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(54) Title: OMICRON CORONAVIRUS VACCINE CONSTRUCTS AND METHODS OF MAKING AND USING SAME

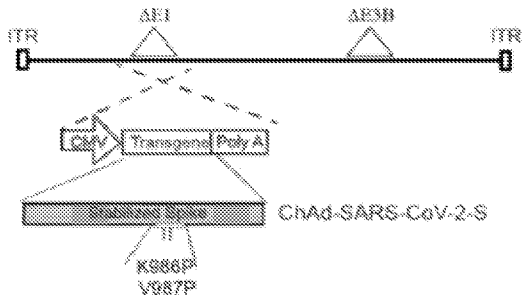


FIG. 1A

(57) Abstract: Provided herein are adenoviral vectors encoding spike protein variants of SARS-CoV2 coronavirus, and compositions comprising the vectors. Also provided are methods of prevention and treatment of SARS-CoV2 using the compositions provided.

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OMICRON CORONAVIRUS VACCINE CONSTRUCTS AND METHODS OF MAKING AND USING SAME

GOVERNMENT SUPPORT

[0001] This invention was made with government support under CA211096 awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD OF THE TECHNOLOGY

[0002] The present disclosure generally relates to the field of biotechnology and medicine and, more particularly, to nucleic acid constructs, polypeptides and vectors that can be used in vaccines for enhanced therapy against respiratory viral infections and methods of use thereof.

CROSS REFERENCE TO RELATED APPLICATIONS

[0003] This application claims the benefit of the U.S. Provisional application number 63/434,815 filed December 22, 2022 and claims the benefit of the U.S. Provisional application number 63/384,075 filed November 16, 2022, and claims the benefit of the U.S. Provisional application number 63/375,803 filed September 15, 2022 and claims the benefit of the U.S. Provisional application number 63/305,979 filed on February 2, 2022, the disclosures of which are herein incorporated by reference in their entirety.

BACKGROUND

[0004] Viral infections are responsible for hundreds of thousands of deaths each year. However, treatment options are limited for many viruses. Additionally, carriers of a virus may be asymptomatic, leading to high transmission rates from infected but asymptomatic individuals. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the etiologic agent of the Coronavirus Disease 2019 (COVID-19) syndrome, which can rapidly progress to pneumonia, respiratory failure, and systemic

inflammatory disease. SARS-CoV-2 is a positive-sense single-stranded RNA virus that was first isolated in late 2019 from patients with severe respiratory illness in Wuhan, China. As a Betacoronavirus, SARS-CoV-2 is related to two other highly pathogenic respiratory viruses, SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV). SARS-CoV-2 infection results in a clinical syndrome that can progress to respiratory failure and also present with cardiac pathology, gastrointestinal disease, coagulopathy, and a hyperinflammatory syndrome. The elderly, immunocompromised, and those with co-morbidities (*e.g.*, obesity, diabetes, and hypertension) are at greatest risk of death from COVID-19. More than 669 million infections and 6.8 million deaths have been recorded worldwide since the start of the pandemic.

[0005] The extensive morbidity, mortality, and destabilizing socioeconomic consequences of COVID-19 highlight the urgent need for deployment of an effective SARS-CoV-2 vaccine to mitigate the severity of infection, curb transmission, end the pandemic, and prevent its return.

SUMMARY

[0006] An aspect of the present technology encompasses an adenoviral vector comprising the genome of a non-human adenovirus. The genome of the adenovirus has been modified such that the vector lacks the native E1 and optionally, the E3 or E3B locus, and comprises a nucleic acid sequence encoding a SARS-CoV-2 spike (S) protein with an amino acid sequence at least 80% identical to any of SEQ ID NOS: 10, 12, 20, or 21 or an immunogenic portion, or fragment thereof.

[0007] Another aspect of the present technology encompasses an adenoviral vector comprising a nucleic acid sequence encoding an amino acid sequence at least 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1 %, 99.2%, 99.3%, 99.4% 99.5%, 99.6%, 99.7%, 99.8% or 99.9% identical to any of SEQ ID NOS: 10-12, 20, 21 or an immunogenic portion, or fragment thereof.

[0008] An additional aspect of the present technology encompasses an adenoviral vector comprising or consisting of a nucleic acid sequence with at least 80%,

90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1%, 99.2%, 99.3%, 99.4% 99.5%, 99.6%, 99.7%, 99.8% or 99.9% sequence identity to any one of SEQ ID NO: 13-19.

[0009] Yet another aspect of the present technology encompasses an adenoviral vector comprising a nucleic acid sequence, wherein the nucleic acid sequence encodes an amino acid sequence at least 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1 %, 99.2%, 99.3%, 99.4% 99.5%, 99.6%, 99.7%, 99.8% or 99.9% identical to SEQ ID NOS: 3 comprising at least 10, or at least 15, or at least 20, or at least 25, or at least 30 mutations from a list consisting of T19I, L24S, del25-27, 69-70del, G142D, V213G, G339D, S371F, S373P, S375F, T376A, K417N, N440K, S477N, T478K, E484A, Q493R, L452R, F486V, Q498R, N501Y, D614G, H655Y, N679K, P681H, R682G, R683S, R685S, N764K, D796Y, A942P Q954H, N969K, K988P and V989P.

[0010] Still another aspect encompasses a pharmaceutical composition, comprising an adenoviral vector described herein. Similarly, an aspect encompasses an immunogenic composition comprising an adenoviral vector described herein.

[0011] Some aspects of the present technology encompass a host cell transduced with an adenoviral vector described herein, or a packaging cell line that produces an adenoviral vector described herein.

[0012] Other aspects of the present technology encompass a kit. Generally speaking, the kit comprises: (i) one or more of, a host cell described herein, a packaging cell line described herein, an adenoviral vector described herein, a pharmaceutical composition described herein, or an immunogenic composition described herein and (ii) instructions for use.

[0013] Certain aspects of the present technology encompass a coronavirus vaccine comprising an adenovirus vector as detailed herein. In particular aspects, the technology encompasses a composition comprising the serum of a first subject previously administered an adenoviral vector described herein, a pharmaceutical composition described herein, or an immunogenic composition

described herein. The present technology further encompasses a method of treating a second subject having a coronavirus infection comprising administering to the second subject an immunogenically effective amount of a composition comprising the serum of the first subject.

[0014] In still another aspect, the present technology encompasses a method for inducing an immune response against a coronavirus in a subject in need thereof. The method comprises administering to the subject an immunogenically effective amount of a composition comprising an adenoviral vector of described herein, a pharmaceutical composition described herein, or an immunogenic composition described herein.

[0015] Other aspects and iterations are described in more detail below.

BRIEF DESCRIPTION OF THE FIGURES

[0016] Those of skill in the art will understand that the drawings, described below, are for illustrative purposes only. The drawings are not intended to limit the scope of the present teachings in any way.

[0017] The application file contains at least one drawing executed in color. Copies of this patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0018] **FIG. 1A** shows a diagram of transgene cassettes: ChAd-control has no transgene insert; ChAd-SARS-CoV-2-S encodes for SARS-CoV-2 S protein with the two indicated proline mutations.

[0019] **FIG. 1B** shows binding of ChAd-SARS-CoV-2-S transduced 293 cells with anti-S mAbs. (Left) Summary: +, ++, +++, - indicate < 25%, 25-50%, > 50%, and no binding, respectively. (Right) Representative flow cytometry histograms of two experiments.

[0020] **FIG. 1C** shows four-week old female BALB/c mice were immunized via intramuscular route with ChAd-control or ChAd-SARS-CoV-2-S and boosted four weeks later. Antibody responses in sera of immunized mice at day 21 after priming or boosting were evaluated.

[0021] **FIG. 1D** shows ELISA measured anti-S and RBD IgG levels.

[0022] **FIG. 1E** shows FRNT determined neutralization activity. Data are pooled from two experiments (n = 15 to 30; Mann-Whitney test: ****, $P < 0.0001$).

[0023] **FIG. 1F** shows cell-mediated responses were analyzed at day 7 post-booster immunization after re-stimulation with an S protein peptide pool. Splenocytes were assayed for IFNγ and granzyme B expression in CD8+ T cells and granzyme B only in $CD4^+$ T cells by flow cytometry.

[0024] **FIG. 1G** shows a summary of frequencies and numbers of positive cell populations (n = 5; Mann-Whitney test: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Bars indicate median values, and dotted lines are the limit of detection (LOD) of the assays.

[0025] **FIG. 1H** shows spleens were harvested at 7 days post-boost, and SARS-CoV-2 spike-specific IgG+ antibody-secreting cells (ASC) frequency was measured by ELISPOT (Mann-Whitney test: ****, $P < 0.0001$). Bars and columns show median values, and dotted lines indicate the limit of detection (LOD) of the assays.

[0026] **FIG. 2A** shows serum from ChAd-control immunized mice collected at day 21 after priming or boosting (as described in **FIG. 1**) was assayed for S-specific IgG responses by ELISA.Four-week old female BALB/c mice were primed or primed and boosted with ChAd-control or ChAd-SARS-CoV-2-S via intramuscular route.

[0027] **FIG. 2B** shows serum samples from ChAd-control or ChAd-SARS-CoV-2 vaccinated mice were collected at day 21 after priming. Four-week old female BALB/c mice were primed or primed and boosted with ChAd-control or ChAd-SARS-CoV-2-S via intramuscular route.

[0028] **FIG. 2C** shows serum samples from ChAd-control or ChAd-SARS-CoV-2 vaccinated mice were collected at day 21 after boosting and assayed for neutralizing activity by FRNT. Serum neutralization curves corresponding to individual mice are shown for the indicated vaccines (n = 15-30 per group). Each point represents the mean of two technical replicates with error bars denoting the standard deviations (SD). ChAd-SARS-CoV-2-S vaccine induces neutralizing antibodies as measured by focus reduction neutralization test (FRNT).

[0029] **FIG. 2D** shows an ELISA measured anti-SARS-CoV-2 NP IgG responses in paired sera obtained 5 days before and 8 days after SARS-CoV-2 challenge of ChAd-control or ChAd-SARS-CoV-2-S mice vaccinated by an intramuscular route (n = 5: ** P < 0.01; *** P < 0.001; paired t test). Dotted lines represent the mean IgG titers from naïve sera.

[0030] **FIG. 3** shows Gating strategy for analyzing T cell responses. Four-week old female BALB/c mice were immunized with ChAd-control or ChAd-SARS-CoV-2-S and boosted four weeks later. T cell responses were analyzed in splenocytes at day 7 post-boost. Cells were gated for lymphocytes (FSC-A/SSC-A), singlets (SSC-W/SSC-H), live cells (Aqua-), CD45+, CD19- followed by CD4+ or CD8+ cell populations expressing IFNγ or granzyme B.

[0031] **FIG. 4A** shows neutralizing activity of Hu-AdV5-hACE2 in the sera from the indicated vaccine groups was determined by FRNT after prime only. Four-week-old female BALB/c mice were primed or primed and boosted. Serum samples were collected one day prior to Hu-AdV5-hACE2 transduction.

[0032] **FIG. 4B** shows neutralizing activity of Hu-AdV5-hACE2 in the sera from the indicated vaccine groups was determined by FRNT after prime and boost. Each symbol represents a single animal; each point represents two technical repeats and bars indicate the range. A positive control (anti-Hu-Adv5 serum) is included as a frame of reference.

[0033] **FIG. 5A** is a scheme of vaccination and challenge for studying the protective efficacy of intramuscularly delivered ChAd-SARS-CoV-2-S against SARS-CoV-2 infection. Four-week-old BALB/c female mice were immunized ChAd-control or ChAd-SARS-CoV-2-S. Some mice received a booster dose of the homologous vaccine. On day 35 post-immunization, mice were challenged with SARS-CoV-2 as follows: animals were treated with anti-Ifnar1 mAb and transduced with Hu-AdV5-hACE2 via an intranasal route one day later. Five days later, mice were challenged with 4×10^5 focus-forming units (FFU) of SARS-CoV-2 via the intranasal route.

[0034] **FIG. 5B** shows tissues were harvested at 4 and 8 dpi for analysis. Infectious virus in the lung was measured by plaque assay.

[0035] **FIC. 5C** shows viral RNA levels were measured in the lung, spleen and heart at 4 and 8 dpi by RT-qPCR (C) (n = 3-7, Mann-Whitney test: *** P < 0.001).

[0036] **FIG. 5D** shows viral RNA in situ hybridization using SARS-CoV-2 probe (brown color) in the lungs harvested at 4 dpi. Images show low-(top; scale bars, 100 μm) and medium-(middle; scale bars, 100 μm) power magnification with a high-power magnification inset (representative images from n = 3 per group).

[0037] **FIG. 5E** shows fold change in gene expression of indicated cytokines and chemokines from lung homogenates at 4 dpi was determined by RT-qPCR after normalization to Gapdh levels and comparison with naïve unvaccinated, unchallenged controls (n = 7; Mann-Whitney test: ***, P < 0.001).

[0038] **FIG. 5F** show mice that received a prime-boost immunization were challenged on day 35 post-booster immunization. Tissues were collected at 4 dpi for analysis. Infectious virus in the lung was determined by plaque assay.

[0039] **FIG. 5G** shows mice that received a prime-boost immunization were challenged on day 35 post-booster immunization and viral RNA was measured in the lung, spleen and heart using RT-qPCR (G) (n = 6-7; Mann-Whitney test: **, P < 0.01). (B-C and E-G) Columns show median values, and dotted lines indicate the LOD of the assays.

[0040] **FIG. 6** show single-dose intramuscular vaccination with ChAd-SARS-CoV-2-S protects mice against SARS-CoV-2-induced inflammation in the lung. Four-week old female BALB/c mice were immunized with ChAd-control and ChAd-SARS-CoV-2-S and challenged following the scheme described in **FIG. 5**. Lungs were harvested at 8 dpi. Sections were stained with hematoxylin and eosin and imaged at 40x (left; scale bar, 250 μm), 200x (middle; scale, 50 μm), and 400x (right; scale bar, 25 μm) magnifications. Each image is representative of a group of 3 mice.

[0041] **FIG. 7A** shows a scheme of experiments to study the immune responses after Intranasal immunization of ChAd-SARS-CoV-2-S. Five-week-old BALB/c female mice were immunized with ChAd-control or ChAd-SARS-CoV-2-S via an intranasal route.

[0042] **FIG. 7B** shows antibody responses in sera of immunized mice at one month after priming were evaluated. An ELISA measured SARS-CoV-2 S-and RBD-specific IgG.

[0043] **FIG. 7C** shows an ELISA measured SARS-CoV-2 S-and RBD-specific IgA levels.

[0044] **FIG. 7D** shows a FRNT determined neutralization activity. Data are pooled from two experiments with n = 10-25 mice per group (Mann-Whitney test: ****, $P < 0.0001$).

[0045] **FIG. 7E** shows mice that received a booster dose were sacrificed one week later to evaluate mucosal and cell-mediated immune responses. SARS-CoV-2 S-and RBD-specific IgG.

[0046] **FIG. 7F** shows SARS-CoV-2 S-and RBD-specific IgA levels in BAL fluid were determined by ELISA.

[0047] **FIG. 7G** shows neutralizing activity of BAL fluid against SARS-CoV-2 was measured by FRNT.

[0048] **FIG. 7H** shows CD8+ T cells in the lung were assayed for IFNγ and granzyme B expression by flow cytometry after re-stimulation with an S protein peptide pool.

[0049] **FIG. 7I** shows CD8+ T cells in the lung also were phenotyped for expression of CD103 and CD69.

[0050] **FIG. 7J** shows SARS-CoV-2 spike-specific IgG+ and IgA+ antibody-secreting cells (ASC) frequency in the spleen harvested one week post-boost was measured by ELISPOT. Data for mucosal and cell-mediated responses are pooled from two experiments (E-I: n = 7-9 per group; Mann-Whitney test: ***, $P < 0.001$); J: n = 5 per group; Mann-Whitney test: **, $P < 0.01$; ***, $P < 0.001$). (B-J) Bars and columns show median values, and dotted lines indicate the LOD of the assays.

[0051] **FIG. 8A** shows serum samples from ChAd-control or ChAd-SARS-CoV-2-S vaccinated mice were tested for neutralizing activity with SARS-CoV-2 strain 2019 n-CoV/USA_WA1/2020 (n = 8-10 per group). Five-week old female BALB/c mice were immunized with ChAd-control or ChAd-SARS-CoV-2-S via an intranasal

inoculation route. Serum samples collected one month after immunization were assayed for neutralizing activity by FRNT. Mice were boosted at day 30 after priming and were sacrificed one week later to evaluate immune responses.

[0052] **FIG. 8B** shows serum samples from ChAd-SARS-CoV-2-S vaccinated mice were tested for neutralization of recombinant luciferase-expressing SARS-CoV-2 viruses (wild-type (left) and D614G variant (middle)). (Right) Paired EC50 values are indicated (n = 5; n.s. not significant, paired t test).

[0053] **FIG. 8C** shows BAL fluid was collected from ChAd-control or ChAd-SARS-CoV-2-S vaccinated mice, and neutralization of SARS-CoV-2 strain 2019 n-CoV/USA_WA1/2020 was measured using a FRNT assay (n = 8-10 per group). Each point represents the mean of two technical replicates with error bars denoting the SD.

[0054] **FIG. 9A** shows tissues and nasal washes were collected at 4 and 8 dpi for analysis. Infectious virus in the lung was measured by plaque assay. Five-week-old BALB/c female mice were immunized with ChAd-control or ChAd-SARS-CoV-2-S via an intranasal route. On day 35 post-immunization, mice were challenged as follows: animals were treated with anti-Ifnar1 mAb and transduced with Hu-AdV5-hACE2 via the intranasal route one day later. Five days later, mice were challenged intranasally with 4 × 105 FFU of SARS-CoV-2.

[0055] **FIG. 9B** shows viral RNA levels in the lung, spleen, heart, nasal turbinates, and nasal washes were measured at 4 and 8 dpi by RT-qPCR. Five-week-old BALB/c female mice were immunized with ChAd-control or ChAd-SARS-CoV-2-S via an intranasal route. On day 35 post-immunization, mice were challenged as follows: animals were treated with anti-Ifnar1 mAb and transduced with Hu-AdV5-hACE2 via the intranasal route one day later. Five days later, mice were challenged intranasally with 4 × 105 FFU of SARS-CoV-2.

[0056] **FIG. 9C** shows fold change in gene expression of indicated cytokines and chemokines was determined by RT-qPCR, normalized to Gapdh, and compared to naïve controls in lung homogenates at 4 dpi (2 experiments, n = 6-9; median values are shown: *, $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; Mann-Whitney test). Columns show median values, and dotted lines indicate the LOD of the

assays. Five-week-old BALB/c female mice were immunized with ChAd-control or ChAd-SARS-CoV-2-S via an intranasal route. On day 35 post-immunization, mice were challenged as follows: animals were treated with anti-Ifnar1 mAb and transduced with Hu-AdV5-hACE2 via the intranasal route one day later. Five days later, mice were challenged intranasally with 4 × 105 FFU of SARS-CoV-2.

[0057] **FIG. 9D** shows lungs were harvested at 8 dpi. Sections were stained with hematoxylin and eosin and imaged at 40x (left; scale bar, 250 μm), 200x (middle; scale, 50 μm), and 400x (right; scale bar, 25 μm) magnifications. Each image is representative of a group of 3 mice. Five-week-old BALB/c female mice were immunized with ChAd-control or ChAd-SARS-CoV-2-S via an intranasal route. On day 35 post-immunization, mice were challenged as follows: animals were treated with anti-Ifnar1 mAb and transduced with Hu-AdV5-hACE2 via the intranasal route one day later. Five days later, mice were challenged intranasally with 4 × 105 FFU of SARS-CoV-2.

[0058] **FIG. 9E** show an ELISA measured anti-SARS-CoV-2 NP IgM (left) and IgG (right) antibody responses in paired sera obtained 5 days before and 8 days after SARS-CoV-2 challenge of ChAd-control or ChAd-SARS-CoV-2-S mice vaccinated by an intranasal route (n = 6: ns; not significant; ** $P < 0.01$, **** $P < 0.0001$; paired t test). Dotted lines represent the mean IgM and IgG titers from naïve sera (n = 6). Five-week-old BALB/c female mice were immunized with ChAd-control or ChAd-SARS-CoV-2-S via an intranasal route. On day 35 post-immunization, mice were challenged as follows: animals were treated with anti-Ifnar1 mAb and transduced with Hu-AdV5-hACE2 via the intranasal route one day later. Five days later, mice were challenged intranasally with 4 × 105 FFU of SARS-CoV-2.

[0059] **FIG. 10A** shows immunization scheme. Five-week old female BALB/c mice were vaccinated via IN or IM route with 10^{10} viral particles of ChAd-control or decreasing doses (10^{10}, 10^{9} and 10^{8} vp) of ChAd-SARS-CoV-2-S.

[0060] **FIG. 10B** shows humoral responses in sera of immunized mice were evaluated (n = 6-14). An ELISA measured anti-S and RBD IgG levels from IN-immunized mice at 100 are provided. One way ANOVA with a Dunnett's post-test comparing vaccine and control groups: ns, not significant; **, $P < 0.01$; ****, $P < 0.0001$).

[0061] **FIG. 10C** shows humoral responses in sera of immunized mice were evaluated (n = 6-14). An ELISA measured IgA levels from IN-immunized mice at 100 are provided. One way ANOVA with a Dunnett's post-test comparing vaccine and control groups: ns, not significant; **, P < 0.01; ****, P < 0.0001). One way ANOVA with a Dunnett's post-test comparing vaccine and control groups: ns, not significant; **, P < 0.01; ****, P < 0.0001). One way ANOVA with a Dunnett's post-test comparing vaccine and control groups: ns, not significant; **, P < 0.01; ****, P < 0.0001).

[0062] **FIG. 10D** shows neutralizing activity of sera determined by FRNT from IN immunized mice at 100 days post-vaccination. One way ANOVA with a Dunnett's post-test comparing vaccine and control groups: ns, not significant; **, P < 0.01; ****, P < 0.0001).

[0063] **FIG. 10E** shows an ELISA measured anti-S and RBD IgG levels from IN-immunized mice at 100 days post-vaccination, or from IM-immunized mice at 100. One way ANOVA with a Dunnett's post-test comparing vaccine and control groups: ns, not significant; **, P < 0.01; ****, P < 0.0001).

[0064] **FIG. 10F** shows an ELISA measured anti-S and RBD IgA levels from IN-immunized mice at 100 days post-vaccination, or from IM-immunized mice at 100. One way ANOVA with a Dunnett's post-test comparing vaccine and control groups: ns, not significant; **, P < 0.01; ****, P < 0.0001).

[0065] **FIG. 10G** shows the neutralizing activity of sera determined by FRNT from IM immunized mice at 100 days post-vaccination. One way ANOVA with a Dunnett's post-test comparing vaccine and control groups: ns, not significant; **, P < 0.01; ****, P < 0.0001).

[0066] **FIG. 10H** shows humoral responses in sera of immunized mice were evaluated (n = 6-14). An ELISA measured anti-S and RBD IgG levels from IN-immunized mice at 200 are provided. One way ANOVA with a Dunnett's post-test comparing vaccine and control groups: ns, not significant; **, P < 0.01; ****, P < 0.0001).

[0067] **FIG. 10I** shows humoral responses in sera of immunized mice were evaluated (n = 6-14). An ELISA measured anti-S and RBD IgA levels from IN-

immunized mice at 200 are provided. One way ANOVA with a Dunnett's post-test comparing vaccine and control groups: ns, not significant; **, P < 0.01; ****, P < 0.0001).

[0068] **FIG. 10J** shows neutralizing activity of sera determined by FRNT from IN immunized mice at 200 days post-vaccination. One way ANOVA with a Dunnett's post-test comparing vaccine and control groups: ns, not significant; **, P < 0.01; ****, P < 0.0001).

[0069] **FIG. 10K** shows an ELISA measured anti-S and RBD IgG levels from IN-immunized mice at 200 days post-vaccination, or from IM-immunized mice at 100. One way ANOVA with a Dunnett's post-test comparing vaccine and control groups: ns, not significant; **, P < 0.01; ****, P < 0.0001).

[0070] **FIG. 10L** shows an ELISA measured anti-S and RBD IgA levels from IN-immunized mice at 200 days post-vaccination, or from IM-immunized mice at 100. One way ANOVA with a Dunnett's post-test comparing vaccine and control groups: ns, not significant; **, P < 0.01; ****, P < 0.0001).

[0071] **FIG. 10M** shows the neutralizing activity of sera determined by FRNT from IM immunized mice at 200 days post-vaccination. One way ANOVA with a Dunnett's post-test comparing vaccine and control groups: ns, not significant; **, P < 0.01; ****, P < 0.0001).

[0072] **FIG. 10N** shows the frequency of S-specific IgG or IgA producing LLPCs in the bone marrow measured by ELISPOT assay (n = 4). Mann-Whitney test: *, P < 0.05. B-N, Bars show median values, and dotted lines indicate the limit of detection (LOD) of the assays.

[0073] **FIG. 11A** shows serum samples from ChAd-SARS-CoV-2-S vaccinated mice were collected at day 100. . Five-week-old female BALB/c mice were immunized via IN or IM route with a single 10^{10}, 10^{9}, or 10^{8} dose of ChAd-SARS-CoV-2-S.

[0074] **FIG. 11B** shows serum samples from ChAd-SARS-CoV-2-S vaccinated mice were collected at day 200 after immunization and assayed for neutralizing activity by FRNT. . Five-week-old female BALB/c mice were immunized via IN or IM route with a single 10^{10}, 10^{9}, or 10^{8} dose of ChAd-SARS-CoV-2-S. Serum

neutralization curves corresponding to individual mice are shown for the indicated vaccines (n = 6-14 per group). Each point represents the mean of two technical replicates.

[0075] **FIG. 12A** shows serum from mice intranasally inoculated ChAd-SARS-CoV-2-S analyzed by Luminex platform to quantify the amount of anti-SARS-CoV-2 (WA1/2020 D614G) spike and RBD IgG1. Bars represent the mean values.

[0076] **FIG. 12B** shows serum was analyzed by luminex to quantify the amount of anti-SARS-CoV-2 IgG1 to different SARS-CoV-2 protein variants. Polar plots represent the IgG1 median percentile rank for each SARS-CoV-2 protein and variant.

[0077] **FIG. 12C** shows a heatmap shows the IgG titer and FcγR binding titer of each vaccine regimen to SARS-CoV-2 Spike or RBD proteins. Each square represents the average z-score within a group for the condition.

[0078] **FIG. 12D** shows serum was incubated with primary mouse neutrophils (mADNP) or J774A.1 cells (mADCP) and SARS-CoV-2 spike-coated beads, and phagocytosis was measured after 1 h. Bars represent the mean and the error bars indicate standard deviations.

[0079] **FIG. 12E** shows serum was incubated with primary mouse neutrophils (mADNP) or J774A.1 cells (mADCP) and WA1/2020 D614G, B.1.1.7, or B1.351 spike-coated beads, and phagocytosis was measured after 1 h. Polar plots represent the mADNP or mADCP median percentile rank for each SARS-CoV-2 protein and variant. For (A and D): one-way ANOVA with a Dunnett's post-test comparing vaccine to control groups: **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$). (A and D): Bars indicate median values.

[0080] **FIG. 13A** show durability of protective efficacy of ChAd-SARS-CoV-2-S against SARS-CoV-2 infection in BALB/c mice. Five-week old female BALB/c mice were immunized via IN route with 10^{10} vp of ChAd-Control or 10^{10}, 10^{9} and 10^{8} vp of ChAd-SARS-CoV-2-S. On day 100, mice were challenged as follows: animals were treated with anti-Ifnar1 mAb and transduced with Hu-AdV5-hACE2 via an IN route one day later. Five days later, mice were inoculated with 5 x 104 FFU of SARS-CoV-2 WA1/2020 via the intranasal route. Tissues as indicated in the figure were harvested at

4 dpi, and viral RNA levels were measured from mice challenged 100 days post-immunization by RT-qPCR (n = 6-14, Kruskal Wallis with Dunn's post-test: ns, not significant; **, P < 0.01; *, P < 0.1; ***, P < 0.001 ****, P < 0.0001). Bars show median values, and dotted lines indicate the LOD of the assays.

[0081] **FIG. 13B** show durability of protective efficacy of ChAd-SARS-CoV-2-S against SARS-CoV-2 infection in BALB/c mice. Five-week old female BALB/c mice were immunized via IN route with 10^{10} vp of ChAd-Control or 10^{10}, 10^{9} and 10^{8} vp of ChAd-SARS-CoV-2-S. On day 100 post-immunization, mice were challenged as follows: animals were treated with anti-Ifnar1 mAb and transduced with Hu-AdV5-hACE2 via an IN route one day later. Five days later, mice were inoculated with 5 x 104 FFU of SARS-CoV-2 WA1/2020 via the intranasal route. Tissues as indicated in the figure were harvested at 4 dpi, and viral RNA levels were measured from mice challenged 100 days post-immunization by RT-qPCR (n = 6-14, Kruskal Wallis with Dunn's post-test: ns, not significant; **, P < 0.01; *, P < 0.1; ***, P < 0.001 ****, P < 0.0001). Bars show median values, and dotted lines indicate the LOD of the assays.

[0082] **FIG. 13C** show durability of protective efficacy of ChAd-SARS-CoV-2-S against SARS-CoV-2 infection in BALB/c mice. Five-week old female BALB/c mice were immunized via IN route with 10^{10} vp of ChAd-Control or 10^{10}, 10^{9} and 10^{8} vp of ChAd-SARS-CoV-2-S. On day 100 post-immunization, mice were challenged as follows: animals were treated with anti-Ifnar1 mAb and transduced with Hu-AdV5-hACE2 via an IN route one day later. Five days later, mice were inoculated with 5 x 10^{4} FFU of SARS-CoV-2 WA1/2020 via the intranasal route. Tissues as indicated in the figure were harvested at 4 dpi, and viral RNA levels were measured from mice challenged 100 days post-immunization by RT-qPCR (n = 6-14, Kruskal Wallis with Dunn's post-test: ns, not significant; **, P < 0.01; *, P < 0.1; ***, P < 0.001 ****, P < 0.0001). Bars show median values, and dotted lines indicate the LOD of the assays.

[0083] **FIG. 13D** show durability of protective efficacy of ChAd-SARS-CoV-2-S against SARS-CoV-2 infection in BALB/c mice. Five-week old female BALB/c mice were immunized via IM route with 10^{10} vp of ChAd-Control or 10^{10} vp of ChAd-Control. On day 100, mice were challenged as follows: animals were treated with anti-Ifnar1

mAb and transduced with Hu-AdV5-hACE2 via an IN route one day later. Five days later, mice were inoculated with 5 x 10^4 FFU of SARS-CoV-2 WA1/2020 via the intranasal route. Tissues as indicated in the figure were harvested at 4 dpi, and viral RNA levels were measured from mice challenged 100 days post-immunization by RT-qPCR (n = 6-14, Kruskal Wallis with Dunn's post-test: ns, not significant; **, P < 0.01; *, P < 0.1; ***, P < 0.001 ****, P < 0.0001). Bars show median values, and dotted lines indicate the LOD of the assays.

[0084] **FIG. 13E** show durability of protective efficacy of ChAd-SARS-CoV-2-S against SARS-CoV-2 infection in BALB/c mice. Five-week old female BALB/c mice were immunized via IM route with 10^{10} vp of ChAd-Control or 10^{10}, 10^9 and 10^8 vp of ChAd-SARS-CoV-2-S. On day 100 post-immunization, mice were challenged as follows: animals were treated with anti-Ifnar1 mAb and transduced with Hu-AdV5-hACE2 via an IN route one day later. Five days later, mice were inoculated with 5 x 104 FFU of SARS-CoV-2 WA1/2020 via the intranasal route. Tissues as indicated in the figure were harvested at 4 dpi, and viral RNA levels were measured from mice challenged 100 days post-immunization by RT-qPCR (n = 6-14, Kruskal Wallis with Dunn's post-test: ns, not significant; **, P < 0.01; *, P < 0.1; ***, P < 0.001 ****, P < 0.0001). Bars show median values, and dotted lines indicate the LOD of the assays.

[0085] **FIG. 13F** show durability of protective efficacy of ChAd-SARS-CoV-2-S against SARS-CoV-2 infection in BALB/c mice. Five-week old female BALB/c mice were immunized via IM route with 10^{10} vp of ChAd-Control or 10^{10}, 10^9 and 10^8 vp of ChAd-SARS-CoV-2-S. On day 100 post-immunization, mice were challenged as follows: animals were treated with anti-Ifnar1 mAb and transduced with Hu-AdV5-hACE2 via an IN route one day later. Five days later, mice were inoculated with 5 x 10^4 FFU of SARS-CoV-2 WA1/2020 via the intranasal route. Tissues as indicated in the figure were harvested at 4 dpi, and viral RNA levels were measured from mice challenged 100 days post-immunization by RT-qPCR (n = 6-14, Kruskal Wallis with Dunn's post-test: ns, not significant; **, P < 0.01; *, P < 0.1; ***, P < 0.001 ****, P < 0.0001). Bars show median values, and dotted lines indicate the LOD of the assays.

[0086] **FIG. 13G** show durability of protective efficacy of ChAd-SARS-CoV-2-S against SARS-CoV-2 infection in BALB/c mice. Five-week old female BALB/c mice were immunized via IN route with 10^{10} vp of ChAd-Control or 10^{10}, 10^{9} and 10^{8} vp of ChAd-SARS-CoV-2-S. On day 200, mice were challenged as follows: animals were treated with anti-Ifnar1 mAb and transduced with Hu-AdV5-hACE2 via an IN route one day later. Five days later, mice were inoculated with 5 x 10^{4} FFU of SARS-CoV-2 WA1/2020 via the intranasal route. Tissues as indicated in the figure were harvested at 4 dpi, and viral RNA levels were measured from mice challenged 200 days post-immunization by RT-qPCR (n = 6-14, Kruskal Wallis with Dunn's post-test: ns, not significant; **, P < 0.01; *, P < 0.1; ***, P < 0.001 ****, P < 0.0001). Bars show median values, and dotted lines indicate the LOD of the assays.

[0087] **FIG. 13H** show durability of protective efficacy of ChAd-SARS-CoV-2-S against SARS-CoV-2 infection in BALB/c mice. Five-week old female BALB/c mice were immunized via IN route with 10^{10} vp of ChAd-Control or 10^{10}, 10^{9} and 10^{8} vp of ChAd-SARS-CoV-2-S. On day 200 post-immunization, mice were challenged as follows: animals were treated with anti-Ifnar1 mAb and transduced with Hu-AdV5-hACE2 via an IN route one day later. Five days later, mice were inoculated with 5 x 104 FFU of SARS-CoV-2 WA1/2020 via the intranasal route. Tissues as indicated in the figure were harvested at 4 dpi, and viral RNA levels were measured from mice challenged 200 days post-immunization by RT-qPCR (n = 6-14, Kruskal Wallis with Dunn's post-test: ns, not significant; **, P < 0.01; *, P < 0.1; ***, P < 0.001 ****, P < 0.0001). Bars show median values, and dotted lines indicate the LOD of the assays.

[0088] **FIG. 13I** show durability of protective efficacy of ChAd-SARS-CoV-2-S against SARS-CoV-2 infection in BALB/c mice. Five-week old female BALB/c mice were immunized via IN route with 10^{10} vp of ChAd-Control or 10^{10}, 10^{9} and 10^{8} vp of ChAd-SARS-CoV-2-S. On day 200 post-immunization, mice were challenged as follows: animals were treated with anti-Ifnar1 mAb and transduced with Hu-AdV5-hACE2 via an IN route one day later. Five days later, mice were inoculated with 5 x 10^{4} FFU of SARS-CoV-2 WA1/2020 via the intranasal route. Tissues as indicated in the figure were harvested at 4 dpi, and viral RNA levels were measured from mice

challenged 200 days post-immunization by RT-qPCR (n = 6-14, Kruskal Wallis with Dunn's post-test: ns, not significant; **, P < 0.01; *, P < 0.1; ***, P < 0.001 ****, P < 0.0001). Bars show median values, and dotted lines indicate the LOD of the assays.

[0089] **FIG. 13J** show durability of protective efficacy of ChAd-SARS-CoV-2-S against SARS-CoV-2 infection in BALB/c mice. Five-week-old female BALB/c mice were immunized via IM route with 10^{10} vp of ChAd-Control or 10^{10} vp of ChAd-Control. On day 200, mice were challenged as follows: animals were treated with anti-Ifnar1 mAb and transduced with Hu-AdV5-hACE2 via an IN route one day later. Five days later, mice were inoculated with 5 x 10^4 FFU of SARS-CoV-2 WA1/2020 via the intranasal route. Tissues as indicated in the figure were harvested at 4 dpi, and viral RNA levels were measured from mice challenged 200 days post-immunization by RT-qPCR (n = 6-14, Kruskal Wallis with Dunn's post-test: ns, not significant; **, P < 0.01; *, P < 0.1; ***, P < 0.001 ****, P < 0.0001). Bars show median values, and dotted lines indicate the LOD of the assays.

[0090] **FIG. 13K** show durability of protective efficacy of ChAd-SARS-CoV-2-S against SARS-CoV-2 infection in BALB/c mice. Five-week-old female BALB/c mice were immunized via IM route with 10^{10} vp of ChAd-Control or 10^{10}, 10^9 and 10^8 vp of ChAd-SARS-CoV-2-S. On day 200 post-immunization, mice were challenged as follows: animals were treated with anti-Ifnar1 mAb and transduced with Hu-AdV5-hACE2 via an IN route one day later. Five days later, mice were inoculated with 5 x 10^4 FFU of SARS-CoV-2 WA1/2020 via the intranasal route. Tissues as indicated in the figure were harvested at 4 dpi, and viral RNA levels were measured from mice challenged 200 days post-immunization by RT-qPCR (n = 6-14, Kruskal Wallis with Dunn's post-test: ns, not significant; **, P < 0.01; *, P < 0.1; ***, P < 0.001 ****, P < 0.0001). Bars show median values, and dotted lines indicate the LOD of the assays.

[0091] **FIG. 13L** show durability of protective efficacy of ChAd-SARS-CoV-2-S against SARS-CoV-2 infection in BALB/c mice. Five-week-old female BALB/c mice were immunized via IM route with 10^{10} vp of ChAd-Control or 10^{10}, 10^9 and 10^8 vp of ChAd-SARS-CoV-2-S. On day 200 post-immunization, mice were challenged as follows: animals were treated with anti-Ifnar1 mAb and transduced with Hu-AdV5-

hACE2 via an IN route one day later. Five days later, mice were inoculated with 5 x 10^4 FFU of SARS-CoV-2 WA1/2020 via the intranasal route. Tissues as indicated in the figure were harvested at 4 dpi, and viral RNA levels were measured from mice challenged 200 days post-immunization by RT-qPCR (n = 6-14, Kruskal Wallis with Dunn's post-test: ns, not significant; **, P < 0.01; *, P < 0.1; ***, P < 0.001 ****, P < 0.0001). Bars show median values, and dotted lines indicate the LOD of the assays.

[0092] **FIG. 14A** shows an ELISA measured SARS-CoV-2 S-and RBD-specific IgG levels. Five-week-old K18-hACE2 female mice were immunized with 10^9 vp ChAd-control or ChAd-SARS-CoV-2-S via an IN route. Antibody responses in sera of mice at 6 weeks after immunization were evaluated. Mann-Whitney test: ***, P < 0.001; ****, P < 0.0001.

[0093] **FIG. 14B** shows an ELISA measured SARS-CoV-2 S-and RBD-specific IgA levels. Five-week-old K18-hACE2 female mice were immunized with 10^9 vp ChAd-control or ChAd-SARS-CoV-2-S via an IN route. Antibody responses in sera of mice at 6 weeks after immunization were evaluated. Mann-Whitney test: ***, P < 0.001; ****, P < 0.0001.

[0094] **FIG. 14C** show an FRNT determined neutralization activity in sera of mice at 6 weeks. Five-week-old K18-hACE2 female mice were immunized with 10^9 vp ChAd-control or ChAd-SARS-CoV-2-S via an IN route. Paired analysis of serum neutralizing activity from immunized mice collected at 6 weeks against WA1/2020 and Wash-B.1.351 is provided. Two-tailed Wilcoxon matched-pairs signed rank test: *, P < 0.05; ****, P < 0.0001.

[0095] **FIG. 14D** show an FRNT determined neutralization activity in sera of mice at 6 weeks. Five-week-old K18-hACE2 female mice were immunized with 10^9 vp ChAd-control or ChAd-SARS-CoV-2-S via an IN route. Paired analysis of serum neutralizing activity from immunized mice collected at 6 weeks against WA1/2020 and Wash-B.1.1.28 is provided. Two-tailed Wilcoxon matched-pairs signed rank test: *, P < 0.05; ****, P < 0.0001.

[0096] **FIG. 14E** shows an ELISA measured SARS-CoV-2 S-and RBD-specific IgG levels. Five-week-old K18-hACE2 female mice were immunized with 10^9 vp

ChAd-control or ChAd-SARS-CoV-2-S via an IN route. Antibody responses in sera of mice at 9 weeks after immunization were evaluated. Mann-Whitney test: ***, $P < 0.001$; ****, $P < 0.0001$.

[0097] **FIG. 14F** shows an ELISA measured SARS-CoV-2 S-and RBD-specific IgA levels. Five-week-old K18-hACE2 female mice were immunized with 10^9 vp ChAd-control or ChAd-SARS-CoV-2-S via an IN route. Antibody responses in sera of mice at 9 weeks after immunization were evaluated. Mann-Whitney test: ***, $P < 0.001$; ****, $P < 0.0001$.

[0098] **FIG. 14G** show an FRNT determined neutralization activity in sera of mice at 6 weeks. Five-week-old K18-hACE2 female mice were immunized with 10^9 vp ChAd-control or ChAd-SARS-CoV-2-S via an IN route. Paired analysis of serum neutralizing activity from immunized mice collected at 9 weeks against WA1/2020 and Wash-B.1.351 is provided. Two-tailed Wilcoxon matched-pairs signed rank test: *, $P < 0.05$; ****, $P < 0.0001$.

[0099] **FIG. 14H** show an FRNT determined neutralization activity in sera of mice at 6 weeks. Five-week-old K18-hACE2 female mice were immunized with 10^9 vp ChAd-control or ChAd-SARS-CoV-2-S via an IN route. Paired analysis of serum neutralizing activity from immunized mice collected at 9 weeks against WA1/2020 and Wash-B.1.1.28 is provided. Two-tailed Wilcoxon matched-pairs signed rank test: *, $P < 0.05$; ****, $P < 0.0001$.

[00100] **FIG. 15A** shows the experimental scheme. Five-week-old K18-hACE2 female mice were immunized via an IN route with 10^{10} vp of ChAd-control or ChAd-SARS-CoV-2-S.

[00101] **FIG. 15B** Five-week-old K18-hACE2 female mice were immunized via an IN route with 10^{10} vp of ChAd-control or ChAd-SARS-CoV-2-S. At 6 weeks post-immunization, mice were challenged with 10^4 FFU of SARS-CoV-2 of Wash-B.1.351. A plot of body weight change over time are shown. Data are the mean ± SEM comparing vaccine to control groups (n = 6-9 for each group; unpaired t test for area under curve, **** $P < 0.0001$).

[00102] **FIG. 15C** Five-week-old K18-hACE2 female mice were immunized via an IN route with 10^{10} vp of ChAd-control or ChAd-SARS-CoV-2-S. At 6 weeks post-immunization, mice were challenged with 10^4 FFU of SARS-CoV-2 of Wash-B.1.351. Viral RNA levels in the lung were measured at 6 dpi by RT-qPCR (n = 6-9; Mann-Whitney test: ** $P < 0.01$, *** $P < 0.001$). Bars show median values, and dotted lines indicate the LOD of the assays.

[00103] **FIG. 15D** Five-week-old K18-hACE2 female mice were immunized via an IN route with 10^{10} vp of ChAd-control or ChAd-SARS-CoV-2-S. At 6 weeks post-immunization, mice were challenged with 10^4 FFU of SARS-CoV-2 of Wash-B.1.351. Viral RNA levels in the heart were measured at 6 dpi by RT-qPCR (n = 6-9; Mann-Whitney test: ** $P < 0.01$, *** $P < 0.001$). Bars show median values, and dotted lines indicate the LOD of the assays.

[00104] **FIG. 15E** Five-week-old K18-hACE2 female mice were immunized via an IN route with 10^{10} vp of ChAd-control or ChAd-SARS-CoV-2-S. At 6 weeks post-immunization, mice were challenged with 10^4 FFU of SARS-CoV-2 of Wash-B.1.351. Viral RNA levels in the nasal washes were measured at 6 dpi by RT-qPCR (n = 6-9; Mann-Whitney test: ** $P < 0.01$, *** $P < 0.001$). Bars show median values, and dotted lines indicate the LOD of the assays.

[00105] **FIG. 15F** Five-week-old K18-hACE2 female mice were immunized via an IN route with 10^{10} vp of ChAd-control or ChAd-SARS-CoV-2-S. At 6 weeks post-immunization, mice were challenged with 10^4 FFU of SARS-CoV-2 of Wash-B.1.351. At 6 weeks post-immunization, mice were challenged with 10^4 FFU of SARS-CoV-2 of Wash-B.1.351. Viral RNA levels in the brain were measured at 6 dpi by RT-qPCR (n = 6-9; Mann-Whitney test: ** $P < 0.01$, *** $P < 0.001$). Bars show median values, and dotted lines indicate the LOD of the assays.

[00106] **FIG. 15G** Five-week-old K18-hACE2 female mice were immunized via an IN route with 10^{10} vp of ChAd-control or ChAd-SARS-CoV-2-S. At 6 weeks post-immunization, mice were challenged with 10^4 FFU of SARS-CoV-2 of Wash-B.1.1.28. A plot of body weight change over time are shown. Data are the mean ± SEM comparing

vaccine to control groups (n = 6-9 for each group; unpaired t test for area under curve, **** $P < 0.0001$).

[00107] **FIG. 15H** Five-week-old K18-hACE2 female mice were immunized via an IN route with 10^{10} vp of ChAd-control or ChAd-SARS-CoV-2-S. At 6 weeks post-immunization, mice were challenged with 10^4 FFU of SARS-CoV-2 of Wash-B.1.1.28. Viral RNA levels in the lung were measured at 6 dpi by RT-qPCR (n = 6-9; Mann-Whitney test: ** $P < 0.01$, *** $P < 0.001$). Bars show median values, and dotted lines indicate the LOD of the assays.

[00108] **FIG. 15I** Five-week-old K18-hACE2 female mice were immunized via an IN route with 10^{10} vp of ChAd-control or ChAd-SARS-CoV-2-S. At 6 weeks post-immunization, mice were challenged with 10^4 FFU of SARS-CoV-2 of Wash-B.1.1.28. Viral RNA levels in the heart were measured at 6 dpi by RT-qPCR (n = 6-9; Mann-Whitney test: ** $P < 0.01$, *** $P < 0.001$). Bars show median values, and dotted lines indicate the LOD of the assays.

[00109] **FIG. 15J** Five-week-old K18-hACE2 female mice were immunized via an IN route with 10^{10} vp of ChAd-control or ChAd-SARS-CoV-2-S. At 6 weeks post-immunization, mice were challenged with 10^4 FFU of SARS-CoV-2 of Wash-B.1.1.28. Viral RNA levels in the nasal washes were measured at 6 dpi by RT-qPCR (n = 6-9; Mann-Whitney test: ** $P < 0.01$, *** $P < 0.001$). Bars show median values, and dotted lines indicate the LOD of the assays.

[00110] **FIG. 15K** Five-week-old K18-hACE2 female mice were immunized via an IN route with 10^{10} vp of ChAd-control or ChAd-SARS-CoV-2-S. At 6 weeks post-immunization, mice were challenged with 10^4 FFU of SARS-CoV-2 of Wash-B.1.1.28. At 6 weeks post-immunization, mice were challenged with 10^4 FFU of SARS-CoV-2 of Wash-B.1.351. Viral RNA levels in the brain were measured at 6 dpi by RT-qPCR (n = 6-9; Mann-Whitney test: ** $P < 0.01$, *** $P < 0.001$). Bars show median values, and dotted lines indicate the LOD of the assays.

[00111] **FIG. 15L** Five-week-old K18-hACE2 female mice were immunized via an IN route with 10^{10} vp of ChAd-control or ChAd-SARS-CoV-2-S. At 6 weeks post-immunization, mice were challenged with 10^4 FFU of SARS-CoV-2 of WA1/2020. A plot

of body weight change over time are shown. Data are the mean ± SEM comparing vaccine to control groups (n = 6-9 for each group; unpaired t test for area under curve, **** P < 0.0001).

[00112] **FIG. 15M** Five-week-old K18-hACE2 female mice were immunized via an IN route with 10^{10} vp of ChAd-control or ChAd-SARS-CoV-2-S. At 6 weeks post-immunization, mice were challenged with 10^4 FFU of SARS-CoV-2 of WA1/2020. Viral RNA levels in the lung were measured at 6 dpi by RT-qPCR (n = 6-9; Mann-Whitney test: ** P < 0.01, *** P < 0.001). Bars show median values, and dotted lines indicate the LOD of the assays.

[00113] **FIG. 15N** Five-week-old K18-hACE2 female mice were immunized via an IN route with 10^{10} vp of ChAd-control or ChAd-SARS-CoV-2-S. At 6 weeks post-immunization, mice were challenged with 10^4 FFU of SARS-CoV-2 of WA1/2020. Viral RNA levels in the heart were measured at 6 dpi by RT-qPCR (n = 6-9; Mann-Whitney test: ** P < 0.01, *** P < 0.001). Bars show median values, and dotted lines indicate the LOD of the assays.

[00114] **FIG. 15O** Five-week-old K18-hACE2 female mice were immunized via an IN route with 10^{10} vp of ChAd-control or ChAd-SARS-CoV-2-S. At 6 weeks post-immunization, mice were challenged with 10^4 FFU of SARS-CoV-2 of WA1/2020. Viral RNA levels in the nasal washes were measured at 6 dpi by RT-qPCR (n = 6-9; Mann-Whitney test: ** P < 0.01, *** P < 0.001). Bars show median values, and dotted lines indicate the LOD of the assays.

[00115] **FIG. 15P** Five-week-old K18-hACE2 female mice were immunized via an IN route with 10^{10} vp of ChAd-control or ChAd-SARS-CoV-2-S. At 9 months post-immunization, mice were challenged with 10^4 FFU of SARS-CoV-2 of Wash-B.1.351. A plot of body weight change over time are shown. Data are the mean ± SEM comparing vaccine to control groups (n = 6-9 for each group; unpaired t test for area under curve, **** P < 0.0001).

[00116] **FIG. 15Q** Five-week-old K18-hACE2 female mice were immunized via an IN route with 10^{10} vp of ChAd-control or ChAd-SARS-CoV-2-S. At 6 weeks post-immunization, mice were challenged with 10^4 FFU of SARS-CoV-2 of Wash-B.1.351.

Viral RNA levels in the lung were measured at 6 dpi by RT-qPCR (n = 6-9; Mann-Whitney test: ** P < 0.01, *** P < 0.001). Bars show median values, and dotted lines indicate the LOD of the assays.

[00117] **FIG. 15R** Five-week-old K18-hACE2 female mice were immunized via an IN route with 10^{10} vp of ChAd-control or ChAd-SARS-CoV-2-S. At 9 months post-immunization, mice were challenged with 10^4 FFU of SARS-CoV-2 of Wash-B.1.351. Viral RNA levels in the heart were measured at 6 dpi by RT-qPCR (n = 6-9; Mann-Whitney test: ** P < 0.01, *** P < 0.001). Bars show median values, and dotted lines indicate the LOD of the assays.

[00118] **FIG. 15S** Five-week-old K18-hACE2 female mice were immunized via an IN route with 10^{10} vp of ChAd-control or ChAd-SARS-CoV-2-S. At 9 months post-immunization, mice were challenged with 10^4 FFU of SARS-CoV-2 of Wash-B.1.351. Viral RNA levels in the nasal washes were measured at 6 dpi by RT-qPCR (n = 6-9; Mann-Whitney test: ** P < 0.01, *** P < 0.001). Bars show median values, and dotted lines indicate the LOD of the assays.

[00119] **FIG. 15T** Five-week-old K18-hACE2 female mice were immunized via an IN route with 10^{10} vp of ChAd-control or ChAd-SARS-CoV-2-S. At 9 months post-immunization, mice were challenged with 10^4 FFU of SARS-CoV-2 of Wash-B.1.351. At 9 months post-immunization, mice were challenged with 10^4 FFU of SARS-CoV-2 of Wash-B.1.351. Viral RNA levels in the brain were measured at 6 dpi by RT-qPCR (n = 6-9; Mann-Whitney test: ** P < 0.01, *** P < 0.001). Bars show median values, and dotted lines indicate the LOD of the assays.

[00120] **FIG. 16A** shows ChAd-SARS-CoV-2-S vaccine induces neutralizing activity against WA1/2020, Wash-B.1.351, or Wash-B.1.1.28 as measured by FRNT. Five-week-old K18-hACE2 female mice were immunized with 10^9 vp ChAd-control or ChAd-SARS-CoV-2-S via an IN route and serum samples were collected at six weeks. Serum neutralization curves corresponding to individual mice are shown for the indicated vaccines (n = 7-20 per group). Each point represents the mean of two technical replicates.

[00121] **FIG. 16B** shows ChAd-SARS-CoV-2-S vaccine induces neutralizing activity against WA1/2020, Wash-B.1.351, or Wash-B.1.1.28 as measured by FRNT. Five-week-old K18-hACE2 female mice were immunized with 10^9 vp ChAd-control or ChAd-SARS-CoV-2-S via an IN route and serum samples were collected at 9 months. Serum neutralization curves corresponding to individual mice are shown for the indicated vaccines (n = 7-20 per group). Each point represents the mean of two technical replicates.

[00122] **FIG. 17** shows a sequence alignment of the stabilized variants of the SARS-Cov2-Omicron S-protein with the SARS-CoV2-Wuhan S-protein.

[00123] **FIG. 18** shows an evaluation of SARS-CoV-2 spike gene expression mediated by ChAd vector derivatives including Omicron BA.5.

[00124] **FIG. 19A** shows the scheme and timing of vaccination, blood sampling, virus challenge, and necropsy.

[00125] **FIG. 19B** shows binding of anti-SARS-CoV-2 IgG to SARS-CoV-2 Wuhan-1 S proteins. Kruskal Wallis with Dunn's post-test: ns, not significant; *, $P < 0.05$, **, $P < 0.01$; ***, $P < 0.001$ ****, $P < 0.0001$).

[00126] **FIG. 19C** shows binding of anti-SARS-CoV-2 IgA to SARS-CoV-2 Wuhan-1 S proteins. Kruskal Wallis with Dunn's post-test: ns, not significant; *, $P < 0.05$, **, $P < 0.01$; ***, $P < 0.001$ ****, $P < 0.0001$).

[00127] **FIG. 19D** shows binding of anti-SARS-CoV-2 IgG to BA.5 S proteins. Kruskal Wallis with Dunn's post-test: ns, not significant; *, $P < 0.05$, **, $P < 0.01$; ***, $P < 0.001$ ****, $P < 0.0001$).

[00128] **FIG. 19E** shows binding of anti-SARS-CoV-2 IgA to BA.5 S proteins. Kruskal Wallis with Dunn's post-test: ns, not significant; *, $P < 0.05$, **, $P < 0.01$; ***, $P < 0.001$ ****, $P < 0.0001$).

[00129] **FIG. 19F** shows binding of anti-SARS-CoV-2 IgG to SARS-CoV-2 BQ.1.1 S proteins. Kruskal Wallis with Dunn's post-test: ns, not significant; *, $P < 0.05$, **, $P < 0.01$; ***, $P < 0.001$ ****, $P < 0.0001$).

[00130] **FIG. 19G** shows binding of anti-SARS-CoV-2 IgA to SARS-CoV-2 BQ.1.1 S proteins. Kruskal Wallis with Dunn's post-test: ns, not significant; *, $P < 0.05$, **, $P < 0.01$; ***, $P < 0.001$ ****, $P < 0.0001$).

[00131]

[00132] **FIG. 20A** shows neutralizing activity against WA1/2020 by FRNT. Seven-week-old K18-hACE2 female mice were immunized with 10^9 vp ChAd-control or ChAd-SARS-CoV-2-S vaccines via an IN route and serum was obtained 28 days later. Each point represents data from an individual mouse and is the mean of two technical replicates.

[00133] **FIG. 20B** shows neutralizing activity against BA.5 by FRNT. Seven-week-old K18-hACE2 female mice were immunized with 10^9 vp ChAd-control or ChAd-SARS-CoV-2-S vaccines via an IN route and serum was obtained 28 days later. Each point represents data from an individual mouse and is the mean of two technical replicates.

[00134] **FIG. 20C** shows neutralizing activity against BF.7 by FRNT. Seven-week-old K18-hACE2 female mice were immunized with 10^9 vp ChAd-control or ChAd-SARS-CoV-2-S vaccines via an IN route and serum was obtained 28 days later. Each point represents data from an individual mouse and is the mean of two technical replicates.

[00135] **FIG. 20D** shows neutralizing activity against BQ.1.1 by FRNT. Seven-week-old K18-hACE2 female mice were immunized with 10^9 vp ChAd-control or ChAd-SARS-CoV-2-S vaccines via an IN route and serum was obtained 28 days later. Each point represents data from an individual mouse and is the mean of two technical replicates.

[00136] **FIG. 20E** shows neutralizing activity against XBB.1.1 by FRNT. Seven-week-old K18-hACE2 female mice were immunized with 10^9 vp ChAd-control or ChAd-SARS-CoV-2-S vaccines via an IN route and serum was obtained 28 days later. Each point represents data from an individual mouse and is the mean of two technical replicates.

[00137] **FIG 20F** show the neutralization data plotted as a direct comparison for a given vaccine (ChAd-SARS-CoV-2 S (Wuhan-1) against the indicated SARS-CoV-2 strains used for infection.

[00138] **Fig 20G** show the neutralization data plotted as a direct comparison for a given vaccine ChAd-SARS-CoV-2-S (BA.5) against the indicated SARS-CoV-2 strains used for infection.

[00139] **Fig 20H** show the neutralization data plotted as a direct comparison for a given vaccine ChAd-SARS-CoV-2-S (bivalent) against the indicated SARS-CoV-2 strains used for infection.

[00140] **FIG. 21A** shows the viral RNA levels in the lung at 6 dpi after challenge with SARS-CoV-2 WA1/2020 D614G (left) or Omicron BQ.1.1 (right). Animals were not vaccinated (control) or immunized via an intransal route with a single dose (10^9 viral particles total) of ChAd-control, ChAd-SARS-CoV-2 S (Wuhan-1), ChAd-SARS-CoV-2-S (BA.5) or bivalent (ChAd-SARS-CoV-2 S (Wuhan-1) + ChAd-SARS-CoV-2-S (BA.5)). Kruskal Wallis with Dunn's post-test: ns, not significant; *, $P < 0.05$, **, $P < 0.01$; ***, $P < 0.001$ ****, $P < 0.0001$).

[00141] **FIG. 21B** shows the viral RNA levels in the nasal turbinates (**FIG. 21B**), and nasal washes at 6 dpi after challenge with SARS-CoV-2 WA1/2020 D614G (left) or Omicron BQ.1.1 (right). Animals were not vaccinated (control) or immunized via an intransal route with a single dose (10^9 viral particles total) of ChAd-control, ChAd-SARS-CoV-2 S (Wuhan-1), ChAd-SARS-CoV-2-S (BA.5) or bivalent (ChAd-SARS-CoV-2 S (Wuhan-1) + ChAd-SARS-CoV-2-S (BA.5)). Kruskal Wallis with Dunn's post-test: ns, not significant; *, $P < 0.05$, **, $P < 0.01$; ***, $P < 0.001$ ****, $P < 0.0001$).

[00142] **FIG. 21C** shows the viral RNA levels in the nasal washes at 6 dpi after challenge with SARS-CoV-2 WA1/2020 D614G (left) or Omicron BQ.1.1 (right) at 6 dpi after challenge with SARS-CoV-2 WA1/2020 D614G (left) or Omicron BQ.1.1 (right). Animals were not vaccinated (control) or immunized via an intransal route with a single dose (10^9 viral particles total) of ChAd-control, ChAd-SARS-CoV-2 S (Wuhan-1), ChAd-SARS-CoV-2-S (BA.5) or bivalent (ChAd-SARS-CoV-2 S (Wuhan-1) + ChAd-SARS-

CoV-2-S (BA.5)). Kruskal Wallis with Dunn's post-test: ns, not significant; *, P < 0.05, **, P < 0.01; ***, P < 0.001 ****, P < 0.0001).

[00143] **FIG 22** shows a heat map of cytokine induction in vaccinated mice at 6 dpi after challenge with SARS-CoV-2 WA1/2020 D614G (left) or Omicron BQ.1.1 (right). On the bottom are indicated the vaccination status of mice (unvaccinated, ChAd-Control, ChAd-SARS-CoV-2 S, ChAd-SARS-CoV-2-S, or bivalent (ChAd-SARS-CoV-2 S (Wuhan-1) + ChAd-SARS-CoV-2-S (BA.5). The color of the map reflects the log2-fold change compared to naïve mice.

[00144] **FIG. 23** shows a sequence alignment of the stabilized variants of the BA.5 S proteins with respect to the Wuhan strain.

DETAILED DESCRIPTION

[00145] The present disclosure is based, at least in part, on the development of recombinant non-human adenoviral vector compositions and immunogenic compositions thereof for treating or preventing coronavirus infection. In addition, the disclosure provides methods of administering the compositions disclosed herein providing durable cellular and humoral mediated immunity against a coronavirus infection.

[00146] Severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) that was responsible for SARS epidemic in 2002-2004, Middle East respiratory syndrome coronavirus (MERS-CoV) that caused MERS first reported in 2012, and SARS-CoV-2 that has been responsible for the more recent coronavirus disease 2019 (Covid-19) pandemic all bind to angiotensin converting enzyme 2 (ACE2) on the surface of the cells in order to infect the cells. Basically ACE-2 is the functional receptor for SARS-CoV-1, SARS-CoV-2, and MERS-COV and most likely future SARS-COV variants. ACE-2 is an important component of Renin–Angiotensin–Aldosterone System (RAAS). ACE-2 converts angiotensin 2 to angiotensin 1-7. High angiotensin 2 is associated with vasoconstriction, inflammation, and acute lung injury. ACE2 is expressed in various organs including lungs, heart, kidney, liver, intestine, and other tissues. SARS-CoV virus bind to ACE-2 and enter the cells.

[00147] The SARS-CoV-2 RNA genome is approximately 30,000 nucleotides in length. The 5' two-thirds encode nonstructural proteins that enable genome replication and viral RNA synthesis. The remaining one-third encode structural proteins such as spike (S), envelope, membrane, and nucleoprotein (NP) that form the spherical virion, and accessory proteins that regulate cellular responses. The S protein forms homotrimeric spikes on the virion and engages the cell-surface receptor angiotensin-converting enzyme 2 (ACE2) to promote coronavirus entry into human cells. The SARS-CoV and SARS-CoV-2 S proteins are cleaved sequentially during the entry process to yield S1 and S2 fragments, followed by further processing of S2 to yield a smaller S2' protein (Hoffmann et al., 2020). The S1 protein includes the receptor binding domain (RBD) and the S2 protein promotes membrane fusion. The structure of a soluble, stabilized prefusion form of the SARS-CoV-2 S protein was solved by cryo-electron microscopy, revealing considerable similarity to the SARS-CoV S protein. This form of the S protein is recognized by potently neutralizing monoclonal antibodies and could serve as a promising vaccine target.

[00148] Release of the SARS-CoV-2 genome sequence prompted academic, government, and industry groups to immediately begin developing vaccine candidates that principally targeted the viral S protein. Improved genomic sequencing capabilities also provided a multitude of information about the variants of SARS-CoV-2 circulating at any given point in time. SARS-CoV-2 mutates over time resulting in emergence of new variants, which differ in sequence, transmissibility, and severity of infection. Various SARS-CoV-2 variants have emerged. The original form of SARS-CoV-2 identified in December 2019; is often referred to as the "original" or "Wuhan" strain. Subsequently variants including alpha (B.1.1.7), beta (B.1.351), gamma (P.1) and delta (B.1.617.2 and AY Sublineages) emerged between December 2020 and April 2021. However, the currently predominant variant is the Omicron variant (B.1.1.529 and BA sublineages) that emerged around November 2021. As used herein, the term "variant" in context of viruses is 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 can emerge. As used here a "lineage" is a group of

closely related viruses with a common ancestor. As used herein the term “sublineage” or “subvariant” is a group of similar viruses within a lineage. Sub-lineages or sub-variants of Omicron and the other variants continue to emerge.

[00149] Multiple platforms have been developed to deliver the SARS-CoV-2 S protein including DNA plasmid, lipid nanoparticle encapsulated mRNA, inactivated virion, and viral-vectored vaccines. Several vaccines have entered clinical trials to evaluate safety, and some have advanced to trials assessing immunogenicity and efficacy. Because of the urgency of the pandemic, most vaccines advanced to human testing without substantive efficacy data in animals. This circumstance occurred in part because vaccine design and development has outpaced the generation of accessible pre-clinical disease models of SARS-CoV-2 infection and pathogenesis.

[00150] Adenovirus (Ad)-based vaccines against Betacoronaviruses have been evaluated previously. A single dose of a chimpanzee Ad-vectored vaccine encoding the full-length S protein of MERS-CoV protected human dipeptidyl peptidase 4 (hDPP4) transgenic mice from infection, reduced virus shedding and enhanced survival in camels, and was safe and immunogenic in humans in a phase 1 clinical trial. A human Ad-based vaccine expressing a MERS S1-CD40L fusion protein also was protective in transgenic hDPP4 mice. An Ad-based SARS-CoV vaccine expressing a full-length S protein prevented pneumonia in ferrets after challenge and was highly immunogenic in rhesus macaques. A chimpanzee Ad vector (Y25, a simian Ad-23) encoding the wild-type SARS-CoV-2 S protein (ChAdOx1 nCoV-19) is currently under evaluation in humans as a single intramuscular injection (NCT04324606). Preliminary pre-print analysis suggests this vaccine protects against lung infection and pneumonia but not against upper respiratory tract infection and nasal virus shedding (doi.org/10.1101/2020.05.13.093195).). However, this vaccine failed as a nasal vaccine.

[00151] Disclosed herein are compositions, methods, and treatment plans for treating an individual who is at risk of having a respiratory viral infection, has mild symptoms of a respiratory viral infection, or has severe symptoms of a respiratory viral infection. A composition of the present disclosure may be used to treat, prevent, or

reduce the infectivity of a respiratory viral infection. A treatment plan may comprise administering a composition of the disclosure to an individual at risk of having a viral infection or who has a viral infection, thereby preventing or treating the viral infection. In some embodiments, a viral transmission may be prevented or reduced by reducing viral infection in the upper respiratory tract. Compositions and methods of the disclosure provide robust antigen-specific antibody, neutralizing antibody, and B and T cell responses. This confers protection against infection with marked reductions in viral yield, inflammation, and pathology in the lung. Compositions and methods of the disclosure generates robust mucosal immunity including high levels of neutralizing and anti-RBD IgA and IgG in the serum and lung and SARS-CoV-2 specific resident memory T cells in the lung. The disclosed compositions and methods completely protect against SARS-CoV-2 infection in the nasal passages, upper airways, lung tissues, and all other sites of possible dissemination. Based on measurements of anti-NP and anti-ORF8 responses, a single intranasal dose of the presently disclosed compositions confers sterilizing immunity, which has not been described before with any COVID-19 vaccine, much less with a single dose administration.

[00152] A composition of the present disclosure may be formulated for locally, for example intra-nasally (*e.g.*, as a nasal spray, or inhalation), or systemically (*e.g.*, intravenous or intraperitoneal) and administered for treating or preventing a respiratory viral infection (*e.g.*, a coronavirus infection such as SARS-CoV-2). The compositions of the present disclosure (*e.g.*, compositions formulated for nasal delivery or inhalation) may be administered to a subject who may be at risk of contracting a viral infection (*e.g.*, SARS-CoV-2). For example, the compositions of the present disclosure may be administered to individuals in high-risk environments (*e.g.*, healthcare workers), individuals who have been or who are suspected to have been exposed to a virus (*e.g.*, SARS-CoV-2), or individuals who have tested positive for a viral infection. A composition of the present disclosure may be administered to an individual who is displaying symptoms of a respiratory infection (*e.g.*, a SARS-CoV-2 infection) or who is asymptomatic at the time of administration. In some embodiments, the compositions of

the present disclosure may be self-administered by the individual (*e.g.*, as a nasal spray or inhalation) and may be administered outside of a medical facility (*e.g.*, at home).

[00153] The methods and compositions disclosed herein may be used to treat, prevent, or reduce the infectivity of a respiratory viral infection. In some embodiments, the viral infection may be a coronavirus infection. Pathogens with long incubation periods, such as SARS-CoV-2 which has a median incubation period of about five days, may have high risk of transmission since many infected individuals may be unaware that they are infected. Additionally, carriers of coronavirus may frequently be asymptomatic or have mild symptoms, leading to unknowing contact between a viral host and other members of a population. A subject at risk for a coronavirus infection may come in contact with an asymptomatic carrier of the coronavirus infection, thereby unknowingly contracting the coronavirus infection. Methods and compositions are needed to prevent coronavirus infections in at-risk individuals (*e.g.*, individuals who have come in contact with a carrier of a coronavirus or who may come in contact with a carrier of a coronavirus). In some embodiments, the compositions, methods, or treatment regiments disclosed herein may treat or prevent a SARS-CoV-2 infection (*e.g.*, COVID-19).

[00154] Discussed below are components to be used to prepare the disclosed compositions as well as the compositions themselves to be used within the methods disclosed herein. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and permutation of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a particular compound is disclosed and discussed and a number of modifications that can be made to a number of molecules of the compound are discussed, specifically contemplated is each and every combination and permutation of the compound and the modifications that are possible unless specifically indicated to the contrary. Thus, if a class of molecules A, B, and C are disclosed as well as a class of molecules D, E, and F and an example of a combination molecule, A-D is disclosed, then even if each is not

individually recited each is individually and collectively contemplated meaning combinations, A-E, A-F, B-D, B-E, B-F, C-D, C-E, and C-F are considered disclosed. Likewise, any subset or combination of these is also disclosed. Thus, for example, the sub-group of A-E, B-F, and C-E would be considered disclosed. This concept applies to all aspects of this application including, but not limited to, steps in methods of making and using the disclosed compositions. Thus, if there are a variety of additional steps that can be performed it is understood that each of these additional steps can be performed with any specific embodiment or combination of embodiments of the disclosed methods.

[00155] Various aspects of the invention are described in further detail in the following sections.

I. Definitions

[00156] So that the present invention may be more readily understood, certain terms are first defined. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which embodiments of the invention pertain. Many methods and materials similar, modified, or equivalent to those described herein can be used in the practice of the embodiments of the present invention without undue experimentation, the preferred materials and methods are described herein. In describing and claiming the embodiments of the present invention, the following terminology will be used in accordance with the definitions set out below.

[00157] The phraseology and terminology employed herein are for the purpose of description and should not be regarded as limiting. For example, the use of a singular term, such as, “a” is not intended as limiting of the number of items. Also, the use of relational terms such as, but not limited to, “top,” “bottom,” “left,” “right,” “upper,” “lower,” “down,” “up,” and “side,” are used in the description for clarity in specific reference to the figures and are not intended to limit the scope of the present inventive concept or the appended claims.

[00158] Any term of degree such as, but not limited to, “substantially” as used in the description and the appended claims, should be understood to include an exact, or a similar, but not exact configuration. For example, “a substantially planar surface” means having an exact planar surface or a similar, but not exact planar surface. Similarly, the terms “about” or “approximately,” as used in the description and the appended claims, should be understood to include the recited values or a value that is three times greater or one third of the recited values. For example, about 3 mm includes all values from 1 mm to 9 mm, and approximately 50 degrees includes all values from 16.6 degrees to 150 degrees. For example, they can refer to less than or equal to ± 5%, such as less than or equal to ± 2%, such as less than or equal to ± 1%, such as less than or equal to ± 0.5%, such as less than or equal to ± 0.2%, such as less than or equal to ± 0.1%, such as less than or equal to ± 0.05%.

[00159] The terms “comprising,” “including” and “having” are used interchangeably in this disclosure. The terms “comprising,” “including” and “having” mean to include, but not necessarily be limited to the things so described.

[00160] The terms “or” and “and/or,” as used herein, are to be interpreted as inclusive or meaning any one or any combination. Therefore, “A, B or C” or “A, B and/or C” mean any of the following: “A,” “B” or “C”; “A and B”; “A and C”; “B and C”; “A, B and C.” An exception to this definition will occur only when a combination of elements, functions, steps or acts are in some way inherently mutually exclusive.

[00161] Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. The following references provide one of skill with a general definition of many of the terms used in this invention: Singleton et al., Dictionary of Microbiology and Molecular Biology (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger et al. (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991), all of which are incorporated by reference herein. As used herein, the following terms have the meanings ascribed to them below, unless specified otherwise.

[00162] The phraseology and terminology employed herein are for the purpose of description and should not be regarded as limiting. When introducing elements of the present disclosure or the preferred aspects(s) thereof, the articles “a”, “an”, “the” and “said” are intended to mean that there are one or more of the elements. The terms “comprising”, “including” and “having” are intended to be inclusive and mean that there may be additional elements other than the listed elements. Wherever the terms “comprising” or “including” are used, it should be understood the disclosure also expressly contemplates and encompasses additional aspects “consisting of” the disclosed elements, in which additional elements other than the listed elements are not included.

[00163] The term “about” or “approximately,” as used herein, can mean within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, e.g., the limitations of the measurement system. For example, “about” can mean within 1 or more than 1 standard deviation, per the practice in the given value. Where particular values are described in the application and claims, unless otherwise stated the term “about” can mean an acceptable error range for the particular value, such as 10% of the value modified by the term “about.” As used herein, the term “about,” can mean relative to the recited value, e.g., amount, dose, temperature, time, percentage, etc., ±10%, ±9%, ±8%, ±7%, ±6%, ±5%, ±4%, ±3%, ±2%, or ±1%.

[00164] Further, as the present inventive concept is susceptible to “aspects” or “embodiments” used interchangeably, of many different forms, it is intended that the present disclosure be considered as an example of the principles of the present inventive concept and not intended to limit the present inventive concept to the specific aspects shown and described. Any one of the features of the present inventive concept may be used separately or in combination with any other feature. References to the terms “aspect,” “aspects,” and/or the like in the description mean that the feature and/or features being referred to are included in, at least, one aspect of the description. Separate references to the terms “aspect,” “aspects,” and/or the like in the description do not necessarily refer to the same aspect and are also not mutually exclusive unless

so stated and/or except as will be readily apparent to those skilled in the art from the description. For example, a feature, structure, process, step, action, or the like described in one aspect may also be included in other aspects but is not necessarily included. Thus, the present inventive concept may include a variety of combinations and/or integrations of the aspects described herein. Additionally, all aspects of the present disclosure, as described herein, are not essential for its practice. Likewise, other systems, methods, features, and advantages of the present inventive concept will be, or become, apparent to one with skill in the art upon examination of the figures and the description. It is intended that all such additional systems, methods, features, and advantages be included within this description, be within the scope of the present inventive concept, and be encompassed by the claims.

[00165] The term “nucleic acid” or “polynucleotide” refers to deoxyribonucleic acids (DNA) or ribonucleic acids (RNA) and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues See, e.g., Batzer et al., Nucleic Acid Res. 19:5081 (1991), the disclosure of which is incorporated in its entirety herein. In some aspects, a nucleic acid sequence may encode a polypeptide sequence.

[00166] As used herein, the term “encode” refers broadly to any process whereby the information in a polymeric macromolecule is used to direct the production of a second molecule that is different from the first. The second molecule may have a chemical structure that is different from the chemical nature of the first molecule. For example, in some aspects, the term “encode” describes the process of semi-

conservative DNA replication, where one strand of a double-stranded DNA molecule is used as a template to encode a newly synthesized complementary sister strand by a DNA-dependent DNA polymerase. In other aspects, a DNA molecule can encode an RNA molecule (e.g., by the process of transcription that uses a DNA-dependent RNA polymerase enzyme). Also, an RNA molecule can encode a polypeptide, as in the process of translation. When used to describe the process of translation, the term "encode" also extends to the triplet codon that encodes an amino acid. In some aspects, an RNA molecule can encode a DNA molecule, e.g., by the process of reverse transcription incorporating an RNA-dependent DNA polymerase. In another aspect, a DNA molecule can encode a polypeptide, where it is understood that "encode" as used in that case incorporates both the processes of transcription and translation.

[00167] A nucleic acid is "operably linked" when it is placed into a structural or functional relationship with another nucleic acid sequence. For example, one segment of DNA may be operably linked to another segment of DNA if they are positioned relative to one another on the same contiguous DNA molecule and have a structural or functional relationship, such as a promoter or enhancer that is positioned relative to a coding sequence so as to facilitate transcription of the coding sequence; a ribosome binding site that is positioned relative to a coding sequence so as to facilitate translation; or a pre-sequence or secretory leader that is positioned relative to a coding sequence so as to facilitate expression of a pre-protein (e.g., a pre-protein that participates in the secretion of the encoded polypeptide). In other examples, the operably linked nucleic acid sequences are not contiguous, but are positioned in such a way that they have a functional relationship with each other as nucleic acids or as proteins that are expressed by them. Enhancers, for example, do not have to be contiguous. Linking may be accomplished by ligation at convenient restriction sites or by using synthetic oligonucleotide adaptors or linkers.

[00168] In some aspects, the nucleic acid encoding a polypeptide may be operably linked to an expression control sequence.

[00169] Expression Control Sequences refer to nucleic acid sequences that regulate the expression of a heterologous nucleic acid sequence to which it is

operatively linked. Expression control sequences are operatively linked to a nucleic acid sequence when the expression control sequences control and regulate the transcription and, as appropriate, translation of the nucleic acid sequence. Thus, expression control sequences can include appropriate promoters, enhancers, transcription terminators, a start codon (ATG) in front of a protein-encoding gene, splicing signal for introns, maintenance of the correct reading frame of that gene to permit proper translation of mRNA, and stop codons. The term "control sequences" is intended to include, at a minimum, components whose presence can influence expression, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences. Expression control sequences can include a promoter.

[00170] A promoter is a minimal sequence sufficient to direct transcription. Also included are those promoter elements which are sufficient to render promoter-dependent gene expression controllable for cell- type specific, tissue-specific, or inducible by external signals or agents; such elements may be located in the 5' or 3' regions of the gene. Both constitutive and inducible promoters are included (see for example, Bitter et al., Methods in Enzymology 153:516-544, 1987). For example, when cloning in bacterial systems, inducible promoters such as pL of bacteriophage lambda, plac, ptrp, ptac (ptrp-lac hybrid promoter) and the like may be used. In one embodiment, when cloning in mammalian cell systems, promoters derived from the genome of mammalian cells (such as metallothionein promoter) or from mammalian viruses (such as the retrovirus long terminal repeat; the adenovirus late promoter; the vaccinia virus 7.5K promoter) can be used. Promoters produced by recombinant DNA or synthetic techniques may also be used to provide for transcription of the nucleic acid sequences. Expression vector: A vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient cis-acting elements for expression; other elements for expression can be supplied by the host cell or in an in vitro expression system. Expression vectors include all those known in the art, such as cosmids, plasmids (*e.g.*, naked or contained in liposomes) and viruses (e.g.,

lentiviruses, retroviruses, adenoviruses, and adeno-associated viruses) that incorporate the recombinant polynucleotide.

[00171] The term “heterologous” refers to originating from a different genetic source. A nucleic acid molecule that is heterologous to a cell originated from a genetic source other than the cell in which it is expressed. In one specific, non-limiting example, a heterologous nucleic acid molecule encoding a recombinant coronavirus S protein is expressed in a cell, such as a mammalian cell. Methods for introducing a heterologous nucleic acid molecule in a cell or organism are well known in the art, for example transformation with a nucleic acid, including electroporation, lipofection, particle gun acceleration, and homologous recombination

[00172] The terms “peptide,” “polypeptide,” and “protein” are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a protein's or peptide's sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. “Polypeptides” include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. A polypeptide includes a natural peptide, a recombinant peptide, or a combination thereof.

[00173] Within the context of the application a protein is represented by an amino acid sequence and correspondingly a nucleic acid molecule or a polynucleotide represented by a nucleic acid sequence. Identity and similarity between sequences: throughout this application, each time one refers to a specific amino acid sequence SEQ ID NO (take SEQ ID NO: Y as example), one may replace it by: a polypeptide represented by an amino acid sequence comprising a sequence that has at least 60%

sequence identity or similarity with amino acid sequence SEQ ID NO: Y. Another preferred level of sequence identity or similarity is 65%. Another preferred level of sequence identity or similarity is 70%. Another preferred level of sequence identity or similarity is 75%. Another preferred level of sequence identity or similarity is 80%. Another preferred level of sequence identity or similarity is 85%. Another preferred level of sequence identity or similarity is 90%. Another preferred level of sequence identity or similarity is 95%. Another preferred level of sequence identity or similarity is 98%. Another preferred level of sequence identity or similarity is 99%.

[00174] Each amino acid sequence described herein by virtue of its identity or similarity percentage with a given amino acid sequence respectively has in a further preferred aspect an identity or a similarity of at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% with the given nucleotide or amino acid sequence, respectively. The terms “homology”, “sequence identity” and the like are used interchangeably herein. Sequence identity is described herein as a relationship between two or more amino acid (polypeptide or protein) sequences or two or more nucleic acid (polynucleotide) sequences, as determined by comparing the sequences. In a preferred aspect, sequence identity is calculated based on the full length of two given SEQ ID NO’s or on a part thereof. Part thereof preferably means at least 50%, 60%, 70%, 80%, 90%, or 100% of both SEQ ID NO’s. In the art, “identity” also refers to the degree of sequence relatedness between amino acid or nucleic acid sequences, as the case may be, as determined by the match between strings of such sequences. The degree of sequence identity between two sequences can be determined, for example, by comparing the two sequences using computer programs commonly employed for this purpose, such as global or local alignment algorithms. Non-limiting examples include BLASTp, BLASTn, Clustal W,

MAFFT, Clustal Omega, AlignMe, Praline, GAP, BESTFIT, or another suitable method or algorithm. A Needleman and Wunsch global alignment algorithm can be used to align two sequences over their entire length or part thereof (part thereof may mean at least 50%, 60%, 70%, 80%, 90% of the length of the sequence), maximizing the number of matches and minimizes the number of gaps. Default settings can be used and preferred program is Needle for pairwise alignment (in an aspect, EMBOSS Needle 6.6.0.0, gap open penalty 10, gap extent penalty: 0.5, end gap penalty: false, end gap open penalty: 10 , end gap extent penalty: 0.5 is used) and MAFFT for multiple sequence alignment (in an aspect, MAFFT v7Default value is: BLOSUM62 [bl62], Gap Open: 1.53, Gap extension: 0.123, Order: aligned, Tree rebuilding number: 2, Guide tree output: ON [true], Max iterate: 2, Perform FFTS: none is used).

[00175] "Similarity" between two amino acid sequences is determined by comparing the amino acid sequence and its conserved amino acid substitutes of one polypeptide to the sequence of a second polypeptide. Similar algorithms used for determination of sequence identity may be used for determination of sequence similarity. Optionally, in determining the degree of amino acid similarity, the skilled person may also take into account so-called conservative amino acid substitutions. As used herein, "conservative" amino acid substitutions refer to the interchangeability of residues having similar side chains.

[00176] For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulphur-containing side chains is cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, and asparagine-glutamine. Substitutional variants of the amino acid sequence disclosed herein are those in which at least one residue in the disclosed sequences has been removed and a different

residue inserted in its place. Preferably, the amino acid change is conservative. Preferred conservative substitutions for each of the naturally occurring amino acids are as follows: Ala to Ser; Arg to Lys; Asn to Gln or His; Asp to Glu; Cys to Ser or Ala; Gln to Asn; Glu to Asp; Gly to Pro; His to Asn or Gln; Ile to Leu or Val; Leu to Ile or Val; Lys to Arg; Gln or Glu; Met to Leu or Ile; Phe to Met, Leu or Tyr; Ser to Thr; Thr to Ser; Trp to Tyr; Tyr to Trp or Phe; and Val to Ile or Leu.

[00177] An adjuvant refers to a vehicle used to enhance antigenicity. In some embodiments, an adjuvant can include a suspension of minerals (alum, aluminum hydroxide, or phosphate) on which antigen is adsorbed; or water - in-oil emulsion, for example, in which antigen solution is emulsified in mineral oil (Freund incomplete adjuvant), sometimes with the inclusion of killed mycobacteria (Freund's complete adjuvant) to further enhance antigenicity (inhibits degradation of antigen and/or causes influx of macrophages). In some embodiments, the adjuvant used in a disclosed immunogenic composition is a combination of lecithin and carbomer homopolymer (such as the ADJUPLEX™ adjuvant available from Advanced BioAdjuvants, LLC, see also Wegmann, Clin Vaccine Immunol, 22(9): 1004-1012, 2015). Additional adjuvants for use in the disclosed immunogenic compositions include the QS21 purified plant extract, Matrix M, AS01, MF59, and ALFQ adjuvants. Immunostimulatory oligonucleotides (such as those including a CpG motif) can also be used as adjuvants. Adjuvants include biological molecules (a "biological adjuvant"), such as costimulatory molecules. Exemplary adjuvants include IL-2, RANTES, GM-CSF, TNF-a, IFN-γ, G-CSF, LFA-3, CD72, B7-1, B7-2, OX-40L, 4-1BBL and toll-like receptor (TLR) agonists, such as TLR-9 agonists. Additional description of adjuvants can be found, for example, in Singh (ed.) Vaccine Adjuvants and Delivery Systems. Wiley-Interscience, 2007). Adjuvants can be used in combination with the disclosed compositions.

[00178] The term “antibody” refers to an immunoglobulin, antigen-binding fragment, or derivative thereof, which specifically binds and recognizes an analyte (antigen) such as a coronavirus S protein, an antigenic fragment thereof, or a dimer or multimer of the antigen. The term "antibody" is used herein in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal

antibodies, polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), and antibody fragments, so long as they exhibit the desired antigen-binding activity. Non-limiting examples of antibodies include, for example, intact immunoglobulins and variants and fragments thereof that retain binding affinity for the antigen. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')2; diabodies; linear antibodies; single-chain antibody molecules (*e.g.* scFv); and multispecific antibodies formed from antibody fragments. Antibody fragments include antigen binding fragments either produced by the modification of whole antibodies or those synthesized de novo using recombinant DNA methodologies (see, e.g., Kontermann and Dubel (Ed), Antibody Engineering, Vols. 1-2, 2nd Ed., Springer Press, 2010).

[00179] Coronavirus is a family of positive-sense, single-stranded RNA viruses that are known to cause severe respiratory illness. Viruses currently known to infect human from the coronavirus family are from the alphacoronavirus and betacoronavirus genera. Additionally, it is believed that the gammacoronavirus and deltacoronavirus genera may infect humans in the future.

[00180] Non-limiting examples of betacoronaviruses include Middle East respiratory syndrome coronavirus (MERS-CoV), Severe Acute Respiratory Syndrome coronavirus (SARS-CoV), Human coronavirus HKU1 (HKUl-CoV), Human coronavirus OC43 (OC43-CoV), Murine Hepatitis Virus (MHV-CoV), Bat SARS- like coronavirus WIV1 (WIVl-CoV), and Human coronavirus HKU9 (HKU9-CoV). Non-limiting examples of alphacoronaviruses include human coronavirus 229E (229E-CoV), human coronavirus NL63 (NL63-CoV), porcine epidemic diarrhea virus (PEDV), and Transmissible gastroenteritis coronavirus (TGEV). A non-limiting example of a deltacoronaviruses is the Swine Delta Coronavirus (SDCV).

[00181] The viral genome is capped, polyadenylated, and covered with nucleocapsid proteins. The coronavirus virion includes a viral envelope containing type I fusion glycoproteins referred to as the spike (S) protein. Most coronaviruses have a common genome organization with the replicase gene included in the 5'-portion of the genome, and structural genes included in the 3'-portion of the genome.

[00182] Coronavirus Spike (S) protein: A class I fusion glycoprotein initially synthesized as a precursor protein. Individual precursor S polypeptides form a homotrimer and undergo glycosylation within the Golgi apparatus as well as processing to remove the signal peptide, and cleavage by a cellular protease to generate separate SI and S2 polypeptide chains, which remain associated as S1/S2 protomers within the homotrimer and is therefore a trimer of heterodimers. The S1 subunit is distal to the virus membrane and contains the receptor-binding domain (RBD) that mediates virus attachment to its host receptor. The S2 subunit contains fusion protein machinery, such as the fusion peptide, two heptad-repeat sequences (HR1 and HR2) and a central helix typical of fusion glycoproteins, a transmembrane domain, and the cytosolic tail domain.

[00183] Coronavirus Spike (S) protein prefusion conformation is a structural conformation adopted by the ectodomain of the coronavirus S protein following processing into a mature coronavirus S protein in the secretory system, and prior to triggering of the fusogenic event that leads to transition of coronavirus S to the postfusion conformation. The three-dimensional structure of an exemplary coronavirus S protein (HKU1- CoV) in a prefusion conformation is disclosed herein and provided in Kirchdoerfer et al., "Pre-fusion structure of a human coronavirus spike protein," Nature, 531 : 118-121, 2016 (incorporated by reference herein).

[00184] A coronavirus S ectodomain trimer "stabilized in a prefusion conformation" comprises one or more amino acid substitutions, deletions, or insertions compared to a native coronavirus S sequence that provide for increased retention of the prefusion conformation compared to coronavirus S ectodomain trimers formed from a corresponding native coronavirus S sequence. The "stabilization" of the prefusion conformation by the one or more amino acid substitutions, deletions, or insertions can be, for example, energetic stabilization (for example, reducing the energy of the prefusion conformation relative to the post-fusion open conformation) and/or kinetic stabilization (for example, reducing the rate of transition from the prefusion conformation to the postfusion conformation). Additionally, stabilization of the coronavirus S ectodomain trimer in the prefusion conformation can include an increase in resistance to denaturation compared to a corresponding native coronavirus S sequence. Methods of

determining if a coronavirus S ectodomain trimer is in the prefusion conformation are provided herein, and include (but are not limited to) negative-stain electron microscopy and antibody binding assays using a prefusion-conformation-specific antibody.

[00185] Degenerate variant: In the context of the present disclosure, a "degenerate variant" refers to a polynucleotide encoding a polypeptide that includes a sequence that is degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences encoding a peptide are included as long as the amino acid sequence of the peptide encoded by the nucleotide sequence is unchanged.

[00186] In one example, a desired response is to inhibit or reduce or prevent CoV (such as SARS-CoV-2) infection. The CoV infection does not need to be completely eliminated or reduced or prevented for the method to be effective. For example, administration of an effective amount of the immunogen can induce an immune response that decreases the CoV infection (for example, as measured by infection of cells, or by number or percentage of subjects infected by the CoV) by a desired amount, for example by at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or even at least 100% (elimination or prevention of detectable CoV infection), as compared to a suitable control. Epitope: An antigenic determinant. These are particular chemical groups or peptide sequences on a molecule that are antigenic, such that they elicit a specific immune response, for example, an epitope is the region of an antigen to which B and/or T cells respond. An antibody can bind to a particular antigenic epitope, such as an epitope on coronavirus S ectodomain, such as a SARS-CoV S ectodomain. Epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein.

[00187] Expression refers to transcription or translation of a nucleic acid sequence. For example, a gene is expressed when its DNA is transcribed into an RNA or RNA fragment, which in some examples is processed to become mRNA. A gene may also be expressed when its mRNA is translated into an amino acid sequence, such as a protein or a protein fragment. In a particular example, a heterologous gene is expressed

when it is transcribed into an RNA. In another example, a heterologous gene is expressed when its RNA is translated into an amino acid sequence. The term "expression" is used herein to denote either transcription or translation. Regulation of expression can include controls on transcription, translation, RNA transport and processing, degradation of intermediary molecules such as mRNA, or through activation, inactivation, compartmentalization or degradation of specific protein molecules after they are produced.

[00188] The term “host cell,” refers to cells into which an exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include “transformants” and “transformed cells,” which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Host cells include cells within the body of a subject (e.g., a mammalian subject (e.g., a human)) into which an exogenous nucleic acid has been introduced.

[00189] By “immunogen” is meant any polypeptide that can induce an immune response in a subject upon administration. In some embodiments, the immunogen is encoded by a nucleic acid molecule that may be incorporated into, for example, a polynucleotide or vector, for subsequent expression of the immunogen (e.g., a gene product of interest, or fragment thereof (e.g., a polypeptide)).

[00190] The term “immunogenic composition” as used herein, is defined as material used to provoke an immune response and may confer immunity after administration of the immunogenic composition to a subject.

[00191] The term “immunostimulatory agent” refers to substances (e.g., drugs and nutrients) that stimulate the immune system by inducing activation or increasing activity of any of its components. An immunostimulatory agent includes a cytokine (e.g., the granulocyte macrophage colony-stimulating factor) and interferon (e.g., IFN-α and/or IFN-γ).

[00192] By “pharmaceutical composition” is meant any composition that contains a therapeutically or biologically active agent, such as an immunogenic composition or vaccine, preferably including a nucleotide sequence encoding an antigenic gene product of interest, or fragment thereof, that is suitable for administration

to a subject and that treats or prevents a disease (e.g., CoV infection) or reduces or ameliorates one or more symptoms of the disease (e.g., CoV viral titer, viral spread, infection, and/or cell fusion)). For the purposes of this invention, pharmaceutical compositions include vaccines, and pharmaceutical compositions suitable for delivering a therapeutic or biologically active agent can include, for example, tablets, gelcaps, capsules, pills, powders, granulates, suspensions, emulsions, solutions, gels, hydrogels, oral gels, pastes, eye drops, ointments, creams, plasters, drenches, delivery devices, suppositories, enemas, injectables, implants, sprays, or aerosols. Any of these formulations can be prepared by well-known and accepted methods of art. See, for example, Remington: The Science and Practice of Pharmacy (21st ed.), ed. A. R. Gennaro, Lippincott Williams & Wilkins, 2005, and Encyclopedia of Pharmaceutical Technology, ed. J. Swarbrick, Informa Healthcare, 2006, each of which is hereby incorporated by reference. An Immune response is a response of a cell of the immune system, such as a B cell, T cell, or monocyte, to a stimulus. In one embodiment, the response is specific for a particular antigen (an "antigen- specific response"). In one embodiment, an immune response is a T cell response, such as a CD4+ response or a CD8+ response. In another embodiment, the response is a B cell response, and results in the production of specific antibodies.

[00193] A prime-boost vaccination is an immunotherapy including administration of a first immunogenic composition (the primary vaccine) followed by administration of a second immunogenic composition (the booster vaccine) to a subject to induce an immune response. The priming vaccine and/or the booster vaccine include a vector (such as a viral vector, RNA, or DNA vector) expressing the antigen to which the immune response is directed. The booster vaccine is administered to the subject after the priming vaccine; a suitable time interval between administration of the priming vaccine and the booster vaccine, and examples of such timeframes are disclosed herein. In some embodiments, the priming vaccine, the booster vaccine, or both primer vaccine and the booster vaccine additionally include an adjuvant.

[00194] A vaccine is a pharmaceutical composition that induces a prophylactic or therapeutic immune response in a subject. In some cases, the immune

response is a protective immune response. Typically, a vaccine induces an antigen-specific immune response to an antigen of a pathogen, for example a viral pathogen, or to a cellular constituent correlated with a pathological condition. A vaccine may include a polynucleotide (such as a nucleic acid encoding a disclosed antigen), a peptide or polypeptide (such as a disclosed antigen), a virus, a cell or one or more cellular constituents. In a non-limiting example, a vaccine induces an immune response that reduces the severity of the symptoms associated with a coronavirus infection (such as a SARS-CoV or MERS-CoV infection) and/or decreases the viral load compared to a control. In another non-limiting example, a vaccine induces an immune response that reduces and/or prevents a coronavirus infection (such as a SARS-CoV or MERS-CoV infection) compared to a control. In some aspects, the vaccine of the current disclosure may be referred to as “bivalent” or “multivalent” because they are as a single vector capable of simultaneous induction of an immune response against two or more viral pathogens, e.g., two isolates of Coronavirus.

[00195] A vector is an entity containing a DNA or RNA molecule bearing a promoter(s) that is operationally linked to the coding sequence of an antigen(s) of interest and can express the coding sequence. Non-limiting examples include a naked or packaged (lipid and/or protein) DNA, a naked or packaged RNA, a subcomponent of a virus or bacterium or other microorganism that may be replication-incompetent, or a virus or bacterium or other microorganism that may be replication-competent. A vector is sometimes referred to as a construct. Recombinant DNA vectors are vectors having recombinant DNA. A vector can include nucleic acid sequences that permit it to replicate in a host cell, such as an origin of replication. A vector can also include one or more selectable marker genes and other genetic elements known in the art. Viral vectors are recombinant nucleic acid vectors having at least some nucleic acid sequences derived from one or more viruses.

[00196] Virus-like particle (VLP) are a non-replicating, viral shell, derived from any of several viruses. VLPs are generally composed of one or more viral proteins, such as, but not limited to, those proteins referred to as capsid, coat, shell, surface and/or envelope proteins, or particle-forming polypeptides derived from these proteins.

VLPs can form spontaneously upon recombinant expression of the protein in an appropriate expression system. Methods for producing particular VLPs are known in the art. The presence of VLPs following recombinant expression of viral proteins can be detected using conventional techniques known in the art, such as by electron microscopy, biophysical characterization, and the like. Further, VLPs can be isolated by known techniques, e.g., density gradient centrifugation and identified by characteristic density banding. See, for example, Baker et al. (1991) Biophys. J. 60: 1445-1456; and Hagensee et al. (1994) /. Virol. 68:4503-4505; Vincente, J Invertebr Pathol., 2011 ; Schneider-Ohrum and Ross, Curr. Top. Microbiol. Immunol, 354: 53073, 2012).

II. COMPOSITIONS

[00197] A composition of the present disclosure may comprise one or more active agents. In some embodiments, an active agent may be an agent to prevent, treat, or reduce the infectivity of a viral infection. In some embodiments, treating a viral infection may comprise reducing the infectivity and/or transmission of the virus. In some embodiments, preventing a viral infection may comprise reducing the infectivity and/or transmission of the virus. A composition of the present disclosure may comprise an active agent to prevent a viral infection, an active agent to treat a viral infection, an active agent to reduce the infectivity of a viral infection, or a combination thereof. A composition of the disclosure may further comprise a pharmaceutically acceptable excipient, carrier, or diluent. Further, a composition of the disclosure may contain preserving agents, solubilizing agents, stabilizing agents, wetting agents, emulsifiers, sweeteners, colorants, odorants, salts (substances of the present invention may themselves be provided in the form of a pharmaceutically acceptable salt), buffers, coating agents, or antioxidants.

[00198] The present disclosure relates to non-human adenoviral vector compositions and methods of using an immunogenic composition comprising the adenovirus vector and optionally one or more additional active ingredients, a pharmaceutically acceptable carrier, diluent, excipient or adjuvant to treat or prevent a respiratory viral infection. Applicant discovered that using a simian adenovirus vector

overcame issues of heterologous vector cross-immunity that were seen with human adenovirus vector platforms (PMID:32450106). Applicant has configured a chimp adenoviral vector expressing a SARS-CoV-2 antigen, such as a stabilized form of the SARS-CoV-2 "S" protein, and have shown it can protect against COVID-19 in an animal model of the disease. A SARS-CoV-2 antigen, or an immunogenic portion thereof, when expressed in an adenovirus vector can, upon infection of a human, stimulate an immune response, and thereby confer immunity to COVID-19 disease. The Applicant has further discovered that this platform of adenoviral vectors can be successfully used to deliver variants of SARS-CoV-2 antigen that are effective in generating an immunogenic response against sub-lineages or sub-variants of SARS-CoV-2.

[00199] Other aspects of the invention are described in further detail below.

a) Adenoviral Vector

[00200] The present disclosure provides an adenoviral vector for delivery of DNA encoding SARS-CoV-2 antigen, such as a stabilized form of the SARS-CoV-2 "S" protein. The nucleotide sequence of a transgene encoding the SARS -CoV2-Wuhan S protein is provided as SEQ ID NO: 1 and the nucleotide sequence of an exemplary adenoviral vector comprising the transgene encoding SARS -CoV2-Wuhan S protein is provided in SEQ ID. NO: 2. The protein coding sequence for the SARS -CoV2-Wuhan S protein is provided in SEQ ID NO: 3.

[00201] Adenoviruses are non-enveloped viruses, approximately 90-100nm in diameter, comprising a nucleocapsid and a linear double stranded DNA genome. The viral nucleocapsid comprises penton and hexon capsomers. A unique fiber is associated with each penton base and aids in the attachment of the virus to the host cell via the Coxsackie-adenovirus receptor on the surface of the host cell. Over 50 serotype strains of adenoviruses have been identified, most of which cause respiratory tract infections, conjunctivitis and gastroenteritis in humans. Rather than integrating into the host genome, adenoviruses normally replicate as episomal elements in the nucleus of the host cell. The genome of adenoviruses comprises 4 early transcriptional units (E1, E2, E3 and E4), which have mainly regulatory functions and prepare the host cell for

viral replication. The genome also comprises 5 late transcriptional units (L1, L2, L3, L4 and L5), which encode structural proteins including the penton (L2), the hexon (L3), the scaffolding protein (L4) and the fiber protein (L5), which are under the control of a single promoter. Each extremity of the genome comprises an Inverted Terminal Repeat (ITR) which is necessary for viral replication.

[00202] Recombinant adenoviruses were originally developed for gene therapy, but the strong and sustained transgene-specific immune responses elicited by these gene delivery agents prompted their use as vaccine carriers. In addition to being highly immunogenic, adenoviruses offer many other advantages for clinical vaccine development. The adenoviral genome is relatively small (between 26 and 45 kbp), well characterized and easy to manipulate. The deletion of a single transcriptional unit, E1 , renders the virus replication-incompetent which increases its predictability and reduces side effects in clinical applications. Recombinant adenoviruses can accommodate relatively large transgenes, in some cases up to 8kb, allowing flexibility in subunit design, and have a relatively broad tropism facilitating transgene delivery to a wide variety of cells and tissues. Importantly for clinical applications, methods for scaled-up production and purification of recombinant adenoviruses to high titre are well established. Thus far, subgroup C serotypes AdHu2 or AdHu5 have predominantly been used as vectors. However, the first generation of vaccine vectors based on the archetypal human adenovirus AdHu5 showed poor efficacy in clinical trials, despite encouraging preclinical data. It was subsequently discovered that a large proportion of human adults harbor significant titers of neutralizing antibodies to common human serotypes such as AdHu2 and AdHu5, as a result of natural infection. Neutralizing antibodies could reduce the potency of viral vector vaccines by blocking viral entry into host cells and hence delivery of the target transgene.

[00203] The compositions described herein include vectors that deliver a heterologous molecule to cells, either for therapeutic or vaccine purposes. As used herein, a vector may include any genetic element including, without limitation, naked DNA, a phage, transposon, cosmid, episome, plasmid, or a virus. In some embodiments such vectors contain simian adenovirus DNA (e.g., SAdV-36), and a transgene or a

nucleic acid sequence encoding a protein (e.g., S-protein). As used herein "transgene" refers to the combination of a selected heterologous gene (nucleic acid sequence encoding a heterologous polypeptide) and the other regulatory elements or expression control sequences necessary to drive translation, transcription and/or expression of the gene product in a host cell. In several embodiments, the viral vector can include an adenoviral vector that expresses a transgene encoding a coronavirus S protein. Adenovirus from various origins, subtypes, or mixture of subtypes can be used as the source of the viral genome for the adenoviral vector. Non-human adenovirus (*e.g.*, simian, chimpanzee, gorilla, avian, canine, ovine, or bovine adenoviruses) can be used to generate the adenoviral vector. For example, a simian adenovirus can be used as the source of the viral genome of the adenoviral vector. A simian adenovirus can be of serotype 1, 3, 7, 11, 16, 18, 19, 20, 27, 33, 36, 38, 39, 48, 49, 50, or any other simian adenoviral serotype. A simian adenovirus can be referred to by using any suitable abbreviation known in the art, such as, for example, SV, SAdV, SAV or sAV. In some examples, a simian adenoviral vector is a simian adenoviral vector of serotype 36.

[00204] Typically, a SAdV-derived adenoviral vector is designed such that the transgene is located in a nucleic acid molecule which contains other adenoviral sequences in the region native to a selected adenoviral gene. The transgene may be inserted into an existing gene region to disrupt the function of that region, if desired. Alternatively, the transgene may be inserted into the site of a partially or fully deleted adenoviral gene. For example, the transgene may be located in the site of such as the site of a functional E1 deletion and/or functional E3 deletion (or E3B) and/or full E3 deletion, among others that may be selected. The term "functionally deleted" or "functional deletion" means that a sufficient amount of the gene region is removed or otherwise damaged, *e.g.*, by mutation or modification, so that the gene region is no longer capable of producing functional products of gene expression. If desired, the entire gene region may be removed. In one embodiment, the adenovirus vector useful according to the disclosure is a simian adenovirus, SAd36, having deletions in the E1 and E3B genes. In one aspect, the vector comprises a full E3 deletion. In one aspect, the adenovirus vector has the nucleic acid sequence of SEQ ID NO: 4. In another

aspect, the adenoviral vector has the nucleic acid sequence with at least 80%, 90%, 91 %, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.1 %, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1%, 99.2%, 99.3%, 99.4% 99.5%, 99.6%, 99.7%, 99.8% or 99.9% sequence identity with SEQ ID NO: 4.

[00205] For example, for a production vector useful for generation of a recombinant virus, the vector may contain the transgene and either the 5' end of the adenoviral genome or the 3' end of the adenoviral genome, or both the 5' and 3' ends of the adenoviral genome. The 5' end of the adenoviral genome contains the 5' cis-elements necessary for packaging and replication, i.e., the 5' inverted terminal repeat (ITR) sequences (which function as origins of replication) and the native 5 ' packaging enhancer domains (that contain sequences necessary for packaging linear Ad genomes and enhancer elements for the E1 promoter). The 3' end of the adenoviral genome includes the 3' cis-elements (including the ITRs) necessary for packaging and encapsidation. Suitably, a recombinant adenovirus contains both 5 ' and 3 ' adenoviral cis-elements and the transgene is located between the 5' and 3' adenoviral sequences. A simian based adenoviral vector (*e.g.* SAdV-36) may also contain additional adenoviral sequences.

[00206] Suitably, these simian-based adenoviral vectors contain one or more adenoviral elements derived from the adenoviral genome. In one embodiment, the vectors contain adenoviral sequences that are derived from a different adenoviral serotype than that which provides the ITRs. As defined herein, a pseudotyped adenovirus refers to an adenovirus in which the capsid protein of the adenovirus is from a different adenovirus than the adenovirus which provides the ITRs. Chimeric or hybrid adenoviruses may be constructed using the adenoviruses described herein using techniques known to those of skill in the art. See, e.g., US 7,291,498.

[00207] The transgene is a nucleic acid sequence, heterologous to the vector sequences flanking the transgene, which encodes a polypeptide, protein, or other product, of interest. The nucleic acid coding sequence is operatively linked to regulatory components in a manner which permits transgene transcription, translation, and/or expression in a host cell.

[00208] In some embodiments, the transgene encodes a SARS-CoV-2 antigen, such as a stabilized form of the SARS-CoV-2 "S" protein. In some embodiments, transgene encodes at least a portion or immunogenic fragment of a coronavirus spike protein (S) protein or an immunogenic fragment or variant thereof having a sequence at least 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.1 %, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1 %, 99.2%, 99.3%, 99.4% 99.5%, 99.6%, 99.7%, 99.8% or 99.9% sequence identity to the coronavirus spike protein portion (or immunogenic portion or variant thereof) of SEQ ID NO: 3; or at least 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.1 %, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1 %, 99.2%, 99.3%, 99.4% 99.5%, 99.6%, 99.7%, 99.8% or 99.9% sequence identity to SEQ ID NO: 3 having K986P and V987P mutations; or SEQ ID NO: 3 having a D614G mutation. In some embodiments, the transgene encodes a spike protein of a SARS-CoV-2 variant, in non-limiting examples a spike protein having a D80G, 144del, F157S, L5F, T95I, A67V, S477N, 144del, Q677H, A701V, F888L, T791I, T859N, D950H, E484Q, D614G, E484K, N501Y, Δ69-70, L452R, or K417N or RBD E484K mutation relative to SEQ ID NO: 3. In some embodiments, the transgene encodes a spike protein from a WA1/2020, B.1.1.7, B.1.351, B.1.1.28, P.1, B.1.427, B.1.526, B.1.526.1, B.1.525, P.2, B.1.617, B.1.617.1, B.1.617.2, B.1.617.3, B.1.429, B.1.429, B.1.529 variants. In each of the above embodiments, the spike protein is further modified to be a prefusion stabilized form (e.g. having double proline substitution between residues 1050 to 1069 or between residues 981 to 999).

[00209] A variant, Omicron (B.1.1.529), having multiple Spike protein mutations was detected initially in Botswana. In particular, the genome of some Omicron variants encode a S protein having the following mutations: A67V, Δ69-70, T95I, G142D/A143-145, A211/L212I, 214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493K, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, and L981F. In some embodiments, this exemplary strain, and other newly emerging strains (e.g., additional variants of concern) are candidates for the methods and formulations

disclosed herein. As such, in some embodiments the current disclosure also encompasses recombinant adenoviral vectors comprising a transgenic stabilized spike protein sequence derived from the Omicron B.1.1.529 strain or a variant thereof. Non-limiting examples of subvariants of the Omicron strain include BA1.1, BA.2, BA.3, BA.4, and BA.5. In an exemplary aspect, the BA.5 variant may have the mutations: T19I, L24S, del25-27, 69-70del, G142D, V213G, G339D, S371F, S373P, S375F, T376A, K417N, N440K, S477N, T478K, E484A, Q493R, L452R, F486V, Q498R, N501Y, D614G, H655Y, N679K, P681H, R682G, R683S, R685S, N764K, D796Y, Q954H, N969K.

[00210] As such, in some embodiments, the transgene nucleic acid encodes a stabilized SARS-CoV2-Omicron-Spike protein (SARS-CoV2-Omicron-S2P) as provided in SEQ ID NO: 10.; or a nucleic acid sequence encoding a protein with at least 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.1 %, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1 %, 99.2%, 99.3%, 99.4% 99.5%, 99.6%, 99.7%, 99.8% or 99.9% sequence identity to SEQ ID NO: 10 (SARS-CoV-2-Omicron-S2P).

[00211] In some embodiments, the transgene nucleic acid encodes a SARS-Cov2-Omicron-Spike protein (SARS-CoV-2-Omicron-S6P) of SEQ ID. NO: 11; or a nucleic acid sequence encoding a protein with at least 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.1 %, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1 %, 99.2%, 99.3%, 99.4% 99.5%, 99.6%, 99.7%, 99.8% or 99.9% sequence identity to SEQ ID NO: 11 (SARS-CoV2-Omicron-S6P).

[00212] In some embodiments, the transgene nucleic acid encodes a SARS-Cov2-Omicron-Spike protein (SARS-CoV-2-Omicron-S6PδF) of SEQ ID NO: 12; or a nucleic acid sequence encoding a protein with at least 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.1 %, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1 %, 99.2%, 99.3%, 99.4% 99.5%, 99.6%, 99.7%, 99.8% or 99.9% sequence identity to SEQ ID NO: 12 (SARS-CoV2-Omicron-S6PdF).

[00213] In some embodiments, the transgene nucleic acid encodes a SARS-Cov2-Omicron-Spike protein (SARS-CoV-2-Omicron-S6PdF) of SEQ ID NO: 20;

or a nucleic acid sequence encoding a protein with at least 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.1 %, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1 %, 99.2%, 99.3%, 99.4% 99.5%, 99.6%, 99.7%, 99.8% or 99.9% sequence identity to SEQ ID NO: 20 (Bivalent BA.5-S6PdF).

[00214] In some embodiments, the transgene nucleic acid encodes a SARS-Cov2-Omicron-Spike protein (SARS-CoV-2-Omicron-S6P) of SEQ ID NO: 21; or a nucleic acid sequence encoding a protein with at least 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.1 %, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1 %, 99.2%, 99.3%, 99.4% 99.5%, 99.6%, 99.7%, 99.8% or 99.9% sequence identity to SEQ ID NO: 21 (Bivalent BA.5-S6P).

[00215] In some aspects, the adenoviral vector comprises a nucleic acid sequence (transgene), wherein the nucleic acid sequence encodes an amino acid sequence at least 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1 %, 99.2%, 99.3%, 99.4% 99.5%, 99.6%, 99.7%, 99.8% or 99.9% identical to SEQ ID NOS: 3 or variants thereof comprising at least 5, or at least 10, or at least 15, or at least 20, or at least 25, or at least 30 or more of the mutations: T19I, L24S, del25-27, 69-70del, G142D, V213G, G339D, S371F, S373P, S375F, T376A, K417N, N440K, S477N, T478K, E484A, Q493R, L452R, F486V, Q498R, N501Y, D614G, H655Y, N679K, P681H, R682G, R683S, R685S, N764K, D796Y, A942P Q954H, N969K, K988P and V989P and further comprising at least 2, or at least 3, or at least 4, or at least 5, or 6 stabilizing mutations as provided in F819P, A894P, A901P, A944P, K988P and V989P. In some aspects, the adenoviral vector encodes a protein at least 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1 %, 99.2%, 99.3%, 99.4% 99.5%, 99.6%, 99.7%, 99.8% or 99.9% identical to SEQ ID. NOS. 3 or Omicron variants as provided in SEQ ID NOS. 10-11 and 20-21 comprising at least 2, or at least 3, or at least 4, or at least 5, or 6 stabilizing mutations as provided in F819P, A894P, A901P, A944P, K988P and V989P. A sequence alignment of the SEQ ID NOS: 10, 11, 20, 21 with respect to the Wuhan sequence is provided in **FIG. 23**.

[00216] In some embodiments, the current disclosure also encompasses adenoviral vectors comprising a nucleic acid sequence encoding more than one coronavirus spike protein variant or an immunogenic fragment of portion thereof. In some embodiments, the current disclosure also encompasses an adenoviral vector comprising a nucleic acid sequence encoding at least a portion or immunogenic fragment of a coronavirus spike protein (S) protein or immunogenic fragment or variant thereof having a sequence at least 80% identical to SEQ ID NOS: 3, 10, 11, 20, or 21, and a nucleic acid sequence encoding at least a portion or immunogenic fragment or variant thereof, having a sequence at least about 80% identical to one or more additional proteins derived from SARS-CoV2. In some aspects, the one or more additional protein may be selected from non-structural proteins 1-16 (nsp1-16), structural proteins including the S protein, envelope (E), membrane (M) and nucleocapsid (N) and eleven accessory proteins: ORF3a, ORF3b, ORF3c, ORF3d, ORF6, ORF7a, ORF7b, ORF8, ORF9b, ORF9c and ORF10. In some embodiments, the current disclosure also encompasses an adenoviral vector comprising a nucleic acid sequence encoding at least a portion or immunogenic fragment of a coronavirus spike protein (S) protein or immunogenic fragment or variant thereof having a sequence at least 80% identical to SEQ ID NOS: 3, 10, 11, 20, or 21, and a nucleic acid sequence encoding at least a portion or immunogenic fragment of a protein derived from another virus, for example rabies, measles, RSV or influenza. Examples of suitable proteins include but are not limited to influenza hemagglutinin, influenza nuclear protein, influenza M2, tetanus toxin C-fragment, anthrax protective antigen, anthrax lethal factor, anthrax germination factors, rabies glycoprotein, HBV surface antigen, HIV gp120, HIV gp160, human carcinoembryonic antigen, malaria CSP, malaria SSP, malaria MSP, malaria pfg, botulinum toxin A, and mycobacterium tuberculosis HSP. In some embodiment, the adenoviral vector is said to express a bivalent, trivalent, or multivalent immunogenic protein/proteins, wherein it elicits an immune response against more than one virus, or more than one SARS-CoV2 lineage, variant, sub lineage or subvariant.

[00217] In some embodiments, the current disclosure also encompasses plasmids and adenoviral vectors (vector) comprising a nucleic acid sequence encoding

the S protein sequence as disclosed herein. In some aspects, the nucleic acid is at least 80% identical to SEQ ID NO: 2, SEQ ID NO: 5, or SEQ ID NO: 6. In some embodiments, the adenoviral vector or plasmid comprises or consists of a nucleic acid sequence at least about 80% identical to SEQ ID NOS: 2, 5 or 6. In some embodiments, the nucleic acid sequence has at least 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1 %, 99.2%, 99.3%, 99.4% 99.5%, 99.6%, 99.7%, 99.8% or 99.9% identity to SEQ ID NOS: 2, 5 or 6.

[00218] In one embodiment, the vector comprises or consists of a nucleic acid sequence as provided in SEQ ID NO:13 (ChAd-S6PdF529). In some embodiments, the vector comprises or consists of a nucleic acid sequence at least about 80% identical to SEQ ID NO: 13. In some embodiments, the nucleic acid sequence has at least 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1 %, 99.2%, 99.3%, 99.4% 99.5%, 99.6%, 99.7%, 99.8% or 99.9% identity to SEQ ID NO: 13.

[00219] In some embodiments, the vector comprises / consists of the nucleic acid sequence as provided in SEQ ID NO: 14 (ChAd-S2P529). In some embodiments, the vector comprises or consists of a nucleic acid sequence at least about 80% identical to SEQ ID NO: 14. In some embodiments, the nucleic acid sequence has at least 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1 %, 99.2%, 99.3%, 99.4% 99.5%, 99.6%, 99.7%, 99.8% or 99.9% identity to SEQ ID NO: 14.

[00220] In some embodiments, the vector comprises or consists of the nucleic acid sequence as provided in SEQ ID NO: 15 (ChAd-S6P529). In some embodiments, the vector comprises or consists of a nucleic acid sequence at least about 80% identical to SEQ ID NO: 15. In some embodiments, the nucleic acid sequence has at least 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1 %, 99.2%, 99.3%, 99.4% 99.5%, 99.6%, 99.7%, 99.8% or 99.9% identity to SEQ ID NO: 15.

[00221] In some embodiments, the plasmid comprises or consists of the nucleic acid sequence as provided in SEQ ID NO: 16 (pSAd36E3E1stS), wherein the entire E3 gene is deleted. In some embodiments, the vector comprises or consists of a nucleic acid sequence at least about 80% identical to SEQ ID NO: 16. In some embodiments, the nucleic acid sequence has at least 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1 %, 99.2%, 99.3%, 99.4% 99.5%, 99.6%, 99.7%, 99.8% or 99.9% identity to SEQ ID NO: 16.

[00222] In some embodiments, the vector comprises or consists of the nucleic acid sequence as provided in SEQ ID NO: 17 (SAd36E3E1stS), wherein the entire E3 gene is deleted. In some embodiments, the vector comprises or consists of a nucleic acid sequence at least about 80% identical to SEQ ID NO: 17. In some embodiments, the nucleic acid sequence has at least 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1 %, 99.2%, 99.3%, 99.4% 99.5%, 99.6%, 99.7%, 99.8% or 99.9% identity to SEQ ID NO: 17.

[00223] In some embodiments, the vector comprises or consists of the nucleic acid sequence as provided in SEQ ID NO: 18 (ChAd.BA.5-S6PdF). In some embodiments, the vector comprises or consists of a nucleic acid sequence at least about 80% identical to SEQ ID NO: 18. In some embodiments, the nucleic acid sequence has at least 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1 %, 99.2%, 99.3%, 99.4% 99.5%, 99.6%, 99.7%, 99.8% or 99.9% identity to SEQ ID NO: 18.

[00224] In some embodiments, the vector comprises or consists of the nucleic acid sequence as provided in SEQ ID NO: 19 (ChAd.BA.5-S6P). In some embodiments, the vector comprises or consists of a nucleic acid sequence at least about 80% identical to SEQ ID NO: 19. In some embodiments, the nucleic acid sequence has at least 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1 %, 99.2%, 99.3%, 99.4% 99.5%, 99.6%, 99.7%, 99.8% or 99.9% identity to SEQ ID NO: 19.

[00225] In addition to the major elements identified above for the transgene, the vector may also include conventional control elements operably linked to the transgene in a manner that permits its transcription, translation and/or expression in a cell transfected with the plasmid vector or infected with the virus produced by the invention. As used herein, "operably linked" sequences include both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in trans or at a distance to control the gene of interest. Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation (polyA) signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance secretion of the encoded product.

[00226] A great number of expression control sequences, including promoters which are native, constitutive, inducible and/or tissue-specific, are known in the art and may be utilized. Examples of constitutive promoters include, without limitation, the retroviral Rous sarcoma virus (RSV) LTR promoter (optionally with the RSV enhancer), the cytomegalovirus (CMV) promoter (optionally with the CMV enhancer) [see, e.g., Boshart *et al.*, Cell, 41:521-530 (1985)], the SV40 promoter, the dihydrofolate reductase promoter, the β-actin promoter, the phosphoglycerol kinase (PGK) promoter, and the EF-Ia promoter [Invitrogen].

[00227] Inducible promoters allow regulation of gene expression and can be regulated by exogenously supplied compounds, environmental factors such as temperature, or the presence of a specific physiological state, e.g., acute phase, a particular differentiation state of the cell, or in replicating cells only. Inducible promoters and inducible systems are available from a variety of commercial sources, including, without limitation, Invitrogen, Clontech and Ariad. Many other systems have been described and can be readily selected by one of skill in the art. For example, inducible promoters include the zinc-inducible sheep metallothionine (MT) promoter and the dexamethasone (Dex)-inducible mouse mammary tumor virus (MMTV) promoter. Other

inducible systems include the T7 polymerase promoter system [WO 98/10088]; the ecdysone insect promoter [No et al, Proc. Natl. Acad. Sci. USA, 93:3346-3351 (1996)], the tetracycline-repressible system [Gossen et al, Proc. Natl. Acad. Sci. USA, 89:5547-5551 (1992)], the tetracycline-inducible system [Gossen et al, Science, 268:1766-1769 (1995), see also Harvey et al, Curr. Opin. Chem. Biol., 2:512-518 (1998)]. Other systems include the FK506 dimer, VP 16 or p65 using castradiol, diphenol murislerone, the RU486-inducible system [Wang et al, Nat. Biotech., 15:239-243 (1997) and Wang et al, Gene Ther., 4:432-441 (1997)] and the rapamycin-inducible system [Magari et al, J. Clin. Invest., 100:2865-2872 (1997)]. The effectiveness of some inducible promoters increases over time. In such cases one can enhance the effectiveness of such systems by inserting multiple repressors in tandem, e.g., TetR linked to a TetR by an IRES. Alternatively, one can wait at least 3 days before screening for the desired function. One can enhance expression of desired proteins by known means to enhance the effectiveness of this system. For example, using the Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE).

[00228] In another embodiment, the native promoter for the transgene may be used. The native promoter may be preferred when it is desired that expression of the transgene should mimic the native expression. The native promoter may be used when expression of the transgene must be regulated temporally or developmentally, or in a tissue-specific manner, or in response to specific transcriptional stimuli. In a further embodiment, other native expression control elements, such as enhancer elements, polyadenylation sites or Kozak consensus sequences may also be used to mimic the native expression. Another embodiment of the transgene includes a transgene operably linked to a tissue-specific promoter. For instance, if expression in skeletal muscle is desired, a promoter active in muscle should be used. These include the promoters from genes encoding skeletal β-actin, myosin light chain 2A, dystrophin, muscle creatine kinase, as well as synthetic muscle promoters with activities higher than naturally occurring promoters (see Li et al , Nat. Biotech., 17:241-245 (1999)). Examples of promoters that are tissue-specific are known for liver (albumin, Miyatake et al, J. Virol., 71:5124-32 (1997); hepatitis B virus core promoter, Sandig et al., Gene Ther., 3:1002-9

(1996); alpha-fetoprotein (AFP), Arbuthnot et al, Hum. Gene Ther., 7:1503-14 (1996)), bone osteocalcin (Stein et al, Mol. Biol Rep., 24:185-96 (1997)); bone sialoprotein (Chen et al, J. Bone Miner. Res., 11:654-64 (1996)), lymphocytes (CD2, Hansal et al, J. Immunol, 161: 1063-8 (1998); immunoglobulin heavy chain; T cell receptor chain), neuronal such as neuron-specific enolase (NSE) promoter (Andersen et al, Cell. Mol Neurobiol, 13:503-15 (1993)), neurofilament light-chain gene (Piccioli et al, Proc. Natl Acad. ScL USA, 88:561 1-5 (1991)), and the neuron-specific vgf gene (Piccioli et al, Neuron, 15:373-84 (1995)), among others. Optionally, vectors carrying transgenes encoding therapeutically useful or immunogenic products may also include selectable markers or reporter genes may include sequences encoding geneticin, hygromicin or purimycin resistance, among others. Such selectable reporters or marker genes (preferably located outside the viral genome to be packaged into a viral particle) can be used to signal the presence of the plasmids in bacterial cells, such as ampicillin resistance. Other components of the vector may include an origin of replication. Selection of these and other promoters and vector elements are conventional, and many such sequences are available [see, e.g., Sambrook et al, and references cited therein]. These vectors are generated using the techniques and sequences provided herein, in conjunction with techniques known to those of skill in the art. Such techniques may include conventional cloning techniques of cDNA such as those described in texts [Sambrook et al, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, NY], use of overlapping oligonucleotide sequences of the adenovirus genomes, polymerase chain reaction, and any suitable method which provides the desired nucleotide sequence.

[00229] In some embodiments, the present disclosure provides a-virus-like particle (VLP) that includes a disclosed recombinant adenoviral vector. VLPs lack the viral components that are required for virus replication and thus represent a highly attenuated, replication-incompetent form of a virus. Virus like particles and methods of their production are known and familiar to the person of ordinary skill in the art, and viral proteins from several viruses are known to form VLPs, including human papillomavirus, HIV (Kang et al., Biol. Chem. 380: 353-64 (1999)), Semliki- Forest virus (Notka et al.,

Biol. Chem. 380: 341-52 (1999)), human polyomavirus (Goldmann et al., J. Virol. 73: 4465-9 (1999)), rotavirus (Jiang et al , Vaccine 17: 1005-13 (1999)), parvovirus (Casal, Biotechnology and Applied Biochemistry, Vol 29, Part 2, pp 141-150 (1999)), canine parvovirus (Hurtado et al., J. Virol. 70: 5422-9 (1996)), hepatitis E virus (Li et al, J. Virol. 71 : 7207-13 (1997)), and Newcastle disease virus. The formation of such VLPs can be detected by any suitable technique. Examples of suitable techniques known in the art for detection of VLPs in a medium include, *e.g.*, electron microscopy techniques, dynamic light scattering (DLS), selective chromatographic separation (e.g., ion exchange, hydrophobic interaction, and/or size exclusion chromatographic separation of the VLPs) and density gradient centrifugation.

b) Components of the Composition

[00230] The present disclosure also provides pharmaceutical compositions. The pharmaceutical composition comprises an adenoviral composition of the present disclosure, as an active ingredient, and at least one pharmaceutically acceptable excipient.

[00231] The pharmaceutically acceptable excipient may be a diluent, a binder, a filler, a buffering agent, a pH modifying agent, a disintegrant, a dispersant, a preservative, a lubricant, taste-masking agent, a flavoring agent, or a coloring agent. The amount and types of excipients utilized to form pharmaceutical compositions may be selected according to known principles of pharmaceutical science.

[00232] In each of the embodiments described herein, a composition of the invention may optionally comprise one or more additional drug or therapeutically active agent in addition to the adenoviral composition of the present disclosure. Thus, in addition to the therapies described herein, one may also provide to the subject other therapies known to be efficacious for treatment of a viral infection. In some embodiments, the secondary agent is selected from a corticosteroid, a non-steroidal anti-inflammatory drug (NSAID), an intravenous immunoglobulin, a kinase inhibitor, a fusion or recombinant protein, a monoclonal antibody, or a combination thereof. In some

embodiments, agents suitable for combination therapy include but are not limited to inhaled bronchodilators and inhaled steroids.

(i) Diluent

[00233] In one embodiment, the excipient may be a diluent. The diluent may be compressible (i.e., plastically deformable) or abrasively brittle. Non-limiting examples of suitable compressible diluents include microcrystalline cellulose (MCC), cellulose derivatives, cellulose powder, cellulose esters (i.e., acetate and butyrate mixed esters), ethyl cellulose, methyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, sodium carboxymethylcellulose, corn starch, phosphated corn starch, pregelatinized corn starch, rice starch, potato starch, tapioca starch, starch-lactose, starch-calcium carbonate, sodium starch glycolate, glucose, fructose, lactose, lactose monohydrate, sucrose, xylose, lactitol, mannitol, malitol, sorbitol, xylitol, maltodextrin, and trehalose. Non-limiting examples of suitable abrasively brittle diluents include dibasic calcium phosphate (anhydrous or dihydrate), calcium phosphate tribasic, calcium carbonate, and magnesium carbonate.

(ii) Binder

[00234] In another embodiment, the excipient may be a binder. Suitable binders include, but are not limited to, starches, pregelatinized starches, gelatin, polyvinylpyrrolidone, cellulose, methylcellulose, sodium carboxymethylcellulose, ethylcellulose, polyacrylamides, polyvinyloxoazolidone, polyvinylalcohols, C_{12}-C_{18} fatty acid alcohol, polyethylene glycol, polyols, saccharides, oligosaccharides, polypeptides, oligopeptides, and combinations thereof.

(iii) Filler

[00235] In another embodiment, the excipient may be a filler. Suitable fillers include, but are not limited to, carbohydrates, inorganic compounds, and polyvinylpyrrolidone. By way of non-limiting example, the filler may be calcium sulfate, both di- and tri-basic, starch, calcium carbonate, magnesium carbonate, microcrystalline cellulose, dibasic calcium phosphate, magnesium carbonate, magnesium oxide, calcium silicate, talc, modified starches, lactose, sucrose, mannitol, or sorbitol.

(iv) Buffering Agent

[00236] In still another embodiment, the excipient may be a buffering agent. Representative examples of suitable buffering agents may include, but are not limited to, phosphates, carbonates, citrates, tris buffers, and buffered saline salts (e.g., Tris buffered saline or phosphate buffered saline).

(v) pH Modifier

[00237] In various embodiments, the excipient may be a pH modifier. By way of non-limiting example, the pH modifying agent may be sodium carbonate, sodium bicarbonate, sodium citrate, citric acid, or phosphoric acid.

(vi) Disintegrant

[00238] In a further embodiment, the excipient may be a disintegrant. The disintegrant may be non-effervescent or effervescent. Suitable examples of non-effervescent disintegrants include, but are not limited to, starches such as corn starch, potato starch, pregelatinized and modified starches thereof, sweeteners, clays, such as bentonite, micro-crystalline cellulose, alginates, sodium starch glycolate, gums such as agar, guar, locust bean, karaya, pecitin, and tragacanth. Non-limiting examples of suitable effervescent disintegrants include sodium bicarbonate in combination with citric acid and sodium bicarbonate in combination with tartaric acid.

(vii) Dispersant

[00239] In yet another embodiment, the excipient may be a dispersant or dispersing enhancing agent. Suitable dispersants may include, but are not limited to, starch, alginic acid, polyvinylpyrrolidones, guar gum, kaolin, bentonite, purified wood cellulose, sodium starch glycolate, isoamorphous silicate, and microcrystalline cellulose.

(viii) Excipient

[00240] In another alternate embodiment, the excipient may be a preservative. Non-limiting examples of suitable preservatives include antioxidants, such as BHA, BHT, vitamin A, vitamin C, vitamin E, or retinyl palmitate, citric acid, sodium citrate; chelators such as EDTA or EGTA; and antimicrobials, such as parabens, chlorobutanol, or phenol.

(ix) Lubricant

[00241] In a further embodiment, the excipient may be a lubricant. Non-limiting examples of suitable lubricants include minerals such as talc or silica; and fats such as vegetable stearin, magnesium stearate, or stearic acid.

(x) Taste-Masking Agent

[00242] In yet another embodiment, the excipient may be a taste-masking agent. Taste-masking materials include cellulose ethers; polyethylene glycols; polyvinyl alcohol; polyvinyl alcohol and polyethylene glycol copolymers; monoglycerides or triglycerides; acrylic polymers; mixtures of acrylic polymers with cellulose ethers; cellulose acetate phthalate; and combinations thereof.

(xi) Flavoring Agent

[00243] In an alternate embodiment, the excipient may be a flavoring agent. Flavoring agents may be chosen from synthetic flavor oils and flavoring aromatics and/or natural oils, extracts from plants, leaves, flowers, fruits, and combinations thereof.

(xii) Coloring Agent

[00244] In still a further embodiment, the excipient may be a coloring agent. Suitable color additives include, but are not limited to, food, drug and cosmetic colors (FD&C), drug and cosmetic colors (D&C), or external drug and cosmetic colors (Ext. D&C).

(xiii) Adjuvants

[00245] In still a further embodiment the formulation may comprise an adjuvant. Adjuvants are known in the art to further increase the immune response to an applied antigenic determinant, and pharmaceutical compositions comprising adenovirus and suitable adjuvants are for instance disclosed in WO 2007/110409, incorporated by reference herein. Examples of suitable adjuvants include aluminum salts such as aluminum hydroxide and/or aluminum phosphate; oil-emulsion compositions (or oil-in-water compositions), including squalene-water emulsions, such as MF59 (see, e.g., WO 90/14837); saponin formulations, such as for example QS21 and Immunostimulating Complexes (ISCOMS); bacterial or microbial derivatives, examples of which are

monophosphoryl lipid A (MPL), 3-O-deacylated MPL (3dMPL), CpG-motif containing oligonucleotides, ADP-ribosylating bacterial toxins or mutants thereof, such as E. coli heat labile enterotoxin LT, cholera toxin CT, and the like. It is also possible to use vector-encoded adjuvant, e.g. by using heterologous nucleic acid that encodes a fusion of the oligomerization domain of C4-binding protein (C4 bp) to the antigen of interest. In certain embodiments the compositions of the invention comprise aluminum as an adjuvant, e.g., in the form of aluminum hydroxide, aluminum phosphate, aluminum potassium phosphate, or combinations thereof, in concentrations of 0.05-5 mg, e.g. from 0.075-1.0 mg, of aluminum content per dose.

[00246] The weight fraction of the excipient or combination of excipients in the composition may be about 99% or less, about 97% or less, about 95% or less, about 90% or less, about 85% or less, about 80% or less, about 75% or less, about 70% or less, about 65% or less, about 60% or less, about 55% or less, about 50% or less, about 45% or less, about 40% or less, about 35% or less, about 30% or less, about 25% or less, about 20% or less, about 15% or less, about 10% or less, about 5% or less, about 2%, or about 1% or less of the total weight of the composition.

[00247] The agents and compositions described herein can be formulated by any conventional manner using one or more pharmaceutically acceptable carriers or excipients as described in, for example, Remington's Pharmaceutical Sciences (A.R. Gennaro, Ed.), 21st edition, ISBN: 0781746736 (2005), incorporated herein by reference in its entirety. Such formulations will contain a therapeutically effective amount of a biologically active agent described herein, which can be in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the subject.

[00248] The term "formulation" refers to preparing a drug in a form suitable for administration to a subject, such as a human. Thus, a "formulation" can include pharmaceutically acceptable excipients, including diluents or carriers.

[00249] The term "pharmaceutically acceptable" as used herein can describe substances or components that do not cause unacceptable losses of pharmacological activity or unacceptable adverse side effects. Examples of

pharmaceutically acceptable ingredients can be those having monographs in United States Pharmacopeia (USP 29) and National Formulary (NF 24), United States Pharmacopeial Convention, Inc, Rockville, Maryland, 2005 ("USP/NF"), or a more recent edition, and the components listed in the continuously updated Inactive Ingredient Search online database of the FDA. Other useful components that are not described in the USP/NF, etc. may also be used.

[00250] The term "pharmaceutically acceptable excipient," as used herein, can include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic, or absorption delaying agents. The use of such media and agents for pharmaceutical active substances is well known in the art (see generally Remington's Pharmaceutical Sciences (A.R. Gennaro, Ed.), 21st edition, ISBN: 0781746736 (2005)). Except insofar as any conventional media or agent is incompatible with an active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

[00251] A "stable" formulation or composition can refer to a composition having sufficient stability to allow storage at a convenient temperature, such as between about 0 ºC and about 60 ºC, for a commercially reasonable period of time, such as at least about one day, at least about one week, at least about one month, at least about three months, at least about six months, at least about one year, or at least about two years.

[00252] The formulation should suit the mode of administration. The agents of use with the current disclosure can be formulated by known methods for administration to a subject using several routes which include, but are not limited to, parenteral, pulmonary, oral, topical, mucosal, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, ophthalmic, buccal, and rectal. The individual agents may also be administered in combination with one or more additional agents or together with other biologically active or biologically inert agents. Such biologically active or inert agents may be in fluid or mechanical communication with the agent(s) or attached to the agent(s) by ionic, covalent, Van der Waals, hydrophobic, hydrophilic or other physical forces.

[00253] In some aspects, the current disclosure encompasses formulations of intranasal delivery. A formulation comprising a composition for intranasal deliver may have a pH corresponding to a physiologically acidic nasal pH. The physiologically acidic nasal pH may depend on intact nasal mucosal function. A composition may comprise a pH of about be 6.5 ± 0.5 (5.9 to 7.3) or about 6.7 ± 0.6 (5.3 to 7.6). A composition may comprise a pH of about 3.8-7.7 (mean ± SD 5.7 ± 0.9). A composition for nasal deliver may be in the slightly acidic range. The average pH may have an acidity of pH 5.7.

[00254] Effective delivery of therapeutic agents via intranasal administration must take into account the decreased transport rate across the protective mucus lining of the nasal mucosa, in addition to drug loss due to binding to glycoproteins of the mucus layer. Normal mucus is a viscoelastic, gel-like substance consisting of water, electrolytes, mucins, macromolecules, and sloughed epithelial cells. It serves primarily as a cytoprotective and lubricative covering for the underlying mucosal tissues. Mucus is secreted by randomly distributed secretory cells located in the nasal epithelium and in other mucosal epithelia. The structural unit of mucus is mucin. This glycoprotein is mainly responsible for the viscoelastic nature of mucus, although other macromolecules may also contribute to this property. In airway mucus, such macromolecules include locally produced secretory IgA, IgM, IgE, lysozyme, and bronchotransferrin, which also play an important role in host defense mechanisms.

[00255] The coordinate administration methods of the instant disclosure optionally incorporate effective mucolytic or mucus-clearing agents, which serve to degrade, thin or clear mucus from intranasal mucosal surfaces to facilitate absorption and/or adsorption of intranasally administered biotherapeutic agents. Within these methods, a mucolytic or mucus-clearing agent is coordinately administered as an adjunct compound to enhance intranasal delivery of the biologically active agent. Alternatively, an effective amount of a mucolytic or mucus-clearing agent is incorporated as a processing agent within a multi-processing method of the invention, or as an additive within a combinatorial formulation of the invention, to provide an improved formulation that enhances intranasal delivery of biotherapeutic compounds by reducing the barrier effects of intranasal mucus.

[00256] A variety of mucolytic or mucus-clearing agents are available for incorporation within the methods and compositions of the invention. Based on their mechanisms of action, mucolytic and mucus clearing agents can often be classified into the following groups: proteases (*e.g.*, pronase, papain) that cleave the protein core of mucin glycoproteins; sulfhydryl compounds that split mucoprotein disulfide linkages; and detergents (e.g., Triton X-100, Tween 20) that break non-covalent bonds within the mucus. Additional compounds in this context include, but are not limited to, bile salts and surfactants, for example, sodium deoxycholate, sodium taurodeoxycholate, sodium glycocholate, and lysophosphatidylcholine.

[00257] The effectiveness of bile salts in causing structural breakdown of mucus is in the order deoxycholate>taurocholate>glycocholate. Other effective agents that reduce mucus viscosity or adhesion to enhance intranasal delivery according to the methods of the invention include, e.g., short-chain fatty acids, and mucolytic agents that work by chelation, such as N-acylcollagen peptides, bile acids, and saponins (the latter function in part by chelating Ca^{2+} and/or Mg^{2+} which play an important role in maintaining mucus layer structure).

[00258] Additional mucolytic agents for use within the methods and compositions of the invention include N-acetyl-L-cysteine (ACS), a potent mucolytic agent that reduces both the viscosity and adherence of bronchopulmonary mucus and is reported to modestly increase nasal bioavailability of human growth hormone in anesthetized rats (from 7.5 to 12.2%). These and other mucolytic or mucus-clearing agents are contacted with the nasal mucosa, typically in a concentration range of about 0.2 to 20 mM, coordinately with administration of the biologically active agent, to reduce the polar viscosity and/or elasticity of intranasal mucus.

[00259] Still other mucolytic or mucus-clearing agents may be selected from a range of glycosidase enzymes, which are able to cleave glycosidic bonds within the mucus glycoprotein. α-amylase and ß-amylase are representative of this class of enzymes, although their mucolytic effect may be limited. In contrast, bacterial glycosidases which allow these microorganisms to permeate mucus layers of their hosts.

[00260] For combinatorial use with the adenoviral composition within the disclosure, non-ionogenic detergents are generally also useful as mucolytic or mucus-clearing agents. These agents typically will not modify or substantially impair the activity of the adenoviral composition.

c) Administration

(i) Dosage Forms

[00261] The composition can be formulated into various dosage forms and administered by a number of different means that will deliver a therapeutically effective amount of the active ingredient. Such compositions can be administered through a suitable route for example oral, parenteral, pulmonary, topical, mucosal, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, ophthalmic, buccal, and rectal in dosage unit formulations containing conventional nontoxic pharmaceutically acceptable carriers, adjuvants, and vehicles as desired. Topical administration may also involve the use of transdermal administration such as transdermal patches or iontophoresis devices. The term parenteral as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, or intrasternal injection, or infusion techniques. Formulation of drugs is discussed in, for example, Gennaro, A. R., Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa. (18th ed, 1995), and Liberman, H. A. and Lachman, L., Eds., Pharmaceutical Dosage Forms, Marcel Dekker Inc., New York, N.Y. (1980). In a specific embodiment, a composition may be a food supplement or a composition may be a cosmetic.

[00262] For parenteral administration (including subcutaneous, intraocular, intradermal, intravenous, intramuscular, intra-articular and intraperitoneal), the preparation may be an aqueous or an oil-based solution. Aqueous solutions may include a sterile diluent such as water, saline solution, a pharmaceutically acceptable polyol such as glycerol, propylene glycol, or other synthetic solvents; an antibacterial and/or antifungal agent such as benzyl alcohol, methyl paraben, chlorobutanol, phenol, thimerosal, and the like; an antioxidant such as ascorbic acid or sodium bisulfite; a chelating agent such as etheylenediaminetetraacetic acid; a buffer such as acetate,

citrate, or phosphate; and/or an agent for the adjustment of tonicity such as sodium chloride, dextrose, or a polyalcohol such as mannitol or sorbitol. The pH of the aqueous solution may be adjusted with acids or bases such as hydrochloric acid or sodium hydroxide. Oil-based solutions or suspensions may further comprise sesame, peanut, olive oil, or mineral oil. The compositions may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carried, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules, and tablets.

[00263] If nasal or respiratory (mucosal) administration is desired, compositions may be in a form and dispensed by a squeeze spray dispenser, pump dispenser or aerosol dispenser. Such dispensers may also be employed to deliver the composition to oral or oral cavity (e.g., buccal or perlingual) mucosa, nasal cavity. Aerosols are usually under pressure by means of a hydrocarbon. Pump dispensers can preferably dispense a metered dose or, a dose having a particular particle size.

[00264] In certain embodiments, the vector compositions disclosed herein are co-formulated, for example, with a pharmaceutically acceptable buffer, carrier, excipient and/or adjuvant, in a single composition for administration, for example admixed, and administered to a subject together at the same time. In other embodiments, the vector and protein are formulated, for example, with a pharmaceutically acceptable buffer, carrier, excipient and/or adjuvant, in separate compositions, and are administered to a subject in separate compositions within 24 hours, such as within 12 hours, 10 hours, 8 hours, 6 hours, 4 hours, 2 hours, or within 1 hour or less.

[00265] Generally, a safe and effective amount of an adenoviral composition is administered, for example, that amount that would cause the desired therapeutic effect in a subject while minimizing undesired side effects. In various embodiments, an effective amount of an adenoviral composition described herein can substantially reduce viral infectivity in a subject suffering from a viral infection. In some embodiments, an effective amount is an amount capable of treating a respiratory viral

infection. In some embodiments, an effective amount is an amount capable of treating one or more symptoms associated with a respiratory viral infection.

[00266] The amount of a composition described herein that can be combined with a pharmaceutically acceptable carrier to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. It will be appreciated by those skilled in the art that the unit content of agent contained in an individual dose of each dosage form need not in itself constitute a therapeutically effective amount, as the necessary therapeutically effective amount could be reached by administration of a number of individual doses.

[00267] Dosages of the adenoviral vector will depend primarily on factors such as the condition being treated, the age, weight and health of the patient, and may thus vary among patients. For example, a therapeutically effective adult human or veterinary dosage of the viral vector is generally in the range of from about 100 µL to about 100 mL of a carrier containing concentrations of from about 1 x 10^6 to about 1 x 10^{15} particles, about 1 x 10^7 to 1 x 10^{13} particles, or about 1 x 10^9 to 1 x 10^{12} particles virus. Dosages will range depending upon the size of the animal and the route of administration. For example, a suitable human or veterinary dosage (for about an 80 kg animal) for intramuscular injection is in the range of about 1 x 10^9 to about 5 x 10^{12} particles per mL, for a single site. Optionally, multiple sites of administration may be delivered. In another example, a suitable human or veterinary dosage may be in the range of about 1 x 10^{11} to about 1 x 10^{15} particles for an oral formulation. One of skill in the art may adjust these doses, depending the route of administration, and the therapeutic or vaccinal application for which the recombinant vector is employed. The levels of expression of the transgene, or for an immunogen, the level of circulating antibody, can be monitored to determine the frequency of dosage administration. Yet other methods for determining the timing of frequency of administration will be readily apparent to one of skill in the art.

[00268] An optional method step involves the co-administration to the patient, either concurrently with, or before or after administration of the viral vector, of a suitable amount of a short acting immune modulator. The selected immune modulator is

defined herein as an agent capable of inhibiting the formation of neutralizing antibodies directed against the recombinant vector of this invention or capable of inhibiting cytolytic T lymphocyte (CTL) elimination of the vector. The immune modulator may interfere with the interactions between the T helper subsets ($T_{H}i$ or T^) and B cells to inhibit neutralizing antibody formation. Alternatively, the immune modulator may inhibit the interaction between $T_{H}i$ cells and CTLs to reduce the occurrence of CTL elimination of the vector. A variety of useful immune modulators and dosages for use of same are disclosed, for example, in Yang et al., J. Virol., 70(9) (Sept., 1996); International Patent Application Publication No. WO 96/12406, published May 2, 1996; and International Patent Application No. PCT/US96/03035, all incorporated herein by reference

[00269] The specific therapeutically effective dose level for any particular subject will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the subject; the time of administration; the route of administration; the rate of excretion of the composition employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts (see e.g., Koda-Kimble et al. (2004) Applied Therapeutics: The Clinical Use of Drugs, Lippincott Williams & Wilkins, ISBN 0781748453; Winter (2003) Basic Clinical Pharmacokinetics, 4th ed., Lippincott Williams & Wilkins, ISBN 0781741475; Sharqel (2004) Applied Biopharmaceutics & Pharmacokinetics, McGraw-Hill/Appleton & Lange, ISBN 0071375503). For example, it is well within the skill of the art to start doses of the composition at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. If desired, the effective daily dose may be divided into multiple doses for purposes of administration. Consequently, single dose compositions may contain such amounts or submultiples thereof to make up the daily dose. It will be understood, however, that the total daily usage of the compounds and compositions of the present disclosure will be decided by an attending physician within the scope of sound medical judgment.

[00270] Administration of a viral composition can occur as a single event or over a time course of treatment. For example, one or more of a nanoparticle composition can be administered daily, weekly, bi-weekly, or monthly. For treatment of acute conditions, the time course of treatment will usually be at least several days. Certain conditions could extend treatment from several days to several weeks. For example, treatment could extend over one week, two weeks, or three weeks. For more chronic conditions, treatment could extend from several weeks to several months or even a year or more.

[00271] Treatment in accord with the methods described herein can be performed prior to, concurrent with, or after conventional treatment modalities for a respiratory virus.

[00272] The present disclosure encompasses pharmaceutical compositions comprising compounds as disclosed above, so as to facilitate administration and promote stability of the active agent. For example, a compound of this disclosure may be admixed with at least one pharmaceutically acceptable carrier or excipient resulting in a pharmaceutical composition which is capably and effectively administered (given) to a living subject, such as to a suitable subject (i.e. “a subject in need of treatment” or “a subject in need thereof”). For the purposes of the aspects and embodiments of the invention, the subject may be a human or any other animal.

III. METHODS

[00273] The present disclosure encompasses methods to treat, prevent, or reduce the infectivity or transmission of a virus in a subject in need thereof. In some embodiments, the methods prevent or reduce the infectivity of a viral infection by preventing internalization of a virus into a cell of the subject or by preventing internalization of a viral genome into a cell of the subject. In some embodiments, administration of a composition provided herein, for instance those described in **Section II**, may create an immune response in a subject which disrupts or prevents an interaction between a viral surface protein (*e.g.*, a spike protein or an envelope protein) and a host receptor protein (*e.g.*, an epithelial angiotensin converting enzyme (ACE)).

Administering a composition of the disclosure to a subject at risk for a viral infection may reduce the risk of coronavirus infection in the subject.

[00274] The disclosed compositions can be administered to a subject to induce an immune response to the corresponding coronavirus spike protein in the subject. In a particular example, the subject is a human. The immune response can be a protective immune response, for example a response that inhibits subsequent infection with the corresponding coronavirus. Elicitation of the immune response can also be used to treat or inhibit infection and illnesses associated with the corresponding coronavirus.

[00275] A subject can be selected for treatment that has, or is at risk for developing, infection with the coronavirus corresponding to the S protein in the immunogen, for example because of exposure or the possibility of exposure to the coronavirus. Following administration of a disclosed immunogen, the subject can be monitored for infection or symptoms associated with the coronavirus, or both.

[00276] Typical subjects intended for treatment with the therapeutics and methods of the present disclosure include humans, as well as non-human primates and other animals. To identify subjects for prophylaxis or treatment according to the methods of the disclosure, accepted screening methods are employed to determine risk factors associated with a targeted or suspected disease or condition, or to determine the status of an existing disease or condition in a subject. These screening methods include, for example, conventional workups to determine environmental, familial, occupational, and other such risk factors that may be associated with the targeted or suspected disease or condition, as well as diagnostic methods, such as various ELISA and other immunoassay methods to detect and/or characterize coronavirus infection. These and other routine methods allow the clinician to select patients in need of therapy using the methods and pharmaceutical compositions of the disclosure. In accordance with these methods and principles, a composition can be administered according to the teachings herein, or other conventional methods, as an independent prophylaxis or treatment program, or as a follow-up, adjunct or coordinate treatment regimen to other treatments.

[00277] The administration of a disclosed compositions can be for prophylactic or therapeutic purpose. When provided prophylactically, the disclosed therapeutic agents are provided in advance of any symptom, for example, in advance of infection. The prophylactic administration of the disclosed therapeutic agents serves to prevent or ameliorate any subsequent infection. When provided therapeutically, the disclosed therapeutic agents are provided at or after the onset of a symptom of disease or infection, for example, after development of a symptom of infection with the coronavirus corresponding to the S protein in the composition, or after diagnosis with the coronavirus infection. The therapeutic agents can thus be provided prior to the anticipated exposure to the coronavirus so as to attenuate the anticipated severity, duration or extent of an infection and/or associated disease symptoms, after exposure or suspected exposure to the virus, or after the actual initiation of an infection.

[00278] The compositions described herein, are provided to a subject in an amount effective to induce or enhance an immune response against the coronavirus S protein in the subject, preferably a human. The actual dosage of disclosed compositions will vary according to factors such as the disease indication and particular status of the subject (for example, the subject' s age, size, fitness, extent of symptoms, susceptibility factors, and the like), time and route of administration, other drugs or treatments being administered concurrently, as well as the specific pharmacology of the composition for eliciting the desired activity or biological response in the subject. Dosage regimens can be adjusted to provide an optimum prophylactic or therapeutic response.

[00279] A composition according to the disclosure can be used in coordinate vaccination protocols or combinatorial formulations. In certain embodiments, compositions and coordinate immunization protocols employ separate transgene or formulations, each directed toward eliciting an anti-viral immune response, such as an immune response to coronavirus S proteins. Separate immunogenic compositions that elicit the anti-viral immune response can be combined in a polyvalent immunogenic composition administered to a subject in a single immunization step, or they can be administered separately (in monovalent immunogenic compositions) in a coordinate (or prime-boost) immunization protocol.

[00280] There can be several boosts, and each boost can be a different disclosed transgene. In some examples that the boost may be the same transgene as another boost, or the prime. The prime and boost can be administered as a single dose or multiple doses, for example two doses, three doses, four doses, five doses, six doses or more can be administered to a subject over days, weeks or months. Multiple boosts can also be given, such one to five (e.g., 1, 2, 3, 4 or 5 boosts), or more. Different dosages can be used in a series of sequential immunizations. For example a relatively large dose in a primary immunization and then a boost with relatively smaller doses.

[00281] In some embodiments, the boost can be administered about two, about three to eight, or about four, weeks following the prime, or about several months after the prime. In some embodiments, the boost can be administered about 5, about 6, about 7, about 8, about 10, about 12, about 18, about 24, months after the prime, or more or less time after the prime. Periodic additional boosts can also be used at appropriate time points to enhance the subject's "immune memory." The adequacy of the vaccination parameters chosen, *e.g.*, formulation, dose, regimen and the like, can be determined by taking aliquots of serum from the subject and assaying antibody titers during the course of the immunization program. In addition, the clinical condition of the subject can be monitored for the desired effect, e.g., prevention of infection or improvement in disease state (e.g., reduction in viral load). If such monitoring indicates that vaccination is sub-optimal, the subject can be boosted with an additional dose of immunogenic composition, and the vaccination parameters can be modified in a fashion expected to potentiate the immune response.

[00282] In some embodiments, a composition of the disclosure is administered in a single-dose.

[00283] Upon administration of a disclosed composition of this disclosure, the immune system of the subject typically responds to the composition by producing antibodies specific for the coronavirus S protein included in the composition. Such a response signifies that an immunologically effective dose was delivered to the subject.

[00284] In some embodiments, the antibody response of a subject will be determined in the context of evaluating effective dosages/immunization protocols. In

most instances it will be sufficient to assess the antibody titer in serum or plasma obtained from the subject. Decisions as to whether to administer booster inoculations and/or to change the amount of the therapeutic agent administered to the individual can be at least partially based on the antibody titer level. The antibody titer level can be based on, for example, an immunobinding assay which measures the concentration of antibodies in the serum which bind to an antigen including, for example, the recombinant coronavirus S protein included in the immunogen.

[00285] Coronavirus infection does not need to be completely eliminated or reduced or prevented for the methods to be effective. For example, elicitation of an immune response to a coronavirus with one or more of the disclosed compositions can reduce or inhibit infection with the coronavirus by a desired amount, for example, by at least 10%, at least 20%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or even at least 100% (elimination or prevention of detectable infected cells), as compared to infection with the coronavirus in the absence of the composition. In additional examples, coronavirus replication can be reduced or inhibited by the disclosed methods. Coronavirus replication does not need to be completely eliminated for the method to be effective. For example, the immune response elicited using one or more of the disclosed compositions can reduce replication of the corresponding coronavirus by a desired amount, for example, by at least 10%, at least 20%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or even at least 100% (elimination or prevention of detectable replication of the coronavirus), as compared to replication of the coronavirus in the absence of the immune response.

[00286] In some embodiments, the disclosed composition is administered to the subject simultaneously with the administration of the adjuvant. Adjuvants are known in the art to further increase the immune response to an applied antigenic determinant, and pharmaceutical compositions comprising adenovirus and suitable adjuvants are for instance disclosed in WO 2007/110409, incorporated by reference herein. Examples of suitable adjuvants are provided herein.

[00287] In some embodiments, administration of a therapeutically effective amount of one or more of the disclosed compositions to a subject induces a neutralizing immune response in the subject. To assess neutralization activity, following immunization of a subject, serum can be collected from the subject at appropriate time points, frozen, and stored for neutralization testing. Methods to assay for neutralization activity are known to the person of ordinary skill in the art and are further described herein, and include, but are not limited to, plaque reduction neutralization (PRNT) assays, microneutralization assays, flow cytometry based assays, single-cycle infection assays. In some embodiments, the serum neutralization activity can be assayed using a panel of coronavirus pseudoviruses. For example, to test the immunogenicity of the vaccine candidates against multiple MERS-CoV strains— without the requirement of a biosafety level 3 facility— a pseudotyped reporter virus neutralization assay was previously developed (Wand et al., Nat Commun, 6:7712, 2015), similar to that previously developed for SARS-CoV (Martin et al, Vaccine 26, 6338, 2008; Yang et al, Nature 428, 561, 2004; Naldini et al, PNAS 93, 11382, 1996; Yang et al, PNAS 102, 797, 2005).

[00288] In other embodiments, the present disclosure provides methods to treat, prevent, or reduce the infectivity of a respiratory viral infection. In some embodiments, the viral infection may be a coronavirus infection. The coronavirus may be SARS-CoV, SARS-CoV-2, MERS-CoV, HKU1, OC43, or 229E. The coronavirus may be a beta-coronavirus. A subject at risk for a coronavirus infection may come in contact with an asymptomatic carrier of the coronavirus infection, thereby unknowingly contracting the coronavirus infection.

[00289] Certain methods of the present disclosure encompass methods of making an adenovirus described herein. Such methods may include transfecting a cell with an adenoviral vector described herein; culturing the cell under conditions such that the cell produces the recombinant adenovirus; and collecting the recombinant adenovirus. Suitable cells are known in the art. In some embodiments, the cell may be a HEK, Vero or PER cell.

[00290] The present disclosure also encompasses methods of treating a second subject with the serum of a first subject. Specifically, the present disclosure encompasses a composition comprising the serum of a first subject previously administered an adenoviral vector detailed herein, a pharmaceutical composition detailed herein, or an immunogenic composition detailed herein. Methods of collecting such serum are known in the art. Methods of treating a second subject with the serum of a first subject generally comprise administering to the second subject an immunogenically effective amount of a composition comprising serum from the first subject. Methods of administering serum to subjects are known, and it is within the skill of one in the art to determine doseage of the serum.

[00291] Generally, the methods as described herein comprise administration of a therapeutically effective amount of a nanoparticle composition of the disclosure to a subject. The methods described herein are generally performed on a subject in need thereof. A subject may be a rodent, a human, a livestock animal, a companion animal, or a zoological animal. In one embodiment, the subject may be a rodent, e.g. a mouse, a rat, a guinea pig, etc. In another embodiment, the subject may be a livestock animal. Non-limiting examples of suitable livestock animals may include pigs, cows, horses, goats, sheep, llamas and alpacas. In still another embodiment, the subject may be a companion animal. Non-limiting examples of companion animals may include pets such as dogs, cats, rabbits, and birds. In yet another embodiment, the subject may be a zoological animal. As used herein, a “zoological animal” refers to an animal that may be found in a zoo. Such animals may include non-human primates, large cats, wolves, and bears. In a preferred embodiment, the subject is a human.

IV. KITS

[00292] Also provided are kits. Such kits can include an agent or composition described herein and, in certain embodiments, instructions for administration. Components may include, but are not limited to one or more of (i) a host cell as described herein, a packaging cell as described herein, an adenoviral vector as described herein, a pharmaceutical composition as described herein, or an

immunogenic composition as described herein, and (ii) instructions for use. In some embodiments, a kit of the present disclosure may comprise a composition as detailed in Section II above. Such kits can facilitate performance of the methods described herein. When supplied as a kit, the different components of the composition can be packaged in separate containers and admixed immediately before use. Such packaging of the components separately can, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the composition. The pack may, for example, comprise metal or plastic foil such as a blister pack. Such packaging of the components separately can also, in certain instances, permit long-term storage without losing activity of the components.

[00293] Kits may also include reagents in separate containers such as, for example, sterile water or saline to be added to a lyophilized active component packaged separately. For example, sealed glass ampules may contain a lyophilized component and in a separate ampule, sterile water, sterile saline or sterile each of which has been packaged under a neutral non-reacting gas, such as nitrogen. Ampules may consist of any suitable material, such as glass, organic polymers, such as polycarbonate, polystyrene, ceramic, metal or any other material typically employed to hold reagents. Other examples of suitable containers include bottles that may be fabricated from similar substances as ampules, and envelopes that may consist of foil-lined interiors, such as aluminum or an alloy. Other containers include test tubes, vials, flasks, bottles, syringes, and the like. Containers may have a sterile access port, such as a bottle having a stopper that can be pierced by a hypodermic injection needle. Other containers may have two compartments that are separated by a readily removable membrane that upon removal permits the components to mix. Removable membranes may be glass, plastic, rubber, and the like.

[00294] In certain embodiments, kits can be supplied with instructional materials. Instructions may be printed on paper or other substrate, and/or may be supplied as an electronic-readable medium, such as a floppy disc, mini-CD-ROM, CD-ROM, DVD-ROM, Zip disc, videotape, audio tape, and the like. Detailed instructions

may not be physically associated with the kit; instead, a user may be directed to an Internet web site specified by the manufacturer or distributor of the kit.

[00295] Compositions and methods described herein utilizing molecular biology protocols can be according to a variety of standard techniques known to the art (see, e.g., Sambrook and Russel (2006) Condensed Protocols from Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, ISBN-10: 0879697717; Ausubel et al. (2002) Short Protocols in Molecular Biology, 5th ed., Current Protocols, ISBN-10: 0471250929; Sambrook and Russel (2001) Molecular Cloning: A Laboratory Manual, 3d ed., Cold Spring Harbor Laboratory Press, ISBN-10: 0879695773; Elhai, J. and Wolk, C. P. 1988. Methods in Enzymology 167, 747-754; Studier (2005) Protein Expr Purif. 41(1), 207–234; Gellissen, ed. (2005) Production of Recombinant Proteins: Novel Microbial and Eukaryotic Expression Systems, Wiley-VCH, ISBN-10: 3527310363; Baneyx (2004) Protein Expression Technologies, Taylor & Francis, ISBN-10: 0954523253).

[00296] Specific embodiments disclosed herein may be further limited in the claims using “consisting of” or “consisting essentially of” language, rather than “comprising”. When used in the claims, whether as filed or added per amendment, the transition term “consisting of” excludes any element, step, or ingredient not specified in the claims. The transition term “consisting essentially of” limits the scope of a claim to the specified materials or steps and those that do not materially affect the basic and novel characteristic(s). Embodiments of the invention so claimed are inherently or expressly described and enabled herein.

[00297] As various changes could be made in the above-described materials and methods without departing from the scope of the invention, it is intended that all matter contained in the above description and in the examples given below, shall be interpreted as illustrative and not in a limiting sense.

EXAMPLES

[00298] The following examples are included to demonstrate various embodiments of the present disclosure. It should be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent techniques

discovered by the inventors to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1: A SINGLE-DOSE CHIMPANZEE ADENOVIRUS VECTORED VACCINE PROTECTS AGAINST SARS-CoV-2 INFECTION AND PNEUMONIA IN MICE EXPRESSING HUMAN ACE2 RECEPTOR

[00299] The present example provides a chimpanzee Ad (simian AdV-36)-based SARS-CoV-2 vaccine (ChAd-SARS-CoV-2-S) encoding a pre-fusion stabilized spike (S) protein after introducing two proline substitutions in the S2 subunit. Intramuscular administration of ChAd-SARS-CoV-2-S induced robust systemic humoral and cell-mediated immune responses against the S protein. One or two vaccine doses protected against lung infection, inflammation, and pathology after SARS-CoV-2 challenge of mice that transiently express the human ACE2 (hACE2) receptor. Despite the induction of high levels of neutralizing antibody in serum, neither dosing regimen completely protected against SARS-CoV-2 infection, as substantial levels of viral RNA were still detected in the lung. In comparison, when a single dose of ChAd-SARS-CoV-2-S by the intranasal route was administered, high levels of neutralizing antibody and anti-SARS-CoV-2 IgA, and complete protection against infection in both the upper and lower respiratory tracts were detected. Moreover, and in contrast to the control ChAd vaccine, 8 days after SARS-CoV-2 challenge, serum antibody responses to the SARS-CoV-2 NP protein were absent in animals immunized with ChAd-SARS-CoV-2-S via intranasal delivery. Thus, ChAd-SARS-CoV-2-S has the potential to confer sterilizing immunity at the site of inoculation, which prevent both virus-induced disease and transmission.

Results

[00300] *Chimpanzee Ad-vectored vaccine induces robust antibody responses against anti-SARS-CoV-2:* Two replication-incompetent ChAd vectors based

on a simian Ad-36 virus were constructed. The ChAd-SARS-CoV-2-S vector encodes the full-length sequence of SARS-CoV-2 S protein as a transgene including the ectodomain, transmembrane domain, and cytoplasmic domain (GenBank: QJQ84760.1) and is stabilized in pre-fusion form by two proline substitutions at residues K986 and V987. The ChAd-control has no transgene. The S protein transgene is controlled transcriptionally by a cytomegalovirus promoter. To make the vector replication-incompetent and enhance packaging capacity, the E1A/B genes were replaced and a deletion in the E3B gene introduced, respectively (**FIG. 1A**). To confirm that the S protein was expressed and antigenically intact, 293T cells were transduced and binding of a panel of 22 neutralizing monoclonal antibodies against the S protein confirmed by flow cytometry (**FIG. 1B**).

[00301] To assess the immunogenicity of ChAd-SARS-CoV-2-S, groups of 4-week-old BALB/c mice were immunized by intramuscular inoculation with 1010 virus particles of ChAd-SARS-CoV-2-S or ChAd-control. Some mice received a booster dose four weeks later. Serum samples were collected 21 days after primary or booster immunization (**FIG. 1C**), and IgG responses against purified S and RBD proteins were evaluated by ELISA. Whereas ChAd-SARS-CoV-2-S induced high levels of S- and RBD-specific IgG, low, if any levels were detected in the ChAd-control-immunized mice (**FIG. 1D** and **FIG. 2A**). Serum samples were assayed *in vitro* for neutralization of infectious SARS-CoV-2 using a focus-reduction neutralization test (FRNT). As expected, serum from ChAd-control-immunized mice did not inhibit SARS-CoV-2 infection after primary immunization or boosting. In contrast, serum from ChAd-SARS-CoV-2-S vaccinated animals strongly neutralized SARS-CoV-2 infection, and boosting enhanced this inhibitory activity (**FIG. 1E** and **FIG. 2B-2C**).

[00302] *Vaccine-induced memory CD8+ T cell and antigen specific B cell responses:* Because optimal vaccine immunity often is comprised of both humoral and cellular responses, the levels of SARS-CoV-2-specific CD4+ and CD8+ T cells after vaccination were measured. Four-week old BALB/c mice were immunized with ChAd-SARS-CoV-2-S or ChAd-control and boosted three weeks later. To assess the vaccine-induced SARS-CoV-2-specific CD4+ and CD8+ T cell responses, splenocytes were

harvested one week after boosting and stimulated *ex vivo* with a pool of 253 overlapping 15-mer S peptides. Subsequently, quantification of intracellular IFNγ and granzyme B expression was determined by flow cytometry. After peptide re-stimulation *ex vivo*, splenic CD8+ T cells expressed IFNγ and both splenic CD4+ and CD8+ T cells expressed granzyme B in mice immunized with ChAd-SARS-CoV-2-S but not the ChAd-control vector (**FIG. 1F-1G** and **FIG. 3**). To assess the antigen-specific B cell responses, splenocytes were harvested and subjected to an ELISPOT analysis with S protein. The ChAd-SARS-CoV-2-S vaccine induced S protein-specific IgG antibody-secreting cells in the spleen whereas the control vaccine did not (**FIG. 1H**).

[00303] *Intramuscular immunization with ChAd-SARS-CoV-2-S vaccine protects against SARS-CoV-2 infection in the lung:* The protective activity of the ChAd vaccine in a recently developed SARS-CoV-2 infection model wherein BALB/c mice express hACE2 in the lung after intranasal delivery of a vectored human Ad (Hu-Ad5-hACE2) was tested. Endogenous mouse ACE2 does not support viral entry, and this system enables productive SARS-CoV-2 infection in the mouse lung. Four-week-old BALB/c mice first were immunized via an intramuscular route with ChAd-control or ChAd-SARS-CoV-2-S vaccines. Approximately thirty days later, mice were administered 10^8 plaque-forming units (PFU) of Hu-Ad5-hACE2 and anti-Ifnar1 monoclonal antibody (mAb) via intranasal and intraperitoneal routes, respectively. A single dose of anti-Ifnar1 mAb was also administered to enhance lung pathogenesis in this model. The absence of cross-immunity between the ChAd and the Hu-Ad5 vector was confirmed. Serum from ChAd-immunized mice did not neutralize Hu-Ad5 infection (**FIG. 4A-4B**).

[00304] Five days after Hu-Ad5-hACE2 transduction, mice were challenged via intranasal route with 4×10^5 focus-forming units (FFU) of SARS-CoV-2 (**FIG. 2A**). At 4 days post-infection (dpi), the peak of viral burden in this model, mice were euthanized, and lungs, spleen, and heart were harvested for viral burden and cytokine analysis. Notably, there was no detectable infectious virus in the lungs of mice immunized with ChAd-SARS-CoV-2-S as determined by plaque assay, whereas high levels were present in mice vaccinated with ChAd-control (**FIG. 5B**). Consistent with this result, reduced viral RNA levels in the lung, heart, and spleen of ChAd-SARS-CoV-2-S

vaccinated animals were observed compared to mice receiving the ChAd-control vector (**FIG. 5C**). *In situ* hybridization staining for viral RNA in lungs harvested at 4 dpi revealed a substantial decrease of SARS-CoV-2 RNA in pneumocytes of animals immunized with ChAd-SARS-CoV-2-S compared to the ChAd-control (**FIG. 5D**). A subset of immunized animals was euthanized at 8 dpi, and tissues were harvested for evaluation. At this time point, viral RNA levels again were lower or absent in the lung and spleen of ChAd-SARS-CoV-2-S immunized mice compared to the control ChAd vector (**FIG. 5C**). Collectively, these data indicate that a single intramuscular immunization with ChAd-SARS-CoV-2-S results in markedly reduced, but not abrogated, SARS-CoV-2 infection in the lungs of challenged mice.

[00305] Next, the effect of the vaccine on lung inflammation and disease were assessed. Several proinflammatory cytokines and chemokine mRNA levels were lower in the lung tissues of animals immunized with ChAd-SARS-CoV-2-S compared to ChAd-control including *CXCL10, IL1β, IL-6, CCL5, IFNβ* and *IFNγ* (**FIG. 5E**). Moreover, mice immunized with ChAd-control vaccine and challenged with SARS-CoV-2 showed evidence of viral pneumonia characterized by immune cell accumulation in perivascular and alveolar locations, vascular congestion, and interstitial edema. In contrast, animals immunized with ChAd-SARS-CoV-2-S showed a marked attenuation of the inflammatory response in the lung that develops in the ChAd-control-immunized mice (**FIG. 6**). Thus, immunization with Ch-Ad-SARS-CoV-2 decreases both viral infection and the consequent lung inflammation and injury associated with SARS-CoV-2 infection.

[00306] Improved protection using a prime-boost vaccine regimen was then assessed. BALB/c mice were immunized via an intramuscular route with ChAd-control or ChAd-SARS-CoV-2-S and received a homologous booster dose four weeks later. At day 29 post-boost, mice were treated with a single dose of anti-Ifnar1 antibody followed by Hu-Ad5-hACE2 and then challenged with SARS-CoV-2 five days later. As expected, the prime-boost regimen protected against SARS-CoV-2 challenge with no infectious virus detected in the lungs (**FIG. 5F**). Although marked reductions of viral RNA in the

lung, spleen, and heart were detected at 4 dpi, residual levels of viral RNA still were present suggesting protection was not complete, even after boosting (**FIG. 5G**).

[00307] *A single intranasal immunization with ChAd-SARS-CoV-2-S induces sterilizing immunity against SARS-CoV-2:* Mucosal immunization through the nasopharyngeal route can elicit local immune responses including secretory IgA antibodies that confer protection at or near the site of inoculation of respiratory pathogens. To assess the immunogenicity and protective efficacy of mucosal vaccination, five-week old BALB/c mice were inoculated via intranasal route with 10^{10} viral particles of ChAd-control or ChAd-SARS-CoV-2-S (**FIG. 7A**). Serum samples were collected at four weeks post-immunization to evaluate humoral immune responses. Intranasal immunization of ChAd-SARS-CoV-2-S but not ChAd-control induced high levels of S- and RBD-specific IgG and IgA (**FIG. 7B-7C**) and SARS-CoV-2 neutralizing antibodies (geometric mean titer of 1/1,574) in serum (**FIG. 7D** and **FIG. 8A**). Serum antibodies from mice immunized with ChAd-SARS-CoV-2-S equivalently neutralized a recombinant, luciferase-expressing variant of SARS-CoV-2 encoding a D614G mutation in the S protein (**FIG. 8B**); this finding is important, because many circulating viruses contain this substitution, which is associated with greater infectivity in cell culture (doi.org/10.1101/2020.06.12.148726). The SARS-CoV-2-specific antibody responses in bronchoalveolar lavage (BAL) fluid of immunized mice was also assessed. BAL fluid from ChAd-SARS-CoV-2-S but not ChAd-control vaccinated mice showed high levels of S- and RBD-specific IgG and IgA antibodies (**FIG. 7E-7F**) including those with neutralizing activity (**FIG. 7G** and **FIG. 8C**).

[00308] To assess T cell responses activated via mucosal immunization, mice were vaccinated via intranasal route with either ChAd-SARS-CoV-2-S or ChAd-control and boosted similarly four weeks later. Lungs were harvested one-week post-boosting, and T cells were analyzed by flow cytometry. Re-stimulation ex vivo with a pool of S peptides resulted in a marked increase in IFNγ and granzyme B producing CD8+ T cells in the lungs of mice that received the ChAd-SARS-CoV-2-S vaccine (**FIG. 7H**). Specifically, a population of IFNγ-secreting, antigen-specific $CD103^{+}CD69^{+}CD8^{+}$ T cells in the lung was identified (**FIG. 7I**) which is phenotypically consistent with vaccine-

induced resident memory T cells. In the spleen, antibody-secreting plasma cells producing IgA or IgG against the S protein were detected after intranasal immunization with ChAd-SARS-CoV-2-S (**FIG. 7J**). Of note, an ~5-fold higher frequency of B cells secreting anti-S IgA than IgG was observed.

[00309] The protective efficacy of the ChAd vaccine after single-dose intranasal immunization was evaluated. At day 30 post-vaccination, mice were administered 10^8 PFU of Hu-Ad5-hACE2 and anti-Ifnar1 mAb as described above. Five days later, mice were challenged with 4 x 10^5 FFU of SARS-CoV-2. At 4 and 8 dpi, lungs, spleen, heart, nasal turbinates, and nasal washes were harvested and assessed for viral burden. Intranasal delivery of the ChAd-SARS-CoV-2-S vaccine demonstrated remarkable protective efficacy as judged by an absence of infectious virus in the lungs (**FIG. 9A**) and almost no measurable viral RNA in the lung, spleen, heart, nasal turbinates, or nasal washes (**FIG. 9B**). The very low viral RNA levels in the lung and nasal turbinates at 4 dpi likely reflects the input, non-replicating virus, as similar levels were measured at this time point in C57BL/6 mice lacking hACE2 receptor expression. Cytokine and chemokine mRNA levels in lung homogenates also were substantially lower in mice immunized with theChAd-SARS-CoV-2-S than the ChAd-control vaccine (**FIG. 9C**), with residual expression likely due to the human Ad vector hACE2 delivery system. Finally, histopathological analysis of lung tissues from animals vaccinated with ChAd-SARS-CoV-2-S by intranasal route and challenged with SARS-CoV-2 showed minimal, if any, perivascular and alveolar infiltrates at 8 dpi compared to the extensive inflammation observed in ChAd-control vaccinated animals (**FIG. 9D**).

[00310] To determine if sterilizing immunity was achieved with intranasal delivery of ChAd-SARS-CoV-2-S, anti-NP antibodies were measured at 8 dpi and compared to responses from 5 days before SARS-CoV-2 infection. Because the NP gene is absent from the vaccine vector, induction of humoral immune responses against NP after SARS-CoV-2 exposure suggests viral protein translation and active infection. After SARS-CoV-2 challenge, anti-NP antibody responses were detected in ChAd-control mice vaccinated by an intranasal route or ChAd-control and ChAd-SARS-CoV-2-S mice vaccinated by an intramuscular route (**FIG. 9E** and **FIG. 2D**). Remarkably, none

of the mice immunized with ChAd-SARS-CoV-2-S via an intranasal route showed significant increases in anti-NP antibody responses after SARS-CoV-2 infection. Combined with our virological analyses, these data suggest that a single intranasal immunization of ChAd-SARS-CoV-2-S induces robust and likely sterilizing mucosal immunity, which prevents SARS-CoV-2 infection in the upper and lower respiratory tracts of mice expressing the hACE2 receptor.

Discussion

[00311] In this example, intramuscular and intranasal administration of a replication-incompetent ChAd vector as a vaccine platforms for SARS-CoV-2 were evaluated. Single dose immunization of a stabilized S protein-based vaccine via an intramuscular route induced S- and RBD-specific binding as well as neutralizing antibodies. Vaccination with one or two doses protected mice expressing human ACE2 against SARS-CoV-2 challenge, as evidenced by an absence of infectious virus in the lungs and substantially reduced viral RNA levels in lungs and other organs. Mice immunized with ChAd-SARS-CoV-2-S also showed markedly reduced if not absent lung pathology, lung inflammation, and evidence of pneumonia compared to the control ChAd vaccine. However, intramuscular vaccination of ChAd-SARS-CoV-2-S did not confer sterilizing immunity, as evidenced by detectable viral RNA levels in several tissues including the lung and induction of anti-NP antibody responses. Mice immunized with a single dose of the ChAd-SARS-CoV-2-S via an intranasal route also were protected against SARS-CoV-2 challenge. Intranasal vaccination, however, generated robust IgA and neutralizing antibody responses that protected against both upper and lower respiratory tract SARS-CoV-2 infection and inhibited infection of both wild-type and D614G variant viruses. The very low viral RNA in upper airway tissues and absence of serological response to NP in the context of challenge strongly suggests that most animals receiving a single intranasal dose of ChAd-SARS-CoV-2-S achieve sterilizing immunity.

[00312] Although several vaccine candidates *(e.g.*, lipid-encapsulated mRNA, DNA, inactivated, and viral-vectored) rapidly advanced to human clinical trials in an expedited effort to control the pandemic, few studies have demonstrated efficacy in

pre-clinical models. Rhesus macaques immunized with two or three doses of a DNA plasmid vaccine encoding full-length SARS-CoV-2 S protein induced neutralizing antibody in sera and reduced viral burden in BAL and nasal mucosa fluids. Moreover, three immunizations over two weeks with purified, inactivated SARS-CoV-2 induced neutralizing antibodies, and depending on the dose administered provided partial or complete protection against infection and viral pneumonia in rhesus macaques. One limitation of these challenge models is that rhesus macaques develop mild interstitial pneumonia after SARS-CoV-2 infection compared to some humans and other non-human primate species. The present example, in hACE2-expressing mice, showed that a single intramuscular or intranasal dose of ChAd-SARS-CoV-2-S vaccine confers substantial and possibly complete protection against viral replication, inflammation, and lung disease.

[00313] The present disclosure supports the use of non-human Ad-vectored vaccines against emerging RNA viruses including SARS-CoV-2. Previous work showed the efficacy of single-dose or two-dose regimens of a gorilla Ad encoding the prM-E genes of Zika virus (ZIKV) in several mouse challenge models including in the context of pregnancy. Others have evaluated ChAd or rhesus macaque Ad vaccine candidates against ZIKV and shown efficacy in mice and non-human primates. A different ChAd encoding the wild-type SARS-CoV-2 S protein (ChAdOx1) is currently in clinical trials in humans (NCT04324606). Although data from the human trials has not yet been reported, studies in rhesus macaques suggest that a single intramuscular dose protects against infection in the lung but not in the upper respiratory tract (biorxiv.org/content/10.1101/2020.05.13.093195v1). None of the vaccines evaluated against SARS-CoV-2 has shown evidence of immune enhancement in any pre-clinical or clinical study, which has been a theoretical risk based on studies with other human and animal coronaviruses. Indeed, and in contrast to data with SARS-CoV vaccines or antibodies, enhanced infection, immunopathology, or disease in animals immunized with ChAd encoding SARS-CoV-2 S proteins or administered passively transferred monoclonal antibodies was not observed.

[00314] ChAd-SARS-CoV-2-S induced SARS-CoV-2 specific $CD8^+$ T cell responses including a high percentage and number of IFNγ and granzyme expressing cells after *ex vivo* S peptide restimulation. The induction of robust $CD8^+$ T cell responses by the ChAd-SARS-CoV-2-S vaccine is consistent with reports with other simian Ad vectors. ChAd vaccine vectors not only overcome issues of pre-existing immunity against human adenoviruses but also have immunological advantages because they do not induce exhausted T cell responses.

[00315] A single intranasal dose of ChAd-SARS-CoV-2-S conferred superior immunity against SARS-CoV-2 challenge, more so than one or two intramuscular immunizations of the same vaccine and dose. Given that the serum neutralizing antibody responses was comparable, it is hypothesized that the greater protection observed after intranasal delivery was because of the mucosal immune responses generated. Indeed, high levels of anti-SARS-CoV-2 IgA were detected in serum and lung, and B cells secreting IgA were detected in the spleen. Moreover, intranasal vaccination also induced SARS-CoV-2-specific CD8+ T cells in the lung including $CD103^+CD69^+$ cells, which are likely of a resident memory phenotype. To our knowledge, none of the SARS-CoV-2 vaccine platforms currently in clinical trials use an intranasal delivery approach. There has been great interest in using intranasal delivery for influenza A virus vaccines because of their ability to elicit local humoral and cellular immune responses. Indeed, sterilizing immunity to influenza A virus re-infection requires local adaptive immune responses in the lung, which is optimally induced by intranasal and not intramuscular inoculation. While there are concerns with administering live-attenuated viral vaccines via an intranasal route, subunit-based or replication-incompetent vectored vaccines are promising for generating mucosal immunity in a safer manner, especially given advances in formulation.

[00316] In summary, the present example established that immunization with ChAd-SARS-CoV-2-S induces both neutralizing antibody and antigen-specific CD8+ T cell responses. While a single intramuscular immunization of ChAd-SARS-CoV-2-S confers protection against SARS-CoV-2 infection and inflammation in the lungs, intranasal delivery of ChAd-SARS-CoV-2-S induces mucosal immunity, provides

superior protection, and likely promotes sterilizing immunity, at least in mice transiently expressing the hACE2 receptor. Thus, the present example supports intranasal delivery of ChAd-SARS-CoV-2-S as a platform for controlling SARS-CoV-2 infection, disease, and transmission.

Methods

[00317] *Viruses and cells:* Vero E6 (CRL-1586, American Type Culture Collection (ATCC), Vero CCL81 (ATCC), and HEK293 cells were cultured at 37°C in Dulbecco's Modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 10 mM HEPES pH 7.3, 1 mM sodium pyruvate, 1X non-essential amino acids, and 100 U/ml of penicillin–streptomycin.

[00318] SARS-CoV-2 strain 2019 n-CoV/USA_WA1/2020 was obtained from the Centers for Disease Control and Prevention (a gift from Natalie Thornburg). The virus was passaged once in Vero CCL81 cells and titrated by focus-forming assay (FFA) on Vero E6 cells. The recombinant luciferase-expressing full-length SARS-CoV-2 reporter virus (2019 n-CoV/USA_WA1/2020 strain) has been reported previously (Zost et al., 2020), and the D614G variant will be described elsewhere. All work with infectious SARS-CoV-2 was performed in Institutional Biosafety Committee approved BSL3 and A-BSL3 facilities using appropriate positive pressure air respirators and protective equipment.

[00319] *Mouse experiments:* Animal studies were carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocols were approved by the Institutional Animal Care and Use Committee at the Washington University School of Medicine (Assurance number A3381-01). Virus inoculations were performed under anesthesia that was induced and maintained with ketamine hydrochloride and xylazine, and all efforts were made to minimize animal suffering.

[00320] Female BALB/c mice were purchased from The Jackson Laboratory (catalog 000651). Four to five-week-old animals were immunized with 1010 viral particles (vp) of ChAdV-empty or ChAd-SARS-CoV-2-S in 50 µl PBS via intramuscular injection in the hind leg or via intranasal inoculation. Subsets of

immunized animals were boosted four weeks after primary immunization using the same route used for primary immunization. Vaccinated mice (10 to 11-week-old) were given a single intraperitoneal injection of 2 mg of anti-Ifnar1 mAb (MAR1-5A3, Leinco) one day before intranasal administration of 10^8 PFU of Hu-AdV5-hACE2. Five days after Hu-AdV5 transduction, mice were inoculated with 4 x 10^5 FFU of SARS-CoV-2 by the intranasal route. Animals were euthanized at 4 or 8 dpi, and tissues were harvested for virological, immunological, and pathological analyses.

[00321] *Construction of chimpanzee adenovirus vectors:* Simian Ad36 vector (ChAd) was obtained from the Penn Vector Core of the University of Pennsylvania. The ChAd genome was engineered with deletions in the E1 and E3B region (GenBank: FJ025917.1; nucleotides 455-3026 and 30072-31869, respectively). A modified human cytomegalovirus major immediate early promoter sequence was incorporated in place of the E1 gene in counterclockwise orientation on the complementary DNA strand. CMV modification included an addition of two copies of the tet operator 2 (TetO2) sequence inserted in tandem (5´-TCT CTA TCA CTG ATA GGG AGA TCT CTA TCA CTG ATA GG GA-3´)(SEQ ID NO: 7) between the TATA box and the mRNA start (GenBank: MN920393, nucleotides 174211-174212). SARS-CoV-2 S (encoding a prefusion stabilized mutant with two proline substitutions at residues K986 and V987 that stabilize the prefusion form of S) was cloned into a unique *PmeI* site under the CMV-tetO2 promoter control in pSAd36 genomic plasmid to generate pSAd36-S. Similarly, the mutant SAR-CoV-2S proteins as disclosed in SEQ ID. NOS: 10,11, 20, 21 were similarly cloned. In parallel, a pSAd36-control carrying an empty CMV-tetO2 cassette with no transgene also was generated. The pSAd36-S and pSAd-control plasmids were linearized with PacI restriction enzyme to liberate viral genomes for transfection into T-Rex 293-HEK cells (Invitrogen). The rescued replication-incompetent ChAd-SARS-CoV-2-S and ChAd-Control vectors were scaled up in 293 cells and purified by CsCl density-gradient ultracentrifugation. Viral particle concentration in each vector preparation was determined by spectrophotometry at 260 nm.

[00322] *Construction of a human adenovirus vector expressing human ACE2*: Codon-optimized hACE2 sequences were cloned into the shuttle vector (pShuttle-CMV, Addgene 240007) to generate pShuttle-hACE2. pShuttle-hACE2 was linearized with *PmeI* and subsequently cotransformed with the HuAdv5 backbone plasmid (pAdEasy-1 vector; Addgene 240005) into *E. coli* strain BJ5183 to generate pAdV5-ACE2 by homologous recombination. The pAdEasy-1 plasmid containing the HuAdV5 genome has deletions in E1 and E3 genes. hACE2 is under transcriptional control of a cytomegalovirus promotor and is flanked at its 3' end by a SV40 polyadenylation signal. The pAd-hACE2 was linearized with *PacI* restriction enzyme before transfection into T-Rex 293 HEK cells (Invitrogen) to generate HuAdv5-hACE2. Recombinant HuAdv5-hACE2 was produced in 293-HEK cells and purified by CsCl density-gradient ultracentrifugation. The viral titer was determined by plaque assay in 293-HEK cells.

[00323] *In situ RNA hybridization and histology*: RNA *in situ* hybridization was performed using RNAscope 2.5 HD (Brown) (Advanced Cell Diagnostics) according to the manufacturer's instructions. Left lung tissues were collected at necropsy, inflated with 10% neutral bufferedformalin (NBF), and thereafter immersion fixed in 10% NBF for seven days before processing. Paraffin-embedded lung sections were deparaffinized by incubating at 60°C for 1 h, and endogenous peroxidases were quenched with H_2O_2 for 10 min at room temperature. Slides were boiled for 15 min in RNAscope Target Retrieval Reagents and incubated for 30 min in RNAscope Protease Plus reagent prior to SARS-CoV2 RNA probe (Advanced Cell Diagnostics 848561) hybridization and signal amplification. Sections were counterstained with Gill's hematoxylin and visualized by brightfield microscopy. Some lung sections were processed for histology after hematoxylin and eosin staining.

[00324] *SARS-CoV-2 Neutralization assays*: Heat-inactivated serum samples were diluted serially and incubated with 10^2 FFU of SARS-CoV-2 for 1 h at 37°C. The virus-serum mixtures were added to Vero cell monolayers in 96-well plates and incubated for 1 h at 37°C. Subsequently, cells were overlaid with 1% (w/v) methylcellulose in MEM supplemented with 2% FBS. Plates were incubated for 30 h

before fixation using 4% PFA in PBS for 1 h at room temperature. Cells were washed then sequentially incubated with anti-SARS-CoV-2 CR3022 antibody (Yuan et al., 2020) (1 μg/mL) and a HRP-conjugated goat anti-human IgG (Sigma) in PBS supplemented with 0.1% (w/v) saponin (Sigma) and 0.1% BSA. TrueBlue peroxidase substrate (KPL) was used to develop the plates before counting the foci on a BioSpot analyzer (Cellular Technology Limited). For neutralization experiments with luciferase expressing SARS-CoV-2, serum samples were diluted 3-fold starting at 1:50 and mixed with 85 PFU of each recombinant virus (wild-type and D614G). Vero E6 cells plated in clear-bottom black-walled 96-well plates (Corning) were inoculated with serum-virus mixtures, and cells were cultured at 37° C for 48 h. Subsequently, cells were lysed and luciferase activity was measured using the Nano-Glo Luciferase Assay System (Promega) according to the manufacturer's specifications.

[00325] *Hu-AdV5 neutralization assays*: One day prior to Hu-AdV5-hACE2 transduction, serum samples were collected from mice immunized intramuscularly with ChAd-Control or ChAd-SARS-CoV-2-S. Sera were heat-inactivated and serially diluted prior to incubation with 10^2 FFU of HuAdV5 for 1 h at 37°C. The virus-serum mixtures were added to HEK293 cell monolayers in 96-well plates and incubated for 1 h at 37°C. Cells were then overlaid with 1% (w/v) methylcellulose in MEM supplemented with 5% FBS. Plates were incubated at 37°C for 48 h before fixation with 2% PFA in PBS for 1 h at room temperature. Subsequently, plates were washed with PBS and incubated overnight at 4°C with biotinylated anti-HuAdV5-hexon antibody (2 μg/mL; Novus Biologicals NB600413) diluted in permeabilization buffer (PBS supplemented with 0.1% (w/v) saponin and 0.1% BSA). Plates were washed again and incubated with streptavidin-HRP (1:3000; Vector Laboratories SA-5004) in permeabilization buffer for 30 min at room temperature. After a final wash series, plates were developed using TrueBlue peroxidase substrate (KPL) and foci were counted on a BioSpot analyzer (Cellular Technology Limited).

[00326] *Protein expression and purification:* Purified RNA from the 2019-nCoV/USA-WA1/2020 SARS-CoV-2 strain was reverse transcribed into cDNA and used as the template for recombinant gene cloning. A full-length SARS-CoV-2 NP (NP-FL)

was cloned into pET21a with a hexahistidine tag and recombinantly expressed using BL21(DE3)-RIL *E. coli* in Terrific Broth (bioWORLD). Following overnight induction with isopropyl β-d-1-thiogalactopyranoside (Goldbio) at 25°C, cells were lysed in 20 mM Tris-HCl pH 8.5, 1 M NaCl, 5 mM β-mercaptopethanol, and 5 mM imidazole for nickel-affinity purification. Following elution in the prior buffer supplemented with 500 mM imidazole, the protein was purified to homogeneity using size exclusion and, in some cases, cation exchange chromatography. SARS-CoV-2 RBD and S ectodomain (S1/S2 furin cleavage site was disrupted, double proline mutations were introduced into the S2 subunit, and foldon trimerization motif was incorporated) were cloned into pFM1.2 with a C-terminal hexahistidine or octahistidine tag, transiently transfected into Expi293F cells, and purified by cobalt-charged resin chromatography (G-Biosciences) as previously described (Alsoussi et al., 2020).

[00327] *ELISA:* Purified antigens (S, RBD, or NP) were coated onto 96-well Maxisorp clear plates at 2 µg/mL in 50 mM Na_2CO_3pH 9.6 (70 µL) overnight at 4°C. Coating buffers were aspirated, and wells were blocked with 200 µL of 1X PBS + 0.05% Tween-20 + 1% BSA + 0.02% NaN_3 (Blocking buffer, PBSTBA) either for 1 h at 37°C or overnight at 4°C. Heat-inactivated serum samples were diluted in PBSTBA in a separate 96-well polypropylene plate. The plates then were washed thrice with 1X PBS + 0.05% Tween-20 (PBST), followed by addition of 50 µL of respective serum dilutions. Sera were incubated in the blocked ELISA plates for at least 1 h at room temperature. The ELISA plates were again washed thrice in PBST, followed by addition of 50 µL of 1:2000 anti- mouse IgG-HRP (Southern Biotech Cat. #1030-05) in PBST or 1:10000 of biotinylated anti-mouse IgG, anti-mouse IgM, or anti-mouse IgA in PBSTBA (SouthernBiotech). Plates were incubated at room temperature for 1 h, washed thrice in PBST, and then 1:5000 dilution of streptavidin-HRP (ThermoFisher) was added to wells. Following a 1 h incubation at room temperature, plates were washed thrice with PBST and 50 µL of 1-Step Ultra TMB-ELISA was added (ThermoFisher Cat. #34028). Following a 12 to 15-min incubation, reactions were stopped with 50 µL of 2 M sulfuric acid. The absorbance of each well at 450 nm was read (Synergy H1) within 2 min of

addition of sulfuric acid. Optical density (450 nm) measurements were determined using a microplate reader (Bio-Rad).

[00328] *ELISpot assay:* MultiScreen-HA filter 96-well plates (Millipore) plates were pre-coated with 3 µg/ml of SARS-CoV-2 S protein overnight at 4° C. After rinsing with PBST, plates were blocked for 4 h at 37° C with culture medium (RPMI, 10% FBS, penicillin-streptomycin, 1 mM sodium pyruvate, 0.1 mM non-essential amino acids, 10 mM HEPES, and 50 mM β-mercaptoethanol). Single cell suspensions of splenocytes in culture medium were added to the S protein coated plates and incubated at 37° C and 5% humidified CO_2 for 4 h. After washing with PBS and PBST, plates were incubated with biotinylated anti-IgG or anti-IgA (Southern Biotech) followed by incubation with streptavidin conjugated horseradish peroxidase (Jackson ImmunoResearch), each for 1 h at room temperature. After additional washes with PBS, 3-amino-9-ethylcarbazole (Sigma) substrate solution was added for spot development. The reaction wasstopped by rinsing with water. Spots were counted using a Biospot plate reader (Cellular Technology).

[00329] *Measurement of viral burden:* SARS-CoV-2 infected mice were euthanized using a ketamine and xylazine cocktail, and organs were collected. Tissues were weighed and homogenized with beads using a MAgNA Lyser (Roche) in 1 ml of Dulbecco's Modified Eagle'sMedium (DMEM) containing 2% fetal bovine serum (FBS). RNA was extracted from clarified tissue homogenates using MagMax mirVana Total RNA isolation kit (Thermo Scientific) and a Kingfisher duo prime extraction machine (Thermo Scientific). SARS-CoV-2 RNA levels were measured by one-step quantitative reverse transcriptase PCR (qRT-PCR) TaqMan assay as described previously (Hassan et al., 2020). SARS-CoV-2 nucleocapsid (N) specific primers and probe set were used: (L Primer: ATGCTGCAATCGTGCTACAA (SEQ ID NO: 8); R primer: GACTGCCGCCTCTGCTC (SEQ ID NO: 9). Viral RNA was expressed as (N) gene copy numbers per milligram on a log10 scale. For some samples, viral titer was determined by plaque assay on Vero E6 cells. Cytokine and chemokine mRNA measurements. RNA extracted from lung homogenates lung homogenates was DNAse-treated and used to synthesize cDNA using the High-Capacity cDNA Reverse

Transcription kit (Thermo Scientific) with the addition of RNase inhibitor according to the manufacturer's protocol. Cytokine and chemokine expression was determined using TaqMan Fast Universal PCR master mix (Thermo Scientific) with commercial primers/probe sets specific for IFN-γ (IDT: Mm.PT.58.41769240), IL-6 (Mm.PT.58.10005566), IL- 1β (Mm.PT.58.41616450), TNF-α (Mm.PT.58.12575861), CXCL10 (Mm.PT.58.43575827), CCL2 (Mm.PT.58.42151692), CCL5 (Mm.PT.58.43548565), CXCL11 (Mm.PT.58.10773148.g), IFN-β (Mm.PT.58.30132453.g), and IFNγ-2/3 (Thermo Scientific Mm04204156_gH) and results werenormalized to GAPDH (Mm.PT.39a.1) levels. Fold change was determined using the $2^{-\Delta\Delta Ct}$ method comparing treated mice to naïve controls.

[00330] *Peptide restimulation and intracellular cytokine staining:* Splenocytes from intramuscularly vaccinated mice were incubated in culture with a pool of 253 overlapping 15-mer SARS-CoV-2 S peptides for 12 h at 37°C before a 4 h treatment with brefeldin A (BioLegend, 420601). Following blocking with FcγR antibody (BioLegend, clone 93), cells were stained on ice with CD45 BUV395 (BD BioSciences clone 30-F11); CD44 PE-Cy7, CD4 PE-Cy5, CD8b PreCP-Cy5.5, and CD19 APC-Cy7 (BioLegend clones, IM7, GK1.5, YTS156.7.7, and 6D5, respectively), and Fixable Aqua Dead Cell Stain (Invitrogen, L34966). Stained cells were fixed and permeabilized with the Foxp3/Transcription Factor Staining Buffer Set (eBiosciences, 00-5523). Subsequently, intracellular staining was performed with anti-IFN-γ Alexa 647 (BD Biosciences, clone XMG1.2), anti-TNFα BV605 (BioLegend, clone MP6-XT22) and anti-GrB PE (Invitrogen, GRB04). Lungs from intranasally immunized mice were harvested and digested for 1 h at 37°C in digestion buffer consisting of RPMI media supplemented with (167 μg/ml) of Liberase DH (Sigma) and (100 g/ml) of DNase I (Sigma). Lung cells were incubated at 37°C with the pool of 253 overlapping 15-mer SARS-CoV-2 S peptides described above in the presence of brefeldin A for 5 h at 37°C. Lung cells then were stained as described above except that CD4-BV421 (BioLegend clone GK1.5) replaced the CD4-PE-Cy5, no CD19 staining was included, and CD103-FITC and CD69-BV711 (BioLegend clones, 2E7, and, H1.2F3, respectively) were added. Analysis was performed on a BD LSRFortessa X-20 cytometer, using FlowJo X 10.0 software.

[00331] *Flow cytometry-based antigen characterization:* HEK-293T cells were seeded at 10^6 cells/well in 6-well plates 24 h prior to transduction with ChAd-SARS-CoV-2-S (MOI of 5). After 20 h, cells were harvested, fixed and permeabilized using Foxp3 Transcription Factor Staining Buffer Set (Thermo Fisher), and stained for viral antigen after incubation with thefollowing anti-SARS-CoV-2 neutralizing murine mAbs: SARS2-01, SARS2-02, SARS2-07, SARS2-11, SARS2-12, SARS2-16, SARS2-18, SARS2-20, SARS2-21, SARS2-22, SARS2-23, SARS2-29, SARS2-31, SARS2-32, SARS2-34, SARS2-38, SARS2-39, SARS2-50, SARS2-55, SARS2-58, SARS2-66, and SARS2-71 (L. VanBlargan and M. Diamond, unpublished results). H77.39, an isotype-matched anti-HCV E2 mAb was used as a negative control. Cells were washed, incubated with Alexa Fluor 647 conjugated goat anti-mouse IgG (Thermo Fisher), and analyzed by flow cytometry using a MACSQuant Analyzer 10 (Miltenyi Biotec). The percentage of cells positive for a given mAb was compared with cells stained with a saturating amount of an oligoclonal mixture of anti-SARS-CoV-2 mAbs.

Example 2: ***AN INTRANASAL VACCINE DURABLY PROTECTS AGAINST SARS-CoV-2 VARIANTS IN MICE***

[00332] SARS-CoV-2 variants that attenuate antibody neutralization could jeopardize vaccine efficacy and the end of the COVID-19 pandemic. Example 1 shows the protective activity of a single-dose intranasally-administered spike protein-based chimpanzee adenovirus-vectored vaccine (ChAd-SARS-CoV-2-S) in animals, which has advanced to human trials. The present example provides the durability, dose-response, and cross-protective activity in mice. A single intranasal dose of ChAd-SARS-CoV-2-S induced durably high neutralizing and Fc effector antibody responses in serum and S-specific IgG and IgA secreting long-lived plasma cells in the bone marrow. Protection against a historical SARS-CoV-2 strain was observed across a 100-fold vaccine dose range and over a 200-day period. At 6 weeks or 9 months after vaccination, serum antibodies neutralized SARS-CoV-2 strains with B.1.351 and B.1.1.28 spike proteins and conferred almost complete protection in the upper and lower respiratory tracts after

challenge. Thus, in mice, intranasal immunization with ChAd-SARS-CoV-2-S provides durable protection against historical and emerging SARS-CoV-2 strains.

[00333] The spike (S) protein of the SARS-CoV-2 virion is the principal target for antibody-based and vaccine countermeasures. The S protein serves as the primary viral attachment and entry factor and engages the cell-surface receptor angiotensin-converting enzyme 2 (ACE2) to promote SARS-CoV-2 entry into human cells. SARS-CoV-2 S proteins are cleaved to yield S1 and S2 fragments, with the S1 protein containing the receptor binding domain (RBD) and the S2 protein promoting membrane fusion and virus penetration into the cytoplasm. The prefusion form of the SARS-CoV-2 S protein is recognized by potently neutralizing monoclonal antibodies or protein inhibitors.

[00334] Many vaccine candidates targeting the SARS-CoV-2 S protein have been developed using DNA plasmid, lipid nanoparticle encapsulated mRNA, inactivated virion, protein subunit, or viral-vectored vaccine platforms. Several vaccines administered by intramuscular (IM) injection (*e.g.*, Pfizer/BioNTech BNT162b2 and Moderna 1273 mRNA and Johnson & Johnson Ad26.COV2 and AstraZeneca ChAdOx1 nCoV-19 adenoviral platforms) have been granted emergency use authorization in many countries with hundreds of millions of doses given worldwide (covid19.who.int).

[00335] While vaccines administered by IM injection induce robust systemic immunity that protects against severe disease and mortality, questions remain as to their ability to curtail SARS-CoV-2 transmission, especially if upper airway infection is not reduced. Indeed, many of the IM-administered vaccines showed variable protection against upper airway infection and transmission in pre-clinical studies and failed to induce substantive mucosal (IgA) immunity. This issue is important because of the emergence of more transmissible SARS-CoV-2 variants including B.1.1.7, B.1.351, and B.1.1.28 with substitutions in the spike protein. Experiments with pseudoviruses and authentic SARS-CoV-2 strains also suggest that neutralization by vaccine-induced sera is diminished against variants expressing mutations in the spike gene at positions L452, E484, and elsewhere. Beyond possible negative impacts on protection, the combination of diminished immunity against certain variants and naturally lower anti-S IgG levels in

the respiratory mucosa could create conditions for further selection of resistance in the upper airway and transmission into the general population.

[00336] As described herein, a single-dose, intranasally (IN)-delivered chimpanzee Adenovirus (simian Ad-36)-based SARS-CoV-2 vaccine (ChAd-SARS-CoV-2-S) encoding a pre-fusion stabilized S protein that induced robust humoral, cell-mediated, and mucosal immune responses and limited upper and lower airway infection in K18-hACE2 transgenic mice, hamsters, and non-human primates. This vaccine, which has advanced to human clinical trials (BBV154, Clinical Trial NCT04751682), differs from ChAdOx1 nCoV-19, a chimpanzee Ad-23-based SARS-CoV-2 vaccine, currently granted emergency use in some countries. Here, as a further step to evaluating the potential utility of ChAd-SARS-CoV-2-S, its dose-response, durability, and cross-protective activity in mice including effects on upper and lower airway infection was assessed. At approximately nine months after IN immunization, neutralizing antibody and anti-S protein IgA levels in serum of ChAd-SARS-CoV-2-S-vaccinated animals remained high and inhibited infection with SARS-CoV-2 strains with B.1.351 and B.1.1.28 spike proteins. At this time, susceptible K18-hACE2 transgenic mice were fully protected against upper and lower respiratory tract infection after challenge with a SARS-CoV-2 virus displaying B.1.351 spike proteins.

Results

[00337] *A single ChAd-SARS-CoV-2-S immunization induces durable anti-spike and neutralizing responses at different doses:* The durability of humoral immune responses in BALB/c mice 100 or 200 days post IM-or IN-immunization with escalating doses of ChAd-SARS-CoV-2-S (10^8, 10^9, and 10^{10} viral particles [vp]) or 10^{10} vp of a ChAd-Control vaccine was assessed (**FIG. 10A**). First, anti-S and anti-RBD IgG and IgA levels were measured by ELISA. Consistent with prior results at a one-month time point 30, at 100 or 200 days post-vaccination, IN immunization with ChAd-SARS-CoV-2-S induced superior antibody responses than IM immunization or vaccination with ChAd-Control (**FIG. 10B-10M** and **FIG. 11**). Anti-S and anti-RBD-specific binding IgG levels in serum were greater after IN than IM immunization at 100 or 200 days. At 100 days after IN immunization with 10^{10}, 10^9, and 10^8 vp of ChAd-SARS-CoV-2-S, geometric mean

titers (GMT) of S-specific IgG responses were 1.1×10^6, 4.8×10^5, and 2.6×10^5, and RBD-specific IgG were 3.2×10^5, 1.8×10^5, and 8.7×10^4, respectively (**FIG. 10B**). In comparison, S- and RBD-specific IgG reponses 100 days after IM immunization with 10^{10}, 10^9, and 10^8 vp of ChAd-SARS-CoV-2-S were 4 to 6-fold lower ($P < 0.0001$) with S-specific IgG titers of 2.1×10^5, 1.1×10^5, and 4.5×10^4 and RBD-specific IgG titers of 5.1×10^4, 2.9×10^4, and 2.3×10^4, respectively (**FIG. 10E**). A similar dose response was observed with S-and RBD-specific IgG titers at 200 days after IN or IM immunization (**FIG. 10H** and **FIG. 10K**). At 200 days after IN immunization with 10^{10}, 10^9, and 10^8 vp of ChAd-SARS-CoV-2-S, GMT of S-specific IgG were 2.8×10^6, 2.4×10^6, and 1.2×10^6, and RBD-specific IgG were 1.1×10^6, 6.1×10^5, and 3.2×10^5, respectively (**FIG. 10H**). At 200 days after IM immunization with 10^{10}, 10^9, and 10^8 vp of ChAd-SARS-CoV-2-S, S-specific IgG GMT were 8.1×10^5, 6.9×10^5, and 2.6×10^5, and RBD-specific IgG GMT were 1.4×10^5, 1.3×10^5, and 8.0×10^4, respectively (**FIG. 10K**). Thus, anti-S and anti-RBD IgG levels were higher after IN than IM immunization and continued to rise in serum even several months after single-dose vaccination.

[00338] We next assessed the induction and durability of serum IgA responses. Although IM immunization failed to induce S- or RBD-specific IgA (Fig 1F and L), substantial levels of anti S- and RBD IgA were detected after IN immunization at 100 or 200 days post-immunization (Fig 1C and I). At 100 days after IN immunization with 1010, 109, and 108 vp of ChAd-SARS-CoV-2-S, the GMT of S-specific IgA were 4.8 × 103, 1.2 × 103, and 8.4 × 102, and RBD-specific IgA were 2.2 × 103, 4.6 × 102, and 2.9 × 102, respectively (Fig 1C). As seen with IgG, the IgA levels continued to increase over time such that at 200 days the GMT of S-specific IgA were 1.1 × 104, 7.4 × 103, and 5.4 × 103, and RBD-specific IgA were 5.2 × 103, 3.8 × 103, and 9.8 × 102 after IN immunization with 1010, 109, and 108 vp of ChAd-SARS-CoV-2-S, respectively (Fig. 1I).

[00339] Next, a functional correlate of the serological response was evaluated by assaying neutralizing activity (**FIG. 10D, FIG. 10G, FIG. 10J, FIG. 10M** and **FIG. 11**) using a focus-reduction neutralization test (FRNT). As expected, neutralizing activity was not detected in sera from ChAd-control treated mice. At 100

days post IN immunization with 10^{10}, 10^{9}, and 10^{8} vp of ChAd-SARS-CoV-2-S, the mean effective half maximal inhibitory titers [EC50] were 39,449, 9,989, and 7,270, respectively (**FIG. 10D**). In comparison, at this time point after IM immunization with 10^{10}, 10^{9}, and 10^{8} vp of ChAd-SARS-CoV-2-S, the EC50 values were 8 to 20-fold lower ($P < 0.0001$) at 4,988, 2,017, and 391, respectively (**FIG. 10G**). At 200 days after IN immunization with 10^{10}, 10^{9}, and 10^{8} vp of ChAd-SARS-CoV-2-S, and consistent with the higher anti-S and RBD titers seen, EC50 values were 45,591, 22,769, and 23,433, respectively (**FIG. 10J**). In comparison, 200 days after IM immunization with 10^{10}, 10^{9}, and 10^{8} vp of ChAd-SARS-CoV-2-S, EC50 values were much lower at 2,524, 940, and 716, respectively (**FIG. 10M**).

[00340] Long-lived plasma cells (LLPCs) reside in the bone marrow and constitutively secrete high levels of antibody that correlate with serum levels. To assess the levels of antigen-specific LLPCs at 200 days after IM or IN immunization with 10^{10} vp of ChAd-SARS-CoV-2-S, $CD138^{+}$ cells were isolated from the bone marrow and assyed for S-specific IgG or IgA production using an ELISPOT assay. A ~4-fold higher frequency of LLPCs secreting S-specific IgG after IN immunization than IM immunization was observed (**FIG. 10N**). Additionally, after IN immunization, higher numbers of LLPCs producing S-specific IgA were detected, which were absent after IM immunization (**FIG. 10N**). Together, these data establish the following: (a) single-dose IN immunization promotes superior humoral immunity than IM immunization; (b) 100-fold lower inoculating doses of ChAd-SARS-CoV-2-S induce robust neutralizing antibody responses in mice; (c) IN but not IM immunization induces serum IgA responses and IgA-specific LLPCs against the SARS-CoV-2 S protein; and (d) the humoral immunity induced by ChAd-SARS-CoV-2-S is durable and rises over a six-month period after vaccination.

[00341] *IN inoculation of ChAd-SARS-CoV-2-S induces broad antibody responses with Fc effector function capacity:* To characterize the humoral response further, antibody binding to SARS-CoV-2 variant proteins and Fc effector functions using serum derived from BALB/c mice were analyzed at 90 days after IN or IM vaccination. Our panel of SARS-CoV-2 proteins included spike (D614G, E484K, N501Y,

Δ69-70, K417N) and RBD (E484K) antigens corresponding to WA1/2020, B.1.1.7, B.1.351, B.1.1.28 strains. First, the anti-SARS-CoV-2 specific antibody response for several isotypes (IgG1, IgG2a, IgG2b, IgG3, IgM, and IgA) and their ability to bind Fcγ receptors (mouse FcγRIIB, FcγRIII, FcγRIV) using a luminex platform were measured. Consistent with data obtained by ELISA (**FIG. 10B** and **FIG. 10E**), IN vaccination of ChAd-SARS-CoV-2-S induced higher levels of IgG1 to D614G spike and WA1/2020 RBD proteins than IM immunization, and as expected, decreasing doses of the vaccine elicited lower antibody titers (**FIG. 12A**). Anti-SARS-CoV-2 IgG1 titers after IN immunization also were higher against all spike and RBD variants than after IM immunization, and titers decreased with vaccine dose (**FIG. 12B**). As shown in a heatmap, this trend was observed for all anti-SARS-CoV-2 specific antibody isotypes and correlated with FcγR binding patterns (**FIG. 12C**). These data suggest that IN vaccination induces a higher magnitude and broader antibody subclass response to SARS-CoV-2 than IM vaccination.

[00342] Antibody effector functions, such as opsonization, are mediated in part by Fcγ receptor engagement. To determine if the observed differences in antibody titers and FcγR binding titers resulted in differences in effector functions, antibody-dependent neutrophil (ADNP) and cellular phagocytosis (ADCP) assays were performed (**FIG. 12D-12E**). Sera from IN vaccinated mice stimulated substantially more ADNP than those obtained from IM vaccinated mice. However, minimal differences in ADCP were apparent from antibodies derived after IN and IM vaccination (**FIG. 12D-12E**). These data demonstrate that IN vaccination with ChAd-SARS-CoV-2-S induces a greater and more functional antibody response than after IM vaccination.

[00343] *Intranasally administered ChAd-SARS-CoV-2-S induces durable protection against SARS-CoV-2 challenge in BALB/c mice:* To assess the efficacy of the ChAd-SARS-CoV-2-S vaccine, immunized BALB/c mice given the dosing regimen described in **FIG. 10A** were challenged with SARS-CoV-2. Virus challenge was preceded by intransal introduction of Hu-Ad5-hACE2, which enables ectopic expression of hACE2 and productive infection of SARS-CoV-2 in BALB/c mice by historical SARS-CoV-2 strains. Animals were immunized once via IN or IM routes with 10^{10} vp of ChAd-

Control or 10^8, 10^9, or 10^{10} vp of ChAd-SARS-CoV-2-S. At day 95 or 195 post-vaccination, mice were given 10^8 plaque-forming units (PFU) of Hu-Ad5-hACE2 and anti-Ifnar1 mAb; the latter attenuates innate immunity and enhances pathogenesis in this model. Five days later, BALB/c mice were challenged with 5 x 10^4 focus-forming units (FFU) of SARS-CoV-2 (strain WA1/2020) via IN route. At 4 days post-infection (dpi), lungs, spleen, and heart were harvested from mice challenged at 100 days post-immunization, and lungs, nasal turbinates, and nasal washes were collected from a second cohort challenged at 200 days post-immunization. Tissues were assessed for viral burden by quantitative reverse transcription PCR (qRT–PCR) using primers for the subgenomic RNA (N gene). IN immunization with all three doses induced remarkable protection at 100 days post-vaccination as evidenced by a virtual absence of viral RNA in lungs, spleen, and heart compared to animals receiving the ChAd-Control vaccine (**FIG. 13A-13C**). At 200 days post-immunization, protection conferred by the IN delivered ChAd-SARS-CoV-2-S remained robust in the upper and lower respiratory tracts compared to ChAd-Control immunized mice. Nevertheless, limited infection breakthrough in the lungs and nasal turbinates in animals immunized with the lowest 10^8 vp dose of ChAd-SARS-CoV-2-S was observed (**FIG. 13G** and **FIG. 13I**). In comparison, protection at 100 days post-IM immunization was less than after IN immunization at the same challenge time point. Although viral RNA was not detected in the heart and spleen (**FIG. 13E-13F**), at least 1,000 to 30,000-fold ($P < 0.0001$) higher levels were measured in the lungs of mice immunized with ChAd-SARS-CoV-2-S by the IM compared to IN route (**FIG. 13A** and **FIG. 13D**). A greater impact of dosing by the IM route was also observed, as the reduction in viral RNA load in the lung at 10^8 vp dose no longer was different than in the ChAd-Control vaccinated mice (**FIG. 13D**). At 200 days post-IM immunization, less protection against SARS-CoV-2 infection in the lungs, nasal washes, and nasal turbinates was observed than after IN immunization (**FIG. 13G-13L**).

[00344] *ChAd-SARS-CoV-2-S induces durable immunity in hACE2 transgenic mice:* Next, the immunogenicity of intransally-delivered ChAd-SARS-CoV-2-S in K18-hACE2 C57BL/6 mice was assessed, which are more vulnerable to SARS-

CoV-2 infection than BALB/c mice. Five-week old K18-hACE2 mice were inoculated via an IN route with 10^9 vp of ChAd-control or ChAd-SARS-CoV-2-S. Serum samples were collected six weeks later, and humoral immune responses were evaluated. IN immunization of ChAd-SARS-CoV-2-S but not ChAd-control induced high levels of S- and RBD-specific IgG and IgA (**FIG. 14A-14B**). Neutralizing antibody titers against WA1/2020 and two other SARS-CoV-2 strains with spike proteins from B.1.351 and B.1.1.28 variants were measured by FRNT assay (**FIG. 14C-14D** and **FIG. 16**). High levels of neutralizing antibody against WA1/2020 were induced after a single IN dose of ChAd-SARS-CoV-2-S (EC50 of 9,591). As seen with vaccine-induced human sera, decreases in neutralizing titers were observed against Wash-B.1.351 (~5-fold, $P < 0.0001$; **FIG. 14C**) and Wash-B.1.1.28 (~3-fold, $P < 0.0001$; **FIG. 4D**) SARS-CoV-2 strains compared to WA1/2020. To assess the durability of humoral responses, a separate cohort of K18-hACE2 mice were immunized via the IN route, and serum samples were collected at nine months. ChAd-SARS-CoV-2-S induced high levels of S- and RBD-specific IgG and IgA and neutralizing antibody against WA1/2020 (EC50 of 12,550) at this time point (**FIG. 14E-14H** and **FIG. 16**). When tested against the Wash-B.1.351 and Wash-B.1.1.28 viruses, a decrease in neutralizing titer was also observed (~6 to 8-fold, $P < 0.05$; **FIG. 14G-14H**) compared to WA1/2020, although they still remained high (EC50 of 1,627 and 1,918, respectively).

[00345] *ChAd-SARS-CoV-2-S confers cross-protection against Wash B.1.351 and Wash-B.1.1.28 challenge in hACE2 transgenic mice:* The protective efficacy of ChAd-SARS-CoV-2-S against WA1/2020 and two chimeric viruses (Wash-B.1.351 and Wash-B.1.1.28) with spike genes corresponding to variants of concern were tested (**FIG. 15A**). Five-week old K18-hACE2 mice were immunized via an IN route with a single 10^9 vp dose of ChAd-control or ChAd-SARS-CoV-2-S. Six weeks later, mice were challenged by an IN route with 10^4 FFU of Wash-B.1.351, Wash B.1.1.28, or WA1/2020. All mice immunized with ChAd-SARS-CoV-2 exhibited no weight loss, whereas most ChAd-Control-vaccinated mice experienced substantial weight loss at 3 to 6 dpi (**FIG. 15B**, **FIG. 15G**, and **FIG. 15L**). Remarkably, vaccination with ChAd-SARS-CoV-2-S resulted in almost no detectable SARS-CoV-2 RNA in the

upper and lower respiratory tract, heart, and brain at 6 dpi (**FIG. 15C-15F**, **FIG. 15H-15K**, and **FIG. 15M-15O**). As a further test of the durability of the cross-protective response, five-week old K18-hACE2 mice were immunized via an IN route with a single 10^{10} vp dose of ChAd-control or ChAd-SARS-CoV-2-S. Nine months later, mice were challenged via IN route with 10^4 FFU of Wash-B.1.351. ChAd-SARS-CoV-2-S-vaccinated mice maintained weight in contrast to ChAd-Control treated mice (**FIG. 15P**). Moreover, substantial virological protection was observed, as only very low amounts of Wash-B.1.351 SARS-CoV-2 RNA were detected in the upper and lower respiratory tracts, heart, and brain in some of the mice (**FIG. 15Q-15T**).

Discussion

[00346] The durability of vaccine-induced immune responses is a key for providing sustained protection against SARS-CoV-2 infection and curtailing the current pandemic. Here, it was shown that a single IN immunization with ChAd-SARS-CoV-2-S induced S-and RBD-specific binding and neutralizing antibodies that continued to rise for several months, suggestive of sustained germinal center reactions. LLPCs in the bone marrow were detected six months after IN vaccination, secreting SARS-CoV-2-specifc IgG and IgA that likely contributed to the durably high antiviral antibody levels in circulation.. In comparison, IM immunization with ChAd-SARS-CoV-2-S induced lower levels of serum neutralizing antibodies, fewer spike-specific IgG secreting LLPCs, and virtually no serum or cellular IgA response. At least in mice, a single IN dose immunization with ChAd-SARS-CoV-2-S produced durable humoral immunity that was observed across a 100-fold dose range. These pre-clinical immunogenicity results compare favorably with studies in humans with mRNA vaccines against SARS-CoV-2, which show humoral immune responses lasting at least several months. In comparison, the durability of antibody responses after natural SARS-CoV-2 infection can vary considerably.

[00347] A single immunization of ChAd-SARS-CoV-2-S conferred durable protection against SARS-CoV-2 (WA1/2020 strain) challenge in hACE2-tranduced BALB/c mice or K18-hACE2 transgenic C57BL/6 mice at multiple time points through six months. IN immunization in particular provided virtually complete virological

protection against upper and lower respiratory tract infection, with only a limited infection breakthrough seen at the 100-fold lower vaccine dose. The abrogation of infection in the upper respiratory tract suggests that IN vaccination could prevent transmission. In comparison, IM immunization reduced the viral RNA levels in the lungs but showed substantially less protection against the homolgous WA1/2020 strain in samples from the upper respiratory tract. While many SARS-CoV-2 vaccine candidates from different platforms have demonstrated immunogenicity and protective efficacy in animals models, to our knowledge, none have established durability or protection against variant viruses. The long-term protection conferred by IN immunization even at 100-fold lower inoculating doses is promising. If results in mice were recapitulated, dose sparing strategies could enable production of a large number of vaccine doses that could curtail infection and transmission of SARS-CoV-2.

[00348] The emergence of SARS-CoV-2 S variants with mutations of amino acids in the receptor binding motif (*e.g.*, B.1.351 and B.1.1.28) is of concern because of their resistance to the inhibitory activity of many neutralizing antibodies. Indeed, human sera from subjects vaccinated with BNT162b2 mRNA or ChAdOx1 nCoV-19 (AZD1222) vaccines showed reduced neutralization against B.1.351. Concerningly, IM-administered ChAdOx1 nCoV-19 (AZD1222) showed reduced protective efficacy against mild to moderate B.1.351 infection in humans. In K18-hACE2 transgenic mice, when we compared the immunogenicity of IN-delivered ChAd-SARS-CoV-2-S against WA1/2020 and chimeric SARS-CoV-2 strains expressing B.1.1.28 or B.1.351 spike proteins, reduced (3 to 8-fold) neutralization of the variant viruses were also observed although the titers remained >1,000. At six weeks post IN immunization of ChAd-SARS-CoV-2-S, K18-hACE2 mice were fully protected mice against weight loss and infection in the upper and lower respiratory tracts and brain by WA1/2020, Wash-B.1.351, and Wash-B.1.1.28. Remarkably, in a separate cohort of K18-hACE2 mice challenged nine months after single IN immunization, animals were fully protected against Wash-B.1.351 challenge. Although correlates of protection are not fully established for SARS-CoV-2 vaccines, the high levels of cross-neutralizing antibodies against the variant viruses combined with robust virus-specific systemic and mucosal $CD8^+$ T cell responses likely

contribute to protection. Beyond this, antibody effector functions also might contribute to prevent SARS-CoV-2 infection and disease. Indeed, enhanced Fc effector functions against SARS-CoV-2 variant proteins were observed in serum derived from IN-delivered ChAd-SARS-CoV-2-S including robust induction of ADNP and ADCP responses.

[00349] In summary, the present example shows that IN immunization with ChAd-SARS-CoV-2-S induces robust and durable binding IgG and IgA antibody, neutralizing antibody, Fc effector functions, and LLPC responses against SARS-CoV-2. In mice, a single IN immunization with ChAd-SARS-CoV-2-S confers cross-protection against SARS-CoV-2 strains displaying spike proteins corresponding to B.1.351 and B.1.1.28 variants, even nine months after vaccination. Given the efficacy of preclinical evaluation in multiple animal models 30, 31, 32 and the durable protective immunity against variants of concern, IN delivery of ChAd-SARS-CoV-2-S is a promising platform for preventing SARS-CoV-2 infection, and curtailing transmission.

Methods

[00350] *Viruses and cells:* Vero E6 (CRL-1586, American Type Culture Collection (ATCC), Vero-TMPRSS2 57, Vero (CCL-81, ATCC) and HEK293 (CRL-1573, ATCC) cells were cultured at 37°C in Dulbecco's Modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 10 mM HEPES pH 7.3, 1 mM sodium pyruvate, 1X non-essential amino acids, and 100 U/ml of penicillin–streptomycin. Vero-TMPRSS2 cells also were supplemented with 5 µg/mL of blasticidin.

[00351] SARS-CoV-2 strain 2019n-CoV/USA_WA1/2020 (WA1/2020) was obtained from the Centers for Disease Control and Prevention. The virus was passaged once in Vero CCL-81 cells and titrated by focus-forming assay (FFA) on Vero E6 cells. The Wash-B.1.351 and Wash-B.1.1.28 chimeric viruses with variant spike genes were described previously 28, 58. All viruses were passaged once in Vero-TMPRSS2 cells and subjected to next-generation sequencing to confirm the introduction and stability of substitutions. All virus experiments were performed in an approved Biosafety level 3 (BSL-3) facility.

[00352] *Mouse experiments:* Animal studies were carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of

the National Institutes of Health. The protocols were approved by the Institutional Animal Care and Use Committee at the Washington University School of Medicine (Assurance number A3381-01). Virus inoculations were performed under anesthesia that was induced and maintained with ketamine hydrochloride and xylazine, and all efforts were made to minimize animal suffering.

[00353] Female BALB/c (catalog 000651) and K18-hACE2 C57BL/6 (catalog 034860) mice were purchased from The Jackson Laboratory. Four to five-week-old animals were immunized with 10^{10} vp of ChAdV-control or 10^{8}, 10^{9}, or 10^{10} vp of ChAd-SARS-CoV-2-S in 50 µl PBS via IM (hind leg) or IN injection. Vaccinated BALB/c mice (10 to 11-week-old) were given a single intraperitoneal injection of 2 mg of anti-Ifnar1 mAb (MAR1-5A3 59 (Leinco) one day before IN administration of 10^{8} PFU of Hu-Ad5-hACE2 37. Five days after Hu-Ad5--hACE2 transduction, mice were inoculated with 4 x 10^{5} FFU of WA1/2020 SARS-CoV-2 by the IN route. K18-hACE2 mice were challenged on indicated days after immunization with 10^{4} FFU of SARS-CoV-2 (WA1/2020, Wash-B.1.351, or Wash-B.1.1.28) via IN route. Animals were euthanized at 6 dpi, and tissues were harvested for virological analysis.

[00354] *Chimpanzee and human adenovirus vectors:* The ChAd-SARS-CoV-2 and ChAd-Control vaccine vectors were derived from simian Ad36 backbones 60, and the constructing and validation has been described herein. The rescued replication-incompetent ChAd-SARS-CoV-2-S and ChAd-Control vectors were scaled up in HEK293 cells and purified by CsCl density-gradient ultracentrifugation. Viral particle concentration in each vector preparation was determined by spectrophotometry at 260 nm. The Hu-AdV5-hACE2 vector also was described above and produced in HEK293 cells. The viral titer was determined by plaque assay in HEK293 cells.

[00355] *SARS-CoV-2 neutralization assays:* Heat-inactivated serum samples were diluted serially and incubated with 10^{2} FFU of different SARS-CoV-2 strains for 1 h at 37°C. The virus-serum mixtures were added to Vero cell monolayers in 96-well plates and incubated for 1 h at 37°C. Subsequently, cells were overlaid with 1% (w/v) methylcellulose in MEM supplemented with 2% FBS. Plates were incubated for 30 h before fixation using 4% PFA in PBS for 1 h at room temperature. Cells were washed

then sequentially incubated with an oligoclonal pool of SARS2-2, SARS2-11, SARS2-16, SARS2-31, SARS2-38, SARS2-57, and SARS2-71 62 anti-S antibodies and HRP-conjugated goat anti-mouse IgG (Sigma, 12-349) in PBS supplemented with 0.1% saponin and 0.1% bovine serum albumin. TrueBlue peroxidase substrate (KPL) was used to develop the plates before counting the foci on a BioSpot analyzer (Cellular Technology Limited).

[00356] *Protein expression and purification:* The cloning and production of purified S and RBD proteins corresponding to the WA1/2020 SARS-CoV-2 strain have been described previously. Briefly, prefusion-stabilized S 64 and RBD were cloned into a pCAGGS mammalian expression vector with a hexahistidine tag and transiently transfected into Expi293F cells. Proteins were purified by cobalt-charged resin chromatography (G-Biosciences).

[00357] *ELISA:* Purified antigens (S or RBD) were coated onto 96-well Maxisorp clear plates at 2 µg/mL in 50 mM Na_2CO_3 pH 9.6 (70 µL) overnight at 4°C. Coating buffers were aspirated, and wells were blocked with 200 µL of 1X PBS + 0.05% Tween-20 + 1% BSA + 0.02% NaN3 (Blocking buffer, PBSTBA) overnight at 4°C. Heat-inactivated serum samples were diluted in PBSTBA in a separate 96-well polypropylene plate. The plates then were washed thrice with 1X PBS + 0.05% Tween-20 (PBST), followed by addition of 50 µL of respective serum dilutions. Sera were incubated in the blocked ELISA plates for at least 1 h at room temperature. The ELISA plates were again washed thrice in PBST, followed by addition of 50 µL of 1:1,000 anti-mouse IgG-HRP (Southern Biotech Cat. #1030-05) in PBST or 1:1000 of anti-mouse IgA-HRP in PBSTBA (SouthernBiotech). Plates were incubated at room temperature for 1 h, washed thrice in PBST, and then 100 µL of 1-Step Ultra TMB-ELISA was added (ThermoFisher Cat. #34028). Following a 10 to 12-min incubation, reactions were stopped with 50 µL of 2 M sulfuric acid. Optical density (450 nm) measurements were determined using a microplate reader (Bio-Rad).

[00358] *ELISPOT assay:* To quantitate S-specific plasma cells in the bone marrow, femurs and tibias were crushed using a mortar and pestle in RPMI 1640, filtered through a 100 µm strainer and subjected to ACK lysis. $CD138^+$ cells were

enriched by positive selection and magnetic beads according to the manufacturer's instructions (EasySep Mouse CD138 Positive Selection, STEMCELL). The enriched $CD138^+$ cells were incubated overnight in RPMI 1640 supplemented with 10% FBS in MultiScreen-HA Filter Plates (Millipore) pre-coated with SARS-CoV-2 S protein. Foci were developed using TruBlue substrate (KPL) following sequential incubation with anti-mouse IgG-biotin or anti-mouse IgA-biotin and streptavidin-HRP. Plates were imaged using a BioSpot instrument, and foci enumerated manually.

[00359] *Measurement of viral burden:* SARS-CoV-2 infected mice were euthanized using a ketamine and xylazine cocktail, and organs were collected. Tissues were weighed and homogenized with beads using a MagNA Lyser (Roche) in 1 ml of Dulbecco's Modified Eagle's Medium (DMEM) containing 2% fetal bovine serum (FBS). RNA was extracted from clarified tissue homogenates using MagMax mirVana Total RNA isolation kit (Thermo Scientific) and the KingFisher Flex extraction system (Thermo Scientific). SARS-CoV-2 RNA levels were measured by one-step quantitative reverse transcriptase PCR (qRT-PCR) TaqMan assay as described previously 37. SARS-CoV-2 nucleocapsid (N) specific primers and probe sets were used as described above. Viral RNA was expressed as (N) gene copy numbers per milligram on a log10 scale.

[00360] *Luminex analysis:* Luminex analhysis was conducted as described previously. Briefly, proteins (Spike: D614G, E484K, N501Δ69-70, K417N, B.1.1.7, B.1.351; Receptor Binding Domain (RBD) (ImmuneTech): WT, E484K, B.1.1.7, B.1.351, B.1.128) were carboxy-coupled to magnetic Luminex microplex carboxylated beads (Luminex Corporation) using NHS-ester linkages with Sulfo-NHS and EDC (Thermo Fisher) and then incubated with serum (IgG1, FcγRIIb, FcγRIII 1:3000; IgG2a, G2b, G3, A, FcγRIV 1:1000, IgM 1:500) for 2 h at 37°C. Isotype analysis was performed by incubating the immune complexes with secondary goat anti-mouse-PE antibody (IgG1 1070-09, IgG2a 1080-09S, IgG2b 1090-09S, IgG3 1100-09, IgM 1020-09, IgA 1040-09 Southern Biotech) for each isotype. FcγR binding was quantified by incubating immune complexes with biotinylated FcγRs (FcγRIIB, FcγRIII, and FcγRIV, courtesy of Duke Protein Production Facility) conjugated to Steptavidin-PE (Prozyme). Flow cytometry

was performed with an IQue (Intellicyt) and analysis was performed on IntelliCyt ForeCyt (v8.1).

[00361] *Antibody-dependent neutrophil or cellular phagocytosis:* Antibody-dependent neutrophil phagocytosis (ADNP) and cellular phagocytosis (ADCP) assays were conducted as described previously. Briefly, spike protein was carboxy coupled to blue, yellow-green, or red FluoSphere™ Carboxylate-modified microsphere, 0.2 µm (ThermoFisher) using NHS-ester linkages with Sulfo-NHS and EDC (Thermo Fisher). Spike-coated beads were incubated with diluted serum (1:150 ADNP, 1:100 ADCP) for 2 hours at 37°C. For the ADNP assay, bone marrow cells were collected from BALB/c mice, and red blood cells were subjected to ACK lysis. The remaining cells were washed with PBS, and aliquoted into 96-well plates (5 x 10^4 cells per well). The bead-antibody complexes were added to cells and incubated for 1 h at 37°C. After washing, cells were stained with the following antibodies: CD11b APC (BioLegend 101212), CD11c A700 (BioLegend 117320), Ly6G Pacific Blue (127628), Ly6C BV605 (BioLegend 128036), Fcblock (BD Bioscience 553142) and CD3 PE/Cy7 (BioLegend 100320). Cells were fixed with 4% PFA, processed on an BD LSRFortessa (BD Biosciences). Neutrophils were defined as $CD3^-$, $CD11b^+$, $Ly6G^+$. The neutrophil phagocytosis score was calculated as (% FITC+) x (geometic mean fluorescent intensity of FITC)/10000. For the ADCP assay, J774A.1 (ATCC TIB-67) murine monocytic cells were incubated with the Spike-coated bead–antibody complexes for 1h at 37°C. Cells were washed in 5 mM EDTA PBS, fixed with 4% PFA, and analyzed on an BD LSRFortessa (BD Biosciences). The cellular phagocytosis score was calculated as (% FITC+) x (geometric mean fluorescent intensity of FITC)/10000.

Example 3: ***EXPRESSION OF SARS-CO-V2 SPIKE GENE (BA. 5) VARIANTS FROM CHAD VECTOR***

[00362] Adenoviral vectors comprising the S6P and S6PdF variants of the SAR-CoV2-Omicron BA.5 (bivalent) were constructed as previously discussed in the construction of chimp adenoviral vectors and tested for expression. These constructs are referred to as ChAd.BA.5-S6P, ChAd.BA.5-S6PdF respectively. Monolayers of

human A549 cells were infected with 3 × 10^3 vp/cell of newly generated ChAd.BA.5-S6P (derived from omicron variant BA.5 and with S6P) and ChAd.BA.5-S6PdF vectors or ChAd-SARS-CoV-2-S(WA1/2020-S2P) and ChAd.BA.1-S6PdF vectors or ChAd vector carrying no transgene. The expression of SARS-CoV-2 spike gene was detected 48 hours post-infection by flow cytometry using cocktail of primary mAb binding different epitopes of spike glycoprotein. As provided in **FIG. 18**, infection with ChAd.BA.5-S6P and ChAd.BA.5-S6PdF vectors resulted in 90 and 92% cells expressing spike gene, respectively, which is very close to the expression levels that were achieved by ChAd-SARS-CoV-2-S(WA1/2020-S2P) (93%) and ChAd.BA.1-S6PdF (96%). Infection with ChAd negative control showed 5% background level of expression similar to control uninfected cells incubated with primary and secondary Alexa Fluor 594-conjugated F(ab')2 fragment of donkey anti-mouse IgG (4.2%). These data suggest that both ChAd.BA.5-S6P and ChAd.BA.5-S6PdF vector should induce efficient spike expression resulting in a strong immune response comparable with that achieved by ChAd-SARS-CoV-2-S and ChAd.BA.1-S6PdF vaccine vectors.

Example 4: ***AN INTRANASAL VACCINATION WITH CHAD-WUHAN-S AND CHAD.BA.5-S OR CHAD-BIVALENT DURABLY PROTECTS AGAINST SARS-CoV-2 VARIANTS IN MICE***

[00363] Intranasal vaccines with the Ch-AD-Control, ChAD-Wuhand-S, ChAd-BA.5 and ChAD-bivalent were tested in vivo for their efficacy using the methods provided herein. FIG. 23 provides a sequence alignment of the relevant S-proteins. **FIG. 19A** provides a schematic of the timing of vaccination, blood sampling, virus challenge and necroscopy. In an initial experiment, binding of binding of anti-SARS-CoV-2 IgG (**FIGs. 19B, 19D, 19F**) or IgA (**FIGs. 19C, 19E, 19G**) to SARS-CoV-2 Wuhan-1 (**FIGs. 19B-C**), BA.5 (**FIGs. 19D-E**), or BQ.1.1 (**FIGs 21F-G**) S proteins was tested. Intranasal immunization with bivalent ChAd-CoV-2 S (Wuhan) and ChAd-CoV-2-S (BA.5) compared to respective monovalent vaccine components provided greater breadth of IgA and IgG responses against Wuhan-1, BA.5, and BQ.1.1 spike proteins based on ELISA tests with serum obtained 28 days after vaccination.

[00364] Next neutralizing activity of intranasal immunization with Ch-AD-Control, ChAD-Wuhand-S, ChAd-BA.5 and ChAD-bivalent against WA1/2020 was tested using FRNT. **FIG. 20A** shows neutralizing activity against WA1/2020 by FRNT. **FIG. 20B** shows neutralizing activity against BA.5 by FRNT. **FIG. 20C** shows neutralizing activity against BF.7 by FRNT. **FIG. 20D** shows neutralizing activity against BQ.1.1 by FRNT. **FIG. 20E** shows neutralizing activity against XBB.1.1 by FRNT. Each point represents data from an individual mouse and is the mean of two technical replicates. **FIG 20F-H** show the neutralization data plotted as a direct comparison for a given vaccine (ChAd-SARS-CoV-2 S (Wuhan-1, **Fig 20F**), ChAd-SARS-CoV-2-S (BA.5, **Fig 20G**) or bivalent (ChAd-SARS-CoV-2 S (Wuhan-1) + ChAd-SARS-CoV-2-S (BA.5), **Fig 20H**) against the indicated SARS-CoV-2 strains used for infection.

[00365] The data provided shows that intranasal immunization with bivalent ChAd-CoV-2 S (Wuhan) and ChAd-CoV-2-S (BA.5) provided greater serum neutralizing antibody responses against Wuhan-1, BA.5, and BQ.1.1 viruses than the respective monovalent vaccines.

[00366] Finally, the viral RNA levels in the lung (**FIG. 21A**), nasal turbinates (**FIG. 21B**), and nasal washes (**FIG. 21C**) at 6 dpi after challenge with SARS-CoV-2 WA1/2020 D614G (left) or Omicron BQ.1.1 (right) were analyzed. Intranasal immunization with bivalent ChAd-CoV-2 S (Wuhan) and ChAd-CoV-2-S (BA.5) provided greater protection against lung infection by WA1/2020 D614G and BQ.1.1 viruses than the respective monovalent vaccines. This corresponded to improved protection against lung inflammation as well as measured by cytokine levels (**FIG. 22**).

EQUIVALENTS

[00367] While several inventive embodiments have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the function and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be within the scope of the inventive embodiments described herein. More generally, those skilled in the art will readily appreciate that all parameters,

dimensions, materials, and configurations described herein are meant to be exemplary and that the actual parameters, dimensions, materials, and/or configurations will depend upon the specific application or applications for which the inventive teachings is/are used. Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific inventive embodiments described herein. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, inventive embodiments may be practiced otherwise than as specifically described and claimed. Inventive embodiments of the present disclosure are directed to each individual feature, system, article, material, kit, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, kits, and/or methods, if such features, systems, articles, materials, kits, and/or methods are not mutually inconsistent, is included within the inventive scope of the present disclosure.

[00368] All references, patents and patent applications disclosed herein are incorporated by reference with respect to the subject matter for which each is cited, which in some cases may encompass the entirety of the document.

[00369] The phrase “and/or,” as used herein in the specification and in the claims, should be understood to mean “either or both” of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with “and/or” should be construed in the same fashion, i.e., “one or more” of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the “and/or” clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to “A and/or B”, when used in conjunction with open-ended language such as “comprising” can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

[00370] As used herein in the specification and in the claims, “or” should be understood to have the same meaning as “and/or” as defined above. For example, when separating items in a list, “or” or “and/or” shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as “only one of” or “exactly one of,” or, when used in the claims, “consisting of,” will refer to the inclusion of exactly one element of a number or list of elements. In general, the term “or” as used herein shall only be interpreted as indicating exclusive alternatives (i.e. “one or the other but not both”) when preceded by terms of exclusivity, such as “either,” “one of,” “only one of,” or “exactly one of.” “Consisting essentially of,” when used in the claims, shall have its ordinary meaning as used in the field of patent law.

[00371] As used herein in the specification and in the claims, the phrase “at least one,” in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase “at least one” refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, “at least one of A and B” (or, equivalently, “at least one of A or B,” or, equivalently “at least one of A and/or B”) can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

[00372] Unless otherwise defined, all technical terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this

invention belongs. As used in this specification and the appended claims, the singular forms “a,” “an,” and “the” include plural references unless the context clearly dictates otherwise. Any reference to “or” herein is intended to encompass “and/or” unless otherwise stated.

CLAIMS

What is claimed is:

1. An adenoviral vector comprising the genome of a non-human adenovirus, wherein the genome of the adenovirus has been modified such that the vector lacks the native E1 and optionally, the E3 or E3B locus, and comprises a nucleic acid sequence encoding a SARS-CoV-2 spike (S) protein with an amino acid sequence at least 80% identical to any of SEQ ID NOS: 10, 12, 20, or 21 or an immunogenic portion, or fragment thereof.
2. The adenoviral vector of claim 1, wherein the non-human adenovirus is a simian adenovirus (SAdV).
3. The adenoviral vector of any one of claims 1 or 2, wherein the nucleic acid sequence encodes an amino acid sequence with at least 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1 %, 99.2%, 99.3%, 99.4% 99.5%, 99.6%, 99.7%, 99.8% or 99.9% sequence identity to one or more of SEQ ID NOS: 10-12, 20 or 21.
4. The adenoviral vector of claim 2, comprising or consisting of a nucleic acid sequence with at least 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1 %, 99.2%, 99.3%, 99.4% 99.5%, 99.6%, 99.7%, 99.8% or 99.9% sequence identity to any one of SEQ ID NO: 13-19.
5. An adenoviral vector comprising a nucleic acid sequence encoding an amino acid sequence at least 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1 %, 99.2%, 99.3%, 99.4% 99.5%, 99.6%, 99.7%, 99.8% or 99.9% identical to any of SEQ ID NOS: 10-12, 20, 21 or an immunogenic portion, or fragment thereof.
6. An adenoviral vector comprising or consisting of a nucleic acid sequence with at least 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1%, 99.2%,

99.3%, 99.4% 99.5%, 99.6%, 99.7%, 99.8% or 99.9% sequence identity to any one of SEQ ID NO: 13-19.

7. An adenoviral vector comprising a nucleic acid sequence, wherein the nucleic acid sequence encodes an amino acid sequence at least 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1 %, 99.2%, 99.3%, 99.4% 99.5%, 99.6%, 99.7%, 99.8% or 99.9% identical to SEQ ID NOS: 3 comprising at least 10, or at least 15, or at least 20, or at least 25, or at least 30 mutations from a list consisting of T19I, L24S, del25-27, 69-70del, G142D, V213G, G339D, S371F, S373P, S375F, T376A, K417N, N440K, S477N, T478K, E484A, Q493R, L452R, F486V, Q498R, N501Y, D614G, H655Y, N679K, P681H, R682G, R683S, R685S, N764K, D796Y, A942P Q954H, N969K, K988P and V989P.
8. The adenoviral vector of claim 7, further comprising at least 2, or at least 3, or at least 4, or at least 5, or 6 stabilizing mutations as provided in F819P, A894P, A901P, A944P, K988P and V989P.
9. The adenoviral vector of any one of claims 5-8, wherein the adenoviral vector has a functional deletion in E1 and optionally, E3 or E3B gene.
10. The adenoviral vector of any one of claims 5-9, wherein the adenoviral vector is a Simian adenoviral vector.
11. The adenoviral vector of claim 10, wherein the adenoviral vector is a Chimpanzee adenoviral vector 36.
12. A pharmaceutical composition, comprising the adenoviral vector of any one of claims 1-11 and a pharmaceutically acceptable carrier, diluent, excipient, or adjuvant.
13. An immunogenic composition comprising the adenoviral vector according to any one of claims 1-11 a pharmaceutically acceptable carrier, diluent, excipient, or adjuvant.
14. The pharmaceutical composition of claim 12 or the immunogenic composition of claim 10, further comprising one or more additional active ingredients.

15. The pharmaceutical composition of claim 12 or the immunogenic composition of claim 13, wherein the composition is formulated for intranasal or intramuscular administration.
16. A host cell transduced with the adenoviral vector or composition of any one of the preceding claims.
17. A packaging cell line producing the composition or viral vector of any one of the preceding claims.
18. The packaging cell line of claim 17, wherein the cell comprises a complement of an adenoviral gene functionally deleted in the adenoviral vector of any one of the preceding claims.
19. The packaging cell line of claim 17, wherein the complement of the adenoviral genes functionally deleted comprises one or more of E1, E3 or E3B genes.
20. A kit comprising: (i) one or more of, the host cell of claim 16, the packaging cell line of claim 18, the adenoviral vector of any one of claims 1-11, the pharmaceutical composition of claim 12 or the immunogenic composition of claim 13 and (ii) instructions for use.
21. A coronavirus vaccine comprising an adenovirus vector of any one of claims 1-11.
22. A composition comprising the serum of a first subject previously administered the adenoviral vector of any one of claims 1-11, the pharmaceutical composition of claim 9 or the immunogenic composition of claim 13.
23. A method of treating a second subject having a coronavirus infection comprising administering to the second subject an immunogenically effective amount of the composition comprising the serum according to claim 22.
24. A method for inducing an immune response against a coronavirus in a subject in need thereof, comprising administering to the subject an immunogenically effective amount of a composition comprising the adenoviral vector of any one of claims 1-11, the pharmaceutical composition of claim 12 or the immunogenic composition of claim 13.

25. A method of treating or preventing a coronavirus infection in a subject in need thereof, comprising administering to the subject an immunogenically effective amount of a composition comprising the adenoviral vector of any one of claims 1-11, the pharmaceutical composition of claim 9 or the immunogenic composition of claim 13 or the serum of claim 22.
26. The method of any one of claims 23-25, wherein the composition is administered intranasally.
27. The method of any one of claims 23-25, wherein the composition is administered intramuscularly.
28. The method of claim 23-27, further comprising one or more additional administration of the composition.
29. The method of any one of claims 24 or 25, wherein the coronavirus is a SARS-CoV-2 virus, or a variant thereof.
30. The method of claim 29, wherein the coronavirus is an alpha, beta, gamma, delta or omicron variant or a subvariant thereof.
31. The method of claim 29, wherein the coronavirus is an omicron strain or a subvariant thereof.
32. The method of 24 or 25, wherein the subject is a human.
33. The method of claim 32, wherein the subject has, is suspected of having, or is at risk for developing a coronavirus infection.
34. A method of making an adenovirus comprising, transfecting a cell with the adenoviral vector according to any one of claims 1-21; culturing the cell under conditions such that the cell produces the recombinant adenovirus; and collecting the recombinant adenovirus.
35. The method of claim 34, wherein the cell is a HEK, Vero or PER cell.

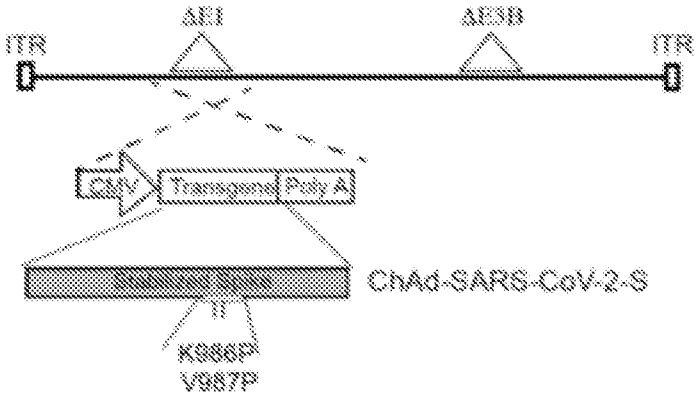

ΔE1
ΔE3B
ITR
ITR
CMV
Transgene
Poly A
ChAd-SARS-CoV-2-S
K986P
V987P

FIG. 1A

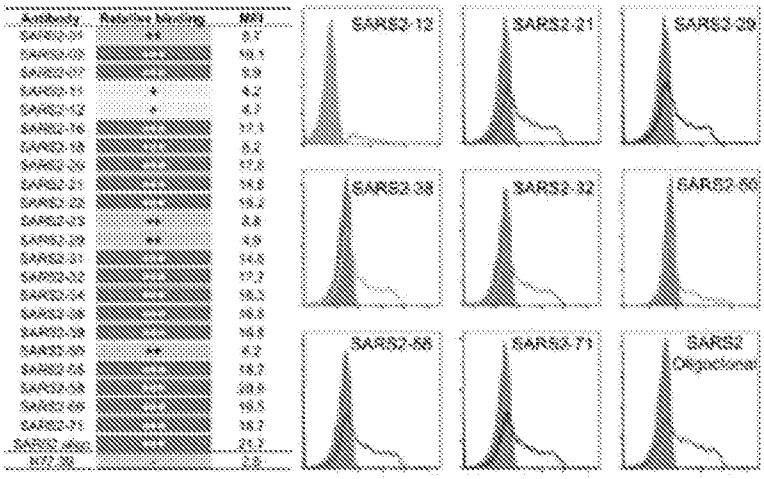

SARS2-13
SARS2-21
SARS2-28
SARS2-38
SARS2-52
SARS2-55
SARS2-56
SARS2-71
SARS2

FIG. 1B

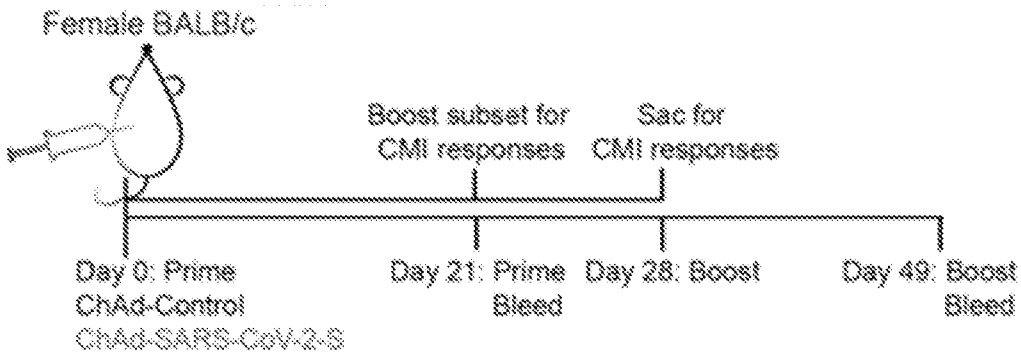

Female BALB/c
Boost subset for CMI responses
Sac for CMI responses
Day 0: Prime ChAd-Control ChAd-SARS-CoV-2-S
Day 21: Prime Bleed
Day 28: Boost
Day 49: Boost Bleed

FIG. 1C

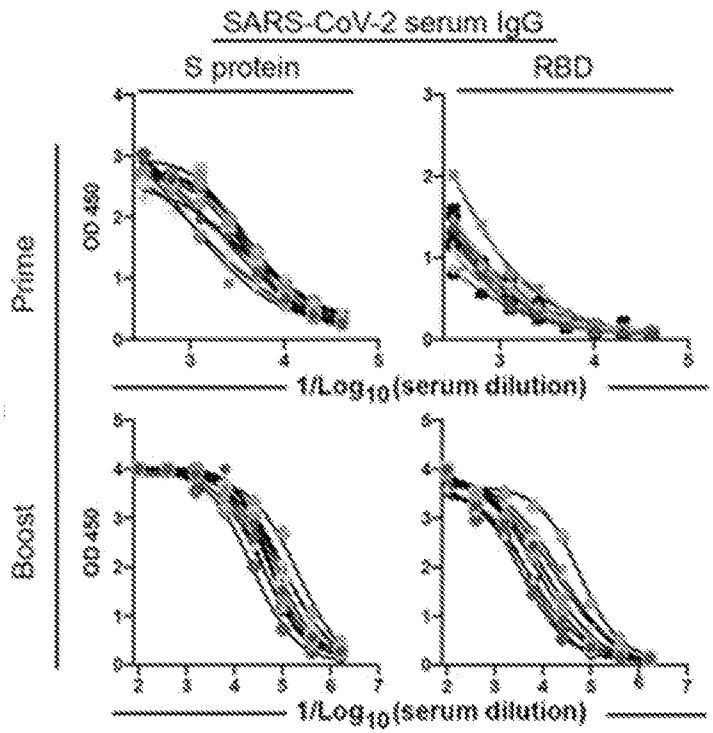

SARS-CoV-2 serum IgG
S protein
RBD
Prime
Boost
OD 450
1/Log10(serum dilution)

FIG. 1D

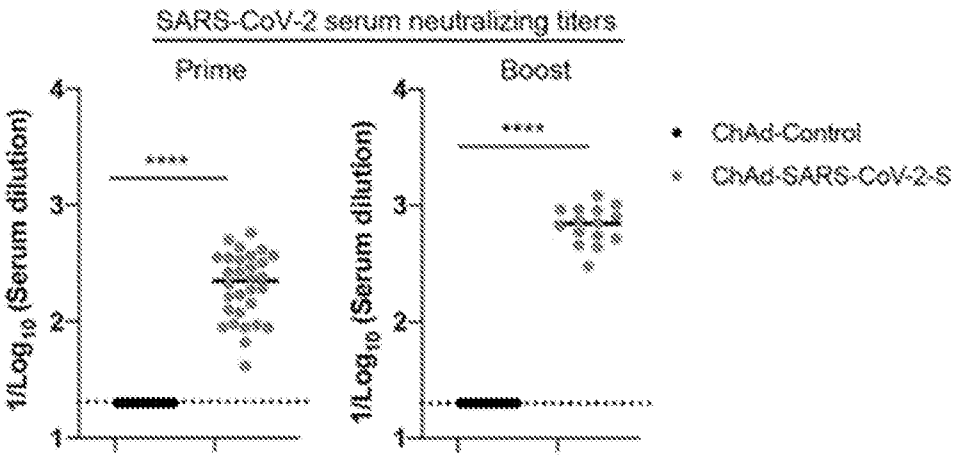

SARS-CoV-2 serum neutralizing titers
Prime
Boost
1/Log10 (Serum dilution)
****
ChAd-Control
ChAd-SARS-CoV-2-S

FIG. 1E

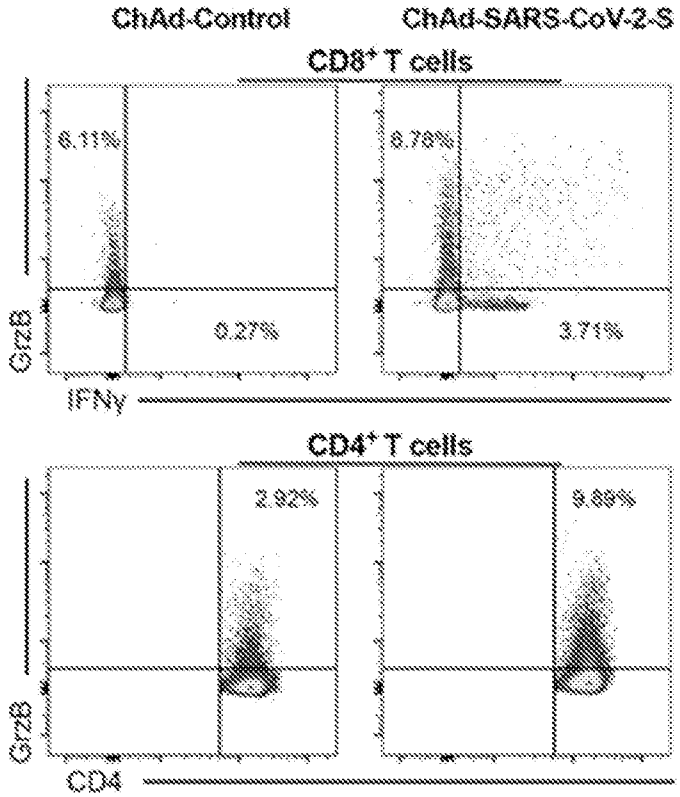

ChAd-Control
ChAd-SARS-CoV-2-S
CD8+ T cells
0.11%
0.78%
0.27%
3.71%
GrzB
IFNγ
CD4+ T cells
2.92%
9.89%
GrzB
CD4

FIG. 1F

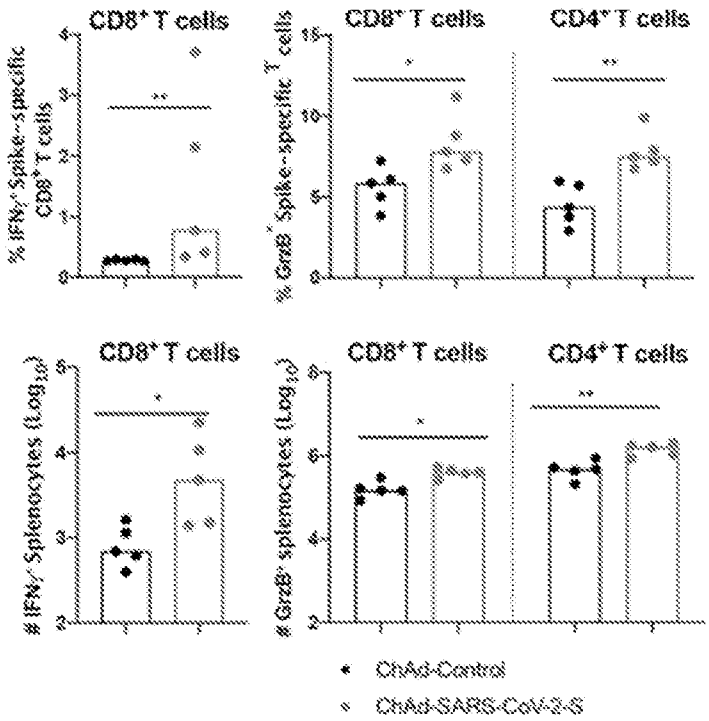

CD8+ T cells
CD8+ T cells
CD4+ T cells
% IFNγ+ Spike-specific CD8+ T cells
% GrzB+ Spike-specific T cells
CD8+ T cells
CD8+ T cells
CD4+ T cells
IFNγ+ Splenocytes (Log10)
GrzB+ splenocytes (Log10)
ChAd-Control
ChAd-SARS-CoV-2-S

FIG. 1G

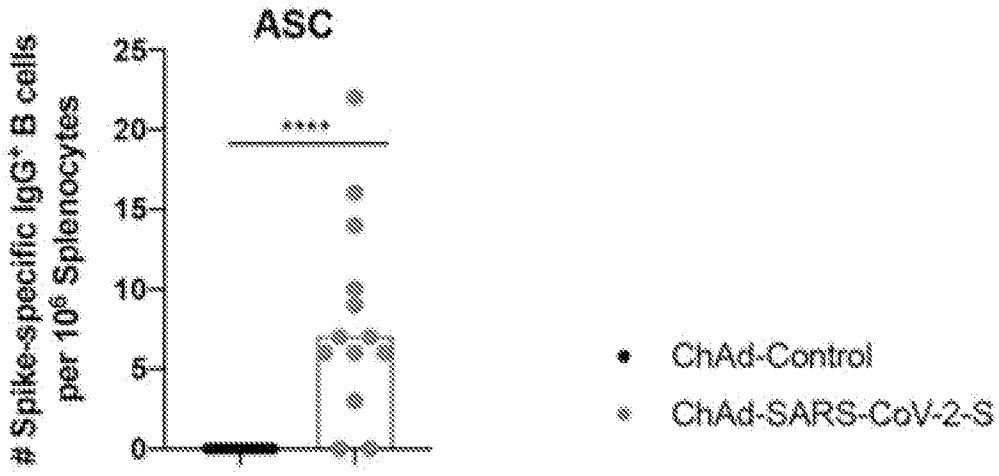

ASC
Spike-specific IgG+ B cells per 10^6 Splenocytes
25
20
15
10
5
0
****
ChAd-Control
ChAd-SARS-CoV-2-S

FIG. 1H

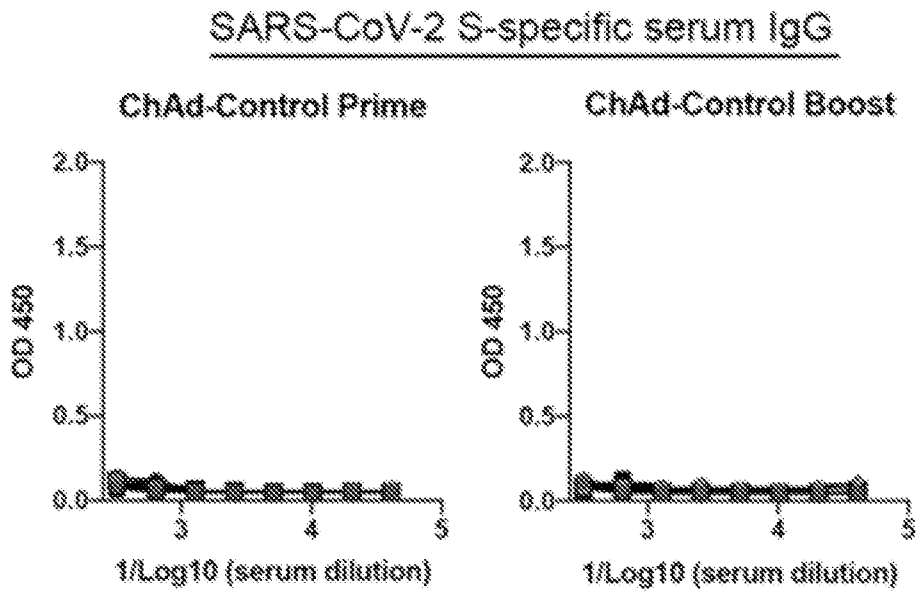

SARS-CoV-2 S-specific serum IgG
ChAd-Control Prime
ChAd-Control Boost
OD 450
2.0
1.5
1.0
0.5
0.0
3
4
5
1/Log10 (serum dilution)

FIG. 2A

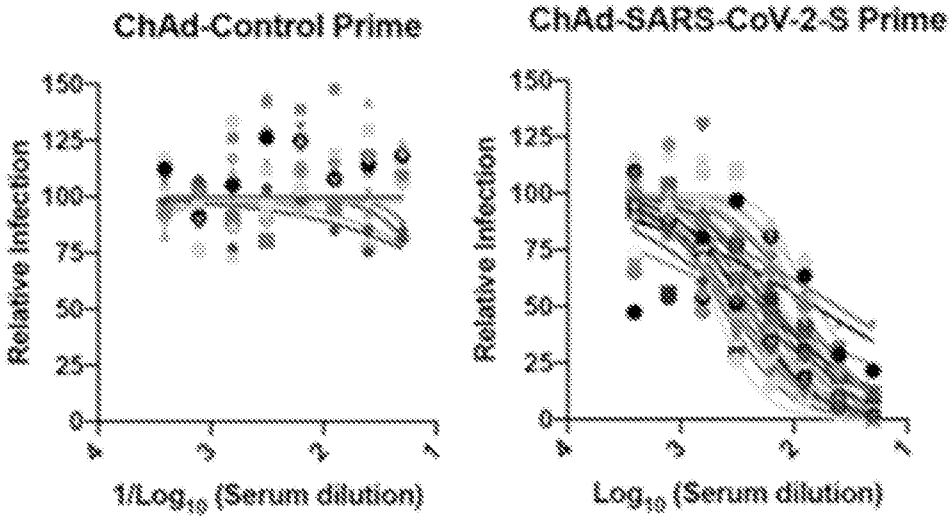

ChAd-Control Prime
ChAd-SARS-CoV-2-S Prime
Relative Infection
150
125
100
75
50
25
0
1/Log10 (Serum dilution)
Log10 (Serum dilution)

FIG. 2B

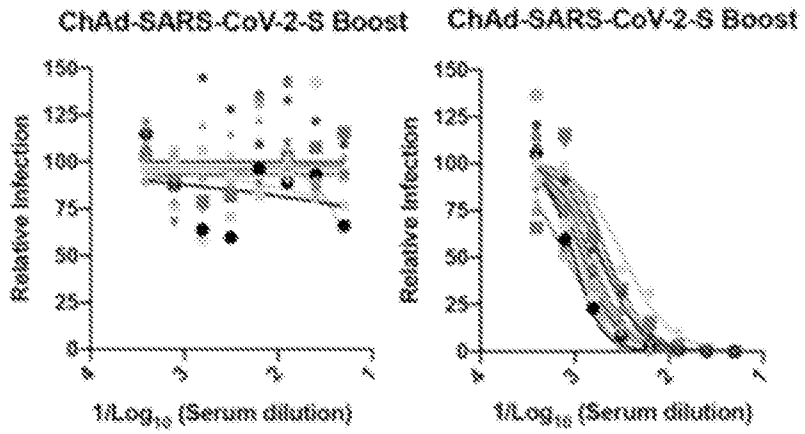

ChAd-SARS-CoV-2-S Boost
ChAd-SARS-CoV-2-S Boost
Relative Infection
Relative Infection
1/Log10 (Serum dilution)
1/Log10 (Serum dilution)

FIG. 2C

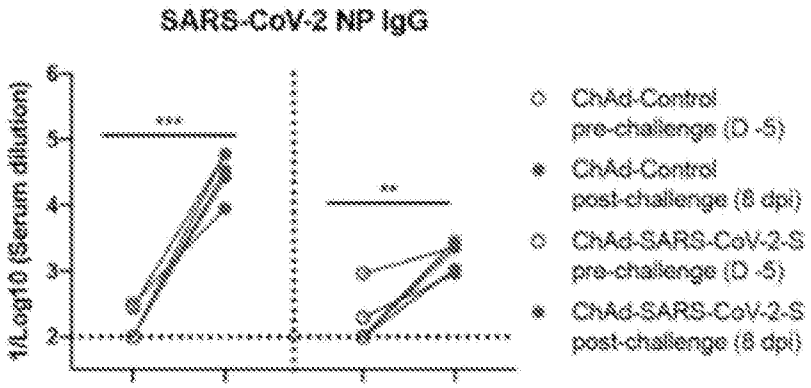

SARS-CoV-2 NP IgG
1/Log10 (Serum dilution)
***
**
ChAd-Control pre-challenge (D -5)
ChAd-Control post-challenge (8 dpi)
ChAd-SARS-CoV-2-S pre-challenge (D -5)
ChAd-SARS-CoV-2-S post-challenge (8 dpi)

FIG. 2D

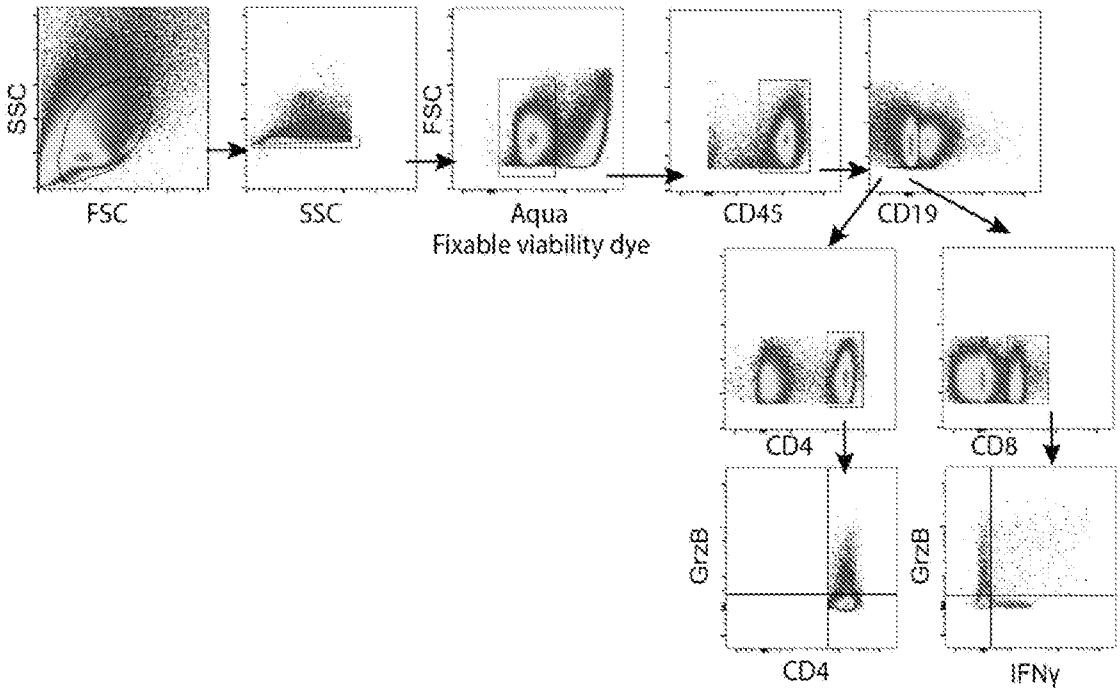

SSC
FSC
SSC
FSC
Aqua
Fixable viability dye
CD45
CD19
CD4
CD8
GrzB
CD4
GrzB
IFNγ

FIG. 3

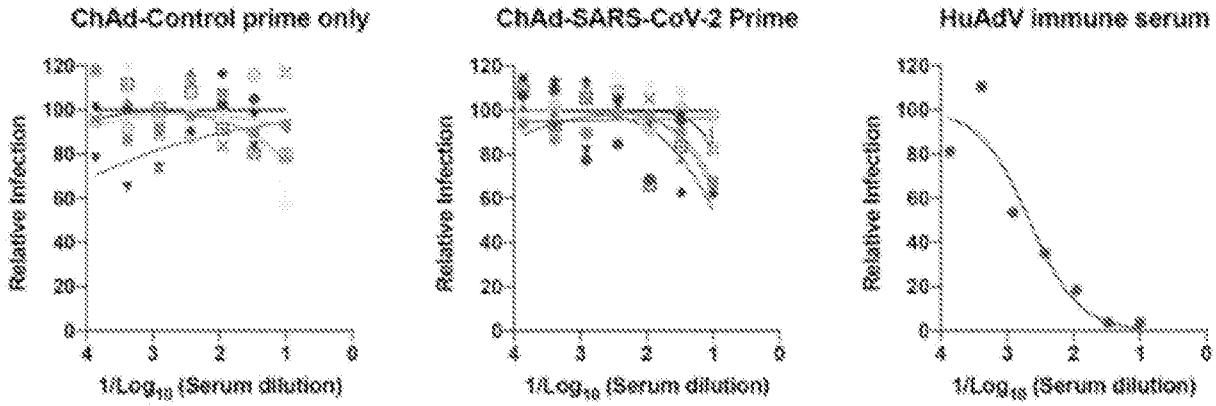

ChAd-Control prime only
ChAd-SARS-CoV-2 Prime
HuAdV immune serum
Relative Infection
1/Log10 (Serum dilution)

FIG. 4A

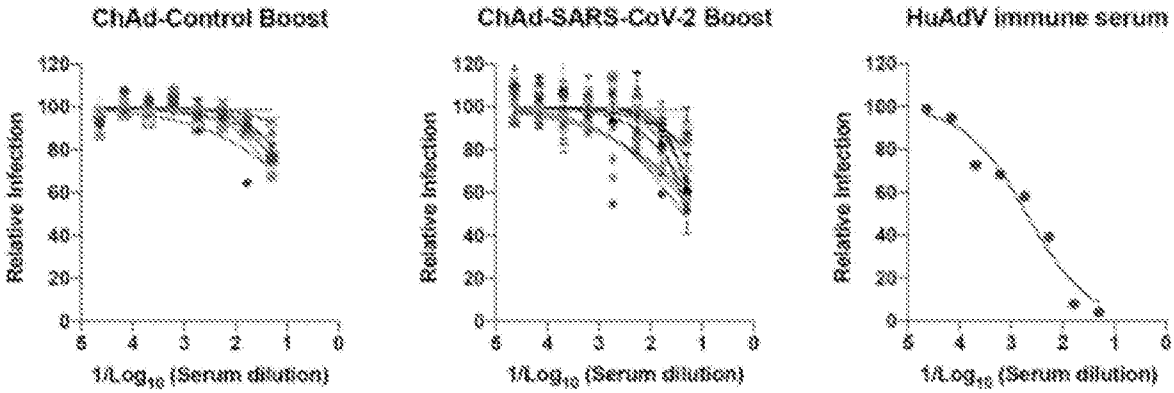

ChAd-Control Boost
ChAd-SARS-CoV-2 Boost
HuAdV immune serum
Relative Infection
1/Log10 (Serum dilution)

FIG. 4B

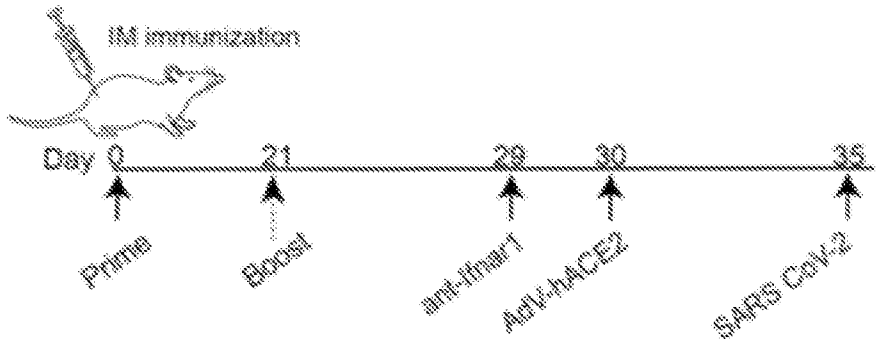

IM immunization
Day 0
21
28
30
35
Prime
Boost
anti-Ifnar1
Ad5-hACE2
SARS CoV-2

FIG. 5A

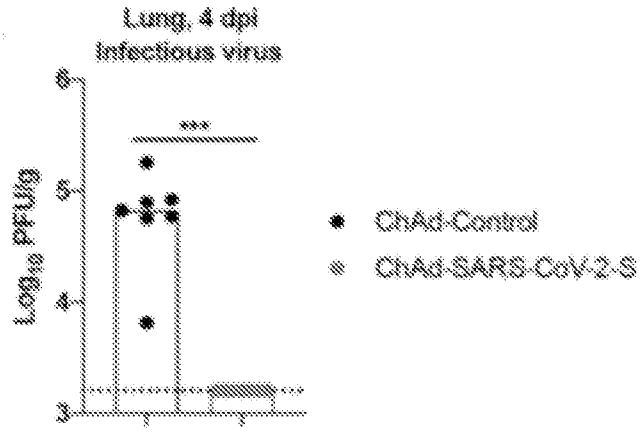

Lung, 4 dpi
Infectious virus
Log10 PFU/g
6
5
4
3
***
ChAd-Control
ChAd-SARS-CoV-2-S

FIG. 5B

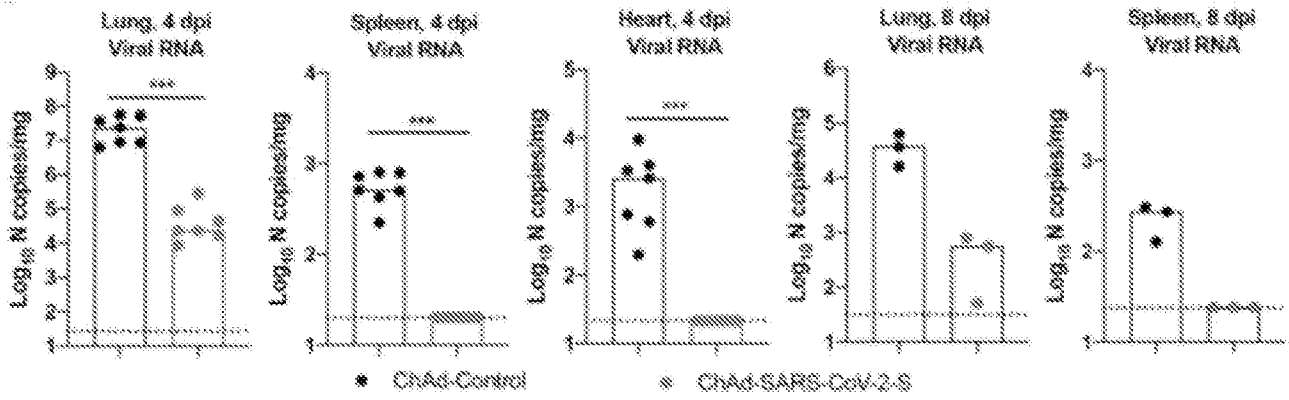

Lung, 4 dpi
Viral RNA
Spleen, 4 dpi
Viral RNA
Heart, 4 dpi
Viral RNA
Lung, 8 dpi
Viral RNA
Spleen, 8 dpi
Viral RNA
Log10 N copies/mg
***
ChAd-Control
ChAd-SARS-CoV-2-S

FIG. 5C

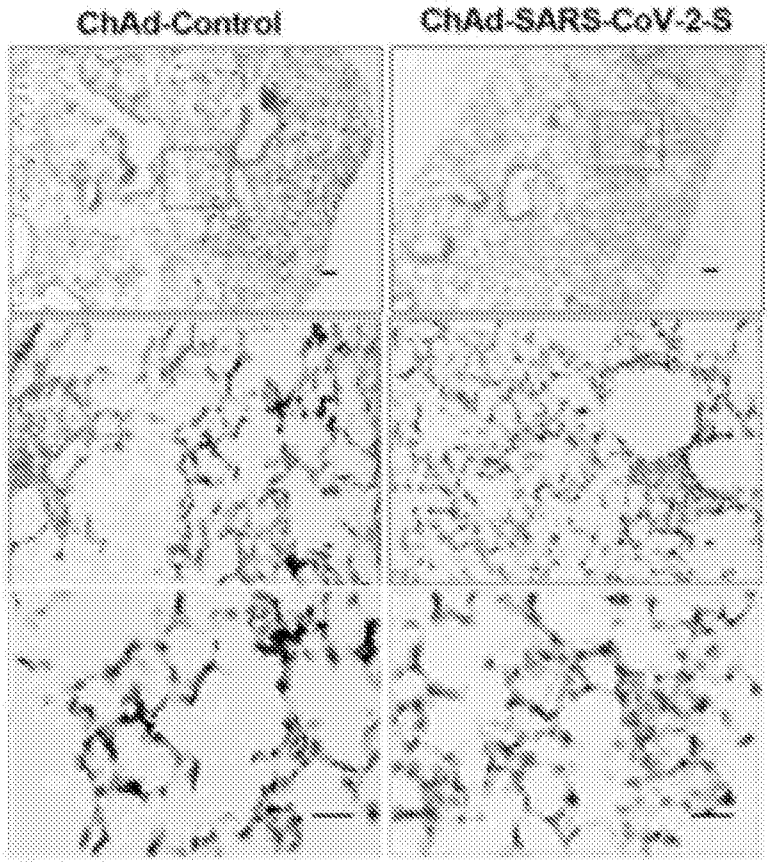

ChAd-Control
ChAd-SARS-CoV-2-S

FIG. 5D

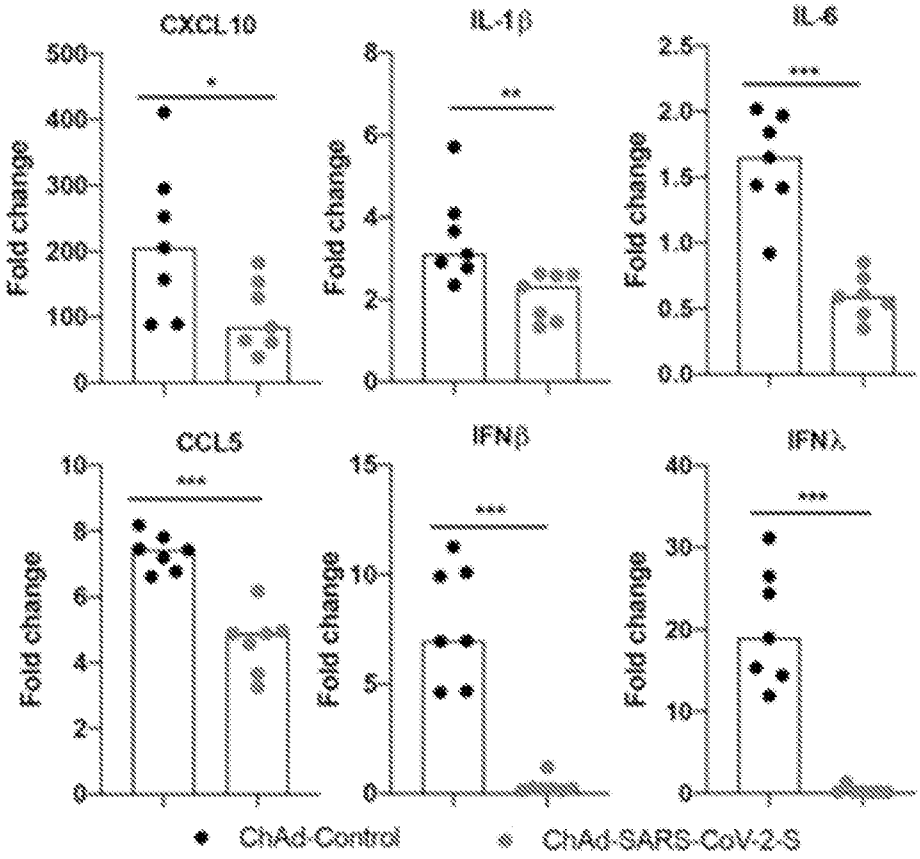

CXCL10
IL-1β
IL-6
CCL5
IFNβ
IFNλ
Fold change
ChAd-Control
ChAd-SARS-CoV-2-S

FIG. 5E

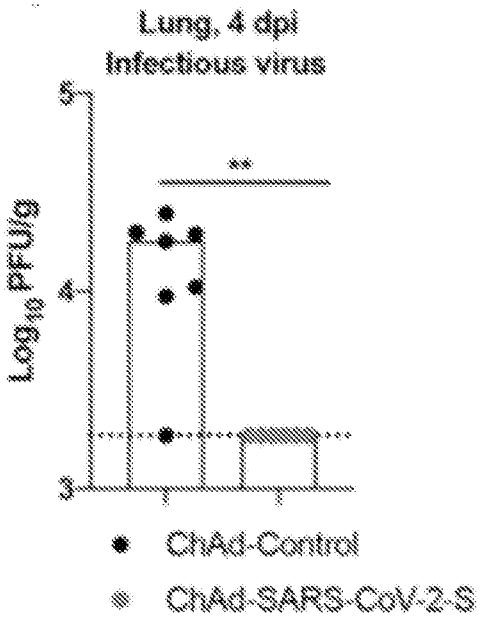

Lung, 4 dpi
Infectious virus
Log10 PFU/g
ChAd-Control
ChAd-SARS-CoV-2-S

FIG. 5F

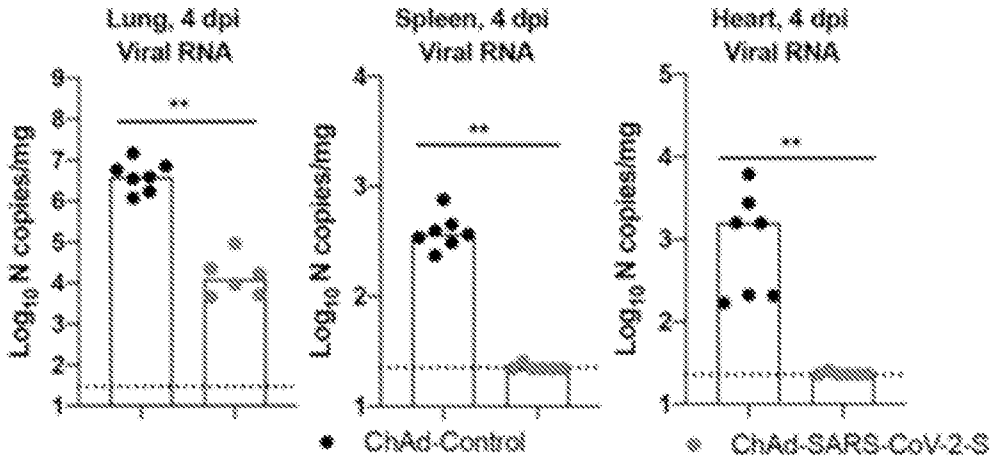

Lung, 4 dpi
Viral RNA
Spleen, 4 dpi
Viral RNA
Heart, 4 dpi
Viral RNA
Log10 N copies/mg
**
ChAd-Control
ChAd-SARS-CoV-2-S

FIG. 5G

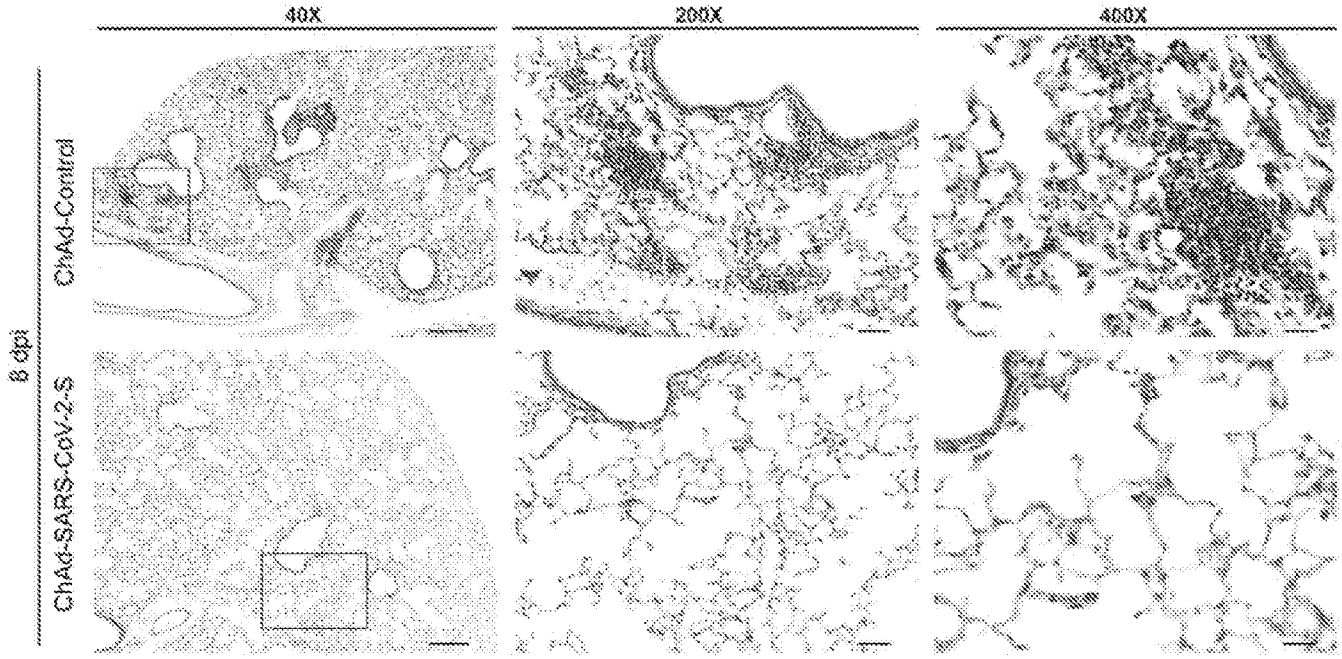


FIG. 6

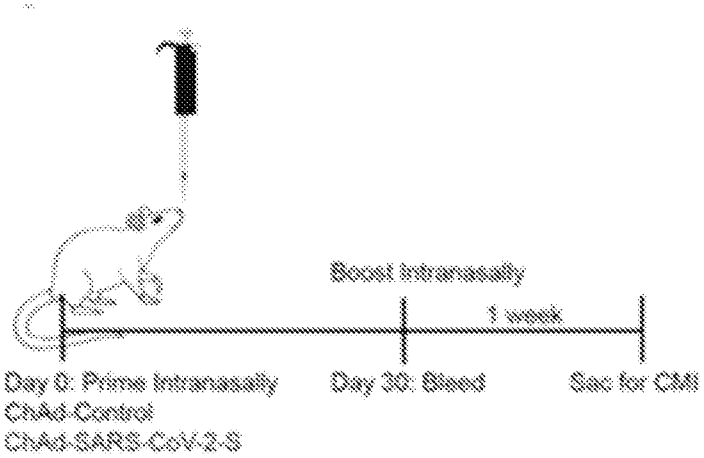

Boost Intranasally
1 week
Day 0: Prime Intranasally
ChAd-Control
ChAd-SARS-CoV-2-S
Day 30: Bleed
Sac for CMI

FIG. 7A

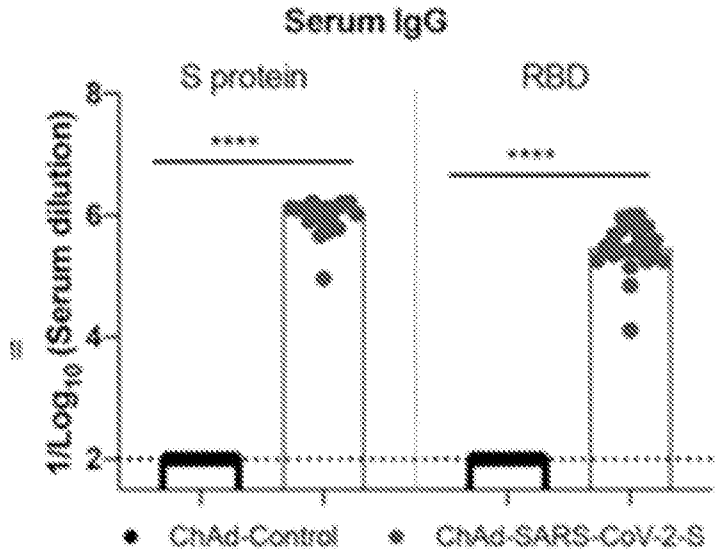

Serum IgG
S protein
RBD
****
****
1/Log10 (Serum dilution)
8
6
4
2
ChAd-Control
ChAd-SARS-CoV-2-S

FIG. 7B

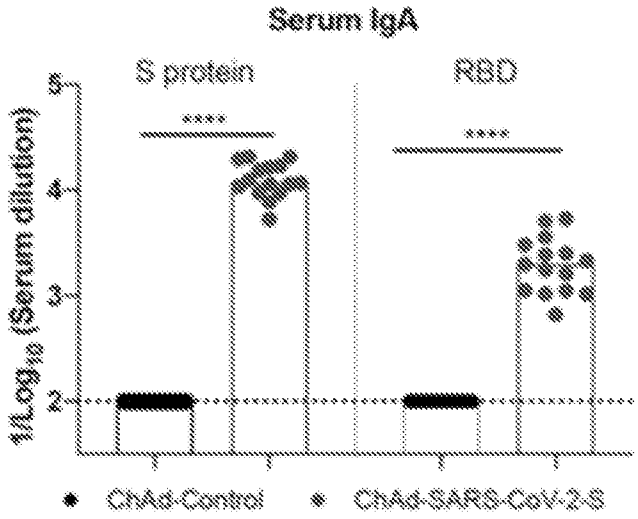

Serum IgA
S protein
RBD
****
****
1/Log10 (Serum dilution)
5
4
3
2
ChAd-Control
ChAd-SARS-CoV-2-S

FIG. 7C

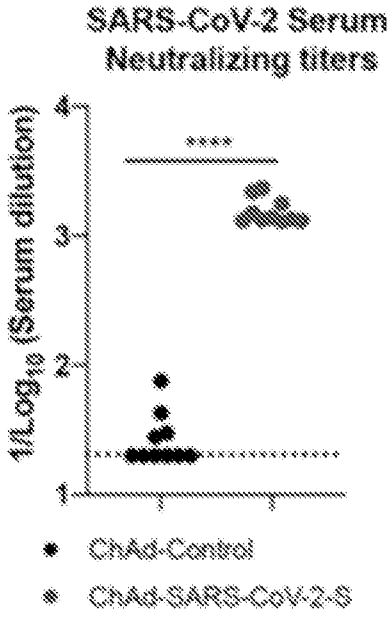

SARS-CoV-2 Serum
Neutralizing titers
****
1/Log10 (Serum dilution)
4
3
2
1
ChAd-Control
ChAd-SARS-CoV-2-S

FIG. 7D

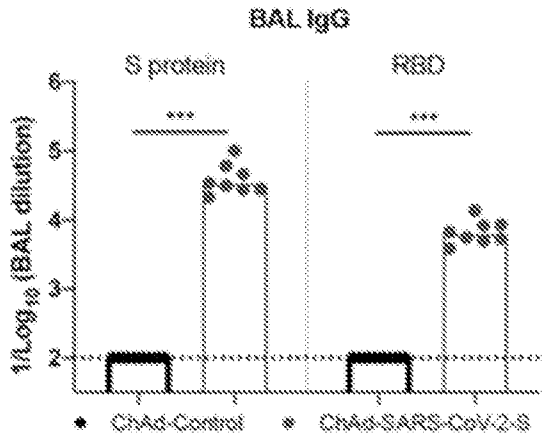

BAL IgG
S protein
RBD
***
***
1/Log10 (BAL dilution)
6
5
4
3
2
ChAd-Control
ChAd-SARS-CoV-2-S

FIG. 7E

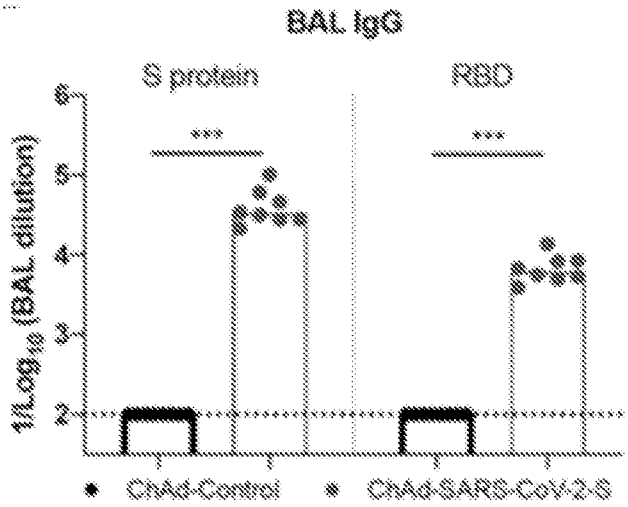

BAL IgG
S protein
RBD
***
***
1/Log10 (BAL dilution)
6
5
4
3
2
ChAd-Control
ChAd-SARS-CoV-2-S

FIG. 7F

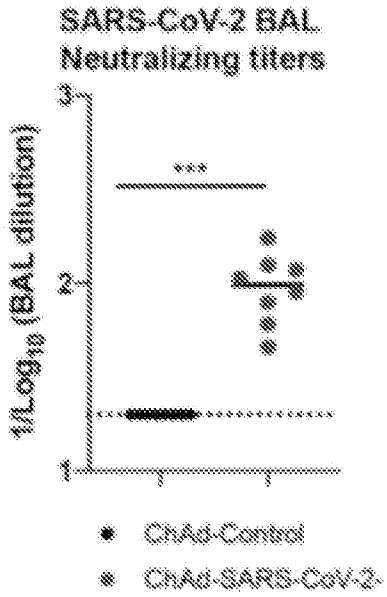

SARS-CoV-2 BAL
Neutralizing titers
1/Log10 (BAL dilution)
ChAd-Control
ChAd-SARS-CoV-2

FIG. 7G

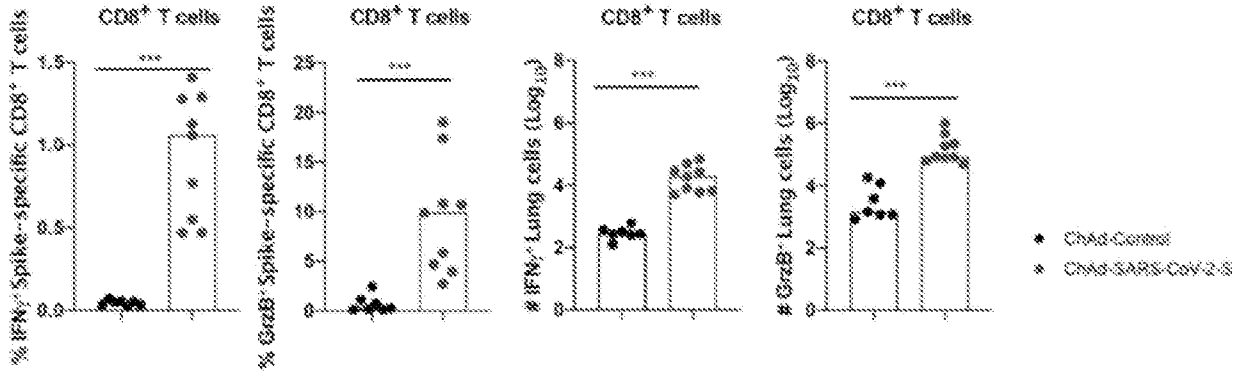

CD8+ T cells
% IFNγ+ Spike-specific CD8+ T cells
% GzB+ Spike-specific CD8+ T cells
# IFNγ+ Lung cells (Log10)
# GzB+ Lung cells (Log10)
ChAd-Control
ChAd-SARS-CoV-2-S

FIG. 7H

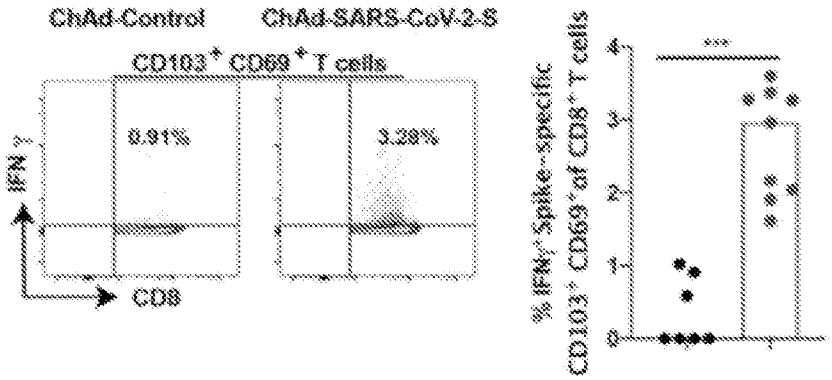

ChAd-Control
ChAd-SARS-CoV-2-S
CD103+ CD69+ T cells
0.91%
3.28%
IFN γ
CD8
% IFNγ+ Spike-specific CD103+ CD69+ of CD8+ T cells
***

FIG. 7I

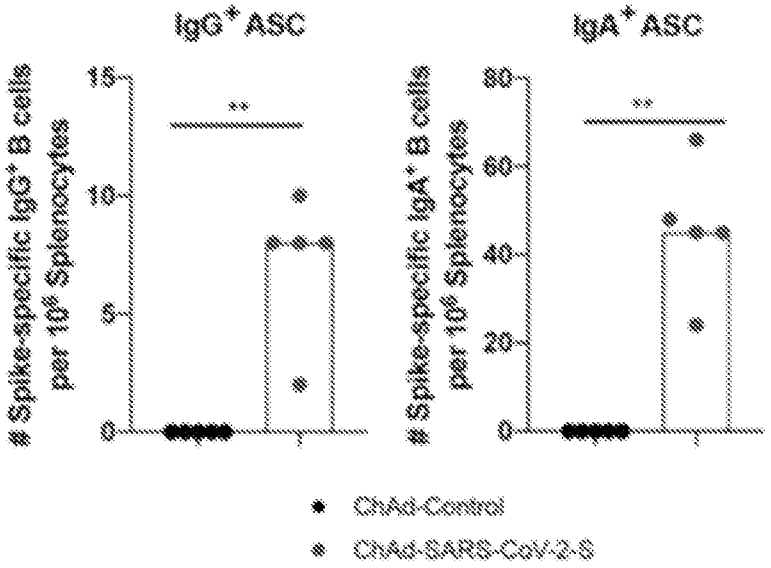

IgG+ ASC
IgA+ ASC
Spike-specific IgG+ B cells per 10^6 Splenocytes
Spike-specific IgA+ B cells per 10^6 Splenocytes
**
**
ChAd-Control
ChAd-SARS-CoV-2-S

FIG. 7J

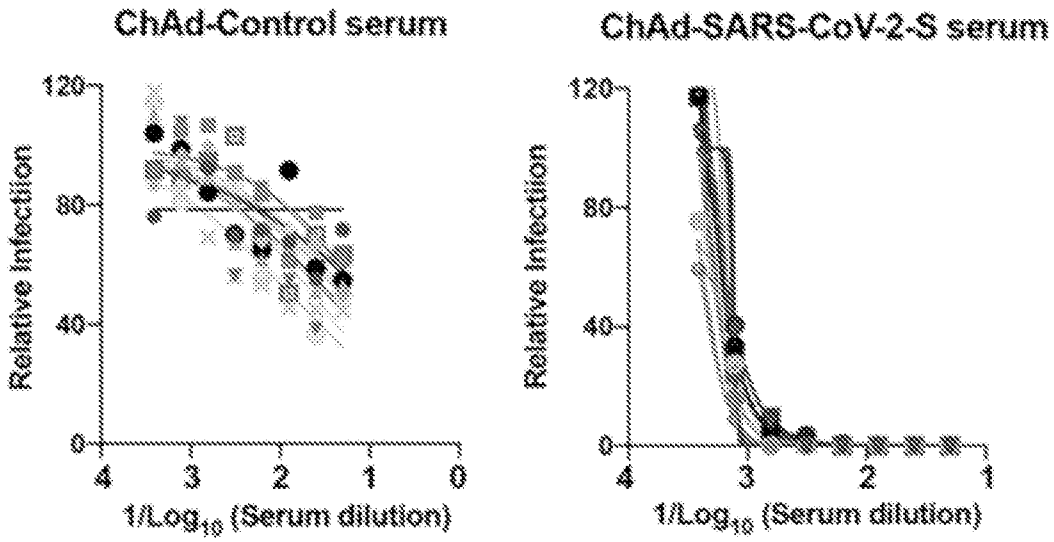

ChAd-Control serum
ChAd-SARS-CoV-2-S serum
Relative Infection
120
80
40
0
4
3
2
1
0
1/Log10 (Serum dilution)

FIG. 8A

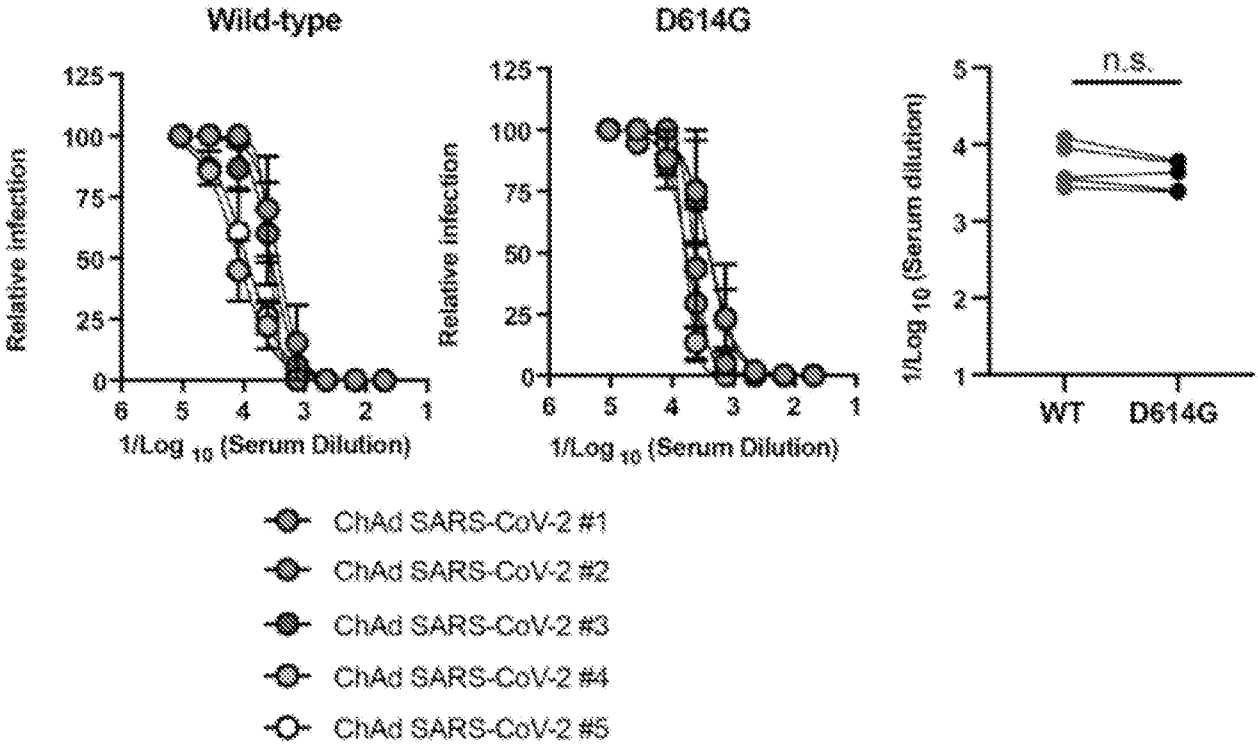

Wild-type
D614G
Relative infection
125
100
75
50
25
0
6
5
4
3
2
1
1/Log 10 (Serum Dilution)
n.s.
1/Log 10 (Serum dilution)
5
4
3
2
1
WT
D614G
ChAd SARS-CoV-2 #1
ChAd SARS-CoV-2 #2
ChAd SARS-CoV-2 #3
ChAd SARS-CoV-2 #4
ChAd SARS-CoV-2 #5

FIG. 8B

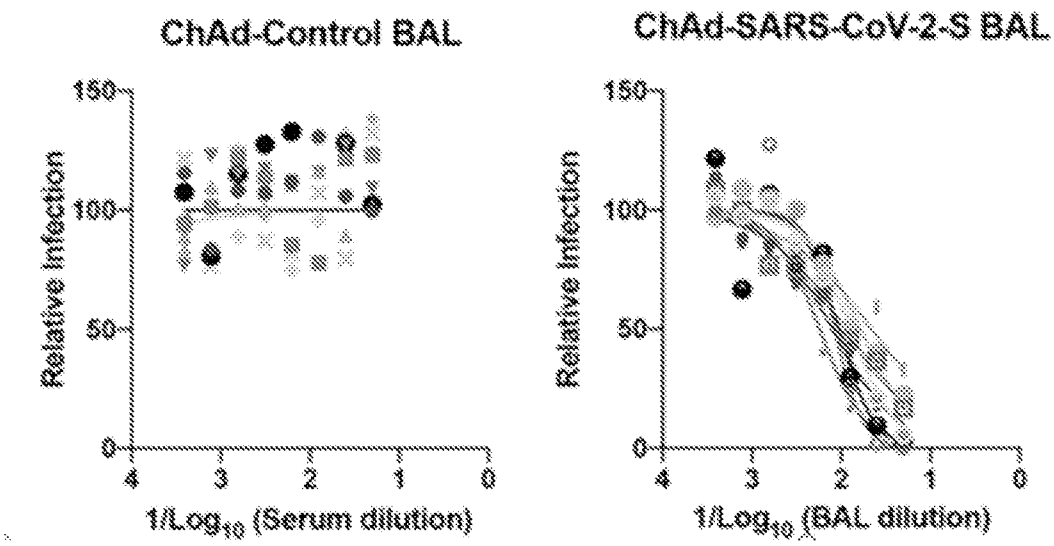

ChAd-Control BAL
ChAd-SARS-CoV-2-S BAL
Relative Infection
150
100
50
0
4
3
2
1
0
1/Log10 (Serum dilution)
1/Log10 (BAL dilution)

FIG. 8C

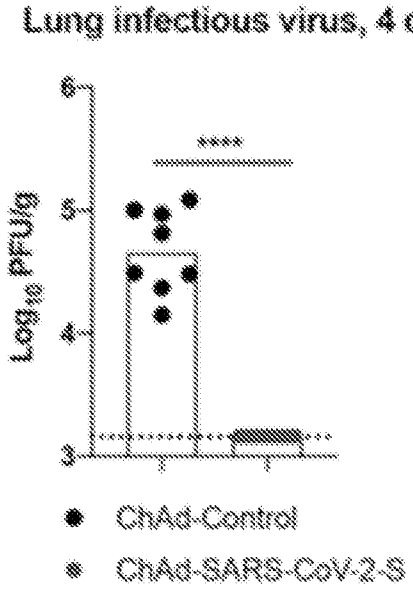

Lung infectious virus, 4 dpi
Log10 PFU/g
****
ChAd-Control
ChAd-SARS-CoV-2-S

FIG. 9A

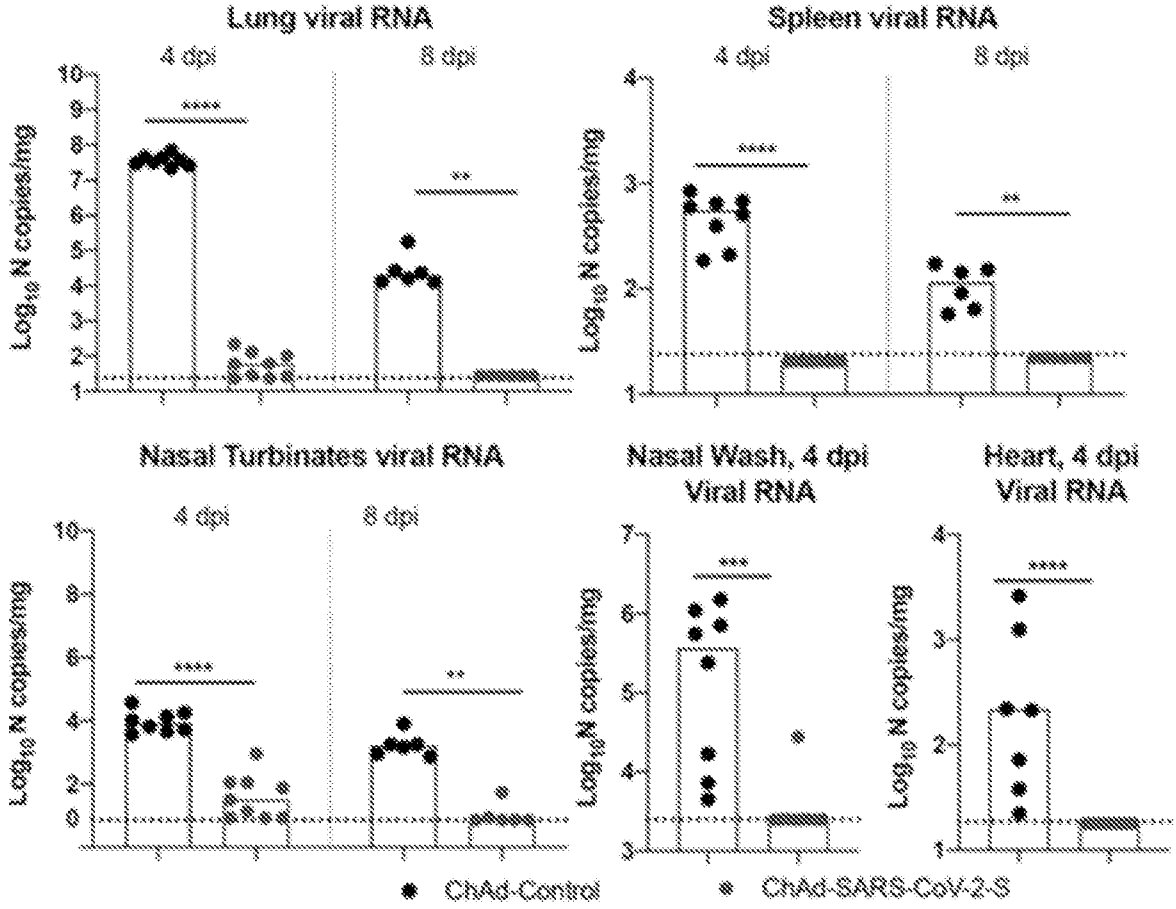

Lung viral RNA
Spleen viral RNA
Nasal Turbinates viral RNA
Nasal Wash, 4 dpi Viral RNA
Heart, 4 dpi Viral RNA
4 dpi
8 dpi
Log10 N copies/mg
ChAd-Control
ChAd-SARS-CoV-2-S

FIG. 9B

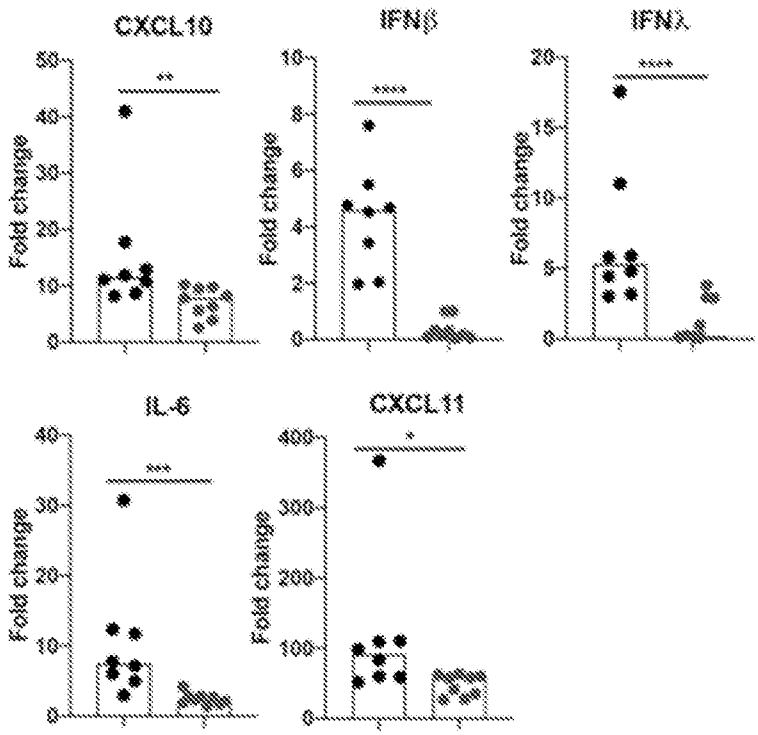

CXCL10
IFNβ
IFNλ
IL-6
CXCL11
Fold change

FIG. 9C

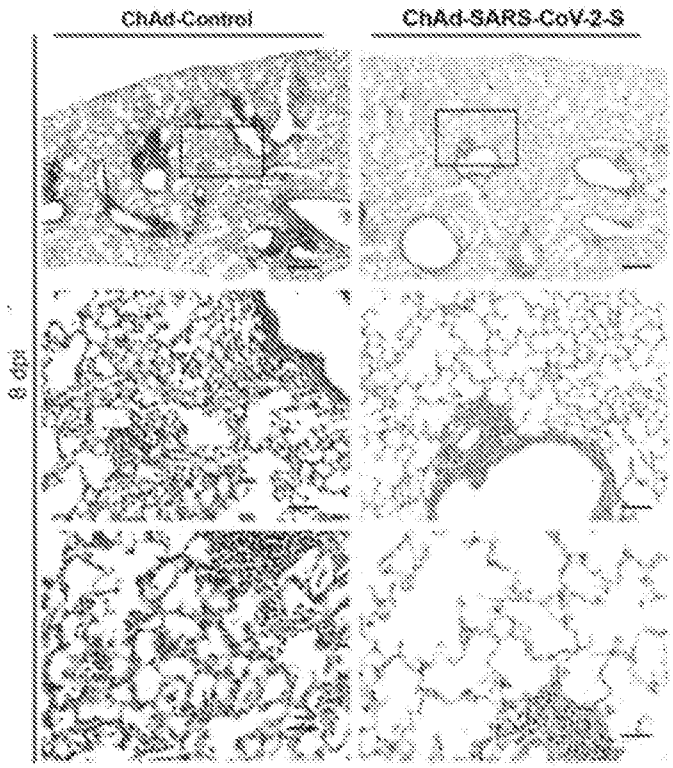

ChAd-Control
ChAd-SARS-CoV-2-S
8 dpi

FIG. 9D

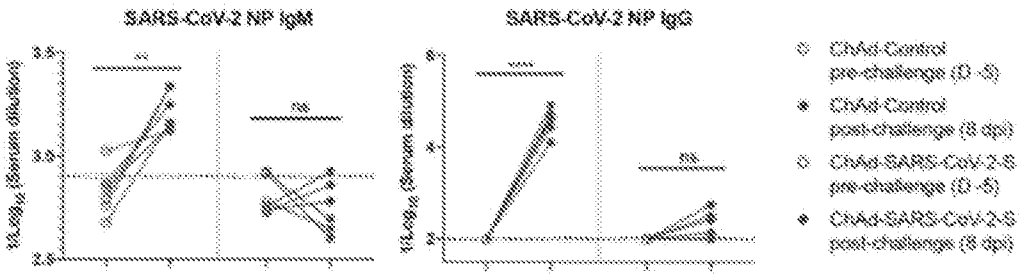

SARS-CoV-2 NP IgM
SARS-CoV-2 NP IgG
ChAd-Control pre-challenge (D -5)
ChAd-Control post-challenge (8 dpi)
ChAd-SARS-CoV-2-S pre-challenge (D -5)
ChAd-SARS-CoV-2-S post-challenge (8 dpi)

FIG. 9E

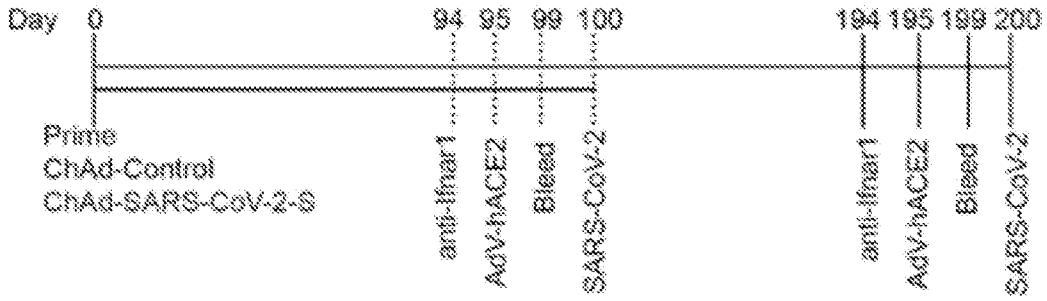


FIG. 10A

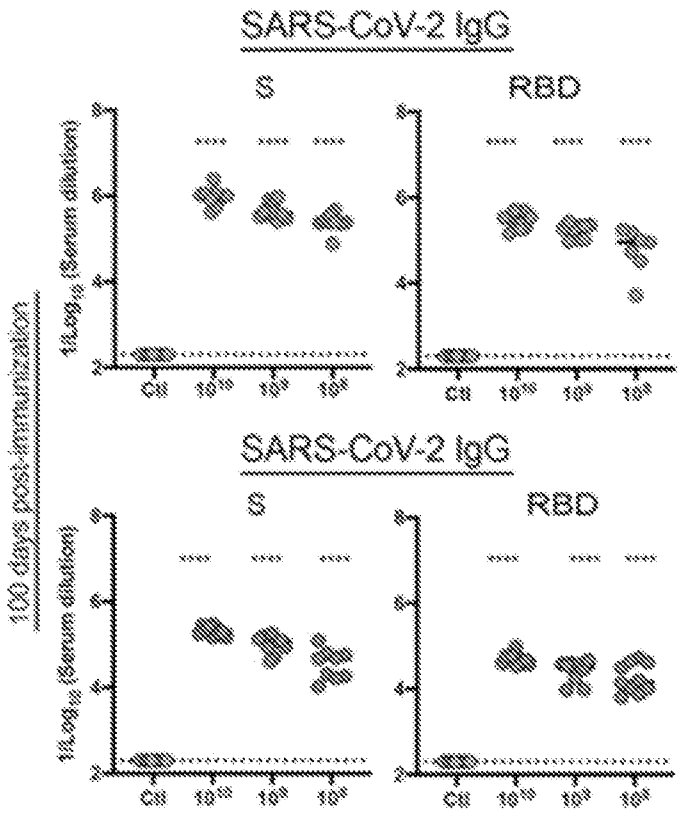


FIG. 10B

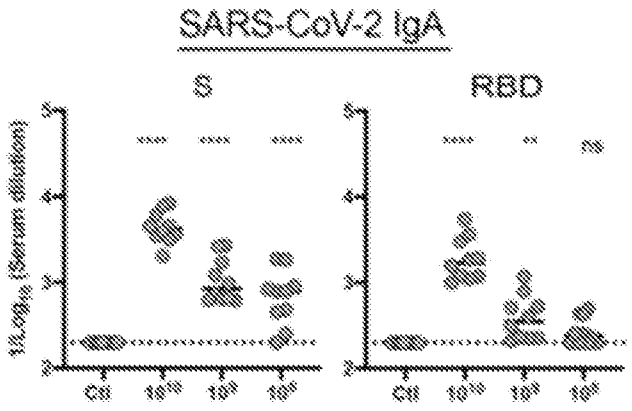


FIG. 10C

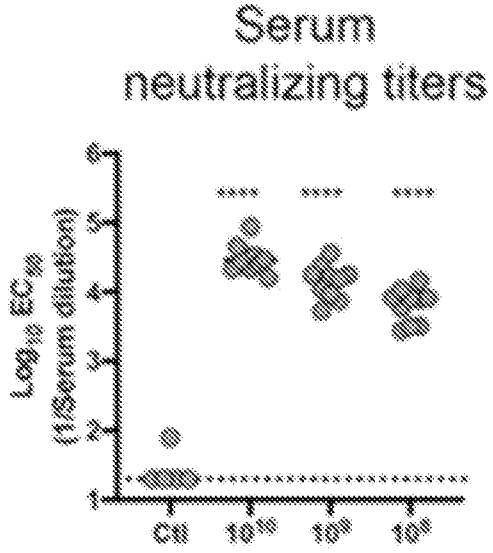


FIG. 10D

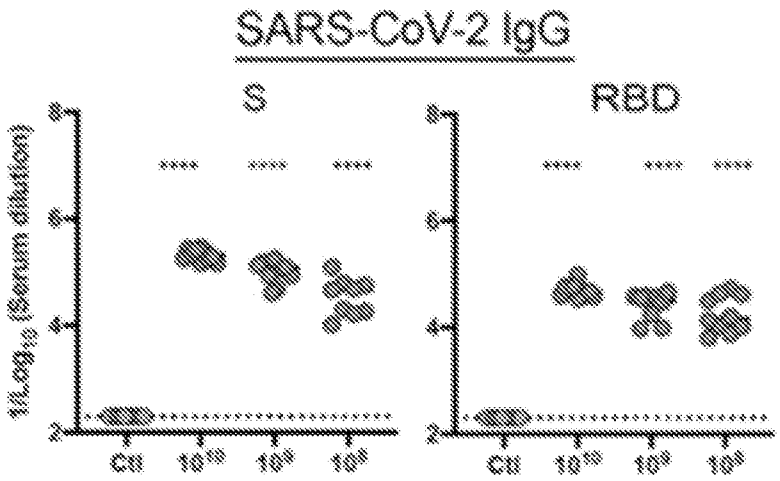


FIG. 10E

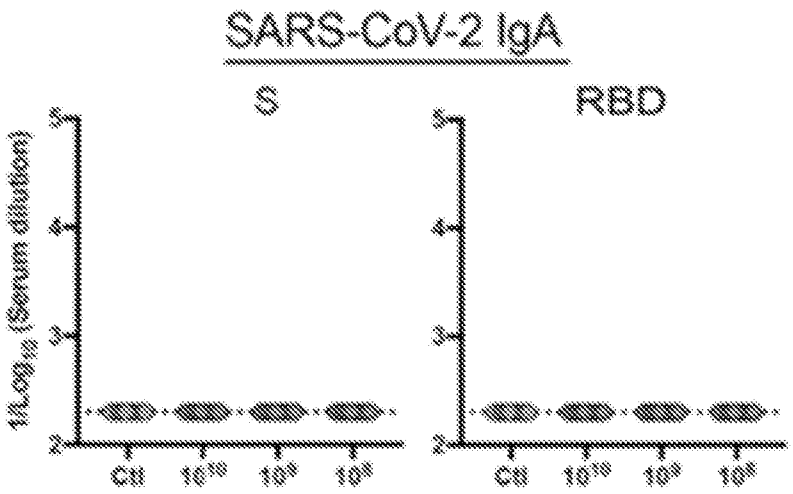


FIG. 10F

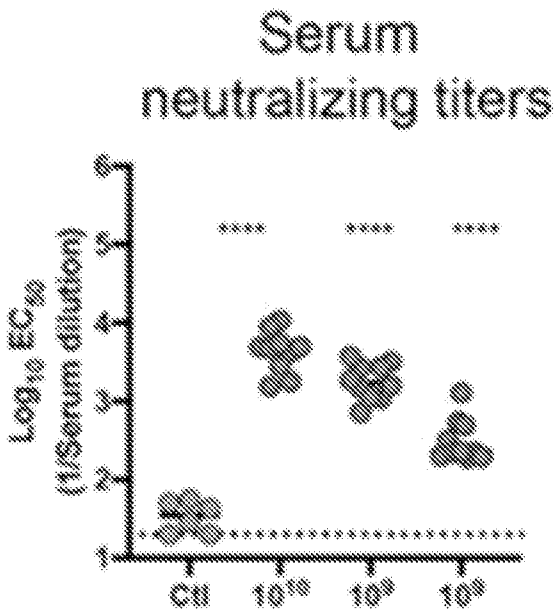


FIG. 10G

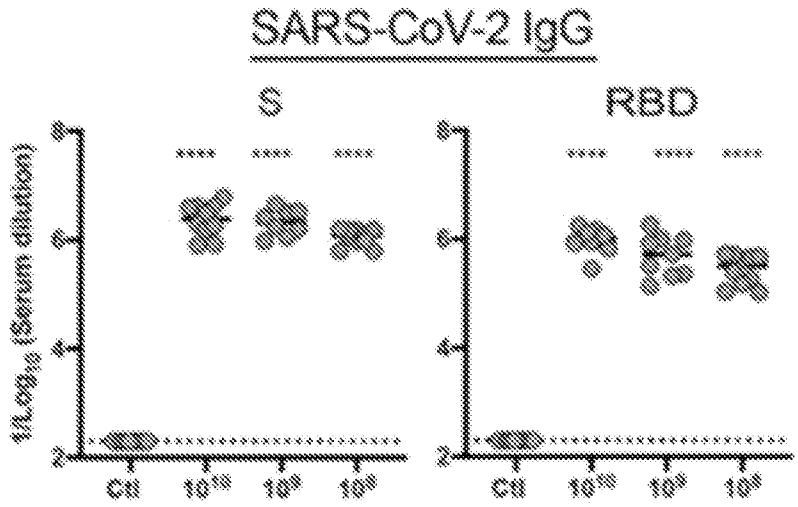


FIG. 10H

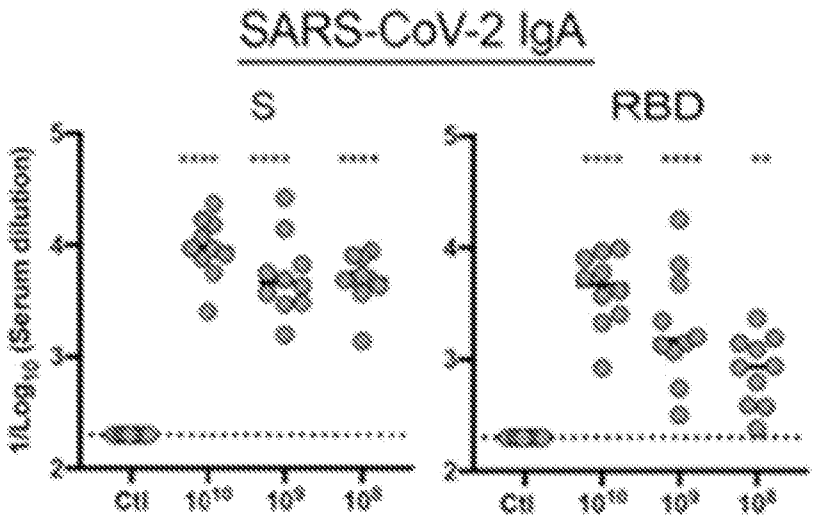


FIG. 10I

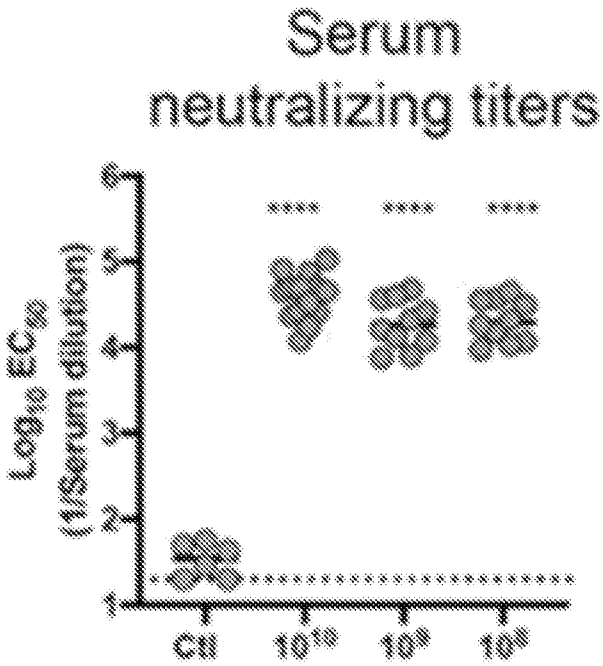


FIG. 10J

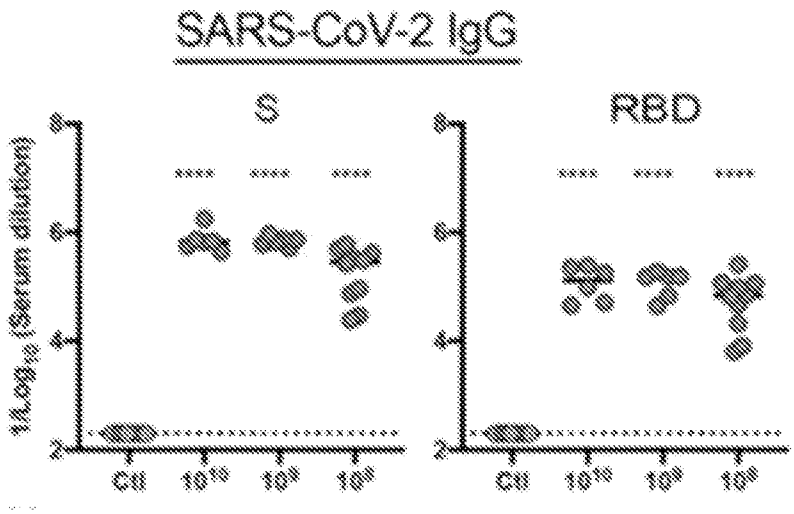


FIG. 10K

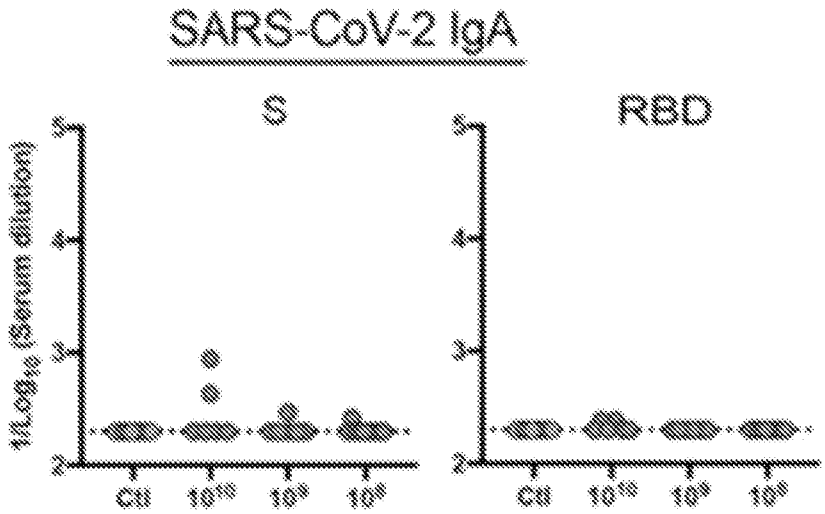


FIG. 10L

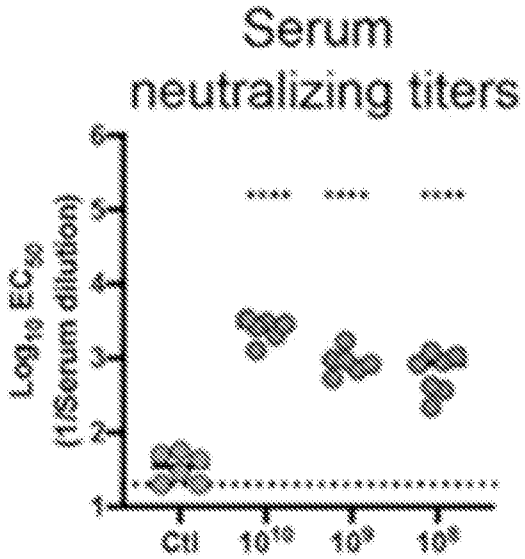


FIG. 10M



FIG. 10N

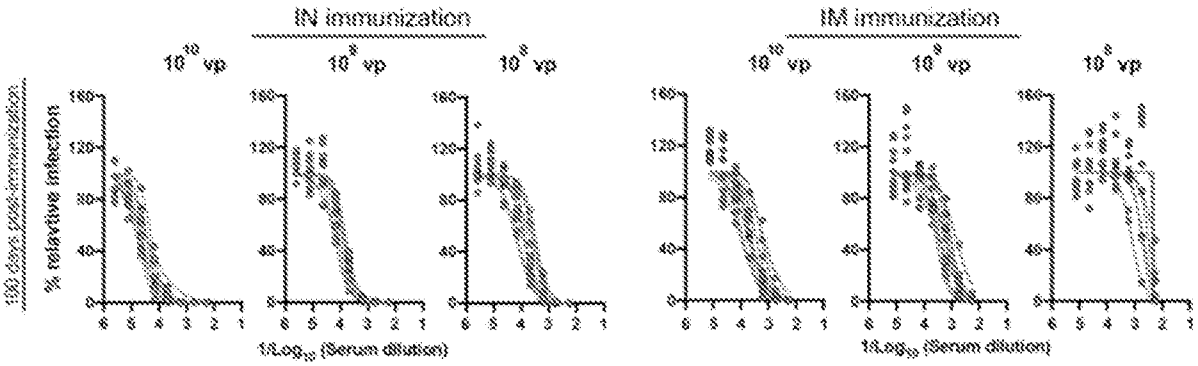


FIG. 11A

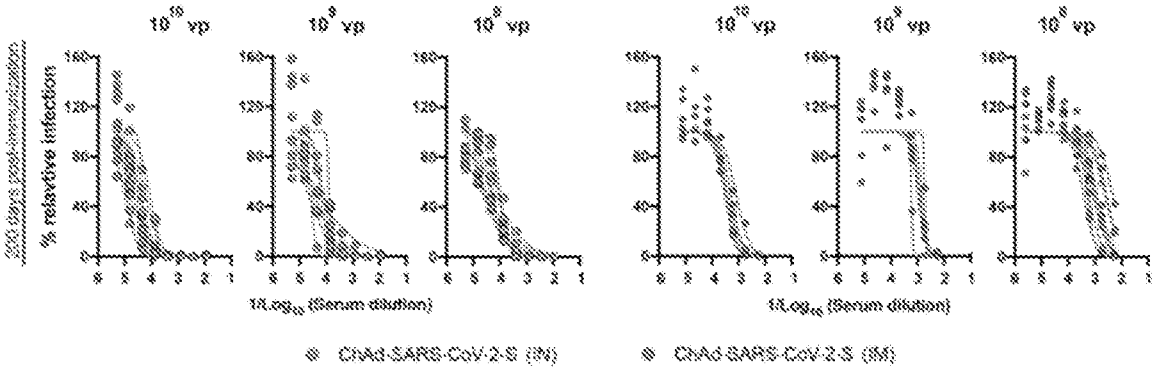


FIG. 11B

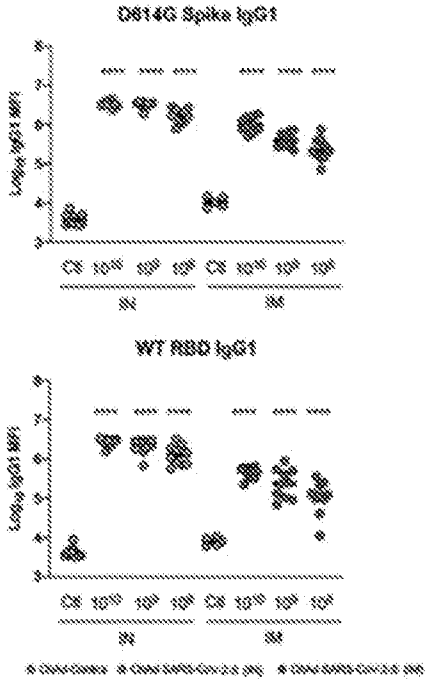


FIG. 12A

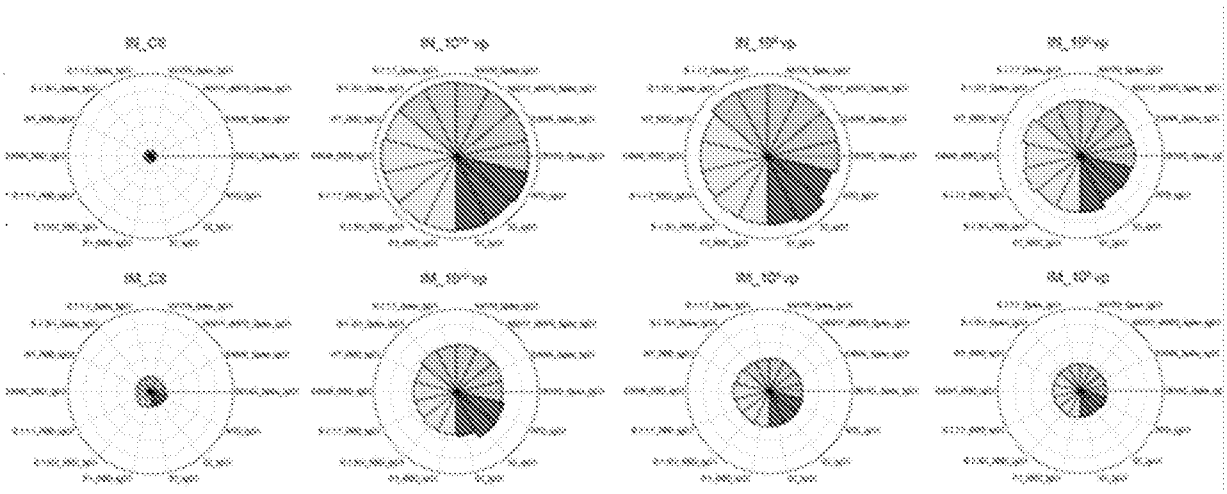


FIG. 12B

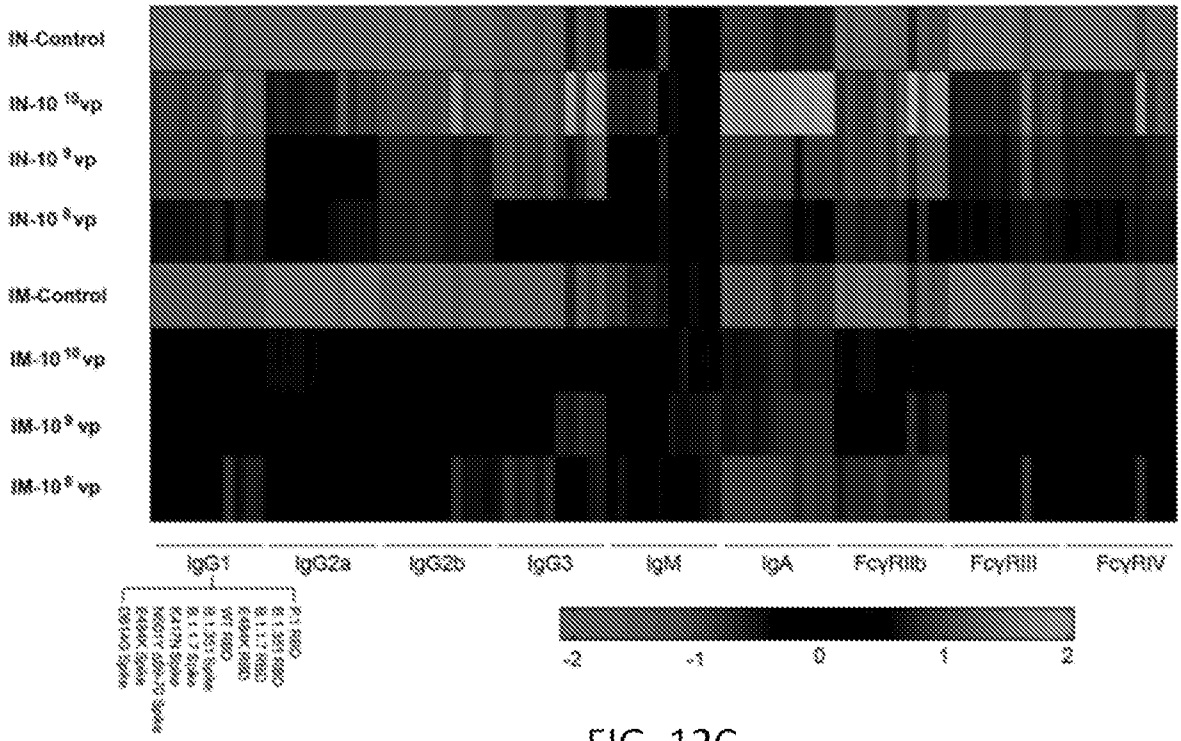


FIG. 12C

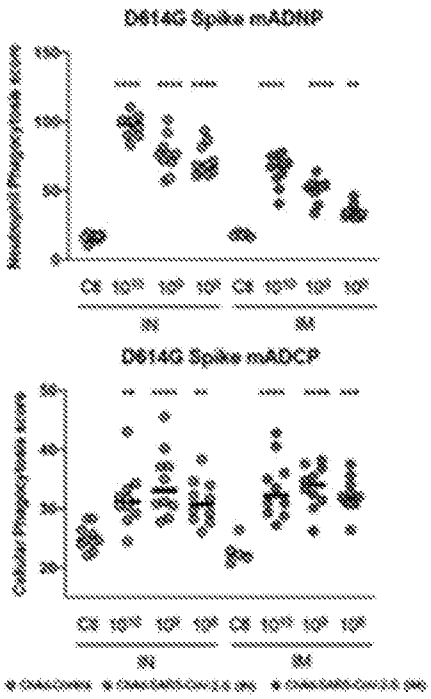


FIG. 12D

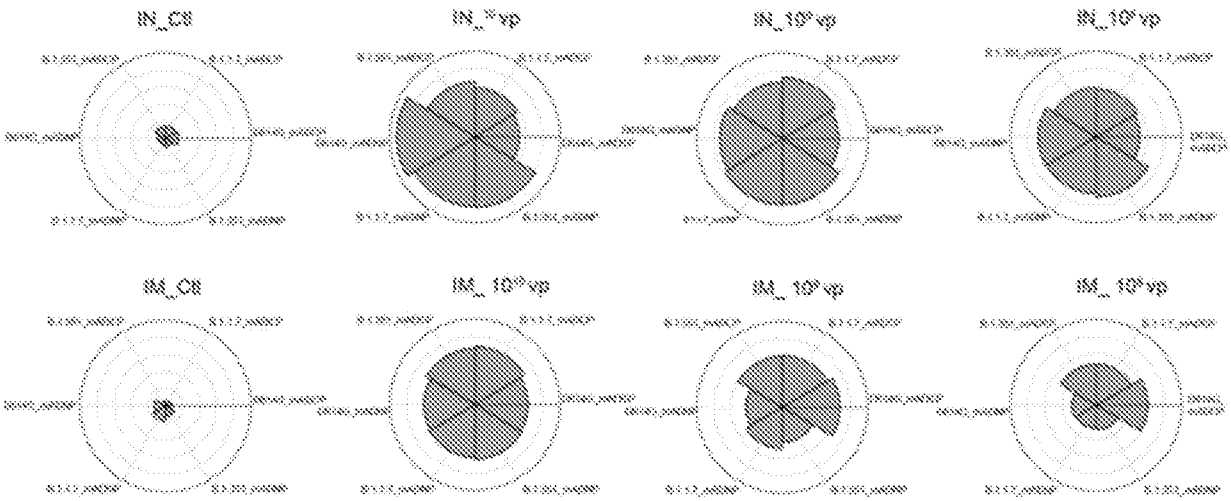


FIG. 12E

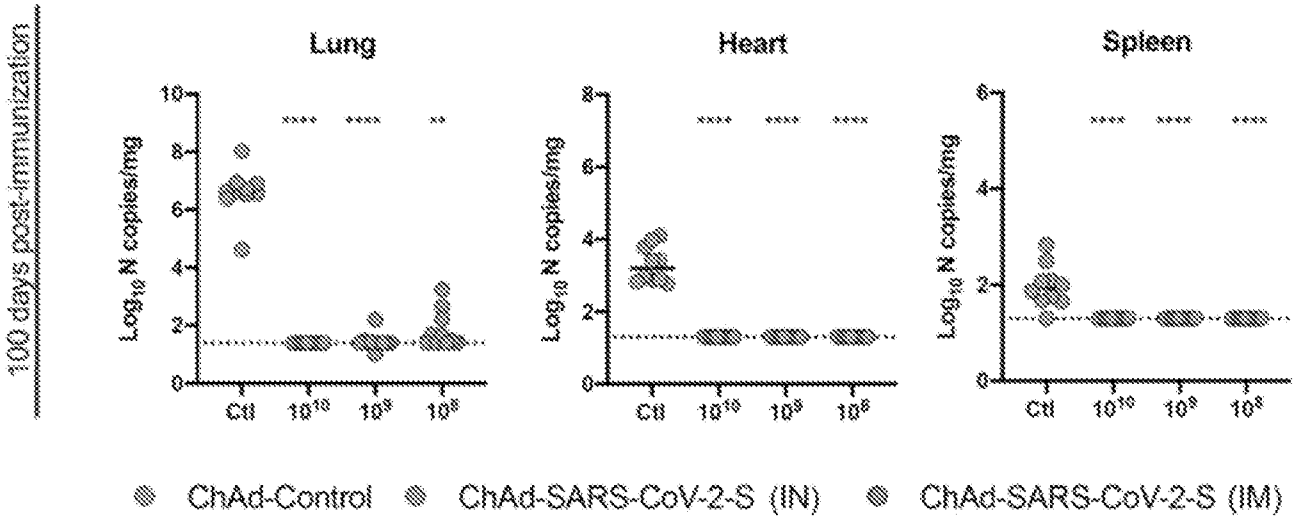


FIG. 13A FIG. 13B FIG. 13C

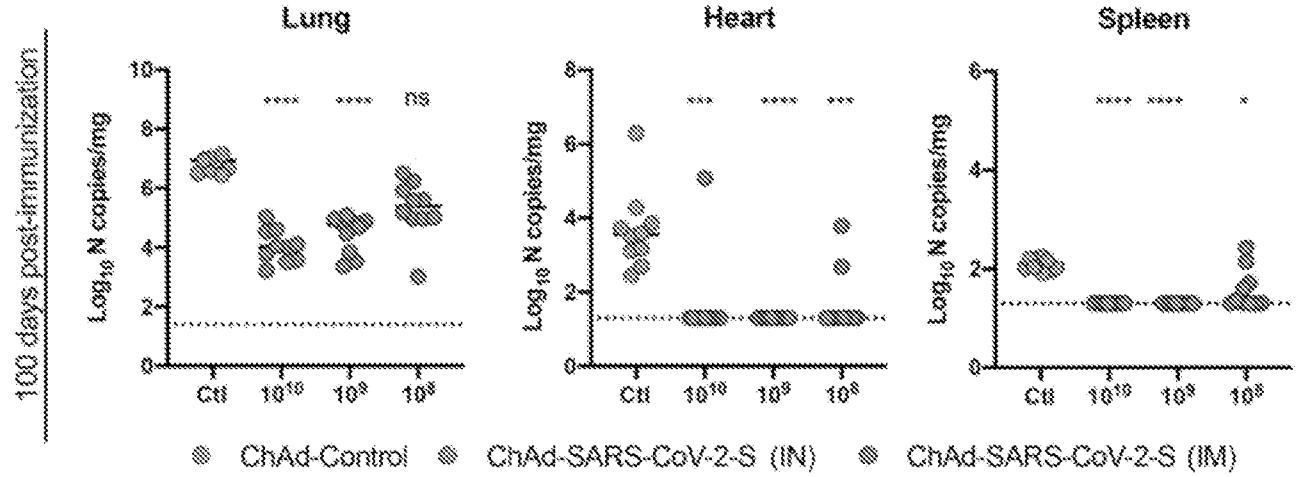


FIG. 13D FIG. 13E FIG. 13F

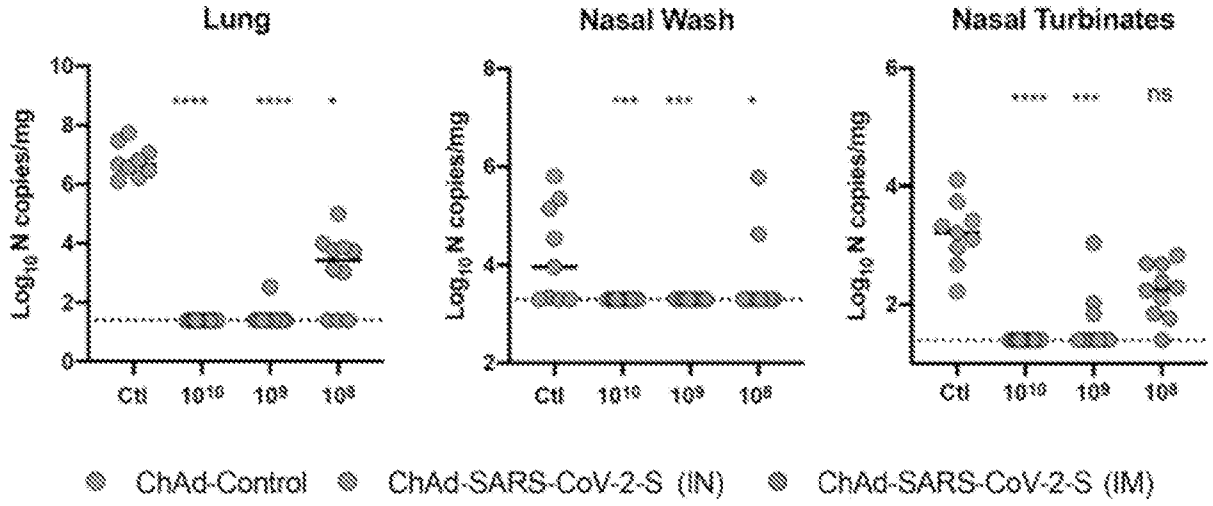


FIG. 13G FIG. 13H FIG. 13I

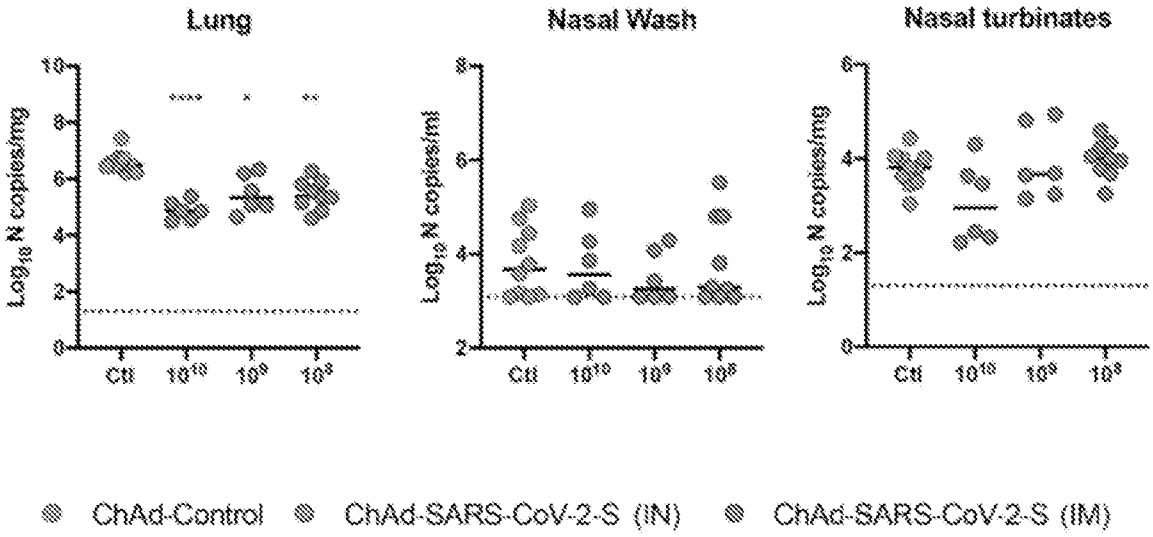


FIG. 13J FIG. 13K FIG. 13L

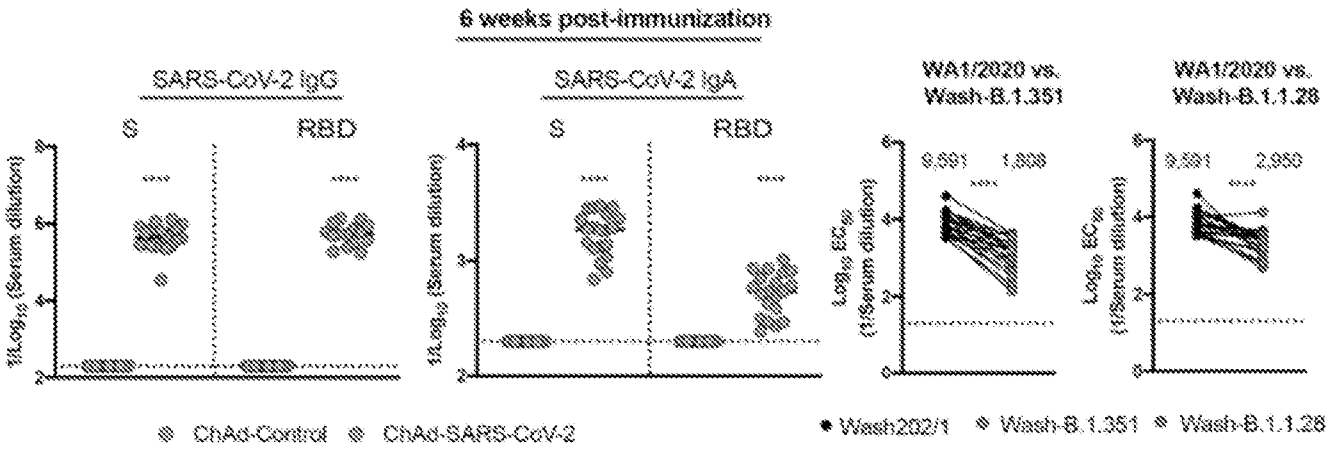


FIG. 14A FIG. 14B FIG. 14C FIG. 14D

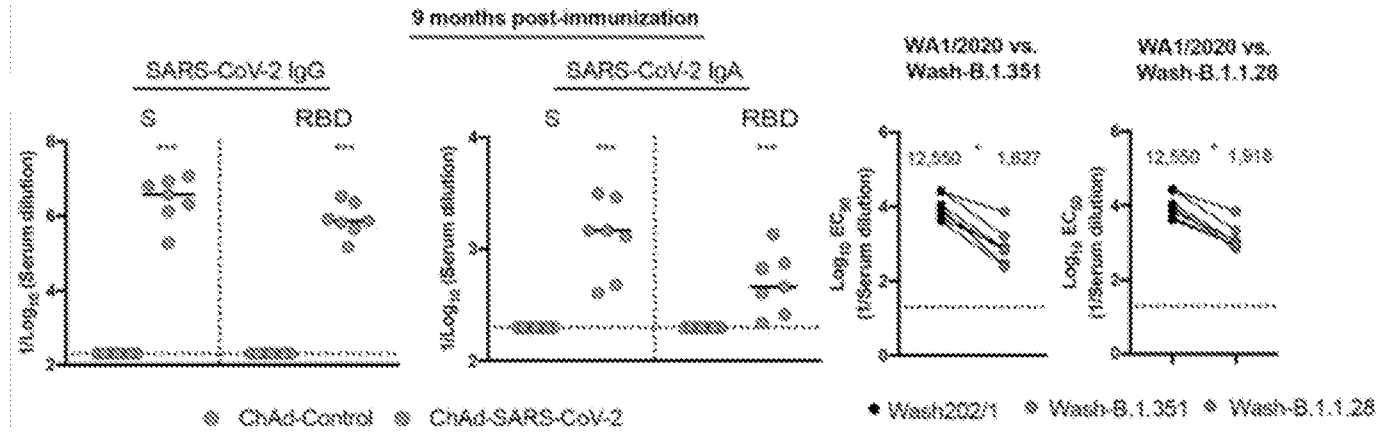


FIG. 14E FIG. 14F FIG. 14G FIG. 14H

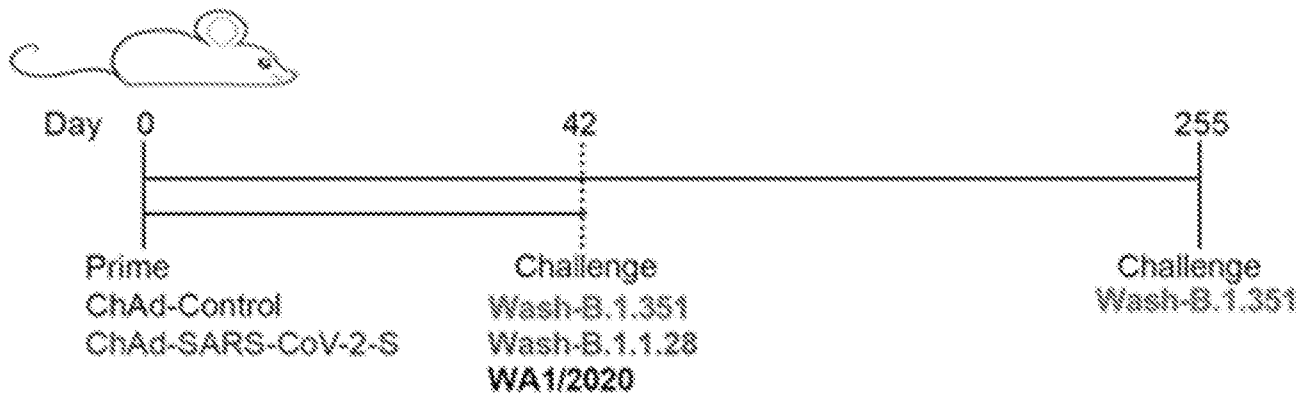


FIG. 15A

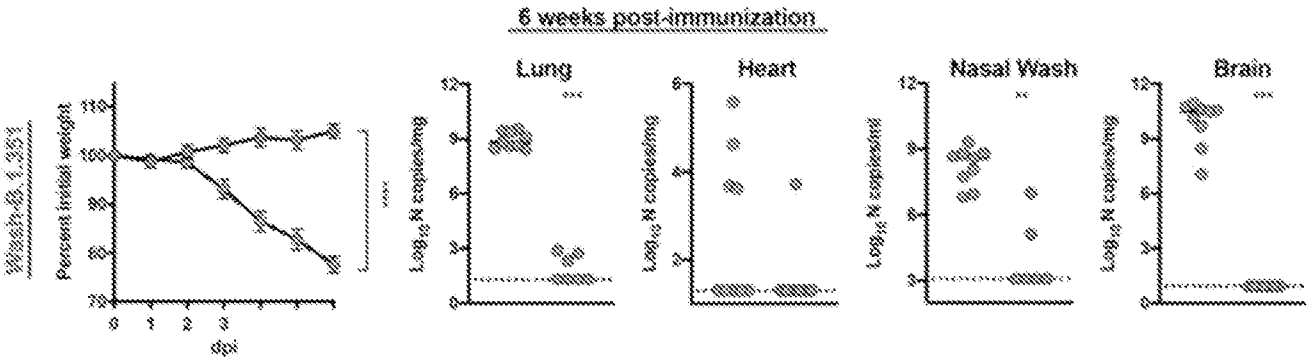


FIG. 15B FIG. 15C FIG. 15D FIG. 15E FIG. 15F

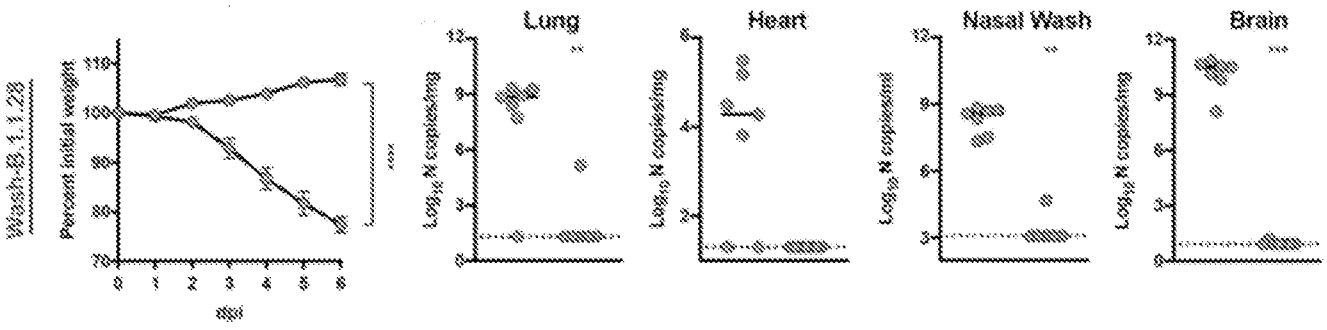


FIG. 15G FIG. 15H FIG. 15I FIG. 15J FIG. 15K

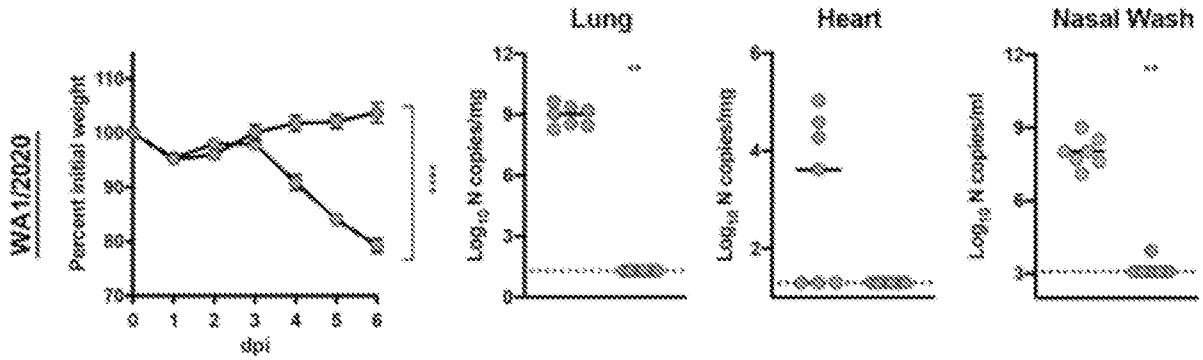


FIG. 15L FIG. 15M FIG. 15N FIG. 15O

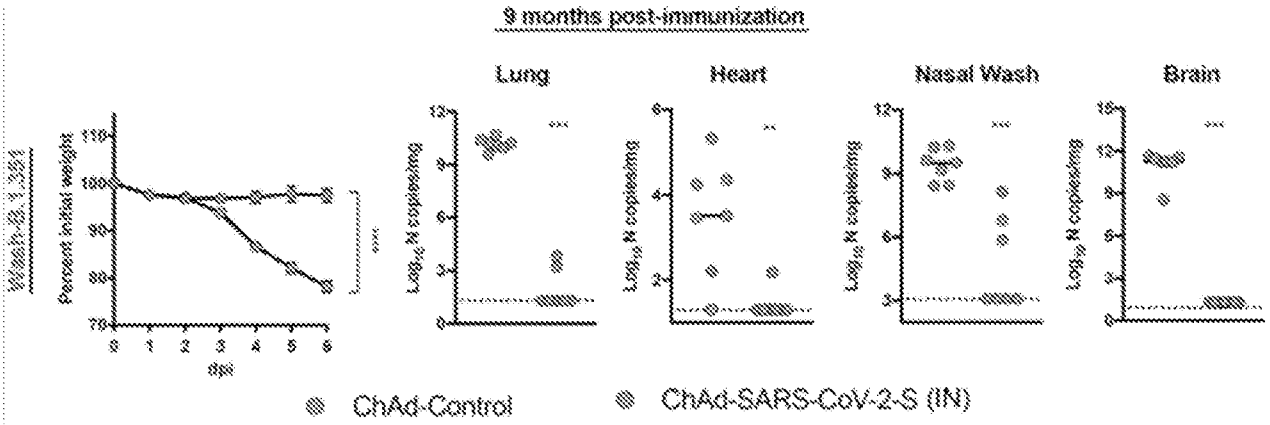


FIG. 15P FIG. 15Q FIG. 15R FIG. 15S FIG. 15T

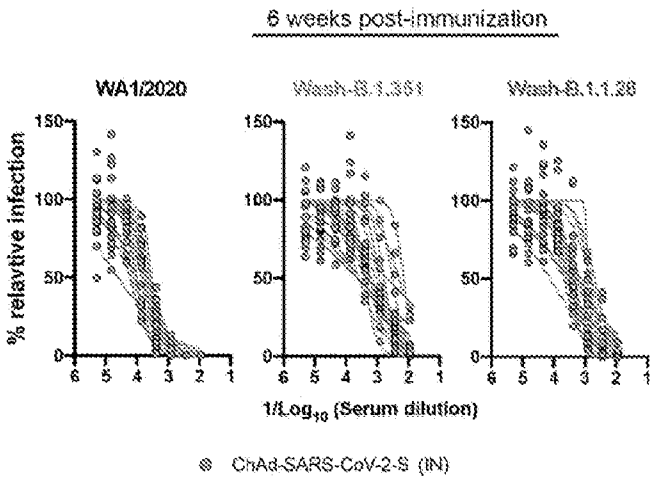


FIG. 16A

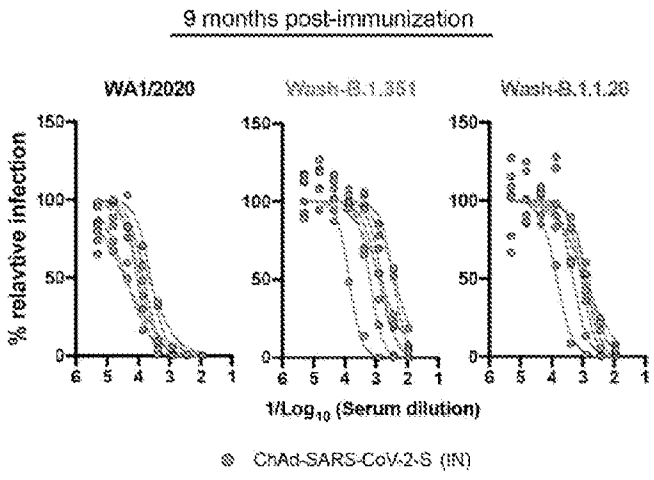


FIG. 16B

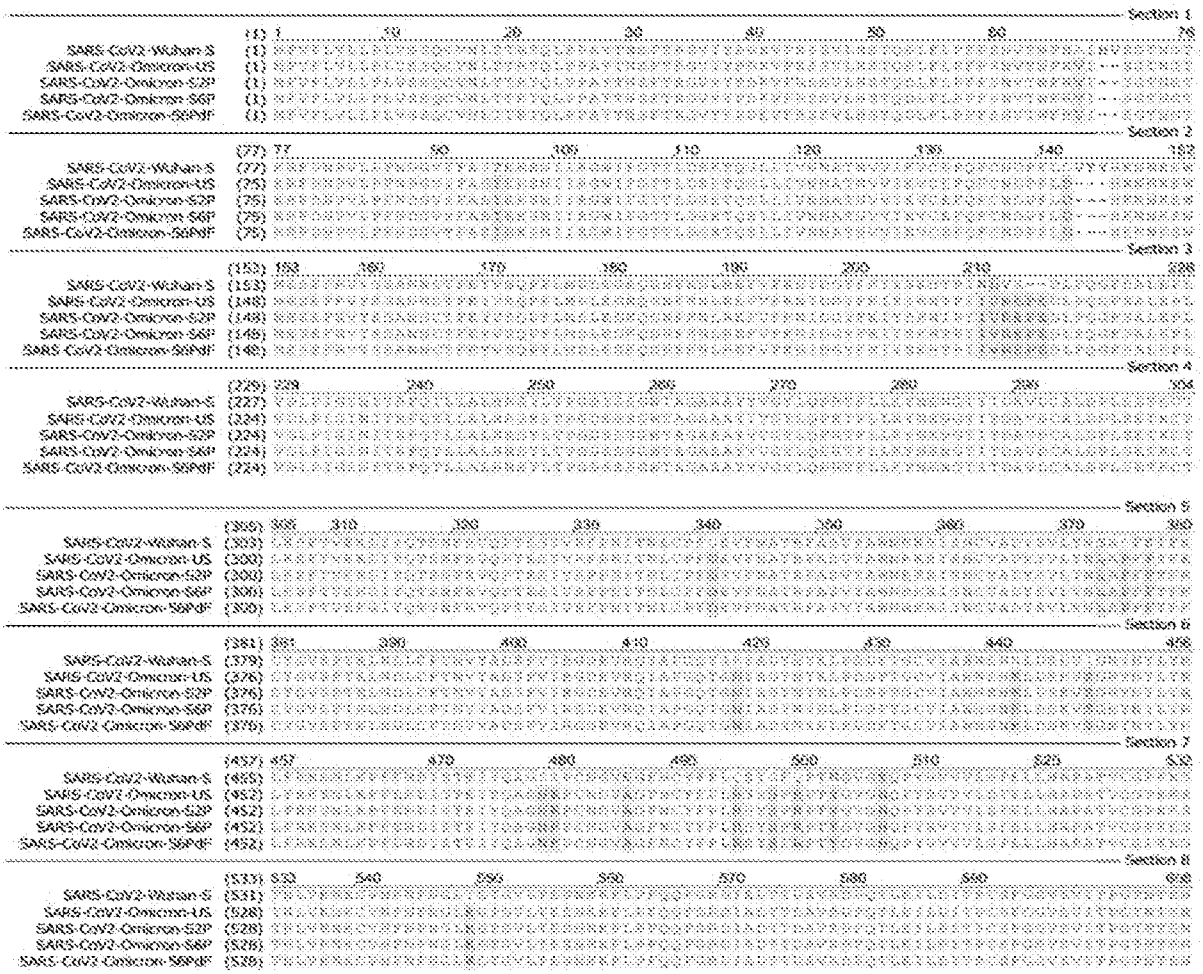

Section 1
SARS-CoV2-Wuhan-S
SARS-CoV2-Omicron-US
SARS-CoV2-Omicron-S2P
SARS-CoV2-Omicron-S6P
SARS-CoV2-Omicron-S6PdF
Section 2
Section 3
Section 4
Section 5
Section 6
Section 7
Section 8

FIG. 17

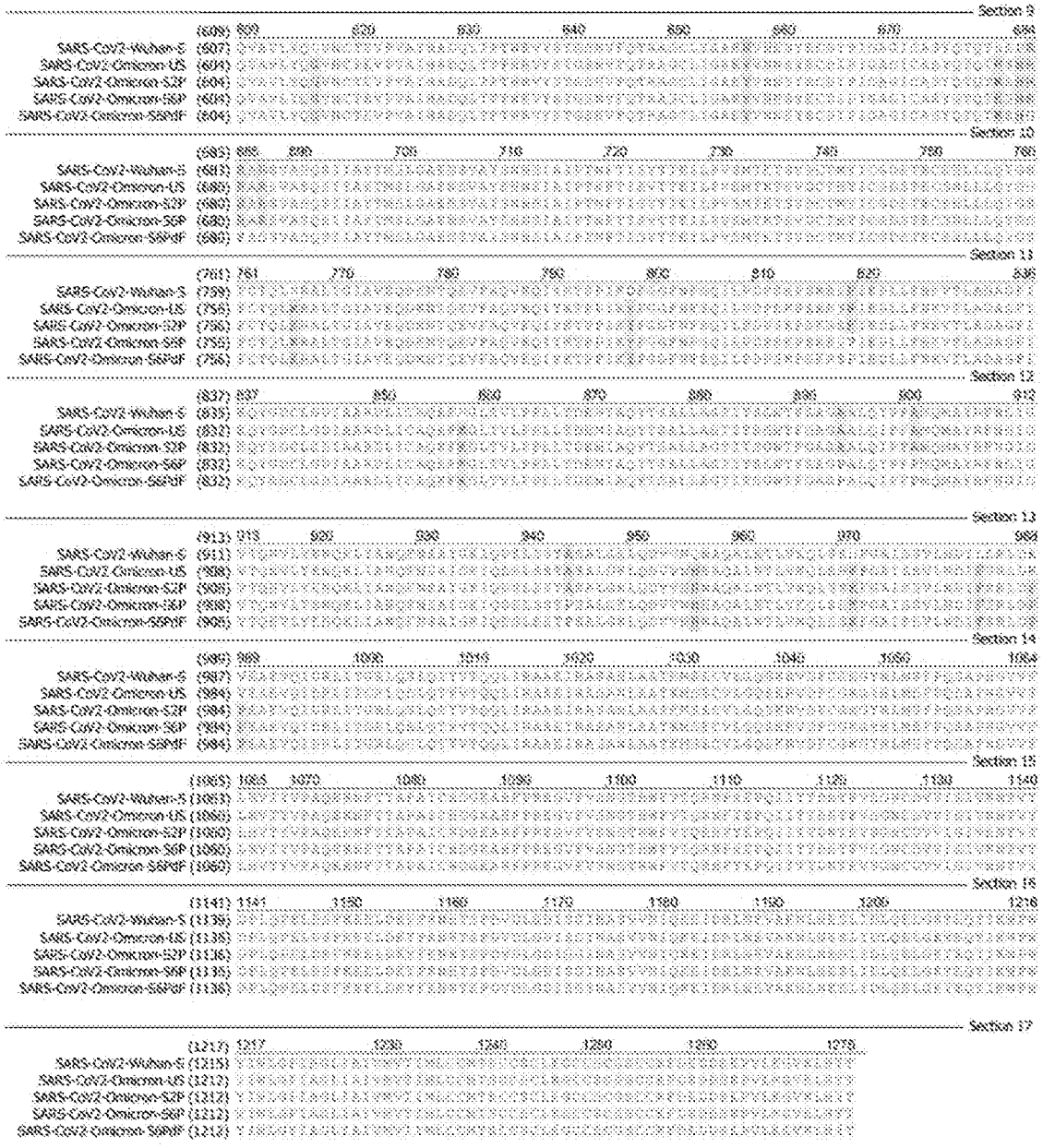

Section 9
Section 10
Section 11
Section 12
Section 13
Section 14
Section 15
Section 16
Section 17
SARS-CoV2-Wuhan-S
SARS-CoV2-Omicron-US
SARS-CoV2-Omicron-S2P
SARS-CoV2-Omicron-S6P
SARS-CoV2-Omicron-S6PdF

FIG. 17 cont.

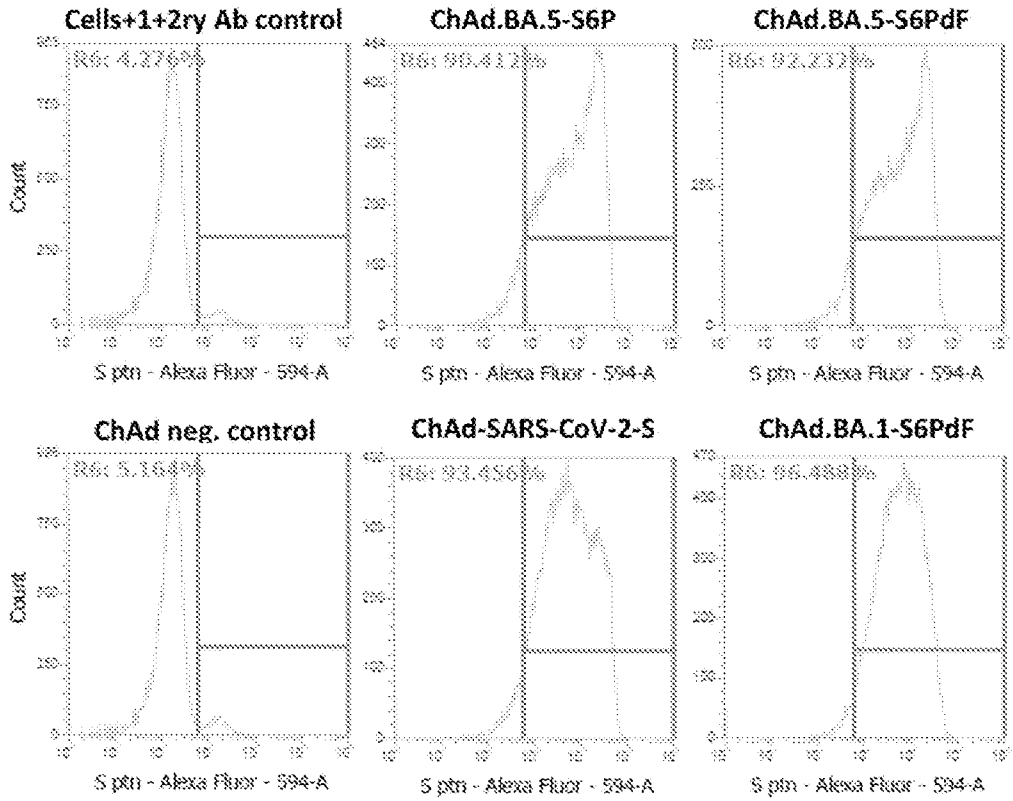

Cells+1+2ry Ab control
ChAd.BA.5-S6P
ChAd.BA.5-S6PdF
ChAd neg. control
ChAd-SARS-CoV-2-S
ChAd.BA.1-S6PdF
Count
S ptn - Alexa Fluor - 594-A

FIG. 18

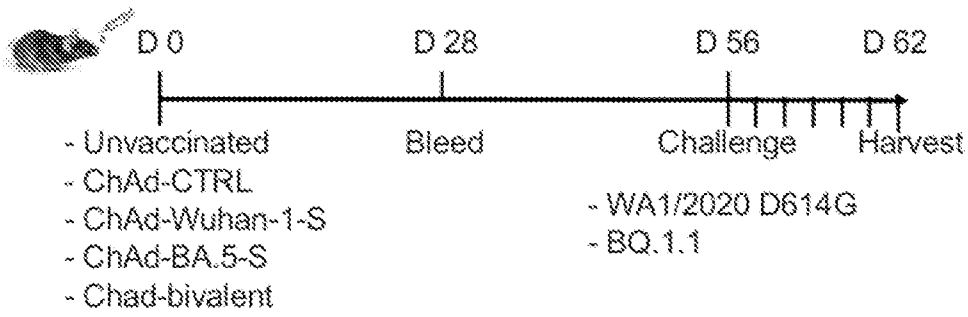


FIG. 19A

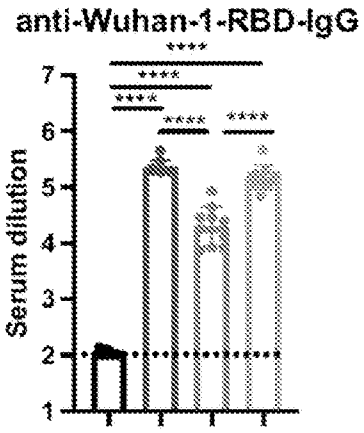


FIG. 19B

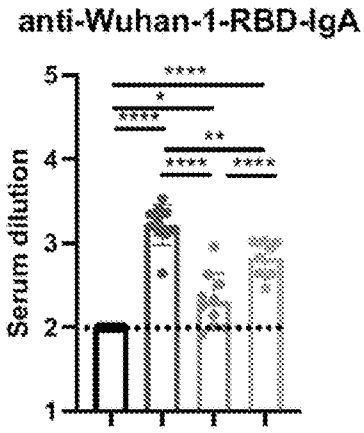


FIG. 19C

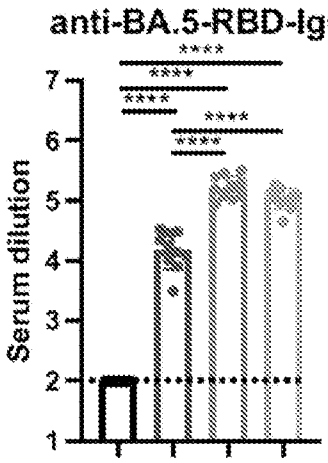


FIG. 19D

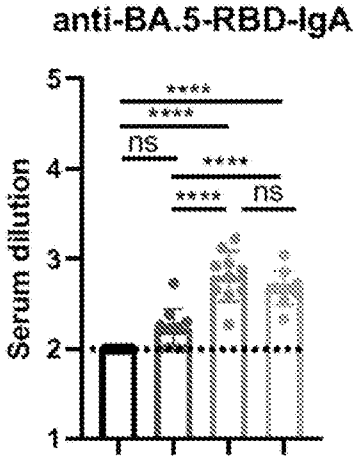


FIG. 19E

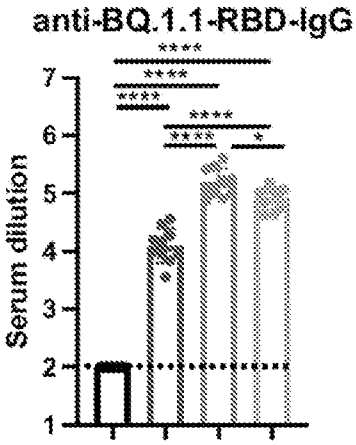


FIG. 19F

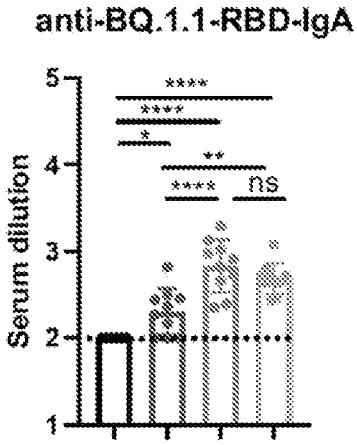


FIG. 19G

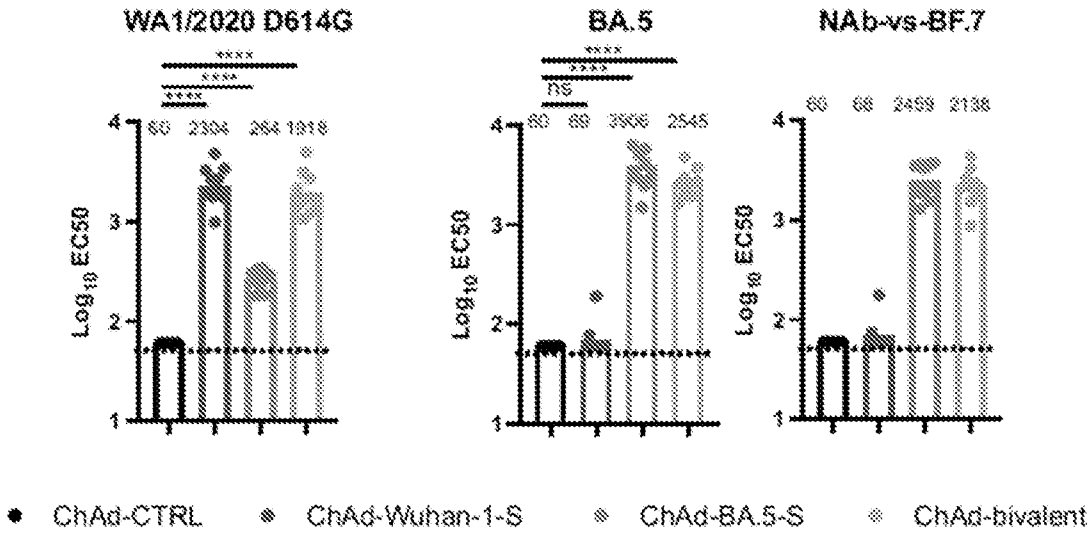


FIG. 20A FIG. 20B FIG. 20C

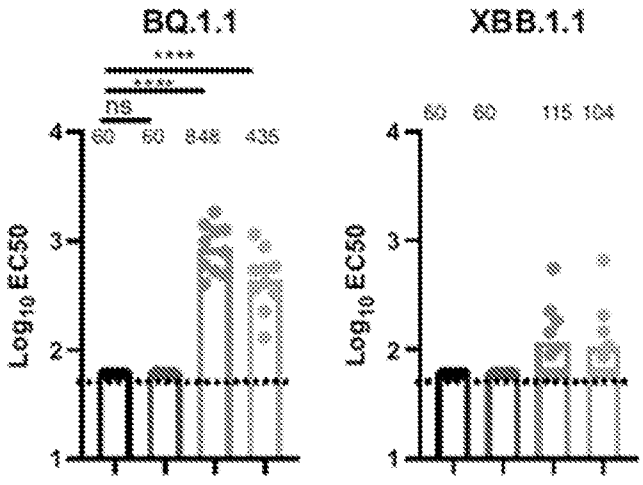


FIG. 20D FIG. 20E

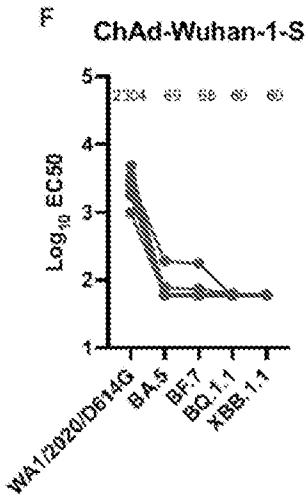


FIG. 20F

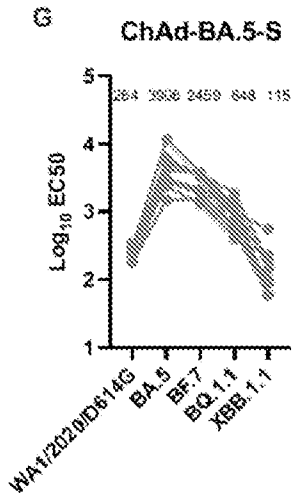


FIG. 20G

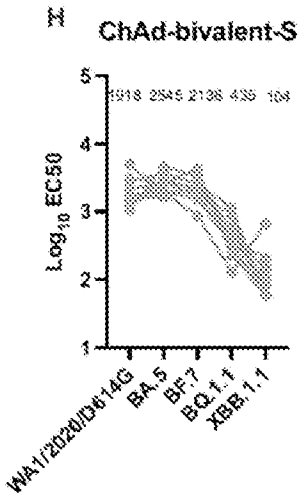


FIG. 20H

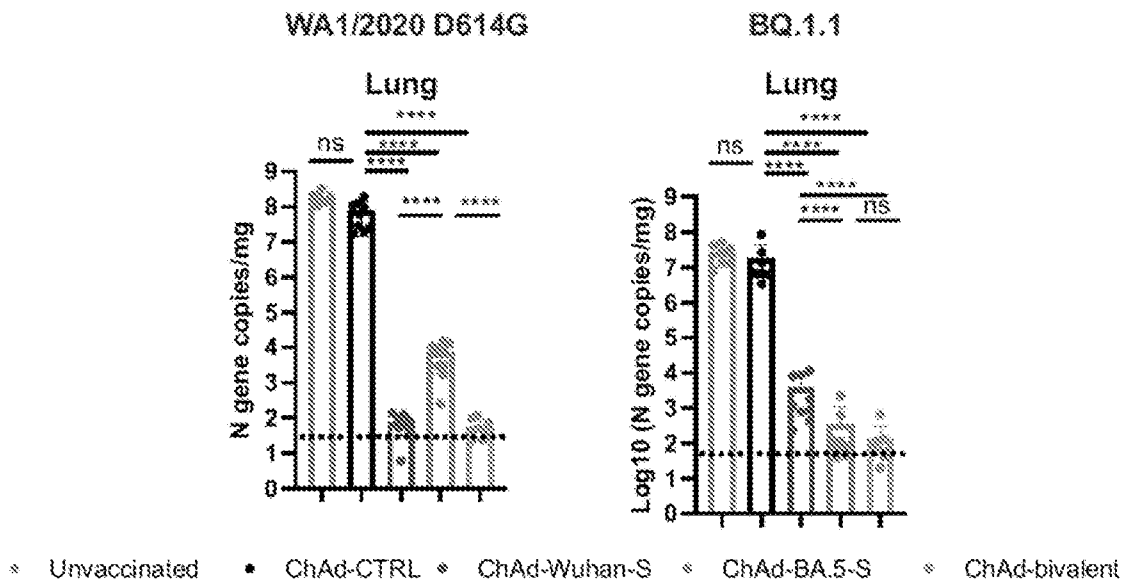


FIG. 21A

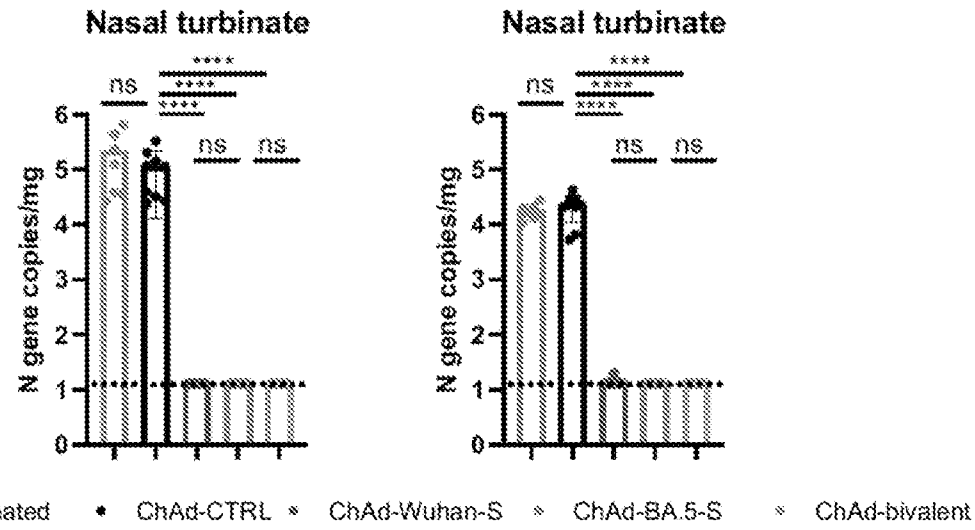


FIG. 21B

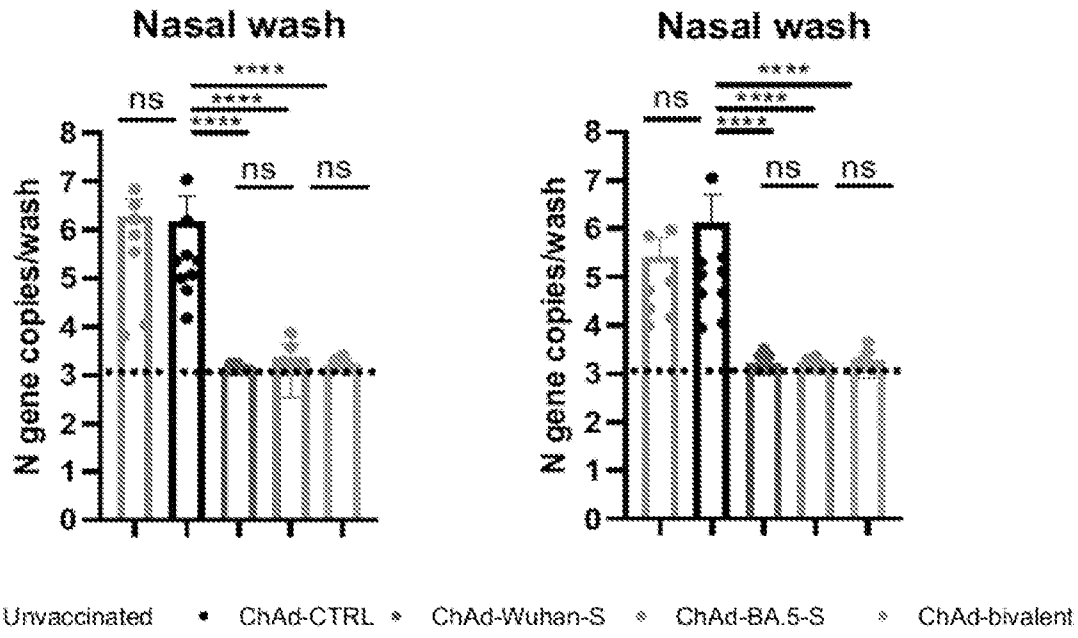

Nasal wash
Nasal wash
N gene copies/wash
ns
****
ns
ns
Unvaccinated
ChAd-CTRL
ChAd-Wuhan-S
ChAd-BA.5-S
ChAd-bivalent

FIG. 21C

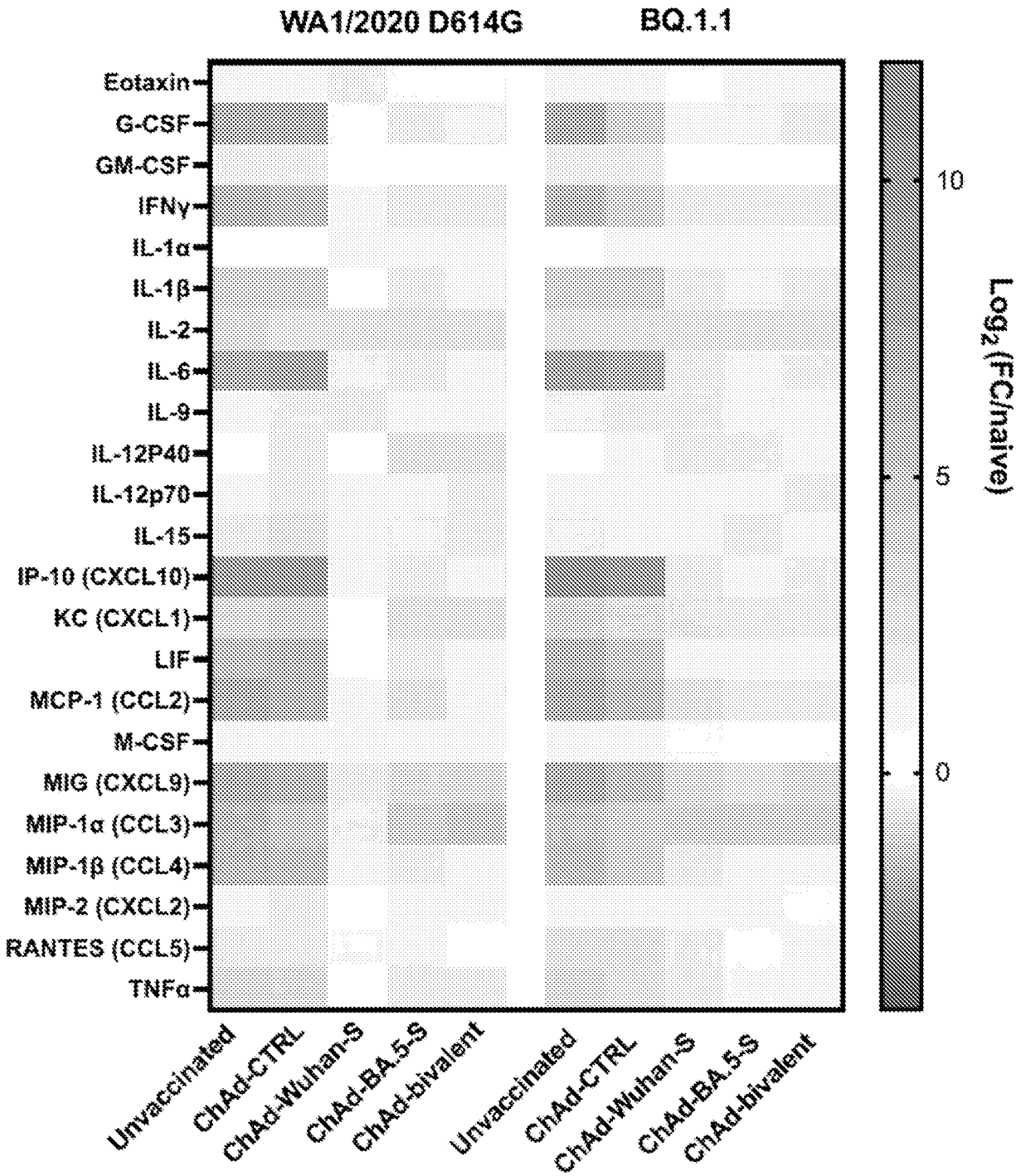

WA1/2020 D614G
BQ.1.1
Eotaxin
G-CSF
GM-CSF
IFNγ
IL-1α
IL-1β
IL-2
IL-6
IL-9
IL-12P40
IL-12p70
IL-15
IP-10 (CXCL10)
KC (CXCL1)
LIF
MCP-1 (CCL2)
M-CSF
MIG (CXCL9)
MIP-1α (CCL3)
MIP-1β (CCL4)
MIP-2 (CXCL2)
RANTES (CCL5)
TNFα
Unvaccinated
ChAd-CTRL
ChAd-Wuhan-S
ChAd-BA.5-S
ChAd-bivalent
Unvaccinated
ChAd-CTRL
ChAd-Wuhan-S
ChAd-BA.5-S
ChAd-bivalent
10
5
0
Log2 (FC/naive)

FIG. 22

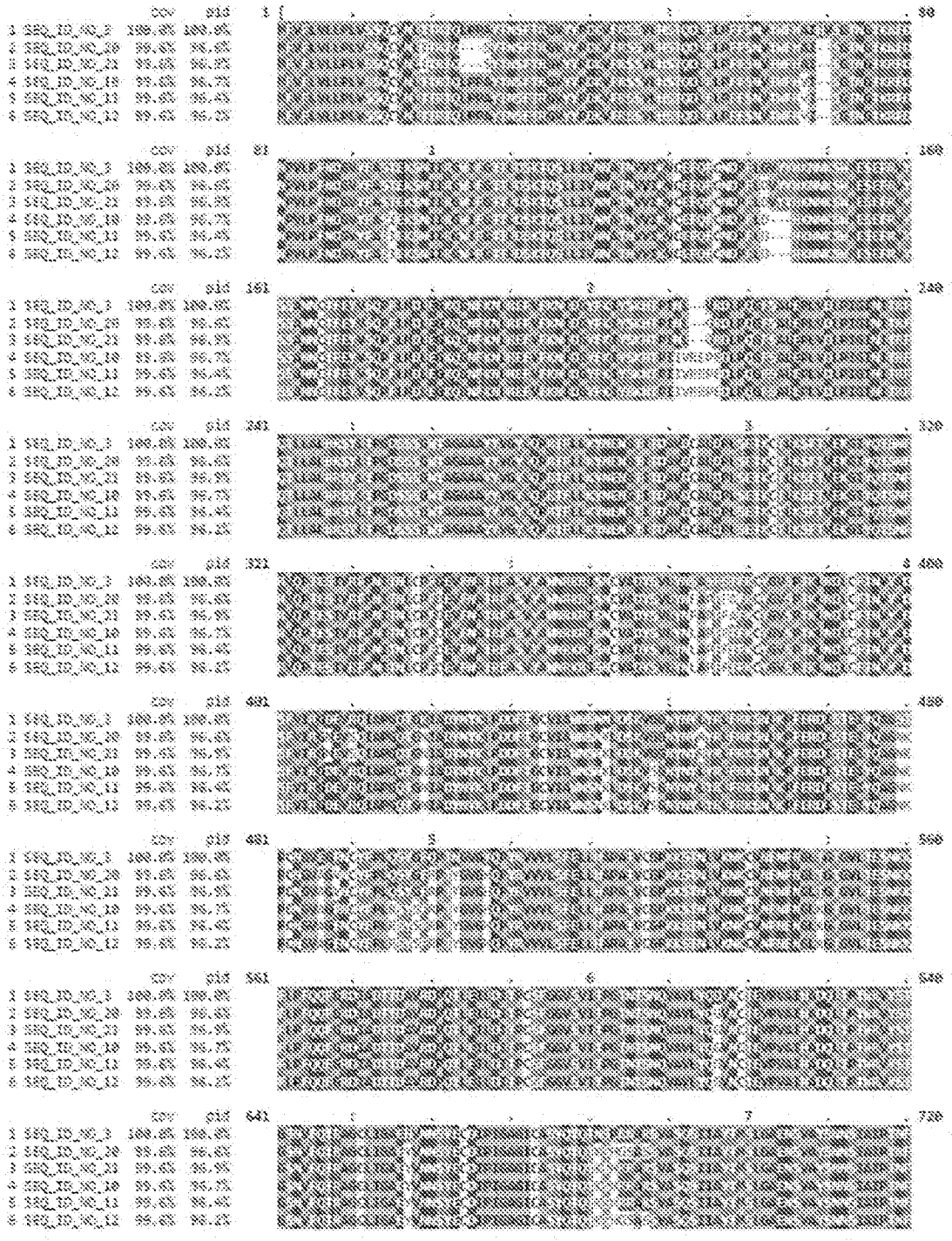


FIG. 23..

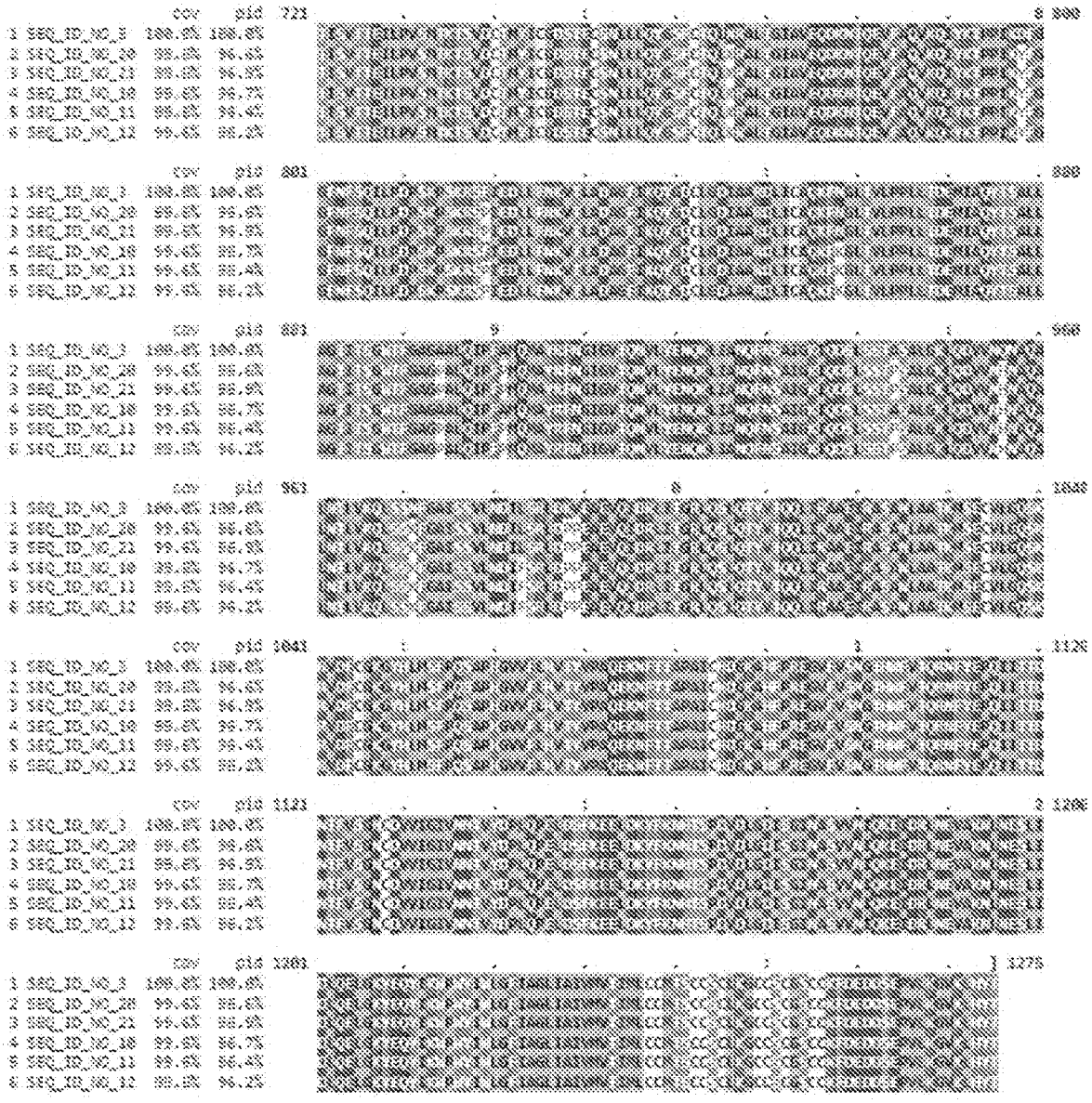


FIG. 23 cont.