed that display of the RBD on the trimeric RBD-8GS-I53-50A component preserved the conformation of the antigen and structural order of several distinct antibody epitopes (Figures 1G and 7D). Finally, we used glycoproteomics to show that all three RBD-I53-50A components were N-glycosylated at positions N331 and N343 similarly to the SARS-CoV-2 S-2P ectodomain trimer (Watanabe et al., 2020), again suggesting that the displayed antigen retained its native antigenic properties (Figure 1H and 7E).

Table 4. Antigenic Characterization of SARS-CoV-2 RBD-I53-50A Components				
Antigen	Binder	k_{on} ($M^{-1} s^{-1}$)	k_{off} (s^{-1})	K_D (nM)
SARS-CoV-2 RBD	hACE2	$7 \times 10^4 \pm 5 \times 10^2$	$5 \times 10^{-3} \pm 1 \times 10^{-5}$	69 ± 0.5
	CR3022 Fab	$2 \times 10^3 \pm 2 \times 10^3$	$9 \times 10^{-3} \pm 3 \times 10^{-5}$	45 ± 0.5
RBD-8GS-I53-50A	hACE2	$6 \times 10^4 \pm 4 \times 10^2$	$4 \times 10^{-3} \pm 1 \times 10^{-5}$	70 ± 0.5
	CR3022 Fab	$2 \times 10^3 \pm 1 \times 10^3$	$1 \times 10^{-2} \pm 3 \times 10^{-5}$	57 ± 0.4
RBD-12GS-I53-50A	hACE2	$6 \times 10^3 \pm 4 \times 10^2$	$5 \times 10^{-3} \pm 1 \times 10^{-5}$	78 ± 0.5
	CR3022 Fab	$2 \times 10^3 \pm 2 \times 10^3$	$9 \times 10^{-3} \pm 2 \times 10^{-5}$	42 ± 0.4
RBD-16GS-I53-50A	hACE2	$6 \times 10^3 \pm 4 \times 10^2$	$4 \times 10^{-3} \pm 1 \times 10^{-5}$	66 ± 0.4
	CR3022 Fab	$2 \times 10^3 \pm 1 \times 10^3$	$1 \times 10^{-2} \pm 2 \times 10^{-5}$	56 ± 0.4

Each experiment was performed at least twice, and the values and fitting errors presented are derived from a representative experiment. The corresponding binding curves and fits are presented in Fig. 8.

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Antigenic Characterization of SARS-CoV-2 RBD-I53-50 Nanoparticle Components and Immunogens

We used recombinant human ACE2 ectodomain and two S-specific mAbs (CR3022 and S309) to characterize the antigenicity of the RBD when fused to I53-50A as well as the accessibility of multiple RBD epitopes in the context of the assembled nanoparticle immunogens. CR3022 and S309 were both isolated from individuals infected with SARS-CoV and cross-react with the SARS-CoV-2 RBD. CR3022 is a weakly neutralizing Ab that binds to a conserved, cryptic epitope in the RBD that becomes accessible upon RBD opening but is distinct from the receptor binding motif (RBM), the surface of the RBD that interacts with ACE2 (Huo et al., 2020; ter Meulen et al., 2006; Yuan et al., 2020). S309 neutralizes both SARS CoV and SARS-CoV-2 by binding to a glycan-containing epitope that is conserved amongst sarbecoviruses and accessible in both the open and closed prefusion S conformational states (Pinto et al., 2020).

We used bio-layer interferometry (BLI) to confirm the binding affinities of the monomeric human ACE2 (hACE2) ectodomain and the CR3022 Fab for the monomeric RBD. Equilibrium dissociation constants (K_D) of these reagents for immobilized RBD-I53-50A fusion proteins closely matched those obtained for the monomeric RBD (Table 4 and Figure 8). These data further confirm that the RBD-I53-50A fusion proteins display the RBD in its native conformation.

To evaluate the possibility that the magnitude and quality of nanoparticle immunogen-elicited Ab responses can be modulated by the accessibility of specific epitopes in the context of a dense, multivalent antigen array, we measured the binding of the nanoparticle immunogens to immobilized dimeric macaque ACE2 (mACE2-Fc) and the CR3022 and S309 mAbs, the latter of which roughly mimics the B cell receptor (BCR)-antigen interaction that is central to B cell activation. This approach does not allow the calculation of K_D values due to the multivalent nature of the interactions, but does enable qualitative comparisons of epitope accessibility in different nanoparticle constructs. We compared the full-valency nanoparticles displaying 60 RBDs to a less dense antigen array by

leveraging the versatility of *in vitro* assembly to prepare nanoparticle immunogens displaying the RBD antigen at 50% valency (~30 RBDs per nanoparticle) (Figure 9). This was achieved by adding pentameric I53-50B to an equimolar mixture of RBD-I53-50A and unmodified I53-50A lacking fused antigen. We found that all of the RBD nanoparticles bound well to the immobilized mACE2-Fc, CR3022, and S309 (Figure 2A). Although there were no consistent trends among the 50% and 100% valency RBD-8GS- and RBD-12GS-I53-50 nanoparticles, the 100% valency RBD-16GS-I53-50 nanoparticles resulted in the highest binding signals against all three binders (Figure 2B). It is possible that the longer linker in the RBD-16GS-I53-50 nanoparticle enables better access to the epitopes targeted by ACE2, CR3022, and S309, although our data cannot rule out other possible explanations. We conclude that multiple distinct epitopes targeted by neutralizing antibodies are exposed and accessible for binding in the context of the RBD antigen array presented on the nanoparticle exterior.

Physical and Antigenic Stability of RBD Nanoparticle Immunogens and S-2P Trimer

We first used chemical denaturation in guanidine hydrochloride (GdnHCl) to compare the stability of the RBD-I53-50A fusion proteins and RBD-12GS-I53-50 nanoparticle immunogen to recombinant monomeric RBD and the S-2P ectodomain trimer (Figure 3A). Fluorescence emission spectra from samples incubated in 0–6.5 M GdnHCl revealed that all three RBD-I53-50A fusion proteins and the RBD-12GS-I53-50 nanoparticle undergo a transition between 4 and 5 M GdnHCl that indicates at least partial unfolding, whereas the S-2P trimer showed a transition at lower [GdnHCl], between 2 and 4 M. The monomeric RBD exhibited a less cooperative unfolding transition over 0–5 M GdnHCl. We then used a suite of analytical assays to monitor physical and antigenic stability over four weeks post-purification at three temperatures: <-70°C, 2-8°C, and 22-27°C (Figure 3B–E). Consistent with previous reports, the monomeric RBD proved quite stable, yielding little change in appearance by SDS-PAGE (Figure 10A), mACE2-Fc and CR3022 binding (Figure 10B), or the ratio of UV/vis absorption at 320/280 nm, a measure of particulate scattering (Figure 10C). The S-2P trimer was unstable at 2-8°C, exhibiting clear signs of unfolding by nsEM even at early time points (Figure 9D). It maintained its structure considerably better at 22-27°C until the latest time point (28 days), when unfolding was apparent by nsEM and UV/vis indicated some aggregation (Figure 10C). All three RBD-I53-50A components were highly stable, exhibiting no substantial change in any readout at any time point (data not shown). Finally, the RBD-12GS-I53-50 nanoparticle was also quite stable over the four-week

study, showing changes only in UV/vis absorbance, where a peak near 320 nm appeared after 7 days at 22-27°C (data not shown). Electron micrographs and DLS of the RBD-12GS-I53-50 nanoparticle samples consistently showed monodisperse, well-formed nanoparticles at all temperatures over the four-week period (Figures 10D, 10E). Collectively, these data show that the RBD-I53-50A components and the RBD-12GS-I53-50 nanoparticle have high physical and antigenic stability, superior to the S-2P ectodomain trimer.

RBD-I53-50 Nanoparticle Immunogens Elicit Potent Neutralizing Antibody Responses in BALB/c and Human Immune Repertoire Mice.

We compared the immunogenicity of the three RBD-I53-50 nanoparticles, each displaying the RBD at either 50% or 100% valency, to the S-2P ectodomain trimer and the monomeric RBD in BALB/c mice. Groups of ten mice were immunized intramuscularly at weeks 0 and 3 with AddaVax™-adjuvanted formulations containing either 0.9 or 5 µg of SARS-CoV-2 antigen in either soluble or particulate form. Three weeks post-prime, all RBD nanoparticles elicited robust S-specific Ab responses with geometric mean reciprocal half-maximal effective concentrations ranging between 8×10^2 and 1×10^4 (Figure 4A). In contrast, the monomeric RBD and the low dose of S-2P trimer did not induce detectable levels of S-specific Abs, while the high dose of S-2P trimer elicited weak responses. Following a second immunization, we observed an enhancement of S-specific Ab titers for all RBD nanoparticle groups, with geometric mean titers (GMT) ranging from 1×10^5 to 2×10^6 (Figure 4B). These levels of S-specific Abs matched or exceeded most samples from a panel of 29 COVID-19 human convalescent sera (HCS) from Washington state and the benchmark 20/130 COVID-19 plasma from NIBSC (Figure 4A–B, Table 5). Immunization with two 5 µg doses of S-2P trimer induced S-specific Ab responses ~1-2 orders of magnitude weaker than the RBD nanoparticles, and the monomeric RBD did not elicit detectable antigen-specific Abs after two immunizations. As expected, we also detected an Ab response to the I53-50 scaffold, which was constant in magnitude across all RBD nanoparticle groups (Figure 11). These data indicate that multivalent display of the RBD on a self-assembling nanoparticle scaffold dramatically improves its immunogenicity.

Table 5. Source of patient convalescent sera

	Hospitalized (N=4)	Not Hospitalized (N=22)	Overall (N=26)
Age			

Mean (SD)	58.0 (15.1)	45.1 (17.2)	47.1 (17.2)
Median [Min, Max]	64.8 [35.5, 67.0]	43.6 [18.1, 76.0]	45.4 [18.1, 76.0]
Sex			
Male	1 (25.0%)	11 (50.0%)	12 (46.2%)
Female	3 (75.0%)	11 (50.0%)	14 (53.8%)
Race			
Asian	1 (25.0%)	2 (9.1%)	3 (11.5%)
Black or African American	1 (25.0%)	0 (0%)	1 (3.8%)
White	2 (50.0%)	20 (90.9%)	22 (84.6%)
Hispanic ethnicity	0 (0%)	1 (4.5%)	1 (3.8%)
Insurance			
Private	3 (75.0%)	17 (77.3%)	20 (76.9%)
Government	1 (25.0%)	5 (22.7%)	6 (23.1%)
Housing Type			
House/condo/townhouse	1 (25.0%)	5 (22.7%)	6 (23.1%)
Apartment	3 (75.0%)	17 (77.3%)	20 (76.9%)
House members			
2 people	1 (25.0%)	10 (45.5%)	11 (42.3%)
3 people	1 (25.0%)	1 (4.5%)	2 (7.7%)
4 people	0 (0%)	2 (9.1%)	2 (7.7%)
5 people	0 (0%)	2 (9.1%)	2 (7.7%)
6 or more people	1 (25.0%)	2 (9.1%)	3 (11.5%)
I live by myself	1 (25.0%)	5 (22.7%)	6 (23.1%)
Smoking			
Nonsmoker	4 (100%)	19 (86.4%)	23 (88.5%)
Tobacco use	0 (0%)	3 (13.6%)	3 (11.5%)
Electronic cigarettes/vapor pen use	0 (0%)	1 (4.5%)	1 (3.8%)
Received 2019-2020 Influenza vaccine (N=23)	2 (50.0%)	16 (72.7%)	18 (69.2%)
Employed			
Retired	1 (25.0%)	0 (0%)	1 (3.8%)
Self-employed	1 (25.0%)	4 (18.2%)	5 (19.2%)
Unemployed	0 (0%)	2 (9.1%)	2 (7.7%)
Yes, and I would be paid for hours missed	2 (50.0%)	12 (54.5%)	14 (53.8%)
Yes, but I would not be paid for hours missed	0 (0%)	4 (18.2%)	4 (15.4%)
Highest Level of Medical Treatment Received			
Outpatient - Testing Only	0 (0%)	15 (68.2%)	15 (57.7%)
Outpatient - Saw Provider**	0 (0%)	7 (31.8%)	7 (26.9%)
Inpatient (General Floor)	2 (50.0%)	0 (0%)	2 (7.7%)
Inpatient (ICU)	2 (50.0%)	0 (0%)	2 (7.7%)
Comorbidities*			
No comorbidities	1 (25.0%)	20 (90.9%)	21 (80.8%)
Hypertension	2 (50.0%)	2 (9.1%)	4 (15.4%)

Diabetes	2 (50.0%)	0 (0%)	2 (7.7%)
Cardiovascular disease	1 (25.0%)	0 (0%)	1 (3.8%)
Chronic kidney disease	1 (25.0%)	0 (0%)	1 (3.8%)
Cardiovascular disease	1 (25.0%)	0 (0%)	1 (3.8%)
HIV	1 (25.0%)	0 (0%)	1 (3.8%)
Highest Level of Respiratory Support			
None	1 (25.0%)	22 (100%)	23 (88.5%)
Non-invasive ventilation (BiPAP)	1 (25.0%)	0 (0%)	1 (3.8%)
Mechanical ventilation/intubation	2 (50.0%)	0 (0%)	2 (7.7%)
Travel out of state in last 30 days (N=23)	0 (0%)	4 (18.2%)	4 (15.4%)
Symptoms*			
Feeling feverish	3 (75.0%)	15 (68.2%)	18 (69.2%)
Cough	4 (100%)	17 (77.3%)	21 (80.8%)
Chills or shivering	3 (75.0%)	15 (68.2%)	18 (69.2%)
Sweats	2 (50.0%)	14 (63.6%)	16 (61.5%)
Sore throat or itchy/scratchy throat	0 (0%)	10 (45.5%)	10 (38.5%)
Nausea or vomiting	1 (25.0%)	3 (13.6%)	4 (15.4%)
Runny or stuffy nose	1 (25.0%)	13 (59.1%)	14 (53.8%)
Muscle or body aches	2 (50.0%)	15 (68.2%)	17 (65.4%)
Increased trouble breathing	3 (75.0%)	5 (22.7%)	8 (30.8%)
Fatigue	2 (50.0%)	17 (77.3%)	19 (73.1%)
Diarrhea	2 (50.0%)	6 (27.3%)	8 (30.8%)
Rash	0 (0%)	1 (4.5%)	1 (3.8%)
Ear pain or ear discharge	0 (0%)	1 (4.5%)	1 (3.8%)
Loss of sense of taste or smell	0 (0%)	7 (31.8%)	7 (26.9%)

*Categories not mutually exclusive

**Includes Primary Care Physician, Urgent care, Emergency Department

- We prototyped potential human antibody responses to the RBD nanoparticle
- 5 immunogens using the Kymab proprietary IntelliSelect™ Transgenic mouse platform (known as 'Darwin') that is transgenic for the non-rearranged human antibody variable and constant region germline repertoire. In contrast to previous mice with chimeric antibody loci that have been described (Lee et al., 2014), the mice in the present study differed in that they were engineered to express fully human kappa light chain Abs. Groups of five Darwin mice were
 - 10 immunized intramuscularly with S-2P trimer, 100% RBD-12GS-, or 100% RBD-16GS-I53-50 nanoparticles at antigen doses of 0.9 µg (nanoparticles only) or 5 µg (Figure 4C). All groups immunized with RBD nanoparticles elicited S-directed Ab responses post-prime (EC_{50} 2×10^3 – 1×10^4) that were substantially boosted by a second immunization at week 3

(EC₅₀ ranging from 4×10^5 to 8×10^5) (Figures 4C and 4D). In this animal model, the S-2P trimer elicited levels of S-specific Abs comparable to the RBD nanoparticles after each immunization.

We then evaluated the neutralizing activity elicited by each immunogen using both pseudovirus and live virus neutralization assays. In BALB/c mice, all RBD nanoparticle immunogens elicited serum neutralizing Abs after a single immunization, with reciprocal half-maximal inhibition dilutions (IC₅₀) ranging from 1×10^2 to 5×10^2 (GMT) in pseudovirus and 3×10^3 to 7×10^3 in live virus neutralization assays (Figure 5A and 5C). No significant differences in pseudovirus or live virus neutralization were observed between low or high doses of RBD-8GS-, RBD-12GS-, or RBD-16GS-I53-50 nanoparticles at 50% (pseudovirus neutralization only) or 100% valency, in agreement with the S-specific Ab data. The GMT of all three 100% valency RBD nanoparticle groups matched or exceeded that of the panel of 29 HCS tested in the pseudovirus neutralization assay (Figure 5A). Immunization with monomeric RBD or S-2P trimer did not elicit neutralizing Abs after a single immunization (Figures 5A and 5C). As in BALB/c mice, both high and low doses of the RBD-I53-50 nanoparticles in Darwin mice elicited pseudovirus neutralizing Ab titers (IC₅₀ 8×10^1 to 2.5×10^2) comparable to HCS (IC₅₀ 1×10^2) after a single immunization, whereas 5 µg of the S-2P trimer did not elicit detectable levels of neutralizing Abs (Figure 5E) despite eliciting similar levels of total S-specific Abs.

In both mouse models, a second immunization with the RBD-I53-50 nanoparticles led to a large increase in neutralizing Ab titers. In BALB/c mice, pseudovirus neutralization GMT reached 2×10^3 to 3×10^4 , exceeding that of the HCS by 1–2 orders of magnitude, and live virus neutralization titers reached 2×10^4 to 3×10^4 (Figures 5B and 5D). A second immunization with 5 µg of the S-2P trimer also strongly boosted neutralizing activity, although pseudovirus and live virus neutralization (GMTs of 3×10^2 and 6×10^3 , respectively) were still lower than in sera from animals immunized with the RBD nanoparticles. The increases between the S-2P trimer and the RBD nanoparticles ranged from 7–90-fold and 4–9-fold in the pseudovirus and live virus neutralization assays, respectively. The 0.9 µg dose of the S-2P trimer and both doses of the monomeric RBD failed to elicit detectable neutralization after two immunizations. Similar increases in pseudovirus neutralization were observed after the second immunization in the Darwin mice, although the titers were lower overall than in BALB/c mice (Figure 5F).

Several conclusions can be drawn from these data. First, the RBD nanoparticles elicit

potent neutralizing Ab responses in two mouse models that exceed those elicited by the prefusion-stabilized S-2P trimer and, after two doses, by infection in humans. Second, linker length and antigen valency did not substantially impact the overall immunogenicity of the RBD nanoparticles, although there is a trend suggesting that RBD-16GS-I53-50 may be more immunogenic than the nanoparticles with shorter linkers. These observations are consistent with the antigenicity and accessibility data presented in Table 4 and Figure 2 showing that multiple epitopes are intact and accessible in all RBD nanoparticle immunogens. Finally, the elicitation of comparable neutralizing Ab titers by both the 0.9 and 5 µg doses of each nanoparticle immunogen suggests that RBD presentation on the I53-50 nanoparticle enables dose sparing, which is a key consideration for vaccine manufacturing and distribution.

Eight mice immunized with AddaVax™ only, monomeric RBD, S-2P trimer, or RBD-8GS- or RBD-12GS- I53-50 nanoparticles were challenged seven weeks post-boost with a mouse-adapted SARS-CoV-2 virus (SARS-CoV-2 MA) to determine whether these immunogens confer protection from viral replication. The RBD-8GS- and RBD-12GS-I53-50 nanoparticles provided complete protection from detectable SARS-CoV-2 MA replication in mouse lung and nasal turbinates (Figure 5G–H). Immunization with the monomeric RBD, 0.9 µg S-2P trimer, and adjuvant control did not protect from SARS-CoV-2 MA replication. These results mirrored our pseudovirus and live virus neutralization data showing that the RBD nanoparticles induce potent anti-SARS-CoV-2 Ab responses at either dose or valency.

RBD Nanoparticle Vaccines Elicit Robust B Cell Responses and Antibodies Targeting Multiple Epitopes in Mice and a Nonhuman Primate

Germinal center (GC) responses are a key process in the formation of durable B cell memory, resulting in the formation of affinity-matured, class-switched memory B cells and long-lived plasma cells. We therefore evaluated the antigen-specific GC B cell responses in mice immunized with the monomeric RBD, S-2P trimer, and RBD-8GS-, RBD-12GS-, or RBD-16GS-I53-50 nanoparticles. The quantity and phenotype of RBD-specific B cells were assessed 11 days after immunization to determine levels of GC precursors and B cells (B220⁺CD3⁺CD138⁺CD38⁺GL7⁺) (Figure 12). Immunization with RBD nanoparticles resulted in an expansion of RBD-specific B cells and GC precursors and B cells (Figure 6A–C). The S-2P trimer resulted in a detectable but lower number and frequency of RBD-specific B cells and GC precursors and B cells compared to the RBD nanoparticles, whereas the monomeric RBD construct did not elicit an appreciable B cell response. Consistent with these

findings, immunization with the three RBD nanoparticles and trimeric S-2P led to the emergence of CD38^{hi}-GL7⁺ IgM⁺ and class-switched (swIg⁺) RBD-specific B cells, indicative of functional GC precursors and GC B cells (Figure 6D). The robust GC B cell responses and increased proportions of IgM⁺ and swIg⁺ RBD-specific B cells in the mice immunized with the RBD-nanoparticles and, to a lesser extent, S-2P constructs is consistent with an ongoing GC reaction, which in time should result in the formation of memory B cells and long-lived plasma cells. To evaluate the durability of humoral responses elicited by the RBD nanoparticle vaccines, we analyzed serum Ab responses 20-24 weeks post-boost. The magnitude of both binding and neutralization titers were similar to their levels two weeks post-boost for all nanoparticle groups (Figures 12B,C), indicating that the designed immunogens elicit not only potent but also durable neutralizing Abs. This is likely due in part to improved induction of long-lived plasma cells by the nanoparticle vaccines, as the number of S-2P-specific Ab secreting cells in the bone marrow was ~3-fold higher for mice immunized with the RBD-16GS-I53-50 nanoparticle compared to the S-2P trimer (Figure 12D).

We compared the ratio of binding to neutralizing antibodies elicited by the S-2P and the RBD-8GS-, RBD-12GS-, and RBD-16GS-I53-50 nanoparticles and HCS as a measure of the quality of the Ab responses elicited by the nanoparticle immunogens. In Kymab DarwinTM mice, the nanoparticle vaccines had lower (better) ratios than S-2P-immunized mice, but higher than HCS (Figure 6E). In BALB/c mice, the ratio of binding to pseudovirus neutralizing titers elicited by RBD-12GS- and RBD-16GS-I53-50 was clearly decreased compared to S-2P and HCS (Figure 6F). This pattern was consistent when ratios were calculated using live virus neutralizing titers, although the magnitude of the differences between groups was smaller due to the high values obtained in the live virus neutralization assay. These results suggest the Ab responses elicited by the RBD-12GS- and RBD-16GS-I53-50 nanoparticle immunogens are of higher quality than that obtained from immunization with the S-2P trimer or acquired during natural infection, perhaps because it is focused on epitopes in the RBD that are the target of most neutralizing Abs.

We set out to identify the epitopes recognized by Abs elicited upon immunization with the nanoparticle immunogens in a nonhuman primate model that more closely resembles humans in their immune response to vaccination. We immunized a pigtail macaque with 250 µg of RBD-12GS-I53-50 (88 µg of RBD antigen) at weeks 0 and 4 and found that serum collected at week 8 had high levels of S-specific Abs (EC₅₀ ~1×10⁶). Polyclonal Fabs were

generated and purified for use in competition BLI with hACE2, CR3022, and S309, which recognize three distinct sites targeted by neutralizing Abs on the SARS-CoV-2 RBD (Figure 6G). The polyclonal sera inhibited binding of hACE2, CR3022 Fab, and S309 Fab at concentrations above their respective dissociation constants in a dose-dependent manner (Figure 6H-J). These data indicate that immunization with 12GS-RBD-I53-50 elicited Abs targeting several non-overlapping epitopes, which we expect to limit the potential for emergence and selection of escape mutants, especially since coronaviruses do not mutate quickly when compared to viruses such as influenza or human immunodeficiency virus (Li et al., 2020; Smith et al., 2014).

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Discussion

Here we showed that two-component self-assembling SARS-CoV-2 RBD nanoparticle vaccine candidates elicit potent neutralizing Ab responses targeting multiple distinct RBD epitopes. The greater neutralizing Ab responses elicited by the RBD nanoparticles compared to the prefusion-stabilized ectodomain trimer are very promising. Our data indicate that RBD-12GS-I53-50 and RBD-16GS-I53-50 elicit nearly ten-fold higher levels of S-specific Abs and, more importantly, roughly ten-fold higher levels of neutralizing activity compared to the S-2P ectodomain trimer. This enhancement in potency is maintained at a more than five-fold lower antigen dose by mass, suggesting that presentation on the nanoparticle also has a dose-sparing effect. Both enhanced potency and dose-sparing could be critical for addressing the need to manufacture an unprecedented number of doses of vaccine to respond to the SARS-CoV-2 pandemic.

Although the RBD is poorly immunogenic as a monomer, our data establish that it can form the basis of a highly immunogenic vaccine when presented multivalently in our designs. The exceptionally low binding:neutralizing ratio elicited upon immunization with the RBD nanoparticles suggests that presentation of the RBD on I53-50 focuses the humoral response on epitopes recognized by neutralizing Abs. This metric is a potentially important indicator of vaccine safety, as high levels of binding yet non-neutralizing or weakly neutralizing Abs may contribute to vaccine-associated enhancement of respiratory disease. Our data further show that RBD-12GS-I53-50 elicited Ab responses targeting several of the non-overlapping epitopes recognized by neutralizing Abs that have been identified in the RBD. Such polyclonal responses targeting multiple distinct epitopes might explain the magnitude of neutralization observed and should minimize the risk of selection or emergence

of escape mutations. Finally, the high production yield of RBD-I53-50A components and the robust stability of the antigen-bearing RBD nanoparticles makes them amenable to large-scale manufacturing.

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Methods

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Table 6. Resources

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
CR3022	(ter Meulen <i>et al.</i> , 2006)	N/A
S309	(Pinto <i>et al.</i> , 2020)	N/A
B38	(Wu <i>et al.</i> , 2020)	N/A
Goat anti-human HRP	Invitrogen	Cat #A18817 Lot #65-180-071919
Goat anti-mouse HRP	Invitrogen	Cat #626520 Lot #TG275230
Horse anti-mouse HRP	Cell Signaling Technology	Cat #7076S
Anti-mouse Fc Block	BD Biosciences	Cat#553142 RRID:AB_394657
Anti-mouse B220 BV737	BD Biosciences	Cat#612838 RRID:AB_2738813
Anti-mouse CD3 PerCP-Cy5.5	BD Biosciences	Cat#551163 RRID:AB_394082
Anti-mouse CD138 BV650	BD Biosciences	Cat#564068 RRID:AB_2738574
Anti-mouse CD38 Alexa TM Fluor 700	Thermo Fisher Scientific	Cat#56-0381-82 RRID:AB_657740
Anti-mouse GL7 ef450	Thermo Fisher Scientific	Cat#48-5902-82 RRID:AB_10870775
Anti-mouse IgM BV786	BD Biosciences	Cat#743328 RRID:AB_2741429
Anti-mouse IgD BV395	BD Biosciences	Cat#565988 RRID:AB_2737433
Anti-mouse CD73 PE-Cy7	Thermo Fisher Scientific	Cat#25-0731-82 RRID:AB_10853348
Anti-mouse CD80 BV605	BD Biosciences	Cat#563052 RRID:AB_273795
Biological Samples		
BALB/c mice	Jackson Laboratory	Cat #000651
Kymice TM	Kymab	
20/130 COVID-19 plasma	NIBSC	20/130
Chemicals, Peptides, and Recombinant Proteins		
AddaVax TM adjuvant	InvivoGen	Cat# vac-adx-10
ABTS	ThermoFisher	Cat# 37615
TMB	SeraCare	Cat# 5120-0083
Thrombin	Sigma	Cat# T9326-150UN
Immobilized Papain	ThermoScientific	Cat# 20341
LysC-endoproteinase	NEB	Cat# P8109S
hACE2-Fc	This study	N/A
EZ-Link TM Sulfo-NHS-LC Biotinylation Kit	Thermo Fisher Scientific	Cat#21435
Streptavidin-APC	Agilent	Cat#PJ27S-1
Streptavidin-PE	Agilent	Cat#PJRS25-1

Anti-PE MicroBeads	Miltenyi Biotec	Cat#130-048-801
Anti-APC MicroBeads	Miltenyi Biotec	Cat#130-090-855
DyLight™ 755 Antibody Labeling Kit	Thermo Fisher Scientific	Cat#84538
AlexaFluor™ 647 Protein Labeling Kit	Thermo Fisher Scientific	Cat#A20173
Experimental Models: Cell Lines		
Expi 293F	ThermoFisher	Cat #A14527
Vero(C1008)E6 adherent	ECACC General Collection	Cat #85020206
HEK-ACE2 adherent	BEI (Gift from Bloom lab)	NR-52511
HEK293T/17 Adherent	ATCC	Cat# CRL-11268
Vero E6	ATCC	Cat# CRL-1586
Recombinant DNA		
pCMV-RBD-12GS-50A	GenScript (this study)	N/A
pCMVR-RBD-16GS-50A	GenScript (this study)	N/A
pCMV-RBD-8GS-50A	GenScript (this study)	N/A
S-2P trimer	GenScript (Walls et al. 2020)	BEI NR-52421
RBD	GenScript (Walls et al. 2020)	BEI NR-52422
SARS-CoV-2 S full length	GenScript (Walls et al. 2020)	BEI NR-52420
Murine leukemia virus gag-pol	Millet and Whittaker 2016	N/A
pTG-Luciferase	Millet and Whittaker 2016	N/A
Software and Algorithms		
UCSF ChimeraX	(Goddard et al., 2018)	https://www.rbvi.ucsf.edu/chimera/x/
Prism™	Graphpad	https://www.graphpad.com/scientific-software/prism/
FlowJo™ v10	FlowJo	https://www.flowjo.com
Other		
Octet™ Biosensors: protein A	Sartorius (FortéBio)	Cat# 18-5010
Octet™ Biosensors: Anti-Penta-HIS (HIS1K)	Sartorius (FortéBio)	Cat# 18-5120
Octet™ Biosensors: NTA	Sartorius (FortéBio)	Cat# 18-5101
EM supplies 300 mesh grids	Ted Pella	Cat# 01843-F
Filter paper	Cytiva	Cat# 1004047
Uranyl formate	SPI Chem	Cat# 02545-AA
Unis™ Capillary Cassettes	Unchained Labs	Cat# 201-1010
Prisma™ Protein A resin	Cytiva	Cat# 17549802
Superdex™ 200 Increase SEC column	Cytiva	Cat# 28-9909-44
Superose™ 6 Increase SEC column	Cytiva	Cat# 29091596
Talon™ resin	TaKaRa	Cat# 635652
VL26 Vantage L column	Millipore	Cat# 96100250
Excel resin	Cytiva	Cat# 17371203
Patterson Veterinary, Isoflurane, USP	Patterson	Cat# 07-893-1389
Eppendorf® Safe-Lock microcentrifuge tubes 1.5-mL	Sigma Millipore	Cat# T9661
BD Luer-Lok™ 1-mL Syringe	BD	Cat# BD309628
BD Single Use Needles 25G x 7/8	VWR	Cat# BD305124
BD PrecisionGlide™ Needle 23Gx1 1/4	BD	Ref# 305120
BD Single Use Needles 27G x 1	VWR	Cat# BD305136

1/4		
EndoSafe™ LAL Test Cartridges	Charles River Labs	Cat # PTS20005F
Lemo21™ (DE3)	New England BioLabs	Cat#C2528J
Isopropyl-B-D-thiogalactoside (IPTG)	Sigma Aldrich	Cat#16758
Kanamycin Sulfate	Sigma-Aldrich	Cat#K1876
HiLoad™ S200 pg	Cytiva	Cat#28989336
Ni Sepharose™ 6 FF	Cytiva	Cat#17531808
HisTrap™ FF	Cytiva	Cat#17525501

Cell lines

HEK293F is a female human embryonic kidney cell line transformed and adapted to grow in suspension (Life Technologies). HEK293F cells were grown in 293FreeStyle™ expression medium (Life Technologies), cultured at 37°C with 8% CO₂ and shaking at 130 rpm. Expi293F™ cells are derived from the HEK293F cell line (Life Technologies). Expi293F™ cells were grown in Expi293™ Expression Medium (Life Technologies), cultured at 36.5°C with 8% CO₂ and shaking at 150 rpm. VeroE6 is a female kidney epithelial cell from African green monkey. HEK293T/17 is a female human embryonic kidney cell line (ATCC). The HEK-ACE2 adherent cell line was obtained through BEI Resources, NIAID, NIH: Human Embryonic Kidney Cells (HEK-293T) Expressing Human Angiotensin-Converting Enzyme 2, HEK-293T-hACE2 Cell Line, NR-52511. All adherent cells were cultured at 37°C with 8% CO₂ in flasks with DMEM + 10% FBS (Hyclone) + 1% penicillin-streptomycin. Cell lines other than Expi293F were not tested for mycoplasma contamination nor authenticated.

Mice

Female BALB/c mice four weeks old were obtained from Jackson Laboratory, Bar Harbor, Maine. Animal procedures were performed under the approvals of the Institutional Animal Care and Use Committee of University of Washington, Seattle, WA, and University of North Carolina, Chapel Hill, NC. Kymab's proprietary IntelliSelect™ Transgenic mouse platform, known as Darwin™, has complete human antibody loci with a non-rearranged human antibody variable and constant germline repertoire. Consequently, the antibodies produced by these mice are fully human.

Pigtail macaques

Two adult male Pigtail macaques (*Macaca nemestrina*) were immunized in this study. All animals were housed at the Washington National Primate Research Center (WaNPRC).

an American Association for the Accreditation of Laboratory Animal Care International (AAALAC)-accredited institution, as previously described (Erasmus et al., 2020). All procedures performed on the animals were with the approval of the University of Washington's Institutional Animal Care and Use Committee (IACUC).

5

Convalescent human sera

Samples collected between 1–60 days post infection from 31 individuals who tested positive for SARS-CoV-2 by PCR were profiled for anti-SARS-CoV-2 S antibody responses and the 29 with anti-S Ab responses were maintained in the cohort (Figures 4 and 5).

- 10 Individuals were enrolled as part of the HAARVI study at the University of Washington in Seattle, WA. Baseline sociodemographic and clinical data for these individuals are summarized in Table 5. This study was approved by the University of Washington Human Subjects Division Institutional Review Board (STUDY00000959 and STUDY00003376). All experiments were performed in at least two technical and two biological replicates (for
- 15 ELISA and pseudovirus neutralization assays). One sample is the 20/130 COVID-19 plasma from NIBSC.

Plasmid construction

- The SARS-CoV-2 RBD (BEI NR-52422) construct was synthesized by GenScript
- 20 into pcDNA3.1- with an N-terminal mu-phosphatase signal peptide and a C-terminal octa-histidine tag (GHHHHHHH) (SEQ ID NO:164). The boundaries of the construct are N-328RFPN331 and 528KKST531-C (Walls et al., 2020). The SARS-CoV-2 S-2P ectodomain trimer (GenBank: YP_009724390.1, BEI NR-52420) was synthesized by GenScript into
- 25 pCMV with an N-terminal mu-phosphatase signal peptide and a C-terminal TEV cleavage site (GSGRENLYFQG) (SEQ ID NO: 165), T4 fibrin foldon (GGGSGYIPEAPRDGQAYVRKDGEWVLLSTFL) (SEQ ID NO:166), and octa-histidine tag (GHHHHHHH) (SEQ ID NO:164) (Walls et al., 2020). The construct contains the 2P mutations (proline substitutions at residues 986 and 987; (Pallesen et al., 2017)) and an
- 682SGAG685 substitution at the furin cleavage site. The SARS-CoV-2 RBD was genetically
- 30 fused to the N terminus of the trimeric I53-50A nanoparticle component using linkers of 8, 12, or 16 glycine and serine residues. RBD-8GS- and RBD-12GS-I53-50A fusions were synthesized and cloned by Genscript into pCMV. The RBD-16GS-I53-50A fusion was cloned into pCMV/R using the XbaI and AvrII restriction sites and Gibson assembly (Gibson

et al., 2009). All RBD-bearing components contained an N-terminal mu-phosphatase signal peptide and a C-terminal octa-histidine tag. The macaque or human ACE2 ectodomain was genetically fused to a sequence encoding a thrombin cleavage site and a human Fc fragment at the C-terminal end. hACE2-Fc was synthesized and cloned by GenScript with a BM40
5 signal peptide. Plasmids were transformed into the NEB 5-alpha strain of *E. coli* (New England Biolabs) for subsequent DNA extraction from bacterial culture (NucleoBond Xtra Midi™ kit) to obtain plasmid for transient transfection into Expi293F cells. The amino acid sequences of all novel proteins used in this study can be found in Table 3.

10 Transient transfection

SARS-CoV-2 S and ACE2-Fc proteins were produced in Expi293F cells grown in suspension using Expi293F expression medium (Life Technologies) at 33°C, 70% humidity, 8% CO₂ rotating at 150 rpm. The cultures were transfected using PEI-MAX™ (Polyscience) with cells grown to a density of 3.0 million cells per mL and cultivated for 3 days.
15 Supernatants were clarified by centrifugation (5 minutes at 4000 rcf), addition of PDADMAC solution to a final concentration of 0.0375% (Sigma Aldrich, #409014), and a second spin (5 minutes at 4000 rcf).

Genes encoding CR3022 heavy and light chains were ordered from GenScript and cloned into pCMV/R. Antibodies were expressed by transient co-transfection of both heavy
20 and light chain plasmids in Expi293F cells using PEI MAX™ (Polyscience) transfection reagent. Cell supernatants were harvested and clarified after 3 or 6 days as described above.

Protein purification

Proteins containing His tags were purified from clarified supernatants via a batch bind
25 method where each clarified supernatant was supplemented with 1 M Tris-HCl pH 8.0 to a final concentration of 45 mM and 5 M NaCl to a final concentration of ~310 mM. Talon cobalt affinity resin (Takara) was added to the treated supernatants and allowed to incubate for 15 minutes with gentle shaking. Resin was collected using vacuum filtration with a 0.2 µm filter and transferred to a gravity column. The resin was washed with 20 mM Tris pH 8.0,
30 300 mM NaCl, and the protein was eluted with 3 column volumes of 20 mM Tris pH 8.0, 300 mM NaCl, 300 mM imidazole. The batch bind process was then repeated and the first and second elutions combined. SDS-PAGE was used to assess purity. RBD-I53-50A fusion protein IMAC elutions were concentrated to >1 mg/mL and subjected to three rounds of

dialysis into 50 mM Tris pH 7, 185 mM NaCl, 100 mM Arginine, 4.5% glycerol, and 0.75% w/v 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) in a hydrated 10K molecular weight cutoff dialysis cassette (Thermo Scientific). S-2P IMAC elution fractions were concentrated to ~1 mg/mL and dialyzed three times into 50 mM Tris pH 8, 150 mM NaCl, 0.25% L-Histidine in a hydrated 10K molecular weight cutoff dialysis cassette (Thermo Scientific). Due to inherent instability, the S-2P trimer was immediately flash frozen and stored at -80°C.

Clarified supernatants of cells expressing monoclonal antibodies and human or macaque ACE2-Fc were purified using a MabSelect Prisma™ 2.6×5 cm column (Cytiva) on an AKTA Avant150 FPLC (Cytiva). Bound antibodies were washed with five column volumes of 20 mM NaPO₄, 150 mM NaCl pH 7.2, then five column volumes of 20 mM NaPO₄, 1 M NaCl pH 7.4 and eluted with three column volumes of 100 mM glycine at pH 3.0. The eluate was neutralized with 2 M Trizma base to 50 mM final concentration. SDS-PAGE was used to assess purity.

Recombinant S309 was expressed as a Fab in expiCHO cells transiently co-transfected with plasmids expressing the heavy and light chain, as described above (see Transient transfection) (Stettler et al., 2016). The protein was affinity-purified using a HiTrap™ Protein A Mab select Xtra™ column (Cytiva) followed by desalting against 20 mM NaPO₄, 150 mM NaCl pH 7.2 using a HiTrap™ Fast desalting column (Cytiva). The protein was sterilized with a 0.22 µm filter and stored at 4°C until use.

Microbial protein expression and purification

The I53-50A and I53-50B.4.PT1 proteins were expressed in Lemo21(DE3) (NEB) in LB (10 g Tryptone, 5 g Yeast Extract, 10 g NaCl) grown in 2 L baffled shake flasks or a 10 L BioFlo 320 Fermenter (Eppendorf). Cells were grown at 37°C to an OD₆₀₀ ~0.8, and then induced with 1 mM IPTG. Expression temperature was reduced to 18°C and the cells shaken for ~16 h. The cells were harvested and lysed by microfluidization using a Microfluidics M110P at 18,000 psi in 50 mM Tris, 500 mM NaCl, 30 mM imidazole, 1 mM PMSE, 0.75% CHAPS. Lysates were clarified by centrifugation at 24,000 g for 30 min and applied to a 2.6×10 cm Ni Sepharose™ 6 FF column (Cytiva) for purification by IMAC on an AKTA Avant150 FPLC system (Cytiva). Protein of interest was eluted over a linear gradient of 30 mM to 500 mM imidazole in a background of 50 mM Tris pH 8, 500 mM NaCl, 0.75% CHAPS buffer. Peak fractions were pooled, concentrated in 10K MWCO centrifugal filters

(Millipore), sterile filtered (0.22 μ m) and applied to either a Superdex™ 200 Increase 10/300, or HiLoad™ S200 pg GL SEC column (Cytiva) using 50 mM Tris pH 8, 500 mM NaCl, 0.75% CHAPS buffer. I53-50A elutes at ~0.6 column volume (CV). I53-50B.4PT1 elutes at ~0.45 CV. After sizing, bacterial-derived components were tested to confirm low levels of
 5 endotoxin before using for nanoparticle assembly.

***In vitro* nanoparticle assembly**

Total protein concentration of purified individual nanoparticle components was determined by measuring absorbance at 280 nm using a UV/vis spectrophotometer (Agilent
 10 Cary 8454) and calculated extinction coefficients (Gasteiger et al., 2005). The assembly steps were performed at room temperature with addition in the following order: RBD-I53-50A trimeric fusion protein, followed by additional buffer as needed to achieve desired final concentration, and finally I53-50B.4PT1 pentameric component (in 50 mM Tris pH 8, 500 mM NaCl, 0.75% w/v CHAPS), with a molar ratio of RBD-I53-50A:I53-B.4PT1 of 1.1:1. In
 15 order to produce partial valency RBD-I53-50 nanoparticles (50% RBD-I53-50), both RBD-I53-50A and unmodified I53-50A trimers (in 50 mM Tris pH 8, 500 mM NaCl, 0.75% w/v CHAPS) were added in a slight molar excess (1.1 $\times$) to I53-50B.4PT1. All RBD-I53-50 *in vitro* assemblies were incubated at 2-8°C with gentle rocking for at least 30 minutes before subsequent purification by SEC in order to remove residual unassembled component.
 20 Different columns were utilized depending on purpose: Superose™ 6 Increase 10/300 GL column was used analytically for nanoparticle size estimation, a Superdex™ 200 Increase 10/300 GL column used for small-scale pilot assemblies, and a HiLoad™ 26/600 Superdex™ 200 pg column used for nanoparticle production. Assembled particles elute at ~11 mL on the Superose™ 6 column and in the void volume of Superdex™ 200 columns. Assembled
 25 nanoparticles were sterile filtered (0.22 μ m) immediately prior to column application and following pooling of fractions.

hACE2-Fc and CR3022 digestion

hACE2-Fc was digested with thrombin protease (Sigma Aldrich) in the presence of
 30 2.5 mM CaCl₂ at a 1:300 w/w thrombin:protein ratio. The reaction was incubated at ambient temperature for 16–18 hours with gentle rocking. Following incubation, the reaction mixture was concentrated using Ultracel™ 10K centrifugal filters (Millipore Amicon Ultra) and sterile filtered (0.22 μ m). Cleaved hACE2 monomer was separated from uncleaved hACE2-

Fc and the cleaved Fc regions using Protein A purification (see Protein purification above) on a HiScreen MabSelect SuRe™ column (Cytiva) using an ÄKTA avant 25 FPLC (Cytiva). Cleaved hACE2 monomer was collected in the flow through, sterile filtered (0.22 µm), and quantified by UV/vis.

- 5 LysC (New England BioLabs) was diluted to 10 ng/µL in 10 mM Tris pH 8 and added to CR3022 IgG at 1:2000 w/w LysC:IgG and subsequently incubated for 18 hours at 37°C with orbital shaking at 230 rpm. The cleavage reaction was concentrated using Ultracel™ 10K centrifugal filters (Millipore Amicon Ultra) and sterile filtered (0.22 µm). Cleaved CR3022 mAb was separated from uncleaved CR3022 IgG and the Fc portion of
- 10 cleaved IgG, using Protein A purification as described above. Cleaved CR3022 was collected in the flow through, sterile filtered (0.22 µm), and quantified by UV/vis.

Bio-layer interferometry (antigenicity)

- Antigenicity assays were performed and analyzed using BLI on an Octet™ Red 96
- 15 System (Pall Forté Bio/Sartorius) at ambient temperature with shaking at 1000 rpm. RBD-153-50A trimeric components and monomeric RBD were diluted to 40 µg/mL in Kinetics buffer (1× HEPES-EP+ (Pall Forté Bio), 0.05% nonfat milk, and 0.02% sodium azide). Monomeric hACE2 and CR3022 Fab were diluted to 750 nM in Kinetics buffer and serially diluted three-fold for a final concentration of 3.1 nM. Reagents were applied to a black 96-
- 20 well Greiner Bio-one microplate at 200 µL per well as described below. RBD-153-50A components or monomeric RBD were immobilized onto Anti-Penta-HIS (HIS1K) biosensors per manufacturer instructions (Forté Bio) except using the following sensor incubation times. HIS1K biosensors were hydrated in water for 10 minutes, and were then equilibrated in Kinetics buffer for 60 seconds. The HIS1K tips were loaded with diluted trimeric RBD-153-
- 25 50A component or monomeric RBD for 150 seconds and washed with Kinetics buffer for 300 seconds. The association step was performed by dipping the HIS1K biosensors with immobilized immunogen into diluted hACE2 monomer or CR3022 Fab for 600 seconds, then dissociation was measured by inserting the biosensors back into Kinetics buffer for 600 seconds. The data were baseline subtracted and the plots fitted using the Pall™
- 30 FortéBio/Sartorius analysis software (version 12.0). Plots in Figure 8 show the association and dissociation steps.

Bio-layer interferometry (accessibility)

Binding of mACE2-Fc, CR3022 IgG, and S309 IgG to monomeric RBD, RBD-I53-50A trimers, and RBD-I53-50 nanoparticles was analyzed for accessibility experiments and real-time stability studies using an Octet™ Red 96 System (Pall™ FortéBio/Sartorius) at ambient temperature with shaking at 1000 rpm. Protein samples were diluted to 100 nM in Kinetics buffer. Buffer, immunogen, and analyte were then applied to a black 96-well Greiner Bio-one microplate at 200 µL per well. Protein A biosensors (FortéBio/Sartorius) were first hydrated for 10 minutes in Kinetics buffer, then dipped into either mACE2-Fc, CR3022, or S309 IgG diluted to 10 µg/mL in Kinetics buffer in the immobilization step. After 500 seconds, the tips were transferred to Kinetics buffer for 60 seconds to reach a baseline. The association step was performed by dipping the loaded biosensors into the immunogens for 300 seconds, and subsequent dissociation was performed by dipping the biosensors back into Kinetics buffer for an additional 300 seconds. The data were baseline subtracted prior to plotting using the FortéBio analysis software (version 12.0). Plots in Figure 2 show the 600 seconds of association and dissociation.

15 Negative stain electron microscopy

RBD-I53-50 nanoparticles were first diluted to 75 µg/mL in 50 mM Tris pH 7, 185 mM NaCl, 100 mM Arginine, 4.5% v/v Glycerol, 0.75% w/v CHAPS, and S-2P protein was diluted to 0.03 mg/mL in 50 mM Tris pH 8, 150 mM NaCl, 0.25% L-Histidine prior to application of 3 µL of sample onto freshly glow-discharged 300-mesh copper grids. Sample was incubated on the grid for 1 minute before the grid was dipped in a 50 µL droplet of water and excess liquid blotted away with filter paper (Whatman). The grids were then dipped into 6 µL of 0.75% w/v uranyl formate stain. Stain was blotted off with filter paper, then the grids were dipped into another 6 µL of stain and incubated for ~70 seconds. Finally, the stain was blotted away and the grids were allowed to dry for 1 minute. Prepared grids were imaged in a Talos model L120C electron microscope at 45,000× (nanoparticles) or 92,000× magnification (S-2P).

Dynamic light scattering

Dynamic Light Scattering (DLS) was used to measure hydrodynamic diameter (Dh) and % Polydispersity (%Pd) of RBD-I53-50 nanoparticle samples on an UNcle Nano-DSF (UNchained Laboratories). Sample was applied to a 8.8 µL quartz capillary cassette (UNI, UNchained Laboratories) and measured with 10 acquisitions of 5 seconds each, using auto-attenuation of the laser. Increased viscosity due to 4.5% v/v glycerol in the RBD nanoparticle

buffer was accounted for by the UNcle™ Client software in Dh measurements.

Guanidine HCl denaturation

Monomeric RBD, RBD-I53-50A fusion proteins, and RBD-I53-50 nanoparticle immunogens were diluted to 2.5 μ M in 50 mM Tris pH 7.0, 185 mM NaCl, 100 mM Arginine, 4.5% v/v glycerol, 0.75% w/v CHAPS, and guanidine chloride [GdnHCl] ranging from 0 M to 6.5 M, increasing in 0.25 M increments, and prepared in triplicate. S-2P trimer was also diluted to 2.5 μ M using 50 mM Tris pH 8, 150 mM NaCl, 0.25% L-Histidine, and the same GuHCl concentration range. Dilutions were mixed 10 $\times$ by pipetting. The samples were then incubated 18–19 hours at ambient temperature. Using a Nano-DSF (UNcle™, UNchained Laboratories) and an 8.8 μ L quartz capillary cassette (UNi™, UNchained Laboratories), fluorescence spectra were collected in triplicate, exciting at 266 nm and measuring emission from 200 nm to 750 nm at 25°C.

Endotoxin measurements

Endotoxin levels in protein samples were measured using the EndoSafe™ Nexgen-MCS System (Charles River). Samples were diluted 1:50 or 1:100 in Endotoxin-free LAL reagent water, and applied into wells of an EndoSafe™ LAL reagent cartridge. Charles River EndoScan™-V software was used to analyze endotoxin content, automatically back-calculating for the dilution factor. Endotoxin values were reported as EU/mL which were then converted to EU/mg based on UV/vis measurements. Our threshold for samples suitable for immunization was <50 EU/mg.

UV/vis

Ultraviolet-visible spectrophotometry (UV/vis) was measured using an Agilent Technologies Cary™ 8454. Samples were applied to a 10 mm, 50 μ L quartz cell (Starna Cells, Inc.) and absorbance was measured from 180 to 1000 nm. Net absorbance at 280 nm, obtained from measurement and single reference wavelength baseline subtraction, was used with calculated extinction coefficients and molecular weights to obtain protein concentration. The ratio of absorbance at 320/280 nm was used to determine relative aggregation levels in real-time stability study samples. Samples were diluted with respective purification/instrument blanking buffers to obtain an absorbance between 0.1 and 1.0. All data produced from the UV/vis instrument was processed in the 845x UV/visible System

software.

Glycan profiling

To identify site-specific glycosylation profiles, including glycoform distribution and occupancy determination, a bottom up mass spectrometry (MS) approach was utilized. Aliquots of 1 mg/mL monomeric, 8GS, 12GS and 16GS RBD protein were prepared to evaluate the glycosylation profiles at N331 and N343 of the four RBD variants. Comprehensive glycoprofiling on the stabilized Spike ectodomain (S-2P) was performed in parallel using 1.5 mg/mL SARS-CoV-2 S-2P protein. All the samples were denatured in a solution containing 25 mM Tris (pH 8.0), 7 M guanidinium chloride (GdnHCl) and 50 mM dithiothreitol (DTT) at 90°C for 30 minutes. Reduced cysteines were alkylated by adding fresh iodoacetamide (IAA) to 100 mM and incubating at room temperature for 1 hour in the dark. 50 mM excess DTT was then added to quench the remaining IAA. The GdnHCl concentration was reduced to 0.6 M by diluting the samples 11-fold with a 10 mM Tris (pH 8.0), 2 mM calcium chloride solution. Each sample was then split in half. One half (275 µL) was mixed with 10 units of recombinant Peptide N-glycanase F (GST-PNGase F) (Krenkova et al., 2013) and incubated at 37°C for 1 hour in order to convert glycosylated Asn into deglycosylated Asp.

Protease digestions were performed in the following manner: all RBD samples and one S-2P sample were digested with Lys-C at a ratio of 1:40 (w/w) for RBD and 1:30 (w/w) for S-2P for 4 hours at 37°C, followed by Glu-C digestion overnight at the same ratios and conditions. The other three S-2P samples were digested with trypsin, chymotrypsin and alpha lytic protease, respectively, at a ratio of 1:30 (w/w) overnight at 37°C. All the digestion proteases used were MS grade (Promega). The next day, the digestion reactions were quenched by 0.02% formic acid (FA, Optima™, Fisher).

The glycoform determination of four S-2P samples was performed by nano LC-MS using an Orbitrap Fusion™ mass spectrometer (Thermo Fisher). The digested samples were desalted by Sep-Pak C18 cartridges (Waters) following the manufacturer's suggested protocol. A 2 cm trapping column and a 35 cm analytical column were freshly prepared in fused silica (100 µm ID) with 5 µm ReproSil-Pur™ C18 AQ beads (Dr. Maisch). 8 µL sample was injected and run by a 60-minute linear gradient from 2% to 30% acetonitrile in 0.1% FA, followed by 10 minutes of 80% acetonitrile. An EThcD method was optimized as followed: ion source: 2.1 kV for positive mode; ion transfer tube temperature: 350 °C;

resolution: $MS^1 = 120000$, $MS^2 = 30000$; AGC target: $MS^1 = 2e^5$, $MS^2 = 1e^5$; and injection time: $MS^1 = 50$ ms, $MS^2 = 60$ ms.

Glycopeptide data were visualized and processed by ByonicTM and ByologicTM (Version 3.8, Protein Metrics Inc.) using a 6 ppm precursor and 10 ppm fragment mass tolerance. Glycopeptides were searched using the N-glycan 309 mammalian database in Protein Metrics PMI-Suite and scored based on the assignment of correct c- and z- fragment ions. The true-positive entities were further validated by the presence of glycan oxonium ions m/z at 204 (HexNAc ions) and 366 (HexNAcHex ions) and the absence in its corresponding spectrum in the deglycosylated sample. The relative abundance of each glycoform was determined by the peak area analyzed in ByologicTM. Glycoforms were categorized in Oligo (Oligomannose), Hybrid, and Complex as well as subtypes in Complex, described in the previous study (Watanabe et al., 2020). HexNAc(2)Hex(9-5) is M(annose)9 to M5; HexNAc(3)Hex(5-6) is classified as Hybrid; HexNAc(3)Hex(3-4)X is A1 subtype; HexNAc(4)X is A2/A1B; HexNAc(5)X is A3/A2B and HexNAc(6)X is A4/A3B subtype. Hybrid and Complex forms with fucosylation are separately listed as FHybrid and FComplex (eg. FA1), respectively.

Glycan occupancy analysis and glycoform determination of the four RBD variants were performed by LC-MS on the Synapt G2-SiTM TOF mass spectrometer coupled to an AcquityTM UPLC system (Waters). Samples were resolved over a Waters CSH C18 1 x 100 mm 1.7 μ m column with a linear gradient from 3% to 40% B over 30 minutes (A: 98% water, 2% acetonitrile, 0.1% FA; B: 100% acetonitrile, 0.1% FA). Data dependent acquisition (DDA) method was utilized with precursor mass range 300-2000, MS/MS mass range 50-2000 and a collision energy ramped from 70 to 100 V. Chromatographic peaks for the most abundant and non-overlapped isotopic peaks were determined and integrated with MassLynxTM (Waters). All the water and organic solvents used, unless specifically stated, were MS grade (OptimaTM, Fisher). The peak area ratio of the non-glycosylated (Asn) to the deglycosylated (Asp) glycopeptide was used to measure the glycan occupancy at each site.

Hydrogen/Deuterium-exchange mass spectrometry

3 μ g of monomeric RBD and RBD-8GS-I53-50A were incubated and H/D exchanged (HDX) in the deuteration buffer (pH* 7.6, 85% D₂O, Cambridge Isotope Laboratories, Inc.) for 3, 60, 1800, and 72000 seconds, respectively, at 23°C. Samples were subsequently mixed 1:1 with ice-cold quench buffer (200 mM tris(2-chlorethyl) phosphate (TCEP), 8 M Urea,

0.2% formic acid) for a final pH 2.5 and immediately flash frozen in liquid nitrogen. Samples were in-line pepsin digested and analyzed by LC-MS-IMS on Synapt G2-Si™ TOF mass spectrometer (Waters) as previously described (Verkerke et al., 2016) with an 18 minute gradient applied. A fully deuteration control was made by collecting the pepsin digest eluate from an undeuterated sample LC-MS run, drying by speedvac, incubating in deuteration buffer for 1 hour at 85°C, and quenching the same as all other HDX samples. Internal exchange standards (Pro-Pro-Pro-Ile [PPPI] and Pro-Pro-Pro-Phe [PPPF]) were added in each sample to ensure consistent labeling conditions for all samples (Zhang et al., 2012). Pepsin digests for undeuterated samples were also analyzed by nano LC-MS using an Orbitrap Fusion™ mass spectrometer (Thermo Fisher) with the settings as described above for glycoprofiling. The data was then processed by Byonic™ to obtain the peptide reference list. Peptides were manually validated using DriftScope™ (Waters) and identified with orthogonal retention time (rt) and drift time (dt) coordinates. Deuterium uptake analysis was performed with HX-Express v2 (Guttman et al., 2013; Weis et al., 2006). Peaks were identified from the peptide spectra with binomial fitting applied. The deuterium uptake level was normalized relative to fully deuterated standards.

Mouse immunizations and challenge

Female BALB/c (Stock: 000651) mice were purchased at the age of four weeks from The Jackson Laboratory, Bar Harbor, Maine, and maintained at the Comparative Medicine Facility at the University of Washington, Seattle, WA, accredited by the American Association for the Accreditation of Laboratory Animal Care International (AAALAC). At six weeks of age, 10 mice per dosing group were vaccinated with a prime immunization, and three weeks later mice were boosted with a second vaccination. Prior to inoculation, immunogen suspensions were gently mixed 1:1 vol/vol with AddaVax™ adjuvant (Invivogen, San Diego, CA) to reach a final concentration of 0.009 or 0.05 mg/mL antigen. Mice were injected intramuscularly into the gastrocnemius muscle of each hind leg using a 27-gauge needle (BD, San Diego, CA) with 50 µL per injection site (100 µL total) of immunogen under isoflurane anesthesia. To obtain sera all mice were bled two weeks after prime and boost immunizations. Blood was collected via submental venous puncture and rested in 1.5 mL plastic Eppendorf tubes at room temperature for 30 minutes to allow for coagulation. Serum was separated from hematocrit via centrifugation at 2000 g for 10 minutes. Complement factors and pathogens in isolated serum were heat-inactivated via

incubating serum at 56°C for 60 minutes. Serum was stored at 4°C or -80°C until use. Six weeks post-boost, mice were exported from Comparative Medicine Facility at the University of Washington, Seattle, WA to an AAALAC accredited Animal Biosafety Level 3 (ABSL3) Laboratory at the University of North Carolina, Chapel Hill. After a 7-day acclimation time, mice were anesthetized with a mixture of ketamine/xylazine and challenged intranasally with 10⁵ plaque-forming units (pfu) of mouse-adapted SARS-CoV-2 MA strain for the evaluation of vaccine efficacy (IACUC protocol 20-114.0). After infection, body weight was monitored daily until the termination of the study two days post-infection, when lung and nasal turbinate tissues were harvested to evaluate the viral load by plaque assay. All experiments were conducted at the University of Washington, Seattle, WA, and University of North Carolina, Chapel Hill, NC according to approved Institutional Animal Care and Use Committee protocols.

Immunization (Kymab Darwin™ mice)

Kymab Darwin™ mice (a mix of males and females, 10 weeks of age), 5 mice per dosing group, were vaccinated with a prime immunization and three weeks later boosted with a second vaccination. Prior to inoculation, immunogen suspensions were gently mixed 1:1 vol/vol with AddaVax™ adjuvant (Invivogen) to reach a final concentration of 0.009 or 0.05 mg/mL antigen. Mice were injected intramuscularly into the tibialis muscle of each hind leg using a 30-gauge needle (BD) with 20 µL per injection site (40 µL total) of immunogen under isoflurane anesthesia. A final boost was administered intravenously (50 µL) with no adjuvant at week 7. Mice were sacrificed 5 days later under UK Home Office Schedule 1 (rising concentration of CO₂) and spleen, lymph nodes, and bone marrow cryopreserved. Whole blood (0.1 ml) was collected 2 weeks after each dose (weeks 0, 2, 5, and week 8 terminal bleed). Serum was separated from hematocrit via centrifugation at 2000 g for 10 minutes. Serum was stored at -20°C and was used to monitor titers by ELISA. All mice were maintained and all procedures carried out under United Kingdom Home Office License 70/8718 and with the approval of the Wellcome Trust Sanger Institute Animal Welfare and Ethical Review Body.

ELISA

For anti-S-2P ELISA, 25 µL of 2 µg/mL S-2P was plated onto 384-well Nunc Maxisorp™ (ThermoFisher) plates in PBS and sealed overnight at 4°C. The next day plates

were washed 4× in Tris Buffered Saline Tween (TBST) using a plate washer (BioTek) and blocked with 2% BSA in TBST for 1 h at 37°C. Plates were washed 4× in TBST and 1:5 serial dilutions of mouse, NHP, or human sera were made in 25 µL TBST starting at 1:25 or 1:50 and incubated at 37°C for 1 h. Plates were washed 4× in TBST, then anti-mouse
5 (Invitrogen) or anti-human (Invitrogen) horseradish peroxidase-conjugated antibodies were diluted 1:5,000 and 25 µL added to each well and incubated at 37°C for 1 h. Plates were washed 4× in TBST and 25 µL of TMB (SeraCare) was added to every well for 5 min at room temperature. The reaction was quenched with the addition of 25 µL of 1N HCl. Plates were immediately read at 450 nm on a VarioSkanLux™ plate reader (ThermoFisher) and
10 data plotted and fit in Prism™ (GraphPad) using nonlinear regression sigmoidal, 4PL, X is log(concentration) to determine EC₅₀ values from curve fits.

Pseudovirus production

MLV-based SARS-CoV-2 S, SARS-CoV S, and WIV-1 pseudotypes were prepared
15 as previously described (Millet and Whittaker, 2016; Walls et al., 2020). Briefly, HEK293T cells were co-transfected using Lipofectamine™ 2000 (Life Technologies) with an S-encoding plasmid, an MLV Gag-Pol packaging construct, and the MLV transfer vector encoding a luciferase reporter according to the manufacturer's instructions. Cells were washed 3× with Opti-MEM and incubated for 5 h at 37°C with transfection medium. DMEM
20 containing 10% FBS was added for 60 h. The supernatants were harvested by a 2,500 g spin, filtered through a 0.45 µm filter, concentrated with a 100 kDa membrane for 10 min at 2,500 g and then aliquoted and placed at -80°C.

Pseudovirus entry and serum neutralization assays

25 HEK-hACE2 cells were cultured in DMEM with 10% FBS (Hyclone) and 1% PenStrep with 8% CO₂ in a 37°C incubator (ThermoFisher). One day prior to infection, 40 µL of poly-lysine (Sigma) was placed into 96-well plates and incubated with rotation for 5 min. Poly-lysine was removed, plates were dried for 5 min then washed 1× with DMEM prior to plating cells. The following day, cells were checked to be at 80% confluence. In a half-area
30 96-well plate a 1:3 serial dilution of sera was made in DMEM starting between 1:3 and 1:66 initial dilution in 22 µL final volume. 22 µL of pseudovirus was then added to the serial dilution and incubated at room temperature for 30-60 min. HEK-hACE2 plate media was removed and 40 µL of the sera/virus mixture was added to the cells and incubated for 2 h at

- 37°C with 8% CO₂. Following incubation, 40 µL 20% FBS and 2% PenStrep containing DMEM was added to the cells for 48 h. Following the 48-h infection, One-Glo-EX™ (Promega) was added to the cells in half culturing volume (40 µL added) and incubated in the dark for 5 min prior to reading on a Varioskan™ LUX plate reader (ThermoFisher).
- 5 Measurements were done on all ten mouse sera samples from each group in at least duplicate. Relative luciferase units were plotted and normalized in Prism™ (GraphPad) using a zero value of cells alone and a 100% value of 1:2 virus alone. Nonlinear regression of log(inhibitor) vs. normalized response was used to determine IC₅₀ values from curve fits. Mann-Whitney tests were used to compare two groups to determine whether they were
- 10 statistically different.

Live virus production

- SARS-CoV-2-nanoLuc virus (WA1 strain) in which ORF7 was replaced by nanoluciferase gene (nanoLuc), and mouse-adapted SARS-CoV-2 (SARS-CoV-2 MA)
- 15 (Dinnon et al., 2020) were generated by the coronavirus reverse genetics system described previously (Hou et al., 2020). Recombinant viruses were generated in Vero E6 cells (ATCC-CRL1586) grown in DMEM high glucose media (Gibco #11995065) supplemented with 10% Hyclone™ Fetal Clone II (GE #SH3006603HI), 1% non-essential amino acid, and 1% Pen/Strep in a 37°C +5% CO₂ incubator. To generate recombinant SARS-CoV-2, seven DNA
- 20 fragments which collectively encode the full-length genome of SARS-CoV-2 flanked by a 5' T7 promoter and a 3' polyA tail were ligated and transcribed *in vitro*. The transcribed RNA was electroporated into Vero E6 cells to generate a P0 virus stock. The seed virus was amplified twice in Vero E6 cells at low moi for 48 h to create a working stock which was titrated by plaque assay (Hou et al., 2020). All the live virus experiments, including the
- 25 ligation and electroporation steps, were performed under biosafety level 3 (BSL-3) conditions at negative pressure, by operators in Tyvek suits wearing personal powered-air purifying respirators.

Luciferase-based serum neutralization assay, SARS-CoV-2-nanoLuc

- 30 Vero E6 cells were seeded at 2x10⁴ cells/well in a 96-well plate 24 h before the assay. One hundred pfu of SARS-CoV-2-nanoLuc virus (Hou et al., 2020) were mixed with serum at 1:1 ratio and incubated at 37°C for 1 h. An 8-point, 3-fold dilution curve was generated for each sample with starting concentration at 1:20 (standard) or 1:2000 (high neutralizer). Virus

and serum mix was added to each well and incubated at 37°C + 5% CO₂ for 48 h. Luciferase activities were measured by Nano-Glo™ Luciferase Assay System (Promega, WI) following manufacturer protocol using SpectraMax™ M3 luminometer (Molecular Device). Percent inhibition and 50% inhibition concentration (IC₅₀) were calculated by the following
5 equation: $[1 - (\text{RLU with sample} / \text{RLU with mock treatment})] \times 100\%$. Fifty percent inhibition titer (IC₅₀) was calculated in GraphPad Prism™ 8.3.0 by fitting the data points using a sigmoidal dose-response (variable slope) curve.

Tetramer production

10 Recombinant SARS-CoV-2 S-2P trimer was biotinylated using the EZ-Link™ Sulfo-NHS-LC Biotinylation Kit (ThermoFisher) and tetramerized with streptavidin-APC (Agilent) as previously described (Krishnamurthy et al., 2016; Taylor et al., 2012). The RBD domain of SARS-CoV-2 S was biotinylated and tetramerized with streptavidin-APC (Agilent). The APC
15 decoy reagent was generated by conjugating SA-APC to DyLight™ 755 using a DyLight 755 antibody labeling kit (ThermoFisher), washing and removing unbound DyLight 755, and incubating with excess of an irrelevant biotinylated His-tagged protein. The PE decoy was generated in the same manner, by conjugating SA-PE to Alexa Fluor 647 with an AF647
antibody labeling kit (ThermoFisher).

20 Mouse immunization, cell enrichment, and flow cytometry

For phenotyping of B cells, 6-week old female BALB/c mice, three per dosing group, were immunized intramuscularly with 50 µL per injection site of vaccine formulations containing 5 µg of SARS-CoV-2 antigen (either S-2P trimer or RBD, but not including mass from the I53-50 nanoparticle) mixed 1:1 vol/vol with AddaVax™ adjuvant on day 0. All
25 experimental mice were euthanized for harvesting of inguinal and popliteal lymph nodes on day 11. The experiment was repeated two times. Popliteal and inguinal lymph nodes were collected and pooled for individual mice. Cell suspensions were prepared by mashing lymph nodes and filtering through 100 µm Nitex™ mesh. Cells were resuspended in PBS containing 2% FBS and Fc block (2.4G2), and were incubated with 10 nM Decoy tetramers at room
30 temperature for 20 min. RBD-PE tetramer and Spike-APC tetramer were added at a concentration of 10 nM and incubated on ice for 20 min. Cells were washed, incubated with anti-PE and anti-APC magnetic beads on ice for 30 min, then passed over magnetized LS columns (Miltenyi Biotec). Bound B cells were stained with anti-mouse B220 (BUV737),

CD3 (PerCP-Cy5.5), CD138 (BV650), CD38 (Alexa Fluor™ 700), GL7 (eFluor™ 450), IgM (BV786), IgD (BUV395), CD73 (PE-Cy7), and CD80 (BV605) on ice for 20 min. Cells were run on the Cytex Aurora™ and analyzed using FlowJo™ software (Treestar). Cell counts were determined using Accucheck™ cell counting beads.

5

NHP immunization

A Pigtail macaque was immunized with 250 µg of RBD-12GS-153-50 nanoparticle (88 µg RBD antigen) at day 0 and day 28. Blood was collected at days 0, 10, 14, 28, 42, and 56 days post-prime. Serum and plasma were collected as previously described (Erasmus et al., 2020). Prior to vaccination or blood collection, animals were sedated with an intramuscular injection (10 mg/kg) of ketamine (Ketaset®; Henry Schein). Prior to inoculation, immunogen suspensions were gently mixed 1:1 vol/vol with AddaVax™ adjuvant (Invivogen, San Diego, CA) to reach a final concentration of 0.250 mg/mL antigen. The vaccine was delivered intramuscularly into both quadriceps muscles with 1 mL per injection site on days 0 and 28. All injection sites were shaved prior to injection and monitored post-injection for any signs of local reactogenicity. At each study timepoint, full physical exams and evaluation of general health were performed on the animals, as previously described (Erasmus et al., 2020), and no adverse events were observed.

20 Competition Bio-layer Interferometry

Purification of Fabs from NHP serum was adapted from (Boyoglu-Barnum et al., 2020). Briefly, 1mL of day 56 serum was diluted to 10 mL with PBS and incubated with 1 mL of 3× PBS washed protein A beads (GenScript) with agitation overnight at 37°C. The next day beads were thoroughly washed with PBS using a gravity flow column and bound antibodies were eluted with 0.1 M glycine pH 3.5 into 1M Tris-HCl (pH 8.0) to a final concentration of 100 mM. Serum and early washes that flowed through were re-bound to beads overnight again for a second, repeat elution. IgGs were concentrated (Amicon 30 kDa) and buffer exchanged into PBS. 2× digestion buffer (40 mM sodium phosphate pH 6.5, 20 mM EDTA, 40 mM cysteine) was added to concentrated and pooled IgGs. 500 µL of resuspended immobilized papain resin (ThermoFisher Scientific) freshly washed in 1× digestion buffer (20 mM sodium phosphate, 10 mM EDTA, 20 mM cysteine, pH 6.5) was added to purified IgGs in 2× digestion buffer and samples were agitated for 5 h at 37°C. The supernatant was separated from resin and resin washes were collected and pooled with the

resin flow through. Pooled supernatants were sterile-filtered at 0.22 μ m and applied 6 $\times$ to PBS-washed protein A beads in a gravity flow column. The column was eluted as described above and the papain procedure repeated overnight with undigested IgGs to increase yield. The protein A flowthroughs were pooled, concentrated (Amicon 10 kDa), and buffer
5 exchanged into PBS. Purity was checked by SDS-PAGE.

Epitope competition was performed and analyzed using BLI on an OctetTM Red 96 System (PallTM Forté Bio/Sartorius) at 30°C with shaking at 1000 rpm. NTA biosensors (PallTM Forté Bio/Sartorius) were hydrated in water for at least 10 minutes, and were then equilibrated in 10 $\times$ Kinetics buffer (KB) (PallTM Forté Bio/Sartorius) for 60 seconds. 10
10 ng/ μ L monomeric RBD in 10 $\times$ KB was loaded for 100 seconds prior to baseline acquisition in 10 $\times$ KB for 300 seconds. Tips were then dipped into diluted polyclonal Fab in 10 $\times$ KB in a 1:3 serial dilution beginning with 5000 nM for 2000 seconds or maintained in 10 $\times$ KB. Tips bound at varying levels depending on the polyclonal Fab concentration. Tips were then dipped into the same concentration of polyclonal Fab plus either 200 nM of hACE2, 400nM
15 CR3022, or 20nM S309 and incubated for 300-2000 seconds. The data were baseline subtracted and aligned to pre-loading with polyclonal Fabs using the PallTM Forté Bio/Sartorius analysis software (version 12.0) and plotted in PRISMTM.

We claim:

1. A polypeptide comprising an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS: 1-84, 138-146, and 167-184, wherein X1
5 is absent or is an amino acid linker, and wherein residues in parentheses are optional and may be present or some or all of the optional residues may be absent.
2. The polypeptide of claim 1, comprising the amino acid sequence selected from the group consisting of SEQ ID NOS:1-12 and 142-151.
10
3. The polypeptide of claim 1, comprising the amino acid sequence selected from the group consisting of SEQ ID NOS: 1-8.
4. The polypeptide of claim 1, comprising the amino acid sequence selected from
15 the group consisting of SEQ ID NOS: 1-4
5. The polypeptide of claim 1, comprising the amino acid sequence selected from the group consisting of SEQ ID NOS:5-8.
- 20 6. The polypeptide of claim 1, comprising the amino acid sequence selected from the group consisting of SEQ ID NOS:1 and 5.
7. The polypeptide of claim 1, comprising the amino acid sequence of SEQ ID
NO:1.
25
8. The polypeptide of claim 1, comprising the amino acid sequence of SEQ ID
NO:5.
9. A nanoparticle comprising a plurality of polypeptides according to any one of
30 claims 1-8.
10. A nanoparticle, comprising:

- (a) a plurality of first assemblies, each first assembly comprising a plurality of identical first proteins; and,
- (b) a plurality of second assemblies, each second assembly comprising a plurality of second proteins;
- 5 wherein the amino acid sequence of the first protein differs from the sequence of the second protein;
- wherein the plurality of first assemblies non-covalently interact with the plurality of second assemblies to form the nanoparticle; and
- wherein the nanoparticle displays on its surface an immunogenic portion of a SARS-
- 10 CoV-2 antigen or a variant or homolog thereof, present in the at least one second protein.

11. The nanoparticle of claim 10, wherein the second proteins comprise an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 100% identical to the amino acid sequence selected from the group consisting of SEQ ID
- 15 NOS:85-124 or 185-193, wherein X1 for at least one second protein comprises an immunogenic portion of a SARS-CoV-2 antigen or a variant or homolog thereof, X2 is absent or an amino acid linker, and residues in parentheses are optional.

12. The nanoparticle of claim 11, wherein the second proteins comprise the amino
- 20 acid sequence selected from the group consisting of SEQ ID NOS:85-88.

13. The nanoparticle of claim 11, wherein the second proteins comprise the amino acid sequence selected from the group consisting of SEQ ID NO:85-86.

- 25 14. The nanoparticle of claim 11, wherein the second proteins comprise the amino acid sequence of SEQ ID NO:85.

15. The nanoparticle of any one of claims 11-14, wherein X1 in at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the second proteins comprises an amino
- 30 acid sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid sequence identity to a Spike (S) protein extracellular domain (ECD) amino acid sequence, an S1 subunit amino acid sequence, an S2 subunit amino acid

sequence, an S1 receptor binding domain (RBD) amino acid sequence, and/or an N-terminal domain (NTD) amino acid sequence, from SARS-CoV-2, or a variant or homolog thereof.

16. The nanoparticle of any one of claims 11-15, wherein X1 in at least 20%,
 5 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the second proteins comprises an amino acid sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid sequence identity to the amino acid sequence selected from the group consisting of SEQ ID NO:125-137.

10 17. The nanoparticle of any one of claims 11-16, wherein X1 in at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the second proteins comprises an amino acid sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid sequence identity to the amino acid sequence of SEQ ID NO:125.

15

18. The nanoparticle of claim 17, wherein:

(a) X1 in at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the second proteins comprise mutations at 1, 2, 3, 4, 5, 6, 7, or all 8 positions relative to SEQ ID NO:125 selected from the group consisting of K90N, K90T, G119S, Y126F, T151I, E157K,
 20 E157A, S167P, N174Y, and L125R, including but not limited to mutations comprising one of the following naturally occurring mutations or combinations of mutations:

N174Y (UK variant);

K90N/E157K/N174Y (South African variant);

K90N or T/E157K/N174Y (Brazil variant); or

25 L125R (LA variant).; or

(b) X1 in at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the second proteins comprise mutations at 1, 2, 3, 4, 5, 6, 7, or all 8 positions relative to SEQ ID NO:130 selected from the group consisting of L18F, T20N, P26S, deletion of residues 69-70, D80A, D138Y, R190S, D215G, K417N, K417T, G446S, L452R, Y453F, T478I, E484K,
 30 S494P, N501Y, A570D, D614G, H655Y, P681H, A701V, T716L including but not limited to mutations comprising one of the following naturally occurring mutations or combinations of mutations:

N501Y, optionally further including 1, 2, 3, 4, or 5 of deletion of one or both of residues 69-70, A570D, D614G, P681H, and/or T716L (UK variant);

K417N/E484K/N501Y, optionally further including 1, 2, 3, 4, or 5 of L18F, D80A, D215G, D614G, and/or A701V (South African variant);

- 5 K417N or T/E484K/N501Y, optionally further including 1, 2, 3, 4, or 5 of L18F, T20N, P26S, D138Y, R190S, D614G, and/or H655Y (Brazil variant); or
L452R (LA variant).

19. The nanoparticle of claim 17, wherein X1 in at least 20%, 30%, 40%, 50%,
10 60%, 70%, 80%, 90%, or 100% of the second proteins comprise 1, 2, 3, or all 4 mutations relative to SEQ ID NO:125 selected from the group consisting of K90N, K90T, E157K, and N174Y.

20. The nanoparticle of any one of claims 11-19, wherein X1 in at least 20%,
15 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the second proteins comprises the amino acid sequence of SEQ ID NO:125.

21. The nanoparticle of any one of claims 11-20, wherein X1 in 100% of the second proteins comprises the amino acid sequence of SEQ ID NO:125, and all second
20 proteins are identical.

22. The nanoparticle of any one of claims 10-21, wherein the plurality of second assemblies in total comprise 2, 3, 4, 5, 6, 7, 8, or more different SARS-CoV-2 antigens.

23. The nanoparticle of any one of claims 10-22, wherein the plurality of second assemblies in total comprise 2, 3, 4, 5, 6, 7, 8, or more polypeptides comprising the amino acid sequence of the polypeptide of any one of claims 1-8.

24. The nanoparticle of any one of claims 10-23, wherein all second assemblies
30 comprise at least one second protein comprising the amino acid sequence of the polypeptide of any one of claims 1-8.

25. The nanoparticle of any one of claims 10-24, wherein all second proteins comprise the amino acid sequence of the polypeptide of any one of claims 1-8.

26. The nanoparticle of any one of claims 10-25, wherein the first protein
5 comprises an amino acid sequence at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected the group consisting of SEQ ID NOS:152-159, wherein residues in parentheses are optional and may be present or some or all of the optional residues may be absent.

10

27. The nanoparticle of any one of claims 10-26, wherein the first protein comprises an amino acid sequence at least at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected the group consisting of SEQ ID NOS:152-159.

15

28. The nanoparticle of any one of claims 10-27, wherein the first protein comprises the amino acid sequence of SEQ ID NO:155.

29. The nanoparticle of claim 28, wherein the at least one second assembly
20 comprises at least one second protein comprising the amino acid sequence selected from the group consisting of SEQ ID NO:85-88.

30. The nanoparticle of claim 28, wherein all second assemblies comprise at least one second protein comprising the amino acid sequence selected from the group consisting of
25 SEQ ID NO:85-88.

31. The nanoparticle of claim 28, wherein all second proteins comprise the amino acid sequence selected from the group consisting of SEQ ID NO:85-88.

32. The nanoparticle of any one of claims 10-31, wherein each first assembly is pentameric and each second assembly is trimeric.

33. The nanoparticle of any one of claims 10-32, wherein:

(a) the first protein comprises the amino acid sequence of SEQ ID NO:155;

(b) all second proteins comprise the amino acid sequence of SEQ ID NO:85, wherein X1 in at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the second proteins comprise an amino acid sequence at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid sequence identity to the amino acid sequence of SEQ ID NO:125.

34. The nanoparticle of any one of claims 10-33, wherein:
 (a) the first protein comprises the amino acid sequence of SEQ ID NO:155;
 (b) all second proteins comprise the amino acid sequence of SEQ ID NO:85,
 wherein X1 in at least 50%, 60%, 70%, 80%, 90%, or 100% of the second proteins comprise an amino acid sequence at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid sequence identity to the amino acid sequence of SEQ ID NO:125.

35. The nanoparticle of any one of claims 10-34, wherein:
 (a) the first protein comprises the amino acid sequence of SEQ ID NO:155;
 (b) all second proteins comprise the amino acid sequence selected from the group consisting of SEQ ID NO:1-8.

36. The nanoparticle of any one of claims 10-35, wherein:
 (a) the first protein comprises the amino acid sequence of SEQ ID NO:155;
 (b) all second proteins comprise the amino acid sequence of SEQ ID NO:1 or 5.

37. A composition, comprising a plurality of nanoparticles of any one of claims 10-36, preferably comprising a plurality of the nanoparticles of any one of claims 33-36.

38. A nucleic acid molecule encoding the polypeptide of any one of claims 1-8, preferably encoding the amino acid sequence of SEQ ID NO:1-12.

39. The nucleic acid molecule of claim 42, wherein the polynucleotide comprises an mRNA.

40. An expression vector comprising the nucleic acid molecule of claim 38 or 39 operatively linked to a suitable control sequence.
41. A cell comprising the polypeptide, the nanoparticle, the composition, the
5 nucleic acid, and/or the expression vector of any preceding claim.
42. A pharmaceutical composition comprising
- (a) the polypeptide, the nanoparticle, the composition, the nucleic acid, the expression vector, and/or the cell of any preceding claim; and
- 10 (b) a pharmaceutically acceptable carrier.
43. The pharmaceutical composition of claim 46, comprising a plurality of the nanoparticles of any one of claims 33-36.
44. The composition or the pharmaceutical composition of any preceding claim,
15 further comprising an adjuvant.
45. A vaccine comprising the polypeptide, the nanoparticle, the composition, the nucleic acid, and/or the composition of any preceding claim.
- 20 46. The vaccine of claim 45, comprising a plurality of the nanoparticles of any one of claims 33-36.
47. A method to treat or limit development of a SARS-CoV-2 infection,
25 comprising administering to a subject in need thereof an amount effective to treat or limit development of the infection the polypeptide, nanoparticle, composition, nucleic acid, pharmaceutical composition, or vaccine of any preceding claim.
48. The method of claim 47, comprising administering to the subject a plurality of
30 the nanoparticles of any one of claims 33-36, the pharmaceutical composition of claim 43, or the vaccine of claim 46.

49. The method of claim 47 or 48, wherein the subject is not infected with SARS-CoV-2, wherein the administering elicits an immune response against SARS-CoV-2 in the subject that limits development of a SARS-CoV-2 infection in the subject.

5 50. The method of claim 49, wherein the administering comprises administering a first dose and a second dose, wherein the second dose is administered about 2 weeks to about 12 weeks, or about 4 weeks to about 12 weeks the first dose is administered.

51. The method of claim 50, wherein the administering comprises
10 (a) administering a prime dose to the subject of a DNA, mRNA, or adenoviral vector vaccine, wherein the DNA, mRNA, or adenoviral vector vaccine encodes an amino acid sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid sequence identity to the amino acid sequence of SEQ ID NO:125-137; and
15 (b) administering a boost dose to the subject of the polypeptide, nanoparticle, composition, nucleic acid, pharmaceutical composition, or vaccine of any preceding claim.

52. The method of claim 50, wherein the administering comprises
(a) administering a prime dose to the subject of the polypeptide, nanoparticle,
20 composition, nucleic acid, pharmaceutical composition, or vaccine of any preceding claim ;
and
(b) administering a boost dose to the subject of a DNA, mRNA, or adenoviral vector vaccine, wherein the DNA, mRNA, or adenoviral vector vaccine encodes an amino acid sequence having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,
25 98%, 99%, or 100% amino acid sequence identity to the amino acid sequence of SEQ ID NO:125-137.

53. The method of any one of claims 47-52, wherein the immune response comprises generation of neutralizing antibodies against SARS-CoV-2.

30 54. The method of any one of claims 47-53, wherein the immune response comprises generation of SARS-CoV-2 spike protein antibody-specific responses with a mean geometric titer of at least 1×10^5 .

55. The method of any one of claims 47-48 or 53-54, wherein the subject is infected with a severe acute respiratory (SARS) virus, including but not limited to SARS-CoV-2, wherein the administering elicits an immune response against the SARS virus in the
5 subject that treats a SARS virus infection in the subject.

56. A kit, comprising:

- (a) the polypeptide of any one of claims 1-8, preferably wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:1 or 5; and
10 (b) a first protein comprising an amino acid sequence at least at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected the group consisting of SEQ ID NOS:152-159, wherein residues in parentheses are optional and may be present or absent, preferably wherein the first protein comprises the amino acid sequence of SEQ ID NO:155.

15

57. A kit, comprising:

- (a) a nucleic acid encoding the polypeptide of any one of claims 1-8, preferably wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:1 or 5; and
(b) a nucleic acid encoding first protein comprising an amino acid
20 sequence at least at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected the group consisting of SEQ ID NOS:152-159, wherein residues in parentheses are optional and may be present or absent, preferably wherein the first protein comprises the amino acid sequence of SEQ ID NO:155.

25

58. A kit, comprising:

- (a) an expression vector comprising a nucleic acid encoding the polypeptide of any one of claims 1-8 operatively linked to a suitable control sequence, preferably wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:1 or 5;
30 and
(b) an expression vector comprising a nucleic acid encoding first protein comprising an amino acid sequence at least at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected the

group consisting of SEQ ID NOS:152-159, wherein residues in parentheses are optional and may be present or absent, wherein the nucleic acid is operatively linked to a suitable control sequence, preferably wherein the first protein comprises the amino acid sequence of SEQ ID NO:155.

5

59. A kit, comprising:

- (a) a cell comprising an expression vector, wherein the expression vector comprises a nucleic acid encoding the polypeptide of any one of claims 1-8 operatively linked to a suitable control sequence, preferably wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:1 or 5; and
- (b) a cell comprising an expression vector, wherein the expression vector comprises a nucleic acid encoding first protein comprising an amino acid sequence at least at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected the group consisting of SEQ ID NOS:152-159, wherein residues in parentheses are optional and may be present or absent, wherein the nucleic acid is operatively linked to a suitable control sequence, preferably wherein the first protein comprises the amino acid sequence of SEQ ID NO:155.

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