



US009884895B2

(12) **United States Patent**  
**Baric et al.**

(10) **Patent No.:** **US 9,884,895 B2**  
(45) **Date of Patent:** **Feb. 6, 2018**

(54) **METHODS AND COMPOSITIONS FOR CHIMERIC CORONAVIRUS SPIKE PROTEINS**

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(\* ) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **15/124,992**

(22) PCT Filed: **Mar. 20, 2015**

(86) PCT No.: **PCT/US2015/021773**

§ 371 (c)(1),

(2) Date: **Sep. 9, 2016**

(87) PCT Pub. No.: **WO2015/143335**

PCT Pub. Date: **Sep. 24, 2015**

(65) **Prior Publication Data**

US 2017/0096455 A1 Apr. 6, 2017

**Related U.S. Application Data**

(60) Provisional application No. 61/968,279, filed on Mar. 20, 2014.

(51) **Int. Cl.**

**C07K 16/10** (2006.01)

**A61K 39/12** (2006.01)

**A61K 39/215** (2006.01)

**A61K 39/395** (2006.01)

**A61K 45/06** (2006.01)

**C07K 14/005** (2006.01)

**C12N 7/00** (2006.01)

**A61K 39/00** (2006.01)

(52) **U.S. Cl.**

CPC ..... **C07K 14/005** (2013.01); **A61K 39/12** (2013.01); **C07K 16/10** (2013.01); **C12N 7/00** (2013.01); **A61K 2039/5256** (2013.01); **A61K 2039/5258** (2013.01); **A61K 2039/70** (2013.01); **C07K 2317/34** (2013.01); **C07K 2317/76** (2013.01); **C12N 2770/20022** (2013.01); **C12N 2770/20034** (2013.01); **C12N 2770/36123** (2013.01); **C12N 2770/36143** (2013.01)

(58) **Field of Classification Search**

CPC .. **A61K 39/12**; **A61K 2039/505**; **C07K 16/10**; **C07K 2317/76**; **C12N 2770/20034**

See application file for complete search history.

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(57) **ABSTRACT**

The present invention provides compositions and methods comprising a chimeric coronavirus spike protein.

**23 Claims, 19 Drawing Sheets**

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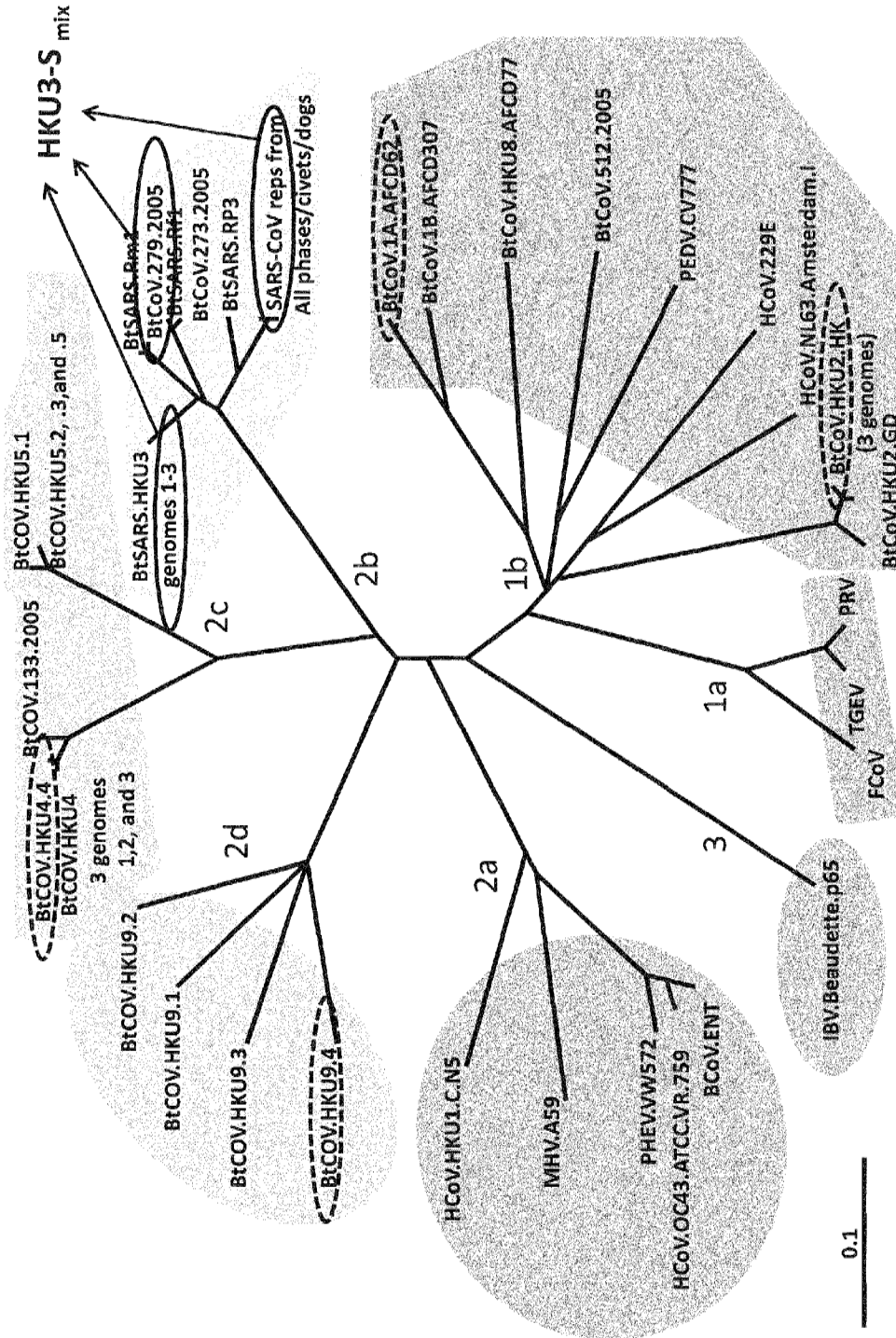
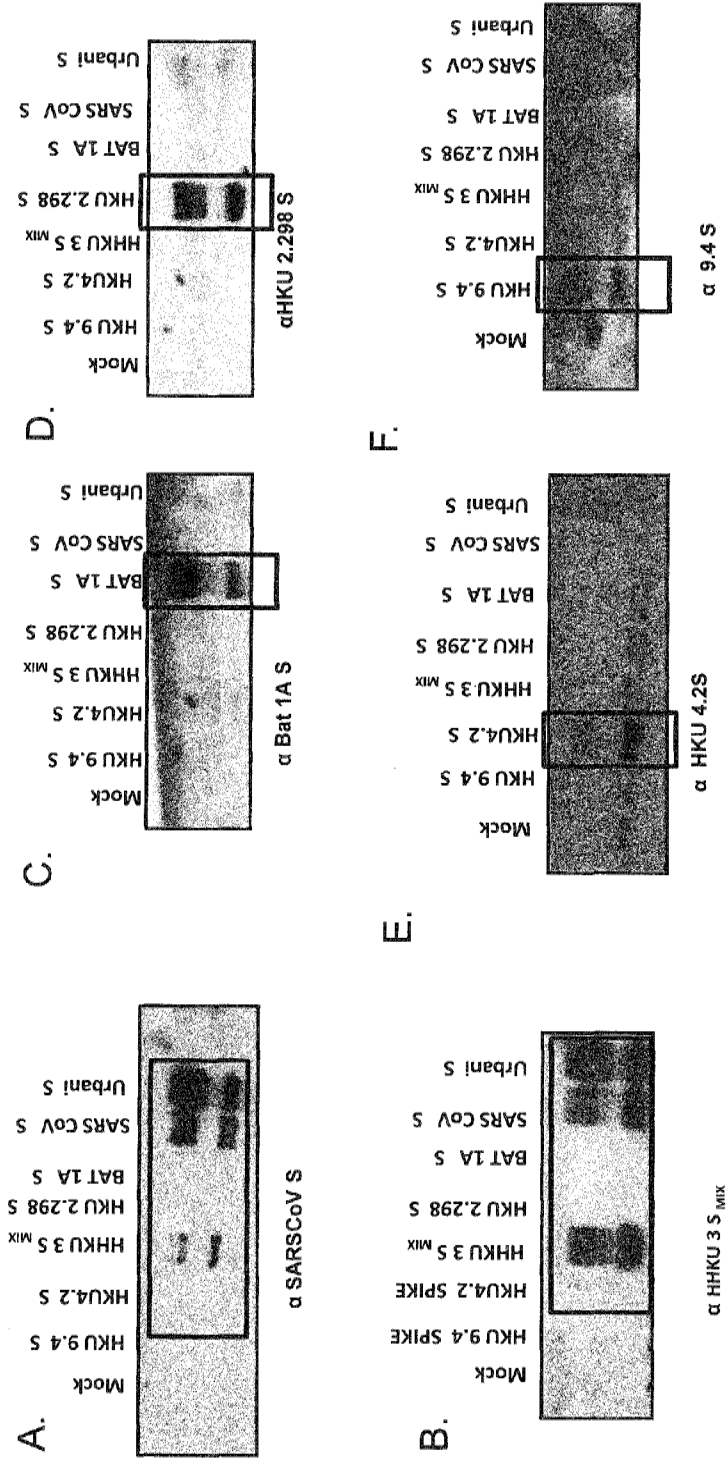


Fig. 1







\* No cross reactivity across groups

Fig. 3

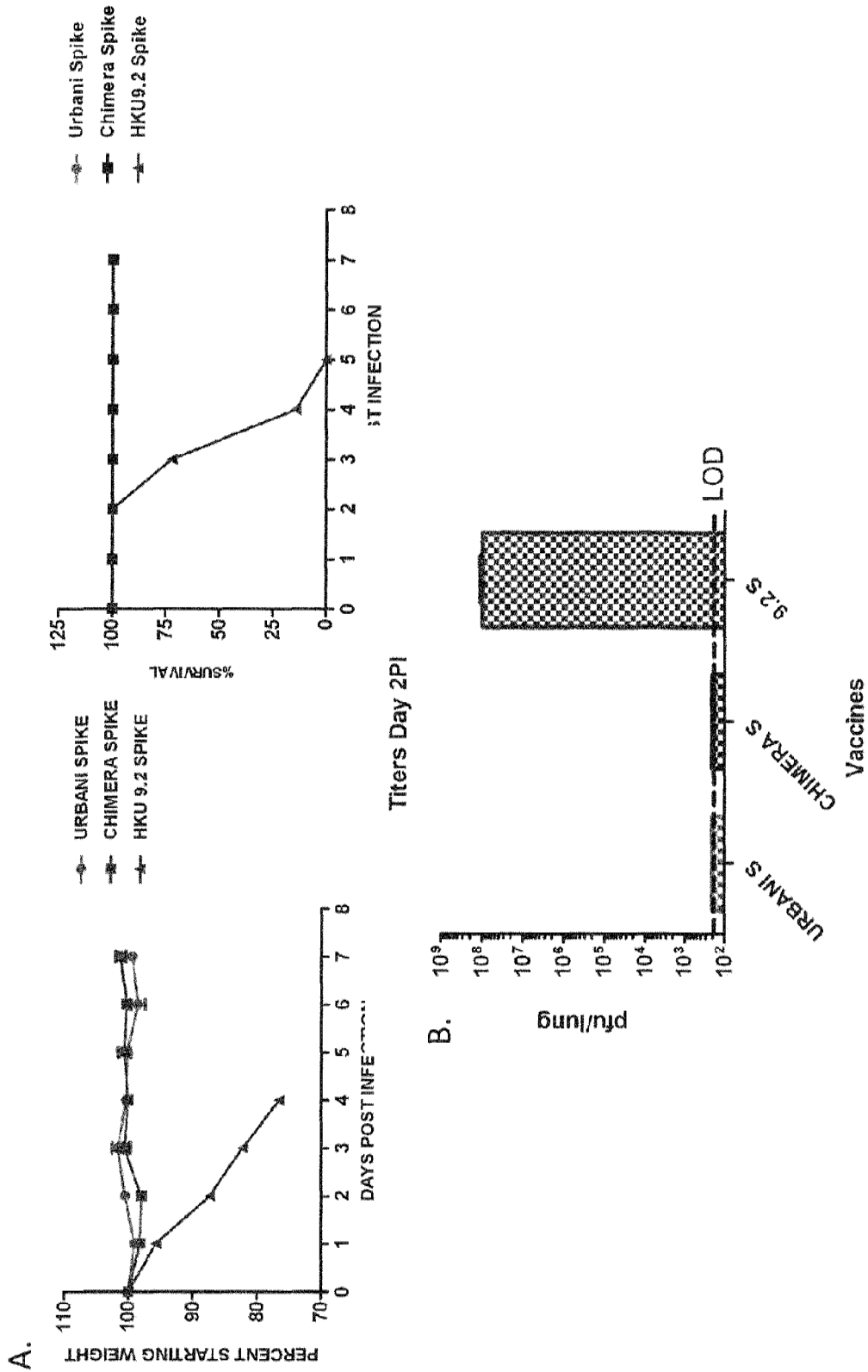


Fig. 4

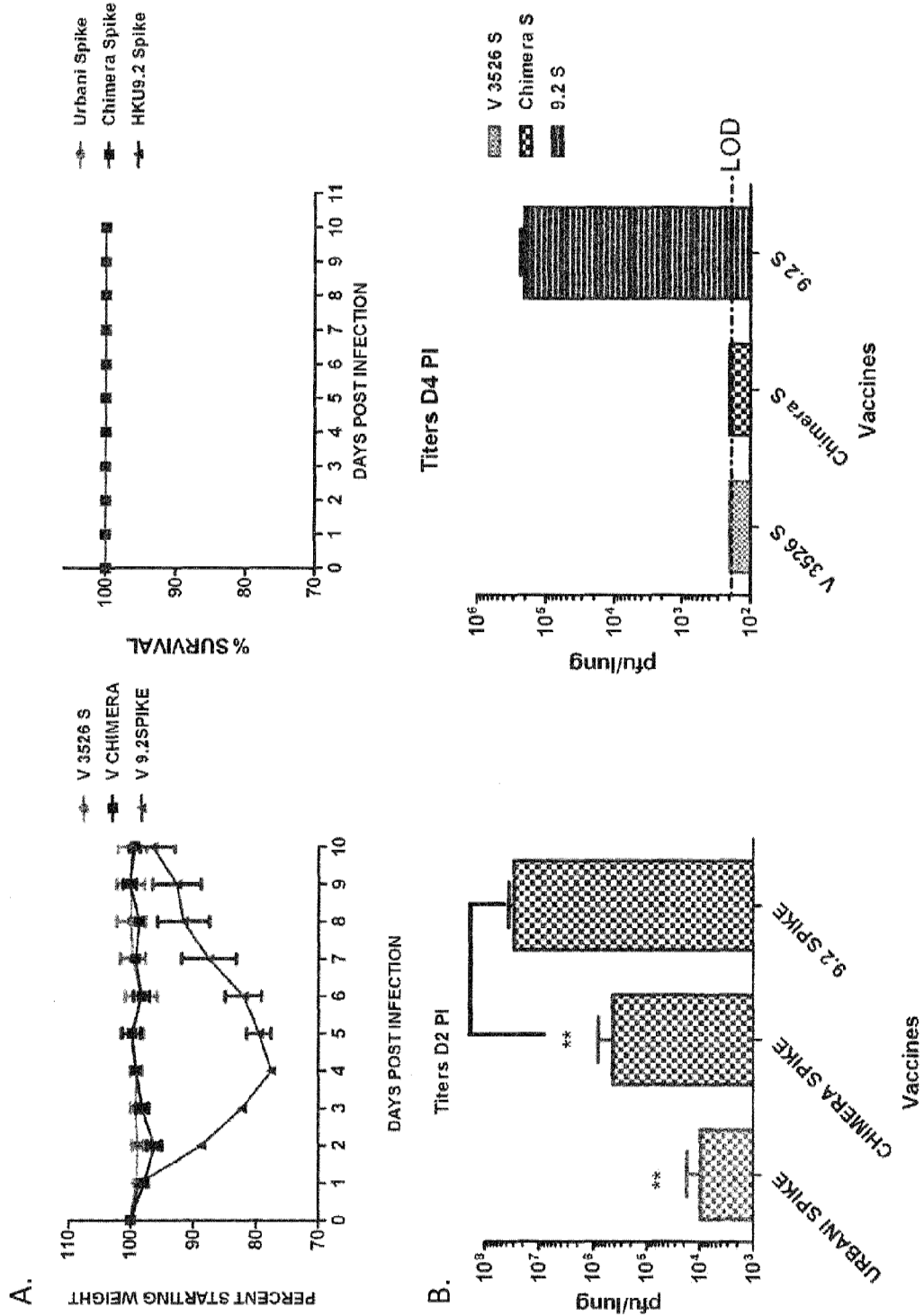


Fig. 5

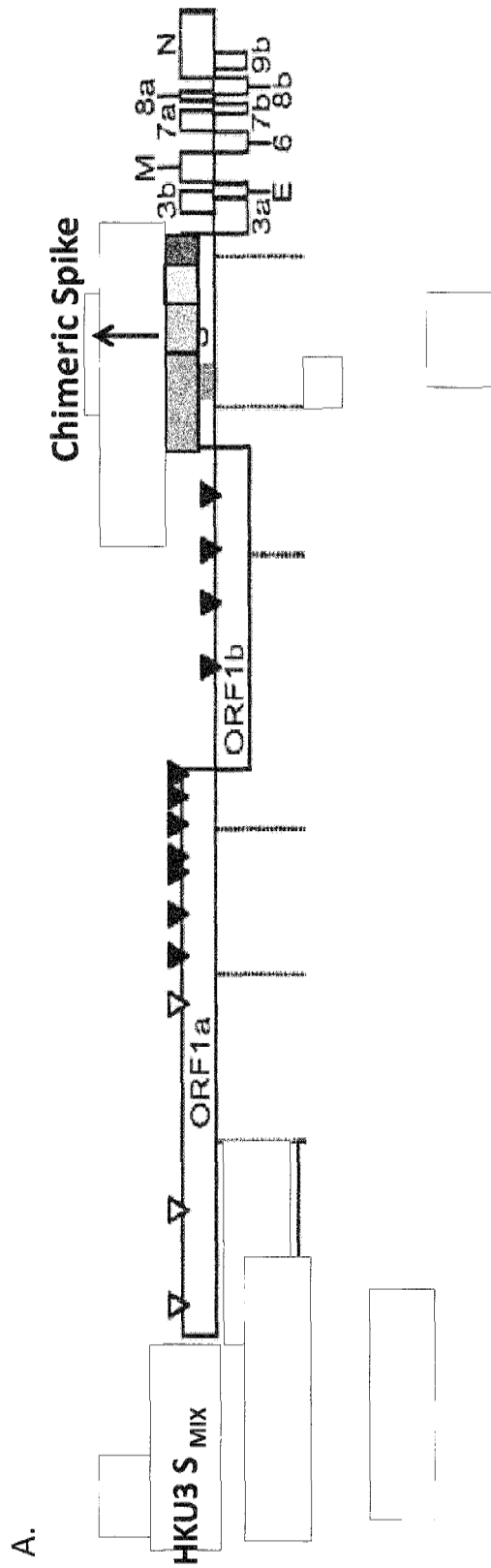


Fig. 6

B.

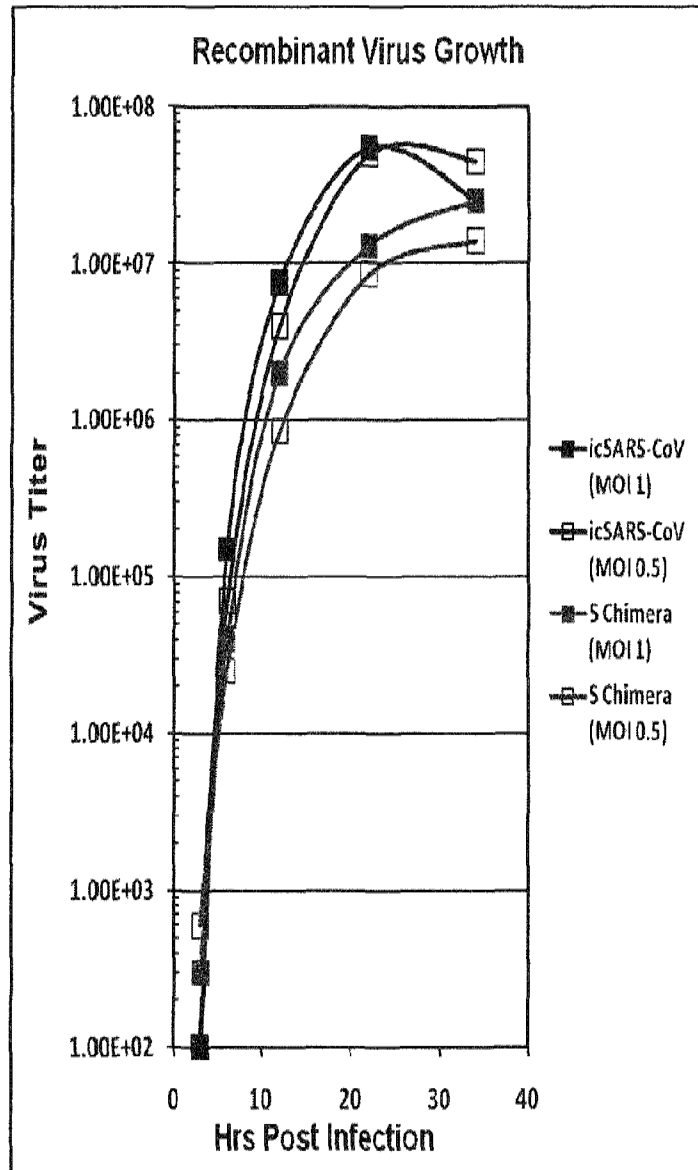
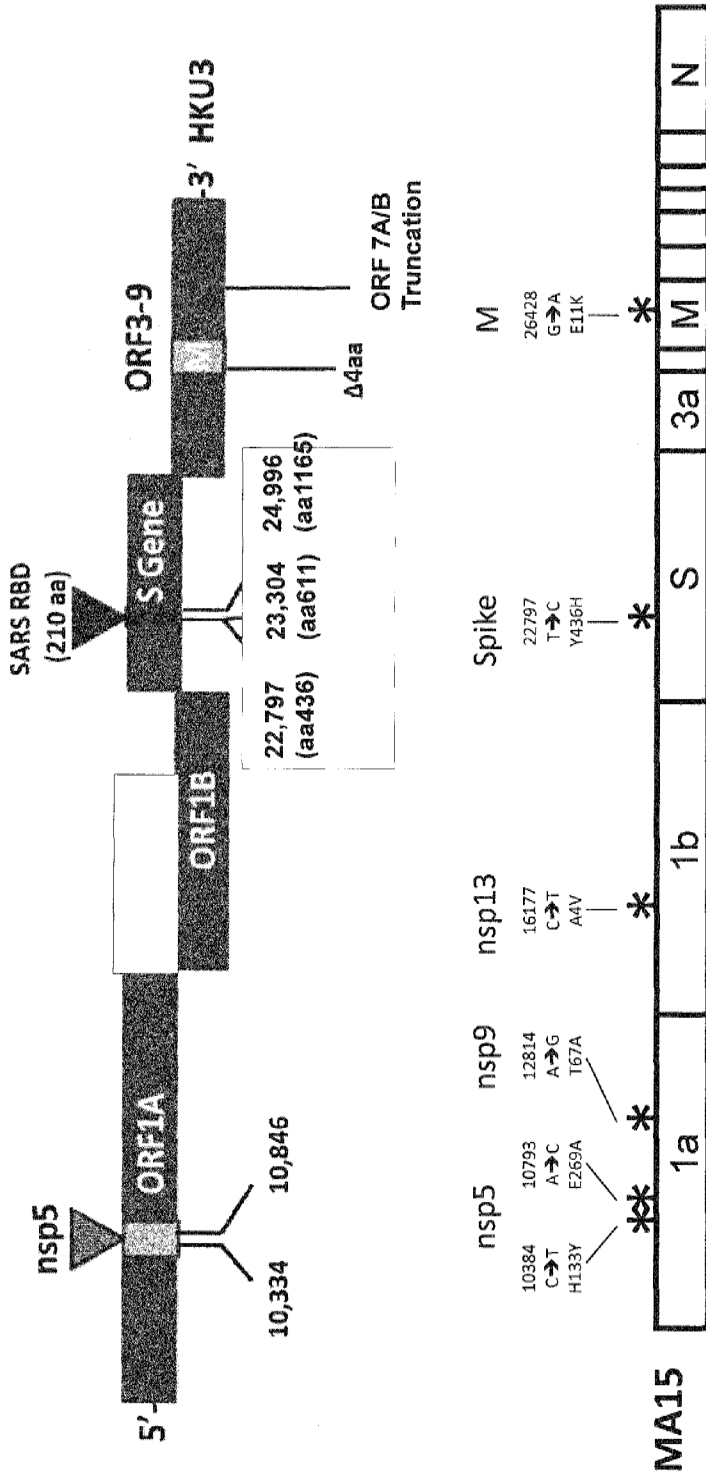


Fig.6 (cont'd.)



- nsp5, S and M common to MA 15
  - Identifies mutation spectra for cross-species transmission
- \*Sequencing in progress

Fig. 7

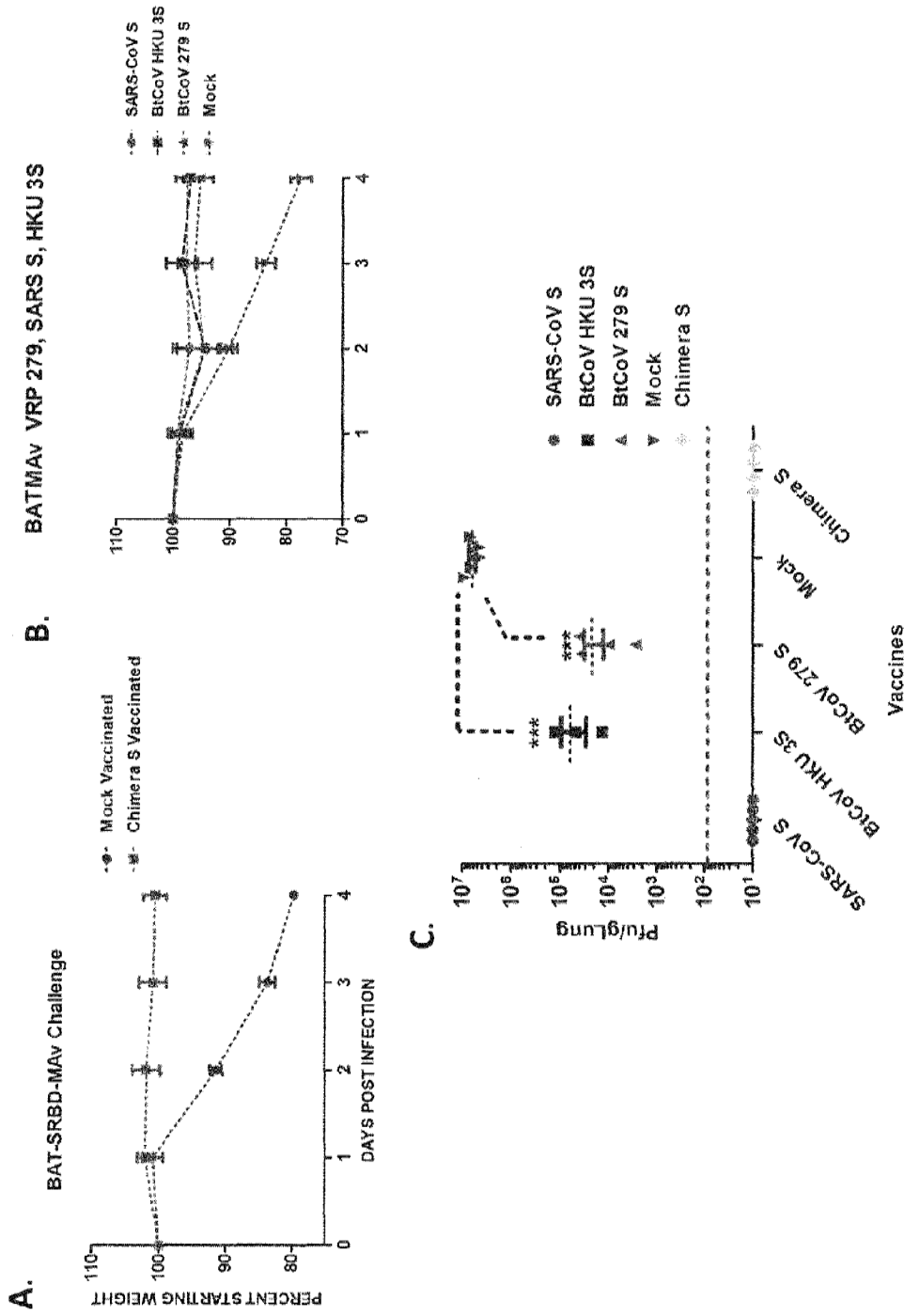


Fig. 8



# Recombinant S Gene Constructs

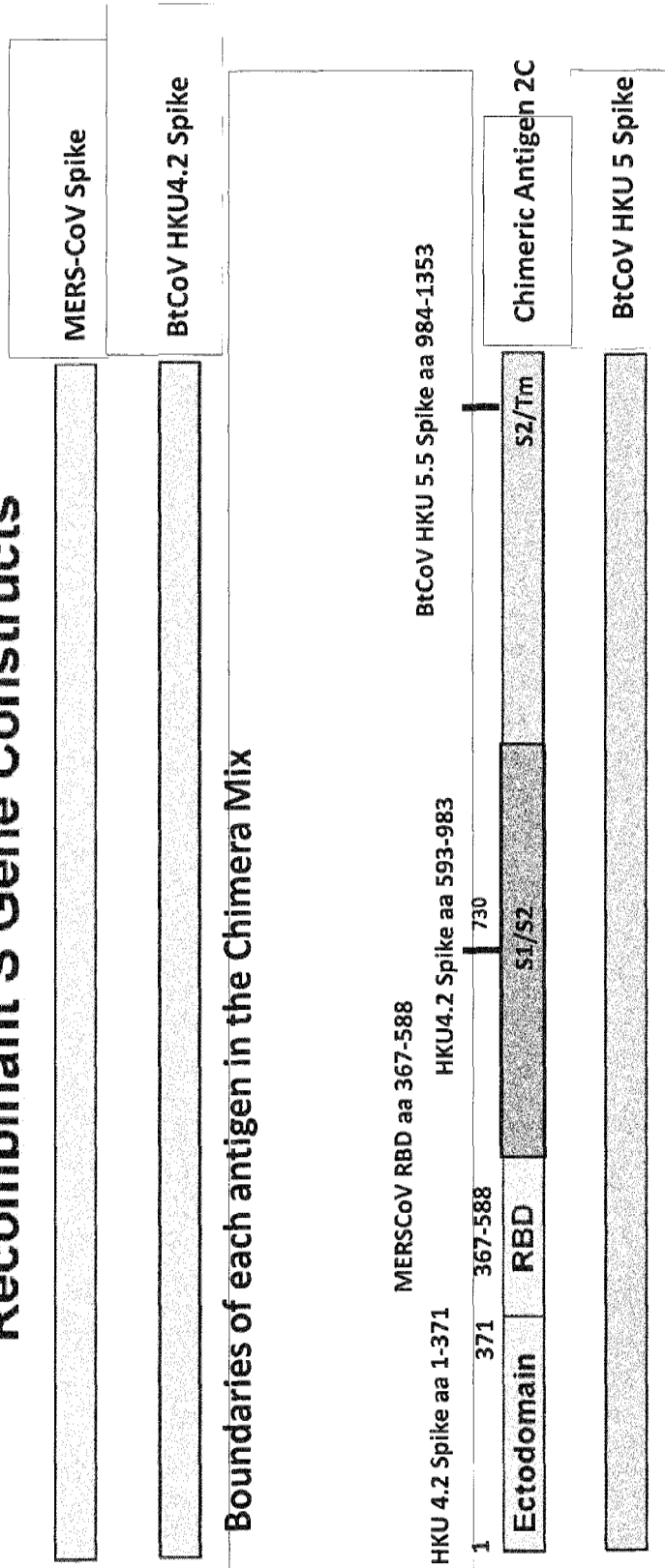


Fig. 9

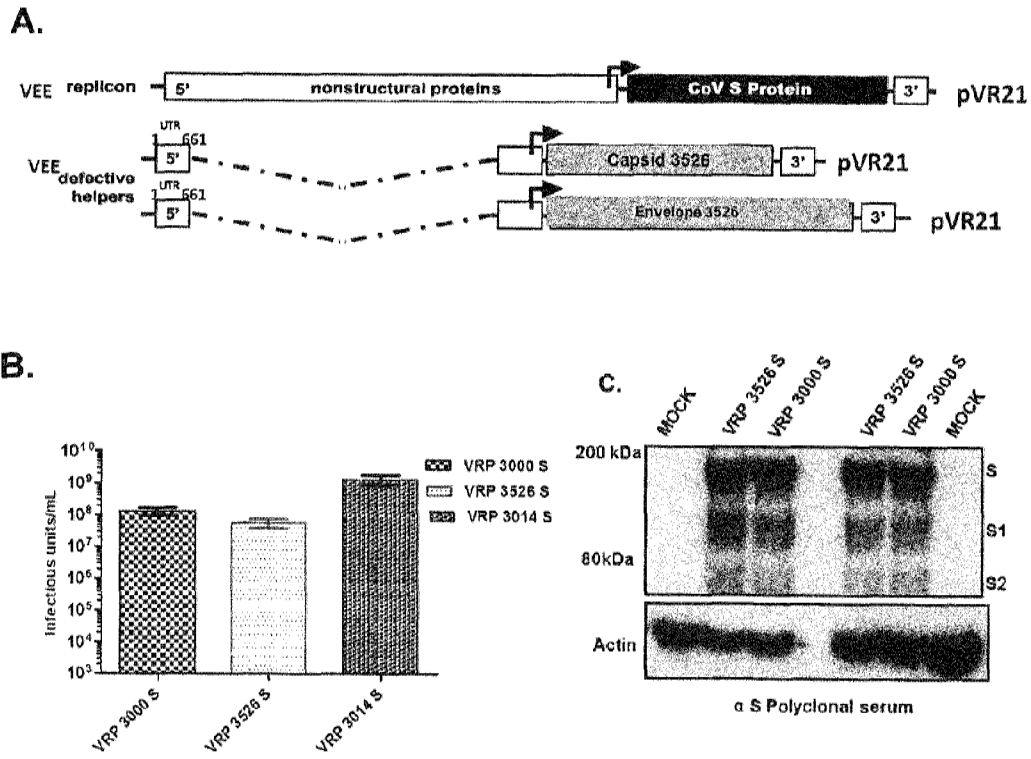


Fig. 10

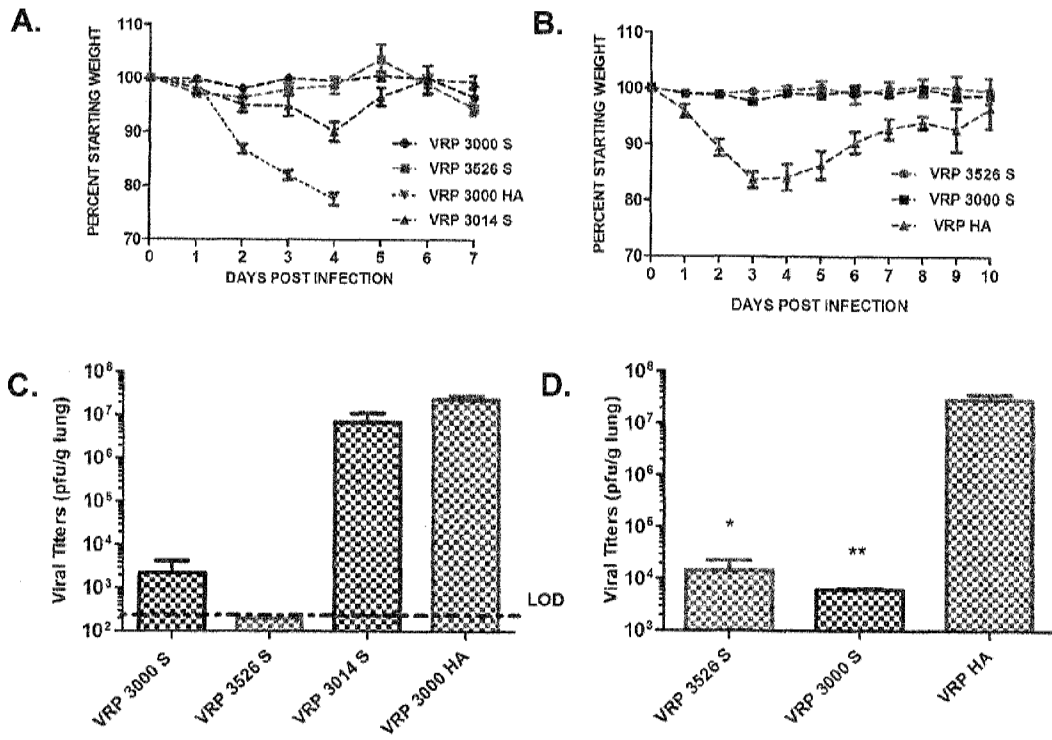


Fig. 11

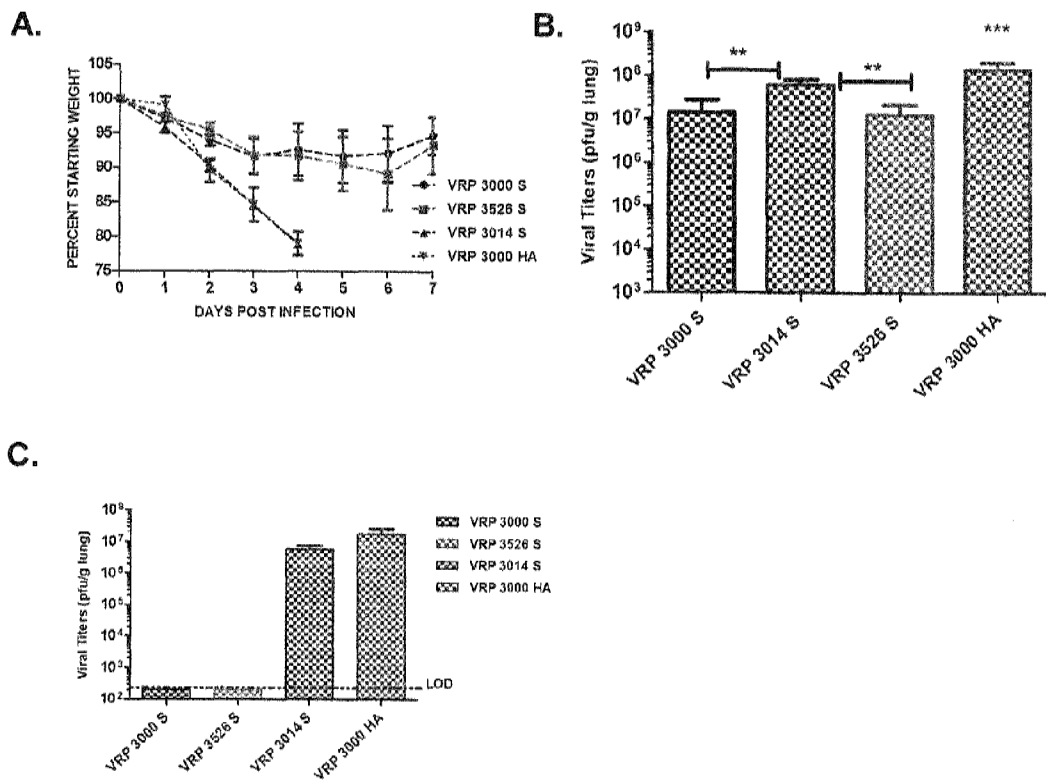


Fig. 12

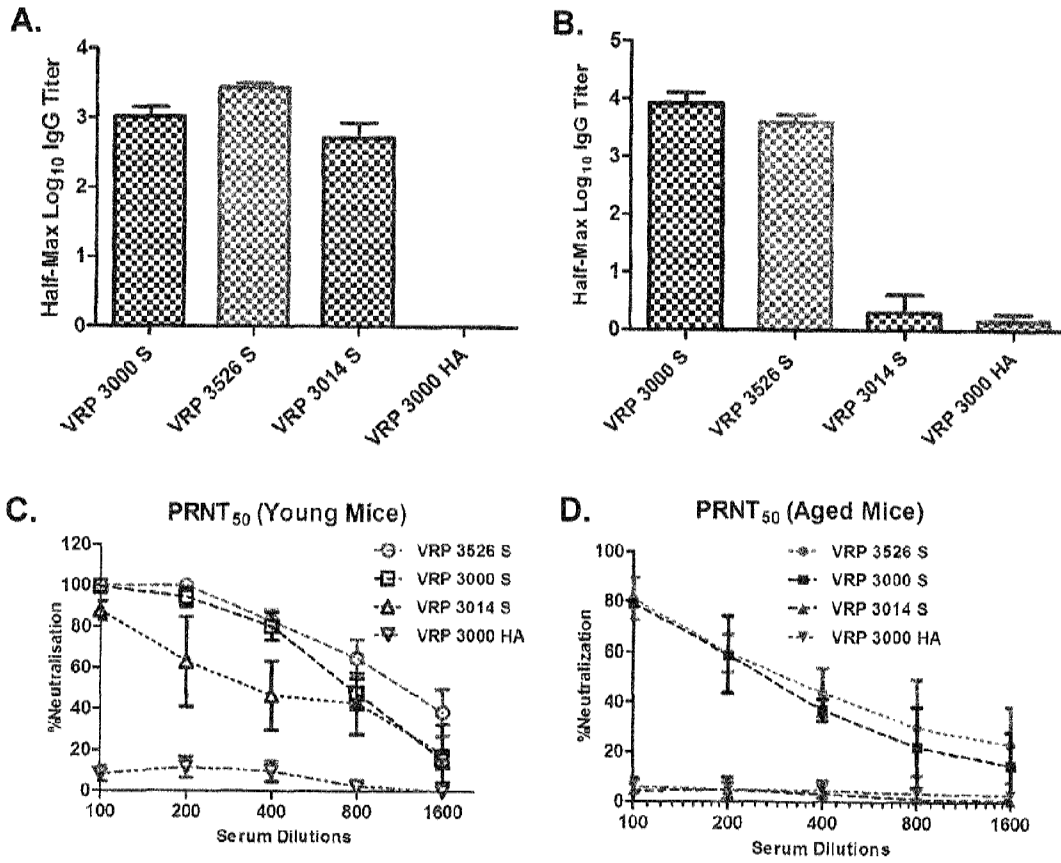


Fig. 13

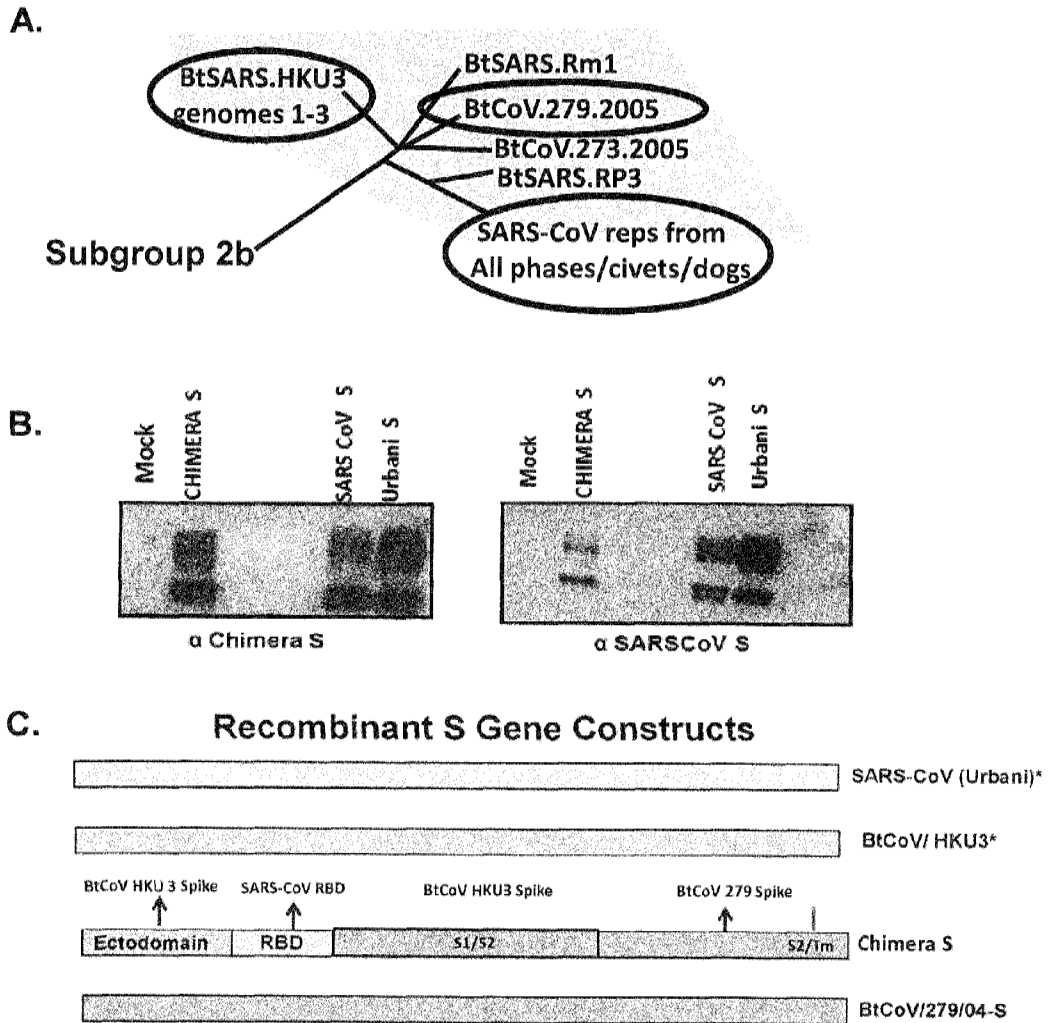


Fig. 14

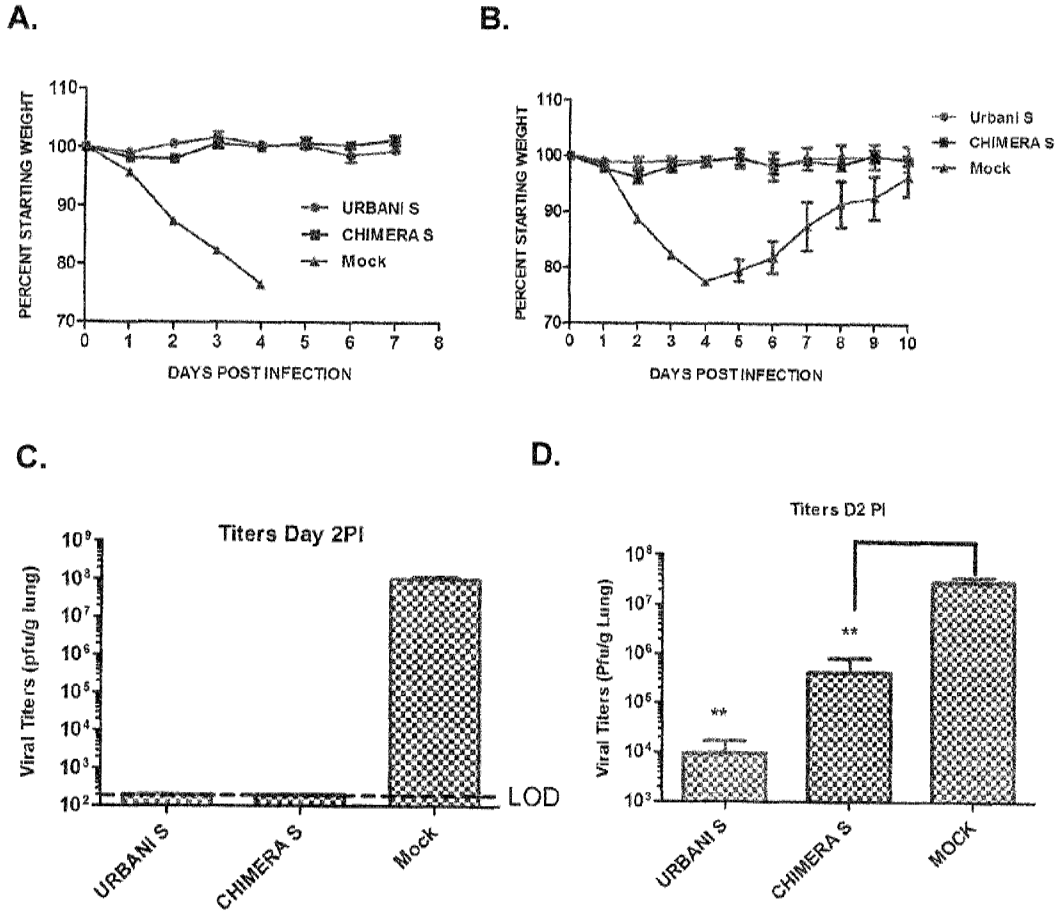


Fig. 15

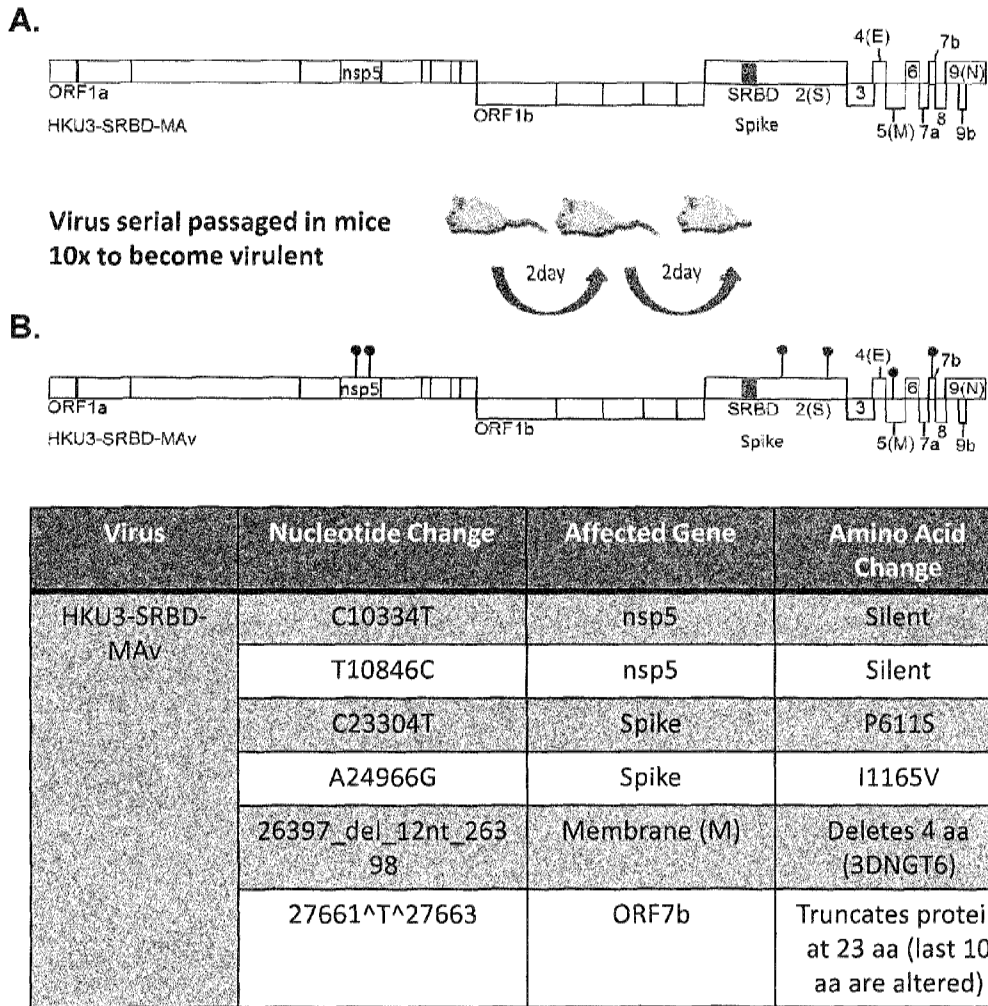


Fig. 16



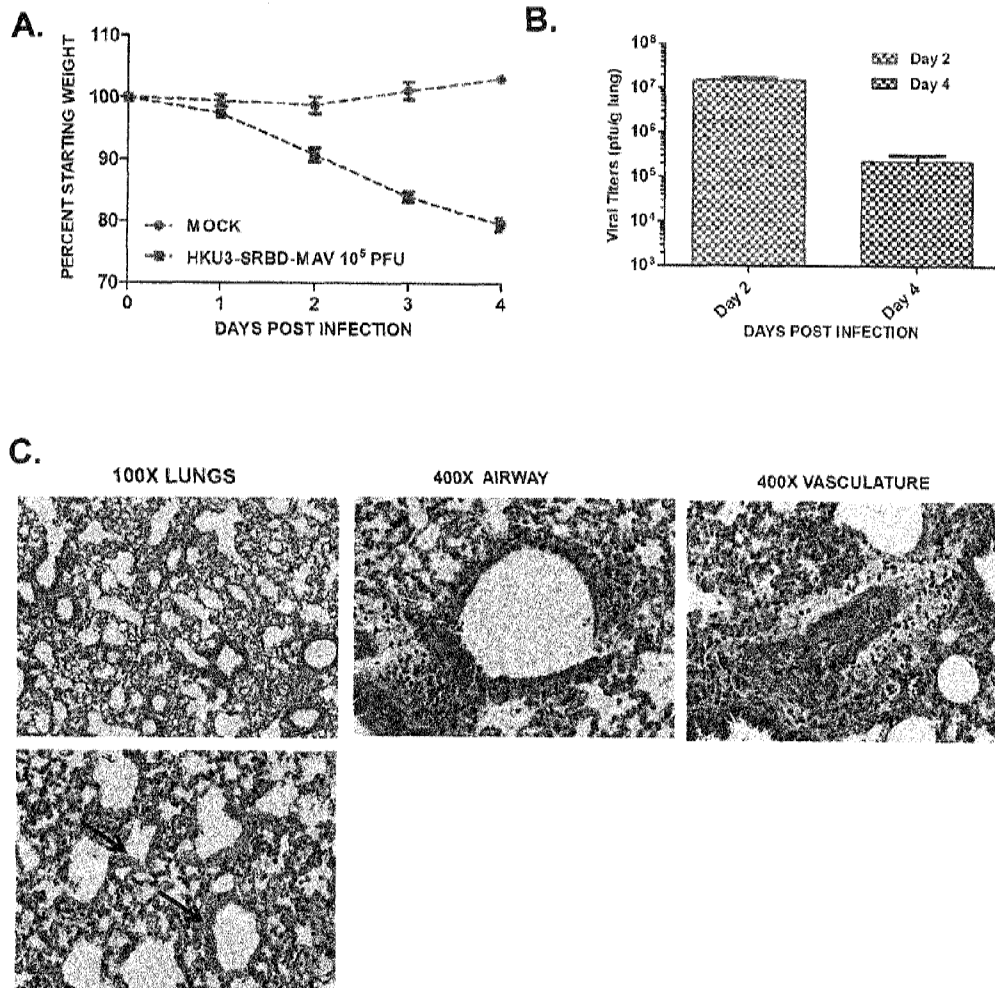
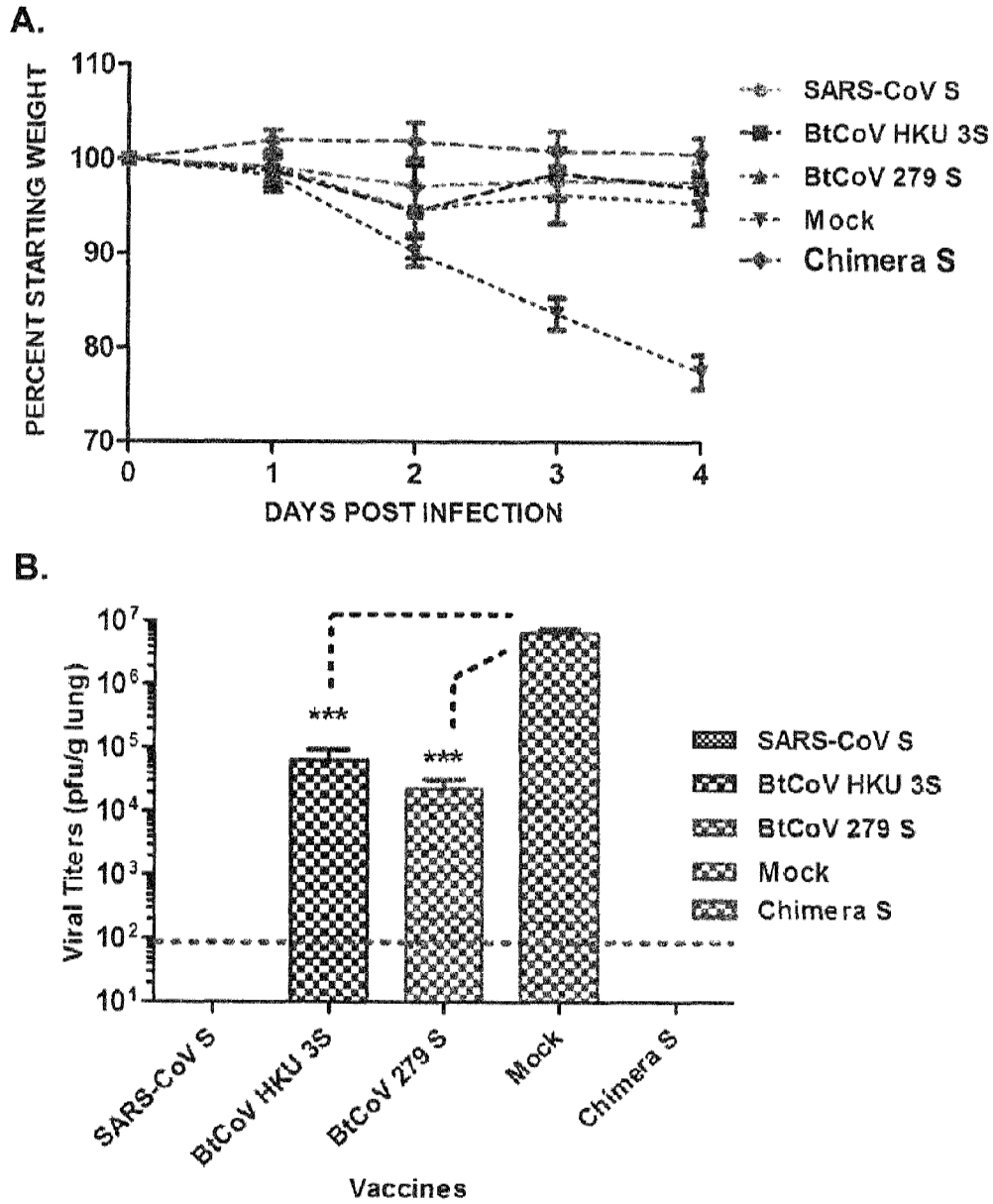


Fig. 17



**Fig. 18**

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## 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-

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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/

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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<sub>MIX</sub>), 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)

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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<sub>MIX</sub> protects against lethal SARS CoV challenge. Panel A. Percent weight loss of young Balb/C mice immunized with chimeric Antigen HKU 3 S<sub>MIX</sub>, 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<sub>MIX</sub> and SARS S.

FIG. 5. Chimeric antigen HKU 3 S<sub>MIX</sub> 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<sub>MIX</sub>, 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<sub>MIX</sub> and SARS S.

FIG. 6. Schematic of the HKU2 virus with the chimeric antigen HKU 3 S<sub>MIX</sub>. Panel A. The HKU3 virus which has the chimeric antigen HKU 3 S<sub>MIX</sub> is shown. The open reading frames are indicated. Panel B. Growth curve of HKU 3 virus with the chimeric spike antigen HKU 3 S<sub>MIX</sub>. The HKU3 virus which has the chimeric spike antigen HKU 3 S<sub>MIX</sub> 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<sub>MIX</sub> 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 VPR 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.

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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 PRNT<sub>50</sub> 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.

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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<sub>v</sub>, 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 51 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

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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

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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 MKILIFAPLA NLAKAQEGCG IISRKQPKM AQVSSRRGV YYNDIFRSD VLHLTQDYFL  
 61 PFDSNLTQYF SLNVDSDRYT YFDNPILDFG DGVYFAATEK SNVIRGWIFG SFDNTTQSA  
 121 VIVNNSTHII IRVCNFNLC EPMYTVSRGT QQANAVYQSA FNCTYDRVEK SFQLDTPKT  
 181 GNFKDLREYV FKNRDGFLSV YQTYTAVNLP RGLPTGFSVL KPIKLPFGI NITSYRVVMA  
 241 MFSQTTSNFL PESAAYYVGN LKYSTFMLRF NENGTITDAV DCSQNPLAEL KCTIKNFNVD  
 301 KGIYQTSNFR VSPTQEVIRF **PNITNLCPPG** EVFNATKFPS *VYAWERKKIS* *NCVADYSVLY*  
 361 NSTPFSTFKC YGVSATKLNLD LCFSNVYADS FVVKGDDVRQ IAPGQTGVIA DYNYKLPDDE  
 421 MGCVLAWNTR NIDATSTGNY NYKYRYLRHG KLRPFERDIS NVPFSPDGKP CTPPALNCYW  
 481 PLNDYGfYTT TGIGYQPYRV VVLSFELLNA PATVCGPKLS TDLVKNQCVN FNFNGLKGTG  
 541 VITSSSKRFQ SFQQFGRDTS DFTDSVRDPQ TLEILDISPC SFGGVSVITP GTNASSEVAV  
 601 LYQDVNCTDV PTAIRADQLT PAWRVYSTGV NVFQTQAGCL IGAHEVNASY ECDIPIGAGI  
 661 CASYHTASVL RSTGQKSIVA YTMSLGAENS IAYANNSIAI PTNFSISVTT EVMPVSMKAT  
 721 AVDCTMYICG DSLECSNLLL QYGSFCTQLN RALTGIAIEQ DKNTQEVFAQ VKQMYKTPAI  
 781 KDFGGFNFSQ ILPDPSKPTK RSFIEDLLFN KVTLADAGFM KQYGDCLGDV SARDLICAQK  
 841 FNGLTVLPL LTDEMVAAYT AALVSGTATA GWTFGAGSAL QIPFAMQMay RFNGIGVTQN  
 901 VLYENQKQIA NQFNKAISQI QESLTTTSTA LGKLQDVVND NAQALNTLVK QLSSNFGAIS  
 961 SVLNDILSRL DKVEAEVQID RLITGRLQSL QTYVTQQLIR AAEIRASANL AATKMSECVL  
 1021 GQSKRVDFCG KGYHLSFPQ AAPHGVVFLH VTYVPSQERN FTTAPAICHE GKAYFPREGV  
 1081 FVSNGTSWFI TQRNFYSPQI ITDNTFVAG NCDVVIGIIN NTVYDPLQPE LDSFKEELDK  
 1141 YFKNHTSPDV DLGDISGINA SVVNIQKEID RLNEVAKNLN ESLIDLQELG KYEQYIKWPW  
 1201 YVWLGFIAGL IAIVMVTILL CCMTSCCSCL KGACSCGSSC KFDEDDSEPV 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 MKILIFAPLA NLAKAQEGCG IISRKQPKM AQVSSRRGV YYNDIFRSD VLHLTQDYFL  
 61 PFDSNLTQYF SLNVDSDRYT YFDNPILDFG DGVYFAATEK SNVIRGWIFG SFDNTTQSA  
 121 VIVNNSTHII IRVCNFNLC EPMYTVSRGT QQANAVYQSA FNCTYDRVEK SFQLDTPKT  
 181 GNFKDLREYV FKNRDGFLSV YQTYTAVNLP RGLPTGFSVL KPIKLPFGI NITSYRVVMA  
 241 MFSQTTSNFL PESAAYYVGN LKYSTFMLRF NENGTITDAV DCSQNPLAEL KCTIKNFNVD  
 301 KGIYQTSNFR VSPTQEVIRF **PNITNRCPPD** *KVFNATRFPN* *VYAWERTKIS* *DCVADYTVLY*

-continued

361 N~~ST~~SFSTFKC YGVSPSKLID LCFTSVYADT FLIRSSSEVRQ VAPGETGVIA DYN~~Y~~KLPPDDF  
 421 TGC~~V~~IAWNTA KHD~~T~~GNYYR SHR~~K~~TKLKP ERDLSSDDGN GVYTLSTYDF NPNVPVAYQA  
 481 TRVVVLSFEL LNAPATVCGP KLSTELVKNO CVNFNFNGLK GTGVLTS~~SS~~K RFQSFQ~~Q~~FGR  
 541 DTSDFTDSVR DPQ~~T~~LEILDI SPCSF~~G~~GVSV ITPGTN~~S~~SE VAVLYQDVNC TDVPTAIRAD  
 601 QLTPAMRVYS TGVNVFQ~~T~~QA GCLIGAEHVN ASYEC~~D~~IPIG AGICASYHTA SVLRSTGQKS  
 661 IVAYTMSLGA ENSIAYANNS IAIP~~T~~NFSIS VTTEVMPVSM AKTAVDCTMY ICGDSLECSN  
 721 LLLQYGSFCT QLN~~R~~ALTGIA IEQDK~~N~~TQEV FAQVKQMYKT PAIKDFGGFN FSQILPDP~~S~~K  
 781 PTKRSFIEDL LFNKVTLADA GFMKQYGDCL GDVSARDLIC AQKFNGLTVL PPLLTDEMVA  
 841 AYTAALVSGT ATAGWTFGAG AALQIPFAMQ MAYRFNGIGV TQNVLYENQK LIANQFNSAI  
 901 GKIQESLSST ASALGKLQDV VNQNAQALNT LVKQLSSNFG AISSVLNDIL SRLDKVEAEV  
 961 QIDRLITGRL QSLQTYVTQQ LIRAAEIRAS ANLAATKMSE CVLGQSKRVD FCGKGYHLMS  
 1021 FPQSAPHGVV FLHVTVVPSQ EKNFTTAPAI CHEGKAYFPR EGVFVSN~~G~~TS WFITQRNFYS  
 1081 PQLITTDNTF VSGNCDVVIG IINNTVYDPL QPELDSFKEE LDKYFNH~~S~~TSDVLDGDISG  
 1141 INASVVNIQK EIDRLNEVAK NLNESLIDLQ ELGKYEQYIK WPWYVWL~~G~~FI AGLIAIVMVT  
 1201 ILLCMTSCC SCLKGACSCG SCCKFDEDDS EPVLKGVK~~L~~H YT

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

1 MFIFLLFLTL TSGSDLDRCT TFDDVQAPNY TQHTSSMRGV YYPDEIFRSD TLYLTQDLFL  
 61 PFYSNVTGFH TINHTFGNPV IPPKDGIFYA ATEKSNVVRG WVFGSTMN~~N~~K SQSVIIINNS  
 121 TNVVIRACNF ELCNPPFFAV SKPMGTQHT MIFDN~~A~~FNCT FEYISDAFSL DVSEKSGNFK  
 181 HLREFVFKNK DGFLVYVYKY QPIDVVRDLP SGFNTL~~K~~PIF KLPLGINITN FRAILTAFSP  
 241 AQDIWGTSA A~~Y~~FGYLKPT TFMLKYDENG TITDAVDCSQ NPLAELKCSV KSF~~E~~IDKGIY  
 301 QTSNFRVVPVSDVVRFPNIT NLC~~P~~FFGEVFN ATK~~F~~PSVYAW ERKKISNCVA DYSVL~~N~~STF  
 361 FSTFKCYGVS ATK~~L~~N~~D~~LCFS NVYADSFVVK GDDVRQIAPG QTGVIADYNY KLPDDFMGCY  
 421 LAWNTRNIDA TSTGN~~Y~~NYKY RYLRHGKLRP FERDISNVPF SPDGK~~P~~CTPP ALN~~C~~YWPLND  
 481 YGFYTTTGIG YQPYRVV~~V~~LS FELLNAPATV CGPKLSTDLI KNQCVN~~F~~NFN GLTGTGVLT~~P~~  
 541 SSKRFQPFQ FGRDVSDFTD SVRDPKTSEI LDISPCSPGG VSVITPGTNA SSEVAVLYQD  
 601 VNCTDVSTAI HADQLTPAWR IYSTGNNVFQ TQAGCLIGAE HVDTSYEC~~D~~I PIGAGICASY  
 661 HTVSLLRSTS QKSIVAYTMS LGADSSIAYS NNTIAIPTNF SISITTEVMP VSMAKTSVDC  
 721 NMYICGDSTE CANLLQYGS FCTQLNRALS GIAAEQDRNT REVFAQVKQM YKTPTLKYFG  
 781 GFNFSQILPD PLKPTKRSFI EDLLENKVTL ADAGFMKQY~~G~~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 FFSQIITTD NTFVSGNCDV VIGIINNTVY DPLQPELDSF KEELDKYFKN  
 1141 HTSPDVLDG ISGINASVVN IQKEIDRLNE VAKNLNESLI DLQELGKYEQ YIKWPWYVWL  
 1201 GFIAGLIAIV MVTILLCCMT SCCSCLKGAC SCGSCCKFDE DDSEPV~~L~~KGV 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 MKVLIFALLF SLAKAQEGCG IISRKPQPKM EKVSSRRGV YNDDIFRSD VLHLTQDYFL  
 61 PFDSNLTQYF SLNIDSNKYT YFDNPILDFG DGVYFAATEK SNVIRGWIFG SSSFDNTTQSA  
 121 IIVNNSTHII IRVCNFNLCK EPMYTVSKGT QQSSWVYQSA FNCTYDRVEK SFQLDTAPKT  
 181 GNFKDLREYV FKNRDGFLSV YQTYTAVNLP RGFPGFVSVL RPILKLPFGI NITSYRVVMT  
 241 MFSQFNSNLF PESAAYYVGN LKYTFMLSF NENGTITDAV DCSQNPLAEL KCTIKNFNVS  
 301 KGIYQTSNFR VTPTQEVVRF PNITNRCFPD KVFNASRFPN VYAWERTKIS DCVADYTVLY  
 361 NSTSFSTFKC YGVSPSKLID LCPTSVMYADT FLIRSSSEVRQ VAPGETGVIA DYNKLPDDF  
 421 TGCVIAWNTA QDQGGQYYR SYRKEKLPKF ERDLSSDENG VYTLSTYDFY PSIPVEYQAT  
 481 RVVVLSEFELL NAPATVCGPK LSTQLVKNQC VNFNENGLRG TGVLTSSKR FQSFQQFGRD  
 541 TSDFTDSVRD PQTLEILDIS PCSFSGVSVI 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 FNKVTLAGAG FMKQYGECLG DISARDLICA QKFNGLTVLP PLLTDEMIAA  
 841 YTAALVSGTA TAGWTFGAGS ALQIPFAMQM AYRFNGIGVT QNVLYENQKQ IANQFNKAIS  
 901 QIQESLTTTS TALGKLQDVV NDNAQALNTL VKQLSSNFGA ISSVLNDILS RLDEVEAEVQ  
 961 IDRLITGRLO SLQTYVTQQL IRAAEIRASA NLAATKMSEC VLGQSKRVDF CGKGYHLMSE  
 1021 PQAAPHGVVF LHVTVPSQE RNFTTAPAIC HEGKAYFPRE GVFVSNGTSW FITQRNFYSP  
 1081 QIITDNTFV AGNCDVVIIGI INNTVYDPLQ PELDSFKEEL DKYFKNHTSP DVDLGDISGI  
 1141 NASVVNIQKE IDRLNEVAKN LNESLIDLQE LGKYEQYIKW PWYVWLGFA 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.



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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

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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 LLIFVRCDS QFVDMSPASN TSECLSQVD AAAPSKLMWP YPIDPSKVDG
61  IYPLGRTYS NITLAYTGLF PLQGDLSQY LYSVSHAVGH DGDPTKAYIS NYSLLVNDPFD
121 NGFVVRIGAA ANSTGTIVIS PSVNTKIKKA YPAFILGSSL TNSAGQPLY ANYSLTIIPD
181 GCGTVLHAFY CILKPRTVNR CPSGTGYVSY FIYETVHNDQ QSTINRNASL NSFKSFDDL
241 NCTFFNSWDI TADEKWEFG ITQDTQGVHL YSSRKGDLYG GNMFRFATLP VYEGIKYYTV
301 IPRSFRSKAN KREAWAAFV YKLHQLTYLL DFSVDGYIRR AIDCGHDDL QLHCSYTSFE
361 VDTGVYSVSS YEAKPSGSV EQAEGVECDF SPLLSGTPPQ VYNFKRLVPT NCNYNLTKLL
421 SLFSSVNDFTC SQISPAAIAS NCYSSLILDY FSYPLSMKSD LSVSSAGPIS QFNKQSFNS
481 PTCLILATVP HNLTTITKPL KYSYINKCSR LLSDDRTEVP QLVNANQYSP CVSIVPSTVW
541 EDGDYRQQL SPLEGGWLV ASGSTVAMTE QLQMGFGITV QYGTDTNSVC PKLDLGDLSL
601 ITNRLGKVD YSLYVTRGRG VFQNTAVGV KQRFVYDSF DNLVGYISDD GNYCYRVCV
661 SVPVSVIYDK STNLHATLFG SVACEHVTM MSQFSRLTQS NLRDRSNIP LQTAVGCVIG
721 LSNNLVSVD CKLPLGQSLC AVPPVSTFRS YSASQFLAV LNYTSPIVVT PINSSGFTAA
781 IPTNFSFSVT QEYIETSIQK VTDCKQYVC NGFTRCEKLL VEYQFCSKI NQALHGANLR
841 QDESVSLSY NIKTTSTQTL EYGLNGDFNL TLLQVPQIGG SSSYSRAIE DLLFDKVTIA
901 DPGYMQYDD CMKQGPQ SAR DLICAYVSG YKVLPPLYDP NMEAYTSSL LGSYIAGAGWT
961 AGLSSFAAIP FAQSMFYRLN GVGITQQVLS ENQKI IANKF NQALGAMQTG FTTNLAFNK
1021 VQDAVNANAM ALSKLAELS NTFGAISSI SDILARLDTV EQEAQIDRLI NGRLTSLNAF
1081 VAQQLVRTEA AARSAQLAQD KVNCEVKSQS KRNGFCGTGT HIVSFAINAP NGLYFFHVGY
1141 QPTSHVNATA AYGLCNTENP PKCIAPIDGY FVLNQTSTA RSSGDQHWY TGSSFFHPEP
1201 ITEANSKYVS MDVKFENLTN KLPPPLLSNS TDLDFKDELE EFFKNVSSQG PNFQEISKIN
1261 TTLNLNTEL MVLSEVVKQL NESYIDLKEL GNYTFYQKWP WYIWLGFAG LVALALCVFF
1321 ILCCTGCGTS CLGKLCNRC CDSYDEYEVE KIHVH
    
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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)

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1  MTLMLCLLMS LLIFVRCDS QFVDMSPASN TSECLSQVD AAAPSKLMWP YPIDPSKVDG
61  IYPLGRTYS NITLAYTGLF PLQGDLSQY LYSVSHAVGH DGDPTKAYIS NYSLLVNDPFD
121 NGFVVRIGAA ANSTGTIVIS PSVNTKIKKA YPAFILGSSL TNSAGQPLY ANYSLTIIPD
181 GCGTVLHAFY CILKPRTVNR CPSGTGYVSY FIYETVHNDQ QSTINRNASL NSFKSFDDL
241 NCTFFNSWDI TADEKWEFG ITQDTQGVHL YSSRKGDLYG GNMFRFATLP VYEGIKYYTV
301 IPRSFRSKAN KREAWAAFV YKLHQLTYLL DFSVDGYIRR AIDCGHDDL QLHCSYTSFE
361 VDTGVYSVSS YEASATGTFI EQPNATECDF SPMLTGVAPO VYNFKRLVPS NCNYNLTKLL
    
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-continued

421 SLFAVDEFSC NGISPDIAIR GCYSTLTVDY FAYPLSMKSY IRPGSAGNIP LYNYKQSFAN  
 481 PTCRVMASVL ANVTITKPHA YGYISKCSRL TGANQDVETP LYINPGEYSI CRDFSPGGFS  
 541 EDGQVFKRTL TQFEGGGLLI GVGTRVPMTD NLQMSFIIISV QYGTGTD SVC PMLDLGDSL  
 601 ITNRLGKCDV YSLYGVTRGR VFQNCVAVGV KQQRVYDSF DNLVGYYSDD GNYICVRPCV  
 661 SVPVSVIYDK STNLHATLFG SVACEHVTTM MSQFSRLTQS NLRRRDSNIP LQAVGCVIG  
 721 LSNNSLVVSD CKLPLGQSLC AVPPVSTFRS YSASQFQLAV LNYTSPIVVT PINSSGPTAA  
 781 IPTNFSFSVT QEYIETSIOK VTVDCQYVC NGFTRCEKLL VEYGFCSKI NQALHGANLR  
 841 QDESVYLSY NIKTTSTQTL EYGLNGDFNL TLLQVPQIGG SSSYSRSAIE DLLFDKVTIA  
 901 DPGYMQGYDD CMKQGPQASR DLICAQVSG YKVLPLPYDP NMEAAYTSSL LGSAGAGWT  
 961 AGLSSFAAIP FAQSMFYRLN GVGITQQVLS ENQKLIANKF NQALGAMQT FTNSLAFSK  
 1021 VQDAVNANAQ ALSKLAELSL NTFGAISSI SDILARLDTV EQDAQIDRLI NGRLTSLNAF  
 1081 VSQQLVRSET AARSAQLASD KVNCEVKSQS KRNGFCGSGT HIVSFVVNAP NGFYFPHVGY  
 1141 VPTNYTNVTA AYGLCANNNP PLCIAPIDGY FITNQTTYS VDEWYTYGS SFYKPEPITQ  
 1201 ANSRVSSDV KFDKLENNLP PPLENSTDV DFKDELEEFF KNVTSHGPNF AEISKINTTL  
 1261 LDLSDMAMML QEVVKQLNDS YIDLKELGNY TYYNKWPWVY WLGFIAGLVA LLLCVFPLLC  
 1321 CTGCGTSCLG KMKCKNCCDS YEEYDVEKIH VH

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

1 MIHSVFLLMF LLTPTESYVD VGPDSVKSAC IEVDIQQTF DKTWRPIDV SKADGIIYPQ  
 61 GRYSNITIT YQGLFPYQGD HGDYVYSAG HATGTPQKL FVANYSQDVK QFANGFVRI  
 121 GAAANSTGTV IISPSTSATI RKIYPAFMLG SSVGNFSDGK MGRFFNHTLV LLPDGCGLL  
 181 RAFYCILEPR SGNHCPAGNS YTSFATYHTP ATDCSDGNYN RNASLNSPKE YFNLRNCTFM  
 241 YTYNITEDEI LEWFGITQTA QGVHLFSSRY VDLGGNMPQ FATLPVYDTI KYYSIIPHSI  
 301 RSIQSDRKAW AAFVYKLPQ LTFLLDFSVD GYIRRAIDCG FNDLSQLHCS YESFDVESGV  
 361 YSVSSFEAKP SGSVVEQAEQ VECDFSPLLS GTPPQVYNFK RLVTNVCNIN LTKLLSLFSV  
 421 NDFTCSQISP AAIASNCYSS LILDYFSYPL SMKSDLVSS AGPISQFNK QSFNPTCLI  
 481 LATVPHNLTT ITKPLKYSYI NKCSRLSDD RTEVPQLVNA NQYSPCVSIV PSTVWEDGDY  
 541 YRKQLSPLEG GGWLVASGST VAMMEQLQMG FGITVQYGTD TNSVCPKLEF ANDTKIASQL  
 601 GNCVEYSLYG VSGRQVQNC TAVGVRQQRV VYDAYQNLVG YYSDDGNYC LRACVSVVPS  
 661 VIYDKETKTH ATLFQSVACE HISSTMSQYS RSTRSMLKRR DSTYGPLQTP VGCVLGLVNS  
 721 SLFVEDCKLP LGQSLCALPD TPSTLTPRSV RSVPGEMRLA SIAFNHPIQV DQLNSSFYKL  
 781 SIPTNFSFGV TQEYIQTIIQ KVTVDCKQYV CNGFQKCEQL LREYGFCSK INQALHGANL  
 841 RQDDSVRNLF ASVKSQSSP IIPGFGGDFN LTLEPVSIS TGSRSARSAI EDLLFDKVTI  
 901 ADPGYMQGYD DCMQGPASA RDLICAQYVA GYKVLPLPMD VNMEAAYTSS LLSIAGVGV  
 961 TAGLSSFAAI PFAQSIFYRL NGVGITQQVL SENQKLIANK FNQALGAMQT GFTTTNEAPQ  
 1021 KVQDAVNNA QALSCLAEL SNTFGAISAS IGDIIQRLDV LEQDAQIDRL INGRLLTNA  
 1081 FVAQQLVRSE SAALSAQLAK DKVNECVKAQ SKRSGFCGQG THIVSFVVNA PNGLYFMHVG  
 1141 YYPSNHIEVV SAYGLCDAAN PTNCIAPVNG YFIKTNNTRI VDEWYTYGS FYAPEPITSL  
 1201 NTKYVAPQVT YQNISTNLPP PLLGNSTGID FQDELDEFFK NVSTSIPNFG SLTQINTLL

- continued

1261 DLTYEMLSLQ QVVKALNESY IDLKELGNYT YYNKWPWYIW LGFIAGLVAL ALCVFFILCC  
 1321 TGCGTNCMGK 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 LANLNAPFLY  
 241 FDLINCTFRY NYTTEDENA EWFGITQDTQ GVHLYSSRKE NVFRNMFHF ATLPVYQKIL  
 301 YTVTIPRSIR SPFNDRKAWA AFYIYKLHPL TYLLNFDVEG YITKAVDCCY DDF AQLCQSY  
 361 ENFDVETGVY SVSSFEASPR GEFIEQATTQ ECDFTPMLTG TPPPIYDFKR LVFTNCNYNL  
 421 TKLLSLFQVS EFSCHQVSPS SLATGCYSSL TVDYFAYSTD MSSYLQPGSA GEIVQFNYKQ  
 481 DFSNPTCRVL ATVPTNLTTI TKSSNYVHLT ECKYSTAYGK NYLYNAPGGY TPCLSLASRG  
 541 FTTNRQSHSL ELPDGYLVTT GSVYPVNGNL QMAFIISVQY GTD TNSVCPM QALRNDTSIE  
 601 DKLDVCEYS LHGITGRGVF HNCTSVGLRN QRFVYDTFDN LVGYHSDNGN YYCVRPCVSV  
 661 PVSVIYDKAS NSHATLFGSV ACSHVTTMMS QFSRMTKTNL PARTTPGPLQ TTVGCAMGFI  
 721 NSSMVVDECQ LPLGQSLCAI PPTTSTRFR R ATSIDPVFQI ATLNFTSPLT LAPINSTGFV  
 781 VAVPTNFTFG VTQEFIETTI QKITVDCKQY VCNQFKKCED LLKEYGQFCS KINQALHGAN  
 841 LRQDESIANL FSSIKTQNTQ PLQAGLNGDF NLTMLQIPQV TTGERKYRST IEDLLFNKVT  
 901 IADPGYMQGY DECMQOQPQS ARDLICAQYV AGYKVLPPLY DPYMEAAYS SLLGSIAGAS  
 961 WTAGLSSFAA IPPFAQSIFYR LNGVGITQOV LSENQKI IAN KFNQALGAMQ TGFTTTNLAF  
 1021 NKVQDAVNAN AMALSKLAAE LSNTFGAISS SISDILARLD TVEQEAQIDR LINGRLTSLN  
 1081 AFVAQQLVRT EAAARSAQLA QDKVNECVKS QSKRNGFCGT GTHIVSFAIN APNGLYFFHV  
 1141 GYQPTSHVNA TAAAYGLCNTE NPPKCIAPID GYFVLNQTTS TARSSGDQHW YTTGSSFFHP  
 1201 EPITEANSKY VSMDEEENL TNKLPPLLS NSTDLDFKDE LEEFFKNVSS QGPNFQEISK  
 1261 INTTLNLNT ELMVLSEVVK QLNESYIDLK ELGNYTFYQK WFWYIWLGFI AGLVALALCV  
 1321 FFILCCTGCG TSCLGKLCN 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), WiV1 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 Pihuh/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 WIV1 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.,  $^{35}\text{S}$ ,  $^{125}\text{I}$ ,  $^{32}\text{P}$ ,  $^{13}\text{H}$ ,  $^{14}\text{C}$ ,  $^{131}\text{I}$ ), 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')<sub>2</sub>, 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')<sub>2</sub> 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')<sub>2</sub> 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, (4<sup>th</sup> 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*, (4<sup>th</sup> 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.), AMPHIGEN™, 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

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<160> NUMBER OF SEQ ID NOS: 8

<210> SEQ ID NO 1
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<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: chimeric coronavirus spike protein

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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

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Asn	Ala	Ser	Ser	Glu	Val	Ala	Val	Leu	Tyr	Gln	Asp	Val	Asn	Cys	Thr	595	600	605	
Asp	Val	Pro	Thr	Ala	Ile	Arg	Ala	Asp	Gln	Leu	Thr	Pro	Ala	Trp	Arg	610	615	620	
Val	Tyr	Ser	Thr	Gly	Val	Asn	Val	Phe	Gln	Thr	Gln	Ala	Gly	Cys	Leu	625	630	635	640
Ile	Gly	Ala	Glu	His	Val	Asn	Ala	Ser	Tyr	Glu	Cys	Asp	Ile	Pro	Ile	645	650	655	
Gly	Ala	Gly	Ile	Cys	Ala	Ser	Tyr	His	Thr	Ala	Ser	Val	Leu	Arg	Ser	660	665	670	
Thr	Gly	Gln	Lys	Ser	Ile	Val	Ala	Tyr	Thr	Met	Ser	Leu	Gly	Ala	Glu	675	680	685	
Asn	Ser	Ile	Ala	Tyr	Ala	Asn	Asn	Ser	Ile	Ala	Ile	Pro	Thr	Asn	Phe	690	695	700	
Ser	Ile	Ser	Val	Thr	Thr	Glu	Val	Met	Pro	Val	Ser	Met	Ala	Lys	Thr	705	710	715	720
Ala	Val	Asp	Cys	Thr	Met	Tyr	Ile	Cys	Gly	Asp	Ser	Leu	Glu	Cys	Ser	725	730	735	
Asn	Leu	Leu	Leu	Gln	Tyr	Gly	Ser	Phe	Cys	Thr	Gln	Leu	Asn	Arg	Ala	740	745	750	
Leu	Thr	Gly	Ile	Ala	Ile	Glu	Gln	Asp	Lys	Asn	Thr	Gln	Glu	Val	Phe	755	760	765	
Ala	Gln	Val	Lys	Gln	Met	Tyr	Lys	Thr	Pro	Ala	Ile	Lys	Asp	Phe	Gly	770	775	780	
Gly	Phe	Asn	Phe	Ser	Gln	Ile	Leu	Pro	Asp	Pro	Ser	Lys	Pro	Thr	Lys	785	790	795	800
Arg	Ser	Phe	Ile	Glu	Asp	Leu	Leu	Phe	Asn	Lys	Val	Thr	Leu	Ala	Asp	805	810	815	
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Pro	Leu	Leu	Thr	Asp	Glu	Met	Val	Ala	Ala	Tyr	Thr	Ala	Ala	Leu	Val	850	855	860	
Ser	Gly	Thr	Ala	Thr	Ala	Gly	Trp	Thr	Phe	Gly	Ala	Gly	Ser	Ala	Leu	865	870	875	880
Gln	Ile	Pro	Phe	Ala	Met	Gln	Met	Ala	Tyr	Arg	Phe	Asn	Gly	Ile	Gly	885	890	895	
Val	Thr	Gln	Asn	Val	Leu	Tyr	Glu	Asn	Gln	Lys	Gln	Ile	Ala	Asn	Gln	900	905	910	
Phe	Asn	Lys	Ala	Ile	Ser	Gln	Ile	Gln	Glu	Ser	Leu	Thr	Thr	Thr	Ser	915	920	925	
Thr	Ala	Leu	Gly	Lys	Leu	Gln	Asp	Val	Val	Asn	Asp	Asn	Ala	Gln	Ala	930	935	940	
Leu	Asn	Thr	Leu	Val	Lys	Gln	Leu	Ser	Ser	Asn	Phe	Gly	Ala	Ile	Ser	945	950	955	960
Ser	Val	Leu	Asn	Asp	Ile	Leu	Ser	Arg	Leu	Asp	Lys	Val	Glu	Ala	Glu	965	970	975	
Val	Gln	Ile	Asp	Arg	Leu	Ile	Thr	Gly	Arg	Leu	Gln	Ser	Leu	Gln	Thr	980	985	990	
Tyr	Val	Thr	Gln	Gln	Leu	Ile	Arg	Ala	Ala	Glu	Ile	Arg	Ala	Ser	Ala				

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Lys Arg Val Asp Phe Cys Gly 1025	Lys Gly Tyr His 1030	Leu Met Ser Phe 1035
Pro Gln Ala Ala Pro His Gly 1040	Val Val Phe Leu 1045	His Val Thr Tyr 1050
Val Pro Ser Gln Glu Arg Asn 1055	Phe Thr Thr Ala 1060	Pro Ala Ile Cys 1065
His Glu Gly Lys Ala Tyr Phe 1070	Pro Arg Glu Gly 1075	Val Phe Val Ser 1080
Asn Gly Thr Ser Trp Phe Ile 1085	Thr Gln Arg Asn 1090	Phe Tyr Ser Pro 1095
Gln Ile Ile Thr Thr Asp Asn 1100	Thr Phe Val Ala 1105	Gly Asn Cys Asp 1110
Val Val Ile Gly Ile Ile Asn 1115	Asn Thr Val Tyr 1120	Asp Pro Leu Gln 1125
Pro Glu Leu Asp Ser Phe Lys 1130	Glu Glu Leu Asp 1135	Lys Tyr Phe Lys 1140
Asn His Thr Ser Pro Asp Val 1145	Asp Leu Gly Asp 1150	Ile Ser Gly Ile 1155
Asn Ala Ser Val Val Asn Ile 1160	Gln Lys Glu Ile 1165	Asp Arg Leu Asn 1170
Glu Val Ala Lys Asn Leu Asn 1175	Glu Ser Leu Ile 1180	Asp Leu Gln Glu 1185
Leu Gly Lys Tyr Glu Gln Tyr 1190	Ile Lys Trp Pro 1195	Trp Tyr Val Trp 1200
Leu Gly Phe Ile Ala Gly Leu 1205	Ile Ala Ile Val 1210	Met Val Thr Ile 1215
Leu Leu Cys Cys Met Thr Ser 1220	Cys Cys Ser Cys 1225	Leu Lys Gly Ala 1230
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<210> SEQ ID NO 2  
 <211> LENGTH: 1242  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial  
 <220> FEATURE:  
 <223> OTHER INFORMATION: SARS coronavirus

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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

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Phe	Asp	Asn	Thr	Thr	Gln	Ser	Ala	Val	Ile	Val	Asn	Asn	Ser	Thr	His
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Thr	Val	Ser	Arg	Gly	Thr	Gln	Gln	Asn	Ala	Trp	Val	Tyr	Gln	Ser	Ala
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Phe	Asn	Cys	Thr	Tyr	Asp	Arg	Val	Glu	Lys	Ser	Phe	Gln	Leu	Asp	Thr
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Thr	Pro	Lys	Thr	Gly	Asn	Phe	Lys	Asp	Leu	Arg	Glu	Tyr	Val	Phe	Lys
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Asn	Arg	Asp	Gly	Phe	Leu	Ser	Val	Tyr	Gln	Thr	Tyr	Thr	Ala	Val	Asn
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Leu	Pro	Arg	Gly	Leu	Pro	Thr	Gly	Phe	Ser	Val	Leu	Lys	Pro	Ile	Leu
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Lys	Leu	Pro	Phe	Gly	Ile	Asn	Ile	Thr	Ser	Tyr	Arg	Val	Val	Met	Ala
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Met	Phe	Ser	Gln	Thr	Thr	Ser	Asn	Phe	Leu	Pro	Glu	Ser	Ala	Ala	Tyr
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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
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Glu	Leu	Lys	Cys	Thr	Ile	Lys	Asn	Phe	Asn	Val	Asp	Lys	Gly	Ile	Tyr
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Gln	Thr	Ser	Asn	Phe	Arg	Val	Ser	Pro	Thr	Gln	Glu	Val	Ile	Arg	Phe
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Pro	Asn	Ile	Thr	Asn	Arg	Cys	Pro	Phe	Asp	Lys	Val	Phe	Asn	Ala	Thr
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Val	Ala	Asp	Tyr	Thr	Val	Leu	Tyr	Asn	Ser	Thr	Ser	Phe	Ser	Thr	Phe
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Val	Ala	Pro	Gly	Glu	Thr	Gly	Val	Ile	Ala	Asp	Tyr	Asn	Tyr	Lys	Leu
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Pro	Asp	Asp	Phe	Thr	Gly	Cys	Val	Ile	Ala	Trp	Asn	Thr	Ala	Lys	His
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Asp	Thr	Gly	Asn	Tyr	Tyr	Tyr	Arg	Ser	His	Arg	Lys	Thr	Lys	Leu	Lys
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Pro	Phe	Glu	Arg	Asp	Leu	Ser	Ser	Asp	Asp	Gly	Asn	Gly	Val	Tyr	Thr
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Leu	Ser	Thr	Tyr	Asp	Phe	Asn	Pro	Asn	Val	Pro	Val	Ala	Tyr	Gln	Ala
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Thr	Arg	Val	Val	Val	Leu	Ser	Phe	Glu	Leu	Leu	Asn	Ala	Pro	Ala	Thr
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Val	Cys	Gly	Pro	Lys	Leu	Ser	Thr	Glu	Leu	Val	Lys	Asn	Gln	Cys	Val
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Gln Ile Asp Arg Leu Ile Thr Gly Arg Leu Gln Ser Leu Gln Thr Tyr
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Val Thr Gln Gln Leu Ile Arg Ala Ala Glu Ile Arg Ala Ser Ala Asn
                               980                       985                       990

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Val Asp Phe Cys Gly Lys Gly Tyr His Leu Met Ser Phe Pro Gln
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Ser Gln Glu Lys Asn Phe Thr Thr Ala Pro Ala Ile Cys His Glu
 1040                               1045                       1050

Gly Lys Ala Tyr Phe Pro Arg Glu Gly Val Phe Val Ser Asn Gly
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Thr Ser Trp Phe Ile Thr Gln Arg Asn Phe Tyr Ser Pro Gln Leu
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Ile Gly Ile Ile Asn Asn Thr Val Tyr Asp Pro Leu Gln Pro Glu
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Ala Lys Asn Leu Asn Glu Ser Leu Ile Asp Leu Gln Glu Leu Gly
 1160                               1165                       1170

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 1190                               1195                       1200

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<223> OTHER INFORMATION: SARS coronavirus
    
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 Val Val Arg Gly Trp Val Phe Gly Ser Thr Met Asn Asn Lys Ser Gln  
 100 105 110  
 Ser Val Ile Ile Ile Asn Asn Ser Thr Asn Val Val Ile Arg Ala Cys  
 115 120 125  
 Asn Phe Glu Leu Cys Asp Asn Pro Phe Phe Ala Val Ser Lys Pro Met  
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 Gly Thr Gln Thr His Thr Met Ile Phe Asp Asn Ala Phe Asn Cys Thr  
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 165 170 175  
 Gly Asn Phe Lys His Leu Arg Glu Phe Val Phe Lys Asn Lys Asp Gly  
 180 185 190  
 Phe Leu Tyr Val Tyr Lys Gly Tyr Gln Pro Ile Asp Val Val Arg Asp  
 195 200 205  
 Leu Pro Ser Gly Phe Asn Thr Leu Lys Pro Ile Phe Lys Leu Pro Leu  
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 Gly Ile Asn Ile Thr Asn Phe Arg Ala Ile Leu Thr Ala Phe Ser Pro  
 225 230 235 240  
 Ala Gln Asp Ile Trp Gly Thr Ser Ala Ala Ala Tyr Phe Val Gly Tyr  
 245 250 255  
 Leu Lys Pro Thr Thr Phe Met Leu Lys Tyr Asp Glu Asn Gly Thr Ile  
 260 265 270  
 Thr Asp Ala Val Asp Cys Ser Gln Asn Pro Leu Ala Glu Leu Lys Cys  
 275 280 285  
 Ser Val Lys Ser Phe Glu Ile Asp Lys Gly Ile Tyr Gln Thr Ser Asn  
 290 295 300  
 Phe Arg Val Val Pro Ser Gly Asp Val Val Arg Phe Pro Asn Ile Thr  
 305 310 315 320  
 Asn Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr Lys Phe Pro Ser  
 325 330 335  
 Val Tyr Ala Trp Glu Arg Lys Lys Ile Ser Asn Cys Val Ala Asp Tyr  
 340 345 350  
 Ser Val Leu Tyr Asn Ser Thr Phe Phe Ser Thr Phe Lys Cys Tyr Gly  
 355 360 365  
 Val Ser Ala Thr Lys Leu Asn Asp Leu Cys Phe Ser Asn Val Tyr Ala  
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 Asp Ser Phe Val Val Lys Gly Asp Asp Val Arg Gln Ile Ala Pro Gly  
 385 390 395 400  
 Gln Thr Gly Val Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe  
 405 410 415  
 Met Gly Cys Val Leu Ala Trp Asn Thr Arg Asn Ile Asp Ala Thr Ser  
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 Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His Gly Lys Leu  
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 Arg Pro Phe Glu Arg Asp Ile Ser Asn Val Pro Phe Ser Pro Asp Gly

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Tyr	Gly	Phe	Tyr	Thr	Thr	Thr	Gly	Ile	Gly	Tyr	Gln	Pro	Tyr	Arg	Val
				485						490					495
Val	Val	Leu	Ser	Phe	Glu	Leu	Leu	Asn	Ala	Pro	Ala	Thr	Val	Cys	Gly
			500						505				510		
Pro	Lys	Leu	Ser	Thr	Asp	Leu	Ile	Lys	Asn	Gln	Cys	Val	Asn	Phe	Asn
		515					520						525		
Phe	Asn	Gly	Leu	Thr	Gly	Thr	Gly	Val	Leu	Thr	Pro	Ser	Ser	Lys	Arg
	530				535						540				
Phe	Gln	Pro	Phe	Gln	Gln	Phe	Gly	Arg	Asp	Val	Ser	Asp	Phe	Thr	Asp
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Ser	Val	Arg	Asp	Pro	Lys	Thr	Ser	Glu	Ile	Leu	Asp	Ile	Ser	Pro	Cys
				565						570					575
Ser	Phe	Gly	Gly	Val	Ser	Val	Ile	Thr	Pro	Gly	Thr	Asn	Ala	Ser	Ser
			580							585				590	
Glu	Val	Ala	Val	Leu	Tyr	Gln	Asp	Val	Asn	Cys	Thr	Asp	Val	Ser	Thr
		595					600						605		
Ala	Ile	His	Ala	Asp	Gln	Leu	Thr	Pro	Ala	Trp	Arg	Ile	Tyr	Ser	Thr
	610					615									620
Gly	Asn	Asn	Val	Phe	Gln	Thr	Gln	Ala	Gly	Cys	Leu	Ile	Gly	Ala	Glu
	625				630					635					640
His	Val	Asp	Thr	Ser	Tyr	Glu	Cys	Asp	Ile	Pro	Ile	Gly	Ala	Gly	Ile
				645						650					655
Cys	Ala	Ser	Tyr	His	Thr	Val	Ser	Leu	Leu	Arg	Ser	Thr	Ser	Gln	Lys
			660							665					670
Ser	Ile	Val	Ala	Tyr	Thr	Met	Ser	Leu	Gly	Ala	Asp	Ser	Ser	Ile	Ala
		675					680							685	
Tyr	Ser	Asn	Asn	Thr	Ile	Ala	Ile	Pro	Thr	Asn	Phe	Ser	Ile	Ser	Ile
	690					695									700
Thr	Thr	Glu	Val	Met	Pro	Val	Ser	Met	Ala	Lys	Thr	Ser	Val	Asp	Cys
	705				710					715					720
Asn	Met	Tyr	Ile	Cys	Gly	Asp	Ser	Thr	Glu	Cys	Ala	Asn	Leu	Leu	Leu
				725						730					735
Gln	Tyr	Gly	Ser	Phe	Cys	Thr	Gln	Leu	Asn	Arg	Ala	Leu	Ser	Gly	Ile
			740							745					750
Ala	Ala	Glu	Gln	Asp	Arg	Asn	Thr	Arg	Glu	Val	Phe	Ala	Gln	Val	Lys
		755					760								765
Gln	Met	Tyr	Lys	Thr	Pro	Thr	Leu	Lys	Tyr	Phe	Gly	Gly	Phe	Asn	Phe
	770					775					780				
Ser	Gln	Ile	Leu	Pro	Asp	Pro	Leu	Lys	Pro	Thr	Lys	Arg	Ser	Phe	Ile
	785				790					795					800
Glu	Asp	Leu	Leu	Phe	Asn	Lys	Val	Thr	Leu	Ala	Asp	Ala	Gly	Phe	Met
				805						810					815
Lys	Gln	Tyr	Gly	Glu	Cys	Leu	Gly	Asp	Ile	Asn	Ala	Arg	Asp	Leu	Ile
			820							825					830
Cys	Ala	Gln	Lys	Phe	Asn	Gly	Leu	Thr	Val	Leu	Pro	Pro	Leu	Leu	Thr
			835							840					845
Asp	Asp	Met	Ile	Ala	Ala	Tyr	Thr	Ala	Ala	Leu	Val	Ser	Gly	Thr	Ala
		850				855					860				
Thr	Ala	Gly	Trp	Thr	Phe	Gly	Ala	Gly	Ala	Ala	Leu	Gln	Ile	Pro	Phe
	865				870						875				880

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Ala Met Gln Met Ala Tyr Arg Phe Asn Gly Ile Gly Val Thr Gln Asn  
885 890 895

Val Leu Tyr Glu Asn Gln Lys Gln Ile Ala Asn Gln Phe Asn Lys Ala  
900 905 910

Ile Ser Gln Ile Gln Glu Ser Leu Thr Thr Thr Ser Thr Ala Leu Gly  
915 920 925

Lys Leu Gln Asp Val Val Asn Gln Asn Ala Gln Ala Leu Asn Thr Leu  
930 935 940

Val Lys Gln Leu Ser Ser Asn Phe Gly Ala Ile Ser Ser Val Leu Asn  
945 950 955 960

Asp Ile Leu Ser Arg Leu Asp Lys Val Glu Ala Glu Val Gln Ile Asp  
965 970 975

Arg Leu Ile Thr Gly Arg Leu Gln Ser Leu Gln Thr Tyr Val Thr Gln  
980 985 990

Gln Leu Ile Arg Ala Ala Glu Ile Arg Ala Ser Ala Asn Leu Ala Ala  
995 1000 1005

Thr Lys Met Ser Glu Cys Val Leu Gly Gln Ser Lys Arg Val Asp  
1010 1015 1020

Phe Cys Gly Lys Gly Tyr His Leu Met Ser Phe Pro Gln Ala Ala  
1025 1030 1035

Pro His Gly Val Val Phe Leu His Val Thr Tyr Val Pro Ser Gln  
1040 1045 1050

Glu Arg Asn Phe Thr Thr Ala Pro Ala Ile Cys His Glu Gly Lys  
1055 1060 1065

Ala Tyr Phe Pro Arg Glu Gly Val Phe Val Phe Asn Gly Thr Ser  
1070 1075 1080

Trp Phe Ile Thr Gln Arg Asn Phe Phe Ser Pro Gln Ile Ile Thr  
1085 1090 1095

Thr Asp Asn Thr Phe Val Ser Gly Asn Cys Asp Val Val Ile Gly  
1100 1105 1110

Ile Ile Asn Asn Thr Val Tyr Asp Pro Leu Gln Pro Glu Leu Asp  
1115 1120 1125

Ser Phe Lys Glu Glu Leu Asp Lys Tyr Phe Lys Asn His Thr Ser  
1130 1135 1140

Pro Asp Val Asp Leu Gly Asp Ile Ser Gly Ile Asn Ala Ser Val  
1145 1150 1155

Val Asn Ile Gln Lys Glu Ile Asp Arg Leu Asn Glu Val Ala Lys  
1160 1165 1170

Asn Leu Asn Glu Ser Leu Ile Asp Leu Gln Glu Leu Gly Lys Tyr  
1175 1180 1185

Glu Gln Tyr Ile Lys Trp Pro Trp Tyr Val Trp Leu Gly Phe Ile  
1190 1195 1200

Ala Gly Leu Ile Ala Ile Val Met Val Thr Ile Leu Leu Cys Cys  
1205 1210 1215

Met Thr Ser Cys Cys Ser Cys Leu Lys Gly Ala Cys Ser Cys Gly  
1220 1225 1230

Ser Cys Cys Lys Phe Asp Glu Asp Asp Ser Glu Pro Val Leu Lys  
1235 1240 1245

Gly Val Lys Leu His Tyr Thr  
1250 1255

&lt;210&gt; SEQ ID NO 4

&lt;211&gt; LENGTH: 1241

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<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: SARS coronavirus

<400> SEQUENCE: 4

Met Lys Val Leu Ile Phe Ala Leu Leu Phe Ser Leu Ala Lys Ala Gln
 1           5           10          15
Glu Gly Cys Gly Ile Ile Ser Arg Lys Pro Gln Pro Lys Met Glu Lys
 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 Ile Asp Ser Asn Lys 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 Ile 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 Lys Gly Thr Gln Gln Ser Ser Trp Val Tyr Gln Ser Ala
 145         150         155         160
Phe Asn Cys Thr Tyr Asp Arg Val Glu Lys Ser Phe Gln Leu Asp Thr
 165         170         175
Ala 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 Phe Pro Ala Gly Phe Ser Val Leu Arg Pro Ile Leu
 210         215         220
Lys Leu Pro Phe Gly Ile Asn Ile Thr Ser Tyr Arg Val Val Met Thr
 225         230         235         240
Met Phe Ser Gln Phe Asn Ser Asn Phe Leu Pro Glu Ser Ala Ala Tyr
 245         250         255
Tyr Val Gly Asn Leu Lys Tyr Thr Thr Phe Met Leu Ser 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 Ser Lys Gly Ile Tyr
 290         295         300
Gln Thr Ser Asn Phe Arg Val Thr Pro Thr Gln Glu Val Val Arg Phe
 305         310         315         320
Pro Asn Ile Thr Asn Arg Cys Pro Phe Asp Lys Val Phe Asn Ala Ser
 325         330         335
Arg Phe Pro Asn Val Tyr Ala Trp Glu Arg Thr Lys Ile Ser Asp Cys
 340         345         350
Val Ala Asp Tyr Thr Val Leu Tyr Asn Ser Thr Ser Phe Ser Thr Phe
 355         360         365
Lys Cys Tyr Gly Val Ser Pro Ser Lys Leu Ile Asp Leu Cys Phe Thr
 370         375         380

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Ser Val Tyr Ala Asp Thr Phe Leu Ile Arg Ser Ser Glu Val Arg Gln  
 385 390 395 400  
 Val Ala Pro Gly Glu Thr Gly Val Ile Ala Asp Tyr Asn Tyr Lys Leu  
 405 410 415  
 Pro Asp Asp Phe Thr Gly Cys Val Ile Ala Trp Asn Thr Ala Gln Gln  
 420 425 430  
 Asp Gln Gly Gln Tyr Tyr Tyr Arg Ser Tyr Arg Lys Glu Lys Leu Lys  
 435 440 445  
 Pro Phe Glu Arg Asp Leu Ser Ser Asp Glu Asn Gly Val Tyr Thr Leu  
 450 455 460  
 Ser Thr Tyr Asp Phe Tyr Pro Ser Ile Pro Val Glu Tyr Gln Ala Thr  
 465 470 475 480  
 Arg Val Val Val Leu Ser Phe Glu Leu Leu Asn Ala Pro Ala Thr Val  
 485 490 495  
 Cys Gly Pro Lys Leu Ser Thr Gln Leu Val Lys Asn Gln Cys Val Asn  
 500 505 510  
 Phe Asn Phe Asn Gly Leu Arg Gly Thr Gly Val Leu Thr Thr Ser Ser  
 515 520 525  
 Lys Arg Phe Gln Ser Phe Gln Gln Phe Gly Arg Asp Thr Ser Asp Phe  
 530 535 540  
 Thr Asp Ser Val Arg Asp Pro Gln Thr Leu Glu Ile Leu Asp Ile Ser  
 545 550 555 560  
 Pro Cys Ser Phe Gly Gly Val Ser Val Ile Thr Pro Gly Thr Asn Ala  
 565 570 575  
 Ser Ser Glu Val Ala Val Leu Tyr Gln Asp Val Asn Cys Thr Asp Val  
 580 585 590  
 Pro Thr Ser Ile His Ala Asp Gln Leu Thr Pro Ala Trp Arg Val Tyr  
 595 600 605  
 Ser Thr Gly Val Asn Val Phe Gln Thr Gln Ala Gly Cys Leu Ile Gly  
 610 615 620  
 Ala Glu His Val Asn Ala Ser Tyr Glu Cys Asp Ile Pro Ile Gly Ala  
 625 630 635 640  
 Gly Ile Cys Ala Ser Tyr His Thr Ala Ser Val Leu Arg Ser Thr Gly  
 645 650 655  
 Gln Lys Ser Ile Val Ala Tyr Thr Met Ser Leu Gly Ala Glu Asn Ser  
 660 665 670  
 Ile Ala Tyr Ala Asn Asn Ser Ile Ala Ile Pro Thr Asn Phe Ser Ile  
 675 680 685  
 Ser Val Thr Thr Glu Val Met Pro Val Ser Ile Ala Lys Thr Ser Val  
 690 695 700  
 Asp Cys Thr Met Tyr Ile Cys Gly Asp Ser Leu Glu Cys Ser Asn Leu  
 705 710 715 720  
 Leu Leu Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu Thr  
 725 730 735  
 Gly Ile Ala Ile Glu Gln Asp Lys Asn Thr Gln Glu Val Phe Ala Gln  
 740 745 750  
 Val Lys Gln Met Tyr Lys Thr Pro Ala Ile Lys Asp Phe Gly Gly Phe  
 755 760 765  
 Asn Phe Ser Gln Ile Leu Pro Asp Pro Ser Lys Pro Thr Lys Arg Ser  
 770 775 780  
 Phe Ile Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala Gly  
 785 790 795 800  
 Phe Met Lys Gln Tyr Gly Glu Cys Leu Gly Asp Ile Ser Ala Arg Asp

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805				810				815							
Leu	Ile	Cys	Ala	Gln	Lys	Phe	Asn	Gly	Leu	Thr	Val	Leu	Pro	Pro	Leu
			820						825				830		
Leu	Thr	Asp	Glu	Met	Ile	Ala	Ala	Tyr	Thr	Ala	Ala	Leu	Val	Ser	Gly
		835					840						845		
Thr	Ala	Thr	Ala	Gly	Trp	Thr	Phe	Gly	Ala	Gly	Ser	Ala	Leu	Gln	Ile
	850					855					860				
Pro	Phe	Ala	Met	Gln	Met	Ala	Tyr	Arg	Phe	Asn	Gly	Ile	Gly	Val	Thr
	865				870					875					880
Gln	Asn	Val	Leu	Tyr	Glu	Asn	Gln	Lys	Gln	Ile	Ala	Asn	Gln	Phe	Asn
				885					890					895	
Lys	Ala	Ile	Ser	Gln	Ile	Gln	Glu	Ser	Leu	Thr	Thr	Thr	Ser	Thr	Ala
			900						905					910	
Leu	Gly	Lys	Leu	Gln	Asp	Val	Val	Asn	Asp	Asn	Ala	Gln	Ala	Leu	Asn
		915					920						925		
Thr	Leu	Val	Lys	Gln	Leu	Ser	Ser	Asn	Phe	Gly	Ala	Ile	Ser	Ser	Val
	930					935					940				
Leu	Asn	Asp	Ile	Leu	Ser	Arg	Leu	Asp	Lys	Val	Glu	Ala	Glu	Val	Gln
	945				950					955					960
Ile	Asp	Arg	Leu	Ile	Thr	Gly	Arg	Leu	Gln	Ser	Leu	Gln	Thr	Tyr	Val
			965						970					975	
Thr	Gln	Gln	Leu	Ile	Arg	Ala	Ala	Glu	Ile	Arg	Ala	Ser	Ala	Asn	Leu
			980						985					990	
Ala	Ala	Thr	Lys	Met	Ser	Glu	Cys	Val	Leu	Gly	Gln	Ser	Lys	Arg	Val
			995				1000						1005		
Asp	Phe	Cys	Gly	Lys	Gly	Tyr	His	Leu	Met	Ser	Phe	Pro	Gln	Ala	
	1010					1015							1020		
Ala	Pro	His	Gly	Val	Val	Phe	Leu	His	Val	Thr	Tyr	Val	Pro	Ser	
	1025					1030							1035		
Gln	Glu	Arg	Asn	Phe	Thr	Thr	Ala	Pro	Ala	Ile	Cys	His	Glu	Gly	
	1040					1045					1050				
Lys	Ala	Tyr	Phe	Pro	Arg	Glu	Gly	Val	Phe	Val	Ser	Asn	Gly	Thr	
	1055					1060					1065				
Ser	Trp	Phe	Ile	Thr	Gln	Arg	Asn	Phe	Tyr	Ser	Pro	Gln	Ile	Ile	
	1070					1075					1080				
Thr	Thr	Asp	Asn	Thr	Phe	Val	Ala	Gly	Asn	Cys	Asp	Val	Val	Ile	
	1085					1090					1095				
Gly	Ile	Ile	Asn	Asn	Thr	Val	Tyr	Asp	Pro	Leu	Gln	Pro	Glu	Leu	
	1100					1105					1110				
Asp	Ser	Phe	Lys	Glu	Glu	Leu	Asp	Lys	Tyr	Phe	Lys	Asn	His	Thr	
	1115					1120					1125				
Ser	Pro	Asp	Val	Asp	Leu	Gly	Asp	Ile	Ser	Gly	Ile	Asn	Ala	Ser	
	1130					1135					1140				
Val	Val	Asn	Ile	Gln	Lys	Glu	Ile	Asp	Arg	Leu	Asn	Glu	Val	Ala	
	1145					1150					1155				
Lys	Asn	Leu	Asn	Glu	Ser	Leu	Ile	Asp	Leu	Gln	Glu	Leu	Gly	Lys	
	1160					1165					1170				
Tyr	Glu	Gln	Tyr	Ile	Lys	Trp	Pro	Trp	Tyr	Val	Trp	Leu	Gly	Phe	
	1175					1180					1185				
Ile	Ala	Gly	Leu	Ile	Ala	Ile	Val	Met	Val	Thr	Ile	Leu	Leu	Cys	
	1190					1195					1200				
Cys	Met	Thr	Ser	Cys	Cys	Ser	Cys	Leu	Lys	Gly	Ala	Cys	Ser	Cys	
	1205					1210					1215				



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Gly Ser Cys Cys Lys Phe Asp Glu Asp Asp Ser Glu Pro Val Leu  
 1220 1225 1230

Lys Gly Val Lys Leu His Tyr Thr  
 1235 1240

<210> SEQ ID NO 5  
 <211> LENGTH: 1355  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial  
 <220> FEATURE:  
 <223> OTHER INFORMATION: chimeric coronavirus spike protein

<400> SEQUENCE: 5

Met Thr Leu Leu Met Cys Leu Leu Met Ser Leu Leu Ile Phe Val Arg  
 1 5 10 15  
 Gly Cys Asp Ser Gln Phe Val Asp Met Ser Pro Ala Ser Asn Thr Ser  
 20 25 30  
 Glu Cys Leu Glu Ser Gln Val Asp Ala Ala Ala Phe Ser Lys Leu Met  
 35 40 45  
 Trp Pro Tyr Pro Ile Asp Pro Ser Lys Val Asp Gly Ile Ile Tyr Pro  
 50 55 60  
 Leu Gly Arg Thr Tyr Ser Asn Ile Thr Leu Ala Tyr Thr Gly Leu Phe  
 65 70 75 80  
 Pro Leu Gln Gly Asp Leu Gly Ser Gln Tyr Leu Tyr Ser Val Ser His  
 85 90 95  
 Ala Val Gly His Asp Gly Asp Pro Thr Lys Ala Tyr Ile Ser Asn Tyr  
 100 105 110  
 Ser Leu Leu Val Asn Asp Phe Asp Asn Gly Phe Val Val Arg Ile Gly  
 115 120 125  
 Ala Ala Ala Asn Ser Thr Gly Thr Ile Val Ile Ser Pro Ser Val Asn  
 130 135 140  
 Thr Lys Ile Lys Lys Ala Tyr Pro Ala Phe Ile Leu Gly Ser Ser Leu  
 145 150 155 160  
 Thr Asn Thr Ser Ala Gly Gln Pro Leu Tyr Ala Asn Tyr Ser Leu Thr  
 165 170 175  
 Ile Ile Pro Asp Gly Cys Gly Thr Val Leu His Ala Phe Tyr Cys Ile  
 180 185 190  
 Leu Lys Pro Arg Thr Val Asn Arg Cys Pro Ser Gly Thr Gly Tyr Val  
 195 200 205  
 Ser Tyr Phe Ile Tyr Glu Thr Val His Asn Asp Cys Gln Ser Thr Ile  
 210 215 220  
 Asn Arg Asn Ala Ser Leu Asn Ser Phe Lys Ser Phe Phe Asp Leu Val  
 225 230 235 240  
 Asn Cys Thr Phe Phe Asn Ser Trp Asp Ile Thr Ala Asp Glu Thr Lys  
 245 250 255  
 Glu Trp Phe Gly Ile Thr Gln Asp Thr Gln Gly Val His Leu Tyr Ser  
 260 265 270  
 Ser Arg Lys Gly Asp Leu Tyr Gly Gly Asn Met Phe Arg Phe Ala Thr  
 275 280 285  
 Leu Pro Val Tyr Glu Gly Ile Lys Tyr Tyr Thr Val Ile Pro Arg Ser  
 290 295 300  
 Phe Arg Ser Lys Ala Asn Lys Arg Glu Ala Trp Ala Ala Phe Tyr Val  
 305 310 315 320  
 Tyr Lys Leu His Gln Leu Thr Tyr Leu Leu Asp Phe Ser Val Asp Gly  
 325 330 335



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Ala Ser Gln Phe Gln Leu Ala Val Leu Asn Tyr Thr Ser Pro Ile Val  
755 760 765

Val Thr Pro Ile Asn Ser Ser Gly Phe Thr Ala Ala Ile Pro Thr Asn  
770 775 780

Phe Ser Phe Ser Val Thr Gln Glu Tyr Ile Glu Thr Ser Ile Gln Lys  
785 790 795 800

Val Thr Val Asp Cys Lys Gln Tyr Val Cys Asn Gly Phe Thr Arg Cys  
805 810 815

Glu Lys Leu Leu Val Glu Tyr Gly Gln Phe Cys Ser Lys Ile Asn Gln  
820 825 830

Ala Leu His Gly Ala Asn Leu Arg Gln Asp Glu Ser Val Tyr Ser Leu  
835 840 845

Tyr Ser Asn Ile Lys Thr Thr Ser Thr Gln Thr Leu Glu Tyr Gly Leu  
850 855 860

Asn Gly Asp Phe Asn Leu Thr Leu Leu Gln Val Pro Gln Ile Gly Gly  
865 870 875 880

Ser Ser Ser Ser Tyr Arg Ser Ala Ile Glu Asp Leu Leu Phe Asp Lys  
885 890 895

Val Thr Ile Ala Asp Pro Gly Tyr Met Gln Gly Tyr Asp Asp Cys Met  
900 905 910

Lys Gln Gly Pro Gln Ser Ala Arg Asp Leu Ile Cys Ala Gln Tyr Val  
915 920 925

Ser Gly Tyr Lys Val Leu Pro Pro Leu Tyr Asp Pro Asn Met Glu Ala  
930 935 940

Ala Tyr Thr Ser Ser Leu Leu Gly Ser Ile Ala Gly Ala Gly Trp Thr  
945 950 955 960

Ala Gly Leu Ser Ser Phe Ala Ala Ile Pro Phe Ala Gln Ser Met Phe  
965 970 975

Tyr Arg Leu Asn Gly Val Gly Ile Thr Gln Gln Val Leu Ser Glu Asn  
980 985 990

Gln Lys Ile Ile Ala Asn Lys Phe Asn Gln Ala Leu Gly Ala Met Gln  
995 1000 1005

Thr Gly Phe Thr Thr Thr Asn Leu Ala Phe Asn Lys Val Gln Asp  
1010 1015 1020

Ala Val Asn Ala Asn Ala Met Ala Leu Ser Lys Leu Ala Ala Glu  
1025 1030 1035

Leu Ser Asn Thr Phe Gly Ala Ile Ser Ser Ser Ile Ser Asp Ile  
1040 1045 1050

Leu Ala Arg Leu Asp Thr Val Glu Gln Glu Ala Gln Ile Asp Arg  
1055 1060 1065

Leu Ile Asn Gly Arg Leu Thr Ser Leu Asn Ala Phe Val Ala Gln  
1070 1075 1080

Gln Leu Val Arg Thr Glu Ala Ala Ala Arg Ser Ala Gln Leu Ala  
1085 1090 1095

Gln Asp Lys Val Asn Glu Cys Val Lys Ser Gln Ser Lys Arg Asn  
1100 1105 1110

Gly Phe Cys Gly Thr Gly Thr His Ile Val Ser Phe Ala Ile Asn  
1115 1120 1125

Ala Pro Asn Gly Leu Tyr Phe Phe His Val Gly Tyr Gln Pro Thr  
1130 1135 1140

Ser His Val Asn Ala Thr Ala Ala Tyr Gly Leu Cys Asn Thr Glu  
1145 1150 1155

Asn Pro Pro Lys Cys Ile Ala Pro Ile Asp Gly Tyr Phe Val Leu

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1160	1165	1170
Asn Gln Thr Thr Ser Thr Ala Arg Ser Ser Gly Asp Gln His Trp		
1175	1180	1185
Tyr Tyr Thr Gly Ser Ser Phe Phe His Pro Glu Pro Ile Thr Glu		
1190	1195	1200
Ala Asn Ser Lys Tyr Val Ser Met Asp Val Lys Phe Glu Asn Leu		
1205	1210	1215
Thr Asn Lys Leu Pro Pro Pro Leu Leu Ser Asn Ser Thr Asp Leu		
1220	1225	1230
Asp Phe Lys Asp Glu Leu Glu Glu Phe Phe Lys Asn Val Ser Ser		
1235	1240	1245
Gln Gly Pro Asn Phe Gln Glu Ile Ser Lys Ile Asn Thr Thr Leu		
1250	1255	1260
Leu Asn Leu Asn Thr Glu Leu Met Val Leu Ser Glu Val Val Lys		
1265	1270	1275
Gln Leu Asn Glu Ser Tyr Ile Asp Leu Lys Glu Leu Gly Asn Tyr		
1280	1285	1290
Thr Phe Tyr Gln Lys Trp Pro Trp Tyr Ile Trp Leu Gly Phe Ile		
1295	1300	1305
Ala Gly Leu Val Ala Leu Ala Leu Cys Val Phe Phe Ile Leu Cys		
1310	1315	1320
Cys Thr Gly Cys Gly Thr Ser Cys Leu Gly Lys Leu Lys Cys Asn		
1325	1330	1335
Arg Cys Cys Asp Ser Tyr Asp Glu Tyr Glu Val Glu Lys Ile His		
1340	1345	1350
Val His		
1355		

<210> SEQ ID NO 6  
 <211> LENGTH: 1352  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Bat coronavirus

<400> SEQUENCE: 6

Met Thr Leu Leu Met Cys Leu Leu Met Ser Leu Leu Ile Phe Val Arg		
1	5	10 15
Gly Cys Asp Ser Gln Phe Val Asp Met Ser Pro Ala Ser Asn Thr Ser		
20	25	30
Glu Cys Leu Glu Ser Gln Val Asp Ala Ala Ala Phe Ser Lys Leu Met		
35	40	45
Trp Pro Tyr Pro Ile Asp Pro Ser Lys Val Asp Gly Ile Ile Tyr Pro		
50	55	60
Leu Gly Arg Thr Tyr Ser Asn Ile Thr Leu Ala Tyr Thr Gly Leu Phe		
65	70	75 80
Pro Leu Gln Gly Asp Leu Gly Ser Gln Tyr Leu Tyr Ser Val Ser His		
85	90	95
Ala Val Gly His Asp Gly Asp Pro Thr Lys Ala Tyr Ile Ser Asn Tyr		
100	105	110
Ser Leu Leu Val Asn Asp Phe Asp Asn Gly Phe Val Val Arg Ile Gly		
115	120	125
Ala Ala Ala Asn Ser Thr Gly Thr Ile Val Ile Ser Pro Ser Val Asn		
130	135	140
Thr Lys Ile Lys Lys Ala Tyr Pro Ala Phe Ile Leu Gly Ser Ser Leu		



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Ile Ile Ser Val Gln Tyr Gly Thr Gly Thr Asp Ser Val Cys Pro Met  
                   580  585  590

Leu Asp Leu Gly Asp Ser Leu Thr Ile Thr Asn Arg Leu Gly Lys Cys  
                   595  600  605

Val Asp Tyr Ser Leu Tyr Gly Val Thr Gly Arg Gly Val Phe Gln Asn  
                   610  615  620

Cys Thr Ala Val Gly Val Lys Gln Gln Arg Phe Val Tyr Asp Ser Phe  
                   625  630  635  640

Asp Asn Leu Val Gly Tyr Tyr Ser Asp Asp Gly Asn Tyr Tyr Cys Val  
                                   645  650  655

Arg Pro Cys Val Ser Val Pro Val Ser Val Ile Tyr Asp Lys Ser Thr  
                   660  665  670

Asn Leu His Ala Thr Leu Phe Gly Ser Val Ala Cys Glu His Val Thr  
                   675  680  685

Thr Met Met Ser Gln Phe Ser Arg Leu Thr Gln Ser Asn Leu Arg Arg  
                   690  695  700

Arg Asp Ser Asn Ile Pro Leu Gln Thr Ala Val Gly Cys Val Ile Gly  
                   705  710  715  720

Leu Ser Asn Asn Ser Leu Val Val Ser Asp Cys Lys Leu Pro Leu Gly  
                                   725  730  735

Gln Ser Leu Cys Ala Val Pro Pro Val Ser Thr Phe Arg Ser Tyr Ser  
                                   740  745  750

Ala Ser Gln Phe Gln Leu Ala Val Leu Asn Tyr Thr Ser Pro Ile Val  
                   755  760  765

Val Thr Pro Ile Asn Ser Ser Gly Phe Thr Ala Ala Ile Pro Thr Asn  
                   770  775  780

Phe Ser Phe Ser Val Thr Gln Glu Tyr Ile Glu Thr Ser Ile Gln Lys  
                   785  790  795  800

Val Thr Val Asp Cys Lys Gln Tyr Val Cys Asn Gly Phe Thr Arg Cys  
                                   805  810  815

Glu Lys Leu Leu Val Glu Tyr Gly Gln Phe Cys Ser Lys Ile Asn Gln  
                   820  825  830

Ala Leu His Gly Ala Asn Leu Arg Gln Asp Glu Ser Val Tyr Ser Leu  
                   835  840  845

Tyr Ser Asn Ile Lys Thr Thr Ser Thr Gln Thr Leu Glu Tyr Gly Leu  
                   850  855  860

Asn Gly Asp Phe Asn Leu Thr Leu Leu Gln Val Pro Gln Ile Gly Gly  
                   865  870  875  880

Ser Ser Ser Ser Tyr Arg Ser Ala Ile Glu Asp Leu Leu Phe Asp Lys  
                                   885  890  895

Val Thr Ile Ala Asp Pro Gly Tyr Met Gln Gly Tyr Asp Asp Cys Met  
                   900  905  910

Lys Gln Gly Pro Gln Ser Ala Arg Asp Leu Ile Cys Ala Gln Tyr Val  
                   915  920  925

Ser Gly Tyr Lys Val Leu Pro Pro Leu Tyr Asp Pro Asn Met Glu Ala  
                   930  935  940

Ala Tyr Thr Ser Ser Leu Leu Gly Ser Ile Ala Gly Ala Gly Trp Thr  
                   945  950  955  960

Ala Gly Leu Ser Ser Phe Ala Ala Ile Pro Phe Ala Gln Ser Met Phe  
                                   965  970  975

Tyr Arg Leu Asn Gly Val Gly Ile Thr Gln Gln Val Leu Ser Glu Asn  
                   980  985  990

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Gln	Lys	Leu	Ile	Ala	Asn	Lys	Phe	Asn	Gln	Ala	Leu	Gly	Ala	Met	Gln
		995					1000					1005			
Thr	Gly	Phe	Thr	Thr	Ser	Asn	Leu	Ala	Phe	Ser	Lys	Val	Gln	Asp	
	1010					1015					1020				
Ala	Val	Asn	Ala	Asn	Ala	Gln	Ala	Leu	Ser	Lys	Leu	Ala	Ser	Glu	
	1025					1030					1035				
Leu	Ser	Asn	Thr	Phe	Gly	Ala	Ile	Ser	Ser	Ser	Ile	Ser	Asp	Ile	
	1040					1045					1050				
Leu	Ala	Arg	Leu	Asp	Thr	Val	Glu	Gln	Asp	Ala	Gln	Ile	Asp	Arg	
	1055					1060					1065				
Leu	Ile	Asn	Gly	Arg	Leu	Thr	Ser	Leu	Asn	Ala	Phe	Val	Ser	Gln	
	1070					1075					1080				
Gln	Leu	Val	Arg	Ser	Glu	Thr	Ala	Ala	Arg	Ser	Ala	Gln	Leu	Ala	
	1085					1090					1095				
Ser	Asp	Lys	Val	Asn	Glu	Cys	Val	Lys	Ser	Gln	Ser	Lys	Arg	Asn	
	1100					1105					1110				
Gly	Phe	Cys	Gly	Ser	Gly	Thr	His	Ile	Val	Ser	Phe	Val	Val	Asn	
	1115					1120					1125				
Ala	Pro	Asn	Gly	Phe	Tyr	Phe	Phe	His	Val	Gly	Tyr	Val	Pro	Thr	
	1130					1135					1140				
Asn	Tyr	Thr	Asn	Val	Thr	Ala	Ala	Tyr	Gly	Leu	Cys	Asn	Asn	Asn	
	1145					1150					1155				
Asn	Pro	Pro	Leu	Cys	Ile	Ala	Pro	Ile	Asp	Gly	Tyr	Phe	Ile	Thr	
	1160					1165					1170				
Asn	Gln	Thr	Thr	Thr	Tyr	Ser	Val	Asp	Thr	Glu	Trp	Tyr	Tyr	Thr	
	1175					1180					1185				
Gly	Ser	Ser	Phe	Tyr	Lys	Pro	Glu	Pro	Ile	Thr	Gln	Ala	Asn	Ser	
	1190					1195					1200				
Arg	Tyr	Val	Ser	Ser	Asp	Val	Lys	Phe	Asp	Lys	Leu	Glu	Asn	Asn	
	1205					1210					1215				
Leu	Pro	Pro	Pro	Leu	Leu	Glu	Asn	Ser	Thr	Asp	Val	Asp	Phe	Lys	
	1220					1225					1230				
Asp	Glu	Leu	Glu	Glu	Phe	Phe	Lys	Asn	Val	Thr	Ser	His	Gly	Pro	
	1235					1240					1245				
Asn	Phe	Ala	Glu	Ile	Ser	Lys	Ile	Asn	Thr	Thr	Leu	Leu	Asp	Leu	
	1250					1255					1260				
Ser	Asp	Glu	Met	Ala	Met	Leu	Gln	Glu	Val	Val	Lys	Gln	Leu	Asn	
	1265					1270					1275				
Asp	Ser	Tyr	Ile	Asp	Leu	Lys	Glu	Leu	Gly	Asn	Tyr	Thr	Tyr	Tyr	
	1280					1285					1290				
Asn	Lys	Trp	Pro	Trp	Tyr	Val	Trp	Leu	Gly	Phe	Ile	Ala	Gly	Leu	
	1295					1300					1305				
Val	Ala	Leu	Leu	Leu	Cys	Val	Phe	Phe	Leu	Leu	Cys	Cys	Thr	Gly	
	1310					1315					1320				
Cys	Gly	Thr	Ser	Cys	Leu	Gly	Lys	Met	Lys	Cys	Lys	Asn	Cys	Cys	
	1325					1330					1335				
Asp	Ser	Tyr	Glu	Glu	Tyr	Asp	Val	Glu	Lys	Ile	His	Val	His		
	1340					1345					1350				

&lt;210&gt; SEQ ID NO 7

&lt;211&gt; LENGTH: 1353

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: MERS coronavirus

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&lt;400&gt; SEQUENCE: 7

Met Ile His Ser Val Phe Leu Leu Met Phe Leu Leu Thr Pro Thr Glu  
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 20 25 30  
 Val Asp Ile Gln Gln Thr Phe Phe Asp Lys Thr Trp Pro Arg Pro Ile  
 35 40 45  
 Asp Val Ser Lys Ala Asp Gly Ile Ile Tyr Pro Gln Gly Arg Thr Tyr  
 50 55 60  
 Ser Asn Ile Thr Ile Thr Tyr Gln Gly Leu Phe Pro Tyr Gln Gly Asp  
 65 70 75 80  
 His Gly Asp Met Tyr Val Tyr Ser Ala Gly His Ala Thr Gly Thr Thr  
 85 90 95  
 Pro Gln Lys Leu Phe Val Val Ala Asn Tyr Ser Gln Asp Val Lys Gln Phe  
 100 105 110  
 Ala Asn Gly Phe Val Val Arg Ile Gly Ala Ala Ala Asn Ser Thr Gly  
 115 120 125  
 Thr Val Ile Ile Ser Pro Ser Thr Ser Ala Thr Ile Arg Lys Ile Tyr  
 130 135 140  
 Pro Ala Phe Met Leu Gly Ser Ser Val Gly Asn Phe Ser Asp Gly Lys  
 145 150 155 160  
 Met Gly Arg Phe Phe Asn His Thr Leu Val Leu Leu Pro Asp Gly Cys  
 165 170 175  
 Gly Thr Leu Leu Arg Ala Phe Tyr Cys Ile Leu Glu Pro Arg Ser Gly  
 180 185 190  
 Asn His Cys Pro Ala Gly Asn Ser Tyr Thr Ser Phe Ala Thr Tyr His  
 195 200 205  
 Thr Pro Ala Thr Asp Cys Ser Asp Gly Asn Tyr Asn Arg Asn Ala Ser  
 210 215 220  
 Leu Asn Ser Phe Lys Glu Tyr Phe Asn Leu Arg Asn Cys Thr Phe Met  
 225 230 235 240  
 Tyr Thr Tyr Asn Ile Thr Glu Asp Glu Ile Leu Glu Trp Phe Gly Ile  
 245 250 255  
 Thr Gln Thr Ala Gln Gly Val His Leu Phe Ser Ser Arg Tyr Val Asp  
 260 265 270  
 Leu Tyr Gly Gly Asn Met Phe Gln Phe Ala Thr Leu Pro Val Tyr Asp  
 275 280 285  
 Thr Ile Lys Tyr Tyr Ser Ile Ile Pro His Ser Ile Arg Ser Ile Gln  
 290 295 300  
 Ser Asp Arg Lys Ala Trp Ala Ala Phe Tyr Val Tyr Lys Leu Gln Pro  
 305 310 315 320  
 Leu Thr Phe Leu Leu Asp Phe Ser Val Asp Gly Tyr Ile Arg Arg Ala  
 325 330 335  
 Ile Asp Cys Gly Phe Asn Asp Leu Ser Gln Leu His Cys Ser Tyr Glu  
 340 345 350  
 Ser Phe Asp Val Glu Ser Gly Val Tyr Ser Val Ser Ser Phe Glu Ala  
 355 360 365  
 Lys Pro Ser Gly Ser Val Val Glu Gln Ala Glu Gly Val Glu Cys Asp  
 370 375 380  
 Phe Ser Pro Leu Leu Ser Gly Thr Pro Pro Gln Val Tyr Asn Phe Lys  
 385 390 395 400  
 Arg Leu Val Phe Thr Asn Cys Asn Tyr Asn Leu Thr Lys Leu Leu Ser



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405				410				415							
Leu	Phe	Ser	Val	Asn	Asp	Phe	Thr	Cys	Ser	Gln	Ile	Ser	Pro	Ala	Ala
			420							425				430	
Ile	Ala	Ser	Asn	Cys	Tyr	Ser	Ser	Leu	Ile	Leu	Asp	Tyr	Phe	Ser	Tyr
		435					440							445	
Pro	Leu	Ser	Met	Lys	Ser	Asp	Leu	Ser	Val	Ser	Ser	Ala	Gly	Pro	Ile
		450				455								460	
Ser	Gln	Phe	Asn	Tyr	Lys	Gln	Ser	Phe	Ser	Asn	Pro	Thr	Cys	Leu	Ile
		465			470					475				480	
Leu	Ala	Thr	Val	Pro	His	Asn	Leu	Thr	Thr	Ile	Thr	Lys	Pro	Leu	Lys
			485						490					495	
Tyr	Ser	Tyr	Ile	Asn	Lys	Cys	Ser	Arg	Leu	Leu	Ser	Asp	Asp	Arg	Thr
			500						505					510	
Glu	Val	Pro	Gln	Leu	Val	Asn	Ala	Asn	Gln	Tyr	Ser	Pro	Cys	Val	Ser
		515					520							525	
Ile	Val	Pro	Ser	Thr	Val	Trp	Glu	Asp	Gly	Asp	Tyr	Tyr	Arg	Lys	Gln
		530				535					540				
Leu	Ser	Pro	Leu	Glu	Gly	Gly	Gly	Trp	Leu	Val	Ala	Ser	Gly	Ser	Thr
		545			550					555					560
Val	Ala	Met	Thr	Glu	Gln	Leu	Gln	Met	Gly	Phe	Gly	Ile	Thr	Val	Gln
			565							570				575	
Tyr	Gly	Thr	Asp	Thr	Asn	Ser	Val	Cys	Pro	Lys	Leu	Glu	Phe	Ala	Asn
			580							585				590	
Asp	Thr	Lys	Ile	Ala	Ser	Gln	Leu	Gly	Asn	Cys	Val	Glu	Tyr	Ser	Leu
		595					600							605	
Tyr	Gly	Val	Ser	Gly	Arg	Gly	Val	Phe	Gln	Asn	Cys	Thr	Ala	Val	Gly
		610				615					620				
Val	Arg	Gln	Gln	Arg	Phe	Val	Tyr	Asp	Ala	Tyr	Gln	Asn	Leu	Val	Gly
		625			630					635					640
Tyr	Tyr	Ser	Asp	Asp	Gly	Asn	Tyr	Tyr	Cys	Leu	Arg	Ala	Cys	Val	Ser
			645						650					655	
Val	Pro	Val	Ser	Val	Ile	Tyr	Asp	Lys	Glu	Thr	Lys	Thr	His	Ala	Thr
			660						665					670	
Leu	Phe	Gly	Ser	Val	Ala	Cys	Glu	His	Ile	Ser	Ser	Thr	Met	Ser	Gln
		675					680							685	
Tyr	Ser	Arg	Ser	Thr	Arg	Ser	Met	Leu	Lys	Arg	Arg	Asp	Ser	Thr	Tyr
		690				695					700				
Gly	Pro	Leu	Gln	Thr	Pro	Val	Gly	Cys	Val	Leu	Gly	Leu	Val	Asn	Ser
		705			710					715				720	
Ser	Leu	Phe	Val	Glu	Asp	Cys	Lys	Leu	Pro	Leu	Gly	Gln	Ser	Leu	Cys
			725						730					735	
Ala	Leu	Pro	Asp	Thr	Pro	Ser	Thr	Leu	Thr	Pro	Arg	Ser	Val	Arg	Ser
			740						745					750	
Val	Pro	Gly	Glu	Met	Arg	Leu	Ala	Ser	Ile	Ala	Phe	Asn	His	Pro	Ile
		755					760							765	
Gln	Val	Asp	Gln	Leu	Asn	Ser	Ser	Tyr	Phe	Lys	Leu	Ser	Ile	Pro	Thr
		770				775					780				
Asn	Phe	Ser	Phe	Gly	Val	Thr	Gln	Glu	Tyr	Ile	Gln	Thr	Thr	Ile	Gln
			785		790					795					800
Lys	Val	Thr	Val	Asp	Cys	Lys	Gln	Tyr	Val	Cys	Asn	Gly	Phe	Gln	Lys
			805							810				815	
Cys	Glu	Gln	Leu	Leu	Arg	Glu	Tyr	Gly	Gln	Phe	Cys	Ser	Lys	Ile	Asn
			820						825					830	



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Glu Leu Asp Glu Phe Phe Lys Asn Val Ser Thr Ser Ile Pro Asn  
 1235 1240 1245  
 Phe Gly Ser Leu Thr Gln Ile Asn Thr Thr Leu Leu Asp Leu Thr  
 1250 1255 1260  
 Tyr Glu Met Leu Ser Leu Gln Gln Val Val Lys Ala Leu Asn Glu  
 1265 1270 1275  
 Ser Tyr Ile Asp Leu Lys Glu Leu Gly Asn Tyr Thr Tyr Tyr Asn  
 1280 1285 1290  
 Lys Trp Pro Trp Tyr Ile Trp Leu Gly Phe Ile Ala Gly Leu Val  
 1295 1300 1305  
 Ala Leu Ala Leu Cys Val Phe Phe Ile Leu Cys Cys Thr Gly Cys  
 1310 1315 1320  
 Gly Thr Asn Cys Met Gly Lys Leu Lys Cys Asn Arg Cys Cys Asp  
 1325 1330 1335  
 Arg Tyr Glu Glu Tyr Asp Leu Glu Pro His Lys Val His Val His  
 1340 1345 1350

<210> SEQ ID NO 8  
 <211> LENGTH: 1357  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Bat coronavirus

<400> SEQUENCE: 8

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 Asn Arg Thr Ser Cys Gln Ser Val Asp Ile Gly Thr Pro Val Thr Gly  
 20 25 30  
 Ser Cys Leu Arg Ser Gln Val Arg Pro Glu Tyr Phe Asp Ile Val His  
 35 40 45  
 Asn Thr Trp Pro Met Pro Ile Asp Thr Ser Lys Ala Glu Gly Val Ile  
 50 55 60  
 Tyr Pro Asn Gly Lys Ser Tyr Ser Asn Ile Ser Leu Thr Tyr Thr Gly  
 65 70 75 80  
 Leu Tyr Pro Lys Ala Lys Asp Leu Gly Lys Gln Tyr Leu Phe Ser Asp  
 85 90 95  
 Gly His Ser Ala Pro Asn Gln Leu Asn Asp Leu Phe Val Ser Asn Tyr  
 100 105 110  
 Ser Ala Gln Val Glu Ser Phe Asp Asp Gly Phe Val Val Arg Ile Gly  
 115 120 125  
 Ala Ala Ser Asn Lys Thr Gly Thr Thr Val Ile Ser Gln Thr Thr Phe  
 130 135 140  
 Lys Pro Ile Lys Lys Ile Tyr Pro Gly Phe Met Leu Gly His Ala Val  
 145 150 155 160  
 Gly Asn Tyr Thr Pro Thr Asn Ile Thr Gly Arg Tyr Leu Asn His Thr  
 165 170 175  
 Leu Val Ile Leu Pro Asp Gly Cys Gly Thr Leu Val His Ala Phe Tyr  
 180 185 190  
 Cys Ile Leu Gln Pro Arg Thr Gln Ala Asn Cys Pro Gly Ala Ser Ser  
 195 200 205  
 Phe Thr Ser Val Thr Leu Trp Asp Thr Pro Ala Thr Asp Cys Ala Pro  
 210 215 220  
 Ser Gly Val Tyr Asn Ser Leu Ala Asn Leu Asn Ala Phe Lys Leu Tyr  
 225 230 235 240

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Phe Asp Leu Ile Asn Cys Thr Phe Arg Tyr Asn Tyr Thr Ile Thr Glu  
245 250 255  
Asp Glu Asn Ala Glu Trp Phe Gly Ile Thr Gln Asp Thr Gln Gly Val  
260 265 270  
His Leu Tyr Ser Ser Arg Lys Glu Asn Val Phe Arg Asn Asn Met Phe  
275 280 285  
His Phe Ala Thr Leu Pro Val Tyr Gln Lys Ile Leu Tyr Tyr Thr Val  
290 295 300  
Ile Pro Arg Ser Ile Arg Ser Pro Phe Asn Asp Arg Lys Ala Trp Ala  
305 310 315 320  
Ala Phe Tyr Ile Tyr Lys Leu His Pro Leu Thr Tyr Leu Leu Asn Phe  
325 330 335  
Asp Val Glu Gly Tyr Ile Thr Lys Ala Val Asp Cys Gly Tyr Asp Asp  
340 345 350  
Phe Ala Gln Leu Gln Cys Ser Tyr Glu Asn Phe Asp Val Glu Thr Gly  
355 360 365  
Val Tyr Ser Val Ser Ser Phe Glu Ala Ser Pro Arg Gly Glu Phe Ile  
370 375 380  
Glu Gln Ala Thr Thr Gln Glu Cys Asp Phe Thr Pro Met Leu Thr Gly  
385 390 395 400  
Thr Pro Pro Pro Ile Tyr Asp Phe Lys Arg Leu Val Phe Thr Asn Cys  
405 410 415  
Asn Tyr Asn Leu Thr Lys Leu Leu Ser Leu Phe Gln Val Ser Glu Phe  
420 425 430  
Ser Cys His Gln Val Ser Pro Ser Ser Leu Ala Thr Gly Cys Tyr Ser  
435 440 445  
Ser Leu Thr Val Asp Tyr Phe Ala Tyr Ser Thr Asp Met Ser Ser Tyr  
450 455 460  
Leu Gln Pro Gly Ser Ala Gly Glu Ile Val Gln Phe Asn Tyr Lys Gln  
465 470 475 480  
Asp Phe Ser Asn Pro Thr Cys Arg Val Leu Ala Thr Val Pro Thr Asn  
485 490 495  
Leu Thr Thr Ile Thr Lys Ser Ser Asn Tyr Val His Leu Thr Glu Cys  
500 505 510  
Tyr Lys Ser Thr Ala Tyr Gly Lys Asn Tyr Leu Tyr Asn Ala Pro Gly  
515 520 525  
Gly Tyr Thr Pro Cys Leu Ser Leu Ala Ser Arg Gly Phe Thr Thr Asn  
530 535 540  
Arg Gln Ser His Ser Leu Glu Leu Pro Asp Gly Tyr Leu Val Thr Thr  
545 550 555 560  
Gly Ser Val Tyr Pro Val Asn Gly Asn Leu Gln Met Ala Phe Ile Ile  
565 570 575  
Ser Val Gln Tyr Gly Thr Asp Thr Asn Ser Val Cys Pro Met Gln Ala  
580 585 590  
Leu Arg Asn Asp Thr Ser Ile Glu Asp Lys Leu Asp Val Cys Val Glu  
595 600 605  
Tyr Ser Leu His Gly Ile Thr Gly Arg Gly Val Phe His Asn Cys Thr  
610 615 620  
Ser Val Gly Leu Arg Asn Gln Arg Phe Val Tyr Asp Thr Phe Asp Asn  
625 630 635 640  
Leu Val Gly Tyr His Ser Asp Asn Gly Asn Tyr Tyr Cys Val Arg Pro  
645 650 655  
Cys Val Ser Val Pro Val Ser Val Ile Tyr Asp Lys Ala Ser Asn Ser

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660				665				670							
His	Ala	Thr	Leu	Phe	Gly	Ser	Val	Ala	Cys	Ser	His	Val	Thr	Thr	Met
	675						680					685			
Met	Ser	Gln	Phe	Ser	Arg	Met	Thr	Lys	Thr	Asn	Leu	Pro	Ala	Arg	Thr
	690				695						700				
Thr	Pro	Gly	Pro	Leu	Gln	Thr	Thr	Val	Gly	Cys	Ala	Met	Gly	Phe	Ile
	705				710					715					720
Asn	Ser	Ser	Met	Val	Val	Asp	Glu	Cys	Gln	Leu	Pro	Leu	Gly	Gln	Ser
				725					730					735	
Leu	Cys	Ala	Ile	Pro	Pro	Thr	Thr	Ser	Thr	Arg	Phe	Arg	Arg	Ala	Thr
			740						745				750		
Ser	Ile	Pro	Asp	Val	Phe	Gln	Ile	Ala	Thr	Leu	Asn	Phe	Thr	Ser	Pro
		755					760					765			
Leu	Thr	Leu	Ala	Pro	Ile	Asn	Ser	Thr	Gly	Phe	Val	Val	Ala	Val	Pro
	770					775					780				
Thr	Asn	Phe	Thr	Phe	Gly	Val	Thr	Gln	Glu	Phe	Ile	Glu	Thr	Thr	Ile
	785				790					795					800
Gln	Lys	Ile	Thr	Val	Asp	Cys	Lys	Gln	Tyr	Val	Cys	Asn	Gly	Phe	Lys
				805					810					815	
Lys	Cys	Glu	Asp	Leu	Leu	Lys	Glu	Tyr	Gly	Gln	Phe	Cys	Ser	Lys	Ile
			820						825				830		
Asn	Gln	Ala	Leu	His	Gly	Ala	Asn	Leu	Arg	Gln	Asp	Glu	Ser	Ile	Ala
		835					840					845			
Asn	Leu	Phe	Ser	Ser	Ile	Lys	Thr	Gln	Asn	Thr	Gln	Pro	Leu	Gln	Ala
	850					855					860				
Gly	Leu	Asn	Gly	Asp	Phe	Asn	Leu	Thr	Met	Leu	Gln	Ile	Pro	Gln	Val
	865				870					875					880
Thr	Thr	Gly	Glu	Arg	Lys	Tyr	Arg	Ser	Thr	Ile	Glu	Asp	Leu	Leu	Phe
				885					890					895	
Asn	Lys	Val	Thr	Ile	Ala	Asp	Pro	Gly	Tyr	Met	Gln	Gly	Tyr	Asp	Glu
			900						905				910		
Cys	Met	Gln	Gln	Gly	Pro	Gln	Ser	Ala	Arg	Asp	Leu	Ile	Cys	Ala	Gln
		915					920					925			
Tyr	Val	Ala	Gly	Tyr	Lys	Val	Leu	Pro	Pro	Leu	Tyr	Asp	Pro	Tyr	Met
	930				935						940				
Glu	Ala	Ala	Tyr	Thr	Ser	Ser	Leu	Leu	Gly	Ser	Ile	Ala	Gly	Ala	Ser
	945				950					955					960
Trp	Thr	Ala	Gly	Leu	Ser	Ser	Phe	Ala	Ala	Ile	Pro	Phe	Ala	Gln	Ser
				965					970					975	
Ile	Phe	Tyr	Arg	Leu	Asn	Gly	Val	Gly	Ile	Thr	Gln	Gln	Val	Leu	Ser
			980				985						990		
Glu	Asn	Gln	Lys	Ile	Ile	Ala	Asn	Lys	Phe	Asn	Gln	Ala	Leu	Gly	Ala
		995					1000					1005			
Met	Gln	Thr	Gly	Phe	Thr	Thr	Thr	Asn	Leu	Ala	Phe	Asn	Lys	Val	
	1010						1015					1020			
Gln	Asp	Ala	Val	Asn	Ala	Asn	Ala	Met	Ala	Leu	Ser	Lys	Leu	Ala	
	1025					1030						1035			
Ala	Glu	Leu	Ser	Asn	Thr	Phe	Gly	Ala	Ile	Ser	Ser	Ser	Ile	Ser	
	1040					1045						1050			
Asp	Ile	Leu	Ala	Arg	Leu	Asp	Thr	Val	Glu	Gln	Glu	Ala	Gln	Ile	
	1055					1060						1065			
Asp	Arg	Leu	Ile	Asn	Gly	Arg	Leu	Thr	Ser	Leu	Asn	Ala	Phe	Val	
	1070					1075						1080			

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Ala	Gln	Gln	Leu	Val	Arg	Thr	Glu	Ala	Ala	Ala	Arg	Ser	Ala	Gln
1085						1090					1095			
Leu	Ala	Gln	Asp	Lys	Val	Asn	Glu	Cys	Val	Lys	Ser	Gln	Ser	Lys
1100						1105					1110			
Arg	Asn	Gly	Phe	Cys	Gly	Thr	Gly	Thr	His	Ile	Val	Ser	Phe	Ala
1115						1120					1125			
Ile	Asn	Ala	Pro	Asn	Gly	Leu	Tyr	Phe	Phe	His	Val	Gly	Tyr	Gln
1130						1135					1140			
Pro	Thr	Ser	His	Val	Asn	Ala	Thr	Ala	Ala	Tyr	Gly	Leu	Cys	Asn
1145						1150					1155			
Thr	Glu	Asn	Pro	Pro	Lys	Cys	Ile	Ala	Pro	Ile	Asp	Gly	Tyr	Phe
1160						1165					1170			
Val	Leu	Asn	Gln	Thr	Thr	Ser	Thr	Ala	Arg	Ser	Ser	Gly	Asp	Gln
1175						1180					1185			
His	Trp	Tyr	Tyr	Thr	Gly	Ser	Ser	Phe	Phe	His	Pro	Glu	Pro	Ile
1190						1195					1200			
Thr	Glu	Ala	Asn	Ser	Lys	Tyr	Val	Ser	Met	Asp	Val	Lys	Phe	Glu
1205						1210					1215			
Asn	Leu	Thr	Asn	Lys	Leu	Pro	Pro	Pro	Leu	Leu	Ser	Asn	Ser	Thr
1220						1225					1230			
Asp	Leu	Asp	Phe	Lys	Asp	Glu	Leu	Glu	Glu	Phe	Phe	Lys	Asn	Val
1235						1240					1245			
Ser	Ser	Gln	Gly	Pro	Asn	Phe	Gln	Glu	Ile	Ser	Lys	Ile	Asn	Thr
1250						1255					1260			
Thr	Leu	Leu	Asn	Leu	Asn	Thr	Glu	Leu	Met	Val	Leu	Ser	Glu	Val
1265						1270					1275			
Val	Lys	Gln	Leu	Asn	Glu	Ser	Tyr	Ile	Asp	Leu	Lys	Glu	Leu	Gly
1280						1285					1290			
Asn	Tyr	Thr	Phe	Tyr	Gln	Lys	Trp	Pro	Trp	Tyr	Ile	Trp	Leu	Gly
1295						1300					1305			
Phe	Ile	Ala	Gly	Leu	Val	Ala	Leu	Ala	Leu	Cys	Val	Phe	Phe	Ile
1310						1315					1320			
Leu	Cys	Cys	Thr	Gly	Cys	Gly	Thr	Ser	Cys	Leu	Gly	Lys	Leu	Lys
1325						1330					1335			
Cys	Asn	Arg	Cys	Cys	Asp	Ser	Tyr	Asp	Glu	Tyr	Glu	Val	Glu	Lys
1340						1345					1350			
Ile	His	Val	His											
1355														

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What is claimed is:

1. 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 a coronavirus spike protein 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 of 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.

2. The chimeric coronavirus spike protein of claim 1, wherein the chimeric coronavirus spike protein is derived from subgroup 1a coronaviruses, subgroup 1b coronaviruses, subgroup 2a coronaviruses, subgroup 2b coronaviruses, subgroup 2c coronaviruses, subgroup 2d coronaviruses or subgroup 3 coronaviruses.

3. The chimeric coronavirus of claim 2, derived from subgroup 2b coronaviruses wherein said first, second and third subgroup 2b coronaviruses are different from one

another and wherein the subgroup 2b coronaviruses are selected from the group consisting of 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.GD01 (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).

4. The chimeric subgroup 2b coronavirus spike protein of claim 3, wherein said first subgroup 2b coronavirus is Bat SARS CoV-HKU3 (GenBank Accession No. FJ211859), said second subgroup 2b coronavirus is SARSCoV.Urbani (GenBank Accession No. AY278741.1), and said third subgroup 2b coronavirus is BtCoV 279.2005 (DQ648857).

5. The chimeric coronavirus spike protein of claim 1, comprising the amino acid sequence:

(SEQ ID NO: 1)

1 MKILIFAPLA NLAKAQEGCG IISRKPQPKM AQVSSRRGV YNDDIFRSD VLHLTQDYFL  
 61 PFDSNLTQYF SLNVDSDRYT YFDNPILDFG DGVYFAATEK SNVIRGWIFG SSFDNTTQSA  
 121 VIVNNSTHII IRVCNFNLCK EPMYTVSRGT QQNAWVYQSA FNCTYDRVEK SFQLDITPKT  
 181 GNFKDLREYV FKNRDGFLSV YQTYTAVNLP RGLPTGFSVL KPILKLPFGI NITSYRVVMA  
 241 MFSQTTSNFL PESAAIYVGN LKYSTFMLRF NENGTITDAV DCSQNPLAEL KCTIKNFNVD  
 301 KGIYQTSNFR VSPTQEVIRF PNITNLCPPG EVFNATKFPS VYAWERKKIS NCVADYSVLY  
 361 NSTFFSTFKC YGVSATKLND LCFSNVYADS FVVKGDVVRQ IAPGQTGVIA DYNKLPDDE  
 421 MGCVLAWNTR NIDATSTGNY NYKYRYLRHG KLRPFERDIS NVPFSPDGKP CTPPALNCYW  
 481 PLNDYGFYTT TGIGYQPYRV VVLSFELLNA PATVCGPKLS TDLVKNQCVN FNFNGLKGTG  
 541 VLTSSSKRFQ SFQQFGRDTS DFTDSVRDPQ TLEILDISPC SFGGVSVITP GTNASSEVAV  
 601 LYQDVNCTDV PTAIRADQLT PAWRVYSTGV NVFQTQAGCL IGAEHVNASY ECDIPIGAGI  
 661 CASYHTASVL RSTGQKSIVA YTMSLGAENS IAYANNSIAI PTNFSISVTT EVMPVSMAKT  
 721 AVDCTMYICG DSLECSNLLL QYGSFCTQLN RALTGIAIEQ DKNTQEVFAQ VKQMYKTPAI  
 781 KDFGGNFSQ ILPDPSPKPK RSFIEDLLFN KVTLADAGFM KQYGDCLGDV SARDLICAQK  
 841 FNGLTVLPLP LTDEMVAAYT AALVSGTATA GWTFGAGSAL QIPFAMQMAY RFNGIGVTQN  
 901 VLYENQKQIA NQFNKAISQI QESLTTTSTA LGKLQDVVND NAQALNTLVK QLSSNFGAIS  
 961 SVLNDILSRL DKVEAEVQID RLITGRLQSL QTYVTQQLIR AAEIRASANL AATKMSECVL  
 1021 GQSKRVDFCG KGYHLMSPFQ AAPHGTVFLH VTYVPSQERN FTTAPAICHE GKAYFPREGV  
 1081 FVSNGTSWFI TQRNFYSPQI ITTDNTFVAG NCDVVIGIIN NTVYDPLQPE LDSFKKELDK  
 1141 YFKNHTSPDV DLGDISGINA SVVNIQKEID RLNEVAKNLN ESLIDLQELG KYEQYIKWPW  
 1201 YVWLGFIAGL IAIVMVTILL CCMTSCCCL KGACSCGCC KFDDEDSEPV LKGVKLHYT.

third subgroup 2c coronaviruses are different from one another and wherein the subgroup 2c coronaviruses are selected from the group consisting of 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.

6. The chimeric coronavirus of claim 2, derived from subgroup 2c coronaviruses wherein said first, second and

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), an isolate of Middle East Respiratory Syndrome Coronavirus having 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, or GenBank Accession No: KF600617.1, Coronavirus Neoromicia/PML-PHE1/RSA/2011 GenBank Accession: KC869678.2, Bat Coronavirus Taper/CILKSA\_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), BtCoV133.2005 (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. KC776174.11; human betacoronavirus 2c EMC/2012 (GenBank Accession No. JX869059.2), and a Pipistrellus bat coronavirus HKU5 isolate having 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.

7. The chimeric subgroup 2c coronavirus spike protein of claim 6, wherein said first subgroup 2c coronavirus is BtCoV HKU4.2 (GenBank Accession No. EF065506.1); said second subgroup 2c coronavirus is MERS-CoV (GenBank Accession No. JX869059.2), and said third subgroup 2c coronavirus is BtCoV HKU5.5 (EF065512.1).

8. The chimeric coronavirus spike protein of claim 1, comprising the amino acid sequence:



(SEQ ID NO: 5)

1 MTLMLCLLMS LLIFVRGCD S QFVDMSPASN TSECLESQVD AAFAFKLMWP YPIDPSKVDG  
 61 IYPLGRITYS NITLAYTGLF PLQGDLSQY LYSVSHAVGH DGDPTKAYIS NYSLLVNDFF  
 121 NGFVVRIGAA ANSTGTIVIS PSVNTKIKKA YPAFILGSSL TNSAGQPLY ANYSLTIIPD  
 181 GCGTVLHAFY CILKPRTVNR CPSGTGYVSY FIYETVHNDQ QSTINRNASL NSFKSFDFLV  
 241 NCTFFNSWDI TADETKEWFG ITQDTQGVHL YSSRKGDLYG GNMFRFATLP VYEGIKYYTV  
 301 IPRSFRRKAN KREAWAAFVY YKLHQLTYLL DFSVDGYIRR AIDCGHDDL S QLHCSYTSFE  
 361 VDTGVYSVSS YEAKPSGSV EQAEGVECDF SPLLSGTPPQ VYNFKRLVFT NCNYNLTKLL  
 421 SLFSVNDFTC SQISPAAIAS NCYSSLILDY FSYPLSMKSD LSVSSAGPIS QFNYKQSFNS  
 481 PTCLILATVP HNLTTITKPL KYSYINKCSR LLSDDRTEVP QLVNANQYSP CVSIVPSTVW  
 541 EDGDYIRKQL SPLEGGGWLV ASGSTVAMTE QLQMGFGITV QYGTDTNSVC PKLDLGDLSLT  
 601 ITNRLGKCDV YSLYGVTVGRG VEQNCTAVGV KQRFVYDSF DNLVGYYSDD GNYICVRPCV  
 661 SVPVSVIYDK STNLHATLFG SVACEHVTTM MSQFSRLTQS NLRRRDSNIP LQTAVGCVIG  
 721 LSNNSLVVSD CKLPLGQSLC AVPPVSTFRS YSASQQLAV LNYTSPIVVT PINSSGFTAA  
 781 IPTNFSFSVT QEYIETSIOK VTVDCQYVC NGFTRCEKLL VEYGGFCSKI NQALHGANLR  
 841 QDESIVSLYS NIKTTSTQTL EYGLNGDFNL TLLQVPQIGG SSSYSRSAIE DLLFDKVTIA  
 901 DPGYMQGYDD CMKQGPQ SAR DLICAQYVSG YKVLPLYDYP NMEAAVTSSL LGSIAGAGWT  
 961 AGLSSFAAIP FAQSMFYRLN GVGITQQVLS ENQKIIANKF NQALGAMQTG FTTTNLAFNK  
 1021 VQDAVNANAM ALSKLAELS NTFGAISSI SDILARLDTV EQEAQIDRLI NGRLTSLNAF  
 1081 VAQQLVRTEA AARSAQLAQD KVNCEKVSQS KRNGFCGTGT HIVSFAINAP NGLYFFHVG Y  
 1141 QPTSHVNATA AYGLCNTENP PKCIAPIDGY FVLNQTSTA RSSGDQHWY Y TGSSFFHPEP  
 1201 ITEANSKYVS MDVKFENLTN KLPPPLLSNS TDLDFKDELE EFFKNVSSQG PNFQEISKIN  
 1261 TLLNLNTEL MVLSEVVKQL NESYIDLKEL GNYTFYQKWP WYIWLGFIA G LVALALCVFF  
 1321 ILCCTGCGTS CLGKLCNRC CDSYDEYEVE KIHVH.

- 9. An isolated nucleic acid molecule encoding the chimeric coronavirus spike protein of claim 1.
- 10. A vector comprising the isolated nucleic acid molecule encoding the chimeric coronavirus spike protein of claim 1.
- 11. A Venezuelan equine encephalitis replicon particle (VRP) comprising the isolated nucleic acid molecule encoding the chimeric coronavirus spike protein of claim 1.
- 12. A virus like particle (VLP) comprising the chimeric coronavirus spike protein of claim 1 and a matrix protein of any virus that can form a VLP.
- 13. A coronavirus particle comprising the chimeric coronavirus spike protein of claim 1.
- 14. A population of VLPs of claim 12.
- 15. A composition comprising the chimeric spike protein of claim 1 in a pharmaceutically acceptable carrier.
- 16. A composition comprising the nucleic acid molecule of claim 9 in a pharmaceutically acceptable carrier.
- 17. A composition comprising the vector of claim 10 in a pharmaceutically acceptable carrier.
- 18. A composition comprising the VRP of claim 11 in a pharmaceutically acceptable carrier.
- 19. A composition comprising the population of claim 14 in a pharmaceutically acceptable carrier.

- 20. A method of producing an immune response to a coronavirus in a subject, comprising administering to the subject an effective amount of the chimeric coronavirus spike protein of claim 1, thereby producing an immune response to a coronavirus in the subject.
- 21. A method of treating a coronavirus infection in a subject in need thereof, comprising administering to the subject an effective amount of the chimeric coronavirus spike protein of claim 1, thereby treating a coronavirus infection in the subject.
- 22. 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 the chimeric coronavirus spike protein of claim 1, thereby preventing a disease or disorder caused by a coronavirus infection in the subject.
- 23. A method of protecting a subject from the effects of coronavirus infection, comprising administering to the subject an effective amount of the chimeric coronavirus spike protein of claim 1, thereby protection the subject from the effects of coronavirus infection.

\* \* \* \* \*