IMPROVED CORONAVIRUS VACCINE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to and benefit of U.S. Provisional Patent Application No. 63/173,752, filed on April 12, 2021, and to U.S. Provisional Patent Application No. 63/63/190,199 filed on May 18, 2021, the contents of which are hereby incorporated by reference in their entirety.

BACKGROUND OF THE PRESENT DISCLOSURE

[0002] Covid-19 outbreak has caused over three millions of deaths worldwide since the first case reported in December, 2019. Currently several COVID-19 vaccines against SARS-CoV-2 infection are being developed in clinical trial stage, and some vaccines have been authorized for human usage. Although a number of approved vaccines demonstrated high efficacy, genetic variants of SARS-CoV-2 have been emerging and circulating around the world throughout the COVID-19 pandemic.

[0003] Therefore, there is an urgent need for improved COVID-19 vaccines for better prevention of the emerging coronavirus infections and/or reducing the severity of life-threatening coronavirus infections.

SUMMARY OF THE PRESENT DISCLOSURE

[0004] The spike protein of SARS-CoV-2 is extensively glycosylated. The present disclosure stems from the recognition that immunization with a modified SARS-CoV-2 spike protein lacking glycan shields or being less shielded by glycans elicited an enhanced immune response against SARS-CoV-2 and the variants of concern (e.g. alpha, gamma, delta and omicron), as compared to a native spike protein of SARS-CoV-2 or a variant thereof. 12 highly conserved epitopes (SEQ ID: 1-12) located in the receptor-binding domain (RBD) and the subunit 2 (S2) including the heptad repeat 2 (HR2) domain were identified based on the alignment of more than 6 million S protein sequences from GISAID. Removal of the glycan shields by N-glycan trimming to better expose these highly conserved epitopes offers an effective approach to developing broadly protective vaccines against SARS-CoV-2 and variants. N-glycan trimming of spike protein can be achieved by in vitro glycoengineering. The glycoengineered spike protein

thereby exposes the highly conserved epitopes shielded by glycans and at the same time preserves the tertiary structure of the spike protein. The present disclosure therefore provides improved immunogens, vaccines, and methods for better prevention and treatment of the emerging coronavirus (*e.g.* SARS-CoV-2) infections.

[0005] Accordingly, the present disclosure provides an immunogen comprising a glycoengineered coronavirus spike protein comprising a plurality of truncated N-glycans and unmodified O-glycans (e.g. O-linked oligosaccharides). In some embodiments, the plurality of truncated N-glycans are located in the receptor-binding domain (RBD), thereby exposing a plurality of highly conserved epitopes having amino acid sequences of TESIVRFPNITNL (SEQ ID NO.: 41), NITNLCPFGEVFNATR (SEQ ID NO: 42), LYNSASFSTFK (SEQ ID NO: 43), LDSKVGGNYN (SEQ ID NO: 44), KSNLKPFERDIST (SEQ ID NO: 45), KPFERDISTEIYQAG (SEQ ID NO: 46) and/or GPKKSTNLVKNKC (SEQ ID NO: 47). In some embodiments, the plurality of truncated N-glycans are located in the heptad repeat 2 (HR2) domain, thereby exposing a plurality of highly conserved epitopes having amino acid sequences of NCDVVIGIVNNTVY (SEQ ID NO: 48), PELDSFKEELDKYFKNHTS (SEQ ID NO: 49), VNIQKEIDRLNEVA (SEQ ID NO: 50), NLNESLIDLQ (SEQ ID NO: 51) and/or LGKYEQYIKWP (SEQ ID NO: 52).

[0006] In some embodiments, the plurality of truncated N-glycans are located in the receptor-binding domain (RBD) and in the heptad repeat 2 (HR2) domain, thereby exposing a plurality of highly conserved epitopes having amino acid sequences of TESIVRFPNITNL (SEQ ID NO.: 41), NITNLCPFGEVFNATR (SEQ ID NO: 42), LYNSASFSTFK (SEQ ID NO: 43), LDSKVGGNYN (SEQ ID NO: 44), KSNLKPFERDIST (SEQ ID NO: 45), KPFERDISTEIYQAG (SEQ ID NO: 46), GPKKSTNLVKNKC (SEQ ID NO: 47), NCDVVIGIVNNTVY (SEQ ID NO: 48), PELDSFKEELDKYFKNHTS (SEQ ID NO: 49), VNIQKEIDRLNEVA (SEQ ID NO: 50), NLNESLIDLQ (SEQ ID NO: 51) and/or LGKYEQYIKWP (SEQ ID NO: 52).

[0007] The glycoengineered coronavirus spike protein described herein comprises the amino acid sequence of SEQ ID NO: 1 or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 1, or an immunologically active fragment of the amino acid sequence or the variant.

[0008] In some embodiments, the glycoengineered coronavirus spike protein comprises a polypeptide consisting of an amino acid sequence of SEQ ID NO: 1, wherein the polypeptide consists of 22 truncated N-glycans, each having a GlcNAc moiety.

[0009] In some embodiments, the glycoengineered coronavirus spike protein comprises a polypeptide consisting of an amino acid sequence of SEQ ID NO: 2, wherein the polypeptide consists of 21 truncated N-glycans, each having a GlcNAc moiety.

[0010] In some embodiments, the truncated N-glycans are monosaccharides, disaccharides or trisaccharides. In some embodiments, the truncated N-glycans are monosaccharides. In preferred embodiments, the monosaccharides are N-acetylglucosamines (GlcNAc).

[0011] In preferred embodiments, the truncated N-glycans described herein are substantially homogeneous. The term "homogeneous" is intended to mean a glycosylation pattern represented by one desired glycan species. The terms "substantially homogeneous" used herein is intended to mean that at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99% of the glycoprotein present in the composition is represented by one desired glycoform (e.g., GlcNAc-decorated) with a trace amount of undesired glycoforms being present in the composition. By "trace amount" is intended that any given undesired glycoform that is present in the glycoprotein composition is present at less than 5%, preferably less than 4%, less than 3%, less than 2%, less than 1%, and even less than 0.5% or even less than 0.1% of the total glycoprotein.

[0012] As described herein, the terms "spike protein" and "spike glycoprotein" and "coronavirus spike protein" are used interchangeable. The glycoengineered spike protein described herein can be generated from a native coronavirus spike protein by glycoengineering (e.g., glycoengineering *in vitro* or *in vivo*). In some embodiments, the glycoengineered spike protein is generated using one or more of chemical or enzymatic methods. In some embodiments, the glycoengineered spike protein is generated using endoglycosidase H (Endo H).

[0013] In some embodiments, the native coronavirus spike protein described herein is the spike protein of severe acute respiratory syndrome coronavirus 2 (SAR-CoV-2) or variants thereof. SARS-CoV-2 described herein is the Wuhan strain of SARS-CoV-2 (hCoV/Wuhan/WH01/2019). The variants of SARS-CoV-2 described herein include, but are not limited to, D614G, Alpha (B.1.1.7 and Q lineages), Beta (B.1.351 and descendent lineages),

Gamma (P.1 and descendent lineages), Epsilon (B.1.427 and B.1.429), Eta (B.1.525), Iota (B.1.526), Kappa (B.1.617.1), 1.617.3, Mu (B.1.621, B.1.621.1), Zeta (P.2), Delta (B.1.617.2 and AY lineages) and Omicron (B.1.1.529 and BA lineages). In some embodiments, the native coronavirus spike protein is the spike protein of bat coronavirus RaTG13 or variants thereof.

[0014] As described herein, the term "native coronavirus spike protein", "native coronavirus spike glycoprotein", "native spike glycoprotein" and "native spike protein" is interchangeable.

[0015] In some embodiments, the glycoengineered spike protein described herein is present as a trimer (e.g., a trimer in solution). The glycoengineered spike protein described herein may retain the same tertiary structure as its native coronavirus spike protein.

[0016] As described herein, the glycoengineered spike protein is capable of inducing an enhanced immune response relative to its native coronavirus spike protein. The enhanced immune response is an increased IgG titer, an increased IgM titer, an increased CD4 T cell response, an increased CD8 T cell response, an increased neutralization titer, or a combination thereof.

[0017] In another aspect, the present invention provides an immunogenic composition, comprising: (a) the immunogen of the disclosure, and (b) optionally, an adjuvant.

[0018] As described herein, the adjuvant may include, but is not limited to, aluminum hydroxide, aluminum phosphate, incomplete Freund's adjuvant (IFA), squalene, Alum, Alhydrogel, MF59, QS-21, CpG 1018, AS03, AS37, Matrix-M or a combination thereof.

[0019] The coronavirus described herein may include SARS-CoV-2 and its variants, and may include bat coronavirus RaTG13 or its variants. In preferred embodiments, the coronavirus infection is caused by SARS-CoV-2 and its variants.

[0020] As described herein, the immunogenic composition is capable of eliciting an enhanced immune response relative to a vaccine using its native SAR-CoV-2 spike protein, thereby serving as an improved COVID-19 vaccine against coronavirus infections caused by SAR-CoV-2 or a variant thereof.

[0021] In another aspect, the present invention provides a method for eliciting an immune response against SAR-CoV-2 or variants in a subject in need thereof, comprising administering to the subject an effective amount of an immunogenic composition of the present invention.

[0022] In another aspect, the present invention provides a method for protecting a subject in need thereof from infection with SAR-CoV-2 or variants, comprising administering to the subject an effective amount of the immunogenic composition of the present invention.

[0023] In another aspect, the present invention provides a method for preventing a subject in need thereof from contracting COVID-19 disease, comprising administering to the subject an effective amount of the immunogenic composition of the present invention.

[0024] In another aspect, the present invention provides use of the immunogenic composition of the present invention for eliciting an immune response against SARS-CoV-2 in a subject in need thereof.

[0025] In another aspect, the present invention provides use of the immunogenic composition of the present invention for protecting a subject in need thereof from infection with SARS-CoV-2 .

[0026] In another aspect, the present invention provides use of the immunogenic composition of the present invention for preventing a subject in need thereof from contracting COVID-19 disease.

[0027] These and other aspects will become apparent from the following description of the preferred embodiment taken in conjunction with the following drawings, although variations and modifications therein may be affected without departing from the spirit and scope of the novel concepts of the disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure, the inventions of which can be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0029] **Figures 1 (a) to (f). Design and characterization of glycoengineered spike protein with mono-GlcNAc-decorated (S_{MG}) protein vaccine.** (a) Schematic representation of the recombinant SARS-CoV-2 Spike glycoprotein construct. Protein domains are illustrated as N-terminal domain (NTD), receptor binding domain (RBD), fusion peptide (FP), heptad repeat 1

(HR1), heptad repeat 2 (HR2), central helix (CH), and connector domain (CD). The C-terminus of soluble Spike protein is linked with fold on sequence and His-tag (His_6). The furin cleavage site was substituted by GSAG residues and the two proline mutations (K986P, and V987P) fix the Spike protein in the prefusion state. The positions of N-linked glycosylation sequons (N-X-S/T, where X ≠ P) are shown as branches (N, Asn; X, any residue; S, Ser; T, Thr; P, Pro). The pound site represents the thrombin cleavage site. (b) Schematic overview of mono-GlcNAc decorated Spike vaccine production. S_{HM}, Spike with high mannose type N-glycans; S_{MG}, Spike with GlcNAc at its N-glycosylation sites. (c) Size-exclusion chromatography of purified S_{MG}. The black curve represents the S_{MG} and the gray curve shows the protein molecular weight markers. (d) SDS-PAGE analysis of S_{FG} (Spike with typical complex type N-glycans), S_{HM} and S_{MG}. (e) Structure model of S_{MG}. Models were created with Protein Data Bank (PDB) ID code 7CN9 by adding glycan using Wincootmand displayed with program ChimeraX. (f) Mass Spectrometry analysis of the N-glycan compositions of the S_{FG} and S_{MG}.

[0030] **Figures 2 (A) to (S).** S_{MG} vaccination provides enhanced protection against SARS-CoV-2 infection *in vivo*. (**A**) The immunization schedule for Syrian hamsters is shown. S_{FG} (blue), S_{MG} (red), and control (gray). (**B**) Weight change was measured in Syrian hamsters after WT SARS-CoV-2 challenge. (**C**) Lung virus titers of challenged hamsters are shown. The dashed line indicates the lower limit of detection. (**D**) Representative images shown histopathology, immunohistochemistry, and immunofluorescence of the lungs from an infected hamster (3 dpi). First row: Hematoxylin and eosin (H&E) staining; scale bar, 50 μm. Second row: Immunohistochemistry (IHC) staining; scale bar, 50 μm. Third row: Immunofluorescence (IF) staining; scale bar, 100 μm. SARS-CoV-2 N-specific polyclonal antibodies were used for virus detection as brown dots in IHC and green dots in IF staining. Blue: 4,6-diamidino-2-phenylindole (DAPI). (**E**) The immunization schedule for CAG-hACE2 or K18-hACE2 transgenic mice is shown. (**F** to **I**) Anti−S IgG titers (F), SARS-CoV-2 WT microneutralization titers (G), and subtype IgG analysis, including IgG1, IgG2c (H), and IgG2c:IgG1 ratio (I), are shown for serum samples collected from immunized CAG-hACE2 transgenic mice (n= 7). (**J**) Representative histopathology, immunohistochemistry, and immunofluorescence of the infected mouse lungs (7 dpi) are shown. Scale bars are the same as in (D). (**K**) Lung virus titers of the infected CAG-hACE2 mice (n = 3). The dashed line indicates the lower limit of detection. (**L** and **M**) Weight change (L) and survival

analysis (M) are shown for WT-SARS-CoV-2–challenged CAG-hACE2 transgenic mice (n = 4). (**N** and **O**) Weight change (N) and survival analysis (O) are shown for SARS-CoV-2 alpha variant–challenged CAG-hACE2 transgenic mice (n = 5). (**P** and **Q**) Weight change (P) and survival analysis (Q) are shown for SARS-CoV-2 gamma variant–challenged CAG-hACE2 transgenic mice (n= 5). (**R** and **S**) Weight change (R) and survival analysis (S) are shown for SARS-CoV-2 delta variant–challenged K18-hACE2 transgenic mice (n= 4). Data are shown as means ± SEM and analyzed by two-sided Mann-Whitney U tests to compare two experimental groups. ns, not significant; *P< 0.05.

[0031] Figures 3 (a) to (e). S_{MG} vaccination provides better protection against a lethal dose SARS-CoV-2 infection in transgenic hACE2 mice. (a) Transgenic mice immunization and challenge schedule. (b) Sera collected at 28 days and 42 days post initial vaccination were examined for anti-Spike specific IgG. (c) Sera collected at 42 days post initial vaccination were examined for neutralizing antibody titer by CPE assay (d) Transgenic mice weight change after challenge. (n = 3) (e) Transgenic mice survival rate after challenge. (n = 3). Data are means ± SEM (standard error of the mean). Comparisons were performed by Student's t-test (unpaired, two tailed). The binding and neutralization fold increases of the antibody elicited by S_{MG} over S_{FG} vaccines are indicated by numbers above the bars. Blue squares, transgenic mice immunized with S_{FG}; Red triangles, transgenic mice immunized with S_{MG}; Gray dots, Control group animals (Alum only group).

[0032] Figure 4 (a) to (f). Antibodies induced by S_{MG} vaccine in mice for enhanced binding and neutralization breadth and potency against SARS-CoV-2 variants. (a) Schematic representations of SARS-CoV-2 spike protein structure and the mutation landscape of variants used in this study are illustrated. RBD, receptor binding domain. In the mutation map, a dot (.) indicates the same amino acid in that position as wild type and a dash (–) indicates a deletion. (b) The SARS-CoV-2-specific IgG antibody titer was determined by ELISA. The binding fold increase of the antibody elicited by S_{MG} over S_{FG} vaccines are indicated by numbers above the bars. (c) SARS-CoV-2 variants pseudovirus neutralization curves are shown. (d) Titers that achieve 50% pseudovirus neutralization (pNT50) of SARS-CoV-2 variants are plotted. The neutralization fold increase of the antibody elicited by S_{MG} over S_{FG} vaccines are indicated by numbers above the bars. (e) Infectious SARS-CoV-2 variants neutralization curves determined

by plaque reduction neutralization test (PRNT) are shown. (f) Titers that achieve 50% neutralization (PRNT50) of SARS-CoV-2 variants are plotted. Data are means ± SEM (standard error of the mean). The curves were fit by nonlinear regression using GraphPrism 9.0 and the comparisons were performed by multiple t-test (paired, two tailed). The titers comparisons were performed by Student's t-test (unpaired, two tailed). The neutralization fold increase of the antibody elicited by S_{MG} over S_{FG} vaccines are indicated by numbers above the bars. Blue squares, transgenic mice immunized with S_{FG}; Red triangles, transgenic mice immunized with S_{MG}; Gray dots, Control group animals (PBS group).

[0033] Figures 5 (A) to (G). S protein glycosylation impacts ACE2 receptor binding and SARS-CoV-2 infection. (**A** to **C**) Binding avidity of ACE2 was measured for differently glycosylated S protein ectodomains (S_{FG}; original fully glycosylated, blue; deS, nonsialylated, light blue; S_{HM}, high-mannose, yellow; and S_{MG}, mono-GlcNAc-decorated, red) from BEAS-2B (A), HEK293T (B), and HEK293S (GnTI^{-}) cells without or with Endo H digestion (C). Data of three technical replicates are shown as means ± SD, and curves fit by nonlinear regression for EC_{50} values. (**D**) Viral infectivity was measured for pseudoviruses carrying differently glycosylated S protein with the same input amount (0.3 μg/ml of p24 equivalent) colored accordingly as in (C). RLU, relative luminescence unit. Data of six technical replicates shown as means ± SD and analyzed with ordinary one-way ANOVA test followed by Tukey's multiple comparisons test. ns, not significant; ****$P<$ 0.0001. (**E**) A schematic view of SARS-CoV-2 S protein [wild type (WT)] is shown colored by domain, including N-terminal domain (NTD; 14–306; orange), receptor binding domain (RBD; 319–541; red), two subdomains (SD1/2; 542–685; yellow), fusion peptide proximal region (FPPR; 816–856; green), heptad repeat 1 (HR1; 912–984; teal), connecting domain (CD; 1063–1162; blue), heptad repeat 2 (HR2; 1163–1211; purple), and transmembrane domain (TM; 1214–1234; white). N-glycan (drawn as branches) and *O*-glycan (circles) sites are marked with residue number. S1 and S2 domains are shown above. (**F**) Viral titers are shown for pseudoviruses carrying WT S protein or mutants with glycans removed at each shown glycosite, normalized by p24 quantification, and colored accordingly as in (E). (**G**) Infectivity of the same panel of pseudoviruses as in (F) tested in five hACE2-expressing cell lines. Values in (F) and (G) are normalized against WT values (defined as 100%, colored in dark gray) with means ± SD of three independent experiments.

[0034] **Figures 6 (A) to (E).** S protein glycan profiles demonstrate differences in two cell lines and correlate with sequence conservation. (**A** and **B**) A comparison of the N-glycosylation profile of recombinant S protein expressed from BEAS-2B lung epithelial cells (A) and HEK293T kidney epithelial cells (B) is shown. Glycans are grouped and colored accordingly: complex-S (sialylated complex type; dark blue), complex (non–sialylated complex type; light blue), hybrid-S (sialylated hybrid type; dark yellow), and hybrid (nonsialylated hybrid type; light yellow), high mannose (green), and unoccupied (gray). The percentage of each group is shown for each glycosite in a pie chart, and the proportion of each glycoform (nos. 1 to 27) in a bar chart. The bar graphs represent the means ± SD of three biological replicates. Fhybrid indicates fucosylated hybrid-type glycans. (**C**) Glycan profiles from (A) and (B) were mapped on the 3D structure of S ectodomain (modeled from 6VSB). Glycans are colored by the highest-abundance group for BEAS-2B (left) or HEK293T (right) data as labeled (complex type, blue; hybrid type, yellow; and high mannose, green). Non–complex-type N-glycosites are labeled with residue number. (**D**) Mapping of relative surface accessibility (RSA) on modeled S structure protein is shown, with buried residues colored in dark yellow, glycan shielded in yellow, and exposed in light yellow. (**E**) Mapping of sequence variation on modeled S protein structure is shown, colored in a heatmap, with darker red indicating higher mutation rates. Several highly conserved glycan-shielded regions are highlighted. More details for (D) and (E) can be found in fig. S9 and data file S1.

[0035] **Figures 7 (A) to (S).** S_{MG} vaccination elicits stronger humoral and cellular immune responses than S_{FG} in BALB/c mice. (**A**) Structural models of S_{FG} and S_{MG} protein vaccine are shown (according to Fig. 2C). Blue: glycans; gray: protein. S_{FG} was expressed by HEK293E without further modification. S_{MG} was obtained by enzymatic digestion to truncate all N-glycans of S_{HM} expressed by HEK293S GnTI^{-} to single GlcNAc, whereas *O*-glycans were unmodified. (**B**) Immunization schedule using proteins as in (A) as immunogens in BALB/c mice (n = 5 in each experiment). S_{FG} (blue), S_{HM} (yellow), S_{MG} (red), and control (gray). Alum, aluminum hydroxide. (**C**) Anti–S protein IgG titers of serum samples were analyzed by ELISA. (**D**) Neutralization titers of serum samples were measured using pseudovirus with WT S protein. (**E** to **G**) IgG subtype analysis of sera, including IgG1 (E), IgG2a (F), and the IgG2a:IgG1 ratio (G). (**H** to **K**) The percentage of Tfh in activated

nonregulatory CD4 T cells (H) and the percentages of IFN-γ (I)–, IL-4 (J)–, and IL-21 (K)–expressing Tfh cells ($CD4^+CD19^-CD44^{hi}Foxp3^-PD\text{-}1^+CXCR5^+$) in the lymph nodes (LNs) of BALB/c mice by flow cytometry. (**L**) The percentage of granzyme B–producing $CD8^+$ T cells ($CD3^+$ $B220^-CD8^+CD49b^-$) in the LN of BALB/c mice analyzed by flow cytometry. (**M**) The ratio of S protein–specific B cells ($CD3^-CD19^+$S protein$^+$) (percentage) normalized to fluorescence minus one (FMO) control staining (stained without S protein) (percentage) in the spleen is shown. (**N**) Kappa and lambda light chain usage is shown. (**O** and **P**) Heavy (O) and kappa (P) chain distribution of B cell repertoire analysis. Less than 5% usage is shown in white. (**Q** to **S**) Anti–S protein IgG titers (Q), pseudovirus neutralization titers (R), and authentic virus neutralization titers (S) are shown for serum isolated from BALB/c mice after three doses of indicated vaccines against SARS-CoV-2 WT (or D614G) and variants (number above each bar indicate fold of increase of S_{MG} compared to S_{FG} group). pNT_{50} represents the reciprocal dilution achieving 50% neutralization. The dotted line in bar charts represents the lower limit of detection. Data are shown as means ± SEM and analyzed by two-sided Mann-Whitney *U* test to compare two experimental groups, except in (N), where five samples were pooled together and a chi-squared test was used. *P* values shown above each bar. $*P < 0.05$; $**P < 0.01$.

[0036] **Figures 8 (A) to (L).** Functional, prophylactic, and structural characterization of antibody m31A7 elicited by S_{MG} vaccination indicates cross-neutralizing capacity. (**A**) ELISA binding of m31A7 to S1, S2, RBD, or the entire S ectodomain. (**B**) Flow cytometry analysis of m31A7 binding to HEK293T cells expressing S protein of SARS-CoV-2 WT and variants. (**C**) Neutralization activity of m31A7 against pseudoviruses carrying WT or variant S proteins. Data of three technical replicates for (A), (B), and (C) are shown as means ± SD and curves fit by nonlinear regression for EC_{50} values. (**D**) Antibody injection and challenge schedule for K18-hACE2 transgenic mice (n= 3) is shown. (**E** and **F**) Weight change (E) and body temperature change (F) are shown for mice treated with m31A7 or PBS. Data are presented as means ± SEM. (**G**) Binding kinetics of m31A7 IgG and Fab to S protein are presented, with dissociation constants (K_d) shown above. (**H**) Epitope mapping by HDX-MS of m31A7 is shown in a time course revealing two peptide candidates, 419–433 and 471–482, with greater than 10% ΔHDX at 15 s. Data are shown as means ± SD and analyzed by multiple t tests at each time point. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$. (**I**)

The cryo-EM map fitted with m31A7-Fab/S protein complex structure is shown. Heavy chain, dark green; light chain, light green; RBD, red; NTD, orange; the rest of S1, light gray; S2, dark gray; and N-glycans, blue. (**J**) An enlarged view of RBD-m31A7 interface is shown. The star marks the vicinity between m31A7 light chain and N165-glycan. (**K**) Superimposition of previously reported mAbs S2E12 (magenta), COV253 (pink), and B1-182.1 (light blue) (PDB 7BEN, 7K4N, and 7MLZ) onto the m31A7-bound RBD (gray). The receptor binding motif and RBD tip are highlighted. (**L**) A footprint comparison of COV253 (pink) and m31A7 (green) on RBD (gray) shows similarity, with residues of VOCs labeled and drawn as red spheres.

[0037] Figures 9 (A) to (C). Delta S_{MG} vaccination elicited more neutralizing antibodies against SARS-CoV-2 variant pseudovirus. **(A)** Scheme of the vaccination. BALB/c mice (n=5) were immunized twice at week 0 and week 2 with Delta S_{FG} or S_{MG} vaccine. Sera were collected at week 6 and subsequently tested the neutralization ability using pseudovirus assay. **(B)** The 50 percent reciprocal neutralization titer (pNT50) of the sera against SARS-CoV-2 variant pseudovirus including wildtype (WH01) and variants of concern (VOCs) including Alpha, Beta, Gamma, Delta, and Omicron. The data were shown as mean (indicated above each bar) ± SEM and analyzed by two-sided Mann–Whitney *U*-test to compare two experimental groups. $^*P < 0.05$. **(C)** The inhibition (%) of the SARS-CoV-2 variant pseudovirus cell infection provided by differently diluted serum. The data were shown as mean ± SEM and the curves were fitted by nonlinear regression using Graph Prism 9.0.

[0038] Figures 10 (A) and (B). Delta or WT S_{MG} vaccination elicited more neutralizing antibodies against SARS-CoV-2 Omicron variant pseudovirus. **(A)** Scheme of the vaccination. BALB/c mice (n=5) were immunized twice at week 0 and week 2 with Delta/WT S_{FG} or S_{MG} vaccine. Sera were collected at week 6 and subsequently tested the neutralization ability using pseudovirus assay. **(B)** The inhibition (%) of the SARS-CoV-2 variant pseudovirus cell infection provided by differently diluted serum. The data were shown as mean ± SEM and the curves were fitted by nonlinear regression using Graph Prism 9.0.

[0039] Figures 11 (A) to (C). Delta S_{MG} protein can be stored at room temperature in solution or in powder. **(A and B)** Delta S_{MG} protein in phosphate-buffered saline additive with two amino acids (pbs-aa), 50 mM L-Arginine and 50 mM L-Glutamate, was filtered with 0.22

μm filter and stored at room temperature (RT) or 4°C. The proteins were collected at different time points including 3, 7, 14, 21 days and 3 months. The collected samples were mixed with 5X SDS-PAGE Loading Dye, heated at 100°C for 5 min and stored in the 4°C until gel running. **(C)** Delta S_{MG} protein storage test in different buffers and lyophilization conditions. The PBS with or without 50 mM L-Arginine and 50 mM L-Glutamate was tested for storing delta S_{HM} or S_{MG} (first 3 lanes) at 4°C. The delta S_{MG} was tested lyophilization with or without excipient and store at RT for over 2 weeks (last 2 lanes).

[0040] **Figure 12** shows a schematic representation of highly conserved epitopes (E1, E2, E3, E4, E5, E6 and E7) present in the receptor-binding domain (RBD) of a SARS-CoV-2 spike protein containing N-terminal domain (NTD), a receptor-binding domain (RBD), a fusion peptide (FP), heptapeptide repeat sequence 1 (HR1), heptapeptide repeat sequence 2 (HR2), transmembrane domain (TM), cytoplasm domain (CD), and S2 subunit.

[0041] **Figure 13** shows a schematic representation of highly conserved epitopes (E8, E9, E10, E11, E12) present in the heptapeptide repeat sequence 2 (HR2) of a SARS-CoV-2 spike protein.

DETAILED DESCRIPTION OF THE INVENTION

[0042] In the following detailed description of embodiments of the present disclosure, reference is made to the accompanying drawings in which like references indicate similar elements, and in which is shown by way of illustration specific embodiments in which the present disclosure may be practiced. These embodiments are described in sufficient detail to enable those skilled in the art to practice the present disclosure, and it is to be understood that other embodiments may be utilized and that logical, structural, functional, and other changes may be made without departing from the scope of the present disclosure. The following detailed description is, therefore, not to be taken in a limiting sense.

[0043] All technical and scientific terms used herein, unless otherwise defined below, are intended to have the same meaning as commonly understood by one of ordinary skill in the art. References to techniques employed herein are intended to refer to the techniques as commonly understood in the art, including variations on those techniques or substitutions of equivalent or later-developed techniques which would be apparent to one of skill in the art. In addition, in

order to more clearly and concisely describe the subject matter which is the invention, the following definitions are provided for certain terms which are used in the specification and appended claims.

[0044] As used herein, and in the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a protein" can refer to one protein or to mixtures of such protein, and reference to "the method" includes reference to equivalent steps and/or methods known to those skilled in the art, and so forth.

[0045] As used herein, the term "mutation" refers to a single change in a virus's genome (genetic code). Mutations happen frequently, but only sometimes change the characteristics of the virus.

[0046] As used herein, the term "lineage" refers to a group of closely related viruses with a common ancestor. SARS-CoV-2 has many lineages; all cause COVID-19.

[0047] As used herein, the term "variant" refers to a viral genome (genetic code) that may contain one or more mutations. In some cases, a group of variants with similar genetic changes, such as a lineage or group of lineages, may be designated by public health organizations as a Variant of Concern (VOC) or a Variant of Interest (VOI) due to shared attributes and characteristics that may require public health action.

[0048] As used herein, the term "adjuvant" refers to a compound that, when used in combination with an immunogen, augments or otherwise alters or modifies the immune response induced against the immunogen. Modification of the immune response may include intensification or broadening the specificity of either or both antibody and cellular immune responses.

[0049] As used herein, the term "about" or "approximately" when preceding a numerical value indicates the value plus or minus a range of 10%. For example, "about 100" encompasses 90 and 110.

[0050] As used herein, an "immunogenic composition" is a composition that comprises an antigen where administration of the composition to a subject results in the development in the subject of a humoral and/or a cellular immune response to the antigen.

[0051] As described herein, the terms "spike protein" and "spike glycoprotein" and "coronavirus spike protein" are used interchangeably.

[0052] The terms "substantially homogeneous" used herein is intended to mean that at least 80%, at least 85%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99% of the glycoprotein present in the composition is represented by one desired glycoform (e.g., mono-GlcNAc-decorated) with a trace amount of undesired glycoforms being present in the composition. By "trace amount" is intended that any given undesired glycoform that is present in the glycoprotein composition is present at less than 5%, preferably less than 4%, less than 3%, less than 2%, less than 1%, and even less than 0.5% or even less than 0.1% of the total glycoprotein.

[0053] The terms "treat," "treatment," and ""reating," as used herein, refer to an approach for obtaining beneficial or desired results, for example, clinical results. For the purposes of this disclosure, beneficial or desired results may include inhibiting or suppressing the initiation or progression of an infection or a disease; ameliorating, or reducing the development of, symptoms of an infection or disease; or a combination thereof.

[0054] The terms "preventing" and "prevention," as used herein, are used interchangeably with "prophylaxis" and can mean complete prevention of an infection, or prevention of the development of symptoms of that infection; a delay in the onset of an infection or its symptoms; or a decrease in the severity of a subsequently developed infection or its symptoms.

[0055] As used herein an "effective amount" refers to an amount of an immunogen sufficient to induce an immune response that reduces at least one symptom of pathogen infection. An effective dose or effective amount may be determined e.g., by measuring amounts of neutralizing secretory and/or serum antibodies, e.g., by plaque neutralization, complement fixation, enzyme-linked immunosorbent (ELISA), or microneutralization assay.

[0056] As used herein, the term "vaccine" refers to an immunogenic composition (with or without an adjuvant), such as an immunogen derived from a coronavirus, which is used to induce an immune response against the coronavirus that provides protective immunity (e.g., immunity that protects a subject against infection with the coronavirus and/or reduces the severity of the condition caused by infection with the coronavirus). The protective immune response may include formation of antibodies and/or a cell-mediated response. Depending on context, the term "vaccine" may also refer to a suspension or solution of an immunogen that is administered to a subject to produce protective immunity.

[0057] As used herein, the term "subject" includes humans and other animals. Typically, the subject is a human. For example, the subject may be an adult, a teenager, a child (2 years to 14 years of age), an infant (birth to 2 year), or a neonate (up to 2 months). In particular aspects, the subject is up to 4 months old, or up to 6 months old. In some aspects, the adults are seniors about 65 years or older, or about 60 years or older. In some aspects, the subject is a pregnant woman or a woman intending to become pregnant. In other aspects, subject is not a human; for example a non-human primate; for example, a baboon, a chimpanzee, a gorilla, or a macaque. In certain aspects, the subject may be a pet, such as a dog or cat.

[0058] As used herein, the term "pharmaceutically acceptable" means being approved by a regulatory agency of a U.S. Federal or a state government or listed in the U.S. Pharmacopeia, European Pharmacopeia or other generally recognized pharmacopeia for use in mammals, and more particularly in humans. These compositions can be useful as a vaccine and/or antigenic compositions for inducing a protective immune response in a vertebrate.

[0059] Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an enveloped, positive-sense, single-stranded RNA virus that causes coronavirus disease 2019 (COVID-19). Virus particles include the RNA genetic material and structural proteins needed for invasion of host cells. Once inside the cell the infecting RNA is used to encode structural proteins that make up virus particles, nonstructural proteins that direct virus assembly, transcription, replication and host control and accessory proteins whose function has not been determined. The structural proteins of SARS-CoV-2 include the envelope protein (E), spike or surface glycoprotein (S), membrane protein (M) and the nucleocapsid protein (N). The spike glycoprotein is found on the outside of the virus particle and gives coronavirus viruses their crown-like appearance. This glycoprotein mediates attachment of the virus particle and entry into the host cell.

[0060] S protein generated by lung epithelial cells has glycoforms associated with increased infectivity. Compared to the fully glycosylated S protein, immunization of S protein with N-glycans trimmed to the mono-GlcNAc decorated state (S_{MG}) elicited stronger immune responses and better protection for human angiotensin-converting enzyme 2 (hACE2) transgenic mice against variants of concern (VOCs). In addition, a broadly neutralizing monoclonal antibody was identified from S_{MG}-immunized mice that could neutralize wild-type SARS-CoV-2 and VOCs with subpicomolar potency.

[0061] A glycoengineered spike protein of the disclosure comprises a polypeptide having an amino acid sequence of SEQ ID NO: 1 or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 1, or an immunologically active fragment of the amino acid sequence or the variant. The amino acid sequence of SEQ ID NO: 1 is shown below.

SEQ ID NO: 1

QCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHV
SGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVV
IKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQG
NFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTL
LALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSE
TKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNR
KRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAP
GQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDIS
TEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCG
PKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLE
ILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGS
NVFQTRAGCLIGAEHVNNSYECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTM
SLGAENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLL
QYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSK
PSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDE
MIAQYTSALLAGTITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIAN
QFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILS
RLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKR
VDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFV
SNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELD
KYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQY

[0062] In one embodiment, the glycoengineered spike protein of the disclosure comprises a polypeptide having the amino acids as shown in SEQ ID NO: 2 below.

SEQ ID NO: 2

QCVNLRTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTK
RFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFL
DVYYHKNNKSWMESGVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKH
TPINLVRDLPQGFSALEPLVDLPIGINITRFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQ
PRTFLLKYNENGTITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNL
CPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADS

FVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYRYRLFRKSNLKP
FERDISTEIYQAGSKPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGP
KKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCS
FGGVSVITPGTNTSNQVAVLYQGVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEH
VNNSYECDIPIGAGICASYQTQTNSRGSAGSVASQSIIAYTMSLGAENSVAYSNNSIAIPTNFT
ISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFA
QVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAAR
DLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQMAYRFNGIGV
TQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQNVVNQNAQALNTLVKQLSSNFGAISS
VLNDILSRLDPPEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKR
VDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGTHWF
VTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLG
DISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQ

[0063] As described herein, a glycoengineered spike protein of SARS-CoV-2 or a variant thereof may include those comprising an amino acid sequence which (i) are substantially identical to the amino acid sequences set forth in SEQ ID NO: 1 (*e.g.*, at least 90%, 95% or 97% identical to SEQ ID NO: 1 such as SEQ ID NO:2) ; and (ii) are encoded by a nucleic acid sequence capable of hybridizing under at least moderately stringent conditions to any nucleic acid sequence encoding the spike protein set forth herein or capable of hybridizing under at least moderately stringent conditions to any nucleic acid sequence encoding the spike protein set forth herein, but for the use of synonymous codons (e.g. a codon which does not have the identical nucleotide sequence, but which encodes the identical amino acid).

[0064] The analysis of cell-specific glycoform distribution, sequence conservation, glycan shielding, and their mutual correlations led to the design of S_{MG} vaccine, in which essentially all glycan shields are removed. SARS-CoV-2 S protein glycosylation has major influence on virus infection, protein integrity, and immune responses. The S protein from lung epithelial cells contains more sialylated complex-type glycans to facilitate receptor binding, and glycosites N801 and N1194 were shown to be essential for S protein folding and viral infection. This made the conserved epitopes better exposed to the immune system so that more effective and broadly protective B cell and T cell responses could be elicited against the virus and variants.

[0065] The glycoengineered coronvirus spike protein of the present disclosure targets the entire S protein ectodomain, particularly the conserved domains shielded by glycans, stimulating the elicitation of both RBD and non–RBD-neutralizing antibodies and CD8 T cell responses that are critical for cross protection.

[0066] In certain aspects, the present disclosure provides a vaccine or a pharmaceutical composition comprising the immunogen as described herein. The present disclosure also provides a method for treating or preventing coronavirus infection in which the method comprises administering to a subject (e.g., a mammal) in need thereof an effective amount of an immunogen, a pharmaceutical composition, or a vaccine as described herein.

[0067] In one embodiment, the vaccine may include an adjuvant. Exemplary adjuvants include, but are not limited to, aluminum hydroxide, aluminum phosphate, incomplete Freund's adjuvant (IFA), squalene, Alum, Alhydrogel, MF59, QS-21, CpG 1018, AS03, AS37, Matrix-M or a combination thereof.

[0068] The vaccine or pharmaceutical composition may be formulated using any suitable method. Formulation of with standard pharmaceutically acceptable carriers and/or excipients may be carried out using routine methods in the pharmaceutical art. The exact nature of a formulation will depend upon several factors including the vaccine to be administered and the desired route of administration. Suitable types of formulation are fully described in Remington's Pharmaceutical Sciences, 19th Edition, Mack Publishing Company, Eastern Pennsylvania, USA.

[0069] The vaccine or pharmaceutical composition as described herein may be administered by any route. Such methods comprise application e.g. parenterally, such as through all routes of injection into or through the skin: e.g. intramuscular, intravenous, intraperitoneal, intradermal, mucosal, submucosal, or subcutaneous. Also, they may be applied by topical application as a drop, spray, gel or ointment to the mucosal epithelium of the eye, nose, mouth, anus, or vagina, or onto the epidermis of the outer skin at any part of the body. Other possible routes of application are by spray, aerosol, or powder application through inhalation via the respiratory tract. Alternatively, application can be via the alimentary route. The effective amount of the vaccine composition may be dependent on any number of variables, including without limitation, the species, breed, size, height, weight, age, overall health of the patient, the type of formulation, or the mode or manner or administration. The appropriate effective amount can be routinely

determined by those of skill in the art using routine optimization techniques and the skilled and informed judgment of the practitioner and other factors evident to those skilled in the art.

[0070] In one embodiment, The composition can comprises an additional therapeutic agent such as an anti-viral agent. The provided pharmaceutical composition is useful for treating a coronavirus infection. Examples of the additional anti-viral agent include, but are not limited to, ribavirin, penciclovir, nitazoxanide, nafamostat, chloroquine, remdesivir (GS-5734) and favipiravir (T-705), interferon, adefovir, tenofovir, acyclovir, brivudin, cidofovir, fomivirsen, foscarnet, ganciclovir, amantadine, rimantadine, zanamivir, remdesivir, molnupiravir and paxlovid.

[0071] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

EXAMPLES

EXAMPLE 1: Gene constructs

[0072] The SARS-CoV-2 spike sequences were designed based on the gene sequences downloaded from GISAID database. A total of 1,117,474 S protein sequences of all available SARS-CoV-2 strains were extracted from the GISAID (Global Initiative on Sharing Avian Influenza Data) database (version: 18 April 2021).

[0073] DNA sequences of spike protein from SARS-CoV-2 Wuhan/WH01/2019 and Delta variant were synthesized with codons optimized for human cell expression. The furine cleavage site were replaced with GSAG and the 2P substitutes were designed for protein to stay in prefusion state. The transmembrane domain was replaced with the thrombin cleavage site, foldon, and the histidine-tag at the C terminus of the spike. The modified HA sequence was cloned into pTT vector for protein expression and purification.

EXAMPLE 2: SARS-CoV-2 S protein expression and purification

[0074] The plasmid that encodes the secreted SARS-CoV-2 spike was transfected into the human embryonic kidney cell lines of either HEK293EBNA (ATCC number CRL-10852) or the HEK293S GnTI$^-$ cells by using transfection reagent (either polyethyleneimine or FectoPRO) and were cultured in Freestyle 293 expression medium (Invitrogen) supplemented with 0.5% bovine calf serum. The supernatant was collected 5 days after transfection and cleared by centrifugation. Then, Spike proteins were purified with Nickel-chelation chromatography and eluted fractions were concentrated by a Millipore Amicon Ultra Filter (100 kDa) and loaded onto a superpose^{TM} 6 gel filtration column (10/300 GL; GE) preequilibrated in tris-based buffer (20mM tris/HCl, 20mM NaCl, 50mM glutamate, 50mM Arginine), and the corresponding trimer fractions were collected. The purified S_{HM} were treated with Endo H (NEB) overnight at Room Temperature to produce Spike protein with a single GlcNAc at the glycosylation sites, the $Spike_{mg}$. For EndoH removal, S_{MG} were further purified by buffer exchange using Millipore Amicon Ultra Filter (100 kDa). Expression and purification method is modified from the lab's previous research published in PNAS.

[0075] The production of glycan-engineered pseudoviruses in (Fig. 1D) followed previous studies (Y. Watanabe, J. D. Allen, D. Wrapp, J. S. McLellan, M. Crispin, Site-specific glycan analysis of the SARS-CoV-2 spike. *Science* **369**, 330–333 (2020); Q. Yang, T. A. Hughes, A. Kelkar, X. Yu, K. Cheng, S. Park, W.-C. Huang, J. F. Lovell, S. Neelamegham, Inhibition of SARS-CoV-2 viral entry upon blocking N- and O-glycan elaboration. *eLife* **9**, e61552 (2020)). For production of glycosite-specific S mutant pseudoviruses (Fig. 1F), HEK293T cells were transiently transfected with pVax-nCoV-SΔ19 construct carrying mutations at each glycosite and luciferase-expressing HIV-1 genome plasmid (pNL4-3.luc.RE).

EXAMPLE 3: S_{MG} Protein characterization

[0076] To characterize the S_{MG} protein, the size-exclusion chromatography using ENrich^{TM} SEC 650 (10 x 300 column; Biorad) preequilibrated in tris-based buffer (20mM tris/HCl, 20mM NaCl, 50mM glutamate, 50mM Arginine) was performed. Then, to check the purity of protein sample, samples were combined with loading buffer, separated by 7.5% SDS-PAGE and stained by Coomassie Brilliant Blue-Plus (EBL).

EXAMPLE 4: Glycopeptide analysis by mass spectrometry

[0077] Two 20 μg aliquots of SARS-CoV-2 Spike protein, from Two biological replicates, were denatured in 55°C for 1h in 50 mM Triethylammonium bicarbonate buffer containing 10 mM of tris(2-carboxyethyl)phosphine. Next, the Spike protein were reduced and alkylated by adding 18 mM iodoacetamide (IAA) and incubated for 30 minutes in the dark. The alkylated Env proteins were digested separately using different combination of chymotrypsin or alpha lytic protease at a ratio of 1:10 (w/w), or trypsin at a ratio of 1:20(w/w). (Mass Spectrometry Grade, Promega) After an overnight digestion, the samples were dried in SpeedVac concentrator and processed for LC-MS/MS determination. Glycans categorization followed by previous study according to the composition detected and visualized by Graphpad Prism 9.0.0.

EXAMPLE 5: Vaccination and challenge experiments

[0078] Female 6- to 7-week-old Golden Syrian Hamster (n= 5) were immunized intramuscularly with 25ug purified S_{FG} or S_{MG} proteins mixed with aluminum hydroxide 250ug at day 0 and day 14. Blood was collected 28 days and 42 days after first immunization, and serum samples were collected from each hamster. Hamsters were challenged at 4 weeks after second vaccination with 1 x 10^4 PFU of SARS-CoV-2 TCDC#4 (hCoV-19/Taiwan/4/2020, GISAID accession ID: EPI_ISL_411927) intranasally in a volume of 100 μL per hamster. Body weight for each hamster were recorded daily after infection. On days 3 after challenge, hamsters were euthanized by carbon dioxide. The right lung was collected for viral load determination (TCID50 assay). The left lung was fixed in 4% paraformaldehyde for histopathological examination. All animal experiments were evaluated and approved by the Institutional Animal Care and Use Committee of Academia Sinica.

[0079] Alternatively, for mice vaccination with a two-dose schedule, female 6- to 8-week-old BALB/c mice (n= 5) were immunized intramuscularly with 10 μg of purified S_{FG}, S_{HM}, or S_{MG} mixed with aluminum hydroxide (50 μg) at days 0 and 14. The serum was collected at day 28 after the first vaccination for evaluation of anti–S IgG abundance, IgG subtype, and neutralizing titers (described in the Supplementary Materials and Methods). The lymph nodes of S_{FG}- or S_{MG}-immunized mice were collected at day 21 after the first vaccination for T cell response analysis (described in the Supplementary Materials and Methods). For B cell repertoire analysis and serum titers against variants, female 6- to 8-week-old BALB/c mice (n= 5) were immunized intramuscularly with 20 μg of purified S_{FG} or S_{MG} mixed with aluminum hydroxide

(20 μg) at days 0, 14, and 56; mice were euthanized at day 84 to collect whole blood for anti–S IgG and neutralizing titer evaluation and spleens for sorting of S protein–specific B cells (described in the Supplementary Materials and Methods).

[0080] For hamster vaccination and virus challenge study, male 6- to 7-week-old golden Syrian hamsters (n= 5) were immunized intramuscularly with 25 μg of purified S_{FG} or S_{MG} mixed with aluminum hydroxide (250 μg) at days 0 and 14. Four weeks after the second immunization, each hamster was intranasally challenged with 1×10^4 $TCID_{50}$ of SARS-CoV-2 (hCoV-19/Taiwan/4/2020) in 100 μl of PBS. Body weight was recorded daily after infection. On day 3 after challenge, hamsters were euthanized by carbon dioxide. The superior lobe of the left lung was fixed in 10% paraformaldehyde for histopathological examination, and the rest of the lung was collected for viral load determination ($TCID_{50}$ assay).

[0081] For transgenic mouse vaccination and virus challenge study, male 6- to 8-week-old CAG-hACE2 transgenic mice or male 12-week-old K18-hACE2 transgenic mice (purchased from the Jackson Laboratory) were immunized intramuscularly with 10 μg of purified S_{FG} or S_{MG} mixed with aluminum hydroxide (50 μg) at days 0 and 14. CAG-hACE2 transgenic mice were challenged intranasally 4 weeks after the second immunization with 1×10^3 $TCID_{50}$ of WT SARS-CoV-2. In the first trial (n= 3), all mice were euthanized at 7 dpi for histopathological examination of superior lobe of the left lung; in the second trial (n= 7), three mice were euthanized at 4 dpi for lung virus titer, and four mice were kept until 14 dpi for survival analysis. Serum was collected 1 day before virus challenge.

[0082] For challenge studies using VOCs, CAG-hACE2 mice were challenged with 1×10^3 $TCID_{50}$ of the alpha variant (hCoV-19/Taiwan/792/2020) (n = 5) or the gamma variant (hCoV-19/Taiwan/906/2021) of SARS-CoV-2 in 50 μl of PBS per mouse. In addition, K18-hACE2 mice were challenged intranasally 4 weeks after the second immunization with 1×10^4 $TCID_{50}$ of the delta SARS-CoV-2 (hCoV-19/Taiwan/1144/2021) (n = 4) in 50 μl of PBS per mice. For all SARS-CoV-2 variant challenge models, body weight for each mouse was recorded daily until 14 dpi.

[0083] For prophylactic protection test of antibody, male 8-week-old K18-hACE2 transgenic mice (n = 3) were injected intraperitoneally with m31A7 (15 mg/kg) or PBS 1 day before being intranasally challenged with 1×10^3 $TCID_{50}$ of WT SARS-CoV-2 (hCoV-19/Taiwan/4/2020).

Body weight and body temperature were recorded daily until 5 dpi. All animal experiments were evaluated and approved by the Institutional Animal Care and Use Committee of Academia Sinica (approval nos. 21-10-1716, 18-12-1272, and 20-10-1522).

EXAMPLE 6: Histology and Immunohistochemistry (IHC) staining

[0084] Hamster lungs at 3 dpi were immediately collected and placed in 10% neutral buffered formalin fixation for 24h, then transferred into 70% ethanol for 72 hours. Paraffin-embedded lungs tissue was trimmed to the thickness of 5 mm. For histological staining, tissue was stained with hematoxylin and eosin (H & E). For immunohistochemistry (IHC) staining, the tissue sectioned were deparaffinized with xylene and rehydrated with ethanol gradient. Antigen retrieval was performed by heating the slides to 95° C for 10 minutes in 10 mM sodium citrate buffer (pH 6.0) in a microwave oven. After cooling at room temperature and washing with PBS, 3% H_2O_2 apply to eliminate endogenous peroxidase activity. The tissue sectioned were blocking with 5% normal goat serum and 1 % BSA in 1X PBST for 1 hour, followed by incubation with rabbit anti-N and anti-S primary antibodies at 1:50 dilution (Anti-SARS-CoV-2 polyclonal antibody) overnight at 4°C. Then the tissue was incubated with goat anti-rabbit HRP secondary antibody at 1:500 dilutions for 1 hour and visualized by incubation with 3,3-diaminobenzidine (DAB) substrate and counterstained with hematoxylin.

EXAMPLE 7: Immunofluorescence (IF) staining

[0085] For immunofluorescence staining, after antigen retrieval steps, tissue was permeabilized with Triton X-100 in PBS. The tissue sectioned were blocking with 5% normal goat serum and 1% BSA in 1X PBST for 1 hour. Then, incubated with an autofluorescence quencher for 5 minutes. The samples were subsequently incubated with rabbit anti-N and anti-S primary antibodies at 1:50 dilution (Anti-SARS-CoV-2 polyclonal antibody) overnight at 4°C, secondary antibody Alexa Fluor-488 (1:500, Thermo Fisher) for 1 hour at room temperature, and 4,6-diamidino-2-phenylindole (DAPI), a nuclear dye for 3 minutes at room temperature. The coverslips were mounted on microscope slides and imaged under a Leica TCS SP8X confocal microscope with HC PL APO CS2 10x/1.40 lens (Leica AG, Wetzlar, Germany).

EXAMPLE 8: Transgenic mice Vaccination and challenge experiments

[0086] Male 8-week-old CAG-hACE2 transgenic mice (n= 3) were immunized intramuscularly with 10ug purified S_{FG} or S_{MG} proteins mixed with aluminum hydroxide 50ug at day 0 and day 14.[14] Blood was collected 28 days and 42 days after first immunization, and serum samples were collected from each transgenic mouse. Hamsters were challenged at 6 weeks after second vaccination with 1 x 10^3 PFU of SARS-CoV-2 TCDC#4 (hCoV-19/Taiwan/4/2020, GISAID accession ID: EPI_ISL_411927) intranasally in a volume of 100 μL per mice. Body weight and survival rate for each transgenic mice were recorded daily after infection. On days 7 after challenge, all transgenic mice were euthanized by carbon dioxide. The lung was fixed in 4% paraformaldehyde for histopathological examination. All animal experiments were evaluated and approved by the Institutional Animal Care and Use Committee of Academia Sinica.

EXAMPLE 9: Quantification of viral titer in lung tissue by cell culture infectious assay (TCID50)

[0087] The middle, inferior, and post-caval lung lobes of hamsters were homogenized in 600 μl of DMEM with 2% FBS and 1% penicillin/streptomycin using a homogenizer. Tissue homogenate was centrifuged at 15,000 rpm for 5 minutes and the supernatant was collected for live virus titration. Briefly, 10-fold serial dilutions of each sample were added onto Vero E6 cell monolayer in quadruplicate and incubated for 4 days. Cells were then fixed with 10% formaldehyde and stained with 0.5% crystal violet for 20 minutes. The plates were washed with tap water and scored for infection. The fifty-percent tissue culture infectious dose (TCID50)/mL was calculated by the Reed and Muench method.

EXAMPLE 10: Mice Vaccination studies

[0088] Female 6- to 8-week-old BALB/c mice (n= 5) were immunized intramuscularly with 20ug purified S_{FG} or S_{MG} proteins mixed with aluminum hydroxide 20ug at day 0, day 14 and day 56. Blood was collected 14 days after third immunization, and serum samples were collected from each mouse. All animal experiments were evaluated and approved by the Institutional Animal Care and Use Committee of Academia Sinica.

EXAMPLE 11: Sera antibody titer evaluation

[0089] Anti-S ELISA were used to determine sera IgG titer. Plates were blocked with 5% skim milk, and mouse polyclonal anti-S primary antibody and HRP-conjugated secondary

antibody were sequentially added. Peroxidase substrate solution (TMB) and 1M H_2SO_4 stop solution were used and absorbance (OD 450 nm) read by a microplate reader. Tested strain included SARS-CoV-2 (wild type, variants B.1.1.7 and B.1.135), RnGT13, and SARS-CoV-1.

[0090] m31A7 antibody was isolated by single B cell screening assay and then characterized. Primers were designed on the basis of a previous publication (T. Tiller, C. E. Busse, H. Wardemann, Cloning and expression of murine Ig genes from single B cells. *J. Immunol. Methods* **350**, 183–193 (2009)). Polymerase chain reaction (PCR) was performed at 50°C for 30 min, 95°C for 15 min, followed by 40 cycles of incubation at 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 10 min. Seminested second-round PCR was performed using KOD One PCR master mix (TOYOBO) with 1 μl of unpurified first-round PCR product at 98°C for 2 min, followed by 45 cycles of incubation at 98°C for 10 s, 55°C for 10 s, and 68°C for 10 s, with a final extension at 68°C for 1 min. PCR products were then analyzed by electrophoresis and sequencing. The Ig V and L genes were identified on the international ImMunoGeneTics information system (http://imgt.org/IMGT_vquest/input). Genes were then amplified from second-round PCR product with single gene-specific V and L gene primers containing restriction sites for cloning into the vectors containing human IgH or IgL expression backbone. The chimeric IgH and IgL expression constructs were cotransfected into Expi293 for antibody production. After m31A7 was isolated, the antibody was subsequently evaluated for S protein binding by ELISA and fluorescence-activated cell sorting, pseudovirus neutralization potency, binding kinetics, epitope mapping, and structure determination.

EXAMPLE 12: Pseudovirus neutralization assay

[0091] To determine the infectious units of pseudotyped lentiviral vectors, we seeded 293T-ACE2 cells at appropriate density in 96-well (100 μL per well) tissue culture plates 1 day prior to infection. After incubate overnight (37 °C, 5% CO_2), 100 mL three premixed pseudovirus supernatant and four-fold serial dilutions of immunized mouse sera were added in the plated cells. Cells were incubated for 48 h at 37 °C/ 5% CO_2 to allow for expression of Nano-Luciferase reporter gene. Luciferase activities were measured by the ELISA reader. Percent inhibition was calculated by the following equation $100*[1-(RLU_{sample} / RLU_{mock\text{-}treatment})]$. Data was analyzed using Graphpad Prism and pNT50 values were calculated by taking the 50% inhibitory concentration value for all samples.

EXAMPLE 13: Plaque reduction assays

[0092] Vero E6 cells were seeded into 24-well culture plates in DMEM with 10% FBS and antibiotics 1 day before infection. SARS-CoV-2 was incubated with antibodies for 1 h at 37°C before adding to the cell monolayer for another hour. Subsequently, virus-antibody mixtures were removed, and the cell monolayer was washed once with PBS before covering with media containing 1% methylcellulose for 5–7 days. The cells were fixed with 10% formaldehyde overnight. After removal of the overlay media, the cells were stained with 0.7% crystal violet, and the plaques were counted. The percentage of inhibition was calculated as [1 − (VD/VC)] × 100%, where VD and VC refer to the virus titers in the presence and absence of the sera, respectively.

[0093] For CPE-Based neutralization assay, Vero E6 cells were plated onto a 6-well plate at 2x105 cells/well overnight for 90% confluence. Serum and viruses were mixed before added onto the monolayer for another hour. The plates are allowed to solidify at room temperature for 30 minutes, then incubated at 37°C until cytopathic effects (CPE) are observed.

[0094] Statistical analysis: All of the data are expressed as the means ± standard errors of the means. For all of the analyses, *P* values were obtained from Student's *t*-test (unpaired, two tailed) except for the curve comparison using Student's *t*-test (paired, two tailed) tests. All of the graphs were generated with GraphPad Prism version 9.0.0 software.

EXAMPLE 14: Design and characterization of glycoengineered mono-GlcNAc-decorated Spike (S_{MG}) protein vaccine

[0095] The recombinant native with the sequence (amino acid 14-1209) from the original SARS-CoV-2 Wuhan strain (hCoV/Wuhan/WH01/2019) was codon optimized for human cell expression, with GSAG residues to replace the original furin cleavage site and 2 proline mutation to fix the spike native in its prefusion state, and at its C-terminus added a foldon trimerization sequence and a His-tag, and expressed with human HEK293S cells (Fig.1). The intermediate high-mannose type spike recombinant native (S_{HM}) where all the N-glycans, indicated by branch sign (Fig. 1a), are of the Man5 N-glycans was first obtained and purified. Endoglycosylase EndoH was then used to removed excess glycans and to generate the end product monoglycosylated Spike native (Fig. 1b). The purified S_{MG} is a trimer with an apparent

molecular weight of ~520 kDa in solution and high purity (Fig. 1c and 1d). The reduction in size due to removal of N-glycans was analyzed by SDS-PAGE, compared with the ordinary S_{FG} and intermediate S_{HM} (Fig. 1d). A mass spectrometry analysis on the S_{MG} indicated that most of the N-glycosylation sites are with near 100% single sugar, N-acetylglucosamine (GlcNAc), with the exception of N603 and N1194 that the GlcNAc is >90% (Fig. 1f). In contrast, the original fully glycosylated Spike native has heterogeneous N-glycans on all its N-glycosylation sites containing complex type, hybrid type and others.

EXAMPLE 15: S_{MG} vaccine provided superior protection against SARS-CoV-2 and variants *in vivo*

[0096] To evaluate the in vivo protective efficacy of S_{MG} vaccine against SARS-CoV-2, we first carried out WT SARS-CoV-2 challenge in Syrian hamsters vaccinated with S_{MG} or S_{FG} (Fig. 2A). S_{MG}-vaccinated hamsters (n= 5) showed less reduction in body weight as compared with the S_{FG} and phosphate-buffered saline (PBS) groups (Fig. 2B), whereas similar virus titer reductions were observed in the lungs of both S_{FG}- and S_{MG}-vaccinated hamsters (Fig. 2C). In addition, according to histopathological staining and anti–nucleocapsid (N) protein immunostaining data, fewer lesions were observed in the lungs of immunized hamsters (Fig. 2D). Because hamsters only showed mild to moderate sickness upon SARS-CoV-2 infection, we then used severe disease models, the highly susceptible CAG-hACE2 (*C.-Y. Tsai, C.-Y. Chen, J.-T. Jan, Y.-C. Chou, M.-L. Chang, L. A. Lu, P.-Y. Huang, M. F. C. Chu, T.-T. Hsu, Y.-P. Hsueh, Sex-biased response to and brain cell infection by SARS-CoV-2 in a highly susceptible human ACE2 transgenic model.*bioRxiv, *2021*) or K18-hACE2 (*E. S. Winkler, A. L. Bailey, N. M. Kafai, S. Nair, B. T. McCune, J. Yu, J. M. Fox, R. E. Chen, J. T. Earnest, S. P. Keeler, J. H. Ritter, L.-I. Kang, S. Dort, A. Robichaud, R. Head, M. J. Holtzman, M. S. Diamond, SARS-CoV-2 infection of human ACE2-transgenic mice causes severe lung inflammation and impaired function.* Nat. Immunol. ***21****, 1327–1335 (2020*) transgenic mice (Fig. 2E). The analysis of anti–S IgG binding titer, neutralizing titers, anti–S subtype IgG, and IgG2c:IgG1 ratio (Fig. 2, F to I) in CAG-hACE2 mice all showed similar results to the BALB/c mice (Fig. 6, C to G). Following challenge with WT SARS-CoV-2 intranasally, virus was not detectable in the lungs of both S_{FG}- and S_{MG}-vaccinated CAG-hACE2 mice (n = 3) by anti-N staining at 7 days postinfection (dpi) (Fig. 2J) or median tissue culture infectious dose ($TCID_{50}$) assay at 4 dpi (Fig. 2K), whereas a viral titer of over 1,000 $TCID_{50}$ was observed in the control group (Fig. 2K). The S_{MG} group (n = 4) exhibited better (75%) survival rate than the S_{FG} (50%) at 14 dpi (Fig. 2, L and M). We then

evaluated the degree of protection conferred by S_{MG} vaccination against challenge with the alpha variant in CAG-hACE2 mice (n = 5). We found that S_{MG} vaccination provided 100% survival rate until 14 dpi (Fig. 2, N and O). S_{MG}-vaccinated mice also showed a 60% survival rate in the gamma variant challenge in CAG-hACE2 mice (n = 5) (Fig. 22 P and Q) and a 75% survival rate in the delta variant challenge in K18-hACE2 mice (n = 4) (Fig. 4, R and S), whereas less than 50% S_{FG}-vaccinated mice survived until 14 dpi in the gamma and delta variant challenge (Fig. 2, Q to S). The improved in vivo protection conferred by S_{MG} vaccination provides further evidence that removal of glycan shields from an immunogen is an advantageous strategy to elicit superior immune response.

EXAMPLE 16: S_{MG} vaccination provides better protection against a lethal dose SARS-CoV-2 infection in transgenic hACE2 mice

[0097] The CAG-hACE2 transgenic mice can develop severe diseases and die when infected with SARS-CoV-2 virus. Two doses of vaccines of S_{FG}, S_{MG} or adjuvant only were given intramuscularly at day 0 and 14, sera were collected at day 28 and 42, $1x10^3$ TCID50 SARS-CoV-2 were used to infect each mice intranasally (Fig. 3a). The spike specific antibody IgG titers of S_{MG} immunized mice are 1.9-fold higher than S_{FG} group (40,000 vs 20.000), and the neutralization titers of S_{MG} are 2.8-fold higher than S_{FG} group (Fig. 3b and 3c). Surprisingly, in this severe-disease model, S_{FG} vaccination failed to protect the CAG-hACE2 transgenic mice from severe disease, as observed from rapid decease of body weights and all of them died before day 7 post virus infection. The disease progression of the S_{FG} vaccinated group was only slightly better than the adjuvant only group (Fig. 3d and 3e). In contract, the S_{MG} vaccinated group exhibited slight weight loss, and all of them survive the SARS-CoV-2 infection. This result demonstrates the superiority of S_{MG} vaccination in the SARS-CoV-2 severe-disease transgenic mouse model.

EXAMPLE 17: Antibodies induced by S_{MG} vaccine in mice for enhanced binding and neutralization breadth and potency against SARS-CoV-2 variants

[0098] We then analyzed whether there are differences in the antibody responses elicited from either vaccination of S_{FG} or S_{MG}, which has the spike native sequence from Wuhan strain, with regard to their ability to neutralize the newly emerged SARS-CoV-2 variants of concern (Fig. 4). Compared with S_{FG}, S_{MG} immunization elicited better spike specific antibody IgG

binding to not only spike native from WT (Wuhan strain), but also variants D614G, B.1.1.7, B.1.351, bat CoV RnGT13 and SARS-CoV-1 (Fig. 4b). Pseudotyped viruses of SARS-CoV-2 variants were used to gauge the ability to neutralize infections from the vaccine immunized sera. Again, the sera from S_{MG} immunization possess superior neutralization ability towards D614G, B.1.1.7 and B.1.351 pseudoviruses, and they are 1.4, 2.7 and 1.5 folds better than S_{FG} induced sera, respectively (Fig. 4c and 4d). Finally, SARS-CoV-2 real virus D614G and B.1.1.7 were used in a plague reduction neutralization assay (Fig. 4e and 4f). S_{MG} vaccination elicits better neutralizing antibody response to D614G and B.1.1.7 variants compared with S_{FG} vaccination, and they are 2.0 and 1.4 folds better, respectively.

EXAMPLE 18: Glycosylation affects pseudovirus S protein interacting with ACE2 on several cell types

[0099] To understand the importance of glycosylation, we expressed S protein from lung epithelial cells, the primary cells for infection, and found that sialylation of S protein is required for higher avidity to the receptor (Fig. 5A). A similar pattern was also observed for HEK293T cell–generated S protein (Fig. 5B), and the avidity was also reduced for the S protein with only high-mannose glycans or in the glycoform with all N-glycans trimmed to a single N-acetylglucosamine (GlcNAc) (Fig. 5C). The impact of its glycosylation was further tested by pseudovirus infection in human angiotensin-converting enzyme 2 (hACE2)–expressing HEK293T cells, revealing a consistent trend, when the same amount of virus was applied (Fig. 5D). This allowed us to conclude that complex-type glycans and sialylation are functionally important for S protein–mediated infectivity. A full panel of 24 lentivirus-based pseudovirus variants (comprising the 22 N- and 2 *O*-glycosites) were also generated for evaluating the viral entry efficiency in five hACE2-expressing cell lines, including HEK293T, Vero-E6, and three human lung cell lines, A549, Calu-1, and Calu-3 cells (Fig. 5, E to G). These pseudoviruses were based on the S construct with C-terminal 19 amino acid deletion, which produced the highest viral titer. Pseudovirus production was quantified by a p24 immunoassay, and results were normalized against the titer of each mutant strain (Fig. 5F). Every N-glycosite asparagine (Asn) was substituted to glutamine (Gln) to minimize the structural influence because of their chemical similarity, and each *O*-glycosite threonine (Thr) or serine (Srn) was substituted to alanine (Ala). Because the mutagenesis did change the amino acids, the resulting change in infectivity would come from collective factors, including the glycosylation-related conformational shifts that affect

receptor engagement and the surface abundance of S protein affected by protein expression, folding, and trafficking. Results showed that disruption of S protein glycosylation reduced infectivity. A substantial reduction was observed for two mutations in the receptor binding domain (RBD), N331Q and N343Q, as well as for mutations of the two *O*-glycosites (T323A and S325A), despite the low occupancy of the latter (Fig. 5G). In addition, deletion of N122 glycosylation in the N-terminal domain (NTD) resulted in reduced infectivity and low protein expression (Fig. 5G). Two NTD mutations, N149Q and N165Q, increased infectivity in Vero-E6 and Calu-1 cells, respectively, although decreased infections were observed in other cells (Fig. 5G). Note that the glycans attached to this N165 residue are structurally proximal to the neighboring RBD in the trimeric S protein, and its mutation reduced ACE2 binding probably because of the conformational shift of RBD toward the "down" state. We identified two mutants, N801Q and N1194Q (Fig. 5F), that universally abolished virus infectivity in all five cells. The glycosite N801 is located near the fusion peptide proximal region (FPPR), and N1194 is near the center of heptad repeat 2 (HR2) helix and is the last N-glycosite preceding the transmembrane domain (Fig. 5E). These mutations both caused low-yield expression. The N801Q mutant was more prone to degradation, and the N1194Q mutant disrupted S protein trimerization, which could be part of the explanation for the reduction of infectivity by pseudoviruses carrying these mutants.

EXAMPLE 19: S protein from lung epithelial cells contains more sialylated complex-type glycans and highly conserved epitopes in S protein are largely shielded by glycans

[00100] The glycan profile analysis of S protein revealed a higher abundance of complex-type glycans (78%), and fewer hybrid-type glycans (less than 1%) for S protein were produced in the human lung epithelial cell line BEAS-2B (Fig. 6A) as compared with S protein produced in the human kidney epithelial cell line, HEK293T (61 and 23%, respectively) (Fig. 6B). Among the high mannose–type glycans, the N-linked mannose-5 glycan (man5) was the predominant type found across the HEK293T-expressed S protein, although it is only seen at site N61 from BEAS-2B cells. In addition, the complex-type glycans at sites N74, N149, N282, and N1194 from BEAS-2B cells were more diversely processed (multiple antennae, galactosylation, fucosylation, or sialylation) than those from HEK293T cells. In contrast, the glycans at sites N122, N331, N1098, and N1134 were less diverse. Furthermore, N149 and N17 harbored no core fucose in

BEAS-2B. We observed an overall higher degree of sialylation on all 22 N-glycosites from BEAS-2B (53%) than that from HEK293T (35%), HEK293E (26%), or the previously reported HEK293F cells (15%) (Y. Watanabe, J. D. Allen, D. Wrapp, J. S. McLellan, M. Crispin, Site-specific glycan analysis of the SARS-CoV-2 spike. *Science* **369**, 330–333 (2020)). Particularly, the two N-glycosites (N331 and N343) of RBD are more sialylated in BEAS-2B (99 and 39%) than in HEK293T (49 and 15%). Despite the differences, the S protein from all cell types contains a non–complex-type glycan belt located around the middle section of the S2 domain (Fig. 6C), where the N-glycosite N801 is critical for infection (Fig. 5G), N1074 contains diverse glycans, and N717 is essential for S protein expression.

[00101] From the modeled SARS-CoV-2 S protein structure and the glycan profile from BEAS-2B cells, we conducted structural analysis of glycan coverage over protein surface areas and overlaid with multiple alignment results using 1,117,474 S protein sequences (S. Elbe, G. Buckland-Merrett, Data, disease and diplomacy: GISAID's innovative contribution to global health. *Global Chall.* **1**, 33–46 (2017)). It revealed several regions that were highly conserved, yet shielded by glycans, including the lower flank of RBD, the S2 stem region with the non–complex-type glycan belt, and the C-terminal part of S2 involving the connecting domain (CD) and HR2 (Fig. 6, D and E). On the primary sequence level, these regions were shown as conserved epitopes. Sequence conservation analysis also showed that most of the glycosite regions were highly conserved, and the most conserved ones (mutation rate lower than 0.02%) included those in the NTD (N61, N122, N165, and N234), the RBD (S325, N331, and N343), the subdomain 1/2 (SD1/2) (N603 and N657), the stem region of subunit 2 (S2) (N709, N1098, N1134, N1158, N1173, and N1194), and N801 near the FPPR. Most of these regions contain around 20 to 40% of conserved surface residues, and among them, a certain percentage of residues were shielded by glycans, 36% in the RBD and about 50% in other regions. Although harboring no N-glycosites, the HR1 region had 69% of its conserved surface residues being covered by glycans stemmed from adjacent domains. These results highlighted the importance of S protein glycosylation, both structurally and evolutionarily, leading to the thought that exposing glycan-shielded conserved regions may elicit immune responses against conserved epitopes. (It is more important to describe the 12 conserved epitopes as targets for vaccine resign. 10 of the 12 epitopes are shielded by glycans; and removal of the shielded glycans exposes the conserved epitopes to elicit broadly protective responses)

EXAMPLE 20: S_{MG} was developed as a vaccine and S_{MG} vaccine elicited better immune response using different antibody subclasses

[00102] Our initial attempt to mutate multiple glycosites led to a markedly reduced expression of S protein. Yet, when we expressed it from GnTI HEK293S cells, we were able to produce a high-mannose glycoform S protein (S_{HM}) with good yield and purity. We then trimmed the glycans using endoglycosidase H (Endo H) to a single GlcNAc at each N-glycosite, generating a soluble trimmer mono-GlcNAc-decorated S protein, which we called S_{MG} (Fig. 7A). S_{MG} was confirmed by mass spectrometry that all N-glycosites mostly occupied with single GlcNAc, and the occupancies of untreated *O*-glycans were too low to be detected. This modified S_{MG}, and S_{HM}, as well as the original fully glycosylated S protein (S_{FG}) were mixed with aluminum hydroxide (alum) as an adjuvant and were then used to immunize BALB/c mice (n = 5) by intramuscular injection (Fig. 7B). The S_{FG} used for comparison in this study was expressed by HEK293E cells and contained diverse glycans; this is similar to the immunogens used in many current COVID-19 vaccines that are either approved or in clinical trials, including the insect cell–expressed S protein vaccines from Sanofi and Novavax (P. J. Klasse, D. F. Nixon, J. P. Moore, Immunogenicity of clinically relevant SARS-CoV-2 vaccines in nonhuman primates and humans. *Sci. Adv.* 7, eabe8065 (2021)), the Chinese hamster ovary (CHO) cell–expressed recombinant S vaccine from Medigen (T.-Y. Kuo, M.-Y. Lin, R. L. Coffman, J. D. Campbell, P. Traquina, Y.-J. Lin, L. T.-C. Liu, J. Cheng, Y.-C. Wu, C.-C. Wu, W.-H. Tang, C.-G. Huang, K.-C. Tsao, C. Chen, Development of CpG-adjuvanted stable prefusion SARS-CoV-2 spike antigen as a subunit vaccine against COVID-19.*Sci. Rep.***10**, 20085 (2020)), the adenovirus-based vaccines from AstraZeneca and Johnson & Johnson, and the mRNA vaccines from Pfizer-BioNTech and Moderna (P. J. Klasse, D. F. Nixon, J. P. Moore, Immunogenicity of clinically relevant SARS-CoV-2 vaccines in nonhuman primates and humans. *Sci. Adv.* 7, eabe8065 (2021)). Both S_{FG} and S_{MG} proteins demonstrate essentially the same trimeric structure in solution by negative staining analysis.

[00103] Mice immunized with S_{MG} induced superior humoral immune response after second immunization as compared with S_{FG}, with a 1.44-fold significantly higher immunoglobulin G (IgG) titer against S protein (end point titer: S_{FG}, 39,408 ± 1,619; S_{MG}, 56,957 ± 5,091; P = 0.0079) (Fig. 7C) and 3.6-fold stronger antibody neutralization potency based on the inhibition of SARS-CoV-2 pseudovirus infection (reciprocal half maximal neutralization titer pNT_{50}: S_{FG}, 1346 ± 285; S_{MG}, 4791 ± 767; P= 0.0159) (Fig. 6D), whereas S_{HM}-immunized group shows

similar anti–S IgG titers (39,086 ± 11,654) and no difference in pNT_{50} titer compared with the S_{FG} group. The analysis of IgG subtype titer and interferon-γ (IFN-γ) or interleukin-4 (IL-4) production by T follicular helper (Tfh) cells revealed that S_{MG} vaccine induced more IgG2a, which is the marker for T helper 1 cell (T_H1) lymphocytes in BALB/c mice, a more balanced T_H1/T_H2 response, and more IFN-γ–expressing Tfh cells compared with the S_{FG}- and S_{HM}-vaccinated groups (Fig. 7, E to J). Furthermore, the S_{MG} vaccine induced higher frequency of IL-21^+ Tfh cells (Fig. 7K) and an elevated frequency of granzyme B–producing $CD8^+$ T cells (Fig. 7L). These data indicated that a more potent humoral and cellular adaptive immune response was elicited by S_{MG}, as compared with that induced by S_{FG}. We then examined the frequency of S protein–specific B cells ($CD3^-CD19^+S^+$) from the spleen of mice immunized after the third dose of S_{FG} or S_{MG} (Fig. 6A) and found that mice immunized with S_{MG} generated more S protein–specific B cells (Fig. 6M). The B cell repertoire analysis from S_{FG}- and S_{MG}-immunized mice (n = 5) indicated that more lambda light chain genes were used in the S_{MG} group compared with that in the S_{FG} group (S_{FG}, 1.92%; S_{MG}, 9.68%) (Fig. 7N). In addition, antibodies derived from several specific loci of the Ig heavy chain variable region (*IGHV*) (Fig. 7O) and the Ig kappa chain variable region (*IGKV*) genes (Fig. 7P) were overrepresented in the S_{MG} group than in the S_{FG} group, especially the *IGHV1-18* gene (Fig. 7O). This finding suggested that B cell epitopes may be processed differently in these two groups, and it remains to be further explored whether and why this difference is immunologically beneficial. In addition, the three-dose S_{MG} vaccination elicited higher end point titer IgG than the two-dose vaccination against wild-type (WT) S protein (end point titer: S_{FG}, 208,911 ± 50,092; S_{MG}, 376,410 ± 80,873). We observed differences between the S_{MG} and S_{FG} groups in serum IgG-binding curves measured by enzyme-linked immunosorbent assay (ELISA) against the S protein from SARS-CoV-2 VOCs (P. R. Krause, T. R. Fleming, I. M. Longini, R. Peto, S. Briand, D. L. Heymann, V. Beral, M. D. Snape, H. Rees, A.-M. Ropero, R. D. Balicer, J. P. Cramer, C. Muñoz-Fontela, M. Gruber, R. Gaspar, J. A. Singh, K. Subbarao, M. D. Van Kerkhove, S. Swaminathan, M. J. Ryan, A.-M. Henao-Restrepo, SARS-CoV-2 variants and vaccines. *N. Engl. J. Med* **385**, 179–186 (2021)), including alpha (B.1.1.7; P = 0.0488), beta (B.1.351;P = 0.0010), gamma (P.1; P = 0.0068), and delta (B.1.617.2; P = 0.0068), but no statistical differences in end point titers analysis (Fig. 7Q). Differences in neutralizing antibody responses against VOCs by pseudovirus neutralization curve, including alpha (P = 0.0156), beta (P = 0.0156), and delta (P = 0.0078), were also observed, but no differences were observed in

the pNT_{50} titer values for pseudovirus or authentic virus neutralization (Fig. 7, R and S), as compared with S_{FG}.

EXAMPLE 21: A broadly neutralizing antibody was isolated from B cells of mice immunized with S_{MG}

[00104] The sorting of S protein–specific B cells from S_{MG}-immunized mice led to the identification of a monoclonal antibody (mAb) m31A7 from the *IGHV1-18* amplified clones, a subset that is uniquely abundant in S_{MG}-immunized B cell repertoire (Fig. 8O). This mAb interacts with the full-length S protein, S1, and RBD, but not S2 (Fig. 8A), and binds to HEK293T cells that express the S protein from different SARS-CoV-2 variants (Fig. 8B). In addition, m31A7 was shown to neutralize various pseudovirus variants (WT, D614G, alpha, beta, and delta) at subpicomolar half-maximal inhibitory concentration (IC_{50}) up to 1000-fold higher than the reported human mAb EY6A (Fig. 8C). A prophylactic study also demonstrated good in vivo efficacy of m31A7 in K18-hACE2 mice ($n = 3$) challenged with WT SARS-CoV-2 (Fig. 8D). Prophylactically treated mice maintained both body weight and temperature (Fig. 8, E and F). Biolayer interferometry (BLI) analysis was used to measure the dissociation constants of m31A7 and its Fab binding to S protein at 70.9 pM and 4.66 nM, respectively (Fig. 8G). Epitope mapping by hydrogen-deuterium exchange mass spectrometry (HDX-MS) revealed its potential binding regions on RBD (Fig. 8H), which overlapped with the observed epitope in the crystal structure of RBD in complex with m31A7-Fab. The cryo–electron microscopy (EM) structure further clarified the binding of m31A7 only to RBD in the "up" state (Fig. 8I), with the N165-glycan from neighboring NTD in the vicinity of the RBD-m31A7 interface (Fig. 8J). The footprint of m31A7 on the RBD is similar to that of human *VH1-58* class mAbs (Fig. 8K), but it approaches the RBD from a different angle, with shifted local contact areas on the tip loop, bypassing most of key mutated residues of VOCs such as E484 and K417, but not T478 (Fig. 8L). The detailed RBD-m31A7 interface and the inhibitory mechanism of this S_{MG}-elicited mAb are under further investigation.

EXAMPLE 22: Delta S_{MG} vaccination elicited more neutralizing antibodies against SARS-CoV-2 variant pseudovirus

[00105] S_{MG} protein expression construct of SARS-CoV-2 Delta variant

SEQ ID NO:3

MKVKLLVLLCTFTATYAGTQCVNLRTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTW
FHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQ
FCNDPFLDVYYHKNNKSWMESGVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSK
HTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFL
LKYNENGTITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATR
FASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTG
KIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYRYRLFRKSNLKPFERDISTEIYQAGSKPCNGVEGF
NCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESN
KKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQGVNCTEVPVAIHA
DQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSYECDIPIGAGICASYQTQTNSRGSAGSVASQSIIAYT
MSLGAENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRA
LTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQ
YGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQMAYRF
NGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQNVVNQNAQALNTLVKQLSSNFGAISSV
LNDILSRLDPPEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKG
YHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIIT
TDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRL
NEVAKNLNESLIDLQELGKYEQDIRSLVPRGSPGSGYIPEAPRDGQAYVRKDGEWVLLSTFLGGSGSLEV
LFQGPHHHHHH

Signal peptide from A/Brisbane/59/2007(H1N1)) : MKVKLLVLLCTFTATYAGT (SEQ ID NO: 4)

Thrombin cleavage site: LVPRGS (SEQ ID NO: 5)

T4 foldon: PGSGYIPEAPRDGQAYVRKDGEWVLLSTFLG (SEQ ID NO: 6)

HRV3C cleavage site: GSGSLEVLFQGP (SEQ ID NO: 7)

His tag : HHHHHH (SEQ ID NO: 8)

BALB/c mice (n=5) were immunized twice at week 0 and week 2 with Delta S_{FG} or S_{MG} vaccine. Sera were collected at week 6 and subsequently tested the neutralization ability using pseudovirus assay (Fig. 9A). Pseudotyped viruses of SARS-CoV-2 Delta variants were used to gauge the ability to neutralize infections from the vaccine immunized sera. Again, the sera from S_{MG} immunization possess superior neutralization ability towards WT, Alpha, Beta, Gamma, Delta and Omicron pseudoviruses. Their respective titers of Delta S_{FG} vs. S_{MG} (pNT50, higher is better) are 1018 vs. 3336 (against WT pseudovirus), 1396 vs. 2680 (against Alpha pseudovirus), 337 vs. 2282 (against Beta pseudovirus), 814 vs 4424 (against Gamma pseudovirus), 1138 vs. 6680 (against Delta pseudovirus) and 144 vs. 2628 (against Omicron pseudovirus), respectively.

EXAMPLE 23: Delta or WT S_{MG} vaccination elicited more neutralizing antibodies against SARS-CoV-2 Omicron variant pseudovirus

[00106] BALB/c mice (n=5) were immunized twice at week 0 and week 2 with Delta/WT S_{FG} or S_{MG} vaccine. Sera were collected at week 6 and subsequently tested the neutralization ability using pseudovirus assay (Fig. 10A). Pseudotyped viruses of SARS-CoV-2 Omicron variant were used to gauge the ability to neutralize infections from the vaccine immunized sera from either WT S_{FG}/S_{MG} or Delta S_{FG}/S_{MG}. WT S_{FG} vaccination elicits no neutralization against Omicron pseudovirus, while WT S_{MG} provides slight protection. Similarly, Delta S_{FG} vaccination elicits little neutralization against Omicron pseudovirus infection, however, Delta S_{MG} provides excellent neutralization.

EXAMPLE 24: Delta S_{MG} protein can be stored at room temperature in solution or in powder

[00107] Delta S_{MG} protein in phosphate-buffered saline additive with two amino acids (pbs-aa), 50 mM L-Arginine and 50 mM L-Glutamate, was filtered with 0.22 μm filter and stored at room temperature (RT) or 4°C. The proteins were collected at different time points including 3, 7, 14, 21 days and 3 months. The collected samples were mixed with 5X SDS-PAGE Loading Dye, heated at 100°C for 5 min and stored in the 4°C until gel running (Fig. 11 A and B). The PBS with or without 50 mM L-Arginine and 50 mM L-Glutamate was tested for storing delta S_{HM} or S_{MG} (first 3 lanes) at 4°C. The delta S_{MG} was tested lyophilization with or without excipient and store at RT for over 2 weeks (last 2 lanes) (Fig. 11 C). The stability test indicates Delta S_{MG} protein can be stored in solution and is stable at either room temperature or 4 degree C for at least 21 days. Moreover, Delta SMG can be lyophilized and stored at RT and remain stable for at least 3 months.

Sequences

SEQ ID NO: 1

[00108] QCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHV

SGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVV

IKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQG

NFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTL

LALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSE

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TKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNR
KRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAP
GQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDIS
TEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCG
PKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLE
ILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGS
NVFQTRAGCLIGAEHVNNSYECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTM
SLGAENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLL
QYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSK
PSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDE
MIAQYTSALLAGTITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIAN
QFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILS
RLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKR
VDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFV
SNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELD
KYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQY

SEQ ID NO: 2

[00109] QCVNLRTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHV
SGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQ
FCNDPFLDVYYHKNNKSWMESGVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGY
FKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLLALHRSYLTPGDSSSGWTAGAAA
YYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVR
FPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCF
TNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYRYRLF
RKSNLKPFERDISTEIYQAGSKPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHA
PATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEI
LDITPCSFGGVSVITPGTNTSNQVAVLYQGVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAG
CLIGAEHVNNSYECDIPIGAGICASYQTQTNSRGSAGSVASQSIIAYTMSLGAENSVAYSNNSI

AIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDK
NTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDC
LGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQMAY
RFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQNVVNQNAQALNTLVKQLSS
NFGAISSVLNDILSRLDPPEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSEC
VLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFV
SNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHT
SPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQ

[00110] S_{MG} protein expression construct of SARS-CoV-2 Delta variant

SEQ ID NO:3

MKVKLLVLLCTFTATYAGTQCVNLRTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQ
DLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSK
TQSLLIVNNATNVVIKVCEFQFCNDPFLDVYYHKNNKSWMESGVYSSANNCTFEYVSQ
PFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGIN
ITRFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCAL
DPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWN
RKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQ
TGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYRYRLFRKSNLKPFERDISTEIY
QAGSKPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKST
NLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCS
FGGVSVITPGTNTSNQVAVLYQGVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGC
LIGAEHVNNSYECDIPIGAGICASYQTQTNSRGSAGSVASQSIIAYTMSLGAENSVAYSN
NSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIA
VEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAG
FIKQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGA
ALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQNV
VNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDPPEAEVQIDRLITGRLQSLQTYVTQQ
LIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVP
AQEKNFTTAPAICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDV
VIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEV

AKNLNESLIDLQELGKYEQDIRSLVPRGSPGSGYIPEAPRDGQAYVRKDGEWVLLSTFLG GSGSLEVLFQGPHHHHHH

[00111] Signal peptide modified from A/Brisbane/59/2007(H1N1)

MKVKLLVLLCTFTATYAGT **(SEQ ID NO: 4)**

[00112] Thrombin cleavage site

LVPRGS **(SEQ ID NO: 5)**

[00113] T4 foldon

PGSGYIPEAPRDGQAYVRKDGEWVLLSTFLG (**SEQ ID NO: 6**)

[00114] HRV3C cleavage site

GSGSLEVLFQGP (**SEQ ID NO:** 7)

[00115] His tag

HHHHHH (SEQ ID NO: 8)

[00116] Highly conserved epitopes

TESIVRFPNITNL (**SEQ ID NO.: 41**)

NITNLCPFGEVFNATR (**SEQ ID NO: 42**)

LYNSASFSTFK (**SEQ ID NO: 43**)

LDSKVGGNYN (**SEQ ID NO: 44**)

KSNLKPFERDIST (**SEQ ID NO: 45**)

KPFERDISTEIYQAG (**SEQ ID NO: 46**)

GPKKSTNLVKNKC (**SEQ ID NO: 47**)

NCDVVIGIVNNTVY (**SEQ ID NO: 48**)

PELDSFKEELDKYFKNHTS (**SEQ ID NO: 49**)

VNIQKEIDRLNEVA (**SEQ ID NO: 50**)

NLNESLIDLQ (**SEQ ID NO: 51**)

LGKYEQYIKWP (**SEQ ID NO: 52**)

CLAIMS

1. An immunogen, comprising a glycoengineered coronavirus spike protein comprising a plurality of truncated N-glycans, and one or more unmodified O-glycans, wherein the glycoengineered coronavirus spike protein consists of the amino acid sequence of SEQ ID NO: 1 or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 1, or an immunologically active fragment thereof.

2. The immunogen of claim 1, wherein the plurality of truncated N-glycans are monosaccharides.

3. The immunogen of claim 2, wherein the monosaccharidesare are N-acetylglucosamines (GlcNAc).

4. The immunogen of claim 1, wherein the plurality of truncated N-glycans are located in the receptor-binding domain (RBD), thereby exposing a plurality of highly conserved epitopes having amino acid sequences of TESIVRFPNITNL (SEQ ID NO.: 41), NITNLCPFGEVFNATR (SEQ ID NO: 42), LYNSASFSTFK (SEQ ID NO: 43), LDSKVGGNYN (SEQ ID NO: 44), KSNLKPFERDIST (SEQ ID NO: 45), KPFERDISTEIYQAG (SEQ ID NO: 46) and/or GPKKSTNLVKNKC (SEQ ID NO: 47).

5. The immunogen of claim 1, wherein the plurality of truncated N-glycans are located in the heptad repeat 2 (HR2) domain, thereby exposing a plurality of highly conserved epitopes having amino acid sequences of NCDVVIGIVNNTVY (SEQ ID NO: 48), PELDSFKEELDKYFKNHTS (SEQ ID NO: 49), VNIQKEIDRLNEVA (SEQ ID NO: 50), NLNESLIDLQ (SEQ ID NO: 51) and/or LGKYEQYIKWP (SEQ ID NO: 52).

6. The immunogen of claim 1, wherein the glycoengineered coronavirus spike protein is derived from a native coronavirus spike protein by glycoengineering in vitro.

7. The immunogen of claim 6, wherein the native coronavirus spike protein is the spike protein of severe acute respiratory syndrome coronavirus 2 (SAR-CoV-2) or a variant thereof.

8. The immunogen of claim 7, wherein the variant is selected from the group consisting of D614G, Alpha (B.1.1.7 and Q lineages), Beta (B.1.351 and descendent lineages), Gamma (P.1

and descendent lineages), Epsilon (B.1.427 and B.1.429), Eta (B.1.525), Iota (B.1.526), Kappa (B.1.617.1), 1.617.3, Mu (B.1.621, B.1.621.1), Zeta (P.2), Delta (B.1.617.2 and AY lineages) and Omicron (B.1.1.529 and BA lineages).

9. The immunogen of claim 1, wherein the glycoengineered coronavirus spike protein is present as a trimer.

10. The immunogen of claim 6, wherein the glycoengineered coronavirus spike protein retained the same tertiary structure as the native coronavirus spike protein.

11. The immunogen of claim 4, wherein the glycoengineered coronavirus spike protein is capable of eliciting an enhanced immune response relative to the native coronavirus spike protein.

12. The immunogen of claim 11, wherein the enhanced immune response is an increased IgG titer, an increased IgM titer, an increased neutralization titer, an increased CD4 T cell response, an increased CD8 T cell response, or a combination thereof.

13. The immunogen of claim 6, wherein the glycoengineered spike protein is generated using one or more of chemical or enzymatic methods.

14. The immunogen of claim 13, wherein the glycoengineered spike protein is generated using endoglycosidase H (Endo H).

15. The immunogen of claim 1, wherein the glycoengineered coronavirus spike protein comprises a polypeptide consisting of an amino acid sequence of SEQ ID NO: 1, wherein the polypeptide consists of 22 truncated N-glycans, each having a GlcNAc moiety.

16. The immunogen of claim 1, wherein the glycoengineered coronavirus spike protein comprises a polypeptide consisting of an amino acid sequence of SEQ ID NO: 2, wherein the polypeptide consists of 21 truncated N-glycans, each having a GlcNAc moiety.

17. An immunogenic composition, comprising: (a) the immunogen of any one of claims 1-16, and (b) optionally, an adjuvant.

18. The immunogenic composition of claim 17, wherein the adjuvant is aluminum hydroxide, aluminum phosphate, incomplete Freund's adjuvant (IFA), squalene, Alum, Alhydrogel, MF59, QS-21, CpG 1018, AS03, AS37, Matrix-M or a combination thereof.

19. The immunogenic composition of claim 17, wherein the immunogenic composition is an improved coronavirus vaccine capable of eliciting an enhanced immune response relative to a vaccine using a native coronavirus spike protein.

20. The immunogenic composition of claim 19, wherein the coronavirus is SAR-CoV-2 or a variant thereof.

21. A method for eliciting an immune response against a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in a subject in need thereof, comprising administering to the subject an effective amount of an immunogenic composition of claim 17 or 18.

22. The method of claim 21, wherein the immune response comprises production of neutralizing antibodies against SARS-CoV-2 or a variant thereof.

23. The method of claim 22, wherein the variant is selected from the group consisting of D614G, Alpha (B.1.1.7 and Q lineages), Beta (B.1.351 and descendent lineages), Gamma (P.1 and descendent lineages), Epsilon (B.1.427 and B.1.429), Eta (B.1.525), Iota (B.1.526), Kappa (B.1.617.1), 1.617.3, Mu (B.1.621, B.1.621.1), Zeta (P.2), Delta (B.1.617.2 and AY lineages) and Omicron (B.1.1.529 and BA lineages).

24. Use of the immunogenic composition of claim 17 or 18 for eliciting an immune response against SARS-CoV-2 or a variant thereof in a subject in need thereof, the method comprising administering to the subject in need thereof an effective amount of the immunogenic composition.

25. The use of claims 24, wherein the immune response comprises production of neutralizing antibodies against SARS-CoV-2 or a variant thereof.

26. The use of claim 25, wherein the variant is selected from the group consisting of D614G, Alpha (B.1.1.7 and Q lineages), Beta (B.1.351 and descendent lineages), Gamma (P.1 and descendent lineages), Epsilon (B.1.427 and B.1.429), Eta (B.1.525), Iota (B.1.526), Kappa (B.1.617.1), 1.617.3, Mu (B.1.621, B.1.621.1), Zeta (P.2), Delta (B.1.617.2 and AY lineages) and Omicron (B.1.1.529 and BA lineages).

27. Use of the immunogenic composition of claim 17 or 18 for protecting a subject in need thereof from infection with SARS-CoV-2 or a variant thereof, the method comprising

administering to the subject in need thereof an effective amount of the immunogenic composition.

28. Use of the immunogenic composition of claim 17 or 18 for preventing a subject in need thereof from contracting COVID-19 disease, the method comprising administering to the subject in need thereof an effective amount of the immunogenic composition.