

Fig. 13

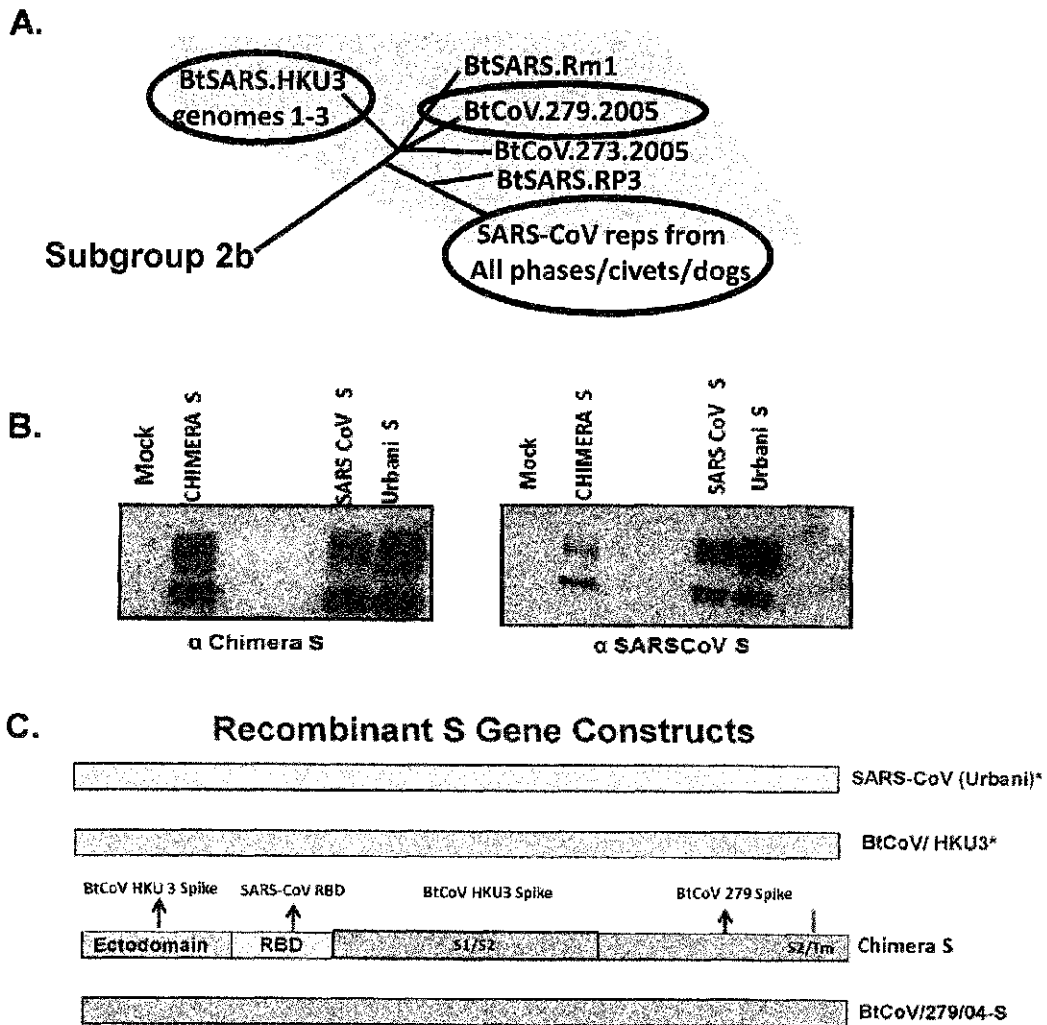


Fig. 14

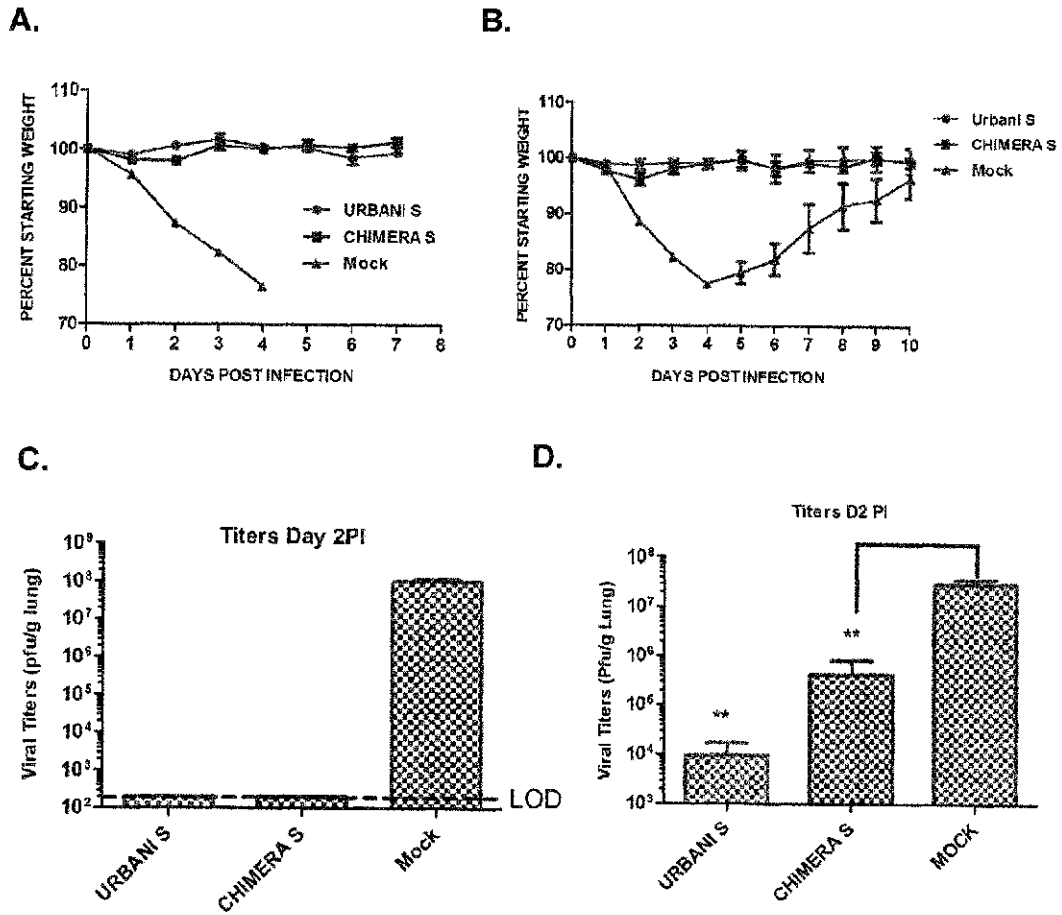


Fig. 15

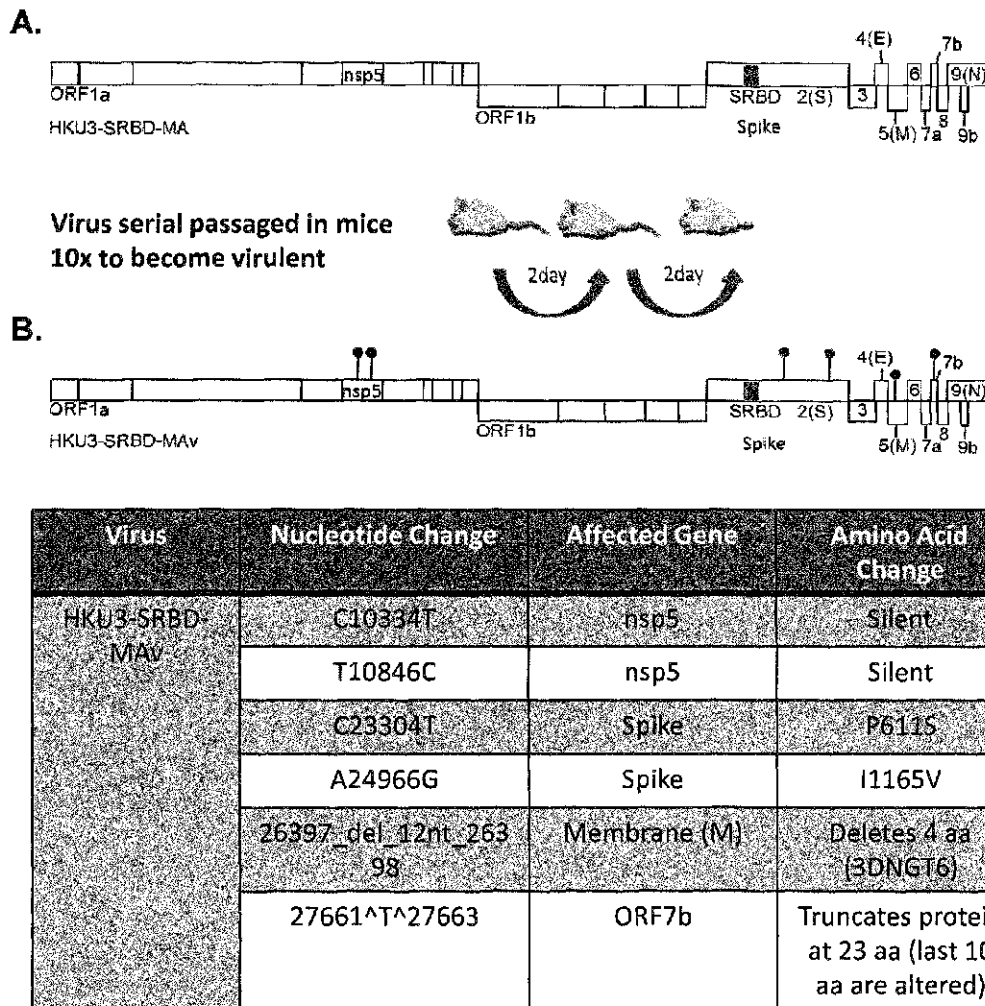


Fig. 16

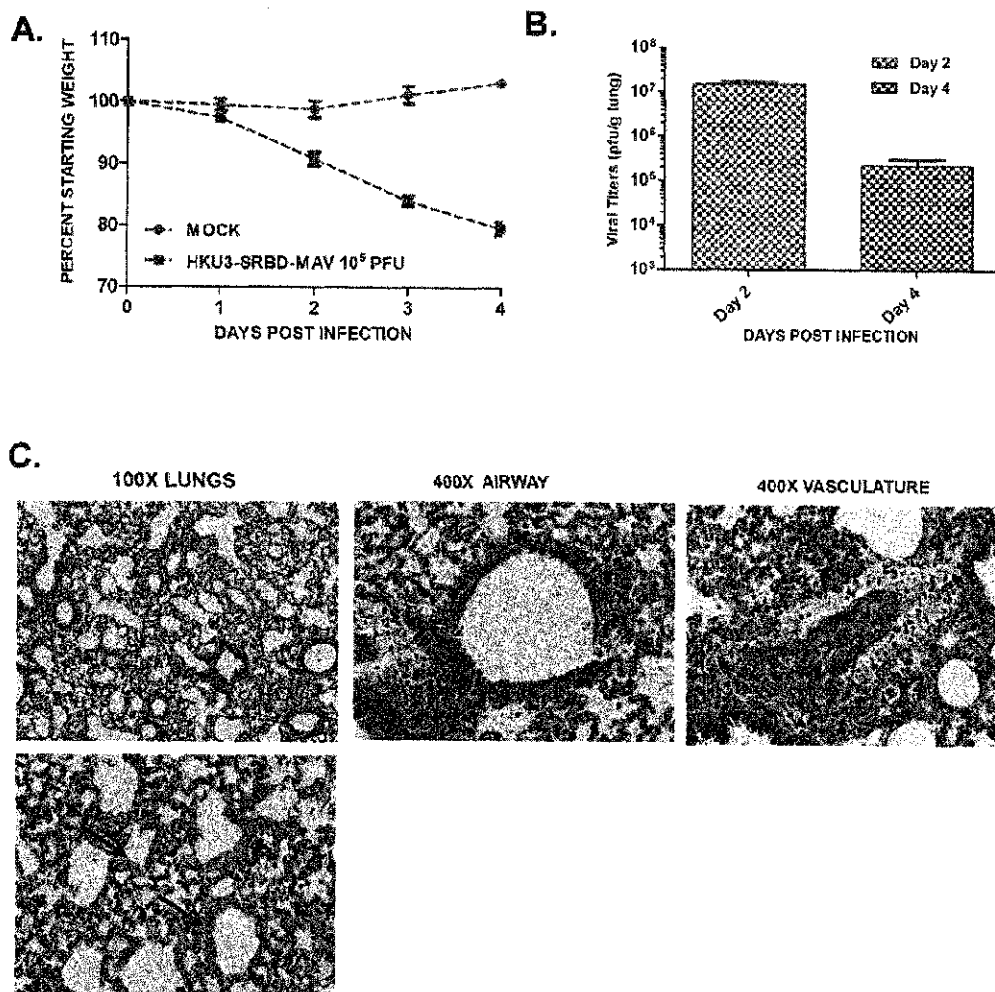


Fig. 17

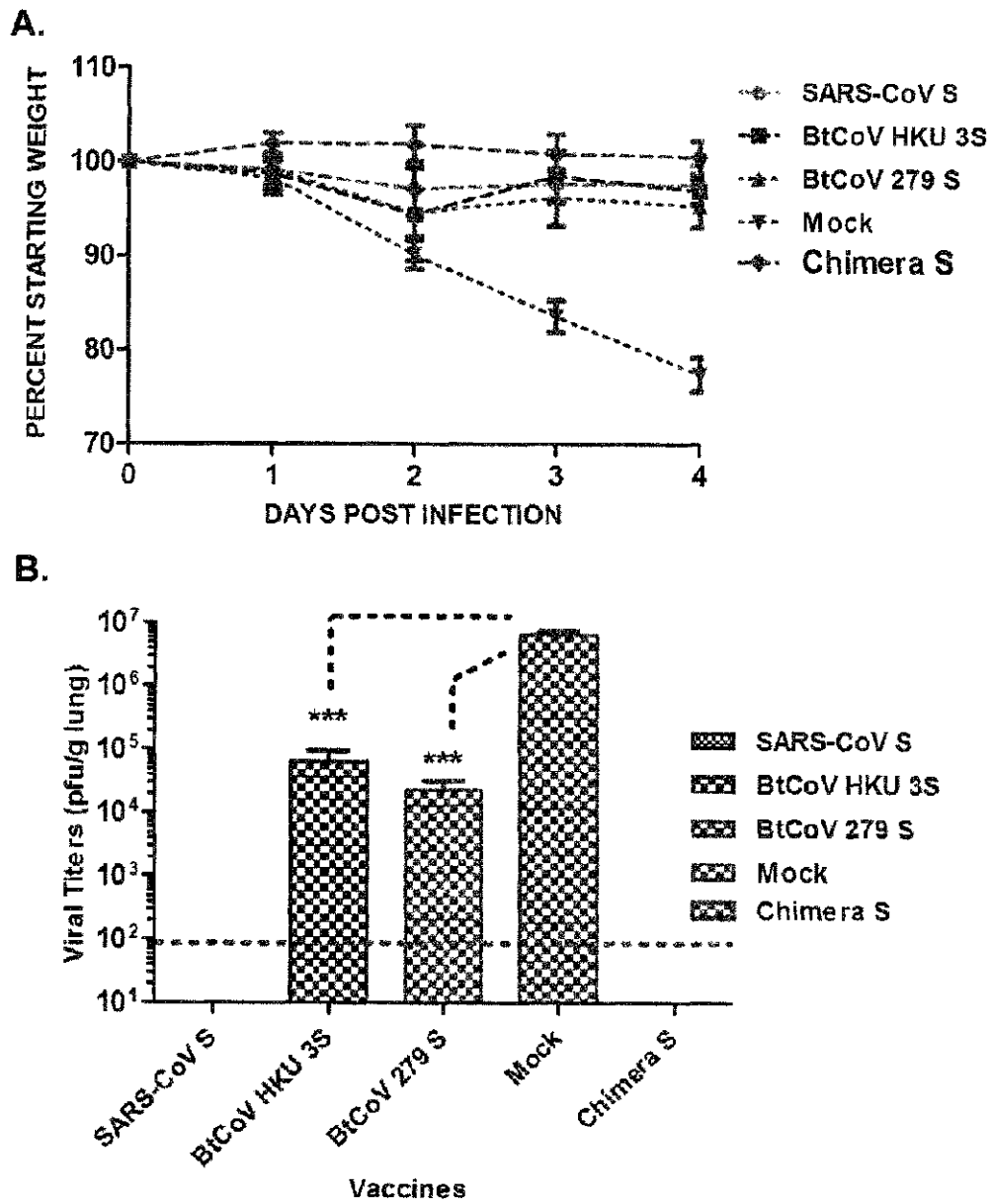


Fig. 18

1

METHODS AND COMPOSITIONS FOR CHIMERIC CORONAVIRUS SPIKE PROTEINS

STATEMENT OF PRIORITY

This application is a 35 U.S.C. §371 national phase application of International Application Serial No. PCT/US2015/021773, filed Mar. 20, 2015, which claims the benefit, under 35 U.S.C. § 119(e), of U.S. Provisional Application Ser. No. 61/968,279, filed Mar. 20, 2014, the entire content of each of which is incorporated by reference herein.

STATEMENT OF FEDERAL SUPPORT

This invention was made with government support under Grant No. U54AI057157 awarded by the National Institutes of Health. The government has certain rights in the invention.

STATEMENT REGARDING ELECTRONIC FILING OF A SEQUENCE LISTING

A Sequence Listing in ASCII text format, submitted under 37 C.F.R. § 1.821, entitled 5470-672_ST25.txt, 90,897 bytes in size, generated on Dec. 9, 2016 and filed via EFS-Web, is provided in lieu of a paper copy. This Sequence Listing is hereby incorporated by reference into the specification for its disclosures.

FIELD OF THE INVENTION

The present invention relates to methods and compositions comprising a chimeric coronavirus spike protein for treating and/or preventing a disease or disorder caused by a coronavirus infection.

BACKGROUND OF THE INVENTION

Updated approaches are needed to rapidly respond to new emerging diseases, especially early in the epidemic when prompt public health intervention strategies can limit mortality and epidemic spread. In particular, emerging respiratory coronaviruses offer a considerable threat to the health of global populations and the economy. Coronaviruses (CoVs) constitute a group of phylogenetically diverse enveloped viruses that encode the largest plus strand RNA genomes and replicate efficiently in most mammals. Human CoV (HCoV-229E, OC43, NL63, and HKU1) infections typically result in mild to severe upper and lower respiratory tract disease. Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) emerged in 2002-2003 causing acute respiratory distress syndrome (ARDS) with 10% mortality overall and up to 50% mortality in aged individuals. Middle Eastern Respiratory Syndrome Coronavirus (MERS-CoV) emerged in the Middle East in April of 2012, manifesting as severe pneumonia, acute respiratory distress syndrome (ARDS) and acute renal failure. The virus is still circulating and has been shown to have a mortality rate of ~49%. Platforms for generating reagents and therapeutics are needed to detect and control the emergence of new strains, especially early in an outbreak prior to the development of type specific serologic reagents and therapeutics.

The present invention overcomes previous shortcomings in the art by providing methods and compositions compris-

2

ing a chimeric coronavirus spike protein for treating/and or preventing diseases and disorders caused by infection by a coronavirus.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides a chimeric coronavirus spike protein comprising, in orientation from amino to carboxy terminus: a) a first region comprising a portion of a coronavirus spike protein ectodomain that precedes the receptor binding domain (RBD) as located in a nonchimeric coronavirus spike protein, of a first coronavirus; b) a second region comprising a coronavirus spike protein receptor binding domain (RBD) of a second coronavirus that is different from said first coronavirus; c) a third region comprising a portion of a coronavirus spike protein S1 domain as located in a nonchimeric coronavirus spike protein immediately downstream of the RBD, contiguous with a portion comprising a coronavirus spike protein S2 domain as located immediately upstream of a fusion protein domain in a nonchimeric coronavirus spike protein, wherein said third region is of said first coronavirus; and d) a fourth region comprising a portion of a coronavirus spike protein from the start of the fusion protein domain through the carboxy terminal end as located in a nonchimeric coronavirus spike protein of a third coronavirus that is different from said first coronavirus and said second coronavirus.

In further aspects, the present invention further provides an isolated nucleic acid molecule encoding the chimeric coronavirus spike protein of this invention, as well as a vector comprising the isolated nucleic acid molecule. Also provided are compositions comprising the chimeric coronavirus spike proteins, isolated nucleic acid molecules and/or vectors of this invention in a pharmaceutically acceptable carrier.

In further aspects, the present invention provides a method of producing an immune response to a coronavirus in a subject, treating a coronavirus infection in a subject, preventing a disease or disorder caused by coronavirus infection in a subject and/or protecting a subject from the effects of coronavirus infection, comprising administering to the subject an effective amount of the chimeric coronavirus spike protein, the isolated nucleic acid molecule the vector and/or the composition of this invention, or any combination thereof, thereby producing an immune response to a coronavirus in the subject, treating a coronavirus infection in the subject, preventing a disease or disorder caused by coronavirus infection in the subject and/or protecting the subject from the effects of coronavirus infection.

In further aspects, the present invention provides a method of identifying a coronavirus spike protein for administration to elicit an immune response to coronavirus in a subject infected by a coronavirus and/or a subject at risk of coronavirus infection and/or to a subject for whom eliciting an immune response to a coronavirus is needed or desired, comprising: a) contacting a sample obtained from a subject infected with a coronavirus with a panel of proteins comprising: 1) one or more chimeric coronavirus spike proteins from a subgroup 2c coronavirus, 2) one or more chimeric coronavirus spike proteins from a subgroup 2b coronavirus, 3) one or more spike proteins from a subgroup 2a coronavirus, 4) one or more chimeric coronavirus spike proteins from a subgroup 2d coronavirus, 5) one or more chimeric coronavirus spike proteins from a subgroup 1a coronavirus, 6) one or more chimeric coronavirus spike proteins from a subgroup 1b coronavirus, and 7) any combination of (1) through (6) above, under conditions whereby an antigen/

3

antibody complex can form; and b) detecting formation of an antigen/antibody complex, whereby detection of formation of the antigen/antibody complex comprising the chimeric coronavirus spike protein(s) of any of (1)-(6) identifies the presence of antibodies to a spike protein of the coronavirus that is infecting the subject of (a), thereby identifying a coronavirus spike protein for administration to the subject of (a) and/or to a subject infected with a coronavirus and/or to a subject at risk of coronavirus infection and/or to a subject for whom eliciting an immune response to a coronavirus is needed or desired.

Also provided herein is a method of identifying an antibody that neutralizes a coronavirus infecting a subject, comprising: a) isolating a coronavirus from a sample of a subject infected with a coronavirus and/or suspected of being infected with a coronavirus; b) contacting the coronavirus of (a) with a panel of antibodies comprising: 1) an antibody reactive with a chimeric coronavirus spike protein from a subgroup 2c coronavirus, 2) an antibody reactive with a chimeric coronavirus spike protein from a subgroup 2b coronavirus, 3) an antibody reactive with a chimeric coronavirus spike protein from a subgroup 2a coronavirus, 4) an antibody reactive with a chimeric coronavirus spike protein from a subgroup 2d coronavirus, 5) an antibody reactive with a chimeric coronavirus spike protein from a subgroup 1a coronavirus, 6) an antibody reactive with a chimeric coronavirus spike protein from a subgroup 1b coronavirus, and 7) any combination of (1) through (6) above, to form respective coronavirus/antibody compositions, each comprising a respective antibody of the panel; c) contacting each of the respective coronavirus/antibody compositions of (b) with cells susceptible to coronavirus infection under conditions whereby coronavirus infection can occur; and d) detecting the presence or absence of infection of the cells, whereby absence of detection of infection of the cells contacted with any of the coronavirus/antibody compositions of (b) identifies the antibody of that coronavirus/antibody composition as an antibody that neutralizes the coronavirus infecting the subject.

The foregoing and other objects and aspects of the present invention are explained in detail in the specification set forth below.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. Phylogenetic tree of the coronaviruses. The chimeric spike antigen HKU3-Smix belongs to subgroup 2b, and the antigenic components of the chimeric antigen are derived from BtCoV HKU 3S, SARS CoV S, and BtCoV 279 S, all of which are circled. The other S antigens representing other subgroups of CoVs are indicated in dashed circles as controls.

FIG. 2. Design of the chimeric spike antigen. The chimeric spike antigen HKU3-Smix has components from HKU3 S, SARS CoV S RBD, and BtCoV 279S. The specific amino acid residues adopted from each of the spike proteins are indicated in the figure. The S1/S2 boundary is indicated (761aa). S2/Tm domain is indicated (1194aa). The top panel represents the spike protein organization in the SARS-CoV Spike, showing the spread of the neutralizing epitope across various domains of the SARS-CoV spike protein.

FIG. 3. Cross reactivity of antisera to chimeric spike antigen, with spike proteins from different CoVs. Mouse antisera to chimeric spike antigen (HKU 3 S_{MIX}), SARS S, BAT 1A S, HKU2.298 S, HKU 4.2 S, and HKU9.4S were analyzed for their cross reactivity with these antigens. Antisera to chimeric spike antigen recognizes SARS S (Panel B)

4

and vice versa (Panel A). Note that there is no cross reactivity between S proteins of other subgroups.

FIG. 4. Chimeric antigen HKU 3 S_{MIX} protects against lethal SARS CoV challenge. Panel A. Percent weight loss of young Balb/C mice immunized with chimeric Antigen HKU 3 S_{MIX}, SARS S and HKU9.4S (negative control) and challenged with lethal dose of mouse adapted SARS CoV (MA 15 virus). Mice immunized with chimeric antigen, SARS S show no weight loss. Panel B. Lung titers on Day 2 post infection of the same groups of mice shown above. Note that there is no virus detected in groups of mice vaccinated with HKU 3 S_{MIX} and SARS S.

FIG. 5. Chimeric antigen HKU 3 S_{MIX} protects against lethal SARS CoV heterologous challenge. Panel A. Percent weight loss of young Balb/C mice immunized with chimeric spike antigen HKU 3 S_{MIX}, SARS S and HKU9.4S (negative control) and challenged with lethal dose of heterologous mouse adapted SARS CoV (GD03 MA virus). Mice immunized with chimeric spike antigen, SARS S show no weight loss. Panel B. Lung titers on Day 2 post infection of the same groups of mice shown above. Viral replication is reduced on D2 and no virus is detected in groups of mice vaccinated with HKU 3 S_{MIX} and SARS S.

FIG. 6. Schematic of the HKU2 virus with the chimeric antigen HKU 3 S_{MIX}. Panel A. The HKU3 virus which has the chimeric antigen HKU 3 S_{MIX} is shown. The open reading frames are indicated. Panel B. Growth curve of HKU 3 virus with the chimeric spike antigen HKU 3 S_{MIX}. The HKU3 virus which has the chimeric spike antigen HKU 3 S_{MIX} grows similar to SARS CoV in Vero cells.

FIG. 7. Schematic of the BAT-SRBDMav. This virus has the HKU3 backbone, with the spike protein containing a chimera of HKU3 spike and receptor binding domain from SARS-CoV spike 210aa. This virus was created by serial passage of the parent virus in 20 week old Balb/C mice, resulting in virulent phenotype. The amino acid mutations essential for mouse adaptation are indicated and for comparison, the mouse adapted SARS-CoV is shown with the mouse adapted mutations.

FIG. 8. Chimeric spike antigen HKU 3 S ma protects against lethal challenge with BAT-SRBD-Mav when compared. Panels A and B. Percent weight loss of young Balb/C mice immunized with chimeric antigen HKU 3 S ma, SARS S, BtCoV 279 S, and BtCoV HKU S and challenged with lethal dose of heterologous mouse adapted BAT-SRBD-Mav. Mice immunized with chimeric antigen, SARS S show no weight loss, whereas there is about 3-5% weight loss with HKU3 S and BtCoV 279 S. Panel C. Lung titers on Day 2 post infection of the same groups of mice shown above. Viral replication is reduced on D2 in BtCoV 279 S and HKU3 S group, but no virus is detected in groups of mice vaccinated with HKU 3 S_{MIX} and SARS S.

FIG. 9. Design of the chimeric spike antigen for subgroup 2c. The chimeric spike antigen 2c has components from HKU4.2 S, MERS-CoV S RBD, and BtCoV 5.5S. The specific amino acid residues adopted from each of the spike proteins are indicated. S1/S2 boundary is indicated (730aa). S2/Tm domain is indicated (~1190aa).

FIG. 10. Characterization of VRP 3526 Platform. Panel A. VEE 3526 replicon CoV S protein expression construct. The capsid and E glycoprotein genes from Venezuelan equine encephalitis virus are replaced with the Coronavirus Spike Protein gene S. The VEE capsid and E glycoproteins are supplied in separate constructs. When cells are transfected with all three constructs, VEE replicons encoding CoV S are formed. Panel B. Titers of S protein vaccines from all three different coats determined on BHK cells by an IFA assay.

5

Panel C. Western blot from independent experiments showing expression of SARS-CoV S protein from VRP 3526 S and VRP 3000 S vaccines in Vero cells. Lower panel indicates actin.

FIG. 11. Young adult mice are protected from homologous (MA15) and heterologous (MA 15 GD03S) SARS-CoV challenge by VRP 3526 S vaccine. Panels A & B. Percent weight loss of young adult mice immunized with indicated vaccines, and challenged with 10^5 pfu of rMA15 (homologous) and rMA15-GD03S (heterologous) respectively. Panels C&D. Lung titers on 2 DPI infection determined by plaque assay Vero cells from experiments in Panel A and B respectively. Error bars indicate SEM. * indicates ($p < 0.05$ in Mann-Whitney Test).

FIG. 12. Aged mice are protected from homologous (MA15) SARS-CoV challenge by VRP 3526 S vaccine. Panel A. Percent weight loss of one year old mice immunized with S protein based vaccines from three different coats, and challenged with 10^5 pfu of rMA15. Panel B and C. Lung titers on 2 DPI (Panel B), and 4 DPI (Panel C) determined by plaque assay Vero cells. Error bars indicate SEM. Significance as determined by Mann-Whitney test ($p < 0.05$, indicated by asterisk).

FIG. 13. VRP 3526 elicits high Antibody response in young and aged animals. Panels A and B. ELISA results showing IgG titers to S protein, elicited in young mice (Panel A) and aged mice (Panel B) by indicated vaccine groups. Panels C and D. Neutralization potential (to SARS-CoV) of antibodies elicited by indicated vaccine groups in young mice (Panel C), and aged mice (Panel D), as measured by PRNT₅₀ assay. Error bars indicate SD.

FIG. 14. Design of a Chimeric Spike based CoV Vaccine. Panel A. Phylogenetic tree showing Coronaviruses in subgroup 2b. The circles represent three viruses from which specific regions of S proteins are combined to form the chimeric spike. Panel B. Western blots showing that serum raised to the Chimera S or SARS-CoV Urbani S recognize the Chimeric Spike due to overlapping epitopes. Panel C. Design of the Chimeric Spike antigen utilizing portions of SARS-COV, BtCoV HKU3 and BtCoV 279 Spike. The Chimera S contains the following epitopes from N terminus: a portion of ectodomain from BtCoV HKU3; a portion of Receptor Binding Domain (RBD) from SARS-CoV; a region from S1/S2 from BtCoV HKU3; followed by a region containing S2/Tm from the BtCoV 279 Spike.

FIG. 15. Chimera S Vaccine Protects from Homologous and Heterologous SARS-CoV Challenge. Panels A & B. Percent weight loss of young adult mice immunized with S protein based or Chimera S vaccine and challenged with 10^5 pfu of rMA15 (homologous) and rMA15-GD03S (heterologous) respectively. Panels C & D. Lung titers on 2 DPI infection determined by plaque assay Vero cells from experiments in Panel A and B respectively. Error bars indicate SEM. * indicates ($p < 0.05$ in Mann-Whitney Test).

FIG. 16. Generation and Mouse Adaptation of a lethal Zoonotic Challenge Virus (BtCoV HKU3) from subgroup 2b. Panel A. Schematic of chimeric HKU3 virus (HKU3-SRBD-MA) containing the Receptor binding domain (green color) from SARS-CoV S protein. The Open Reading Frames are Indicated. The asterisk indicates Y436H mutation which enhances replication in mice. HKU3-SRBD-MA was serially passaged in 20 week old BALB/c mice (schematic below) at 2 day intervals to create a lethal challenge virus. Panel B. Mouse adaptation leads to mutations in nsp5, Spike, Membrane and ORF 7b. The mutations are indicated by lollipops, and the table shows the exact nucleotide and amino acid mutations are indicated in the table.

6

FIG. 17. HKU3-SRBD-MAv causes severe respiratory disease in 20 wk old Balb/c mice culminating in lethality. Panel A. Percent weight loss of 20 wk old Balb/c mice infected with 10^5 pfu of HKU3-SRBD-MA_v, through 4 days post infection. Note that the infected mice lose 20% of their body weight 4 days post infection. Panel B. Viral titers in lungs of mice at Day 2 and 4 post infection. Panel C. Histopathology of H&E stained lung sections at day 4 P.I. showing denuded airways, perivascular cuffing and formation of hyaline membranes (black arrow), which are markers of severe lung disease.

FIG. 18. Chimera S vaccine protects mice from HKU3-SRBD-MAv Challenge. Panel A. Percent weight loss of 20 wk old Balb/C mice immunized with SARS CoV S, BtCoV HKU3S, BtCoV 279S, Chimera S or mock vaccinated and challenged with 10^5 pfu of HKU3-SRBD-MAv. Panel B. Lung titers on 2 DPI infection determined by plaque assay Vero cells Error bars indicate SEM. * indicates ($p < 0.05$ in Mann-Whitney Test).

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on the production and development of a chimeric coronavirus spike protein which induces a neutralizing immune response to coronavirus, for use for example, in the treatment and/or prevention of a disease or disorder caused by infection by a variety of different coronavirus strains.

Thus, in one aspect, the present invention provides a chimeric coronavirus spike protein comprising, in orientation from amino to carboxy terminus: a) a first region comprising a portion of a coronavirus spike protein ectodomain that precedes the receptor binding domain (RBD) as located in a nonchimeric coronavirus spike protein, of a first coronavirus; b) a second region comprising a coronavirus spike protein receptor binding domain (RBD) of a second coronavirus that is different from said first coronavirus; c) a third region comprising a portion of a coronavirus spike protein S1 domain as located in a nonchimeric coronavirus spike protein immediately downstream of the RBD, contiguous with a portion comprising a coronavirus spike protein S2 domain as located immediately upstream of a fusion protein domain in a nonchimeric coronavirus spike protein, wherein said third region is of said first coronavirus; and d) a fourth region comprising a portion of a coronavirus spike protein from the start of the fusion protein domain through the carboxy terminal end as located in a nonchimeric coronavirus spike protein of a third coronavirus that is different from said first coronavirus and said second coronavirus.

By "orientation from amino to carboxy terminus" it is meant that the regions of the chimeric coronavirus spike protein are present from left to right in the same orientation as the amino terminus and carboxy terminus of a protein. This term is intended to describe orientation only and does not mean that the first region as described in the chimeric coronavirus structural protein is present at the exact amino terminus in all embodiments although that could be the case in some embodiments. Similarly this term does not mean that the fourth region as described in the chimeric coronavirus structural protein is present at the exact carboxy terminus in all embodiments although that could be the case in some embodiments.

Representative nonlimiting examples of a chimeric coronavirus spike protein of this invention are shown in FIGS. 2 and 9, each of which show a schematic of a subgroup b

7

coronavirus spike protein and a subgroup c coronavirus spike protein, respectively with the regions described above shown in their locations in a nonchimeric (e.g., wild type) coronavirus spike protein.

The chimeric coronavirus spike protein of this invention can be produced by combining domains or portions of coronavirus spike proteins as described above from subgroup 1a coronaviruses, subgroup 1b coronaviruses, subgroup 2a coronaviruses, subgroup 2b coronaviruses, subgroup 2c coronaviruses, or subgroup 2d coronaviruses. As one nonlimiting example, the present invention provides a chimeric subgroup 2b coronavirus spike protein comprising, in orientation from amino to carboxy terminus: a) a first region comprising amino acids 1-325 of a spike protein of a

8

first subgroup 2b coronavirus; b) a second region comprising amino acids 322-500 of a spike protein of a second subgroup 2b coronavirus; c) a third region comprising amino acids 488-842 of a spike protein of said first subgroup 2b coronavirus; and) a fourth region comprising amino acids 842-1241 of a spike protein of a third subgroup 2b coronavirus. The amino acid sequence of the chimeric coronavirus spike protein of this example is shown below, with these four regions identified (first and third regions from said first subgroup 2b coronavirus shown in bold; second region from said second subgroup 2b coronavirus shown with underline; and fourth region from said third subgroup 2b coronavirus shown in italics).

(SEQ ID NO: 1)

1 MKILIFAFLA NLAKAQEGCG IISRKQPKM AQVSSRRGV YYNDIFRSD VLHLTQDYFL
 61 PFDSNLTQYF SLNVDSDRYT YFDNPILDFG DGVYFAATEK SNVIRGWIFG SSFDNTTQSA
 121 VIVNNSTHII IRVCNPNLCK EPMYTVSRGT QQANAVYQSA FNCTYDRVEK SFQLDTPPKT
 181 GNFKDLREYV FKNRDGFLSV YQTYTAVNLP RGLPTGFSVL KPILKLPFGI NITSYRVVMA
 241 MFSQTTSNFL PESAAYYVGN LKYSTFMLRF NENGTITDAV DCSQNPLAEL KCTIKNFNVD
 301 KGIYQTSNFR VSPTQEVIRF PNITNLCPPG EVFNATKFPF VYAWERKKIS NCVADYSVLY
 361 NSTPFSTPKC YGVSATKLNLD LCFSNVYADS FVVKGDDVRQ IAPGQTGVIA DYNKYKLPDDE
 421 MGCVLAWNTR NIDATSTGNY NYKYRYLRHG KLRPPERDIS NVPFSPDGKP CTPPALNCYW
 481 PLNDYGFYTT TGIGYQPYRV VVLSFELLNA PATVCGPKLS TDLVKNQCVN FNFNGLKGTG
 541 VITSSSKRFQ SFQQFGRDTS DFTDSVRDPQ TLEILDISPC SFGGVSVITP GTNASSEVAV
 601 LYQDVNCTDV PTAIRADQLT PANRVYSTGV NVFQTQAGCL IGAEHVNASY ECDIPIGAGI
 661 CASYHTASVL RSTGQKSIVA YTMSLGAENS IAYANNSIAI PTNFSISVTT EVMFVSMARK
 721 AVDCTMYICG DSLECSNLLL QYGSFCTQLN RALTGIAIEQ DKNTQEVFAQ VKQMYKTPAI
 781 KDFGGFNFSQ ILPDPKPTK RSFIEDLLFN KVTLADAGFM KQYGDCLGDV SARDLICAQR
 841 FNGLTVLPLP LTDEMVAAYT AALVSGTATA *GNTFGAGSAL QIPFAMQ MAY RFNGIGVTQN*
 901 VLYENQKQIA NQFNKAISQI *QESLTTTSTA LGKLQDVVND NAQALNTLVK QLSSNFGAIS*
 961 SVLNDILSRL DKVEAEVQID RLITGRLQSL QTYVTQQLIR AAEIRASANL AATKMSECVL
 1021 GQSKRVDFCG KGYHLMSPFQ AAPHGVVFLH VTYVPSQERN FTTAPAICHE GKAYFPREGV
 1081 FVSNGTSWFI TQRNFYSPQI ITTDTNFVAG NCDVVIGIIN NTVYDPLQPE LDSFKKELDK
 1141 YFKNHTSPDV DLGDISGINA SVVNIQKEID RLNEVAKNLN ESLIDLQBLG KYEQYIKWPW
 1201 YVWLGFIAGL IAIVMVTILL CCMTSCCCL KGACSCGSCC KFEDEDSFV LKGVKLHYT
 50

The exemplary chimeric coronavirus spike protein shown above was produced from the following three subgroup 2b coronaviruses:

Bat SARS CoV-HKU3 spike protein (GenBank® Accession No. ACJ60694.1) (first coronavirus) (SEQ ID NO:2)

1 MKILIFAFLA NLAKAQEGCG IISRKQPKM AQVSSRRGV YYNDIFRSD VLHLTQDYFL
 61 PFDSNLTQYF SLNVDSDRYT YFDNPILDFG DGVYFAATEK SNVIRGWIFG SSFDNTTQSA
 121 VIVNNSTHII IRVCNPNLCK EPMYTVSRGT QQANAVYQSA FNCTYDRVEK SFQLDTPPKT
 181 GNFKDLREYV FKNRDGFLSV YQTYTAVNLP RGLPTGFSVL KPILKLPFGI NITSYRVVMA
 241 MFSQTTSNFL PESAAYYVGN LKYSTFMLRF NENGTITDAV DCSQNPLAEL KCTIKNFNVD
 301 KGIYQTSNFR VSPTQEVIRF PNITNRCPPD KVFNATRFPM VYAWERTKIS DCVADYTVLY

-continued

361 NSTSPSTFKC YGVSPSKLID LCFTSVYADT FLIRSSSEVRQ VAPGETGVIA DYNKLPDDF
 421 TGCVIAMNTA KHDGTNYYYR SHRKTCLKPF ERDLSSDDGN GVYTLSTYDF NPNVPVAYQA
 481 TRVVVLSFEL LNAPATVCGP KLSTELVKNQ CVNENFNGLK GTGVLTSSEK RFQSFQQFGR
 541 DTSDFTDSVR DPQLEILDI SPCSFGGVSV ITPGTNASSE VAVLYQDVNC TDVPTAIRAD
 601 QLTPAMRVYS TGVNVFQTQA GCLIGAEHVN ASYECDIPIG AGICASYHTA SVLRSTGQKS
 661 IVAYTMSLGA ENSIAYANNS IAIPTNFSIS VTTEVMPVSM AKTAVDCTMY ICGDSLECSN
 721 LLLQYGSFCT QLNRLTQIA IEQDKNTQEV FAQVKQMYKT PAIKDFGGFN FSQILPDPSE
 781 PTKRSEFIEDL LFNKVTLADA GFMKQYGDCL GDVSARDLIC AQKFNGLTVL PPLLTDEMTA
 841 AYTAALVSGT ATAGWTFGAG AALQIPFAMQ MAYRFNGIGV TQNVLYENQK LIANQFNSAI
 901 GKIQESLSST ASALGKQLQDV VNQNAQALNT LVKQLSSENFQ AISSVLNDIL SRLDKVEAEV
 961 QIDRLITGRL QSLQTYVTQQ LIRAAEIRAS ANLAATKMSE CVLGQSKRVD FCGKGYHLMS
 1021 FPQSAPHGVV FLHVTVVPSQ EKNFTTAPAI CHEGKAYFPR EGVFVSNQTS WFITQRNFYS
 1081 PQLITTDNTF VSGNCDVVIG IINNTVYDPL QPELDSFKEE LDKYFKNHTS PDVDLGDISG
 1141 INASVVNIQK EIDRLNEVAK NLNESLIDLQ ELGKYEQYIK WPWYVWLQFI AGLIAIVMVT
 1201 ILLCCMTSCC SCLKGACSCG SCCKFDEDDS EPVLKGVKLVH YT

SARS CoV Urbani spike protein (Accession No. AAP13441.1) (second coronavirus) (SEQ ID NO:3)

1 MFIFLLFLTL TSGSDLDRCT TFDDVQAPNY TQHTSSMRGV YYPDEIFRSD TLYLTQDLFL
 61 PFYSNVTGPH TINHTFGNPV IPPKDGIFYA ATEKSNVVRG WVFGSTMNKK SQSVIIINNS
 121 TNVVIRACNF ELCNPPFAV SKPMGTQHT MIFDNFNCT FEYISDAFSL DVSEKSGNFK
 181 HLRPFVFNK DGFLVYVYGY QPIDVVRDLP SGENTLKPIF KLPLGINITN FRAILTAFSP
 241 AQDIWGTSA AAYFVGYLKPT TFMLKYDENG TITDAVDCSQ NPLAELKCSV KSFEDKGIY
 301 QTSNFRVVPV GDVVRFPNIT NLCFFGEVFN ATKFPSVYAW ERKKISNCVA DYSVLYNSTF
 361 FSTFKCYGVS ATKLNLCFS NVYADSFVVE GDDVRQIAPG QTGVIADYNY KLPDDFMGCY
 421 LAWNTRNIDA TSTGNYNYKY RYLRHGKLRP FERDISNVPE SPDGKCTPP ALNCYWPLND
 481 YGFYTTTGIG YQPYRVVLS FELLNAPATV CGPKLSTDLI KNQCVNFNFN GLTGTGVLTTP
 541 SSKRFQPPQQ FGRDVSDFTD SVRDPKTSEI LDISPCSPGG VSVITPGTNA SSEVAVLYQD
 601 VNCTDVSTAI HADQLTPAWR IYSTGNNVFQ TQAGCLIGAE HVDTSYECDI PIGAGICASY
 661 HTVSLLRSTS QKSIVAYTMS LGADSSIAYS NNTIAIPTNF SISITTEVMP VSMAKTSVDC
 721 NMYICGDSTE CANLLLQYGS FCTQLNRALS GIAAEQDRNT REVFAQVKQM YKTPTLKYFG
 781 GFNFSQILPD PLKPTKRSFI EDLLFNKVTL ADAGFMKQYG ECLGDINARD LICAQKFNGL
 841 TVLPPLLTDD MIAAYTAALV SGTATAGWTF GAGAALQIPF AMQMAYRFNG IGVTONVLYE
 901 NQKQIANQFN KAISIQESL TTTSTALGKL QDVVNQNAQA LNTLVKQLSS NFGAISSVLN
 961 DILSRLDKVE AEVQIDRLIT GRLQSLQTYV TQQLIRAAEI RASANLAATK MSECVLGQSK
 1021 RVDFCGKGYH LMSFPQAAPH GVVFLHVTVV PSQERNFTTA PAICHEGKAY FPREGVVFVN
 1081 GTSWFITQRN FESPQIITTD NTFVSGNCDV VIGIINNTVY DPLQPELDSF KEELDKYFKN
 1141 HTSPDVLGDL ISGINASVVN IQKEIDRLNE VAKNLNESLI DLQELGKYEQ YIKWPWYVWL
 1201 GFIAGLIAIV MVTILLCCMT SCCSCLKGAC SCGSCCKFDE DDSEPVKGV KLHYT

Bt SARS CoV 279/2005 spike protein (Accession No. 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, ABG47069) (third coronavirus) (SEQ ID NO:4) 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519,

1 MKVLIFALLE SLAKAQEGCG IISFKPQPKM EKVSSRRGV YNDDIFRSD VLHLTQDYFL
 61 PFDSNLTQYF SLNIDSNKYT YFDNPILDFG DGVYFAATEK SNVIRGWIFG SSFDNTTQSA
 121 IIVNNSTHII IRVCNPNLCK EPMYTVSKGT QQSSWVYQSA FNCTYDRVEK SFQLDTAPKT
 181 GNFKDLREYV FKNRDGFLSV YQTYTAVNLP RGFPAGFSVL RPILKLPFGI NITSYRVVMT
 241 MFSQFNSNLF PESAAVYVGN LKYTTFMLSF NENGTITDAV DCSQNPLAEL KCTIKNFNVS
 301 KGIYQTSNFR VTPTQEVVRF PNITNRCPPD KVFNASRFPN VYAWERTKIS DCVADYTVLY
 361 NSTSFSSTFKC YGVSPSKLID LCPTSVMYADT FLIRSSSEVRQ VAPGETGVIA DYNKLPDDF
 421 TGCVIAWNTA QDQDQYVYR SYRKEKLPKPF ERDLSSDENG VYTLSTYDFY PSIPVEYQAT
 481 RVVVLSEFELL NAPATVCGPK LSTQLVKNQC VNFNENGLRG TGVLTSSKR FQSPQPFGRD
 541 TSDFTDSVRD PQTLEILDIS PCSFGGVSVI TPGTNASSEV AVLYQDVNCT DVPTSIHADQ
 601 LTPAWRVYST GVNVPQTQAG CLIGAEHVNA SYECDIPIGA GICASYHTAS VLRSTGQKSI
 661 VAYTMSLGAE NSIAYANNSI AIPTNFSISV TTEVMPVISA KTSVDCTMYI CGDSLECSNL
 721 LLQYGSFCTQ LNRALTGIAI EQDKNTQEVF AQVKQMYKTP AIKDFGGFNF SQILPDPSPK
 781 TKRSFIEDLL FNKVTLDAG FMKQYGECLG DISARDLICA QKFNGLTVLP PLLTDEMIAA
 841 YTAALVSGTA TAGWTFGAGS ALQIPFAMQM AYRFNGIGVT QNVLYENQKQ IANQFNKAIS
 901 QIQESLTTTS TALGKLOQDVV NDNAQALNTL VKQLSSNFGA ISSVLNDILS RLDEVEAEVQ
 961 IDRLITGRLO SLQTYVTQQL IRAAEIRASA NLAATKMSEC VLGQSKRVDF CGKGYHLMSE
 1021 POAAPHGVVF LHVTVPSQE RNFTTAPAIC HEGKAYFPRE GVFVSNGTSW FITQRNFYSP
 1081 QIITTDNTFV AGNCDVVIIGI INNTVYDPLQ PELDSFKEEL DKYFKNHTSP DVLDGDISGI
 1141 NASVVNIQKE IDRLNEVAKN LNESLIDLQE LGKYEYQIKW PNYVWLGPIA GLIAIVMVTI
 1201 LLCCMTSCCS CLKGACSCGS CCKFDEDDSE PVLKGVKLHY T

It is to be understood that this example is not intended to be limiting and any of these three subgroup 2b coronaviruses can be combined with any other subgroup 2b coronavirus in any combination of first coronavirus, second coronavirus and third coronavirus, provided that they are all different from one another.

Furthermore, the length in amino acid residues of the respective regions of the chimeric subgroup 2b coronavirus spike protein can vary. For example, the first region can comprise amino acid 1 through amino acid 320, amino acid 1 through amino acid 321, amino acid 1 through amino acid 322, amino acid 1 through amino acid 323, amino acid 1 through amino acid 324, amino acid 1 through amino acid 325, amino acid 1 through amino acid 326, amino acid 1 through amino acid 327, amino acid 1 through amino acid 328, amino acid 1 through amino acid 329 or amino acid 1 through amino acid 330 of a subgroup 2b coronavirus spike protein, which is a first coronavirus Amino acid numbering is based on the numbering of amino acid residues in a subgroup 2b coronavirus spike protein, representative examples of which are provided herein.

For the second region of the chimeric subgroup 2b coronavirus spike protein of this invention, the amino end of the second region can begin at amino acid 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329 or 330 of a subgroup 2b coronavirus spike protein and be contiguous through amino acid 490, 491, 492, 493, 494, 495,

520, 521, 522, 523, 524 or 525 of a subgroup 2b coronavirus spike protein of a second coronavirus that is different from the first coronavirus.

For the third region of the chimeric subgroup 2b coronavirus spike protein of this invention, the amino end of the third region can begin at amino acid 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509 or 510 of a subgroup 2b coronavirus spike protein and be contiguous through amino acid 825, 826, 827, 828, 829, 830, 831, 832, 833, 834, 835, 836, 837, 838, 839, 840, 841, 842, 843, 844, 845, 846, 847, 848, 849 or 850 of a subgroup 2b coronavirus spike protein. As noted above, the third region of the chimeric coronavirus spike protein is from the coronavirus that is the first coronavirus.

For the fourth region of the chimeric subgroup 2b coronavirus spike protein of this invention, the amino end of the fourth region can begin at amino acid 825, 826, 827, 828, 829, 830, 831, 832, 833, 834, 835, 836, 837, 838, 839, 840, 841, 843, 844, 845, 846, 847, 848, 849 or 850 of a subgroup 2b coronavirus spike protein and be contiguous through amino acid 1225, 1226, 1227, 1228, 1229, 1230, 1231, 1232, 1233, 1234, 1235, 1236, 1237, 1238, 1240, 1241, 1242 or the final amino acid at the carboxy terminus of a subgroup 2b coronavirus spike protein. As noted above the fourth region of the chimeric coronavirus spike protein is from a third coronavirus that is different from the first coronavirus and the second coronavirus used to produce the chimeric coronavirus spike protein.

As a further nonlimiting example, the present invention provides a chimeric subgroup 2c coronavirus spike protein comprising, in orientation from amino to carboxy terminus: a) a first region comprising amino acids 1-371 of a spike protein of a first subgroup 2c coronavirus; b) a second region comprising amino acids 367-588 of a spike protein of a second subgroup 2c coronavirus; c) a third region comprising amino acids 594-983 of the spike protein of said first subgroup 2c coronavirus; and) a fourth region comprising

amino acids 986-1357 of a spike protein of a third subgroup 2c coronavirus. The amino acid sequence of the chimeric coronavirus spike protein of this example is shown below, with these four regions identified (first and third regions from said first subgroup 2c coronavirus shown in bold; second region from said second subgroup 2c coronavirus shown with underline; and fourth region from said third subgroup 2c coronavirus shown in italics).

(SEQ ID NO: 5)

```

1  MTLMLCLLMS LLIFVRGCD S QFVDMSPASN TSECL ESQVD AAAPSKLMWP YPIDPSKVDG
61  IYPLGR TYS NITLAYTGLF PLQGD LGSQY LYSVSHAVGH DGDPTKAYIS NYSLLVNDFD
121 NGFVVRIGAA ANSTGTIVIS PSVNTKIKKA YPAFILGSSL TNSAGQPLY ANYSLTIIPD
181 GCGTVLHAFY CILKPR TVNR CPSGTGYVSY FIYETVHND C QSTINRNASL NSFKSFDDL V
241 NCTFFNSWDI TADETKEWFG ITQDTQGVHL YSSRKG DLYG GNMFRFATLP VYEGIKYYTV
301 IPRSFRSKAN KREAWAAFYV YKLHQLTYLL DFSVDGYIRR AIDCGHDDL S QLHC SYTSFE
361 VDTGVYSVSS YEAKPSGSV EQAEGVECDF SPLLSGTPPQ VYNFKRLVFT NCNYNLTKLL
421 SLFSSVNDFTC SQISPAAIAS NCYSSLILDY FSYPLSMKSD LSVSSAGPIS QFNKYQSFSN
481 PTCLILATVP HNLTTITKPL KYSYINKCSR LLSDDRTEVP QLVNANQYSP CVSIVPSTVW
541 EDGDYYRKQL SPLEGGWLV ASGSTVAMTE QLQMGFGITV QYGTDTNSVC PKLDLGDLSLT
601 ITNRLGKCDV YSLYGV TGRG VFQNC TAVGV KQRFVYDSF DNLVGYISDD GNYCYVRPCV
661 SVPVSVIYDK STNLHATLFG SVACEHVTTM MSQFSRLTQS NLRRRDSNIP LQTAVGCVIG
721 LSNNSLVVSD CKLPLGQSLC AVPPVSTFRS YSASQFQLAV LNYTSPIVVT PINSSGF TAA
781 IPTNFSFSVT QEYIETS IQK VTDCKQYVC NGFTRCEKLL VEYQGFCSKI NQALHGANLR
841 QDESVSLSYS NIKTTSTQTL EYGLNGDFNL TLLQVPQIGG SSSYSRSAIE DLLFDKVTIA
901 DPGYMQGYDD CMKQGPQSAR DLICAQYVSG YKVLPPLYDP NMEAA YTSSL LGS IAGAGWT
961 AGLSSFAAIP FAQSMFYRLN GVGITQQVLS ENQKI LANKF NQALGAMQTG FTTNLA FNK
1021 VQDAVNANAM ALSKLAELS NTFGAISSI SDILARLDTV EQEAQIDRLI NGR L TSLNAF
1081 VAQQLVRTEA AARSAQLAQD KVN EC VKSQS KRNGFCGTGT HIVSFAINAP NGLYFFHVGY
1141 QPTSHVNATA AYGLCNTENP PKCIAPIDGY FVLNQT TSTA RSSGDQH WY Y TGSSFFHPEP
1201 ITEANSKYVS MDVKFENLTN KLPPPLLSNS TDLDFKDELE EFFKNVSSQG PNFQEISKIN
1261 TTLNLNTEL MVLSEVVKQL NESYIDLKEL GNYTPYQKWP WYIWLGF IAG LVALALCVFF
1321 ILCCTGCGTS CLGRLKCNRC CDSYDEYEVE KIHVH
    
```

50 The exemplary chimeric coronavirus spike protein shown above was produced from the following three subgroup 2c coronaviruses:
 Bat CoV HKU4-2 spike protein (Accession No. ABN10848.1) (SEQ ID NO:6)

```

1  MTLMLCLLMS LLIFVRGCD S QFVDMSPASN TSECL ESQVD AAAPSKLMWP YPIDPSKVDG
61  IYPLGR TYS NITLAYTGLF PLQGD LGSQY LYSVSHAVGH DGDPTKAYIS NYSLLVNDFD
121 NGFVVRIGAA ANSTGTIVIS PSVNTKIKKA YPAFILGSSL TNSAGQPLY ANYSLTIIPD
181 GCGTVLHAFY CILKPR TVNR CPSGTGYVSY FIYETVHND C QSTINRNASL NSFKSFDDL V
241 NCTFFNSWDI TADETKEWFG ITQDTQGVHL YSSRKG DLYG GNMFRFATLP VYEGIKYYTV
301 IPRSFRSKAN KREAWAAFYV YKLHQLTYLL DFSVDGYIRR AIDCGHDDL S QLHC SYTSFE
361 VDTGVYSVSS YEASATGTFI EQPNATECDF SPMLTGVAPQ VYNFKRLVFS NCNYNLTKLL
    
```

-continued

421 SLFAVDEFSC NGISPDAIAR GCYSTLTVDY FAXPLSMKSY IRPGSAGNIP LYNYKQSFAN
 481 PTCRVMASVL ANVTITKPHA YGYISKCSRL TGANQDVETP LYINPGEYSI CRDFSPGGFS
 541 EDGQVFKRTL TQFEGGGLLI GVGTRVPMTD NLQMSFIIISV QYGTGTDSVC PMLDLGDSLT
 601 ITNRLGKCDV YSLYGVTRGR VFQNCTAVGV KQQRFVYDSF DNLVGYSDD GNYYCVRPCV
 661 SVPVSVIYDK STNLHATLFG SVACEHVTTM MSQFSRLTQS NLRRRDSNIP LQTAVGCVIG
 721 LSNNSLVVSD CKLPLGQSLC AVPPVSTFRS YSASQQLAV LNYTSPIVVT PINSSGFTAA
 781 IPTNFSFSVT QEIYETSIQ VTVDCKQYVC NGFTRCEKLL VEYGQFCSKI NQALHGANLR
 841 QDESVYLSY NIKTTSTQTL EYGLNGDFNL TLLQVPQIGG SSSYRSAIE DLLPDKVTIA
 901 DPGYMQGYDD CMKQGPOSAR DLICAQYVSG YKVLPPLYDP NMEAAYTSSL LGSIAGAGWT
 961 AGLSSFAAIP FAQSMFYRLN GVGITQQVLS ENQKLIANKF NQALGAMQTG FTTSLAFSK
 1021 VQDAVNANAQ ALSKLAELSL NTFGAISSSI SDILARLDTV EQDAQIDRLI NGRLTSLNAP
 1081 VSQQLVRSET AARSAQLASD KVNCEVKSQS KRNGFCGSGT HIVSFVVNAP NGFYFPHVGY
 1141 VPTNYTNVTA AYGLCNMNNP PLCIAPIDGY FITNQTTTYS VDTEWYITGS SFYKPEPITQ
 1201 ANSRYVSSDV KFDKLENNLP PPLENSTDV DFKDELEEFF KNVTSHGPNF AEISKINTTL
 1261 LDLSDMAML QEVVKQLNDS YIDLKELGNY TYNKWPWYV WLGFIAGLVA LLLCVFLLC
 1321 CTGCGTSCLG KMKCKNCCDS YEEYDVEKIH VH

MERS-CoV spike protein (GenBank Accession No. AFS88936.1) (SEQ ID NO:7)

1 MIHVSFLLMF LLTPTESYVD VGPDSVKSAC IEVDIQQTFE DKTWPRPIDV SKADGIIYPQ
 61 GRTYSNITIT YQGLFPYQGD HGDYVYSAG HATGTPQKL FVANYSQDVK QFANGFVVRI
 121 GAAANSTGTV IISPTSATI RKIYPAFMLG SSVGNFSDGK MGRFFNHTLV LLPDGCGLL
 181 RAYFCILEPR SGNHCAPAGNS YTSFATYHTP ATDCSDGNYN RNASLNSPKE YFNLRNCTFM
 241 YTYNITEDEI LEWFGITQTA QGVHLFSSRY VDLYGGNMPQ FATLPVYDTI KYYSIIPHSI
 301 RSIQSDRKAW AAFVYVKLQP LTFLLDFSVD GYIRRAIDCG FNDLSQLHCS YESFDVESGV
 361 YSVSSFEAKP SGSVVEQAEG VECDFSPLLS GTPPQVYNFK RLVFTNCNIN LTKLLSLFSV
 421 NDFTCSQISF AAIASNCYSS LILDYFSYPL SMKSDLSVSS AGPISQFNYK QSFSNPTCLI
 481 LATVPHNLTT ITKPLKYSYI NKCSRLSDD RTEVPQLVNA NQYSPCVSIV PSTVWEDGDY
 541 YRKQLSPLEG GGWLVASGST VAMMEQLQMG FGITVQYGTD TNSVCPKLEF ANDTKIASQL
 601 GNCVEYSLYG VSGRQVFNQ TAVGVRQORF VYDAYQNLVG YYSDDGNYYC LRACVSVVPS
 661 VIYDKETKTH ATLFQSVACE HISSTMSQYS RSTRMLKRR DSTYGPLQTP VGCVLGLVNS
 721 SLFVEDCKLP LGQSLCALPD TPSTLTPRSV RSVPGEMRLA SIAFNHPIQV DQLNSSYFKL
 781 SIPTNFSEGV TQEYIQTITQ KVTVDCKQYV CNGFQKCEQL LREYGQFCSK INQALHGANL
 841 RQDDSVRNLF ASVKSQSSP IIPGFGDFN LTLEPVSIS TGRSARSASAI EDLLEDFKVTI
 901 ADPGYMQGYD DCMQQGPASA RDLICAQYVA GYKVLPLPMD VNMEAAYTSS LLGSIAGVGV
 961 TAGLSSFAAI PFAQSIFYRL NGVGITQQVL SENQKLIANK FNQALGAMQT GFTTTNEAPQ
 1021 KVQDAVNNA QALSKLASEL SNTFGAISAS IGDIIQRLDV LEQDAQIDRL INGRLTTLNA
 1081 FVAQQLVRSE SAALSAQLAK DKVNECVKAQ SKRSGFCGQG THIVSFVVNA PNGLYFMHVG
 1141 YYPSNHIEVV SAYGLCDAAN PTNCIAPVNG YFIKTNNTRI VDEWYSYGSS FYAPEPITSL
 1201 NTKYVAPQVT YQNI~~ST~~NLPP PLLGNSTGID FQDELDEFFK NVSTSIPNFG SLTQINTTLL

- continued

1261 DLTYEMLSLQ QVVKALNESY IDLKELGNYT YYNKWPWYIW LGFIAGLVAL ALCVFFILCC
 1321 TGCCTNMGK LKCNRCDDRY EYDLEPHKV HVH

5

Bat CoV HKU5-5 spike protein (GenBank Accession No. ABN10902.1) (SEQ ID NO:8)

For the second region of the chimeric subgroup 2c coronavirus spike protein of this invention, the amino end of the

1 MIRSVLVLMC SLTFIGNRTS CQSVDIGTPV TGSCLRSQVR PEYFDIVHNT WPMPIDTSKA
 61 EGVIIYPNGKS YSNISLTYTG LYPKAKDLGK QYLFSDGHS A PNQLNDLFVS NYS AQVESFD
 121 DGFVVRIGAA SNKTGTTVIS QTTFKPIKKI YPGFMLGHAV GNYTPTNITG RYLNHTLVIL
 181 PDGCGTLVHA FYCILQPR TQ ANCPGASSFT SVTLWDTPAT DCAPSGVYNS LANLNAFKLY
 241 FDLINCTFRY NYTTITEDENA EWFGITQDTQ GVHLYSSRKE NVFRNMFMHF ATLPVYQKIL
 301 YTTVIPRSIR SPFNDRKAWA AFYIYKLHPL TYLLNFDVEG YITKAVDCCGY DDF AQLCQSY
 361 ENFDVETGVY SVSSFEASPR GEFIEQATTQ ECDFTPMLTG TPPPIYDFKR LVFTNCNYNL
 421 TKLLSLFQVS EFSCHQVSPS SLATGCYSSL TVDYFAYSTD MSSYLQPGSA GEIVQPFNYKQ
 481 DFSNPTCRVL ATVPNTLTTI TKSSNVVHLT ECKYSTAYGK NYLYNAPGGY TPCLSLASRG
 541 FTTNRQSHSL ELPDGYLVTT GSVYPVNGNL QMAFIISVQY GTDTSNVCPM QALRNDTSIE
 601 DKLDVCEYS LHGITGRGVF HNCTSVGLRN QRFVYDTFDN LVGYHSDNGN YYCVRPCVSV
 661 PVSVIYDKAS NSHATLFGSV ACSHVTTMMS QFSRMTKTNL PARTTPGPLQ TTVGCAMGFI
 721 NSSMVVDECQ LPLGQSLCAI PPTTSTRFRR ATSIPDV FQI ATLNFTSPLT LAPINSTGFV
 781 VAVPTNFTFG VTQEFIE TTI QKITVDCKQY VCN GFKKCED LLKEYGQFCS KINQALHGAN
 841 LRQDESANL FSSIKTQNTQ PLQAGLNGDF NLTMLQIPQV TTGERKYRST IEDLLENKVT
 901 IADPGYMQGY DECMQQGPQS ARDLICAQYV AGYKVLPPLY D FYMEAA YTS SLLGSIAGAS
 961 WTAGLSSFAA IPFAQSIF YR LNGVGITQOV LSENOKILAN KFNQALGAMQ TGFTTTNLAF
 1021 NKVQDAVNAN AMALS KLAEE LSNTFGAISS SISDILARLD TVEQEAQIDR LINGRLTSLN
 1081 AFVAQQLVRT EAAARSAQLA QDKVNECVKS QSKRNGFCGT GTHIVSFAIN APNGLYFFHV
 1141 GYOPTSHVNA TAA YGLCNTE NPPKCIAPID GYFVLNQTTS TARSSGDQHW YTTGSSFFHP
 1201 EPITEANSKY VSM DVEEENL TNKLP PPLLS NSTDLDFKDE LEEFFKNVSS QGPNFQEISK
 1261 INTTLNLNT ELMVLSEVVK QLNESYIDLE ELGN YTFYQK WFWYIWL GFI AGLVALALCV
 1321 FFILCCTGCG TSCLGK LKCN RCCDSYDEYE VEKIHVH

It is to be understood that this example is not intended to be limiting and any of these three subgroup 2c coronaviruses can be combined with any other subgroup 2c coronavirus in any combination of first coronavirus, second coronavirus and third coronavirus, provided that they are all different from one another.

Furthermore, the length in amino acid residues of the respective regions of the chimeric subgroup 2c coronavirus spike protein can vary. For example, the first region can comprise amino acid 1 through amino acid 365, amino acid 1 through amino acid 366, amino acid 1 through amino acid 367, amino acid 1 through amino acid 368, amino acid 1 through amino acid 369, amino acid 1 through amino acid 370, amino acid 1 through amino acid 371, amino acid 1 through amino acid 372, amino acid 1 through amino acid 373, amino acid 1 through amino acid 374 or amino acid 1 through amino acid 375 of a subgroup 2c coronavirus spike protein, which is a first coronavirus. Amino acid numbering is based on the numbering of amino acid residues in a subgroup 2c coronavirus spike protein, representative examples of which are provided herein.

second region can begin at amino acid 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374 or 375 of a subgroup 2c coronavirus spike protein and be contiguous through amino acid 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599 or 600 of a subgroup 2c coronavirus spike protein of a second coronavirus that is different from the first coronavirus.

For the third region of the chimeric subgroup 2c coronavirus spike protein of this invention, the amino end of the third region can begin at amino acid 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599 or 600 of a subgroup 2c coronavirus spike protein and be contiguous through amino acid 975, 976, 977, 978, 979, 980, 981, 982, 983, 984, 985, 986, 987, 988, 989, 990, 991, 992, 993, 994, 995, 996, 997, 998, 999 or 1000 of a subgroup 2c coronavirus spike protein. As noted above, the third region of the chimeric coronavirus spike protein is from the subgroup 2c coronavirus that is the first coronavirus.

For the fourth region of the chimeric subgroup 2c coronavirus spike protein of this invention, the amino end of the fourth region can begin at amino acid 975, 976, 977, 978, 979, 980, 981, 982, 983, 984, 985, 986, 987, 988, 989, 990, 991, 992, 993, 994, 995, 996, 997, 998, 999 or 1000 of a subgroup 2c coronavirus spike protein and be contiguous through amino acid 1345, 1346, 1347, 1348, 1349, 1350, 1351, 1352, 1353, 1354, 1355, 1356, 1357, 1358, 1359, 1360, 1361, 1362, 1363, 1364, 1365, 1366, 1367, 1368, 1369, 1370 or the final amino acid at the carboxy terminus of a subgroup 2c coronavirus spike protein. As noted above the fourth region of the chimeric coronavirus spike protein is from a third subgroup 2c coronavirus that is different from the first subgroup 2c coronavirus and the second subgroup 2c coronavirus used to produce this chimeric coronavirus spike protein.

Although the examples set forth above describe a chimeric spike protein produced from subgroup 2b coronaviruses and a chimeric spike protein produced from subgroup 2c coronaviruses, it is to be understood that a chimeric coronavirus spike protein of this invention can be made from any combination of three different coronaviruses from any subgroup, including subgroup 1a, subgroup 1b, subgroup 2a, subgroup 2d and subgroup 3 in addition to subgroup 2b and subgroup 2c. The same arrangement of the first, second, third and fourth regions as described above would be applicable to a chimeric coronavirus spike protein of any subgroup and the same variability with regard to the amino acids that define the beginning and end of each of these four regions would be applicable to a chimeric coronavirus spike protein of any subgroup.

Furthermore, the chimeric coronavirus spike proteins produced from the respective coronavirus subgroups 1a, 1b, 2a, 2b, 2c, 2d and 3 can be included in the methods and compositions of this invention in any combination and/or in any ratio relative to one another, as would be well understood to one of ordinary skill in the art.

Nonlimiting examples of subgroup 2b coronaviruses that can be used to produce the chimeric coronavirus spike protein of this invention include Bat SARS CoV (GenBank Accession No. FJ211859), SARS CoV (GenBank Accession No. FJ211860), BtSARS.HKU3.1 (GenBank Accession No. DQ022305), BtSARS.HKU3.2 (GenBank Accession No. DQ084199), BtSARS.HKU3.3 (GenBank Accession No. DQ084200), BtSARS.Rm1 (GenBank Accession No. DQ412043), BtCoV.279.2005 (GenBank Accession No. DQ648857), BtSARS.Rf1 (GenBank Accession No. DQ412042), BtCoV.273.2005 (GenBank Accession No. DQ648856), BtSARS.Rp3 (GenBank Accession No. DQ071615), SARS CoV.A022 (GenBank Accession No. AY686863), SARSCoV.CUHK-W1 (GenBank Accession No. AY278554), SARSCoV.GDO1 (GenBank Accession No. AY278489), SARSCoV.HC.SZ.61.03 (GenBank Accession No. AY515512), SARSCoV.SZ16 (GenBank Accession No. AY304488), SARSCoV.Urbani (GenBank Accession No. AY278741), SARSCoV.civet010 (GenBank Accession No. AY572035), and SARSCoV.MA.15 (GenBank Accession No. DQ497008), Rs SHC014 (GenBank® Accession No. KC881005), Rs3367 (GenBank® Accession No. KC881006), WtV1 S (GenBank® Accession No. KC881007) as well as any other subgroup 2b coronavirus now known (e.g., as can be found in the GenBank® Database) or later identified, and any combination thereof.

Nonlimiting examples of subgroup 2c coronaviruses that can be used to produce the chimeric coronavirus capsid protein of this invention include: Middle East respiratory syndrome coronavirus isolate Riyadh_2_2012 (GenBank

Accession No. KF600652.1), Middle East respiratory syndrome coronavirus isolate Al-Hasa_18_2013 (GenBank Accession No. KF600651.1), Middle East respiratory syndrome coronavirus isolate Al-Hasa_17_2013 (GenBank Accession No. KF600647.1), Middle East respiratory syndrome coronavirus isolate Al-Hasa_15_2013 (GenBank Accession No. KF600645.1), Middle East respiratory syndrome coronavirus isolate Al-Hasa_16_2013 (GenBank Accession No. KF600644.1), Middle East respiratory syndrome coronavirus isolate Al-Hasa_21_2013 (GenBank Accession No. KF600634), Middle East respiratory syndrome coronavirus isolate Al-Hasa_19_2013 (GenBank Accession No. KF600632), Middle East respiratory syndrome coronavirus isolate Buraidah_1_2013 (GenBank Accession No. KF600630.1), Middle East respiratory syndrome coronavirus isolate Hafr-Al-Batin_1_2013 (GenBank Accession No. KF600628.1), Middle East respiratory syndrome coronavirus isolate Al-Hasa_12_2013 (GenBank Accession No. KF600627.1), Middle East respiratory syndrome coronavirus isolate Bisha_1_2012 (GenBank Accession No. KF600620.1), Middle East respiratory syndrome coronavirus isolate Riyadh_3_2013 (GenBank Accession No. KF600613.1), Middle East respiratory syndrome coronavirus isolate Riyadh_1_2012 (GenBank Accession No. KF600612.1), Middle East respiratory syndrome coronavirus isolate Al-Hasa_3_2013 (GenBank Accession No. KF186565.1), Middle East respiratory syndrome coronavirus isolate Al-Hasa_1_2013 (GenBank Accession No. KF186567.1), Middle East respiratory syndrome coronavirus isolate Al-Hasa_2_2013 (GenBank Accession No. KF186566.1), Middle East respiratory syndrome coronavirus isolate Al-Hasa_4_2013 (GenBank Accession No. KF186564.1), Middle East respiratory syndrome coronavirus (GenBank Accession No. KF192507.1), Betacoronavirus England 1-N1 (GenBank Accession No. NC_019843), MERS-CoV_SA-N1 (GenBank Accession No. KC667074), following isolates of Middle East Respiratory Syndrome Coronavirus (GenBank Accession No: KF600656.1, GenBank Accession No: KF600655.1, GenBank Accession No: KF600654.1, GenBank Accession No: KF600649.1, GenBank Accession No: KF600648.1, GenBank Accession No: KF600646.1, GenBank Accession No: KF600643.1, GenBank Accession No: KF600642.1, GenBank Accession No: KF600640.1, GenBank Accession No: KF600639.1, GenBank Accession No: KF600638.1, GenBank Accession No: KF600637.1, GenBank Accession No: KF600636.1, GenBank Accession No: KF600635.1, GenBank Accession No: KF600631.1, GenBank Accession No: KF600626.1, GenBank Accession No: KF600625.1, GenBank Accession No: KF600624.1, GenBank Accession No: KF600623.1, GenBank Accession No: KF600622.1, GenBank Accession No: KF600621.1, GenBank Accession No: KF600619.1, GenBank Accession No: KF600618.1, GenBank Accession No: KF600616.1, GenBank Accession No: KF600615.1, GenBank Accession No: KF600614.1, GenBank Accession No: KF600641.1, GenBank Accession No: KF600633.1, GenBank Accession No: KF600629.1, GenBank Accession No: KF600617.1), Coronavirus Neoromicia/PML-PHE1/RSA/2011 (GenBank Accession: KC869678.2, Bat Coronavirus Taper/CII_KSA_287/Bisha/Saudi Arabia/GenBank Accession No: KF493885.1, Bat coronavirus Rhhar/CII_KSA_003/Bisha/Saudi Arabia/2013 (GenBank Accession No: KF493888.1, Bat coronavirus Piku/CII_KSA_001/Riyadh/Saudi Arabia/2013 (GenBank Accession No: KF493887.1, Bat coronavirus Rhhar/CII_KSA_002/Bisha/Saudi Arabia/2013 (GenBank Accession No: KF493886.1, Bat Coronavirus Rhhar/

CII_KSA_004/Bisha/Saudi Arabia/2013 GenBank Accession No: KF493884.1, BtCoV.HKU4.2 (GenBank Accession No. EF065506), BtCoV.HKU4.1 (GenBank Accession No. NC_009019), BtCoV.HKU4.3 (GenBank Accession No. EF065507), BtCoV.HKU4.4 (GenBank Accession No. EF065508), BtCoV 133.2005 (GenBank Accession No. NC_008315), BtCoV.HKU5.5 (GenBank Accession No. EF065512); BtCoV.HKU5.1 (GenBank Accession No. NC_009020), BtCoV.HKU5.2 (GenBank Accession No. EF065510), BtCoV.HKU5.3 (GenBank Accession No. EF065511), human betacoronavirus 2c Jordan-N3/2012 (GenBank Accession No. KC776174.1; human betacoronavirus 2c EMC/2012 (GenBank Accession No. JX869059.2), Pipistrellus bat coronavirus HKU5 isolates (GenBank Accession No:KC522089.1, GenBank Accession No:KC522088.1, GenBank Accession No:KC522087.1, GenBank Accession No:KC522086.1, GenBank Accession No:KC522085.1, GenBank Accession No: KC522084.1, GenBank Accession No:KC522083.1, GenBank Accession No:KC522082.1, GenBank Accession No:KC522081.1, GenBank Accession No:KC522080.1, GenBank Accession No:KC522079.1, GenBank Accession No:KC522078.1, GenBank Accession No: KC522077.1, GenBank Accession No:KC522076.1, GenBank Accession No:KC522075.1, GenBank Accession No:KC522104.1, GenBank Accession No:KC522104.1, GenBank Accession No:KC522103.1, GenBank Accession No:KC522102.1, GenBank Accession No: KC522101.1, GenBank Accession No:KC522100.1, GenBank Accession No:KC522099.1, GenBank Accession No:KC522098.1, GenBank Accession No:KC522097.1, GenBank Accession No:KC522096.1, GenBank Accession No:KC522095.1, GenBank Accession No: KC522094.1, GenBank Accession No:KC522093.1, GenBank Accession No:KC522092.1, GenBank Accession No:KC522091.1, GenBank Accession No:KC522090.1, GenBank Accession No:KC522119.1 GenBank Accession No:KC522118.1 GenBank Accession No: KC522117.1 GenBank Accession No:KC522116.1 GenBank Accession No:KC522115.1 GenBank Accession No:KC522114.1 GenBank Accession No:KC522113.1 GenBank Accession No:KC522112.1 GenBank Accession No:KC522111.1 GenBank Accession No: KC522110.1 GenBank Accession No:KC522109.1 GenBank Accession No:KC522108.1, GenBank Accession No:KC522107.1, GenBank Accession No:KC522106.1, GenBank Accession No:KC522105.1) Pipistrellus bat coronavirus HKU4 isolates (GenBank Accession No:KC522048.1, GenBank Accession No:KC522047.1, GenBank Accession No:KC522046.1, GenBank Accession No:KC522045.1, GenBank Accession No: KC522044.1, GenBank Accession No:KC522043.1, GenBank Accession No:KC522042.1, GenBank Accession No:KC522041.1, GenBank Accession No:KC522040.1 GenBank Accession No:KC522039.1, GenBank Accession No:KC522038.1, GenBank Accession No:KC522037.1, GenBank Accession No:KC522036.1, GenBank Accession No:KC522048.1 GenBank Accession No:KC522047.1 GenBank Accession No:KC522046.1 GenBank Accession No:KC522045.1 GenBank Accession No:KC522044.1 GenBank Accession No:KC522043.1 GenBank Accession No:KC522042.1 GenBank Accession No:KC522041.1 GenBank Accession No:KC522040.1, GenBank Accession No:KC522039.1 GenBank Accession No:KC522038.1 GenBank Accession No:KC522037.1 GenBank Accession No:KC522036.1, GenBank Accession No:KC522061.1 GenBank Accession No:KC522060.1 GenBank Accession No:KC522059.1 GenBank Accession No:KC522058.1 GenBank Accession No:KC522057.1 GenBank Accession

No:KC522056.1 GenBank Accession No:KC522055.1 GenBank Accession No:KC522054.1 GenBank Accession No:KC522053.1 GenBank Accession No:KC522052.1 GenBank Accession No:KC522051.1 GenBank Accession No:KC522050.1 GenBank Accession No:KC522049.1 GenBank Accession No:KC522074.1, GenBank Accession No:KC522073.1 GenBank Accession No:KC522072.1 GenBank Accession No:KC522071.1 GenBank Accession No:KC522070.1 GenBank Accession No:KC522069.1 GenBank Accession No:KC522068.1 GenBank Accession No:KC522067.1, GenBank Accession No:KC522066.1 GenBank Accession No:KC522065.1 GenBank Accession No:KC522064.1, GenBank Accession No:KC522063.1, or GenBank Accession No:KC522062.1, as well as any other subgroup 2b coronavirus now known (e.g., as can be found in the GenBank® Database) or later identified, and any combination thereof.

Nonlimiting examples of a subgroup 1a coronavirus of this invention include FCov.FIPV.79.1146.VR.2202 (GenBank Accession No. NV_007025), transmissible gastroenteritis virus (TGEV) (GenBank Accession No. NC_002306; GenBank Accession No. Q811789.2; GenBank Accession No. DQ811786.2; GenBank Accession No. DQ811788.1; GenBank Accession No. DQ811785.1; GenBank Accession No. X52157.1; GenBank Accession No. AJ011482.1; GenBank Accession No. KC962433.1; GenBank Accession No. AJ271965.2; GenBank Accession No. JQ693060.1; GenBank Accession No. KC609371.1; GenBank Accession No. JQ693060.1; GenBank Accession No. JQ693059.1; GenBank Accession No. JQ693058.1; GenBank Accession No. JQ693057.1; GenBank Accession No. JQ693052.1; GenBank Accession No. JQ693051.1; GenBank Accession No. JQ693050.1), porcine reproductive and respiratory syndrome virus (PRRSV) (GenBank Accession No. NC_001961.1; GenBank Accession No. DQ811787), as well as any other subgroup 1a coronavirus now known (e.g., as can be found in the GenBank® Database) or later identified, and any combination thereof.

Nonlimiting examples of a subgroup 1b coronavirus of this invention include BtCoV.1A.AFCD62 (GenBank Accession No. NC_010437), BtCoV.1B.AFCD307 (GenBank Accession No. NC_010436), BtCoV.HKU8.AFCD77 (GenBank Accession No. NC_010438), BtCoV.512.2005 (GenBank Accession No. DQ648858), porcine epidemic diarrhea virus PEDV.CV777 (GenBank Accession No. NC_003436, GenBank Accession No. DQ355224.1, GenBank Accession No. DQ355223.1, GenBank Accession No. DQ355221.1, GenBank Accession No. JN601062.1, GenBank Accession No. N601061.1, GenBank Accession No. JN601060.1, GenBank Accession No. JN601059.1, GenBank Accession No. JN601058.1, GenBank Accession No. JN601057.1, GenBank Accession No. JN601056.1, GenBank Accession No. JN601055.1, GenBank Accession No. JN601054.1, GenBank Accession No. JN601053.1, GenBank Accession No. JN601052.1, GenBank Accession No. JN400902.1, GenBank Accession No. JN547395.1, GenBank Accession No. FJ687473.1, GenBank Accession No. FJ687472.1, GenBank Accession No. FJ687471.1, GenBank Accession No. FJ687470.1, GenBank Accession No. FJ687469.1, GenBank Accession No. FJ687468.1, GenBank Accession No. FJ687467.1, GenBank Accession No. FJ687466.1, GenBank Accession No. FJ687465.1, GenBank Accession No. FJ687464.1, GenBank Accession No. FJ687463.1, GenBank Accession No. FJ687462.1, GenBank Accession No. FJ687461.1, GenBank Accession No. FJ687460.1, GenBank Accession No. FJ687459.1, GenBank Accession No. FJ687458.1, GenBank Accession No.

FJ687457.1, GenBank Accession No. FJ687456.1, GenBank Accession No. FJ687455.1, GenBank Accession No. FJ687454.1, GenBank Accession No. FJ687453, GenBank Accession No. FJ687452.1, GenBank Accession No. FJ687451.1, GenBank Accession No. FJ687450.1, GenBank Accession No. FJ687449.1, GenBank Accession No. AF500215.1, GenBank Accession No. KF476061.1, GenBank Accession No. KF476060.1, GenBank Accession No. KF476059.1, GenBank Accession No. KF476058.1, GenBank Accession No. KF476057.1, GenBank Accession No. KF476056.1, GenBank Accession No. KF476055.1, GenBank Accession No. KF476054.1, GenBank Accession No. KF476053.1, GenBank Accession No. KF476052.1, GenBank Accession No. KF476051.1, GenBank Accession No. KF476050.1, GenBank Accession No. KF476049.1, GenBank Accession No. KF476048.1, GenBank Accession No. KF177258.1, GenBank Accession No. KF177257.1, GenBank Accession No. KF177256.1, GenBank Accession No. KF177255.1), HCoV.229E (GenBank Accession No. NC_002645), HCoV.NL63.Amsterdam.I (GenBank Accession No. NC_005831), BtCoV.HKU2.HK.298.2006 (GenBank Accession No. EF203066), BtCoV.HKU2.HK.33.2006 (GenBank Accession No. EF203067), BtCoV.HKU2.HK.46.2006 (GenBank Accession No. EF203065), BtCoV.HKU2.GD.430.2006 (GenBank Accession No. EF203064), as well as any other subgroup 1b coronavirus now known (e.g., as can be found in the GenBank® Database) or later identified, and any combination thereof.

Nonlimiting examples of a subgroup 2a coronavirus of this invention include HCoV.HKU1.C.N5 (GenBank Accession No. DQ339101), MHV.A59 (GenBank Accession No. NC_001846), PHEV.VW572 (GenBank Accession No. NC_007732), HCoV.OC43.ATCC.VR.759 (GenBank Accession No. NC_005147), bovine enteric coronavirus (BCoV.ENT) (GenBank Accession No. NC_003045), as well as any other subgroup 2a coronavirus now known (e.g., as can be found in the GenBank® Database) or later identified, and any combination thereof.

Nonlimiting examples of a subgroup 2d coronavirus of this invention include BtCoV.HKU9.2 (GenBank Accession No. EF065514), BtCoV.HKU9.1 (GenBank Accession No. NC_009021), BtCoV.HKU9.3 (GenBank Accession No. EF065515), BtCoV.HKU9.4 (GenBank Accession No. EF065516), as well as any other subgroup 2d coronavirus now known (e.g., as can be found in the GenBank® Database) or later identified, and any combination thereof.

Nonlimiting examples of a subgroup 3 coronavirus of this invention include Nonlimiting examples of a subgroup 3 coronavirus of this invention include IBV.Beaudette.IBV.p65 (GenBank Accession No. DQ001339), as well as any other subgroup 3 coronavirus now known (e.g., as can be found in the GenBank® Database) or later identified, and any combination thereof.

The present invention further provides an isolated nucleic acid molecule encoding the chimeric coronavirus spike protein of this invention. In some embodiments, a nucleic acid molecule of this invention can be a cDNA. Also provided is a vector (e.g., a viral or bacterial vector), plasmid or other nucleic acid construct comprising the isolated nucleic acid molecule of this invention.

Further provided herein is a Venezuelan equine encephalitis replicon particle (VRP) comprising the isolated nucleic acid molecule encoding the chimeric coronavirus spike protein of this invention.

In addition, the present invention provides a virus like particle (VLP) comprising the chimeric coronavirus spike protein of any of this invention and a matrix protein of any virus that can form a VLP.

The present invention also provides a coronavirus particle comprising the chimeric coronavirus spike protein of this invention.

Also provided are cells (e.g., isolated cells) comprising the vectors, nucleic acid molecules, VLPs, VRPs, and/or coronavirus particles of the invention.

Additionally provided herein is a population of any of the VLPs, VRPs and for coronavirus particles of this invention, as well as a population of virus particles that are used as viral vectors encoding the chimeric coronavirus spike protein of this invention.

The chimeric coronavirus spike proteins of this invention can be produced as recombinant proteins, e.g., in a eukaryotic cell system for recombination protein production.

The invention also provides immunogenic compositions comprising the cells, vectors, nucleic acid molecules, VLPs, VRPs, coronavirus particles and/or populations of the invention. The composition can further comprise a pharmaceutically acceptable carrier.

The present invention further provides a method of producing an immune response to a coronavirus in a subject, comprising administering to the subject an effective amount of a chimeric coronavirus spike protein, a nucleic acid molecule, a vector, a VRP, a VLP, a coronavirus particle, population and/or a composition of this invention, including any combination thereof, thereby producing an immune response to a coronavirus in the subject.

In further embodiments, the present invention provides a method of treating a coronavirus infection in a subject in need thereof, comprising administering to the subject an effective amount of a chimeric coronavirus spike protein, a nucleic acid molecule, a vector, a VRP, a VLP, a coronavirus particle, population and/or a composition of this invention, including any combination thereof, thereby treating a coronavirus infection in the subject.

Additionally provided herein is a method of preventing a disease or disorder caused by a coronavirus infection in a subject, comprising administering to the subject an effective amount of a chimeric coronavirus spike protein, a nucleic acid molecule, a vector, a VRP, a VLP, a coronavirus particle, population and/or a composition of this invention, including any combination thereof, thereby preventing a disease or disorder caused by a coronavirus infection in the subject.

Furthermore the present invention provides a method of protecting a subject from the effects of coronavirus infection, comprising administering to the subject an effective amount of a chimeric coronavirus spike protein, a nucleic acid molecule, a vector, a VRP, a VLP, a coronavirus particle, population and/or a composition of this invention, including any combination thereof, thereby protecting the subject from the effects of coronavirus infection.

The chimeric coronavirus spike proteins of this invention can be used to immunize a subject against infection by a newly emerging coronavirus, as well as treat a subject infected with a newly emerging coronavirus. For example, the chimeric subgroup 2b coronavirus spike proteins of this invention can be used to immunize against and/or treat infection by bat SARS CoV like virus strains such as Rs SHC014 (GenBank® Accession No. KC881005), Rs3367 (GenBank® Accession No. KC881006) and/or WtV1 S (GenBank® Accession No. KC881007).

In yet further embodiments, the present invention provides a method of identifying a coronavirus spike protein for administration to elicit an immune response to coronavirus in a subject infected by a coronavirus and/or a subject at risk of coronavirus infection and/or to a subject for whom eliciting an immune response to a coronavirus is needed or desired, comprising: a) contacting a sample obtained from a subject infected with a coronavirus with a panel of proteins comprising: 1) one or more chimeric coronavirus spike proteins from a subgroup 2c coronavirus, 2) one or more chimeric coronavirus spike proteins from a subgroup 2b coronavirus, 3) one or more spike proteins from a subgroup 2a coronavirus, 4) one or more chimeric coronavirus spike proteins from a subgroup 2d coronavirus, 5) one or more chimeric coronavirus spike proteins from a subgroup 1a coronavirus, 6) one or more chimeric coronavirus spike proteins from a subgroup 1b coronavirus, 7) one or more chimeric coronavirus spike proteins from a subgroup 3 coronavirus and 8) any combination of (1) through (7) above, under conditions whereby an antigen/antibody complex can form; and b) detecting formation of an antigen/antibody complex, whereby detection of formation of the antigen/antibody complex comprising the chimeric coronavirus spike protein(s) of any of (1)-(6) identifies the presence of antibodies to a spike protein of the coronavirus that is infecting the subject of (a), thereby identifying a coronavirus spike protein for administration to the subject of (a) and/or to a subject infected with a coronavirus and/or to a subject at risk of coronavirus infection and/or to a subject for whom eliciting an immune response to a coronavirus is needed or desired.

In some embodiments, the method set forth above can further comprise the step of administering the coronavirus spike protein identified according to the method to the subject of (a) and/or to a subject at risk of coronavirus infection and/or to a subject infected with a coronavirus and/or to a subject for whom eliciting an immune response to a coronavirus is needed or desired.

A method is also provided herein of identifying an antibody that neutralizes a coronavirus infecting a subject, comprising: a) isolating a coronavirus from a sample of a subject infected with a coronavirus and/or suspected of being infected with a coronavirus; b) contacting the coronavirus of (a) with a panel of antibodies comprising: 1) an antibody reactive with a chimeric coronavirus spike protein from a subgroup 2c coronavirus, 2) an antibody reactive with a chimeric coronavirus spike protein from a subgroup 2b coronavirus, 3) an antibody reactive with a chimeric coronavirus spike protein from a subgroup 2a coronavirus, 4) an antibody reactive with a chimeric coronavirus spike protein from a subgroup 2d coronavirus, 5) an antibody reactive with a chimeric coronavirus spike protein from a subgroup 1a coronavirus, 6) an antibody reactive with a chimeric coronavirus spike protein from a subgroup 1b coronavirus, 7) an antibody reactive with a chimeric coronavirus spike protein from a subgroup 3 coronavirus, and 8) any combination of (1) through (7) above, to form respective coronavirus/antibody compositions, each comprising a respective antibody of the panel; c) contacting each of the respective coronavirus/antibody compositions of (b) with cells susceptible to coronavirus infection under conditions whereby coronavirus infection can occur; and d) detecting the presence or absence of infection of the cells, whereby absence of detection of infection of the cells contacted with any of the coronavirus/antibody compositions of (b) identi-

fies the antibody of that coronavirus/antibody composition as an antibody that neutralizes the coronavirus infecting the subject.

In some embodiments, the method set forth above can further comprise the step of administering the antibody identified according to the method to the subject of (a) and/or to a subject infected with a coronavirus and/or to a subject at risk of coronavirus infection and/or to a subject for whom eliciting an immune response to a coronavirus is needed or desired.

As used herein, "a" or "an" or "the" can mean one or more than one. For example, "a" cell can mean one cell or a plurality of cells.

Also as used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative ("or").

Furthermore, the term "about," as used herein when referring to a measurable value such as an amount of a compound or agent of this invention, dose, time, temperature, and the like, is meant to encompass variations of $\pm 20\%$, $\pm 10\%$, $\pm 5\%$, $\pm 1\%$, $\pm 0.5\%$, or even $\pm 0.1\%$ of the specified amount.

As used herein, the transitional phrase "consisting essentially of" means that the scope of a claim is to be interpreted to encompass the specified materials or steps recited in the claim, "and those that do not materially affect the basic and novel characteristic(s)" of the claimed invention. See, *In re Herz*, 537 F.2d 549, 551-52, 190 USPQ 461, 463 (CCPA 1976) (emphasis in the original); see also MPEP §2111.03.

A "sample" or "biological sample" of this invention can be any biological material, such as a biological fluid, an extract from a cell, an extracellular matrix isolated from a cell, a cell (in solution or bound to a solid support), a tissue, a tissue homogenate, and the like as are well known in the art.

In the methods of this invention in which formation of an antigen/antibody complex is detected, a variety of assays can be employed for such detection. For example, various immunoassays can be used to detect antibodies or proteins (antigens) of this invention. Such immunoassays typically involve the measurement of antigen/antibody complex formation between a protein or peptide (i.e., an antigen) and its specific antibody.

The immunoassays of the invention can be either competitive or noncompetitive and both types of assays are well-known and well-developed in the art. In competitive binding assays, antigen or antibody competes with a detectably labeled antigen or antibody for specific binding to a capture site bound to a solid surface. The concentration of labeled antigen or antibody bound to the capture agent is inversely proportional to the amount of free antigen or antibody present in the sample.

Noncompetitive assays of this invention can be, for example, sandwich assays, in which, for example, the antigen is bound between two antibodies. One of the antibodies is used as a capture agent and is bound to a solid surface. The other antibody is labeled and is used to measure or detect the resultant antigen/antibody complex by e.g., visual or instrument means. A number of combinations of antibody and labeled antibody can be used, as are well known in the art. In some embodiments, the antigen/antibody complex can be detected by other proteins capable of specifically binding human immunoglobulin constant regions, such as protein A, protein L or protein G. These proteins are normal constituents of the cell walls of streptococcal bacteria. They exhibit a strong nonimmunogenic reactivity with immunoglobulin

constant regions from a variety of species. (See, e.g., Kronval et al. *J. Immunol.* 111:1401-1406 (1973); Akerstrom et al. *J. Immunol.* 135:2589-2542 (1985)).

In some embodiments, the non-competitive assays need not be sandwich assays. For instance, the antibodies or antigens in the sample can be bound directly to the solid surface. The presence of antibodies or antigens in the sample can then be detected using labeled antigen or antibody, respectively.

In some embodiments, antibodies and/or proteins can be conjugated or otherwise linked or connected (e.g., covalently or noncovalently) to a solid support (e.g., bead, plate, slide, dish, membrane or well) in accordance with known techniques. Antibodies can also be conjugated or otherwise linked or connected to detectable groups such as radiolabels (e.g., ³⁵S, ¹²⁵I, ³²P, ¹³H, ¹⁴C, ¹³¹I), enzyme labels (e.g., horseradish peroxidase, alkaline phosphatase), gold beads, chemiluminescence labels, ligands (e.g., biotin) and/or fluorescence labels (e.g., fluorescein) in accordance with known techniques.

A variety of organic and inorganic polymers, both natural and synthetic can be used as the material for the solid surface. Nonlimiting examples of polymers include polyethylene, polypropylene, poly(4-methylbutene), polystyrene, polymethacrylate, poly(ethylene terephthalate), rayon, nylon, poly(vinyl butyrate), polyvinylidene difluoride (PVDF), silicones, polyformaldehyde, cellulose, cellulose acetate, nitrocellulose, and the like. Other materials that can be used include, but are not limited to, paper, glass, ceramic, metal, metalloids, semiconductive materials, cements and the like. In addition, substances that form gels, such as proteins (e.g., gelatins), lipopolysaccharides, silicates, agarose and polyacrylamides can be used. Polymers that form several aqueous phases, such as dextrans, polyalkylene glycols or surfactants, such as phospholipids, long chain (12-24 carbon atoms) alkyl ammonium salts and the like are also suitable. Where the solid surface is porous, various pore sizes can be employed depending upon the nature of the system.

A variety of immunoassay systems can be used, including but not limited to, radio-immunoassays (RIA), enzyme-linked immunosorbent assays (ELISA) assays, enzyme immunoassays (EIA), "sandwich" assays, gel diffusion precipitation reactions, immunodiffusion assays, agglutination assays, immunofluorescence assays, fluorescence activated cell sorting (FACS) assays, immunohistochemical assays, protein A immunoassays, protein G immunoassays, protein L immunoassays, biotin/avidin assays, biotin/streptavidin assays, immunoelectrophoresis assays, precipitation/flocculation reactions, immunoblots (Western blot; dot/slot blot); immunodiffusion assays; liposome immunoassay, chemiluminescence assays, library screens, expression arrays, immunoprecipitation, competitive binding assays and immunohistochemical staining. These and other assays are described, among other places, in Hampton et al. (*Serological Methods, a Laboratory Manual*, APS Press, St Paul, Minn. (1990)) and Maddox et al. (*J. Exp. Med.* 158:1211-1216 (1993)); the entire contents of which are incorporated herein by reference for teachings directed to immunoassays).

The methods of this invention can also be carried out using a variety of solid phase systems, such as described in U.S. Pat. No. 5,879,881, as well as in a dry strip lateral flow system (e.g., a "dipstick" system), such as described, for example, in U.S. Patent Publication No. 20030073147, the entire contents of each of which are incorporated by reference herein.

Embodiments of the present invention include monoclonal antibodies produced from B cells isolated from a subject of this invention that has produced an immune response against the chimeric coronavirus spike protein of this invention, wherein said monoclonal antibodies are specific to epitopes present on the chimeric coronavirus spike protein. Such monoclonal antibodies can be specific for an epitope in any of the first, second, third or fourth regions of the chimeric coronavirus spike protein of this invention as described herein.

The term "antibody" or "antibodies" as used herein refers to all types of immunoglobulins, including IgG, IgM, IgA, IgD, and IgE. The antibody can be monoclonal or polyclonal and can be of any species of origin, including, for example, mouse, rat, rabbit, horse, goat, sheep or human, or can be a chimeric or humanized antibody. See, e.g., Walker et al., *Molec. Immunol.* 26:403-11 (1989). The antibodies can be recombinant monoclonal antibodies produced according to the methods disclosed in U.S. Pat. No. 4,474,893 or U.S. Pat. No. 4,816,567. The antibodies can also be chemically constructed according to the method disclosed in U.S. Pat. No. 4,676,980. The antibody can further be a single chain antibody or bispecific antibody. The antibody can also be humanized for administration to a human subject.

Antibody fragments included within the scope of the present invention include, for example, Fab, F(ab')₂, and Fc fragments, and the corresponding fragments obtained from antibodies other than IgG. Such fragments can be produced by known techniques. For example, F(ab')₂ fragments can be produced by pepsin digestion of the antibody molecule, and Fab fragments can be generated by reducing the disulfide bridges of the F(ab')₂ fragments. Alternatively, Fab expression libraries can be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (Huse et al., (1989) *Science* 254:1275-1281).

Monoclonal antibodies can be produced in a hybridoma cell line according to the technique of Kohler and Milstein, (1975) *Nature* 265:495-97. For example, a solution containing the appropriate antigen can be injected into a mouse and, after a sufficient time, the mouse sacrificed and spleen cells obtained. The spleen cells are then immortalized by fusing them with myeloma cells or with lymphoma cells, typically in the presence of polyethylene glycol, to produce hybridoma cells. The hybridoma cells are then grown in a suitable medium and the supernatant screened for monoclonal antibodies having the desired specificity. Monoclonal Fab fragments can be produced in bacterial cell such as *E. coli* by recombinant techniques known to those skilled in the art. See, e.g., W. Huse, (1989) *Science* 246:1275-81.

Antibodies can also be obtained by phage display techniques known in the art or by immunizing a heterologous host with a cell containing an epitope of interest.

"Nidovirus" as used herein refers to viruses within the order Nidovirales, including the families Coronaviridae and Arteriviridae. All viruses within the order Nidovirales share the unique feature of synthesizing a nested set of multiple subgenomic mRNAs. See M. Lai and K. Holmes, *Coronaviridae: The Viruses and Their Replication*, in Fields Virology, pg 1163, (4th Ed. 2001). Particular examples of Coronaviridae include, but are not limited to, toroviruses and coronaviruses.

"Coronavirus" as used herein refers to a genus in the family Coronaviridae, which family is in turn classified within the order Nidovirales. The coronaviruses are large, enveloped, positive-stranded RNA viruses. They have the largest genomes of all RNA viruses and replicate by a unique

mechanism that results in a high frequency of recombination. The coronaviruses include antigenic groups I, II, and III. Nonlimiting examples of coronaviruses include SARS coronavirus, MERS coronavirus, transmissible gastroenteritis virus (TGEV), human respiratory coronavirus, porcine respiratory coronavirus, canine coronavirus, feline enteric coronavirus, feline infectious peritonitis virus, rabbit coronavirus, murine hepatitis virus, sialodacryoadenitis virus, porcine hemagglutinating encephalomyelitis virus, bovine coronavirus, avian infectious bronchitis virus, and turkey coronavirus, as well as chimeras of any of the foregoing. See Lai and Holmes "Coronaviridae: The Viruses and Their Replication" in Fields *Virology*, (4th Ed. 2001).

A "nidovirus permissive cell" or "coronavirus permissive cell" as used herein can be any cell in which a coronavirus can at least replicate, including both naturally occurring and recombinant cells. In some embodiments the permissive cell is also one that the nidovirus or coronavirus can infect. The permissive cell can be one that has been modified by recombinant means to produce a cell surface receptor for the nidovirus or coronavirus.

An "isolated" nucleic acid molecule is one that is chemically synthesized (e.g., derived from reverse transcription) or is separated from other nucleic acid molecules that are present in the natural source of the nucleic acid molecule. Preferably, an "isolated" nucleic acid molecule is free of sequences (preferably protein encoding sequences) that naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated nucleic acid molecule can contain less than about 5 kB, 4 kB, 3 kB, 2 kB, 1 kB, 0.5 kB or 0.1 kB of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. (e.g., as described in Sambrook et al., eds., "Molecular Cloning: A Laboratory Manual," 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1989).

In particular embodiments, a nucleic acid of this invention has at least about 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more nucleic acid sequence homology with the sequences specifically disclosed herein. The term "homology" as used herein refers to a degree of similarity between two or more sequences. There can be partial homology or complete homology (i.e., identity). A partially homologous sequence that at least partially inhibits an identical sequence from hybridizing to a target nucleic acid is referred to using the functional term "substantially homologous." The inhibition of hybridization to the target sequence can be examined using a hybridization assay (Southern or northern blot, solution hybridization and the like) under conditions of low stringency. A substantially homologous sequence or hybridization probe will compete for and inhibit the binding of a completely homologous sequence to the target sequence under conditions of low stringency. This is not to say that conditions of low stringency are such that non-specific binding is permitted; low stringency conditions require that the binding of two sequences to one another be a specific (i.e., selective) interaction. The absence of non-specific binding can be tested by the use of a second target sequence, which lacks even a partial degree of complementarity (e.g.,

less than about 30% identity). In the absence of non-specific binding, the probe will not hybridize to the second non-complementary target sequence.

Alternatively stated, in particular embodiments, nucleic acids encoding a cDNA of a coronavirus that hybridize under the conditions described herein to the complement of the sequences specifically disclosed herein can also be used according to the present invention. The term "hybridization" as used herein refers to any process by which a first strand of nucleic acid binds with a second strand of nucleic acid through base pairing.

The term "stringent" as used here refers to hybridization conditions that are commonly understood in the art to define the commodities of the hybridization procedure. High stringency hybridization conditions that will permit homologous nucleotide sequences to hybridize to a nucleotide sequence as given herein are well known in the art. As one example, hybridization of such sequences to the nucleic acid molecules disclosed herein can be carried out in 25% formamide, 5×SSC, 5×Denhardt's solution and 5% dextran sulfate at 42° C., with wash conditions of 25% formamide, 5×SSC and 0.1% SDS at 42° C., to allow hybridization of sequences of about 60% homology. Another example includes hybridization conditions of 6×SSC, 0.1% SDS at about 45° C., followed by wash conditions of 0.2×SSC, 0.1% SDS at 50-65° C. Another example of stringent conditions is represented by a wash stringency of 0.3 M NaCl, 0.03M sodium citrate, 0.1% SDS at 60-70° C. using a standard hybridization assay (see SAMBROOK et al., EDS., MOLECULAR CLONING: A LABORATORY MANUAL 2d ed. (Cold Spring Harbor, N.Y. 1989, the entire contents of which are incorporated by reference herein).

The nucleic acids, proteins, peptides, viruses, vectors, particles, antibodies and populations of this invention are intended for use as therapeutic agents and immunological reagents, for example, as antigens, immunogens, vaccines, and/or nucleic acid delivery vehicles. Thus, in various embodiments, the present invention provides a composition comprising the nucleic acid, virus, vector, particle, antibody and/or population of this invention in a pharmaceutically acceptable carrier. The compositions described herein can be formulated for use as reagents (e.g., to produce antibodies) and/or for administration in a pharmaceutical carrier in accordance with known techniques. See, e.g., Remington, *The Science And Practice of Pharmacy* (latest edition).

In embodiments of this invention wherein a chimeric coronavirus spike protein is being administered, delivered and/or introduced into a subject, e.g., to elicit or induce an immune response, the protein can be administered, delivered and/or introduced into the subject as a protein present in an inactivated (e.g., inactivated through UV irradiation or formalin treatment) coronavirus. The protein or active fragment thereof of this invention can be administered, delivered and/or introduced into the subject according to any method now known or later identified for administration, introduction and/or delivery of protein or active fragment thereof, as would be well known to one of ordinary skill in the art. Nonlimiting examples include administration of the protein or fragment with a protease inhibitor or other agent to protect it from degradation and/or with a polyalkylene glycol moiety (e.g., polyethylene glycol).

In some embodiments, the coronavirus protein or active fragment thereof can be administered to a subject as a nucleic acid molecule, which can be a naked nucleic acid molecule or a nucleic acid molecule present in a vector (e.g., a delivery vector, which in some embodiments can be a VRP). The nucleic acids and vectors of this invention can be

administered orally, intranasally, parenterally (e.g., intravenously), by intramuscular injection, by intraperitoneal injection, transdermally, extracorporeally, topically or the like. In the methods described herein which include the administration and uptake of exogenous DNA into the cells of a subject (i.e., gene transduction or transfection), the nucleic acids of the present invention can be in the form of naked DNA or the nucleic acids can be in a vector for delivering the nucleic acids to the cells for expression of the polypeptides and/or fragments of this invention. The vector can be a commercially available preparation or can be constructed in the laboratory according to methods well known in the art.

Delivery of the nucleic acid or vector to cells can be via a variety of mechanisms, including but not limited to recombinant vectors including bacterial, viral and fungal vectors, liposomal delivery agents, nanoparticles, and gene gun related mechanisms.

As one example, delivery can be via a liposome, using commercially available liposome preparations such as LIPOFECTIN, LIPOFECTAMINE (GIBCO-BRL, Inc., Gaithersburg, Md.), SUPERFECT (Qiagen, Inc. Hilden, Germany) and TRANSFECTAM (Promega Biotec, Inc., Madison, Wis.), as well as other liposomes developed according to procedures standard in the art. In addition, the nucleic acid or vector of this invention can be delivered in vivo by electroporation, the technology for which is available from Genetronics, Inc. (San Diego, Calif.) as well as by means of a SONOPORATION machine (ImaRx Pharmaceutical Corp., Tucson, Ariz.).

As one example, vector delivery can be via a viral system, such as a retroviral vector system, which can package a recombinant retroviral genome. The recombinant retrovirus can then be used to infect and thereby deliver to the infected cells nucleic acid encoding the polypeptide and/or fragment of this invention. The exact method of introducing the exogenous nucleic acid into mammalian cells is, of course, not limited to the use of retroviral vectors. Other techniques are widely available for this procedure including the use of adenoviral vectors, alphaviral vectors (e.g., VRPs), adeno-associated viral (AAV) vectors, lentiviral vectors, pseudotyped retroviral vectors and vaccinia viral vectors, as well as any other viral vectors now known or developed in the future. Physical transduction techniques can also be used, such as liposome delivery and receptor-mediated and other endocytosis mechanisms. This invention can be used in conjunction with any of these or other commonly used gene transfer methods.

If ex vivo methods are employed, cells or tissues can be removed and maintained outside the body according to standard protocols well known in the art. The nucleic acids and vectors of this invention can be introduced into the cells via any gene transfer mechanism, such as, for example, virus-mediated gene delivery, calcium phosphate mediated gene delivery, electroporation, microinjection or proteoliposomes. The transduced cells can then be infused (e.g., in a pharmaceutically acceptable carrier) or transplanted back into the subject per standard methods for the cell or tissue type. Standard methods are known for transplantation or infusion of various cells into a subject.

Parenteral administration of the peptides, polypeptides, nucleic acids and/or vectors of the present invention, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution of suspension in liquid prior to injection, or as emulsions. As used herein, "parenteral administration" includes intradermal, intranasal, subcutaneous, intramuscular, intraperitoneal, intravenous

and intratracheal routes, as well as a slow release or sustained release system such that a constant dosage is maintained. See, e.g., U.S. Pat. No. 3,610,795, which is incorporated by reference herein in its entirety.

In the manufacture of a pharmaceutical composition according to embodiments of the present invention, the composition of this invention is typically admixed with, inter alia, a pharmaceutically acceptable carrier. By "pharmaceutically acceptable carrier" is meant a carrier that is compatible with other ingredients in the pharmaceutical composition and that is not harmful or deleterious to the subject. A "pharmaceutically acceptable" component such as a salt, carrier, excipient or diluent of a composition according to the present invention is a component that (i) is compatible with the other ingredients of the composition in that it can be combined with the compositions of the present invention without rendering the composition unsuitable for its intended purpose, and (ii) is suitable for use with subjects as provided herein without undue adverse side effects (such as toxicity, irritation, and allergic response). Side effects are "undue" when their risk outweighs the benefit provided by the composition. Non-limiting examples of pharmaceutically acceptable components include, without limitation, any of the standard pharmaceutical carriers such as phosphate buffered saline solutions, water, emulsions such as oil/water emulsion, microemulsions and various types of wetting agents. A pharmaceutically acceptable carrier can comprise, consist essentially of or consist of one or more synthetic components (e.g., components that do not naturally occur in nature), as are known in the art.

The carrier may be a solid or a liquid, or both, and is preferably formulated with the composition of this invention as a unit-dose formulation. The pharmaceutical compositions are prepared by any of the well-known techniques of pharmacy including, but not limited to, admixing the components, optionally including one or more accessory ingredients. Exemplary pharmaceutically acceptable carriers include, but are not limited to, sterile pyrogen-free water and sterile pyrogen-free physiological saline solution. Such carriers can further include protein (e.g., serum albumin) and sugar (sucrose, sorbitol, glucose, etc.)

The pharmaceutical compositions of this invention include those suitable for oral, rectal, topical, inhalation (e.g., via an aerosol) buccal (e.g., sub-lingual), vaginal, parenteral (e.g., subcutaneous, intramuscular, intradermal, intraarticular, intrapleural, intraperitoneal, intracerebral, intraarterial, or intravenous), topical (i.e., both skin and mucosal surfaces, including airway surfaces) and transdermal administration. The compositions herein may also be administered via a skin scarification method, or transdermally via a patch or liquid. The compositions may be delivered subdermally in the form of a biodegradable material that releases the compositions over a period of time. The most suitable route in any given case will depend, as is well known in the art, on such factors as the species, age, gender and overall condition of the subject, the nature and severity of the condition being treated and/or on the nature of the particular composition (i.e., dosage, formulation) that is being administered.

A subject of this invention is any animal that is capable of producing an immune response against a coronavirus. A subject of this invention can also be any animal that is susceptible to infection by coronavirus and/or susceptible to diseases or disorders caused by coronavirus infection. A subject of this invention can be a mammal and in particular embodiments is a human, which can be an infant, a child, an adult or an elderly adult. A "subject at risk of infection by a

coronavirus” or a “subject at risk of coronavirus infection” is any subject who may be or has been exposed to a coronavirus.

As used herein, an “effective amount” refers to an amount of a compound or composition that is sufficient to produce a desired effect, which can be a therapeutic, prophylactic and/or beneficial effect.

Thus, the present invention provides a method of inducing or eliciting an immune response in a subject, comprising administering to the subject an effective amount of a virus, vector, particle, population and/or composition of this invention.

The present invention also provides a method of treating and/or preventing a coronavirus infection in a subject, comprising administering to the subject an effective amount of a virus, vector, particle, population and/or composition of this invention.

Also as used herein, the terms “treat,” “treating” and “treatment” include any type of mechanism, action or activity that results in a change in the medical status of a subject, including an improvement in the condition of the subject (e.g., change or improvement in one or more symptoms and/or clinical parameters), delay in the progression of the condition, delay of the onset of a disease or illness, etc.

One nonlimiting example of an effective amount of a virus or virus particle (e.g., VRP) of this invention is from about 10^4 to about 10^{10} , preferably from about 10^5 to about 10^9 , and in particular from about 10^6 to about 10^8 infectious units (IU, as measured by indirect immunofluorescence assay), or virus particles, per dose, which can be administered to a subject, depending upon the age, species and/or condition of the subject being treated. For subunit vaccines (e.g., purified antigens) a dose range of from about 1 to about 100 micrograms can be used. As would be well known to one of ordinary skill in the art, the optimal dosage would need to be determined for any given antigen or vaccine, e.g., according to the method of production and resulting immune response.

As one example, if the nucleic acid of this invention is delivered to the cells of a subject in an adenovirus vector, the dosage for administration of adenovirus to humans can range from about 10^7 to 10^9 plaque forming units (pfu) per injection, but can be as high as 10^{12} , 10^{15} and/or 10^{20} pfu per injection. Ideally, a subject will receive a single injection. If additional injections are necessary, they can be repeated at daily/weekly/monthly intervals for an indefinite period and/or until the efficacy of the treatment has been established. As set forth herein, the efficacy of treatment can be determined by evaluating the symptoms and clinical parameters described herein and/or by detecting a desired immunological response.

The exact amount of the nucleic acid or vector required will vary from subject to subject, depending on the species, age, weight and general condition of the subject, the particular nucleic acid or vector used, its mode of administration and the like. Thus, it is not possible to specify an exact amount for every nucleic acid or vector. However, an appropriate amount can be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein.

For administration of serum or antibodies, as one non-limiting example, a dosage range of from about 20 to about 40 international Units/Kilogram can be used, although it would be well understood that optimal dosage for administration to a subject of this invention needs to be determined, e.g., according to the method of production and resulting immune response.

In some embodiments of the present invention, the compositions can be administered with an adjuvant. As used herein, “adjuvant” describes a substance, which can be any immunomodulating substance capable of being combined with the polypeptide or nucleic acid vaccine to enhance, improve or otherwise modulate an immune response in a subject without deleterious effect on the subject.

Non-limiting examples of adjuvants that can be used in the vaccine of the present invention include the RIBI adjuvant system (Ribi Inc., Hamilton, Mont.), alum, mineral gels such as aluminum hydroxide gel, oil-in-water emulsions, water-in-oil emulsions such as, e.g., Freund’s complete and incomplete adjuvants, Block copolymer (CytRx, Atlanta Ga.), QS-21 (Cambridge Biotech Inc., Cambridge Mass.), SAF-M (Chiron, Emeryville Calif.), AMPHIGENT™, adjuvant, saponin, Quil A or other saponin fraction, monophosphoryl lipid A, and Avridine lipid-amine adjuvant. Non-limiting examples of oil-in-water emulsions useful in the vaccine of the invention include modified SEAM62 and SEAM 1/2 formulations. Modified SEAM62 is an oil-in-water emulsion containing 5% (v/v) squalene (Sigma), 1% (v/v) SPAN™ 85 detergent (ICI Surfactants), 0.7% (v/v) TWEEN™ 80 detergent (ICI Surfactants), 2.5% (v/v) ethanol, 200 µg/ml Quil A, 100 µg/ml cholesterol, and 0.5% (v/v) lecithin. Modified SEAM 1/2 is an oil-in-water emulsion comprising 5% (v/v) squalene, 1% (v/v) SPAN™ 85 detergent, 0.7% (v/v) Tween 80 detergent, 2.5% (v/v) ethanol, 100 µg/ml Quil A, and 50 µg/ml cholesterol. Other immunomodulatory agents that can be included in the vaccine include, e.g., one or more interleukins, interferons, or other known cytokines.

In some embodiments, VEE replicon vectors can be used to express coronavirus structural genes in producing combination vaccines. Dendritic cells, which are professional antigen-presenting cells and potent inducers of T-cell responses to viral antigens, are preferred targets of VEE and VEE replicon particle infection, while SARS coronavirus targets the mucosal surfaces of the respiratory and gastrointestinal tract. As the VEE and coronavirus replicon RNAs synergistically interact, two-vector vaccine systems are feasible that may result in increased immunogenicity when compared with either vector alone. Combination prime-boost vaccines (e.g., DNA immunization and vaccinia virus vectors) have dramatically enhanced the immune response (notably cellular responses) against target papillomavirus and lentivirus antigens compared to single-immunization regimens (Chen et al. (2000) *Vaccine* 18:2015-2022; Gonzalo et al. (1999) *Vaccine* 17:887-892; Hanke et al. (1998) *Vaccine* 16:439-445; Pancholi et al. (2000). *J. Infect. Dis.* 182:18-27). Using different recombinant viral vectors (influenza and vaccinia) to prime and boost may also synergistically enhance the immune response, sometimes by an order of magnitude or more (Gonzalo, et al. (1999) *Vaccine* 17:887-892). Thus, the present invention also provides methods of combining different recombinant viral vectors (e.g., VEE and coronavirus) in prime boost protocols.

Examples

A Multivalent Vaccine that Elicits Broader Protection Against Emerging Human Coronaviruses

Replicon particles (VRPs) based on Venezuelan Equine Encephalitis Virus (VEEV) have been successfully used as vector platforms to deliver a variety of antigens. However, the requirement of wild type VEEV proteins for packaging restricts their production to biological safety laboratory level

3 (BSL3) containment and the risk of generation of wild-type VEEV through recombination imposes a high risk for use of these VRPs in humans. To circumvent this issue, we constructed VRPs using attenuated VEEV strain 3526, which can be packaged under biological safety laboratory level 2 (BSL2). Using Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) Spike protein (S) as a model antigen, we show that the VRP 3526 vaccine platform (VRP 3526 S) is equally efficacious in antigen production, antibody induction and protecting young and aged mice from lethal SARS disease caused by homologous and heterologous strains of SARS-CoV.

SARS-CoV originated from a pool of heterologous viruses circulating in bats, confounding vaccine and therapeutic design should future outbreaks emerge. To address this issue, the VRP 3526 platform was used and a synthetically designed chimeric S protein containing different regions of S proteins from of BtCoV HKU3, SARS CoV S and BtCoV 279 S was constructed in V3526 backbone (Chimera S). Chimera S was efficiently expressed and was recognized by polyclonal serum to SARS-CoV. Chimera S was also effective in protecting mice from SARS disease induced by several divergent strains of SARS CoV belonging to subgroup 2b. A zoonotic lethal challenge HKU3 virus from subgroup 2b (HKU3-SRBD-MAv) was then created where receptor binding domain (RBD) from HKU3 Spike was replaced by SARS-CoV RBD. Serial passage of this virus in mice resulted in severe airway disease and lethality. The Chimera S vaccine and SARS-CoV S vaccine was successful in eliciting complete protection from weight loss

and viral replication caused by HKU3-SRBD-MAv, where as BtCoV 279 S and BtCoV HKU 3 S elicited partial protection.

Collectively, these studies describe the generation of a safe VRP platform that can be manufactured under BSL2 and also demonstrate a strategy for broadening vaccine efficacy for epidemic and closely related zoonotic pools which may emerge in the future.

The results as shown in FIGS. 10-18 demonstrate: 1) the generation of a VRP 3526 platform that can be prepared under BSL2; 2) that the VRP 3526 platform has efficacy in young and aged models of SARS disease; 3) the generation of a subgroup specific Chimeric S protein vaccine for coronaviruses; 4) the creation of a subgroup specific lethal zoonotic challenge virus (HKU3-SRBD-MAv) that is representative of a virus that may emerge into the human population in the future; 5) the generation of a Chimera S vaccine that is effective in protection from divergent strains of lethal SARS CoV and HKU3-SRBD-MAv; 6) that a Chimeric Spike vaccine design can be effectively applied to coronaviruses from other subgroups; and 7) that the VRP 3526 platform and chimeric spike vaccine design can be broadly applicable to other zoonotic viruses that may emerge into humans.

All publications, patent applications, patents and other references cited herein are incorporated by reference in their entirety for the teachings relevant to the sentence and/or paragraph in which the reference is presented.

The invention is described by the following claims, with equivalents of the claims to be included therein.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 8

<210> SEQ ID NO 1
 <211> LENGTH: 1259
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: chimeric coronavirus spike protein

<400> SEQUENCE: 1

```

Met Lys Ile Leu Ile Phe Ala Phe Leu Ala Asn Leu Ala Lys Ala Gln
1             5             10             15

Glu Gly Cys Gly Ile Ile Ser Arg Lys Pro Gln Pro Lys Met Ala Gln
20             25             30

Val Ser Ser Ser Arg Arg Gly Val Tyr Tyr Asn Asp Asp Ile Phe Arg
35             40             45

Ser Asp Val Leu His Leu Thr Gln Asp Tyr Phe Leu Pro Phe Asp Ser
50             55             60

Asn Leu Thr Gln Tyr Phe Ser Leu Asn Val Asp Ser Asp Arg Tyr Thr
65             70             75             80

Tyr Phe Asp Asn Pro Ile Leu Asp Phe Gly Asp Gly Val Tyr Phe Ala
85             90             95

Ala Thr Glu Lys Ser Asn Val Ile Arg Gly Trp Ile Phe Gly Ser Ser
100            105            110

Phe Asp Asn Thr Thr Gln Ser Ala Val Ile Val Asn Asn Ser Thr His
115            120            125

Ile Ile Ile Arg Val Cys Asn Phe Asn Leu Cys Lys Glu Pro Met Tyr
130            135            140

Thr Val Ser Arg Gly Thr Gln Gln Asn Ala Trp Val Tyr Gln Ser Ala
145            150            155            160

```


-continued

Phe Asn Cys Thr Tyr Asp Arg Val Glu Lys Ser Phe Gln Leu Asp Thr
 165 170 175

Thr Pro Lys Thr Gly Asn Phe Lys Asp Leu Arg Glu Tyr Val Phe Lys
 180 185 190

Asn Arg Asp Gly Phe Leu Ser Val Tyr Gln Thr Tyr Thr Ala Val Asn
 195 200 205

Leu Pro Arg Gly Leu Pro Thr Gly Phe Ser Val Leu Lys Pro Ile Leu
 210 215 220

Lys Leu Pro Phe Gly Ile Asn Ile Thr Ser Tyr Arg Val Val Met Ala
 225 230 235 240

Met Phe Ser Gln Thr Thr Ser Asn Phe Leu Pro Glu Ser Ala Ala Tyr
 245 250 255

Tyr Val Gly Asn Leu Lys Tyr Ser Thr Phe Met Leu Arg Phe Asn Glu
 260 265 270

Asn Gly Thr Ile Thr Asp Ala Val Asp Cys Ser Gln Asn Pro Leu Ala
 275 280 285

Glu Leu Lys Cys Thr Ile Lys Asn Phe Asn Val Asp Lys Gly Ile Tyr
 290 295 300

Gln Thr Ser Asn Phe Arg Val Ser Pro Thr Gln Glu Val Ile Arg Phe
 305 310 315 320

Pro Asn Ile Thr Asn Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr
 325 330 335

Lys Phe Pro Ser Val Tyr Ala Trp Glu Arg Lys Lys Ile Ser Asn Cys
 340 345 350

Val Ala Asp Tyr Ser Val Leu Tyr Asn Ser Thr Phe Phe Ser Thr Phe
 355 360 365

Lys Cys Tyr Gly Val Ser Ala Thr Lys Leu Asn Asp Leu Cys Phe Ser
 370 375 380

Asn Val Tyr Ala Asp Ser Phe Val Val Lys Gly Asp Asp Val Arg Gln
 385 390 395 400

Ile Ala Pro Gly Gln Thr Gly Val Ile Ala Asp Tyr Asn Tyr Lys Leu
 405 410 415

Pro Asp Asp Phe Met Gly Cys Val Leu Ala Trp Asn Thr Arg Asn Ile
 420 425 430

Asp Ala Thr Ser Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg
 435 440 445

His Gly Lys Leu Arg Pro Phe Glu Arg Asp Ile Ser Asn Val Pro Phe
 450 455 460

Ser Pro Asp Gly Lys Pro Cys Thr Pro Pro Ala Leu Asn Cys Tyr Trp
 465 470 475 480

Pro Leu Asn Asp Tyr Gly Phe Tyr Thr Thr Thr Gly Ile Gly Tyr Gln
 485 490 495

Pro Tyr Arg Val Val Val Leu Ser Phe Glu Leu Leu Asn Ala Pro Ala
 500 505 510

Thr Val Cys Gly Pro Lys Leu Ser Thr Asp Leu Val Lys Asn Gln Cys
 515 520 525

Val Asn Phe Asn Phe Asn Gly Leu Lys Gly Thr Gly Val Leu Thr Ser
 530 535 540

Ser Ser Lys Arg Phe Gln Ser Phe Gln Gln Phe Gly Arg Asp Thr Ser
 545 550 555 560

Asp Phe Thr Asp Ser Val Arg Asp Pro Gln Thr Leu Glu Ile Leu Asp
 565 570 575