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(57) Abstract: The present invention relates to antibodies including human antibodies and antigen-binding portions thereof that specifically bind to human SARS-CoV S protein, and that function to neutralize SARS-CoV. The invention also relates to antibodies that are bispecific, derivatized, single chain antibodies or portions of fusion proteins. The invention also relates to isolated heavy and light chain immunoglobulins derived from human anti- SARS-CoV S protein antibodies and nucleic acid molecules encoding such immunoglobulins. The present invention also relates to methods of using the antibodies and compositions for diagnosis and treatment. The invention also provides gene therapy methods using nucleic acid molecules encoding the heavy and/or light immunoglobulin molecules that comprise the human anti-SARS-CoV S protein antibodies. The invention also relates to transgenic animals or plants comprising nucleic acid molecules of the present invention.



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## ANTIBODIES TO SARS CORONAVIRUS

### BACKGROUND OF THE INVENTION

[0001] Coronaviruses (CoV) historically are known to cause relatively mild upper  
5 respiratory tract infections, and account for approximately 30% of the cases of the common cold in humans. However, a recently identified CoV, severe acute respiratory syndrome coronavirus (SARS-CoV) causes severe respiratory distress in humans leading to mortality in 9.6% of individuals infected (1). In the year 2003, SARS-CoV established efficient human to human transmission resulting in several super-spreading events. By the end of the  
10 outbreak in July of 2003, SARS-CoV was responsible for more than 774 deaths and 8096 cases worldwide involving 29 countries (see World Health Organization website, Epidemic and Pandemic Alert and Response, Diseases, SARs). Since the conclusion of the SARS outbreak several reports of confirmed cases of SARS of unknown origin (29, See World Health Organization website) indicate that the environmental threat of SARS-CoV still  
15 exists. SARS-CoV-like virus can be isolated from horseshoe bats in China, and researchers postulate that this is the natural reservoir for the virus (18). SARS-CoV-like virus remains present in intermediate wild animal hosts, such as the Himalayan palm civet, raising the possibility of re-emergence of SARS-CoV infection in humans. Because of the remaining threat, it is prudent to develop effective modalities of pre- and post-exposure treatments  
20 against SARS-CoV infection.

[0002] During the SARS outbreak, isolation measures proved effective in bringing the outbreak under control. In addition, corticosteroids and antiviral treatments, such as ribavirin, were used to treat infected patients although the efficacy of these treatments for SARS has not been established (5). Therefore, a targeted and effective treatment for SARS-  
25 CoV remains highly desirable. In humans, SARS-CoV peak viral load is reached by about 10 days post-infection, thus offering an opportunity for effective post-exposure treatment (6). One modality of treatment that may limit virus replication and thus the spread of the virus is passive immunization with pre-formed neutralizing human monoclonal antibodies

(mAbs). Such a treatment during the prodromal phase of the disease could aid in rapid clearance of virus and limit poor clinical outcome and person to person spread, without the adverse effects associated with use of corticosteroids, animal sera, or human sera.

[0003] SARS-CoV mediates infection of target cells via the spike (S) protein expressed on the surface. SARS-CoV S protein (Genbank accession number: AY525636; nucleotide sequence SEQ ID NO: 93; amino acid sequence SEQ ID NO: 94) is a type one transmembrane glycoprotein divided into two functional domains S1 (amino acids 15-680) and S2 (amino acids 681-1255) (13). The S1 domain mediates the interaction of the S protein with its receptor, angiotensin-converting enzyme 2 (ACE2) (17). A region of S1 consisting of 193 amino acids forms the receptor binding domain (RBD) which is responsible for ACE2 binding (30). More recently, a receptor binding motif (RBM) within the RBD, consisting of 70 amino acids, has been shown to come in direct contact with the tip of ACE2 (16). The S2 domain of the S protein contributes to infection of the target cell by mediating fusion of viral and host membranes through a conformational change in which two conserved helical regions (HR1 and HR2) of the S protein are brought together to form a six-helix bundle fusion core (11).

[0004] The S protein serves as the main antigen that elicits protective immune responses, including neutralizing antibodies in infected humans and animals (3, 4, 6, 9, 12, 14). Intranasal or intramuscular application of a modified vaccinia virus Ankara (MVA) expressing S protein into mice elicits SARS-CoV neutralizing antibodies (3). Immunization of mice with a DNA vaccine encoding the S sequence, devoid of the cytoplasmic domain and/or the transmembrane domain, results in the development of neutralizing antibodies as well as both CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses (31). However, it is not the cellular, but the humoral (IgG) component of immunity that inhibits viral replication (31). In fact, transfer of immune serum from immunized mice to naive mice reduces SARS-CoV titers following viral challenge (25). Together, these studies show that primarily Abs are responsible for protection against SARS-CoV replication, and indicate the potential therapeutic value of passive transfer of neutralizing Abs against SARS-CoV. The immunogenic property of the S protein, including its ability to induce neutralizing antibodies and its essential role in viral attachment and fusion, make it an ideal target for developing effective immunotherapy against SARS-CoV infection.

[0005] During an outbreak, the SARS-CoV can mutate and exhibit antigenic variation. In fact sequence analysis indicated that the clinical isolates could be divided into early, middle, and late isolates (27). The significance of this is demonstrated in the ability of later isolates to escape neutralization by a monoclonal antibody that effectively neutralized an earlier isolate (32). Therefore, it is important to produce neutralizing mAbs that are effective against a wide range of clinical isolates with antigenic diversity. Because of the potential evolution of antigenic variants an effective passive therapy against SARS-CoV will likely contain a cocktail of neutralizing Abs that target different epitopes and/or steps in the entry process, such as blocking receptor binding and fusion.

[0006] Passive therapy with human immunoglobulin can confer immediate protection without the deleterious effects associated with the use of animal or chimeric Abs containing animal derived amino acid sequences. Accordingly, there remains an urgent need for potent, broad spectrum antibody therapeutics for use in treating SARS- CoV infection.

#### SUMMARY OF THE INVENTION

[0007] In certain aspects, the disclosure provides a neutralizing human monoclonal antibody or antigen-binding portion thereof that specifically binds to a region of human severe acute respiratory syndrome (SARS) Corona Virus (SARS-CoV) Spike (S) protein and blocks S protein binding to a receptor, wherein said region is selected from the group consisting of: amino acid residues 1-1255 (SEQ ID NO: 94), a region that is at least 85% identical to SEQ ID NO: 94, amino acid residues 12-261 (SEQ ID NO: 95), a region that is at least 85% identical to SEQ ID NO: 95, amino acid residues 318-510 (SEQ ID NO: 96), a region that is at least 85% identical to SEQ ID NO: 96, amino acid residues 15-680 (SEQ ID NO: 97), and a region that is at least 85% identical to SEQ ID NO: 97.

[0008] In certain embodiments, said antibody or antigen-binding portion binds to the S protein in the region defined by amino acid residues 15-680 (SEQ ID NO: 97). In certain embodiments, said antibody or antigen-binding portion binds to the S protein in a region that is at least 85% identical to SEQ ID NO: 97. In certain embodiments, said antibody or antigen-binding portion binds to the S protein in a region that is at least 80%, 90% or 95%

identical to SEQ ID NO: 97. In certain embodiments, said antibody or antigen-binding portion blocks binding of the S protein to angiotensin converting enzyme 2 (Ace2).

[0009] In certain embodiments, said antibody or antigen-binding portion binds to the S protein in the region defined by amino acid residues 12-261 (SEQ ID NO: 95). In certain  
5       embodiments, said antibody or antigen-binding portion binds to the S protein in a region that is at least 85% identical to SEQ ID NO: 95. In certain embodiments, said antibody or antigen-binding portion binds to the S protein in a region that is at least 80%, 90% or 95% identical to SEQ ID NO: 95. In certain embodiments, said antibody or antigen-binding portion blocks binding of the S protein to angiotensin converting enzyme 2 (Ace2).

10       [0010] In certain embodiments, said antibody or an antigen-binding portion according to any of the preceding embodiments binds to the S protein in the region defined by amino acid residues 318-510 (SEQ ID NO: 96). In certain embodiments, said antibody or antigen-binding portion according to any of the preceding embodiments binds to the S protein in a region that is at least 85% identical to SEQ ID NO: 96. In certain embodiments, said  
15       antibody or antigen-binding portion according to any of the preceding embodiments binds to the S protein in a region that is at least 80%, 90% or 95% identical to SEQ ID NO: 96. In certain embodiments, said antibody or antigen-binding portion blocks binding of the S protein to angiotensin converting enzyme 2 (Ace2).

[0011] In certain embodiments, said antibody or antigen-binding portion comprises a  
20       heavy chain that utilizes a human VH 4-59 gene, a human VH 1-18 gene, a human VH 3-33 gene, or a human VH 1-2 gene. In certain embodiments, said antibody or antigen-binding portion comprises a light chain that utilizes a human VK A30 gene, a human VK L5 gene, or a human VK A1 gene.

[0012] In certain aspects, the disclosure provides a human monoclonal antibody or  
25       antigen-binding portion that specifically binds to human severe acute respiratory syndrome (SARS) Corona Virus (SARS-CoV) S protein, wherein said antibody or antigen-binding portion neutralizes at least 50% of 200 times the tissue culture infectious dose (200xTCID<sub>50</sub>) of the virus at an antibody concentration of 12.5 µg/ml or less. In some  
30       embodiments, neutralizing antibodies are effective at antibody concentrations of <3.125 µg/ml, <.8 µg/ml, <.2 µg/ml, or <.1 µg/ml.

**[0013]** In certain aspects, the disclosure provides a human monoclonal antibody or antigen-binding portion thereof that specifically binds SARS-CoV S protein comprising VL and VH domains that are at least 90% identical in amino acid sequence to the VL and VH domains, respectively, of a monoclonal antibody selected from the group consisting of: 1B5,  
 5 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2.

**[0014]** In certain aspects, the disclosure provides a human monoclonal antibody or  
 10 antigen-binding portion thereof that specifically binds SARS-CoV S protein comprising VL and VH domains that are at least 80%, 85% or 95% identical in amino acid sequence to the VL and VH domains, respectively, of a monoclonal antibody selected from the group consisting of: 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1,  
 15 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2.

**[0015]** In certain aspects, the disclosure provides a human monoclonal antibody or antigen-binding portion thereof that specifically binds SARS-CoV S protein comprising:  
 (a) a heavy chain variable domain amino acid sequence that comprises the amino acid  
 20 sequence of the heavy chain variable domain of an antibody selected from: 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2;  
 25 (b) a light chain variable domain amino acid sequence that comprises the amino acid sequence of the light chain variable domain of an antibody selected from: 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2;  
 30 (c) a heavy chain variable domain of (a) and a light chain variable domain of (b); or

(d) heavy chain and light chain variable domain amino acid sequences comprising the heavy chain and light chain variable domain amino acid sequences, respectively, from the same antibody selected from: 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2.

**[0016]** In certain aspects, the disclosure provides a monoclonal antibody or an antigen-binding portion thereof that specifically binds human SARS-CoV S protein, comprising:

- (a) a heavy chain variable domain amino acid sequence that comprises the heavy chain CDR1, CDR2 and CDR3 amino acid sequences of an antibody selected from: 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2;
- (b) a light chain variable domain amino acid sequence that comprises the light chain CDR1, CDR2 and CDR3 amino acid sequences of an antibody selected from: 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2;
- (c) a heavy chain variable domain of (a) and a light chain variable domain of (b); or
- (d) the heavy chain variable domain and light chain variable domain of (c), comprising heavy chain and light chain CDR amino acid sequences from the same antibody selected from: 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2.

**[0017]** In certain aspects, the disclosure provides a monoclonal antibody or an antigen-binding portion thereof that specifically binds SARS-CoV S protein, wherein the antibody comprises FR1, FR2, FR3 and FR4 amino acid sequences from an antibody selected from:

1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4,

5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2.

[0018] In certain aspects, the disclosure provides a monoclonal antibody that specifically binds SARS-CoV S protein, wherein said antibody comprises a heavy chain of an antibody  
5 selected from the group consisting of: 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2.

[0019] In certain aspects, the disclosure provides a monoclonal antibody that specifically  
10 binds SARS-CoV S protein, wherein said antibody comprises a light chain of an antibody selected from the group consisting of: 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2.

[0020] In certain aspects, the disclosure provides a monoclonal antibody that specifically  
15 binds SARS-CoV S protein, wherein said antibody comprises a heavy chain and a light chain of the same antibody which is selected from the group consisting of: 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1,  
20 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2.

[0021] In certain aspects, the disclosure provides a composition comprising at least one neutralizing human monoclonal antibody or antigen-binding portion thereof that specifically binds to a region of human SARS-CoV S protein,

wherein said region is selected from the group consisting of: amino acid residues 1-1255

25 (SEQ ID NO: 94), a region that is at least 85% identical to SEQ ID NO: 94, amino acid residues 12-261 (SEQ ID NO: 95), a region that is at least 85% identical to SEQ ID NO: 95, amino acid residues 318-510 (SEQ ID NO: 96), a region that is at least 85% identical to SEQ ID NO: 96, amino acid residues 15-680 (SEQ ID NO: 97), and a region that is at least 85% identical to SEQ ID NO: 97 and a pharmaceutically-acceptable carrier.

[0022] In certain embodiments, said composition further comprising at least one additional  
30 therapeutic agent selected from the group consisting of:



(a) one or more antibodies or an antigen binding portion thereof, wherein said antibody is from the group consisting of: 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2;

(b) one or more antibodies that specifically bind SARS-CoV S protein of a different SARS-CoV strain;

(c) one or more SARS-CoV S protein neutralizing antibodies, wherein said antibodies do not bind SARS-CoV S protein;

(d) one or more agents that bind a SARS-CoV S protein receptor and blocks binding of S protein to the receptor; and

(e) one or more anti-viral agents.

**[0023]** In certain embodiments, at least one additional SARS-CoV neutralizing human monoclonal antibody or antigen-binding portion thereof comprises at least two antibodies that specifically bind to different regions of human SARS-CoV S protein selected from the group consisting of: amino acid residues 1-1255 (SEQ ID NO: 94), a region that is at least 85% identical to SEQ ID NO: 94, amino acid residues 12-261 (SEQ ID NO: 95), a region that is at least 85% identical to SEQ ID NO: 95, amino acid residues 318-510 (SEQ ID NO: 96), a region that is at least 85% identical to SEQ ID NO: 96, amino acid residues 15-680 (SEQ ID NO: 97), and a region that is at least 85% identical to SEQ ID NO: 97.

**[0024]** In certain aspects, the disclosure provides an isolated cell line that produces (i) the antibody or antigen-binding portion according to any one of the preceding embodiments; or (ii) the heavy chain or light chain of said antibody or antigen-binding portion.

**[0025]** In certain aspects, the disclosure provides an isolated nucleic acid molecule comprising a nucleotide sequence that encodes the heavy chain or an antigen-binding portion or the light chain or an antigen-binding portion thereof of an antibody according to any one of the preceding embodiments.

**[0026]** In certain aspects, the disclosure provides a vector comprising the nucleic acid molecule according to any one of the preceding embodiments, wherein the vector optionally comprises an expression control sequence operably linked to the nucleic acid molecule.

[0027] In certain aspects, the disclosure provides a host cell comprising a vector according to any one of the preceding embodiments or a nucleic acid molecule according to any one of the preceding embodiments.

[0028] In certain aspects, the disclosure provides a non-human transgenic animal or transgenic plant comprising the nucleic acid according to any one of the preceding  
5       embodiments, wherein the non-human transgenic animal or transgenic plant expresses said nucleic acid. In certain embodiments, said non-human transgenic animal is a mammal.

[0029] In certain aspects, the disclosure provides a method for isolating an antibody or antigen-binding portion thereof that specifically binds to human SARS-CoV S protein,  
10       comprising the step of isolating the antibody from the non-human transgenic animal or transgenic plant according to any one of the preceding embodiments.

[0030] In certain aspects, the disclosure provides a method for producing a human monoclonal antibody according to any one of the preceding embodiments comprising the step of expressing the antibody in a host cell according to any one of the preceding  
15       embodiments.

[0031] In certain aspects, the disclosure provides a method for decreasing S protein-mediated SARS-CoV binding to cells, the method comprising the step of contacting the S protein with an antibody or antigen-binding portion according to any one of the preceding  
20       embodiments. In certain embodiments, said cells express angiotensin converting enzyme 2 (Ace2).

[0032] In certain aspects, the disclosure provides a method for decreasing a SARS-CoV S protein-mediated activity, comprising contacting the S protein with an antibody or antigen-binding portion according to any one of the preceding embodiments; or a composition according to any one of the preceding embodiments. In certain embodiments, said SARS-  
25       CoV S protein-mediated activity is selected from: viral attachment to a cell, fusing of viral membrane with a cell, or combinations thereof. In certain embodiments, said virus is in a subject.

[0033] In certain aspects, the disclosure provides a method for decreasing the SARS-CoV viral load in a subject in need thereof comprising the step of administering an antibody  
30       according to any one of the preceding embodiments.

[0034] In certain aspects, the disclosure provides a method for treating, preventing or alleviating the symptoms of a SARS-CoV-mediated disorder in a subject in need thereof, comprising the step of administering to said subject an antibody or antigen-binding portion according to any one of the preceding embodiments or a composition according to any one  
5 of the preceding embodiments. In certain embodiments, said SARS-CoV-mediated disorder is severe acute respiratory syndrome (SARS).

[0035] In certain aspects, the disclosure provides a method for treating, preventing or alleviating the symptoms of a SARS-CoV-mediated disorder in a subject in need thereof, comprising the step of administering to said subject an antibody or antigen-binding portion  
10 according to any one of the preceding embodiments, further comprising at least one additional therapeutic agent selected from the group consisting of:

(a) one or more antibodies from the group consisting of: 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1,  
15 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2;

(b) one or more antibodies that specifically bind SARS-CoV S protein of a plurality of SARS-CoV strains;

(c) one or more neutralizing antibodies that do not bind SARS-CoV S protein;

(d) one or more agents that bind SARS-CoV S protein receptor; and

20 (e) one or more anti-viral agents.

[0036] The invention contemplates combinations of any of the foregoing aspects and embodiments of the invention.

#### BRIEF DESCRIPTION OF THE DRAWINGS

[0037] **Figures 1A-1B** show expression of overlapping fragments of the S1 domain of  
25 SARS-CoV S protein. (a) Four plasmid constructs encoding different fragments of the S1 protein (12-672, 12-510, 261-672, 318-510) were transformed into MC 1061/P3 cells and insert size confirmed by digestion with NheI and BamHI and analyzed on a 1% agarose gel. (b) Protein expression in transiently transfected 293T cells was confirmed by Coomassie Blue staining of a 4-20% SDS/PAGE gel.

[0038] **Figure 2** shows reactivity of anti-SARS-CoV antibodies produced from hybridomas generated from immunized XenoMouse<sup>®</sup> mice against S1-Ig fragments. All S-V5-HIS reacting monoclonal antibodies were tested against S1-Ig (12-672) fragments in an ELISA. A total of 165 human monoclonal antibodies reacted with S1-Ig (12-672). These monoclonal antibodies were then further examined for their reactivity against the other three S1-Ig fragments (12-510, 261-510, and 318-510). Plates were coated with indicated protein fragments at 50ng/well, 50μl of hybridoma supernatant containing S specific human antibodies were used. HRP conjugated anti-human antibody was used to detect the binding of human antibodies. An OD of  $\geq 0.5$  was considered to be highly reactive.

[0039] **Figure 3** shows neutralizing monoclonal antibodies were purified and examined for S1-Ig fragment reactivity. Following identification of neutralizing monoclonal antibodies, purified antibodies were examined by ELISA for their reactivity against relevant S1-Ig fragments (318-510 or 12-510). Plates were coated with the indicated protein fragments at 50ng/well and indicated amounts of human monoclonal antibodies were added. HRP conjugated anti-human antibody was used to detect the binding of human antibodies.

[0040] **Figure 4** shows an alignment of CDR sequences of neutralizing monoclonal antibodies. Immunoglobulin genes of neutralizing antibodies were sequenced. Alignment of the amino acid sequences of the heavy chain variable region (left) and light chain variable region (right) of all human mAbs are depicted and arranged by common gene segment usage. Additions in antibody sequences not contained in germline sequence are annotated (#) in germline sequence.

[0041] **Figures 5A-5F** show receptor binding inhibition of neutralizing human anti-SARS-CoV monoclonal antibodies. (a) Group 1A1, (b) Group 1b1, (c) Group 1B2, (d) Group 1B3 and 1B4, (e) Group 1D, and (f) Group 2B. The results of antibodies 3A7, 3F3, and 3C7 of groups 1B1, 2, and 4 respectively are low because recombinant S1 binding in those experiments was unusually low.

## DETAILED DESCRIPTION OF THE INVENTION

Definitions and General Techniques

[0042] Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclature used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well known and commonly used in the art.

[0043] The methods and techniques of the present invention are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, *e.g.*, Sambrook *et al.* Molecular Cloning: A Laboratory Manual, second ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) and Ausubel *et al.*, Current Protocols in Molecular Biology, Greene Publishing Associates (1992), and Harlow and Lane Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1990), incorporated herein by reference. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein.

The nomenclature used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

[0044] The following terms, unless otherwise indicated, shall be understood to have the following meanings:

[0045] The term "polypeptide" encompasses native or artificial proteins, protein fragments and polypeptide analogs of a protein sequence. A polypeptide may be monomeric or polymeric.

[0046] The term “isolated protein”, “isolated polypeptide” or “isolated antibody” is a protein, polypeptide or antibody that by virtue of its origin or source of derivation (1) is not associated with naturally associated components that accompany it in its native state, (2) is free of other proteins from the same species, (3) is expressed by a cell from a different species, or (4) does not occur in nature. Thus, a polypeptide that is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates will be “isolated” from its naturally associated components. A protein may also be rendered substantially free of naturally-associated components by isolation, using protein purification techniques well known in the art.

[0047] Examples of isolated antibodies include an anti- SARS-CoV S protein antibody that has been affinity purified using SARS-CoV S protein or a portion thereof, an anti- SARS-CoV S protein antibody that has been synthesized by a hybridoma or other cell line *in vitro*, and a human anti- SARS-CoV S protein antibody derived from a transgenic mouse.

[0048] A protein or polypeptide is “substantially pure,” “substantially homogeneous,” or “substantially purified” when at least about 60 to 75% of a sample exhibits a single species of polypeptide. The polypeptide or protein may be monomeric or multimeric. A substantially pure polypeptide or protein will typically comprise about 50%, 60%, 70%, 80% or 90% W/W of a protein sample, more usually about 95%, and preferably will be over 99% pure. Protein purity or homogeneity may be indicated by a number of means well known in the art, such as polyacrylamide gel electrophoresis of a protein sample, followed by visualizing a single polypeptide band upon staining the gel with a stain well known in the art. For certain purposes, higher resolution may be provided by using HPLC or other means well known in the art for purification.

[0049] The term “polypeptide fragment” as used herein refers to a polypeptide that has an amino-terminal and/or carboxy-terminal deletion, but where the remaining amino acid sequence is identical to the corresponding positions in the naturally-occurring sequence. In some embodiments, fragments are at least 5, 6, 8 or 10 amino acids long. In other embodiments, the fragments are at least 14, at least 20, at least 50, or at least 70, 80, 90, 100, 150 or 200 amino acids long.

[0050] The term “polypeptide analog” as used herein refers to a polypeptide that comprises a segment that has substantial identity to a portion of an amino acid sequence and

that has at least one of the following properties: (1) specific binding to SARS-CoV S protein under suitable binding conditions, (2) ability to inhibit SARS-CoV S protein.

Typically, polypeptide analogs comprise a conservative amino acid substitution (or insertion or deletion) with respect to the native sequence. Analogs typically are at least 20 or 25 amino acids long, preferably at least 50, 60, 70, 80, 90, 100, 150 or 200 amino acids long or longer, and can often be as long as a full-length polypeptide. Some embodiments of the invention include polypeptide fragments or polypeptide analog antibodies with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 or 17 substitutions from the germline amino acid sequence.

**[0051]** In certain embodiments, amino acid substitutions to an anti- SARS-CoV S protein antibody or antigen-binding portion thereof are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, and (4) confer or modify other physicochemical or functional properties of such analogs, but still retain specific binding to SARS-CoV S protein. Analogs can include various muteins of a sequence other than the normally-occurring peptide sequence. For example, single or multiple amino acid substitutions, preferably conservative amino acid substitutions, may be made in the normally-occurring sequence, preferably in the portion of the polypeptide outside the domain(s) forming intermolecular contacts. A conservative amino acid substitution should not substantially change the structural characteristics of the parent sequence; *e.g.*, a replacement amino acid should not alter the anti-parallel  $\beta$ -sheet that makes up the immunoglobulin binding domain that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence. In general, glycine and proline would not be used in an anti-parallel  $\beta$ -sheet. Examples of art-recognized polypeptide secondary and tertiary structures are described in *Proteins, Structures and Molecular Principles* (Creighton, Ed., W. H. Freeman and Company, New York (1984)); Introduction to Protein Structure (C. Branden and J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); and Thornton *et al.*, *Nature* 354:105 (1991), incorporated herein by reference.

**[0052]** Non-peptide analogs are commonly used in the pharmaceutical industry as drugs with properties analogous to those of the template peptide. These types of non-peptide compound are termed "peptide mimetics" or "peptidomimetics." Fauchere, *J. Adv. Drug*

Res. 15:29 (1986); Veber and Freidinger, *TINS* p.392 (1985); and Evans *et al.*, *J. Med. Chem.* 30:1229 (1987), incorporated herein by reference. Such compounds are often developed with the aid of computerized molecular modeling. Peptide mimetics that are structurally similar to therapeutically useful peptides may be used to produce an equivalent therapeutic or prophylactic effect. Generally, peptidomimetics are structurally similar to a paradigm polypeptide (*i.e.*, a polypeptide that has a desired biochemical property or pharmacological activity), such as a human antibody, but have one or more peptide linkages optionally replaced by a linkage selected from the group consisting of: --CH<sub>2</sub>NH--, --CH<sub>2</sub>S--, --CH<sub>2</sub>-CH<sub>2</sub>--, --CH=CH--(cis and trans), --COCH<sub>2</sub>--, --CH(OH)CH<sub>2</sub>--, and --CH<sub>2</sub>SO--, by methods well known in the art. Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (*e.g.*, D-lysine in place of L-lysine) may also be used to generate more stable peptides. In addition, constrained peptides comprising a consensus sequence or a substantially identical consensus sequence variation may be generated by methods known in the art (Rizo and Gierasch, *Ann. Rev. Biochem.* 61:387 (1992), incorporated herein by reference); for example, by adding internal cysteine residues capable of forming intramolecular disulfide bridges which cyclize the peptide.

**[0053]** Where an "antibody" is referred to herein with respect to the invention, it is normally understood that an antigen-binding portion thereof may also be used. An antigen-binding portion competes with the intact antibody for specific binding. See generally, Fundamental Immunology, Ch. 7 (Paul, W., ed., second ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety for all purposes). Antigen-binding portions may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. In some embodiments, antigen-binding portions include Fab, Fab', F(ab')<sub>2</sub>, Fd, Fv, dAb, and complementarity determining region (CDR) fragments, single-chain antibodies (scFv), chimeric antibodies, diabodies and polypeptides that contain at least a portion of an antibody that is sufficient to confer specific antigen binding to the polypeptide.

**[0054]** From N-terminus to C-terminus, both the mature light and heavy chain variable domains comprise the regions FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain herein is in accordance with the definitions of



Kabat, Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987 and 1991)), Chothia & Lesk, *J. Mol. Biol.* 196:901-917 (1987) or Chothia *et al.*, *Nature* 342:878-883 (1989).

[0055] As used herein, an antibody that is referred to by number is the same as a

5 monoclonal antibody that is obtained from the hybridoma of the same number. For example, monoclonal antibody 5C12 is the same antibody as one obtained from hybridoma 5C12, or a subclone thereof.

[0056] As used herein, a Fd fragment means an antibody fragment that consists of the V<sub>H</sub> and C<sub>H</sub>1 domains; an Fv fragment consists of the V<sub>L</sub> and V<sub>H</sub> domains of a single arm of an  
10 antibody; and a dAb fragment (Ward *et al.*, *Nature* 341:544-546 (1989)) consists of a V<sub>H</sub> domain.

[0057] In some embodiments, the antibody is a single-chain antibody (scFv) in which a V<sub>L</sub> and V<sub>H</sub> domains are paired to form a monovalent molecules via a synthetic linker that enables them to be made as a single protein chain. (Bird *et al.*, *Science* 242:423-426 (1988)  
15 and Huston *et al.*, *Proc. Natl. Acad. Sci. USA* 85:5879-5883 (1988).) In some embodiments, the antibodies are diabodies, *i.e.*, are bivalent antibodies in which V<sub>H</sub> and V<sub>L</sub> domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding  
20 sites. (See *e.g.*, Holliger P. *et al.*, *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993), and Poljak R. J. *et al.*, *Structure* 2:1121-1123 (1994).) In some embodiments, one or more CDRs from an antibody of the invention may be incorporated into a molecule either covalently or noncovalently to make it an immunoadhesin that specifically binds to SARS-CoV S protein. In such embodiments, the CDR(s) may be incorporated as part of a larger  
25 polypeptide chain, may be covalently linked to another polypeptide chain, or may be incorporated noncovalently.

[0058] In embodiments having one or more binding sites, the binding sites may be identical to one another or may be different.

[0059] As used herein, the term "human antibody" means any antibody in which the  
30 variable and constant domain sequences are human sequences. The term encompasses antibodies with sequences derived from human genes, but which have been changed, *e.g.* to

decrease possible immunogenicity, increase affinity, eliminate cysteines that might cause undesirable folding, etc. The term encompasses such antibodies produced recombinantly in non-human cells, which might impart glycosylation not typical of human cells. These antibodies may be prepared in a variety of ways, as described below.

5   **[0060]** The term “chimeric antibody” as used herein means an antibody that comprises regions from two or more different antibodies. In one embodiment, one or more of the CDRs of the chimeric antibody are derived from a human anti- SARS-CoV S protein antibody. In another embodiment, all of the CDRs are derived from a human anti- SARS-CoV S protein antibodies. In another embodiment, the CDRs from more than one human  
10 anti- SARS-CoV S protein antibodies are combined in a chimeric antibody. For instance, a chimeric antibody may comprise a CDR1 from the light chain of a first human anti- SARS-CoV S protein antibody, a CDR2 from the light chain of a second human anti- SARS-CoV S protein antibody and a CDR3 from the light chain of a third human anti- SARS-CoV S protein antibody, and CDRs from the heavy chain may be derived from one or more other  
15 anti- SARS-CoV S protein antibodies. Further, the framework regions may be derived from one of the anti- SARS-CoV S protein antibodies from which one or more of the CDRs are taken or from one or more different human antibodies.

**[0061]** In some embodiments, a chimeric antibody of the invention is a humanized anti-SARS-CoV S protein antibody. A humanized anti- SARS-CoV S protein antibody of the  
20 invention comprises the amino acid sequence of one or more framework regions and/or the amino acid sequence from at least a portion of the constant region of one or more human anti- SARS-CoV S protein antibodies of the invention and CDRs derived from a non-human anti- SARS-CoV S protein antibody.

**[0062]** A “neutralizing antibody”, an antibody with “neutralizing activity”, “antagonistic  
25 antibody”, or “inhibitory antibody” as used herein means an antibody that neutralizes 200 times the tissue culture infectious dose required to infect 50% of cells (200xTCID<sub>50</sub>) of the SARS-Corona virus. In some embodiments, neutralizing antibodies are effective at antibody concentrations of <12.5 µg/ml, <3.125 µg/ml, <.8 µg/ml. In preferred  
embodiments, neutralizing antibodies are effective at antibody concentrations of <.2 µg/ml.  
30 In the most preferred embodiments, neutralizing antibodies are effective at antibody concentrations of <.1 µg/ml.

[0063] Fragments or analogs of antibodies or immunoglobulin molecules can be readily prepared by those of ordinary skill in the art following the teachings of this specification. Preferred amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid sequence data to public or proprietary sequence databases. Preferably, computerized comparison methods are used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known. See Bowie *et al.*, *Science* 253:164 (1991).

[0064] The term “surface plasmon resonance”, as used herein, refers to an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIACORE™ system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.). For further descriptions, see Jonsson U. *et al.*, *Ann. Biol. Clin.* 51:19-26 (1993); Jonsson U. *et al.*, *Biotechniques* 11:620-627 (1991); Jonsson B. *et al.*, *J. Mol. Recognit.* 8:125-131 (1995); and Johnsson B. *et al.*, *Anal. Biochem.* 198:268-277 (1991).

[0065] The term “TCID<sub>50</sub>” refers to the amount of virus necessary to infect 50% of cells in tissue culture. The 100x and 200x refer to 100 or 200 times the concentration of virus compared to the TCID<sub>50</sub>.

[0066] The term “K<sub>D</sub>” refers to the equilibrium dissociation constant of a particular antibody-antigen interaction.

[0067] The term “epitope” includes any protein determinant capable of specific binding to an immunoglobulin or T-cell receptor or otherwise interacting with a molecule. Epitopic determinants generally consist of chemically-active surface groupings of molecules such as amino acids or carbohydrate or sugar side chains and generally have specific three dimensional structural characteristics, as well as specific charge characteristics. An epitope may be “linear” or “conformational.” In a linear epitope, all of the points of interaction between the protein and the interacting molecule (such as an antibody) occur linearly along the primary amino acid sequence of the protein. In a conformational epitope, the points of interaction occur across amino acid residues on the protein that are separated from one another. An antibody is said to specifically bind an antigen when the dissociation constant

is  $\leq 1$  mM, preferably  $\leq 100$  nM and most preferably  $\leq 10$  nM. In certain embodiments, the  $K_D$  is 1 pM to 500 pM. In other embodiments, the  $K_D$  is between 500 pM to 1  $\mu$ M. In other embodiments, the  $K_D$  is between 1  $\mu$ M to 100 nM. In other embodiments, the  $K_D$  is between 100 mM to 10 nM. Once a desired epitope on an antigen is determined, it is possible to generate antibodies to that epitope, *e.g.*, using the techniques described in the present invention. Alternatively, during the discovery process, the generation and characterization of antibodies may elucidate information about desirable epitopes. From this information, it is then possible to competitively screen antibodies for binding to the same epitope. An approach to achieve this is to conduct cross-competition studies to find antibodies that competitively bind with one another, *e.g.*, the antibodies compete for binding to the antigen. A high throughput process for “binning” antibodies based upon their cross-competition is described in International Patent Application No. WO 03/48731.

[0068] As used herein, the twenty conventional amino acids and their abbreviations follow conventional usage. See Immunology - A Synthesis (second Edition, E.S. Golub and D.R. Gren, Eds., Sinauer Associates, Sunderland, Mass. (1991)), incorporated herein by reference.

[0069] The term “polynucleotide” as referred to herein means a polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms.

[0070] The term “isolated polynucleotide” as used herein means a polynucleotide of genomic, cDNA, or synthetic origin or some combination thereof, which by virtue of its origin the “isolated polynucleotide” (1) is not associated with all or a portion of a polynucleotides with which the “isolated polynucleotide” is found in nature, (2) is operably linked to a polynucleotide to which it is not linked in nature, or (3) does not occur in nature as part of a larger sequence.

[0071] The term “naturally occurring nucleotides” as used herein includes deoxyribonucleotides and ribonucleotides. The term “modified nucleotides” as used herein includes nucleotides with modified or substituted sugar groups and the like. The term “oligonucleotide linkages” referred to herein includes oligonucleotides linkages such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate,

phosphoroanilothioate, phosphoraniladate, phosphoroamidate, and the like. See *e.g.*, LaPlanche *et al.*, *Nucl. Acids Res.* 14:9081 (1986); Stec *et al.*, *J. Am. Chem. Soc.* 106:6077 (1984); Stein *et al.*, *Nucl. Acids Res.* 16:3209 (1988); Zon *et al.*, *Anti-Cancer Drug Design* 6:539 (1991); Zon *et al.*, Oligonucleotides and Analogues: A Practical Approach, pp. 87-108 (F. Eckstein, Ed., Oxford University Press, Oxford England (1991)); U.S. Patent No. 5,151,510; Uhlmann and Peyman, *Chemical Reviews* 90:543 (1990), the disclosures of which are hereby incorporated by reference. An oligonucleotide can include a label for detection, if desired.

**[0072]** “Operably linked” sequences include both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in *trans* or at a distance to control the gene of interest. The term “expression control sequence” as used herein means polynucleotide sequences that are necessary to effect the expression and processing of coding sequences to which they are ligated. Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (*i.e.*, Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance protein secretion. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence; in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term “control sequences” is intended to include, at a minimum, all components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

**[0073]** The term “vector”, as used herein, means a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. In some embodiments, the vector is a plasmid, *i.e.*, a circular double stranded piece of DNA into which additional DNA segments may be ligated. In some embodiments, the vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. In some embodiments, the vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian

vectors). In other embodiments, the vectors (*e.g.*, non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to  
5 herein as “recombinant expression vectors” (or simply, “expression vectors”).

[0074] The term “recombinant host cell” (or simply “host cell”), as used herein, means a cell into which a recombinant expression vector has been introduced. It should be understood that “recombinant host cell” and “host cell” mean not only the particular subject cell but also the progeny of such a cell. Because certain modifications may occur in  
10 succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term “host cell” as used herein.

[0075] The term “selectively hybridize” referred to herein means to detectably and specifically bind. Polynucleotides, oligonucleotides and fragments thereof in accordance  
15 with the invention selectively hybridize to nucleic acid strands under hybridization and wash conditions that minimize appreciable amounts of detectable binding to nonspecific nucleic acids. “High stringency” or “highly stringent” conditions can be used to achieve selective hybridization conditions as known in the art and discussed herein. One example of “high stringency” or “highly stringent” conditions is the incubation of a polynucleotide with  
20 another polynucleotide, wherein one polynucleotide may be affixed to a solid surface such as a membrane, in a hybridization buffer of 6X SSPE or SSC, 50% formamide, 5X Denhardt’s reagent, 0.5% SDS, 100 µg/ml denatured, fragmented salmon sperm DNA at a hybridization temperature of 42°C for 12-16 hours, followed by twice washing at 55°C using a wash buffer of 1X SSC, 0.5% SDS. See also Sambrook *et al.*, *supra*, pp. 9.50-9.55.

[0076] The term “percent sequence identity” in the context of nucleotide sequences means  
25 the residues in two sequences that are the same when aligned for maximum correspondence. The length of sequence identity comparison may be over a stretch of at least about nine nucleotides, usually at least about 18 nucleotides, more usually at least about 24 nucleotides, typically at least about 28 nucleotides, more typically at least about 32  
30 nucleotides, and preferably at least about 36, 48 or more nucleotides. There are a number of different algorithms known in the art which can be used to measure nucleotide sequence

identity. For instance, polynucleotide sequences can be compared using FASTA, Gap or Bestfit, which are programs in Wisconsin Package Version 10.0, Genetics Computer Group (GCG), Madison, Wisconsin. FASTA, which includes, *e.g.*, the programs FASTA2 and FASTA3, provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson, *Methods Enzymol.* 183:63-98 (1990); Pearson, *Methods Mol. Biol.* 132:185-219 (2000); Pearson, *Methods Enzymol.* 266:227-258 (1996); Pearson, *J. Mol. Biol.* 276:71-84 (1998); incorporated herein by reference). Unless otherwise specified, default parameters for a particular program or algorithm are used. For instance, percent sequence identity between nucleotide sequences can be determined using FASTA with its default parameters (a word size of 6 and the NOPAM factor for the scoring matrix) or using Gap with its default parameters as provided in GCG Version 6.1, incorporated herein by reference.

**[0077]** A reference to a nucleotide sequence encompasses its complement unless otherwise specified. Thus, a reference to a nucleic acid having a particular sequence should be understood to encompass its complementary strand, with its complementary sequence.

**[0078]** As used herein, the terms “percent sequence identity” and “percent sequence homology” are used interchangeably.

**[0079]** The term “substantial similarity” or “substantial sequence similarity,” when referring to a nucleic acid or fragment thereof, means that when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 85%, preferably at least about 90%, and more preferably at least about 95%, 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or Gap, as discussed above.

**[0080]** As applied to polypeptides, the term “substantial identity” means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights as supplied with the programs, share at least 70%, 75% or 80% sequence identity, preferably at least 90% or 95% sequence identity, and more preferably at least 97%, 98% or 99% sequence identity. In certain embodiments, residue positions that are not identical differ by conservative amino acid substitutions. A “conservative amino acid substitution” is one in which an amino acid residue is substituted by another amino acid

residue having a side chain R group with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. See, e.g., Pearson, *Methods Mol. Biol.* 243:307-31 (1994). Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine, and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; 6) acidic side chains: aspartic acid and glutamic acid; and 7) sulfur-containing side chains: cysteine and methionine. Conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine.

**[0081]** Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet *et al.*, *Science* 256:1443-45 (1992), incorporated herein by reference. A “moderately conservative” replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

**[0082]** Sequence identity for polypeptides is typically measured using sequence analysis software. Protein analysis software matches sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG contains programs such as “Gap” and “Bestfit” which can be used with default parameters as specified by the programs to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. See, e.g., GCG Version 6.1 (University of Wisconsin, WI). Polypeptide sequences also can be compared using FASTA using default or recommended parameters, see GCG Version 6.1. FASTA (e.g., FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson, *Methods Enzymol.* 183:63-98 (1990);



Pearson, *Methods Mol. Biol.* 132:185-219 (2000)). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially blastp or tblastn, using default parameters as supplied with the programs. See, *e.g.*, Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990); Altschul *et al.*, *Nucleic Acids Res.* 25:3389-402 (1997).

[0083] The length of polypeptide sequences compared for homology will generally be at least about 16 amino acid residues, usually at least about 20 residues, more usually at least about 24 residues, typically at least about 28 residues, and preferably more than about 35 residues. When searching a database containing sequences from a large number of different organisms, it is preferable to compare amino acid sequences.

[0084] As used herein, the terms "label" or "labeled" refers to incorporation of another molecule in the antibody. In one embodiment, the label is a detectable marker, *e.g.*, incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (*e.g.*, streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). In another embodiment, the label or marker can be therapeutic, *e.g.*, a drug conjugate or toxin. Various methods of labeling polypeptides and glycoproteins are known in the art and may be used. Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (*e.g.*,  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{15}\text{N}$ ,  $^{35}\text{S}$ ,  $^{90}\text{Y}$ ,  $^{99}\text{Tc}$ ,  $^{111}\text{In}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$ ), fluorescent labels (*e.g.*, FITC, rhodamine, lanthanide phosphors), enzymatic labels (*e.g.*, horseradish peroxidase,  $\beta$ -galactosidase, luciferase, alkaline phosphatase), chemiluminescent markers, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (*e.g.*, leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags), magnetic agents, such as gadolinium chelates, toxins such as pertussis toxin, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. In some embodiments, the labels for polypeptides include fluorophore. The term "fluorophore" refers to compounds with a

fluorescent emission maximum between about 400 and 900 nm. In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.

[0085] Throughout this specification and claims, the word “comprise,” or variations such as “comprises” or “comprising,” will be understood to imply the inclusion of a stated

5 integer or group of integers but not the exclusion of any other integer or group of integers.

#### Human Anti-SARS-CoV S protein Antibodies and Characterization Thereof

[0086] In one embodiment, the invention provides humanized anti-SARS-CoV S protein antibodies. In another embodiment, the invention provides human anti-SARS-CoV S protein antibodies. In some embodiments, human anti-SARS-CoV S protein antibodies are  
10 produced by immunizing a non-human transgenic animal, *e.g.*, a rodent, whose genome comprises human immunoglobulin genes so that the transgenic animal produces human antibodies. In some embodiments, the anti-SARS-CoV S protein antibodies and antigen-binding portions include, but are not limited to, antibodies or antigen-binding portions which bind to (i) the S1 domain of SARS-CoV S protein; (ii) the S2 domain of SARS-CoV  
15 S protein; or (iii) both (i) and (ii).

[0087] An anti-SARS-CoV S protein antibody of the invention can comprise a human kappa or a human lambda light chain or an amino acid sequence derived therefrom. In some embodiments comprising a kappa light chain, the light chain variable domain ( $V_L$ ) is encoded in part by a human  $V_{K1}$  or  $V_{K2}$  family gene. In certain embodiments, the light  
20 chain utilizes a human  $V_K A30$ , a human  $V_K L5$  or a human  $V_K A1$  gene.

[0088] In some embodiments, the  $V_L$  of the SARS-CoV S protein antibody comprises one or more amino acid substitutions relative to the germline amino acid sequence of the human gene. In some embodiments, the  $V_L$  of the anti-SARS-CoV S protein antibody comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions relative to the germline amino acid  
25 sequence. In some embodiments, one or more of those substitutions from germline is in the CDR regions of the light chain. In some embodiments, the amino acid substitutions relative to germline are at one or more of the same positions as the substitutions relative to germline in any one or more of the  $V_L$  of antibodies 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1,  
30 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2,

5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2. For example, the V<sub>L</sub> of an anti-SARS-CoV S protein antibody of the invention may contain one or more amino acid substitutions compared to germline found in the V<sub>L</sub> of antibody 4E2. In some embodiments, the amino acid changes are at one or more of the same positions, but involve a different substitution than in the reference antibody.

[0089] In some embodiments, amino acid changes relative to germline occur at one or more of the same positions as in any of the V<sub>L</sub> of antibodies 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2, but the changes may represent conservative amino acid substitutions at such position(s) relative to the amino acid in the reference antibody. For example, if a particular position in one of these antibodies is changed relative to germline and is glutamate, one may substitute aspartate at that position. Similarly, if an amino acid substitution compared to germline is serine, one may conservatively substitute threonine for serine at that position. Conservative amino acid substitutions are discussed *supra*.

[0090] In some embodiments, the light chain of the human anti-SARS-CoV S protein antibody comprises the V<sub>L</sub> amino acid sequence of monoclonal antibody 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2 or said amino acid sequence having up to 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 conservative amino acid substitutions and/or a total of up to 3 non-conservative amino acid substitutions. In some embodiments, the light chain comprises the amino acid sequence from the beginning of the CDR1 to the end of the CDR3 of any one of the foregoing antibodies.

[0091] In some embodiments, the light chain may comprise CDR1, CDR2 and CDR3 regions independently selected from the light chain CDR1, CDR2 and CDR3, respectively, of the light chain of monoclonal antibody 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2, or CDR regions each having less

than 4 or less than 3 conservative amino acid substitutions and/or a total of three or fewer non-conservative amino acid substitutions. In some embodiments, the light chain of the anti-SARS-CoV S protein antibody comprises a light chain CDR1, CDR2, and CDR3, each of which are independently selected from the light chain CDR1, CDR2 and CDR3 regions of monoclonal antibody 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2. In certain embodiments, the light chain of the anti-SARS-CoV S protein antibody comprises the light chain CDR1, CDR2 and CDR3 regions of an antibody comprising the amino acid sequence of the V<sub>L</sub> region of an antibody selected from 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2 or said CDR regions each having less than 4 or less than 3 conservative amino acid substitutions and/or a total of three or fewer non-conservative amino acid substitutions.

**[0092]** With regard to the heavy chain, in some embodiments, the variable domain (V<sub>H</sub>) is encoded in part by a human V<sub>H</sub> 1, V<sub>H</sub>3 or V<sub>H</sub> 4 family gene. In certain embodiments, the heavy chain utilizes a human V<sub>H</sub> 1-2, V<sub>H</sub> 1-18, V<sub>H</sub> 3-33 or V<sub>H</sub> 4-49 gene. In some embodiments, the V<sub>H</sub> sequence of the anti-SARS-CoV S protein antibody contains one or more amino acid substitutions, deletions or insertions (additions) relative to the germline amino acid sequence. In some embodiments, the variable domain of the heavy chain comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17 mutations from the germline amino acid sequence. In some embodiments, the mutation(s) are non-conservative substitutions compared to the germline amino acid sequence. In some embodiments, the mutations are in the CDR regions of the heavy chain. In some embodiments, the amino acid changes are made at one or more of the same positions as the mutations from germline in any one or more of the V<sub>H</sub> of antibodies 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2. In other embodiments, the amino

acid changes are at one or more of the same positions but involve a different mutation than in the reference antibody.

[0093] In some embodiments, the heavy chain comprises the V<sub>H</sub> amino acid sequence of monoclonal antibody; 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2 or said V<sub>H</sub> amino acid sequence having up to 1, 2, 3, 4, 6, 8, or 10 conservative amino acid substitutions and/or a total of up to 3 non-conservative amino acid substitutions. In some embodiments, the heavy chain comprises the amino acid sequence from the beginning of the CDR1 to the end of the CDR3 of any one of the foregoing antibodies.

[0094] In some embodiments, the heavy chain comprises the heavy chain CDR1, CDR2 and CDR3 regions of monoclonal antibody 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2 or said CDR regions each having less than 8, less than 6, less than 4, or less than 3 conservative amino acid substitutions and/or a total of three or fewer non-conservative amino acid substitutions.

[0095] In some embodiments, the heavy chain CDR regions are independently selected from the CDR regions of monoclonal antibodies 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2. In another embodiment, the antibody comprises a light chain as disclosed above and a heavy chain as disclosed above. In a further embodiment, the light chain CDRs and the heavy chain CDRs are from the same antibody.

[0096] One type of amino acid substitution that may be made is to change one or more cysteines in the antibody, which may be chemically reactive, to another residue, such as, without limitation, alanine or serine. In one embodiment, there is a substitution of a non-canonical cysteine. The substitution can be made in a CDR or framework region of a

variable domain or in the constant domain of an antibody. In some embodiments, the cysteine is canonical.

[0097] Another type of amino acid substitution that may be made is to change any potential proteolytic sites in the antibody. Such sites may occur in a CDR or framework region of a variable domain or in the constant domain of an antibody. Substitution of cysteine residues and removal of proteolytic sites may decrease the risk of any heterogeneity in the antibody product and thus increase its homogeneity. Another type of amino acid substitution is to eliminate asparagine-glycine pairs, which form potential deamidation sites, by altering one or both of the residues.

[0098] In some embodiments, the C-terminal lysine of the heavy chain of the anti SARS-CoV S protein antibody of the invention is cleaved. In various embodiments of the invention, the heavy and light chains of the anti-SARS-CoV S protein antibodies may optionally include a signal sequence.

[0099] In one aspect, the invention relates to nineteen inhibitory human anti-SARS-CoV S protein monoclonal antibodies and the hybridoma cell lines that produce them, 4E2, 4G2, 6C1, 3A7, 5A7, 5D3, 5D6, 6B8, 4A10, 6C2, 3F3, 5A5, 6B5, 5E4, 3C7, 6B1, 3H12, 4D4 or 1B5. The nucleic acids encoding the full-length, or variable domain-comprising portions, of heavy and light chains, and the corresponding deduced amino acid sequences can be found in the sequence listing.

[0100] The invention further provides heavy and/or light chain variants of certain of the above-listed human anti-SARS-CoV S protein antibodies, comprising one or more amino acid substitutions. In still further embodiments, the invention includes antibodies comprising variable domain amino acid sequences with more than 80%, more than 85%, more than 90%, more than 95%, more than 96%, more than 97%, more than 98% or more than 99% sequence identity to an variable domain amino acid sequence of any of the above-listed human anti-SARS-CoV S protein antibodies.

#### Class and Subclass of Anti-SARS-CoV S protein Antibodies

[0101] The class and subclass of anti-SARS-CoV S protein antibodies may be determined by any method known in the art. In general, the class and subclass of an antibody may be determined using antibodies that are specific for a particular class and subclass of antibody.

Such antibodies are commercially available. The class and subclass can be determined by ELISA, or Western Blot as well as other techniques. Alternatively, the class and subclass may be determined by sequencing all or a portion of the constant domains of the heavy and/or light chains of the antibodies, comparing their amino acid sequences to the known amino acid sequences of various class and subclasses of immunoglobulins, and determining the class and subclass of the antibodies.

[0102] In some embodiments, the anti-SARS-CoV S protein antibody is a monoclonal antibody. The anti-SARS-CoV S protein antibody can be an IgG, an IgM, an IgE, an IgA, or an IgD molecule. In one embodiment, the anti-SARS-CoV S protein antibody is an IgG and is an IgG1, IgG2, IgG3, IgG4 subclass. In still another embodiment, the antibody is subclass IgG1.

#### Binding Affinity of Anti-SARS-CoV S protein Antibodies to SARS-CoV S protein

[0103] In some embodiments of the invention, the anti-SARS-CoV S protein antibodies bind to SARS-CoV S protein with high affinity.

[0104] In some embodiments, the anti-SARS-CoV S protein antibodies bind with high affinity to the S1 domain of SARS-CoV S protein.

[0105] In some embodiments, the anti-SARS-CoV S protein antibodies bind to the S2 domain of SARS-CoV S protein.

[0106] In another embodiment, the anti-SARS-CoV S protein antibody binds to SARS-CoV S protein.

[0107] The binding affinity and dissociation rate of an anti-SARS-CoV S protein antibody to SARS-CoV S protein can be determined by methods known in the art. The binding affinity can be measured by ELISAs, RIAs, flow cytometry, surface plasmon resonance, such as BIACORE™. The dissociate rate can be measured by surface plasmon resonance. Preferably, the binding affinity and dissociation rate is measured by surface plasmon resonance. More preferably, the binding affinity and dissociation rate are measured using BIACORE™. One can determine whether an antibody has substantially the same  $K_D$  as an anti-SARS-CoV S protein antibody by using methods known in the art. Example V exemplifies a method for determining affinity constants of anti-SARS-CoV S protein monoclonal antibodies.

Identification of SARS-CoV S protein Epitopes Recognized by Anti-SARS-CoV S protein Antibodies

[0108] The invention provides a human anti-SARS-CoV S protein monoclonal antibody that binds to SARS-CoV S protein and competes or cross-competes with and/or binds the same epitope as an antibody selected from 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2; If two antibodies reciprocally compete with each other for binding to SARS-CoV S protein, they are said to cross-compete.

[0109] One can determine whether an antibody binds to the same epitope or cross competes for binding with an anti-SARS-CoV S protein antibody by using methods known in the art. In one embodiment, one allows the anti-SARS-CoV S protein antibody of the invention to bind to SARS-CoV S protein under saturating conditions and then measures the ability of the test antibody to bind to SARS-CoV S protein. If the test antibody is able to bind to SARS-CoV S protein at the same time as the anti-SARS-CoV S protein antibody, then the test antibody binds to a different epitope as the anti-SARS-CoV S protein antibody. However, if the test antibody is not able to bind to SARS-CoV S protein at the same time, then the test antibody binds to the same epitope, an overlapping epitope, or an epitope that is in close proximity to the epitope bound by the human anti-SARS-CoV S protein antibody. This experiment can be performed using ELISA, RIA, BIACORE™, or flow cytometry.

[0110] To test whether an anti-SARS-CoV S protein antibody cross-competes with another anti-SARS-CoV S protein antibody, one may use the competition method described above in two directions *i.e.* determining if the reference antibody blocks the test antibody and vice versa. In one embodiment, the experiment is performed using ELISA. Methods of determining  $K_D$  are discussed further below.



Inhibition of SARS-CoV S Protein Activity by Anti-SARS-CoV S protein Antibody

[0111] In another embodiment, the invention provides an anti-SARS-CoV S protein antibody that inhibits, blocks, or decreases SARS-CoV S protein binding to a receptor, in particular, to angiotensin-converting enzyme 2 (ACE2). In another embodiment, the invention provides an anti-SARS-CoV S protein antibody that inhibits, blocks, or decreases SARS-CoV S protein-mediated viral entry into cells. In another embodiment, the invention provides an anti-SARS-CoV S protein antibody that inhibits, blocks, or decreases fusion of viral and cell membranes. In another embodiment, the invention provides an anti-SARS-CoV S protein antibody that decreases viral load. In another embodiment, the invention provides an anti-SARS-CoV S protein antibody that inhibits, blocks, or decreases in severity for any period of time symptoms or conditions resulting from SARS-CoV infection. In certain embodiments, the invention provides an anti-SARS-CoV S protein antibody that inhibits, blocks, or decreases in severity for a day, a week, a month, 6 months, a year, or for the remainder of the subjects life symptoms or conditions resulting from SARS-CoV infection by 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100%. In certain embodiments, the invention provides an anti-SARS-CoV S protein antibody that may perform any combination of the preceding embodiments.

Methods of Producing Antibodies and Antibody Producing Cell Lines20 *Immunization*

[0112] In some embodiments, human antibodies are produced by immunizing a non-human, transgenic animal comprising within its genome some or all of human immunoglobulin heavy chain and light chain loci with a SARS-CoV S protein antigen. In one embodiment, the non-human animal is a XENOMOUSE™ animal. (Abgenix, Inc., Fremont, CA).

[0113] XENOMOUSE™ mice are engineered mouse strains that comprise large fragments of human immunoglobulin heavy chain and light chain loci and are deficient in mouse antibody production. See, e.g., Green *et al.*, *Nature Genetics* 7:13-21 (1994) and

U.S. Patents 5,916,771, 5,939,598, 5,985,615, 5,998,209, 6,075,181, 6,091,001, 6,114,598, 6,130,364, 6,162,963 and 6,150,584. See also WO 91/10741, WO 94/02602, WO 96/34096, WO 96/33735, WO 98/16654, WO 98/24893, WO 98/50433, WO 99/45031, WO 99/53049, WO 00/09560, and WO 00/037504.

5 [0114] In another aspect, the invention provides a method for making anti-SARS-CoV S protein antibodies from non-human, non-mouse animals by immunizing non-human transgenic animals that comprise human immunoglobulin loci with a SARS-CoV S protein antigen. One can produce such animals using the methods described in the above-cited documents. The methods disclosed in these documents can be modified as described in  
10 U.S. Patent 5,994,619, which is hereby incorporated by reference. U.S. Patent 5,994,619 describes methods for producing novel cultured inner cell mass (CICM) cells and cell lines, derived from pigs and cows, and transgenic CICM cells into which heterologous DNA has been inserted. CICM transgenic cells can be used to produce cloned transgenic embryos, fetuses, and offspring. The '619 patent also describes methods of producing transgenic  
15 animals that are capable of transmitting the heterologous DNA to their progeny. In preferred embodiments of the current invention, the non-human animals are mammals, particularly rats, sheep, pigs, goats, cattle or horses.

[0115] XENOMOUSE™ mice produce an adult-like human repertoire of fully human antibodies and generate antigen-specific human antibodies. In some embodiments, the  
20 XENOMOUSE™ mice contain approximately 80% of the human antibody V gene repertoire through introduction of megabase sized, germline configuration fragments of the human heavy chain loci and kappa light chain loci in yeast artificial chromosome (YAC). In other embodiments, XENOMOUSE™ mice further contain approximately all of the human lambda light chain locus. See Mendez *et al.*, *Nature Genetics* 15:146-156 (1997),  
25 Green and Jakobovits, *J. Exp. Med.* 188:483-495 (1998), and WO 98/24893, the disclosures of which are hereby incorporated by reference.

[0116] In some embodiments, the non-human animal comprising human immunoglobulin genes are animals that have a human immunoglobulin "minilocus". In the minilocus approach, an exogenous Ig locus is mimicked through the inclusion of individual genes  
30 from the Ig locus. Thus, one or more V<sub>H</sub> genes, one or more D<sub>H</sub> genes, one or more J<sub>H</sub> genes, a mu constant domain, and a second constant domain (preferably a gamma constant

domain) are formed into a construct for insertion into an animal. This approach is described, *inter alia*, in U.S. Patent Nos. 5,545,807, 5,545,806, 5,569,825, 5,625,126, 5,633,425, 5,661,016, 5,770,429, 5,789,650, 5,814,318, 5,591,669, 5,612,205, 5,721,367, 5,789,215, and 5,643,763, hereby incorporated by reference.

5 [0117] In another aspect, the invention provides a method for making humanized anti-SARS-CoV S protein antibodies. In some embodiments, non-human animals are immunized with a SARS-CoV S protein antigen as described below under conditions that permit antibody production. Antibody-producing cells are isolated from the animals, fused with myelomas to produce hybridomas, and nucleic acids encoding the heavy and light  
10 chains of an anti-SARS-CoV S protein antibody of interest are isolated. These nucleic acids are subsequently engineered using techniques known to those of skill in the art and as described further below to reduce the amount of non-human sequence, *i.e.*, to humanize the antibody to reduce the immune response in humans

[0118] In some embodiments, the SARS-CoV S protein antigen is isolated and/or purified  
15 SARS-CoV S protein or an antigenic portion thereof, for example, the ectodomain. In some embodiments, the SARS-CoV S protein antigen is a fragment of SARS-CoV S protein. In some embodiments, the SARS-CoV S protein fragment is the S1 domain of SARS-CoV S protein. In some embodiments, the SARS-CoV S protein fragment is the S2 domain of SARS-CoV S protein. In certain embodiments, the SARS-CoV S protein fragment  
20 comprises the S1 or S2 domain of SARS-CoV S protein. In some embodiments, the SARS-CoV S protein fragment comprises at least one epitope of SARS-CoV S protein. In other embodiments, the SARS-CoV S protein antigen is a cell that expresses or overexpresses SARS-CoV S protein or an immunogenic fragment thereof on its surface. In some embodiments, the SARS-CoV S protein antigen is a SARS-CoV S protein fusion protein.  
25 In some embodiments, the SARS-CoV S protein is a synthetic peptide immunogen.

[0119] Immunization of animals can be by any method known in the art. See, *e.g.*, Harlow and Lane, Antibodies: A Laboratory Manual, New York: Cold Spring Harbor Press, 1990. Methods for immunizing non-human animals such as mice, rats, sheep, goats, pigs, cattle and horses are well known in the art. See, *e.g.*, Harlow and Lane, *supra*, and U.S.  
30 Patent 5,994,619. In one embodiment, the SARS-CoV S protein antigen is administered with an adjuvant to stimulate the immune response. Exemplary adjuvants include complete

or incomplete Freund's adjuvant, RIBI (muramyl dipeptides) or ISCOM (immunostimulating complexes). Such adjuvants may protect the polypeptide from rapid dispersal by sequestering it in a local deposit, or they may contain substances that stimulate the host to secrete factors that are chemotactic for macrophages and other components of the immune system. Preferably, if a polypeptide is being administered, the immunization schedule will involve two or more administrations of the polypeptide, spread out over several weeks. Example I exemplifies a method for producing anti-SARS-CoV S protein monoclonal antibodies in XenoMouse™ mice.

*Production of Antibodies and Antibody-Producing Cell Lines*

10 [0120] After immunization of an animal with a SARS-CoV S protein antigen, antibodies and/or antibody-producing cells can be obtained from the animal. In some embodiments, anti-SARS-CoV S protein antibody-containing serum is obtained from the animal by bleeding or sacrificing the animal. The serum may be used as it is obtained from the animal, an immunoglobulin fraction may be obtained from the serum, or the anti-SARS-  
15 CoV S protein antibodies may be purified from the serum.

[0121] In some embodiments, antibody-producing immortalized cell lines are prepared from cells isolated from the immunized animal. After immunization, the animal is sacrificed and lymph node and/or splenic B cells are immortalized by any means known in the art. Methods of immortalizing cells include, but are not limited to, transfecting them  
20 with oncogenes, infecting them with an oncogenic virus and cultivating them under conditions that select for immortalized cells, subjecting them to carcinogenic or mutating compounds, fusing them with an immortalized cell, e.g., a myeloma cell, and inactivating a tumor suppressor gene. See, e.g., Harlow and Lane, supra. If fusion with myeloma cells is used, the myeloma cells preferably do not secrete immunoglobulin polypeptides (a non-  
25 secretory cell line). Immortalized cells are screened using SARS-CoV S protein, a portion thereof, or a cell expressing SARS-CoV S protein. In one embodiment, the initial screening is performed using an enzyme-linked immunoassay (ELISA) or a radioimmunoassay. An example of ELISA screening is provided in WO 00/37504, incorporated herein by reference.

[0122] Anti-SARS-CoV S protein antibody-producing cells, *e.g.*, hybridomas, are selected, cloned and further screened for desirable characteristics, including robust growth, high antibody production and desirable antibody characteristics, as discussed further below. Hybridomas can be expanded *in vivo* in syngeneic animals, in animals that lack an immune system, *e.g.*, nude mice, or in cell culture *in vitro*. Methods of selecting, cloning and expanding hybridomas are well known to those of ordinary skill in the art.

[0123] In one embodiment, the immunized animal is a non-human animal that expresses human immunoglobulin genes and the splenic B cells are fused to a myeloma cell line from the same species as the non-human animal. In a more preferred embodiment, the immunized animal is a XENOMOUSE<sup>TM</sup> mouse and the myeloma cell line is a non-secretory mouse myeloma. In an even more preferred embodiment, the myeloma cell line is P3-X63-Ag8.653 (American Type Culture Collection. See, *e.g.*, Example I.

[0124] Thus, in one embodiment, the invention provides methods for producing a cell line that produces a human monoclonal antibody or a fragment thereof directed to SARS-CoV S protein comprising (a) immunizing a non-human transgenic animal described herein with SARS-CoV S protein, a portion of SARS-CoV S protein or a cell or tissue expressing SARS-CoV S protein ; (b) allowing the transgenic animal to mount an immune response to SARS-CoV S protein ; (c) isolating antibody-producing cells from the transgenic animal; (d) immortalizing the antibody-producing cells; (e) creating individual monoclonal populations of the immortalized antibody-producing cells; and (f) screening the immortalized antibody-producing cells to identify an antibody directed to SARS-CoV S protein. In one embodiment, step (f) comprises screening the immortalized antibody-producing cells to identify an antibody directed to the S1 domain of SARS-CoV S protein; the S2 domain of SARS-CoV S protein; or (iii) both (i) and (ii).

[0125] Where it is desired to identify a monoclonal antibody directed to the S1 or S2 domains of SARS-CoV S protein, one may screen the antibodies for binding to a peptide comprising the amino acid sequence of the S1 or S2 domain of SARS-CoV S protein.

[0126] In another aspect, the invention provides hybridomas that produce a human anti-SARS-CoV S protein antibody. In one embodiment, the human anti-SARS-CoV S protein antibody produced by the hybridoma is an antagonist of SARS-CoV S protein. In some embodiments, the anti-SARS-CoV S protein monoclonal antibody does not mediate

antibody dependent enhancement of viral infection. In one embodiment, the hybridomas are mouse hybridomas, as described above. In other embodiments, the hybridomas are produced in a non-human, non-mouse species such as rats, sheep, pigs, goats, cattle or horses. In another embodiment, the hybridomas are human hybridomas.

5 [0127] In one embodiment of the invention, antibody-producing cells are isolated and expressed in a host cell, for example myeloma cells. In still another embodiment, a transgenic animal is immunized with a SARS-CoV S protein immunogen as described herein, primary cells, *e.g.*, spleen or peripheral blood cells, are isolated from an immunized transgenic animal and individual cells producing antibodies specific for the desired antigen  
10 are identified. Polyadenylated mRNA from each individual cell is isolated and reverse transcription polymerase chain reaction (RT-PCR) is performed using sense primers that anneal to variable region sequences, *e.g.*, degenerate primers that recognize most or all of the FR1 regions of human heavy and light chain variable region genes and anti-sense primers that anneal to constant or joining region sequences. cDNAs of the heavy and light  
15 chain variable domains are then cloned and expressed in any suitable host cell, *e.g.*, a myeloma cell, as chimeric antibodies with respective immunoglobulin constant regions, such as the heavy chain and  $\kappa$  or  $\lambda$  constant domains. See Babcook, J.S. *et al.* (1996) *Proc. Natl. Acad. Sci. USA* 93: 7843-48, incorporated herein by reference. Anti SARS-CoV S protein antibodies may then be identified and isolated as described herein.

20 [0128] In another embodiment, phage display techniques can be used to provide libraries containing a repertoire of antibodies with varying affinities for SARS-CoV S protein. For production of such repertoires, it is unnecessary to immortalize the B cells from the immunized animal. Rather, the primary B cells can be used directly as a source of DNA. The mixture of cDNAs obtained from B cell, *e.g.*, derived from spleens, is used to prepare  
25 an expression library, for example, a phage display library transfected into *E.coli*. The resulting cells are tested for immunoreactivity to SARS-CoV S protein. Techniques for the identification of high affinity human antibodies from such libraries are described by Griffiths *et al.*, (1994) *EMBO J.*, 13:3245-3260 ; Nissim *et al.*, *ibid*, pp. 692-698 and by Griffiths *et al.*, *ibid*, 12:725-734, which are incorporated by reference. Ultimately, clones  
30 from the library are identified that produce binding affinities of a desired magnitude for the antigen and the DNA encoding the product responsible for such binding is recovered and

manipulated for standard recombinant expression. Phage display libraries may also be constructed using previously manipulated nucleotide sequences and screened in a similar fashion. In general, the cDNAs encoding heavy and light chains are independently supplied or linked to form Fv analogs for production in the phage library.

- 5 [0129] The phage library is then screened for the antibodies with the highest affinities for SARS-CoV S protein and the genetic material recovered from the appropriate clone. Further rounds of screening can increase affinity of the original antibody isolated.

Nucleic Acids, Vectors, Host Cells, and  
Recombinant Methods of Making Antibodies

10 *Nucleic Acids*

- [0130] The present invention also encompasses nucleic acid molecules encoding anti-SARS-CoV S protein antibodies or antigen-binding portions thereof. In some embodiments, different nucleic acid molecules encode a heavy chain and a light chain of an anti-SARS-CoV S protein immunoglobulin. In other embodiments, the same nucleic acid molecule encodes a heavy chain and a light chain of an anti-SARS-CoV S protein immunoglobulin. In one embodiment, the nucleic acid encodes a SARS-CoV S protein antibody, or antigen-binding portion thereof, of the invention.

- [0131] In some embodiments, the nucleic acid molecule encoding the variable domain of the light chain ( $V_L$ ) comprises a human  $V_{\kappa 1}$  or  $V_{\kappa 2}$  gene, and a  $J_{\kappa 2}$ ,  $J_{\kappa 3}$ ,  $J_{\kappa 4}$ , or  $J_{\kappa 5}$  gene with or without mutations from the germline. In various embodiments, the  $V_L$  utilizes a human  $V_{\kappa 1}$  gene and a human  $J_{\kappa 3}$ ,  $J_{\kappa 4}$ , or  $J_{\kappa 5}$  gene. In some embodiments, the human  $V_{\kappa}$  gene is a human A30 gene and the human  $J_{\kappa}$  gene is a human  $J_{\kappa 3}$ ,  $J_{\kappa 4}$  or  $J_{\kappa 5}$  gene. In other embodiments, the human  $V_{\kappa}$  gene is a human L5 gene and the human  $J_{\kappa}$  gene is a human  $J_{\kappa 4}$  gene. In still other embodiments, the human  $V_{\kappa}$  gene is a human A1 gene and the human  $J_{\kappa}$  gene is a human  $J_{\kappa 2}$  gene.

[0132] In some embodiments, the nucleic acid molecule encoding the light chain, encodes an amino acid sequence comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 substitutions from the germline amino acid sequence(s). In some embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes a  $V_L$  amino acid sequence comprising 1, 2, 3,

4, 5, 6, 7, 8, 9, or 10 conservative amino acid substitutions and/or 1, 2, or 3 non-conservative substitutions compared to germline V<sub>K</sub>, J<sub>K</sub> and J<sub>L</sub> sequences. Substitutions may be in the CDR regions, the framework regions, or in the constant domain.

**[0133]** In some embodiments, the nucleic acid molecule encodes a V<sub>L</sub> amino acid

5 sequence comprising one or more variants compared to germline sequence that are identical to the variations found in the V<sub>L</sub> of one of the antibodies 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2.

10 **[0134]** In some embodiments, the nucleic acid molecule encodes at least three amino acid substitutions compared to the germline sequence found in the V<sub>L</sub> of one of the antibodies

1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2.

**[0135]** In some embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes the V<sub>L</sub> amino acid sequence of 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2,

2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2,

20 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2, or a variant or portion thereof. In some embodiments, the nucleic acid encodes an amino acid sequence

comprising the light chain CDRs of one of said above-listed antibodies. In some embodiments, said portion is a contiguous portion comprising CDR1-CDR3.

**[0136]** In some embodiments, the nucleic acid encodes the amino acid sequence of the

25 light chain CDRs of said antibody. In some embodiments, said portion encodes a contiguous region from CDR1-CDR3 of the light chain of an anti-SARS-CoV S protein antibody.

**[0137]** In some embodiments, the nucleic acid molecule encodes a V<sub>L</sub> amino acid

sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% identical to a

30 V<sub>L</sub> amino acid sequence of a V<sub>L</sub> region of any one of antibodies 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3,



4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2. Nucleic acid molecules of the invention include nucleic acids that hybridize under highly stringent conditions, such as those described above, to a nucleotide sequence encoding the amino acid sequence of a  $V_L$  region.

[0138] In another embodiment, the nucleic acid encodes a full-length light chain of an antibody selected 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2, or a light chain comprising a mutation, such as one disclosed herein.

[0139] In still another embodiment, the nucleic acid molecule encodes the variable domain of the heavy chain ( $V_H$ ) that comprises a human  $V_{H1}$ ,  $V_{H3}$  or  $V_{H4}$  family gene sequence or a sequence derived therefrom. In various embodiments, the nucleic acid molecule encoding the  $V_H$  domain utilizes a human  $V_H$  1-2 gene, a human D3-10 gene and a human  $J_H$  4B gene; human  $V_H$  1-18 gene, a D1-26 gene and a human  $J_H$  4B gene; a human  $V_H$  3-33 gene, a human D2-2 gene and a human  $J_H$  4B gene; a human  $V_H$  3-33 gene, a human D4-17 gene and a human  $J_H$  5B gene; or a human  $V_H$  4-59 gene, a human D3-9 gene and a human  $J_H$  6B gene.

[0140] In some embodiments, the nucleic acid molecule encodes an amino acid sequence comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or 18 mutations compared to the germline amino acid sequence of the human V, D or J genes. In some embodiments, said mutations are in the  $V_H$  region. In some embodiments, said mutations are in the CDR regions.

[0141] In some embodiments, the nucleic acid molecule encodes one or more amino acid mutations compared to the germline sequence that are identical to amino acid mutations found in the  $V_H$  of one of monoclonal antibodies 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2. In some embodiments, the nucleic acid encodes at least three amino acid mutations compared to the germline

sequences that are identical to at least three amino acid mutations found in one of the above-listed monoclonal antibodies.

[0142] In some embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes at least a portion of the V<sub>H</sub> amino acid sequence of a monoclonal antibody  
5 selected from monoclonal antibodies 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2, a variant thereof, or said sequence having conservative amino acid mutations and/or a total of three or fewer non-conservative  
10 amino acid substitutions. In various embodiments the sequence encodes one or more CDR regions, preferably a CDR3 region, all three CDR regions, a contiguous portion including CDR1-CDR3, or the entire V<sub>H</sub> region, with or without a signal sequence.

[0143] In some embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes the amino acid sequence of one of 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2,  
15 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2, or said sequence lacking the signal sequence. In some preferred embodiments, the nucleic acid molecule comprises at least a portion of the nucleotide sequence of 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2,  
20 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2, or said sequence lacking the signal sequence. In some embodiments, said portion encodes the V<sub>H</sub> region (with or without a signal sequence), a CDR3 region, all three CDR regions, or a contiguous region  
25 including CDR1-CDR3.

[0144] In some embodiments, the nucleic acid molecule encodes a V<sub>H</sub> amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% identical to the V<sub>H</sub> amino acid sequences of any one of 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2,  
30 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2. Nucleic acid molecules of

the invention include nucleic acids that hybridize under highly stringent conditions, such as those described above, to a nucleotide sequence encoding the amino acid sequence of 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2, or to a V<sub>H</sub> region thereof, or that has the nucleotide sequence of 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2 or that encodes a V<sub>H</sub> region thereof.

**[0145]** In another embodiment, the nucleic acid encodes a full-length heavy chain of an antibody selected from 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2, or a heavy chain having the amino acid sequence of 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2, with or without a signal sequence, or a heavy chain comprising a mutation, such as one of the variants discussed herein. Further, the nucleic acid may comprise the nucleotide sequence of 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2, with or without a signal sequence, or a nucleic acid molecule encoding a heavy chain comprising a mutation, such as one of the variants discussed herein.

**[0146]** A nucleic acid molecule encoding the heavy or light chain of an anti-SARS-CoV S protein antibody or portions thereof can be isolated from any source that produces such antibody. In various embodiments, the nucleic acid molecules are isolated from a B cell isolated from an animal immunized with SARS-CoV S protein or from an immortalized cell derived from such a B cell that expresses an anti-SARS-CoV S protein antibody. Methods

of isolating mRNA encoding an antibody are well-known in the art. See, *e.g.*, Sambrook *et al.* The mRNA may be used to produce cDNA for use in the polymerase chain reaction (PCR) or cDNA cloning of antibody genes. In one embodiment, the nucleic acid molecule is isolated from a hybridoma that has as one of its fusion partners a human

5 immunoglobulin-producing cell from a non-human transgenic animal. In an even more preferred embodiment, the human immunoglobulin producing cell is isolated from a XENOMOUSE<sup>™</sup> animal. In another embodiment, the human immunoglobulin-producing cell is from a non-human, non-mouse transgenic animal, as described above. In another embodiment, the nucleic acid is isolated from a non-human, non-transgenic animal. The  
10 nucleic acid molecules isolated from a non-human, non-transgenic animal may be used, *e.g.*, for humanized antibodies.

[0147] In some embodiments, a nucleic acid encoding a heavy chain of an anti-SARS-CoV S protein antibody of the invention can comprise a nucleotide sequence encoding a V<sub>H</sub> domain of the invention joined in-frame to a nucleotide sequence encoding a heavy chain  
15 constant domain from any source. Similarly, a nucleic acid molecule encoding a light chain of an anti-SARS-CoV S protein antibody of the invention can comprise a nucleotide sequence encoding a V<sub>L</sub> domain of the invention joined in-frame to a nucleotide sequence encoding a light chain constant domain from any source.

[0148] In a further aspect of the invention, nucleic acid molecules encoding the variable  
20 domain of the heavy (V<sub>H</sub>) and/or light (V<sub>L</sub>) chains are “converted” to full-length antibody genes. In one embodiment, nucleic acid molecules encoding the V<sub>H</sub> or V<sub>L</sub> domains are converted to full-length antibody genes by insertion into an expression vector already encoding heavy chain constant (C<sub>H</sub>) or light chain constant (C<sub>L</sub>) domains, respectively, such that the V<sub>H</sub> segment is operatively linked to the C<sub>H</sub> segment(s) within the vector, and/or the  
25 V<sub>L</sub> segment is operatively linked to the C<sub>L</sub> segment within the vector. In another embodiment, nucleic acid molecules encoding the V<sub>H</sub> and/or V<sub>L</sub> domains are converted into full-length antibody genes by linking, *e.g.*, ligating, a nucleic acid molecule encoding a V<sub>H</sub> and/or V<sub>L</sub> domains to a nucleic acid molecule encoding a C<sub>H</sub> and/or C<sub>L</sub> domain using standard molecular biological techniques. Nucleotide sequences of human heavy and light  
30 chain immunoglobulin constant domain genes are known in the art. See, *e.g.*, Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed., NIH Publ. No. 91-3242, 1991.

Nucleic acid molecules encoding the full-length heavy and/or light chains may then be expressed from a cell into which they have been introduced and the anti-SARS-CoV S protein antibody isolated.

[0149] The nucleic acid molecules may be used to recombinantly express large quantities of anti-SARS-CoV S protein antibodies. The nucleic acid molecules also may be used to produce chimeric antibodies, bispecific antibodies, single chain antibodies, immunoadhesins, diabodies, mutated antibodies and antibody derivatives, as described further below. If the nucleic acid molecules are derived from a non-human, non-transgenic animal, the nucleic acid molecules may be used for antibody humanization, also as described below.

[0150] In another embodiment, a nucleic acid molecule of the invention is used as a probe or PCR primer for a specific antibody sequence. For instance, the nucleic acid can be used as a probe in diagnostic methods or as a PCR primer to amplify regions of DNA that could be used, inter alia, to isolate additional nucleic acid molecules encoding variable domains of anti-SARS-CoV S protein antibodies. In some embodiments, the nucleic acid molecules are oligonucleotides. In some embodiments, the oligonucleotides are from highly variable domains of the heavy and light chains of the antibody of interest. In some embodiments, the oligonucleotides encode all or a part of one or more of the CDRs of antibodies 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2, or variants thereof as described herein.

#### *Vectors*

[0151] The invention provides vectors comprising nucleic acid molecules that encode the heavy chain of an anti-SARS-CoV S protein antibody of the invention or an antigen-binding portion thereof. The invention also provides vectors comprising nucleic acid molecules that encode the light chain of such antibodies or antigen-binding portion thereof. The invention further provides vectors comprising nucleic acid molecules encoding fusion proteins, modified antibodies, antibody fragments, and probes thereof.

[0152] In some embodiments, the anti-SARS-CoV S protein antibodies or antigen-binding portions of the invention are expressed by inserting DNAs encoding partial or full-length light and heavy chains, obtained as described above, into expression vectors such that the genes are operatively linked to necessary expression control sequences such as

5 transcriptional and translational control sequences. Expression vectors include plasmids, retroviruses, adenoviruses, adeno-associated viruses (AAV), plant viruses such as cauliflower mosaic virus, tobacco mosaic virus, cosmids, YACs, EBV derived episomes, and the like. The antibody gene is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating  
10 the transcription and translation of the antibody gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene and the antibody heavy chain gene can be inserted into separate vectors. In one embodiment, both genes are inserted into the same expression vector. The antibody genes are inserted into the expression vector by standard methods (*e.g.*, ligation of  
15 complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present).

[0153] A convenient vector is one that encodes a functionally complete human C<sub>H</sub> or C<sub>L</sub> immunoglobulin sequence, with appropriate restriction sites engineered so that any V<sub>H</sub> or V<sub>L</sub> sequence can easily be inserted and expressed, as described above. In such vectors,  
20 splicing usually occurs between the splice donor site in the inserted J region and the splice acceptor site preceding the human C domain, and also at the splice regions that occur within the human C<sub>H</sub> exons. Polyadenylation and transcription termination occur at native chromosomal sites downstream of the coding regions. The recombinant expression vector also can encode a signal peptide that facilitates secretion of the antibody chain from a host  
25 cell. The antibody chain gene may be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the immunoglobulin chain. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (*i.e.*, a signal peptide from a non-immunoglobulin protein).

[0154] In addition to the antibody chain genes, the recombinant expression vectors of the  
30 invention carry regulatory sequences that control the expression of the antibody chain genes in a host cell. It will be appreciated by those skilled in the art that the design of the

expression vector, including the selection of regulatory sequences may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. Preferred regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as  
5 promoters and/or enhancers derived from retroviral LTRs, cytomegalovirus (CMV) (such as the CMV promoter/enhancer), Simian Virus 40 (SV40) (such as the SV40 promoter/enhancer), adenovirus, (*e.g.*, the adenovirus major late promoter (AdMLP)), polyoma and strong mammalian promoters such as native immunoglobulin and actin promoters. For further description of viral regulatory elements, and sequences thereof, see  
10 *e.g.*, U.S. Patent No. 5,168,062, U.S. Patent No. 4,510,245 and U.S. Patent No. 4,968,615. Methods for expressing antibodies in plants, including a description of promoters and vectors, as well as transformation of plants is known in the art. See, *e.g.*, United States Patent 6,517,529, incorporated herein by reference. Methods of expressing polypeptides in bacterial cells or fungal cells, *e.g.*, yeast cells, are also well known in the art.

15 [0155] In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors of the invention may carry additional sequences, such as sequences that regulate replication of the vector in host cells (*e.g.*, origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see *e.g.*, U.S. Patent Nos. 4,399,216, 4,634,665 and 5,179,017,  
20 incorporated herein by reference). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr-host cells with methotrexate selection/amplification), the neo gene (for G418 selection), and the glutamate synthetase  
25 gene.

*Non-Hybridoma Host Cells and Methods of Recombinantly Producing Protein*

[0156] Nucleic acid molecules encoding anti-SARS-CoV S protein antibodies and vectors comprising these nucleic acid molecules can be used for transfection of a suitable mammalian, plant, bacterial or yeast host cell. Transformation can be by any known  
30 method for introducing polynucleotides into a host cell. Methods for introduction of

heterologous polynucleotides into mammalian cells are well known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene-mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei. In addition, nucleic acid

5 molecules may be introduced into mammalian cells by viral vectors. Methods of transforming cells are well known in the art. See, *e.g.*, U.S. Patent Nos. 4,399,216, 4,912,040, 4,740,461, and 4,959,455, incorporated herein by reference). Methods of transforming plant cells are well known in the art, including, *e.g.*, *Agrobacterium*-mediated transformation, biolistic transformation, direct injection, electroporation and viral  
10 transformation. Methods of transforming bacterial and yeast cells are also well known in the art.

[0157] Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC). These include, *inter alia*, Chinese hamster ovary (CHO) cells, N50  
15 cells, SP2 cells, HEK-293T cells, NIH-3T3 cells, HeLa cells, baby hamster kidney (BHK) cells, African green monkey kidney cells (COS), human hepatocellular carcinoma cells (*e.g.*, Hep G2), A549 cells, and a number of other cell lines. Cell lines of particular preference are selected through determining which cell lines have high expression levels. Other cell lines that may be used are insect cell lines, such as Sf9 or Sf21 cells. When  
20 recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown.

Antibodies can be recovered from the culture medium using standard protein purification  
25 methods. Plant host cells include, *e.g.*, *Nicotiana*, *Arabidopsis*, duckweed, corn, wheat, potato, etc. Bacterial host cells include *E. coli* and *Streptomyces* species. Yeast host cells include *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae* and *Pichia pastoris*.

[0158] Further, expression of antibodies of the invention from production cell lines can be enhanced using a number of known techniques. For example, the glutamine synthetase  
30 gene expression system (the GS system) is a common approach for enhancing expression



under certain conditions. The GS system is discussed in whole or part in connection with European Patent Nos. 0 216 846, 0 256 055, 0 323 997 and 0 338 841.

[0159] It is likely that antibodies expressed by different cell lines or in transgenic animals will have different glycosylation from each other. However, all antibodies encoded by the nucleic acid molecules provided herein, or comprising the amino acid sequences provided  
5 herein are part of the instant invention, regardless of the glycosylation of the antibodies.

#### *Transgenic Animals and Plants*

[0160] Anti-SARS-CoV S protein antibodies of the invention also can be produced transgenically through the generation of a mammal or plant that is transgenic for the immunoglobulin heavy and light chain sequences of interest and production of the antibody  
10 in a recoverable form therefrom. In connection with the transgenic production in mammals, anti-SARS-CoV S protein antibodies can be produced in, and recovered from, the milk of goats, cows, or other mammals. See, *e.g.*, U.S. Patent Nos. 5,827,690, 5,756,687, 5,750,172, and 5,741,957, incorporated herein by reference. In some embodiments, non-  
15 human transgenic animals that comprise human immunoglobulin loci are immunized with SARS-CoV S protein or an immunogenic portion thereof, as described above. Methods for making antibodies in plants are described, *e.g.*, in U.S. patents 6,046,037 and 5,959,177, incorporated herein by reference.

[0161] In some embodiments, non-human transgenic animals or plants are produced by  
20 introducing one or more nucleic acid molecules encoding an anti-SARS-CoV S protein antibody of the invention into the animal or plant by standard transgenic techniques. See Hogan and United States Patent 6,417,429, *supra*. The transgenic cells used for making the transgenic animal can be embryonic stem cells or somatic cells or a fertilized egg. The transgenic non-human organisms can be chimeric, nonchimeric heterozygotes, and  
25 nonchimeric homozygotes. See, *e.g.*, Hogan *et al.*, Manipulating the Mouse Embryo: A Laboratory Manual second ed., Cold Spring Harbor Press (1999); Jackson *et al.*, Mouse Genetics and Transgenics: A Practical Approach, Oxford University Press (2000); and Pinkert, Transgenic Animal Technology: A Laboratory Handbook, Academic Press (1999), all incorporated herein by reference. In some embodiments, the transgenic non-human  
30 animals have a targeted disruption and replacement by a targeting construct that encodes a

heavy chain and/or a light chain of interest. In one embodiment, the transgenic animals comprise and express nucleic acid molecules encoding heavy and light chains that specifically bind to SARS-CoV S protein, and preferably to (i) the S1 domain of SARS-CoV S protein; (ii) the S2 domain of SARS-CoV S protein; or (iii) both (i) and (ii). In one  
5 embodiment, the transgenic animals comprise and express nucleic acid molecules encoding heavy and light chains that specifically bind to human SARS-CoV S protein. In some embodiments, the transgenic animals comprise nucleic acid molecules encoding a modified antibody such as a single-chain antibody, a chimeric antibody or a humanized antibody. The anti-SARS-CoV S protein antibodies may be made in any transgenic animal. In one  
10 embodiment, the non-human animals are mice, rats, sheep, pigs, goats, cattle or horses. The non-human transgenic animal expresses said encoded polypeptides in blood, milk, urine, saliva, tears, mucus and other bodily fluids.

#### *Phage Display Libraries*

**[0162]** The invention provides a method for producing an anti-SARS-CoV S protein  
15 antibody or antigen-binding portion thereof comprising the steps of synthesizing a library of human antibodies on phage, screening the library with SARS-CoV S protein or a portion thereof, isolating phage that bind SARS-CoV S protein, and obtaining the antibody from the phage. By way of example, one method for preparing the library of antibodies for use in phage display techniques comprises the steps of immunizing a non-human animal  
20 comprising human immunoglobulin loci with SARS-CoV S protein or an antigenic portion thereof to create an immune response, extracting antibody-producing cells from the immunized animal; isolating RNA encoding heavy and light chains of antibodies of the invention from the extracted cells, reverse transcribing the RNA to produce cDNA, amplifying the cDNA using primers, and inserting the cDNA into a phage display vector  
25 such that antibodies are expressed on the phage. Recombinant anti-SARS-CoV S protein antibodies of the invention may be obtained in this way.

**[0163]** Recombinant anti-SARS-CoV S protein human antibodies of the invention can be isolated by screening a recombinant combinatorial antibody library. Preferably the library is a scFv phage display library, generated using human V<sub>L</sub> and V<sub>H</sub> cDNAs prepared from  
30 mRNA isolated from B cells. Methods for preparing and screening such libraries are known

in the art. Kits for generating phage display libraries are commercially available (e.g., the Pharmacia Recombinant Phage Antibody System, catalog no. 27-9400-01; and the Stratagene SurfZAP™ phage display kit, catalog no. 240612). There also are other methods and reagents that can be used in generating and screening antibody display libraries (see, e.g., U.S. Patent No. 5,223,409; PCT Publication Nos. WO 92/18619, WO 91/17271, WO 92/20791, WO 92/15679, WO 93/01288, WO 92/01047, WO 92/09690; Fuchs *et al.*, *Bio/Technology* 9:1370-1372 (1991); Hay *et al.*, *Hum. Antibod. Hybridomas* 3:81-85 (1992); Huse *et al.*, *Science* 246:1275-1281 (1989); McCafferty *et al.*, *Nature* 348:552-554 (1990); Griffiths *et al.*, *EMBO J.* 12:725-734 (1993); Hawkins *et al.*, *J. Mol. Biol.* 226:889-896 (1992); Clackson *et al.*, *Nature* 352:624-628 (1991); Gram *et al.*, *Proc. Natl. Acad. Sci. USA* 89:3576-3580 (1992); Garrad *et al.*, *Bio/Technology* 9:1373-1377 (1991); Hoogenboom *et al.*, *Nuc. Acid Res.* 19:4133-4137 (1991); and Barbas *et al.*, *Proc. Natl. Acad. Sci. USA* 88:7978-7982 (1991), all incorporated herein by reference.

**[0164]** In one embodiment, to isolate and produce human anti-SARS-CoV S protein antibodies with the desired characteristics, a human anti-SARS-CoV S protein antibody as described herein is first used to select human heavy and light chain sequences having similar binding activity toward SARS-CoV S protein, using the epitope imprinting methods described in PCT Publication No. WO 93/06213, incorporated herein by reference. The antibody libraries used in this method are preferably scFv libraries prepared and screened as described in PCT Publication No. WO 92/01047, McCafferty *et al.*, *Nature* 348:552-554 (1990); and Griffiths *et al.*, *EMBO J.* 12:725-734 (1993), all incorporated herein by reference. The scFv antibody libraries preferably are screened using human SARS-CoV S protein as the antigen.

**[0165]** Once initial human V<sub>L</sub> and V<sub>H</sub> domains are selected, "mix and match" experiments are performed, in which different pairs of the initially selected V<sub>L</sub> and V<sub>H</sub> segments are screened for SARS-CoV S protein binding to select preferred V<sub>L</sub>/V<sub>H</sub> pair combinations. Additionally, to further improve the quality of the antibody, the V<sub>L</sub> and V<sub>H</sub> segments of the preferred V<sub>L</sub>/V<sub>H</sub> pair(s) can be randomly mutated, preferably within the CDR3 region of V<sub>H</sub> and/or V<sub>L</sub>, in a process analogous to the in vivo somatic mutation process responsible for affinity maturation of antibodies during a natural immune response. This in vitro affinity maturation can be accomplished by amplifying V<sub>H</sub> and V<sub>L</sub> domains using PCR primers

complimentary to the V<sub>H</sub> CDR3 or V<sub>L</sub> CDR3, respectively, which primers have been “spiked” with a random mixture of the four nucleotide bases at certain positions such that the resultant PCR products encode V<sub>H</sub> and V<sub>L</sub> segments into which random mutations have been introduced into the V<sub>H</sub> and/or V<sub>L</sub> CDR3 regions. These randomly mutated V<sub>H</sub> and V<sub>L</sub> segments can be re-screened for binding to SARS-CoV S protein.

[0166] Following screening and isolation of an anti-SARS-CoV S protein antibody of the invention from a recombinant immunoglobulin display library, nucleic acids encoding the selected antibody can be recovered from the display package (*e.g.*, from the phage genome) and subcloned into other expression vectors by standard recombinant DNA techniques. If desired, the nucleic acid can further be manipulated to create other antibody forms of the invention, as described below. To express a recombinant human antibody isolated by screening of a combinatorial library, the DNA encoding the antibody is cloned into a recombinant expression vector and introduced into mammalian host cells, as described above.

#### *Class switching*

[0167] Another aspect of the invention provides a method for converting the class or subclass of an anti-SARS-CoV S protein antibody to another class or subclass. In some embodiments, a nucleic acid molecule encoding a V<sub>L</sub> or V<sub>H</sub> that does not include sequences encoding C<sub>L</sub> or C<sub>H</sub> is isolated using methods well-known in the art. The nucleic acid molecule then is operatively linked to a nucleotide sequence encoding a C<sub>L</sub> or C<sub>H</sub> from a desired immunoglobulin class or subclass. This can be achieved using a vector or nucleic acid molecule that comprises a C<sub>L</sub> or C<sub>H</sub> chain, as described above. For example, an anti-SARS-CoV S protein antibody that was originally IgM can be class switched to an IgG. Further, the class switching may be used to convert one IgG subclass to another, *e.g.*, from IgG1 to IgG2. Another method for producing an antibody of the invention comprising a desired isotype comprises the steps of isolating a nucleic acid encoding a heavy chain of an anti-SARS-CoV S protein antibody and a nucleic acid encoding a light chain of an anti-SARS-CoV S protein antibody, isolating the sequence encoding the V<sub>H</sub> region, ligating the V<sub>H</sub> sequence to a sequence encoding a heavy chain constant domain of the desired isotype,

expressing the light chain gene and the heavy chain construct in a cell, and collecting the anti-SARS-CoV S protein antibody with the desired isotype.

#### *Deimmunized Antibodies*

[0168] In another aspect of the invention, the antibody may be deimmunized to reduce its immunogenicity using the techniques described in, *e.g.*, PCT Publication Nos. WO98/52976 and WO00/34317 (incorporated herein by reference).

#### *Mutated Antibodies*

[0169] In another embodiment, the nucleic acid molecules, vectors and host cells may be used to make mutated anti-SARS-CoV S protein antibodies. The antibodies may be mutated in the variable domains of the heavy and/or light chains, *e.g.*, to alter a binding property of the antibody. For example, a mutation may be made in one or more of the CDR regions to increase or decrease the  $K_D$  of the antibody for SARS-CoV S protein, to increase or decrease  $k_{off}$ , or to alter the binding specificity of the antibody. Techniques in site-directed mutagenesis are well-known in the art. See, *e.g.*, Sambrook *et al.* and Ausubel *et al.*, *supra*. In another embodiment, one or more mutations are made at an amino acid residue that is known to be changed compared to the germline in monoclonal antibody 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2. The mutations may be made in a CDR region or framework region of a variable domain, or in a constant domain. In one embodiment, the mutations are made in a variable domain. In some embodiments, one or more mutations are made at an amino acid residue that is known to be changed compared to the germline in a CDR region or framework region of a variable domain of an amino acid sequence selected from 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2.

[0170] In another embodiment, the framework region is mutated so that the resulting framework region(s) have the amino acid sequence of the corresponding germline gene. A mutation may be made in a framework region or constant domain to increase the half-life of the anti-SARS-CoV S protein antibody. See, *e.g.*, PCT Publication No. WO 00/09560, incorporated herein by reference. A mutation in a framework region or constant domain also can be made to alter the immunogenicity of the antibody, to provide a site for covalent or non-covalent binding to another molecule, or to alter such properties as complement fixation, FcR binding and antibody-dependent cell-mediated cytotoxicity (ADCC).

According to the invention, a single antibody may have mutations in any one or more of the CDRs or framework regions of the variable domain or in the constant domain.

[0171] In some embodiments, there are from 1 to 8, including any number in between, amino acid mutations in either the V<sub>H</sub> or V<sub>L</sub> domains of the mutated anti-SARS-CoV S protein antibody compared to the anti-SARS-CoV S protein antibody prior to mutation. In any of the above, the mutations may occur in one or more CDR regions. Further, any of the mutations can be conservative amino acid substitutions. In some embodiments, there are no more than 5, 4, 3, 2, or 1 amino acid changes in the constant domains.

#### *Modified Antibodies*

[0172] In another embodiment, a fusion antibody or immunoadhesin may be made that comprises all or a portion of an anti-SARS-CoV S protein antibody of the invention linked to another polypeptide. In one embodiment, only the variable domains of the anti-SARS-CoV S protein antibody are linked to the polypeptide. In still another embodiment, the V<sub>H</sub> domain of an anti-SARS-CoV S protein antibody is linked to a first polypeptide, while the V<sub>L</sub> domain of an anti-SARS-CoV S protein antibody is linked to a second polypeptide that associates with the first polypeptide in a manner such that the V<sub>H</sub> and V<sub>L</sub> domains can interact with one another to form an antigen binding site. In still another embodiment, the V<sub>H</sub> domain is separated from the V<sub>L</sub> domain by a linker such that the V<sub>H</sub> and V<sub>L</sub> domains can interact with one another (see below under Single Chain Antibodies). The V<sub>H</sub>-linker-V<sub>L</sub> antibody is then linked to the polypeptide of interest. The fusion antibody is useful for directing a polypeptide to a SARS-CoV S protein -expressing cell or tissue. The polypeptide may be a therapeutic agent, such as a toxin, chemokine or other regulatory

protein, or may be a diagnostic agent, such as an enzyme that may be easily visualized, such as horseradish peroxidase. In addition, fusion antibodies can be created in which two (or more) single-chain antibodies are linked to one another. This is useful if one wants to create a divalent or polyvalent antibody on a single polypeptide chain, or if one wants to create a bispecific antibody.

**[0173]** To create a single chain antibody, (scFv) the  $V_H$ - and  $V_L$ -encoding DNA fragments are operatively linked to another fragment encoding a flexible linker, *e.g.*, encoding the amino acid sequence (Gly<sub>4</sub>-Ser)<sub>3</sub>, such that the  $V_H$  and  $V_L$  sequences can be expressed as a contiguous single-chain protein, with the  $V_L$  and  $V_H$  domains joined by the flexible linker.

See, *e.g.*, Bird *et al.*, *Science* 242:423-426 (1988); Huston *et al.*, *Proc. Natl. Acad. Sci. USA* 85:5879-5883 (1988); McCafferty *et al.*, *Nature* 348:552-554 (1990). The single chain antibody may be monovalent, if only a single  $V_H$  and  $V_L$  are used, bivalent, if two  $V_H$  and  $V_L$  are used, or polyvalent, if more than two  $V_H$  and  $V_L$  are used. Bispecific or polyvalent antibodies may be generated that bind specifically to SARS-CoV S protein and to another molecule.

**[0174]** In other embodiments, other modified antibodies may be prepared using anti-SARS-CoV S protein antibody encoding nucleic acid molecules. For instance, "Kappa bodies" (Ill *et al.*, *Protein Eng.* 10: 949-57 (1997)), "Minibodies" (Martin *et al.*, *EMBO J.* 13: 5303-9 (1994)), "Diabodies" (Holliger *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993)), or "Janusins" (Traunecker *et al.*, *EMBO J.* 10:3655-3659 (1991) and Traunecker *et al.*, *Int. J. Cancer* (Suppl.) 7:51-52 (1992)) may be prepared using standard molecular biological techniques following the teachings of the specification.

**[0175]** Bispecific antibodies or antigen-binding fragments can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. See, *e.g.*, Songsivilai & Lachmann, *Clin. Exp. Immunol.* 79: 315-321 (1990), Kostelny *et al.*, *J. Immunol.* 148:1547-1553 (1992). In addition, bispecific antibodies may be formed as "diabodies" or "Janusins." In some embodiments, the bispecific antibody binds to two different epitopes of SARS-CoV S protein. In some embodiments, the bispecific antibody has a first heavy chain and a first light chain from monoclonal antibody 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2,

5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2 and an additional antibody heavy chain and light chain. In some embodiments, the additional light chain and heavy chain also are from one of the above-identified monoclonal antibodies, but are different from the first heavy and light chains.

- 5 [0176] In some embodiments, the modified antibodies described above are prepared using one or more of the variable domains or CDR regions from a human anti-SARS-CoV S protein monoclonal antibody provided herein.

#### *Derivatized and Labeled Antibodies*

- 10 [0177] An anti-SARS-CoV S protein antibody or antigen-binding portion of the invention can be derivatized or linked to another molecule (*e.g.*, another peptide or protein). In general, the antibodies or portion thereof are derivatized such that the SARS-CoV S protein binding is not affected adversely by the derivatization or labeling. Accordingly, the antibodies and antibody portions of the invention are intended to include both intact and modified forms of the human anti-SARS-CoV S protein antibodies described herein. For  
15 example, an antibody or antibody portion of the invention can be functionally linked (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (*e.g.*, a bispecific antibody or a diabody), a detection agent, a cytotoxic agent, a pharmaceutical agent, and/or a protein or peptide that can mediate association of the antibody or antibody portion with another molecule (such as  
20 a streptavidin core region or a polyhistidine tag).

- [0178] One type of derivatized antibody is produced by crosslinking two or more antibodies (of the same type or of different types, *e.g.*, to create bispecific antibodies). Suitable crosslinkers include those that are heterobifunctional, having two distinctly reactive groups separated by an appropriate spacer (*e.g.*, *m*-maleimidobenzoyl-N-  
25 hydroxysuccinimide ester) or homobifunctional (*e.g.*, disuccinimidyl suberate). Such linkers are available from Pierce Chemical Company, Rockford, IL.

- [0179] Another type of derivatized antibody is a labeled antibody. Useful detection agents with which an antibody or antigen-binding portion of the invention may be derivatized include fluorescent compounds, including fluorescein, fluorescein  
30 isothiocyanate, rhodamine, phycoerythrin, 5-dimethylamine-1-naphthalenesulfonyl chloride,



lanthanide phosphors and the like. An antibody can also be labeled with enzymes that are useful for detection, such as horseradish peroxidase,  $\beta$ -galactosidase, luciferase, alkaline phosphatase, glucose oxidase and the like. When an antibody is labeled with a detectable enzyme, it is detected by adding additional reagents that the enzyme uses to produce a reaction product that can be discerned. For example, when the agent horseradish peroxidase is present, the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is detectable. An antibody can also be labeled with biotin, and detected through indirect measurement of avidin or streptavidin binding. An antibody can also be labeled with a predetermined polypeptide epitope recognized by a secondary reporter (*e.g.*, leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.

[0180] An anti-SARS-CoV S protein antibody can also be labeled with a radiolabeled amino acid. The radiolabel can be used for both diagnostic and therapeutic purposes. For instance, the radiolabel can be used to detect SARS-CoV S protein -expressing tumors by x-ray or other diagnostic techniques. Further, the radiolabel can be used therapeutically as a toxin for cancerous cells or tumors. Examples of labels for polypeptides include, but are not limited to, the following radioisotopes or radionuclides --  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{15}\text{N}$ ,  $^{35}\text{S}$ ,  $^{90}\text{Y}$ ,  $^{99}\text{Tc}$ ,  $^{111}\text{In}$ ,  $^{125}\text{I}$ , and  $^{131}\text{I}$ .

[0181] An anti-SARS-CoV S protein antibody can also be derivatized with a chemical group such as polyethylene glycol (PEG), a methyl or ethyl group, or a carbohydrate group. These groups are useful to improve the biological characteristics of the antibody, *e.g.*, to increase serum half-life or to increase tissue binding.

[0182] In some embodiments, the anti-SARS-CoV S protein antibody can be labeled with a paramagnetic, radioactive or fluorogenic ion that is detectable upon imaging. In some embodiments, the paramagnetic ion is chromium (III), manganese (II), iron (III), iron (II), cobalt (II), nickel (II), copper (II), neodymium (III), samarium (III), ytterbium (III), gadolinium (III), vanadium (II), terbium (III), dysprosium (III), holmium (III) or erbium (III). In other embodiments, the radioactive ion is iodine123, technetium99, indium111, rhenium188, rhenium186, copper67, iodine131, yttrium90, iodine125, astatine211, and

gallium<sup>67</sup>. In other embodiments, the anti-SARS-CoV S protein antibody is labeled with an X-ray imaging agent such as lanthanum (III), gold (III) lead (II) and bismuth (III).

#### Compositions and Kits

[0183] The invention relates to compositions comprising a human anti-SARS-CoV S protein antibody with antagonist properties for the treatment of patients infected with SARS-CoV. In certain embodiments, a composition may comprise antibodies of any of the preceding embodiments. In some embodiments, the subject of treatment is a human. In other embodiments, the subject is a veterinary subject.

[0184] Antagonist anti-SARS-CoV S protein antibodies of the invention and compositions comprising them can be administered in combination with one or more other therapeutic, diagnostic, or prophylactic agents. In some embodiments, more than one antagonist SARS-CoV S protein antibody of the invention can be used in treatment of a subject. In some embodiments, an antagonist anti-SARS-CoV S protein antibody that binds to the S1 domain and one that binds to the S2 domain or antigen-binding portions of either or both, are both administered to a subject, either together or separately. In certain embodiments the antibodies are in a composition comprising a pharmaceutically acceptable carrier. In another embodiment, one or more of the antagonist SARS-CoV S protein antibodies of the invention are administered in combination with one or more additional antagonistic antibodies that bind different epitopes on the S protein, that bind the S protein from different isolates of SARS-CoV and/or that bind different stages of SARS-CoV (i.e., early, middle or late stage virus).

[0185] As used herein, "pharmaceutically acceptable carrier" means any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Some examples of pharmaceutically acceptable carriers are water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Additional examples of pharmaceutically acceptable substances are wetting agents or minor amounts of auxiliary substances such as

wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody.

[0186] The compositions of this invention may be in a variety of forms, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (*e.g.*, injectable and

5 infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Typical preferred compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for passive immunization of humans. The preferred mode of administration is parenteral (*e.g.*, intravenous, 10 subcutaneous, intraperitoneal, intramuscular). In one embodiment, the antibody is administered by intravenous infusion or injection. In still another embodiment, the antibody is administered by intramuscular or subcutaneous injection.

[0187] Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution,

15 microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the anti-SARS-CoV S protein antibody in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization.

Generally, dispersions are prepared by incorporating the active compound into a sterile 20 vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a

25 solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

30 [0188] The antibodies of the present invention can be administered by a variety of methods known in the art, although for many therapeutic applications, the preferred

route/mode of administration is subcutaneous, intramuscular, or intravenous infusion. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. Other modes of administration include intraperitoneal, intrabronchial, transmucosal, intraspinal, intrasynovial, intraaortic, intranasal, ocular, otic, topical and buccal.

[0189] In certain embodiments, the active compound of the antibody compositions may be prepared with a carrier that will protect the antibody against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, *e.g.*, Sustained and Controlled Release Drug Delivery Systems (J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978).

[0190] The invention also provides compositions suitable for administration by inhalation, which comprise the anti-SARS-CoV S protein antibodies described herein. The anti-SARS-CoV S protein antibodies may be conveniently delivered to a subject in the form of an aerosol spray presentation from pressurized packs or from a nebuliser, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of *e.g.*, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch. Dellamary *et al.* (2004) *J Control Release*;95(3): 489-500 describes formulations for the pulmonary delivery of antibodies. Such inhalational formulations may be particular useful for the treatment of asthma or for reducing inflammation in the pulmonary mucosa.

[0191] The invention also provides compositions, suitable for administration through the oral mucosa, which comprise the anti-SARS-CoV S protein antibody described herein. Oral transmucosal delivery refers to the delivery of a delivery vehicle across a mucous membrane in the oral cavity, pharyngeal cavity, or esophagus, and may be contrasted, for example, with traditional oral delivery, in which absorption of a drug occurs in the intestine.

Accordingly, routes of administration in which the anti-SARS-CoV S protein antibodies are absorbed through the buccal, sublingual, gingival, pharyngeal, and/or esophageal mucosa are all encompassed within "oral transmucosal delivery," as that term is used herein. For administration through the transmucosal mucosa, the anti-SARS-CoV S protein antibody  
5 may be formulated, for example, into chewing gums (see U.S. Pat No. 5,711,961) or buccal patches (see *e.g.* U.S. Patent No. 5,298,256).

[0192] The invention also provides compositions suitable for administration through the vaginal mucosa, which comprise the anti-SARS-CoV S protein antibodies described herein. The anti-SARS-CoV S protein antibodies of the invention may be formulated into a vaginal  
10 suppository, foam, cream, tablet, capsule, ointment, or gel.

[0193] In certain embodiments, the compositions comprising the anti-SARS-CoV S protein antibodies are formulated with permeants appropriate to the transmucosal barrier to be permeated. Such penetrants are generally known in the art, and include, for example, for transmucosal administration bile salts and fusidic acid derivatives

[0194] In certain embodiments, an anti-SARS-CoV S protein antibody of the invention can be orally administered, for example, with an inert diluent or an assimilable edible carrier. The compound (and other ingredients, if desired) can also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the anti-SARS-CoV S protein antibodies can be  
15 incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. To administer a compound of the invention by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation.

[0195] Additional active compounds also can be incorporated into the compositions. In certain embodiments, an inhibitory anti-SARS-CoV S protein antibody of the invention is co-formulated with and/or co-administered with one or more additional therapeutic agents, particularly anti-viral agents. These therapeutic agents include, without limitation, antibodies that bind other targets, photosensitizers, androgen, estrogen, nonsteroidal  
20 antiinflammatory agents, antihypertensive agents, analgesic agents, antidepressants, antibiotics, anticancer agents, anesthetics, antiemetics, antiinfectants, contraceptives,  
30

antidiabetic agents, steroids, anti-allergy agents, chemotherapeutic agents, anti-migraine agents, agents for smoking cessation, anti-viral agents, immunosuppressants, thrombolytic agent, cholesterol-lowering agents and anti-obesity agents.

[0196] Therapeutic agents also include peptide analogues that inhibit SARS-CoV S

5 protein activity, antibodies or other molecules that prevent SARS-CoV entry into a cell, including but not limited to preventing S protein binding to a receptor such as the ACE2 receptor, and agents that inhibit SARS-CoV S protein expression. In one embodiment, the additional agents that inhibit SARS-CoV S protein expression comprise an antisense nucleic acid capable of hybridizing to a SARS-CoV S protein mRNA, such as a hairpin RNA or  
10 siRNA, locked nucleic acids (LNA) or ribozymes. Sequence-specific nucleic acids capable of inhibiting gene function by RNA interference are well-known in the art. Such combination therapies may require lower dosages of the inhibitory anti-SARS-CoV S protein antibody as well as the co-administered agents, thus avoiding possible toxicities or complications associated with the various monotherapies.

15 [0197] In certain specific embodiments, the therapeutic agent(s) that is co-formulated with and/or co-administered with an inhibitory anti-SARS-CoV S protein antibody of the invention is an antimicrobial agent. Antimicrobial agents include antibiotics (e.g. antibacterial), antiviral agents, antifungal agents, and anti-protozoan agents. Non-limiting examples of antimicrobial agents are sulfonamides, trimethoprim-sulfamethoxazole,  
20 quinolones, penicillins, and cephalosporins.

[0198] The compositions of the invention may include a “therapeutically effective amount” or a “prophylactically effective amount” of an antibody or antigen-binding portion of the invention. A “therapeutically effective amount” refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A  
25 therapeutically effective amount of the antibody or antibody portion may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody or antibody portion to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects. A

30 “prophylactically effective amount” refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a

prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount may be less than the therapeutically effective amount.

[0199] Dosage regimens can be adjusted to provide the optimum desired response (*e.g.*, a therapeutic or prophylactic response). For example, a single bolus can be administered, several divided doses can be administered over time or the dose can be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the anti-SARS-CoV S protein antibody or portion thereof and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an antibody for the treatment of sensitivity in individuals.

[0200] An exemplary, non-limiting range for a therapeutically or prophylactically-effective amount of an antibody or antibody portion of the invention is 0.025 to 50 mg/kg, more preferably 0.1 to 50 mg/kg, more preferably 0.1-25, 0.1 to 10 or 0.1 to 3 mg/kg. In some embodiments, a formulation contains 5 mg/ml of antibody in a buffer of 20mM sodium citrate, pH 5.5, 140mM NaCl, and 0.2mg/ml polysorbate 80. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

[0201] Another aspect of the present invention provides kits comprising an anti-SARS-CoV S protein, or antigen-binding portion, of the invention or a composition comprising such an antibody or antigen-binding fragment. A kit may include, in addition to the antibody or composition, diagnostic or therapeutic agents. A kit can also include

instructions for use in a diagnostic or therapeutic method, as well as packaging material such as, but not limited to, ice, dry ice, styrofoam, foam, plastic, cellophane, shrink wrap, bubble wrap, cardboard and starch peanuts. In one embodiment, the kit includes the antibody or a composition comprising it and a diagnostic agent that can be used in a method described below. In still another embodiment, the kit includes the antibody or a composition comprising it and one or more therapeutic agents that can be used in a method described below.

[0202] The invention also relates to compositions for inhibiting viral infection, and in particular SARS infection, in a mammal comprising an amount of an antibody of the invention in combination with an amount of an antiviral agent, wherein the amounts of the anti-SARS-CoV S protein antibody and of antiviral agent are together effective in inhibiting viral replication, viral infection of new cells or viral loads. Many antiviral agents are presently known in the art, including nucleoside analogues (*e.g.*, AZT, 3TC, ddI), protease inhibitors and chemokine receptor antagonists.

#### Diagnostic Methods of Use

[0203] In another aspect, the invention provides diagnostic methods. The anti-SARS-CoV S protein antibodies can be used to detect SARS-CoV S protein in a biological sample *in vitro* or *in vivo*. In one embodiment, the invention provides a method for diagnosing the presence or location of SARS-CoV viruses in a subject in need thereof.

[0204] The anti-SARS-CoV S protein antibodies can be used in a conventional immunoassay, including, without limitation, an ELISA, an RIA, flow cytometry, tissue immunohistochemistry, Western blot or immunoprecipitation. The anti-SARS-CoV S protein antibodies of the invention can be used to detect SARS-CoV S protein from humans.

[0205] The invention provides a method for detecting SARS-CoV S protein in a biological sample comprising contacting the biological sample with an anti-SARS-CoV S protein antibody of the invention and detecting the bound antibody. In one embodiment, the anti-SARS-CoV S protein antibody is directly labeled with a detectable label. In another embodiment, the anti-SARS-CoV S protein antibody (the first antibody) is unlabeled and a second antibody or other molecule that can bind the anti-SARS-CoV S protein antibody is



labeled. As is well known to one of skill in the art, a second antibody is chosen that is able to specifically bind the particular species and class of the first antibody. For example, if the anti-SARS-CoV S protein antibody is a human IgG, then the secondary antibody could be an anti-human-IgG. Other molecules that can bind to antibodies include, without limitation, Protein A and Protein G, both of which are available commercially, *e.g.*, from Pierce Chemical Co.

[0206] Suitable labels for the antibody or secondary antibody have been disclosed supra, and include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase,  $\beta$ -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{35}\text{S}$  or  $^3\text{H}$ .

[0207] In other embodiments, SARS-CoV S protein can be assayed in a biological sample by a competition immunoassay utilizing SARS-CoV S protein standards labeled with a detectable substance and an unlabeled anti-SARS-CoV S protein antibody. In this assay, the biological sample, the labeled SARS-CoV S protein standards and the anti-SARS-CoV S protein antibody are combined and the amount of labeled SARS-CoV S protein standard bound to the unlabeled antibody is determined. The amount of SARS-CoV S protein in the biological sample is inversely proportional to the amount of labeled SARS-CoV S protein standard bound to the anti-SARS-CoV S protein antibody.

[0208] One can use the immunoassays disclosed above for a number of purposes. For example, the anti-SARS-CoV S protein antibodies can be used to detect SARS-CoV S protein in cultured cells or as a diagnostic assay in samples from a subject.

#### Therapeutic Methods of Use

[0209] In another embodiment, the invention provides a method for neutralizing SARS-CoV by administering an anti-SARS-CoV S protein antibody to a patient in need thereof.

Any of the types of antibodies described herein may be used therapeutically. In various

embodiments, the anti-SARS-CoV S protein antibody is a human antibody. In some embodiments, the antibody, or antigen-binding portion thereof, binds to the S1 domain of SARS-CoV S protein.

[0210] In some embodiments, the patient is a human patient. Alternatively, the patient may be a mammal infected with SARS-CoV. The antibody may be administered to a non-human mammal infected with SARS for veterinary purposes or as an animal model of human disease. Such animal models may be useful for evaluating the therapeutic efficacy of antibodies of this invention.

[0211] In one embodiment, the invention provides methods of treating, aiding in the treatment, preventing or aiding in the prevention of, SARS-CoV infection and conditions or disorders resulting from such infection, in a subject by administering to the subject a therapeutically-effective amount of an anti-SARS-CoV S protein antibody of the invention.

[0212] Antibodies and antigen-binding fragments thereof which are antagonists of SARS-CoV S protein can be used as therapeutics for SARS-CoV infection. SARS-CoV infects target cells via the spike (S) protein expressed on the virus surface. SARS-CoV S protein is a type one transmembrane glycoprotein divided into two functional domains, S1 (amino acids 15-680) and S2 (amino acids 681-1255). The S1 subunit mediates the interaction of the S protein with its receptor, angiotensin converting enzyme 2 (Ace2). A region of S1 consisting of 193 amino acids named receptor binding domain (RBD) is responsible for ACE2 binding. The S2 subunit of the S protein mediates fusion of viral and host membranes through a conformational change in which two conserved helical regions of the S protein are brought together to form a six-helix bundle fusion core.

[0213] The antibody may be administered once, but more preferably is administered multiple times. The antibody may be administered from three times daily to once every six months or longer. The administering may be on a schedule such as three times daily, twice daily, once daily, once every two days, once every three days, once weekly, once every two weeks, once every month, once every two months, once every three months and once every six months. The antibody may also be administered continuously via a minipump. The antibody may be administered via an oral, mucosal, buccal, intranasal, inhalable, intravenous, subcutaneous, intramuscular, parenteral, intratumor or topical route. The antibody may be administered locally or systemically.

[0214] The therapeutic compositions comprising anti-SARS-CoV S protein antibodies may be administered to the subject, for example, orally, nasally, vaginally, buccally, rectally, via the eye, or via the pulmonary route, in a variety of pharmaceutically acceptable dosing forms, which will be familiar to those skilled in the art.

5 [0215] For example, the anti-SARS-CoV S protein antibodies may be administered via the nasal route using a nasal insufflator device. Examples of these are already employed for commercial powder systems intended for nasal application (*e.g.* Fisons Lomudal System). Details of other devices can be found in the pharmaceutical literature (see for example Bell, A. Intranasal Delivery devices, in Drug Delivery Devices Fundamentals and Applications,  
10 Tyle P. (ed), Dekker, New York, 1988).

[0216] The anti-SARS-CoV S protein antibodies can be administered to the vagina in a freeze dried powder formulation. Anti-SARS-CoV S protein antibodies may be administered in a vaginal applicator and once in the vagina, the formulation comprising the anti-SARS-CoV S protein antibodies are released by pressing a syringe-type piston or  
15 similar release mechanism on the applicator. Alternatively, the anti-SARS-CoV S protein antibodies may be formulated as a powder using a powder device, formulated into a vagina suppository or pessary or vaginal tablet or vaginal gel.

[0217] The anti-SARS-CoV S protein antibodies can also be administered to the eye in a gel formulation. For example, before administration, a formulation containing the anti-  
20 SARS-CoV S protein antibodies may be conveniently contained in a two compartment unit dose container, one compartment containing a freeze-dried anti-SARS-CoV S protein antibody preparation and the other compartment containing normal saline. Prior to application, the two compartments are mixed and a gel is formed, which is then administered to the eye.

25 [0218] Other delivery routes for the anti-SARS-CoV S protein antibodies include via the pulmonary route using a powder inhaler or metered dose inhaler, via the buccal route formulated into a tablet or a buccal patch, via the rectal route formulated into suppositories; and via the oral route in the form of a tablet, a capsule or a pellet (which compositions may administer agent via the stomach, the small intestine or the colon), all of which may be  
30 formulated in accordance with techniques which are well known to those skilled in the art.

[0219] The antibody may be administered once, at least twice or for at least the period of time until the condition is treated, palliated or cured. The antibody will generally be administered as part of a composition as described *supra*. The dosage of antibody will generally be in the range of 0.1-100 mg/kg, more preferably 0.5-50 mg/kg, more preferably 1-20 mg/kg, and even more preferably 1-10 mg/kg. The serum concentration of the antibody may be measured by any method known in the art.

[0220] In another embodiment, the antibodies of the present invention are administered to the subject in combination with other therapeutic agents. In one embodiment, the additional therapeutic agents may be treat the symptoms of the SARS-CoV infection on their own, and may optionally synergize with the effects of the antibodies. The additional agent that is administered may be selected by one skilled in the art for treating the infection.

[0221] Co-administration of the antibody with an additional therapeutic agent (combination therapy) encompasses administering a composition comprising the anti-SARS-CoV S protein antibody and the additional therapeutic agent as well as administering two or more separate compositions, one comprising the anti-SARS-CoV S protein antibody and the other(s) comprising the additional therapeutic agent(s). Further, although co-administration or combination therapy generally means that the antibody and additional therapeutic agents are administered at the same time as one another, it also encompasses instances in which the antibody and additional therapeutic agents are administered at different times. For instance, the antibody may be administered once every three days, while the additional therapeutic agent is administered once daily. Alternatively, the antibody may be administered prior to or subsequent to treatment with the additional therapeutic agent, for example after a patient has failed therapy with the additional agent. Similarly, administration of the anti-SARS-CoV S protein antibody may be administered prior to or subsequent to other therapy.

[0222] The antibody and one or more additional therapeutic agents (the combination therapy) may be administered once, twice or at least the period of time until the condition is treated, palliated or cured. Preferably, the combination therapy is administered multiple times. The combination therapy may be administered from three times daily to once every six months. The administering may be on a schedule such as three times daily, twice daily, once daily, once every two days, once every three days, once weekly, once every two

weeks, once every month, once every two months, once every three months and once every six months, or may be administered continuously via a minipump. The combination therapy may be administered via an oral, mucosal, buccal, intranasal, inhalable, intravenous, subcutaneous, intramuscular, or parenteral.

5 [0223] In certain aspects, the disclosure provides a method for treating, preventing or alleviating the symptoms of a SARS-CoV-mediated disorder in a subject in need thereof, comprising the step of administering to said subject an antibody or antigen-binding portion according to any one of the preceding embodiments, further comprising at least one additional therapeutic agent selected from the group consisting of:

10 (a) one or more antibodies from the group consisting of: 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2;

(b) one or more antibodies that specifically bind SARS-CoV S protein of a plurality of  
15 SARS-CoV strains;

(c) one or more neutralizing antibodies that do not bind SARS-CoV S protein;

(d) one or more agents that bind SARS-CoV S protein receptor; and

(e) one or more anti-viral agents.

[0224] In certain embodiments, antibodies with different binding specificities may be used  
20 in combination to simultaneously target several neutralizing epitopes and prevent emergence of escape mutants. In certain embodiments, neutralizing epitopes may include regions of S1 or S2, other SARS-CoV proteins, or S protein receptors. In certain embodiments, the neutralizing epitopes are in the S1 RBD domain or upstream of the RBD.

[0225] In certain embodiments, antibodies with binding specificities to a plurality of viral  
25 strains may be used in combination to simultaneously target multiple viral strains. In certain embodiments, an antibody may bind to a single strain or multiple strains. A number of SARS-CoV strains have been described and are known to one of skill in the art, for example some common SARS-CoV strains include: TWJ, Urbani, and Tor2.

[0226] In order that this invention may be better understood, the following examples are  
30 set forth. These examples are for purposes of illustration only and are not to be construed as limiting the scope of the invention in any manner.

## EXAMPLE I

### Production of Human Anti-SARS-CoV S Protein Antibodies

#### Preparation of purified S protein ectodomain

- 5 [0227] A cDNA encoding amino acids 1-1193 of the ectodomain of the S protein (Tor2; Genbank accession number: AY274119) (kind gift from Marco Marra and Caroline Astell at British Columbia Cancer Agency Genome Sciences Centre) was cloned into the BaculoDirect™ Baculovirus Expression System (Invitrogen) in frame with a V5-HIS-tag. The protein was expressed in Sf9 cells and purified using Probond™ Nickel-Chelating Resin  
10 (Invitrogen).

#### Immunization and hybridoma production

- [0228] Five micrograms of purified S protein was emulsified in Titermax Gold adjuvant (Sigma) and 6-10 week old IgG2K XenoMouse® animals were immunized intraperitoneally. Subsequent boosts were performed sequentially using TiterMax Gold or alum (Sigma) as  
15 adjuvants. When the animals developed an anti-S antibody response, a final boost in PBS was performed, four days later the spleen and lymph cells were harvested, fused with P3 myeloma cells and HPRT+ hybridomas were selected in hypoxanthine-azaserine (HA) using a standard protocol (Davis).

[0229] Hybridoma supernatants from a total of 11,520 wells were individually screened for S reactivity by ELISA against S-V5-HIS with a counter screen against OVA-V5-HIS as a control. Hybridoma supernatants yielding OD values above 0.7 when tested against S-V5-HIS (Tor2) were further tested against various S1-Ig fragments by ELISA.

5

## EXAMPLE II

### Epitope Mapping

#### Production and purification of S1-Ig protein and fragments

[0230] The cDNAs encoding different S1-Ig fragments (i.e. aa 12-672, 12-510, 261-672, 318-510) were transformed into MC1061/P3 cells and the bacteria were grown on  
10 tetracycline and ampicillin agar plates. The constructs were confirmed by restriction analysis using NheI and BamHI (Figure 1A).

[0231] The S1-Ig fragments consisted of amino acids 12-672, 12-510, 261-672, or 318-510, the C5 signal sequence and a human Ig Fc lacking the transmembrane domain to allow secretion of the protein. The cDNAs encoding S1-Ig fragments were transfected into 293T  
15 cells using a CaPO<sub>3</sub> transfection kit (Invitrogen). Briefly, 293T cells were seeded 1 day prior to transfection, and the media changed the following morning. The CaPO<sub>3</sub> transfection procedure was performed as follows: 10µg DNA+ 50µl CaCl<sub>2</sub> (2.5M) + 450µl sterile H<sub>2</sub>O was mixed and added to 500µl of HBS while aerating (values are per transfected plate.). This mixture was incubated for 20 minutes at room temperature and subsequently was  
20 added dropwise to 293T cells. Next day the cells were washed with PBS+1mM CaCl<sub>2</sub>+ 0.5M MgCl<sub>2</sub> and medium replaced with 293T serum free medium supplemented with 2mM L-glutamine and antibiotic/antimycotic (Gibco). Cells were incubated at 37°C for two days at which time the medium was harvested and protease inhibitor tablets added (Roche). The supernatant was then spun at 1500rpm for 5 minutes to remove any cell debris, and protein  
25 purified from the supernatant using Protein-A sepharose beads (Santa Cruz Biotechnology) by rocking overnight at 4°C. Beads were then washed with PBS+CaCl<sub>2</sub>+ MgCl<sub>2</sub>+ 0.5M

NaCl<sub>2</sub> one time followed by two additional washes with PBS+CaCl<sub>2</sub>+ MgCl<sub>2</sub>. Protein was eluted using 50mM sodium citrate/50mM glycine at pH 2 and neutralized immediately using Tris-HCl (pH 9.5). Protein was concentrated on Centricon filters (Amicon) spinning at 3000rpm for 1 hour at 4°C. Protein was then dialyzed overnight at 4°C against PBS.

5 Reactivity of hybridoma supernatants with S1 fragments

[0232] As described above, hybridoma supernatants were tested against a recombinant S protein (S-V5-HIS Tor2 isolate), and counter-screened against OVA-V5-HIS protein as a control. This led to the identification of 666 hybridomas producing human monoclonal antibodies against the SARS-CoV S protein (Table 1). From the initial screening, 576 anti-  
10 SARS-CoV S protein monoclonal antibodies with OD values above ~0.7 were selected, further tested and characterized. These monoclonal antibodies were examined for their reactivity with the S1 domain of the S protein containing amino acids 12-672.

[0233] Initial screening was carried out using the full-length S1-Ig (12-672). Briefly, plates were coated with 50ng/well of S1-IgG protein overnight at 4°C. The plates were  
15 blocked using 5% non-fat milk, 0.05% Tween-20 for 1 hour at room temperature, washed and 50µl of hybridoma supernatant (diluted 1:3.5) was added to each well and incubated at room temperature for 1 hour. After washing, 50µl/well of HRP conjugated goat-anti-human antibody was added and incubated for 1 hour at room temperature. Following washing, the antibody binding was detected using 50µl/well of substrate and the reaction stopped using  
20 25µl of 10% HCl; the absorbance was then read on an ELISA plate reader (BioRad) at 450nm. The same procedure was followed for screening of hybridoma supernatants (used at a dilution of 1:6) and purified human monoclonal antibodies (HmAbs) using the other S1-Ig fragments.

[0234] This screening identified 165 monoclonal antibodies that were specific to the S1  
25 domain of the S protein with reactivity ranging from 0.171 to 1.817 (Figure 2). Samples with OD values greater than 2X the average background value (0.0825) were considered positive (Figure 2). These 165 S1 reacting antibodies were further analyzed for their ELISA reactivity with additional S1 protein fragments (i.e. 12-510, 261-672 and 318-510). The smallest fragment encoding the minimal RBD 318-510 often yielded the highest reactivity  
30 for most of the monoclonal antibodies tested relative to the other fragments. Fragments



consisting of amino acids 12-672 and 261-672 demonstrated the least reactivity. One explanation for this observation is that the region between 511 and 672 could possibly partially mask epitopes within 318-510 in these S1 fragments (Figure 2).

[0235] The antibodies were grouped based on their reactivity with various S1 fragments (Table 2). Comparison of antibody (Ab) reactivity across all S1 fragments indicated that most Abs react within the receptor binding domain 318-510. Antibodies that bound to 318-510 however fell into four groups (group designation 1A-1D), based on their differential reactivity with the other S1 fragments. The differences in S1 fragment reactivity suggest that the epitope(s) recognized by the monoclonal antibodies in each group, though still within 318-510, are different.

[0236] Further epitope mapping experiments were conducted using overlapping peptides derived from the 318-510 region of S1 domain (provided by NIH). These peptides consist of 18 amino acids with 10 amino acid overlap. None of the antibodies showed significant reactivity with any of the peptides indicating that the antibodies recognized either conformational epitopes and/or require glycosylation

[0237] SARS coronavirus (SARS-CoV) Urbani strain (Genbank accession number: AY278741) was obtained from the CDC. Virus was propagated in VeroE6 cells in OptiPro serum free medium (SFM). The TCID<sub>50</sub> value was then determined by infecting  $5 \times 10^3$  VeroE6 cells/well in a 96 well plate with serial 1:10 dilution of SARS-CoV, 8 wells were infected per dilution. After 3 days of incubation at 37°C in a 5%CO<sub>2</sub> humidified incubator cells were evaluated for cytopathic effect (CPE). The TCID<sub>50</sub> value was calculated as follows:  $-\log\text{TCID}_{50} = -\log \text{dilution above 50\%} + (-\text{proportionate distance})$ .

[0238] Because mice were immunized with full-length S protein, monoclonal antibodies that did not react with the S1 domain were examined for their reactivity with HR1 and HR2 domains in the S2 region. None of the antibodies reacted with HR1 and three showed significant reactivity with HR2. Two of the three HR2 binding Abs resulted in high OD values (1.281 and 1.26), however, none of these three Abs showed neutralizing activity (data not shown).

### EXAMPLE III

#### Identification of Neutralizing Monoclonal Antibodies

[0239] ELISA positive monoclonal antibodies were tested for their ability to neutralize SARS-CoV in a microneutralization assay.

5 [0240] VeroE6 cells were seeded at  $5 \times 10^3$  cells per well in a 96 well plate a few hours before the neutralization assay was performed in OptiPro SFM (Gibco). Neutralizing ability of the Abs in hybridoma supernatants was tested by mixing 50 $\mu$ l of hybridoma supernatant with 200xTCID<sub>50</sub> of virus in 50 $\mu$ l of medium for 1 hour at 37°C. Following incubation, the antibody/virus mixture was added to VeroE6 cells and incubated at 37°C for 3 days. At this  
10 time, cells were visually observed for cytopathic effect (CPE; indicated by rounding of VeroE6 cells) as an indicator of SARS-CoV infection. A similar assay was performed using 1:4 serial dilutions of purified human monoclonal antibodies (HmAbs).

[0241] VeroE6 cells were seeded at  $5 \times 10^3$  cells per well in a 96 well plate a few hours before the neutralization assay was performed in OptiPro SFM (Gibco). Neutralizing ability  
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20 1:4 serial dilutions of purified human monoclonal antibodies (HmAbs).

[0242] Of the 165 strongly S1 positive HmAbs, 27 antibodies completely neutralized 200TCID<sub>50</sub> SARS-CoV as indicated by a total lack of CPE (Table 2). A significant proportion of the neutralizing monoclonal antibodies reacted with the RBD of the S protein consisting of amino acids 318-510. Though fewer Abs belonged to group 1A, the majority  
25 of these Abs were neutralizing and suggested that they are reacting with a dominant neutralizing domain containing one or more epitope(s). The same can be said for Abs in group 1B, the majority of neutralizing Abs belonged to this group indicating that they are also reacting with a dominant neutralizing domain, but most likely distinct from that recognized by Abs in group 1A.

[0243] Three additional neutralizing HmAbs were found that most likely bind to a region between amino acids 12 and 261 of the S1 domain. This is analogous to other studies which have shown neutralizing ability of Abs that bind upstream of the known RBD (i.e. 130-150). However, how these Abs prevent SARS-CoV infection is yet to be determined.

5

#### EXAMPLE IV

##### Characterization of Purified Human Anti-SARS-CoV Monoclonal Antibodies

[0244] Neutralizing HmAbs were then cloned by limiting dilution and 24 out of 27 antibodies were purified by protein A/G affinity columns, 19 of these were subsequently confirmed as monoclonal by Ig gene sequencing. Following purification, the reactivity of HmAbs was re- tested against the 318-510 fragment, or 12-510 of the S1-Ig for those that failed to bind to 318-510. The range and pattern of reactivity noted in the initial screening was maintained after the purification of HmAbs. Most HmAbs demonstrated a dose dependent binding in which OD values decreased with increasing dilution of the antibody. Other Abs, such as 6B5 and 3H12, maintained high OD values indicating their relative high affinity. Often, the degree of reactivity in ELISA did not correlate with the neutralization titer (Figure 3 and Table 3). This perhaps suggests limited availability of relevant epitopes in the virally expressed native S protein relative to their availability in a particular recombinant S1-Ig fragment, thus limiting neutralizing ability of certain HmAbs.

[0245] Different dilutions of purified HmAbs were tested for their ability to neutralize 200TCID<sub>50</sub> of SARS-CoV. The titer of the antibody was defined as the lowest concentration of HmAb capable of neutralizing 200TCID<sub>50</sub> of SARS-CoV. The HmAbs varied in their neutralizing potential. Some HmAbs neutralized the virus at concentrations as low as 0.195µg/ml. However, some HmAbs could not neutralize virus below a concentration of 12.5µg/ml (Table 3). This variance in neutralizing ability between HmAbs may be due to differences in affinities, fine binding specificities and/or the extent of availability of the targeted epitope on virally expressed native S protein.

[0246] Hybridomas that were positive for the production of neutralizing antibodies were cloned by limiting dilution and the clones were cultured to produce larger quantities of

human monoclonal antibodies. Supernatants from these hybridomas were purified using Protein A/G affinity columns.

#### EXAMPLE V

##### 5 Ig gene Utilization in the Human Anti-SARS-CoV Monoclonal Antibodies

[0247] Each of the purified HmAbs was sequenced, and previous group designations now can be further divided based on the sequence data suggesting that there are at least 10 different binding specificities among the panel of HmAbs. Unique binding specificities were deduced from the usage of different V and J, and also D gene sequences in the case of heavy chains. There is preferential usage of A30, JK4 rearrangement in the light chain and the VH1-2, D3-10, JH4B rearrangement in the heavy chain. However, several other V(D)J segments were also used (Table 3). Group 1B for example can be divided into likely four different specificities based on different V(D)J usage in the heavy and light chains.

15 [0248] The CDR3 region is formed by the heavy and light chain and is particularly important in determining binding specificity of Abs. Our data demonstrate sequence differences within the CDR3 regions of 4A10 and 4G2 although they both contain the same heavy and light chain genes and both fall into group 1A. Differences seen in the CDR3 region of the heavy chain may be responsible for the higher neutralizing titer of 4G2 by allowing better binding affinity and/or specificity. Two HmAbs showed changes in the CDR1 and CDR2 regions. Although these regions are not as important as the CDR3 region in determining binding specificity they do contribute to the overall binding specificity of the antibody. Therefore, changes within these regions may also affect binding specificity and/or affinity. For example, 4G2 and 6C1 have three amino acid differences within the CDR1 as compared to 4E2 although they all fall into group 1A and have the same V(D)J usage (Figure 4). Though all antibodies seem to bind within the 318-510 RBD, small changes in amino acid sequence may alter the fine specificity and/or affinity of their binding. HmAb 6B1 has a single amino acid change in the CDR2 region when compared to 3C7; again this change may alter the affinity of 6B1 for the binding region within the S protein (Figure 4).

25

[0249] Total RNA was purified from approximately  $10^5$  hybridoma cells using an RNeasy Mini Kit Qiagen (Mississauga, Ontario) as per the manufacturer's instructions. The PCR amplification protocol and primers have previously been described (33, 34). Primers specific for the Ig variable (V) gene family members were pooled or used individually.

5 Sequencing was performed by Lone Star Labs (Houston, Texas) using the BigDye™ Terminator Version 3.0 DNA sequencing kit (Applied Biosystems, Foster City, Ca) and ABI 3730 or 3100 automated sequencers (Applied Biosystems, Foster City, Ca).

## EXAMPLE VI

### 10 Receptor Binding Inhibition of the Human Anti-SARS-CoV Monoclonal Antibodies

[0250] To understand the mechanism of neutralizing action of the human anti-SARS-CoV monoclonal antibodies, we used a receptor binding inhibition assay determine whether the antibodies inhibit receptor binding and thereby neutralize the virus. The assay was

15 performed by preincubating the antibodies with an S1 fragment (aa 12-510) carrying an IgG1 Fc tag, incubating the mixture with VeroE6 cells expressing S protein receptor, and measuring the percentage of fragment bound cells using flow cytometry via an anti-human IgG FITC tagged secondary antibody. The antibodies were preincubated (starting concentration 10 µg/ml and 1:2 serial dilutions of Ab thereafter) with the S1 protein  
20 fragment (10 µg/ml, remains constant) for 1 hour at 4C. If the antibodies prevented binding of the S protein to Ace2, then there was a decrease in the fluorescent signal as measured by anti-human IgG FITC because unbound protein and antibody was washed away during the experiment.

[0251] Eighteen of the nineteen neutralizing antibodies tested reduced S protein binding to  
25 cells that were positive for the SARS receptor (Figures 5A-5F). All of the antibodies that bind to S protein in the region of aa 318-510 reduced receptor binding.

[0252] Of the two antibodies that bind upstream of the RBD, one inhibited receptor binding (1B5) and the second did not, and may even slightly enhance binding (4D4).

## EXAMPLE VII

Analysis of Epitopes of the Human Anti-SARS-CoV Monoclonal Antibodies

[0253] Neutralizing human anti-SARS-CoV monoclonal antibodies were assayed under native or denatured conditions to determine if they recognized conformational or linear epitopes. At least five antibodies appeared to recognize conformational epitopes (Table 4).

## EXAMPLE VIII

Pseudotyping Assay

[0254] We are establishing a pseudotyping assay in order to test neutralizing antibodies against changes seen in the S protein during an outbreak. An HIV core (HIV-deltaE-GFP) that expresses GFP is used so transfection efficiency during the production of the pseudovirus and infection by the pseudovirus can be measured using the GFP reporter. The mutations that are found in the S protein are introduced and tested to determine if these antibodies can inhibit entry of the pseudotyped virus. In addition, the pseudotyped virus is used to assay the antibodies for inhibition of fusion.

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TABLE 1. Reactivity to the ectodomain of S protein determined by ELISA reactivity to recombinant S protein (a.a. 12-1193)

Plate Breakdown	V5-S-HIS	V5-OVA-HIS
OD > 0.0	1152	1152
0.1	893	439
0.2	739	94
0.3	688	22
0.4	657	11
0.5	620	7
0.6	593	6
0.7	565	6
0.8	541	6
0.9	520	6
1.0	508	4
1.5	438	3
2.0	401	3
2.5	375	3
3.0	352	2
3.5	298	2
4.0	136	0

<sup>a</sup>numbers of positive reacting supernatants within OD value range at left as determined by ELISA

TABLE 2. Likely binding region of human hybridoma supernatants determined by ELISA reactivity with S1-IgG protein fragments

ELISA reactivity	Group Designation	Number of Supernatants	Number Neutralizing	Likely Binding Region
All S1-IgG fragments	1A	13	9	318-510
12-672	3A	1	0	510-672
12-672, 12-510	2A	4	1	12-261
12-672, 12-510, 318-510	1B	45	14	318-510
12-672, 261-672	3B	2	0	510-672
12-510	2B	29	2	12-261
12-510, 318-510	1C	41	0	318-510
318-510	1D	29	1	318-510
Total		165	27	

TABLE 3. Summary of HmAbs reactivity, neutralizing titer and heavy (H) and light (L) chain usage.

HmAb	Group	Reactivity S <sub>H</sub> G <sub>G</sub> (12472, 12510, 261672, 318510)	Binding region	Neutralizing titer 200TCID <sub>50</sub> (μg/mL)	H chain	L chain	H CDR3	L CDR3
4-E2	1A1	0.919, 1.518, 0.551, 1.839	318-510	0.781	VH1-2, D3-10, JH4B	A30, JK4	GPHSFGSGSYPPFDY	QQYNSYPLT
4-G2	1A1	1.200, 1.662, 0.665, 1.811	318-510	0.781	VH1-2, D3-10, JH4B	A30, JK4	GPHSFGSGSYPPFDY	QQYNSYPLT
6-C1	1A1	1.226, 1.586, 0.649, 2.405	318-510	0.781	VH1-2, D3-10, JH4B	A30, JK4	GPHSFGSGSYPPFDY	QQYNSYPLT
3-A7	1B1	1.307, 1.523, 0.379, 1.964	318-510	0.195	VH1-18, D1-26, JH4B	A30, JK4	GRYLDY	LQYNSYPLT
5-A7	1B1	1.111, 1.449, 0.366, 1.997	318-510	0.781	VH1-18, D1-26, JH4B	A30, JK4	GRYLDY	LQYNSYPLT
5-D3	1B1	0.968, 1.316, 0.403, 2.020	318-510	0.195	VH1-18, D1-26, JH4B	A30, JK4	GRYLDY	LQYNSYPLT
5-D6	1B1	0.747, 1.313, 0.355, 2.117	318-510	0.195	VH1-18, D1-26, JH4B	A30, JK4	GRYLDY	LQYNSYPLT
6-B8	1B1	1.045, 1.704, 0.497, 2.133	318-510	0.781	VH1-18, D1-26, JH4B	A30, JK4	GRYLDY	LQYNSYPLT
4-A10	1B2	1.013, 1.524, 0.567, 1.792	318-510	3.125	VH1-2, D3-10, JH4B	A30, JK4	GPHTFGSGSYPPFDY	QQYNSYPLT
6-C2	1B2	1.005, 1.603, 0.586, 1.849	318-510	0.781	VH1-2, D3-10, JH4B	A30, JK4	GPHTFGSGSYPPFDY	QQYNSYPLT
3-F3	1B2	1.075, 1.349, 0.325, 1.887	318-510	0.781	VH1-2, D3-10, JH4B	A30, JK4	GPHTFGSGSYPPFDY	QQYNSYPLT
5-A5	1B2	0.986, 1.187, 0.337, 2.310	318-510	0.195	VH1-2, D3-10, JH4B	A30, JK4	GPHTFGSGSYPPFDY	QQYNSYPLT
6-B5	1B2	1.040, 1.324, 0.430, 2.087	318-510	3.125	VH1-2, D3-10, JH4B	A30, JK4	GPHTFGSGSYPPFDY	QQYNSYPLT
5-E4	1B3	0.735, 1.199, 0.298, 2.275	318-510	12.5	VH1-2, N/A, JH4B	A30, JK5	GRYLDY	LQYNSYPIT
3-C7	1B4	1.092, 1.422, 0.357, 2.193	318-510	12.5	VH3-33, D2-2, JH4B	L5, JK4	DPLGYCSSTSCSYFDY	QQANNFPLT
6-B1	1B4	1.128, 1.166, 0.369, 2.093	318-510	3.125	VH3-33, D2-2, JH4B	L5, JK4	DPLGYCSSTSCSYFDY	QQANNFPLT
3-H12	1D	0.185, 0.318, 0.090, 1.304	318-510	3.125	VH4-59, D3-9, JH6B	A30, JK3	DYDILTGYSNYYGMDV	LQHNSYPFT
4-D4	2B1	0.258, 0.761, 0.103, 0.101	12-261	12.5	VH3-33, D4-17, JH4B	A1, JK2	GGDGERFDY	MQGTHWPPYVQ
1-B5	2B2	0.463, 1.292, 0.110, 0.191	12-261	0.195	VH3-33, N/A, JH5B	A30, JK4	GDFYWFDP	QQYNSYPLT

Table 4. Evaluation of epitope type for selected neutralizing human anti-SARS-CoV monoclonal antibodies

	Native						Denatured					
	1G3	5C12	2B10	2E8	5E1	Cont	1G3	5C12	2B10	2E8	5E1	Cont
10 µg/ml	1.378	1.242	1.562	1.426	1.231	0.738	0.062	0.069	0.062	0.049	0.094	0.146
	1.315	1.308	1.615	1.379	1.197	0.587	0.053	0.062	0.061	0.07	0.076	0.157
1:5	1.348	1.066	1.608	1.225	1.071	0.052	0.059	0.058	0.057	0.053	0.054	0.06
	1.276	1.137	1.53	1.184	1.199	0.049	0.053	0.053	0.056	0.065	0.049	0.045
1:25	0.91	1.053	1.107	0.921	0.828		0.052	0.051	0.054	0.05	0.045	
	1.051	0.499	1.077	0.999	0.687		0.051	0.055	0.065	0.052	0.046	
1:125	0.322	0.337	0.385	0.4	0.264		0.054	0.05	0.051	0.047	0.04	
	0.298	0.161	0.395	0.342	0.251		0.048	0.043	0.039	0.054	0.035	

**ANTIBODY SEQUENCES****5 SEQ ID NO: 1****Antibody 5D6 Nucleotide sequence of heavy chain variable region**

5' CAGGTT CAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAGCCTGGGGCCTCAG  
TGAAGGTCTCCTGCAAGGCTTCTGGTTACACCTTTACCAGCTATGGTATCAGCT  
10 GGGTGC GACAGGCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCAGCGCT  
TATAATGGTAACACAACTATGCACAGAAGCTCCAGGGCAGAGTCACCATGAC  
CACAGACACATCCACTAACACAGCCTACATGGAGCTGAGGAGCCTGAGATCTG  
ACGACACGGCCGTGTATTACTGTGCGGTTGGGAGGTACCTTGACTACTGGGGCC  
AGGGAACCCTGGTCACCGTCTCCTCA3'

15

**SEQ ID NO: 2****Antibody 5D6 Amino acid sequence of heavy chain variable region**

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISAY  
20 NGNTNYAQKLQGRVTMTTDTSTNTAYMELRSLRSDDTAVYYCAVGRYLDYWGQ  
GTLVTVSS

**SEQ ID NO: 3****Antibody 5D6 Nucleotide sequence of light chain variable region**

25

5' GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAG  
AGTCACCATCACTTGCCGGGCAAGTCAGGACATTAGAAATGATTTAGGCTGGTA  
TCAGCAGAAACCAGGGAAAGCCCCCTAAGCGCCTGATCTATGCTGCATCCAGTTT  
GCAAAGTGGGGTCCCATCAAGGTTCAAGCGGCAGTGGATCTGGGACAGAATTCA  
30 CTCTCACAATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCTAC  
AGTATAATAGTTACCCTCTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAA3

**SEQ ID NO: 4****35 Antibody 5D6 Amino acid sequence of light chain variable region**

DIQMTQSPSSLSASVGDRVTITCRASQDIRNDLGWYQQKPGKAPKRLIYAASSLQSG  
VPSRFSGSGSGTEFTLTISLQPEDFATYYCLQYNSYPLTFGGGTKEIK

40

**SEQ ID NO: 5**

**Antibody 5D3 = 6B8 = 3A7 = 5A7 = 2E8.1 = 2E8.2 = 5D1.2 Nucleotide sequence of heavy chain variable region**

5 5'CAGGTTTCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAGCCTGGGGCCTCAG  
TGAAGGTCTCCTGCAAGGCTTCTGGTTACACCTTTACCAGCTATGGTATCAGCT  
GGGTGCGACAGGCCCTGGACAAGGTCTTGAGTGGATGGGATGGATCAGCGCA  
TATAATGGTAACACAACTATGCACAGAAGCTCCAGGGCAGAGTCACCATGAC  
CACAGACACATCCACGAGCACAGCCTACATGGAGCTGAGGAGCCTGAGATCTG  
10 ACGACACGGCCGTGTATTACTGTGCGGTTGGGAGGTACCTTGACTACTGGGGCC  
AGGGAACCCTGGTCACCGTCTCCTCA3'

**SEQ ID NO: 6**

**Antibody 5D3 = 6B8 = 3A7 = 5A7 = 2E8.1 = 2E8.2 = 5D1.2 Amino acid sequence of heavy chain variable region**

15 QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISAY  
NGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCAVGRYLDYWGQ  
GTLVTVSS  
20

**SEQ ID NO: 7**

**Antibody 5D3 = 6B8 = 3A7 = 5A7 = 2E8.1 = 2E8.2 = 5D1.2 Nucleotide sequence of light chain variable region**

25 5'GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAG  
AGTCACCATCACTTGCCGGGCAAGTCAGGACATTAGAAATGATTTAGGCTGGTA  
TCAGCAGAAACCAGGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCAGTTT  
GCAAAGTGGGGTCCCATCAAGGTTTCAGCGGCAGTGGATCTGGGACAGAATTCA  
30 CTCTCACAATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCTAC  
AGTATAATAGTTACCCTCTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAA3  
,

**SEQ ID NO: 8**

**Antibody 5D3 = 6B8 = 3A7 = 5A7 = 2E8.1 = 2E8.2 = 5D1.2 Amino acid sequence of light chain variable region**

35 DIQMTQSPSSLSASVGDRTITCRASQDIRNDLGWYQQKPGKAPKRLIYAASSLQSG  
VPSRFSGSGSGTEFTLTISLQPEDFATYYCLQYNSYPLTFGGGTKVEIK  
40

**SEQ ID NO: 9****Antibody 5E4 Nucleotide sequence of heavy chain variable region**

5 5'CAGGTACACCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAG  
TGAAGGTCTCCTGCAAGGCTTCTGGATACACCTTCACCGGCTACTATGTACACT  
GGGTGCGACAGGCCCCCTGGACAGGGGCTTGAGTGGATGGGATGGATCAACCCT  
AACAGTGGTGGCACAACCTATGCACAGAAGTTTCAGGGCAGGGTCACCATGAC  
CAGGGACACGTCCATCAGCACAGCCTACATGGAGCTGAGCAGGCTGAGATCTG  
10 ACGACACGGCCGTGTATTTCTGTGCGGGTGGGCGCTACTTGGACTACTGGGGCC  
AGGGAACCCTGGTCACCGTCTCCTCA3'

**SEQ ID NO: 10****Antibody 5E4 Amino acid sequence of heavy chain variable region**

15 QVHLVQSGAEVKKPGASVKVSCKASGYFTGYVHWVRQAPGQGLEWMGWINP  
NSGGTNYAQKFQGRVTMTRDTSISTAYMELSRRLRSDDTAVYFCAGGRYLDYWGQ  
GTLVTVSS

20

**SEQ ID NO: 11****Antibody 5E4 Nucleotide sequence of light chain variable region**

5'GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAG  
25 AGTCACCATCACTTGCCGGGCAAGTCAGGGCATTAGAAATGATTTAGGCTGGTA  
TCAGCAGAAACCAGGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCAGTTT  
GCAAAGTGGGGTCCCATCAAGGTTCAAGCGGCAGCGGATCTGGGACAGAATTCA  
CTCTCACAATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCTAC  
AGTATAATAGTTACCCGATCACCTTCGGCCAAGGGACACGACTGGAGATTAAA3  
30 '

**SEQ ID NO: 12****Antibody 5E4 Amino acid sequence of light chain variable region**

35 DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSG  
VPSRFSGSGSGTEFTLTISLQPEDFATYYCLQYNSYPITFGQGTREIK



**SEQ ID NO: 13****Antibody 5E1.1.1 = 5E1.2 = 5E1.3 Nucleotide sequence of heavy chain variable region**

5' CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAG  
5 TGAAGGTCTCCTGCAAGGCTTCTGGATACACCTTCACCGGCTACTATATGCACT  
GGGTGCGACAGGCCCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCAACCCT  
AACAGTGGTGGCACA AACTATGCACAGAAGTTTCAGGGCAGGGTCACCATGAC  
CAGGGACACGTCCATCAGCACAGCCTACATGGAGCTGAGCAGGCTGAGATCTG  
ACGACACGGCCGTGTTTTACTGTGCGGGTGGGACCTACTTGGACTACTGGGGCC  
10 AGGGAACCCTGGTCACCGTCTCCTCA3'

**SEQ ID NO: 14****Antibody 5E1.1.1 = 5E1.2 = 5E1.3 Amino acid sequence of heavy chain variable region**

15 QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINP  
NSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVFYCAGGTYLDYWGQ  
GTLVTVSS

**SEQ ID NO: 15****Antibody 5E1.1.1 = 5E1.2 = 5E1.3 Nucleotide sequence of light chain variable region**

5' GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAG  
AGTCACCATCACTTGCCGGGCAAGTCAGGGCATTAGAAATGATTTAGGCTGGTA  
25 TCAGCAGAAACCAGGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCAGTTT  
GCAAAGTGGGGTCCCATCAAGGTT CAGCGGCAGTGGATCTGGGACAGAATTCA  
CTCTCACAATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCTAC  
AGTATAATAGTTACCCGATCACCTTCGGCCAAGGGACACGACTGGAGATTAAA3  
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**SEQ ID NO: 16****Antibody 5E1.1.1 = 5E1.2 = 5E1.3 Amino acid sequence of light chain variable region**

DIQMTQSPSSLSASVGDRTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSG  
35 VPSRFSGSGSGTEFTLTISLQPEDFATYYCLQYNSYPITFGQGTRLEIK

**SEQ ID NO: 17****Antibody 5E1.1.2 Nucleotide sequence of heavy chain variable region**

5' CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAG  
5 TGAAGGTCTCCTGCAAGGCTTCTGGATACACCTTCACCGGCTACTATATGCACT  
GGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCAACCCT  
AACAGTGGTGGCACA AACTATGCACAGAAGTTTCAGGGCAGGGTCACCATGAC  
CAGGGACACGTCCATCAGCACAGCCTACATGGAGCTGAGCAGGCTGAGATCTG  
ACGACACGGCCGTGTTTTACTGTGCGGGTGGGACCTACTTGGACTACTGGGGCC  
10 AGGGAACCCTGGTCACCGTCTCCTCA3'

**SEQ ID NO: 18****Antibody 5E1.1.2 Amino acid sequence of heavy chain variable region**

15 QVQLVQSGAEVKKPGASVKVSC KASGYTFTGYYMHWVRQAPGQGLEWMGWINP  
NSGGTNYAQKFQGRVTMTRDTSISTAYMELSR LRSDDTAVFYCAGGTYLDYWGQ  
GTLVTVSS

**20 SEQ ID NO: 19****Antibody 5E1.1.2 Nucleotide sequence of light chain variable region**

5' GACATCGTGATGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAG  
GGCCACCATCAACTGCAAGTCCAGCCAGAGTGTTTTATACAGCTCCAACAATAA  
25 GAACTACTTAGCTTGGTACCAGCAGAAACCAGGACAGCCTCCTAAGCTGCTCAT  
T TACTGGGCATCTACCCGGGAATCCGGGGTCCCTGACCGATT CAGTGGCAGCGG  
GTCTGGGACAGATTTCACTCTCACCATCAGCAGCCTGCAGGCTGAAGATGTGGC  
AGTTTATTACTGTCAGCAATATTATAGTACTCCTCGGACGTTCCGGCCAAGGGAC  
CAAGGTGGAAATCAAA3'

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**SEQ ID NO: 20****Antibody 5E1.1.2 Amino acid sequence of light chain variable region**

DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLA WYQQKPGQP PKLLIYW  
35 ASTRESGV PDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSTPRTFGQGTKVEIK

**SEQ ID NO: 21****5 Antibody 3F3 = 6C2 Nucleotide sequence of heavy chain variable region**

5' CAGGTGCAACTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAG  
TGAAGGTCTCCTGCAAGGCTTCTGGATACACCTTCACCGGCTACTATATGCACT  
GGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCAACCCT  
10 AACAGTGGTGGCACA AACTATGCACAGAAGTTTCAGGGCAGGGTCACCATGAC  
CAGGGACACGTCCATCAGCTCGGCCTACATGGA ACTGAGCAGCCTGAGATCTG  
ACGACACGGCCGTTTATTACTGTGCGAGAGGGCCCCATACCTTCGGTTCGGGGA  
GTTACCCCTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA3'

**15 SEQ ID NO: 22****Antibody 3F3 = 6C2 Amino acid sequence of heavy chain variable region**

QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINP  
NSGGTNYAQKFQGRVTMTRDTSISSAYMELSSLRSDDTAVYYCARGPHTFGSGSYP  
20 FDYWGQGTLVTVSS

**SEQ ID NO: 23****Antibody 3F3 = 6C2 Nucleotide sequence of light chain variable region**

25 5' GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAG  
AGTCACCATCACTTGCCGGGCAAGTCAGGGCATTAGAAGTGATTTAGGCTGGTA  
TCAGCAGAGACCAGGGAACACCCCTAAGCGCCTGATCTATGCTGCATCCAGTTT  
ACAAAGTGGGGTCCCATCAAGGTT CAGCGGCAGTGGATCTGGGACAGAATTCA  
30 CTCTCACAATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCAAC  
AGTATAATAGTTACCCGCTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAA3  
,

**SEQ ID NO: 24****35 Antibody 3F3 = 6C2 Amino acid sequence of light chain variable region**

DIQMTQSPSSLSASVGDRVTITCRASQGIRSDLGWYQQRPGNTPKRLIYAASSLQSG  
VPSRFSGSGSGTEFTLTISLQPEDFATYYCQQYN SYPLTFGGG TKVEIK

**SEQ ID NO: 25****Antibody 6C1 = 4G2 = 5C12.2 Nucleotide sequence of heavy chain variable region**

5' CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAG  
5 TGAAGGTCTCCTGCAAGGCTTCTGGATTCCCCTTCACCGACTACTATATGCACTG  
GGTGCACAGGCCCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCAACTCTA  
ACAGTGGTGGCACA AACTATGCACAGAAGTTTCAGGGCAGGGTCACCATGACC  
AGGGACACGTCCATCAGCACAGCCTACATGGAGCTGAGCAGGCTGAGATCTGA  
CGACACGGCCGTGTATTACTGTGCGAGAGGGCCCCATTCTTTGGTTTCGGGGAG  
10 TTACCCCTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA3'

**SEQ ID NO: 26****Antibody 6C1 = 4G2 = 5C12.2 Amino acid sequence of heavy chain variable region**

15 QVQLVQSGAEVKKPGASVKVSCKASGFPFTDYMHWRQAPGQGLEWMGWINS  
NSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGPHSFGSGSY  
PFDYWGGQGLTVSS

**SEQ ID NO: 27****Antibody 6C1 = 4G2 = 5C12.2 Nucleotide sequence of light chain variable region**

5' GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAG  
AGTCACCATCACTTGCCGGGCAAGTCAGGGCATTAGAAGTGATTTAGGCTGGTA  
25 TCAGCAGAGACCAGGGAAAACCCCTAAGCGCCTGATCTATGCTGCATCCAGTTT  
GCAAAGTGGGGTCCCATCAAGGTTCAAGCGGCAGTGGATCTGGGACAGAATTCA  
CTCTCACAATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCAAC  
AGTATAATAGTTACCCGCTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAA3  
,

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**SEQ ID NO: 28****Antibody 6C1 = 4G2 = 5C12.2 Amino acid sequence of light chain variable region**

DIQMTQSPSSLSASVGDRVTITCRASQGIRSDLGWYQQRPGKTPKRLIYAASSLQSG  
35 VPSRFSGSGSGTEFTLTISLQPEDFATYYCQYNSYPLTFGGGTKVEIK

**SEQ ID NO: 29****Antibody 4E2 Nucleotide sequence of heavy chain variable region**

5' CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAG  
5 TGAAGGTCTCCTGCAAGGCTTCTGGATACACCTTCACCGGCTACTATATGCACT  
GGGTGCGACAGGCCCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCAACTCT  
AACAGTGGTGGCACA AACTATGCACAGAAGTTTCAGGGCAGGGTCACCATGAC  
CAGGGACACGTCCATCAGCACAGCCTACATGGAGCTGAGCAGGCTGAGATCTG  
ACGACACGGCCGTGTATTACTGTGCGAGAGGGCCCCATTCTTTGGTTTCGGGGA  
10 GTTACCCCTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA3'

**SEQ ID NO: 30****Antibody 4E2 Amino acid sequence of heavy chain variable region**

15 QVQLVQSGAEVKKPGASVKV SCKASGYTFTGYMHVVRQAPGQGLEWMGWINS  
NSGGTNYAQKFQGRVTMTRDTSISTAYMELSR LRSDDTAVYYCARGPHSFGSGSY  
PFDYWGQGTLVTVSS

**20 SEQ ID NO: 31****Antibody 4E2 Nucleotide sequence of light chain variable region**

5' GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAG  
AGTCACCATCACTTGCCGGGCAAGTCAGGGCATTAGAAGTGATTTAGGCTGGTA  
25 TCAGCAGAGACCAGGGAAAACCCCTAAGCGCCTGATCTATGCTGCATCCAGTTT  
GCAAAGTGGGGTCCCATCAAGGTT CAGCGGCAGTGGATCTGGGACAGAATTCA  
CTCTCACAATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCAAC  
AGTATAATAGTTACCCGCTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAA3  
,

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**SEQ ID NO: 32****Antibody 4E2 Amino acid sequence of light chain variable region**

DIQMTQSPSSLSASVGDRVTITCRASQGIRSDLGWYQQRPGKTPKRLIYAASSLQSG  
35 VPSRFSGSGSGTEFTLTISLQPEDFATYYCQQYNSYPLTFGGGTKVEIK

**SEQ ID NO: 33****Antibody 5A5 = 4A10 Nucleotide sequence of heavy chain variable region**

5' CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAG  
 5 TGAAGGTCTCCTGCAAGGCTTCTGGATACACCTTCACCGGCTACTATATGCACT  
 GGGTGCAGACAGGCCCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCAACCCT  
 AACAGTGGTGGCACA AACTATGCACAGAAAGTTTCAGGGCAGGGTCACCATGAC  
 CAGGGACTCGTCCATCAGCACAGCCTACATGGAAGT GAGCAGGCTGAGATCTG  
 ACGACACGGCCGTGTATTACTGTGCGAGAGGGCCCCATACCTTTGGTTCGGGGA  
 10 GTTACCCCTTTGACTACTGGGGCCAGGGAACCCTGGTCCCCGTCTCCTCA3'

**SEQ ID NO: 34****Antibody 5A5 = 4A10 Amino acid sequence of heavy chain variable region**

15 QVQLVQSGAEVKKPGASVKV SCKASGYTFTGYMHVVRQAPGQGLEWMGWINP  
 NSGGTNYAQKFQGRVTMTRDSSISTAYMELSR LRSDDTAVYYCARGPHTFGSGSY  
 PFDYWGQGTLPVSS

**SEQ ID NO: 35****20 Antibody 5A5 = 4A10 Nucleotide sequence of light chain variable region**

5' GACATCCAGTTGACCCAGTCTCCATCTTCCCTGTCTGCATCTGTAGGAGACAG  
 AGTCACCATCACTTGCCGGGCAAGTCAGGGCATTAGAAGTGATTTAGGCTGGTA  
 TCAGCAGAGACCAGGGAAAACCCCTAAGCGCCTGATCTATGCTGCATCCAGTTT  
 25 GCAAAGTGGGGTCCCATCAAGGTT CAGCGGCAGTGGATCTGGGACAGAATTCA  
 CTCTACAATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCAAC  
 AGTATAATAGTTACCCGCTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAA3  
 ,

**30 SEQ ID NO: 36****Antibody 5A5 = 4A10 Amino acid sequence of light chain variable region**

DIQLTQSPSSLSASVGDRV TITCRASQGIRSDLGWYQQRPGKTPKR LIYAASSLQSG  
 VPSRFSGSGSGTEFTLTIS SLQPEDFATYYCQQYNSYPLTFGGG TKVEIK

**SEQ ID NO: 37****Antibody 6B5 = 5C12.1 Nucleotide sequence of heavy chain variable region**

5' CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAG  
5 TGAAGGTCTCCTGCAAGGCTTCTGGATACACCTTCACCGGCTACTATATGCACT  
GGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCAACCCT  
AACAGTGGTGGCACA AACTATGCACAGAAGTTTCAGGGCAGGGTCACCATGAC  
CAGGGACACGTCCATCAGCACAGCCTACATGGAGCTGAGCAGGCTGAGATCTG  
ACGACACGGCCGTGTATTACTGTGCGAGAGGGCCCCATACCTTTGGTTCGGGGA  
10 GTTACCCCTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA3'

**SEQ ID NO: 38****Antibody 6B5 = 5C12.1 Amino acid sequence of heavy chain variable region**

15 QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYMHVVRQAPGQGLEWMGWINP  
NSGGTNYAQKFQGRVTMTRDTSISTAYMELSR LRSDDTAVYYCARGPHTFGSGSY  
PFDYWGQGTLVTVSS

**SEQ ID NO: 39****Antibody 6B5 = 5C12.1 Nucleotide sequence of light chain variable region**

5' GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAG  
AGTCACCATCACTTGCCGGGCAAGTCAGGGCATTAGAAGTGATTTAGGCTGGTA  
25 TCAGCAGAGACCAGGGAAAACCCCTAAGCGCCTGATCTATGCTGCATCCAGTTT  
GCAAAGTGGGGTCCCATCAAGGTT CAGCGGCAGTGGATCTGGGACAGAATTCA  
CTCTCACAATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCAAC  
AGTATAATAGTTACCCGCTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAA3  
,

30

**SEQ ID NO: 40****Antibody 6B5 = 5C12.1 Amino acid sequence of light chain variable region**

35 DIQMTQSPSSLSASVGDRVTITCRASQGIRSDLGWYQQRPGKTPKRLIYAASSLQSG  
VPSRFSGSGSGTEFTLTISLQPEDFATYYCQYNSYPLTFGGGTKVEIK

**SEQ ID NO: 41****Antibody 1B5 Nucleotide sequence of heavy chain variable region**

5 5'CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCC  
TGAGACTCTCCTGTGCAGCGTCTGGATTACCTTCAGTAGCTATGGCATGCACT  
GGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATGGTAT  
GATGGAAGTAATAAATACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCC  
AGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGA  
10 GGACACGGCTGTGTATTACTGTGCGAGAGGGGACTTTTACTGGTTCGACCCCTG  
GGGCCAGGGAACCCTGGTCACCGTCTCCTCA3'

**SEQ ID NO: 42****Antibody 1B5 Amino acid sequence of heavy chain variable region**

15 QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWYD  
GSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGDFYWFDPWG  
QGTLVTVSS

20

**SEQ ID NO: 43****Antibody 1B5 Nucleotide sequence of light chain variable region**

25 5'GACATCCAGATGACCCAGTCTCCATCTTCCCTGTCTGCATCTGTAGGAGACAG  
AGTCACCATCACTTGCCGGGCAAGTCAGGGCATTAGAAGTGATTTAGGCTGGTA  
TCAGCAGAGACCAGGGAAAACCCCTAAGCGCCTGATCTATGCTGCATCCAGTTT  
GCAAAGTGGGGTCCCATCAAGGTTCAAGCGGCAGTGGATCTGGGACAGAATTCA  
CTCTCACAATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCAAC  
AGTATAATAGTTACCCGCTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAA3  
30 '

**SEQ ID NO: 44****Antibody 1B5 Amino acid sequence of light chain variable region**

35 DIQMTQSPSSLSASVGDRVTITCRASQGIRSDLGWYQQRPGKTPKRLIYAASSLQSG  
VPSRFSGSGSGTEFTLTISLQPEDFATYYCQYNSYPLTFGGGTKVEIK



**SEQ ID NO: 45****Antibody 2B10.1 = 2B10.2 = 2B10.3 Nucleotide sequence of heavy chain variable region**

5 5'CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAG  
TGAAGGTCTCCTGCAAGGCTTCTGGATACACCTTCACCGGCTACTATATGCACT  
GGGTGCGACAGGCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCAACCCT  
AACAGTGGTGGCACAACTATGCACAGAAGTTTCAGAGCAGGGTCACCATGAC  
CAGGGACACGTCCATCAGCACAGCCTACATGGAGCTGAGCAGGCTGAGATCTG  
10 ACGACACGGCCGTGTATTACTGTGCGGGGGGGATGGGGGACGTCTGGGGCCAA  
GGGACCACGGTCACCGTCTCCTCA3'

**SEQ ID NO: 46****Antibody 2B10.1 = 2B10.2 = 2B10.3 Amino acid sequence of heavy chain variable region**

15 QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINP  
NSGGTNYAQKFQSRVTMTRDTSISTAYMELSRRLRSDDTAVYYCAGGMGDVWGQG  
TTVTVSS  
20

**SEQ ID NO: 47****Antibody 2B10.1 = 2B10.2 = 2B10.3 Nucleotide sequence of light chain variable region**

25 5'GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAG  
AGTCACCATCACTTGCCGGGCAAGTCAGGGGCATTAGAAATGATTTAGGCTGGTA  
TCAGCAGAAACCAGGGAAAGCCCCCTAAGCGCCTGATCTATGCTGCATCCAGTTT  
GCAAAGTGGGGTCCCATCAAGGTTTCAGCGGCAGTGGATCTGGGACAGAATTCA  
30 CTCTCACAATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCTAC  
AGTATAATAGTTACCCGTGGACGTTTCGGCCAAGGGACCAAGGTGGAAATCAAA  
3'

**SEQ ID NO: 48****Antibody 2B10.1 = 2B10.2 = 2B10.3 Amino acid sequence of light chain variable region**

35 DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSG  
VPSRFSGSGSGTEFTLTISLQPEDFATYYCLQYNSYPWTFGQGTKVEIK

**SEQ ID NO: 49****Antibody 2B10.4 Nucleotide sequence of heavy chain variable region**

5 5'CAGGTGCAGCTGGTGGAGTCCGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCC  
TGAGACTCTCCTGTGCAGCGTCTGGATTACCTTCAGTAGCTATGGCATGCACT  
GGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATGGTAT  
GATGGAAGTAATAAATACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCC  
10 AGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGA  
GGACACGGCTGTGTATTACTGTGCGAGAGGGGACTTTTACTGGTTCGACCCCTG  
GGGCCAGGGAACCCTGGTCACCGTCTCCTCA3'

**SEQ ID NO: 50****Antibody 2B10.4 Amino acid sequence of heavy chain variable region**

15 QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWYD  
GSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGDFYWFDPWG  
QGTLVTVSS

20

**SEQ ID NO: 51****Antibody 2B10.4 Nucleotide sequence of light chain variable region**

25 5'GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAG  
AGTCACCATCACTTGCCGGGCAAGTCAGGGCATTAGAAATGATTTAGGCTGGTA  
TCAGCAGAAACCAGGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCAGTTT  
GCAAAGTGGGGTCCCATCAAGGTTCAGCGGCAGTGGATCTGGGACAGAATTCA  
CTCTCACAATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCTAC  
30 AGTATAATAGTTACCCGTGGACGTTCCGGCCAAGGGACCAAGGTGGAAATCAAA  
3'

**SEQ ID NO: 52****Antibody 2B10.4 Amino acid sequence of light chain variable region**

35 DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSG  
VPSRFSGSGSGTEFTLTISSLQPEDFATYYCLQYNSYPWTFGQGTKVEIK

**SEQ ID NO: 53****Antibody 6B1 Nucleotide sequence of heavy chain variable region**

5  
5'CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGACGTCCC  
TGAGACTCTCCTGTGCAGCGTCTGGATTACCTTCAGTAGCTATGGCATACT  
GGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATGGTAT  
GATGGAAGTAATAAATTCCATGCAGACTCCGTGAAGGGCCGATTCACCATCTCC  
10 AGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGA  
GGACACGGCTGTGTATTACTGTGCGAGAGACCCACTAGGATATTGTAGTAGTAC  
CAGCTGCTCTTACTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTC  
A3'

**15 SEQ ID NO: 54****Antibody 6B1 Amino acid sequence of heavy chain variable region**

QVQLVESGGGVVQPGTSLRLSCAASGFTFSSYGIHWVRQAPGKGLEWVAVIWDYD  
SNKFHADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARDPLGYCSSTSCSY  
20 FDYWGQGT LTVSS

**SEQ ID NO: 55****25 Antibody 6B1 Nucleotide sequence of light chain variable region**

5'GACATCCAGATGACCCAGTCTCCATCTTCCGTGTCTGCATCTGTAGGAGACAG  
AGTCACCATCACTTGTCGGGCGAGTCAGGGTATTAGCAACTGGTTAGCCTGGTA  
TCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTT  
30 GCAAAGTGGGGTCCCATCAAGGTTTCAGCGGCAGTGGATCTGGGACAGATTTCA  
CTCTCACCATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTACTATTGTCAAC  
AGGCTAACAATTTCCCGCTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAA3  
,

**35 SEQ ID NO: 56****Antibody 6B1 Amino acid sequence of light chain variable region**

DIQMTQSPSSVSASVGDRVTITCRASQGISNWLAWYQQKPGKAPKLLIYAASSLQS  
GVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCQQANNFPLTFGGGTKVEIK

**SEQ ID NO: 57****Antibody 3C7 Nucleotide sequence of heavy chain variable region**

5' CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGACGTCCC  
5 TGAGACTCTCCTGTGCAGCGTCTGGATTACCTTCAGTAGCTATGGCATACT  
GGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATGGTAT  
GATGGAAGTAATAAATTCTATGCAGACTCCGTGAAGGGCCGATTCACTATCTCC  
AGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGA  
GGACACGGCTGTGTATTACTGTGCGAGAGACCCACTAGGATATTGTAGTAGTAC  
10 CAGCTGCTCTTACTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTC  
A3'

**SEQ ID NO: 58****Antibody 3C7 Amino acid sequence of heavy chain variable region**

15 QVQLVESGGGVVQPGTSLRLSCAASGFTFSSYGIHWVRQAPGKGLEWVAVIWDG  
SNKFYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARDPLGYCSSTSCSY  
FDYWGQGT LTVSS

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**SEQ ID NO: 59****Antibody 3C7 Nucleotide sequence of light chain variable region**

5' GACATCCAGATGACCCAGTCTCCATCTTCCGTGTCTGCATCTGTAGGAGACAG  
25 AGTCACCATCACTTGTCGGGCGAGTCAGGGTATTAGCAACTGGTTAGCCTGGTA  
TCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTT  
GCAAAGTGGGGTCCCATCACGGTTCAGCGGCAGTGGATCTGGGACAGATTTCA  
CTCTCACCATCCGCAGCCTGCAGCCTGAAGATTTTGCAACTTACTATTGTCAAC  
AGGCTAACAATTTCCCGCTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAA3  
30 '

**SEQ ID NO: 60****Antibody 3C7 Amino acid sequence of light chain variable region**

35 DIQMTQSPSSVSASVGDRVITCRASQGISNWLAWYQQKPGKAPKLLIYAASSLQS  
GVPSRFSGSGSGTDFTLTIRSLQPEDFATYYCQQANNFPLTFGGGTKVEIK

**SEQ ID NO: 61****Antibody 4D4.1 = 4D4.2 = 4D4.3 Nucleotide sequence of heavy chain variable region**

5' CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCC  
5 TGAGACTCTCCTGTGCAGCGTCTGGATTACCTTCAGTAGCTATGGCATGCACT  
GGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATGGTAT  
GATGGAAGTAATAAATACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCC  
AGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGA  
GGACACGGCTGTGTATTACTGTGCGAGAGGAGGGGACGGTGAACGGTTTGACT  
10 ACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA3'

**SEQ ID NO: 62****Antibody 4D4.1 = 4D4.2 = 4D4.3 Amino acid sequence of heavy chain variable region**

15 QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWYD  
GSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGGDGERFDYW  
GQGLTVTVSS

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**SEQ ID NO: 63****Antibody 4D4.1 = 4D4.2 = 4D4.3 Nucleotide sequence of light chain variable region**

5' GATGTTGTGATGACTCAGTCTCCACTCTCCCTGCCCCGTCACCCTTGGACAGCC  
25 GGCCTCCATCTCCTGCAGGTCTAGTCAAAGCCGCGTATACAGTGATGGAAACAC  
CTTCTTGAATTGGTTTCAGCAGAGGCCAGGCCAATTTCCAAGGCGCCTAATTTA  
TAAGGTTTCCA ACTGGGACTCTGGGGTCCCAGACAGATTCAGCGGCAGTGGGTC  
AGGCACTGATTT CACACTGAAAATCAGCAGGGTGGAGGCTGAGGATGTTGGGG  
TTTATTACTGCATGCAAGGTACACACTGGCCTCCTTATGTGCAGTTTTGGCCAGG  
30 GGACCAAGCTGGAGATCAA3'

**SEQ ID NO: 64****Antibody 4D4.1 = 4D4.2 = 4D4.3 Amino acid sequence of light chain variable region**

35 DVVMTQSPLSLPVTLGQPASISCRSSQSRVYSDGNTFLNWFQQRPGQFPRRLIYKVS  
NWD SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQGT HWPPYVQFWPGDQA  
GDQ

**SEQ ID NO: 65****Antibody 5E10.1 = 5E10.2 Nucleotide sequence of heavy chain variable region**

5' CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCC  
5 TGAGACTCTCCTGTGCAGCGTCTGGATTACCTTCAGAAAGCTATGGCATGCACT  
GGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATAAGGCAT  
GATGGAAGTAATAAATATAATGCAGACTCCGTGAAGGGCCGATTCACCATCTCC  
AGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGTCTGAGAGCCGA  
GGACACGGCTGTGTATTACTGTGCGAGAGACCCACTAGGATATTGTAGTAGTAC  
10 CAGCTGCTCTTACTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTC  
A3'

**SEQ ID NO: 66****15 Antibody 5E10.1 = 5E10.2 Amino acid sequence of heavy chain variable region**

QVQLVESGGGVVQPGRSLRLSCAASGFTFRSYGMHWVRQAPGKGLEWVAVIRHD  
GSNKYNADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARDPLGYCSSTSCS  
YFDYWGQGTLVTVSS  
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**SEQ ID NO: 67****Antibody 5E10.1 = 5E10.2 Nucleotide sequence of light chain variable region**

25 5' GACATCCAGATGACCCAGTCTCCATCTTCCGTGTCTGCATCTGTAGGAGACAG  
AGTCACCATCACTTGTCTGGGCGAGTCAGGGTATTAGCAGCTGGTTAGCCTGGTA  
TCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTACATCCAGTTT  
GCAAAGTGGGGTCCCATCAAGGTTTCAGCGGCAGTGGATCTGGGACAGATTTCA  
CTCTCACCATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTACTATTGTCAAC  
30 AGGCTAACAATTTCCCGCTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAA3  
,

**SEQ ID NO: 68****Antibody 5E10.1 = 5E10.2 Amino acid sequence of light chain variable region**

35 DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPGKAPKLLIYATSSLQSG  
VPSRFSGSGSGTDFTLTISLQPEDFATYYCQQANFPLTFGGGTKVEIK

**SEQ ID NO: 69****Antibody 3H12 Nucleotide sequence of heavy chain variable region**

5 5'CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCC  
 TGTCCTTCACCTGCACTGTCTCTGATGGCTCCATCAGTAGTTTCTACTGGAGCTG  
 GATCCGGCAGCCCCCAGGGAAGGGACTGGAGTGGATTGGGTATATCTATTACA  
 GTGGGAGCACCAACTACAACCCCTCCCTCAAGAGTCGAGTCACCATATCAGTAG  
 ACACGTCCAAGAACCAGTTCTCCCTGAAGCTGAGCTCTGTGACCGCTGCGGACA  
 10 CGGCCGTGTATTACTGTGCGAGAGATTACGATATCTTGACTGGTTATTCCAAC  
 TACTACGGTATGGACGTCTGGGGCCAAGGGACACGGTCACCGTCTCCTCA3'

**SEQ ID NO: 70****Antibody 3H12 Amino acid sequence of heavy chain variable region**

15 QVQLQESGPGLVKPSETLSLTCTVSDGSISSFYWSWIRQPPGKGLEWIGYIYYSGST  
 NYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARDYDILTGYSNYYGMDV  
 WGQGTTVTVSS

**SEQ ID NO: 71****Antibody 3H12 Nucleotide sequence of light chain variable region**

5'GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAG  
 AGTCACCATCACTTGCCGGGCAAGTCAGGGCATTAGAAATGATTTAGGCTGGTA  
 25 TCAGCAGAAACCAGGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCAGTTT  
 GCAAAGTGGGGTCCCATCAAGGTTCAAGCGGCAGTGGATCTGGGACAGAATTCA  
 CTCTCACAATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCTGC  
 AGCATAATAGTTACCCATTCACTTTCGGCCCTGGGACCAAACCTGGATATCAAA3'

**SEQ ID NO: 72****Antibody 3H12 Amino acid sequence of light chain variable region**

DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSG  
 VPSRFSGSGSGTEFTLTISLQPEDFATYYCLQHNSYPFTFGPGTKLDIK

**SEQ ID NO: 73****Antibody 1G3 Nucleotide sequence of heavy chain variable region**

5' CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAG  
5 TGAAGGTCTCCTGCAAGGCTTCTGGATACACCTTCACCGGCTACTATATGCACT  
GGGTGCGACAGGCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCAACCCT  
AACAGTGGTGGCACA AACTATGCACAGAAGTTTCAGGGCAGGGTCACCATGAC  
CAGGGACACGTCCATCAGCACAGCCTACATGGAGCTGAGCAGGCTGAGATCTG  
ACGACACGGCCGTGTTTTACTGTGCGGGTGGGACCTACTTGGACTACTGGGGCC  
10 AGGGAACCCTGGTCACCGTCTCCTCA3'

**SEQ ID NO: 74****Antibody 1G3 Amino acid sequence of heavy chain variable region**

15 QVQLVQSGAEVKKPGASVKV SCKASGYTFTGYMHVVRQAPGQGLEWMGWINP  
NSGGTNYAQKFQGRVTMTRDTSISTAYMELSR LRSDDTAVFYCAGGT YLDYWGQ  
GTLVTVSS

**SEQ ID NO: 75****Antibody 1G3 Nucleotide sequence of light chain variable region**

5' GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAG  
AGTCACCATCACTTGCCGGGCAAGTCAGGGCATTAGAAATGATTTAGGCTGGTA  
25 TCAGCAGAAACCAGGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCAGTTT  
GCAAAGTGGGGTCCCATCAAGGTT CAGCGGCAGTGGATCTGGGACAGAATTCA  
CTCTCACAATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCTAC  
AGTATAATAGTTACCCGATCACCTTCGGCCAAGGGACACGACTGGAGATTAAA3  
,

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**SEQ ID NO: 76****Antibody 1G3 Amino acid sequence of light chain variable region**

DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSG  
35 VPSRFSGSGSGTEFTLTISSLQPEDFATYYCLQYNSYPITFGQGTRLEIK



**SEQ ID NO: 77****Antibody 5D1.1 Nucleotide sequence of heavy chain variable region**

5 5'CAGGTTTCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAGCCTGGGGCCTCAG  
TGAAGGTCTCCTGCAAGGCTTCTGGTTACACCTTTACCAGCTATGGTATCAGCT  
GGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCAGCGCT  
TATAATGGTAACACAACTATGCACAGAAGCTCCAGGGCAGAGGCACCATGGC  
CACAGACACATCCACGAGCACAGCCTACATGGAGCTGAGGAGCCTGAGATCTG  
10 ACGACACGGCCGTGTATTACTGTGCGGTTGGGAGGTACCTTGACTACTGGGGCC  
AGGGAACCCTGGTCACCGTCTCCTCA3'

**SEQ ID NO: 78****Antibody 5D1.1 Amino acid sequence of heavy chain variable region**

15 QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISAY  
NGNTNYAQKLQGRGTMTDTSTSTAYMELRSLRSDDTAVYYCAVGRYLDYWGQ  
GTLVTVSS

**SEQ ID NO: 79****Antibody 5D1.1 Nucleotide sequence of light chain variable region**

5'GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAG  
AGTCACCATCACTTGCCGGGCAAGTCAGGACATTAGAAATGATTTAGGCTGGTA  
25 TCAGCAGAAACCAGGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCAGTTT  
GCAAAGTGGGGTCCCATCAAGGTTTCAGCGGCAGTGGATCTGGGACAGAATTCA  
CTCTCACAATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCTAC  
AGTATAATAGTTACCCTCTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAA3  
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**SEQ ID NO: 80****Antibody 5D1.1 Amino acid sequence of light chain variable region**

DIQMTQSPSSLSASVGDRVTITCRASQDIRNDLGWYQQKPGKAPKRLIYAASSLQSG  
35 VPSRFSGSGSGTEFTLTISLQPEDFATYYCLQYNSYPLTFGGGTKVEIK

**SEQ ID NO: 81****Antibody 5D1.3 Nucleotide sequence of heavy chain variable region**

5 5'CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCC  
TGAGACTCTCCTGTGCAGCGTCTGGATTACCTTCAGTAGCTATGGCATGCACT  
GGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATGGTAT  
GATGGAAGTAATAAATACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCC  
AGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGA  
10 GGACACGGCTGTGTATTACTGTGCGAGAGGGGACTTTTACTGGTTCGACCCCTG  
GGGCCAGGGAACCCTGGTCACCGTCTCCTCA3'

**SEQ ID NO: 82****Antibody 5D1.3 Amino acid sequence of heavy chain variable region**

15 QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWYD  
GSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGDFYWFDPWG  
QGTLVTVSS

**SEQ ID NO: 83****Antibody 5D1.3 Nucleotide sequence of light chain variable region**

5'GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAG  
AGTCACCATCACTTGCCGGGCAAGTCAGGACATTAGAAATGATTTAGGCTGGTA  
25 TCAGCAGAAACCAGGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCAGTTT  
GCAAAGTGGGGTCCCATCAAGGTTCAGCGGCAGTGGATCTGGGACAGAATTCA  
CTCTCACAATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCTAC  
AGTATAATAGTTACCCTCTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAA3  
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**SEQ ID NO: 84****Antibody 5D.3 Amino acid sequence of light chain variable region**

DIQMTQSPSSLSASVGDRVTITCRASQDIRNDLGWYQQKPGKAPKRLIYAASSLQSG  
35 VPSRFSGSGSGTEFTLTISLQPEDFATYYCLQYNSYPLTFGGGTKVEIK

**SEQ ID NO: 85****Antibody 5D1.4 Nucleotide sequence of heavy chain variable region**

5 5'CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAG  
TGAAGGTCTCCTGCAAGGCTTCTGGATACACCTTCACCGGCTACTATATGCACT  
GGGTGCGACAGGCCCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCAACCCT  
AACAGTGGTGGCACAACCTATGCACAGAAGTTTCAGGGCAGGGTCACCATGAC  
CAGGGACACGTCCATCAGCACAGCCTACATGGAGCTGAGCAGGCTGAGATCTG  
10 ACGACACGGCCGTGTATTACTGTGCGAGAGGGCCCCATACCTTTGGTTCGGGGA  
GTTACCCCTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA3'

**SEQ ID NO: 86****Antibody 5D1.4 Amino acid sequence of heavy chain variable region**

15 QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINP  
NSGGTNYAQKFQGRVTMTRDTSISTAYMELSRRLRSDDTAVYYCARGPHTFGSGSY  
PFDYWGQGTLVTVSS

**SEQ ID NO: 87****Antibody 5D1.4 Nucleotide sequence of light chain variable region**

5'GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAG  
AGTCACCATCACTTGCCGGGCAAGTCAGGACATTAGAAATGATTTAGGCTGGTA  
25 TCAGCAGAAACCAGGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCAGTTT  
GCAAAGTGGGGTCCCATCAAGGTTCAAGCGGCAGTGGATCTGGGACAGAATTCA  
CTCTCACAATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCTAC  
AGTATAATAGTTACCCTCTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAA3  
,

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**SEQ ID NO: 88****Antibody 5D1.4 Amino acid sequence of light chain variable region**

DIQMTQSPSSLSASVGDRVTITCRASQDIRNDLGWYQQKPGKAPKRLIYAASSLQSG  
35 VPSRFSGSGSGTEFTLTISLQPEDFATYYCLQYNSYPLTFGGGTKVEIK

**SEQ ID NO: 89****Antibody 5D1.5 Nucleotide sequence of heavy chain variable region**

5 5'CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAG  
 TGAAGGTCTCCTGCAAGGCTTCTGGATACACCTTCACCGGCTACTATATGCACT  
 GGGTGCAGACAGGCCCCCGGACAAGGGCTTGAGTGGATGGGATGGATCAACCCT  
 AACAGTGGTGGCACAACACTATGCACAGAAGTTTCAGGGCAGGGTCACCATGAC  
 CAGGGACACGTCCATCAGCACAGCCTACATGGAGCTGAGCAGGCTGAGATCTG  
 10 ACGACACGGCCGTGTTTTACTGTGCGGGTGGGACCTACTTGGACTACTGGGGCC  
 AGGGAACCCTGGTCACCGTCTCCTCA3'

**SEQ ID NO: 90****Antibody 5D1.5 Amino acid sequence of heavy chain variable region**

15 QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINP  
 NSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLSDDTAVFYCAGGTYL DYWGQ  
 GTLVTVSS

**SEQ ID NO: 91****Antibody 5D1.5 Nucleotide sequence of light chain variable region**

5'GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAG  
 AGTCACCATCACTTGCCGGGCAAGTCAGGACATTAGAAATGATTTAGGCTGGTA  
 25 TCAGCAGAAACCAGGGAAAGCCCCTAAGCGCCTGATCTATGCTGCATCCAGTTT  
 GCAAAGTGGGGTCCCATCAAGGTTTCAGCGGCAGTGGATCTGGGACAGAATTCA  
 CTCTCACAATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCTAC  
 AGTATAATAGTTACCCTCTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAA3  
 ,

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**SEQ ID NO: 92****Antibody 5D1.5 Amino acid sequence of light chain variable region**

DIQMTQSPSSLSASVGDRVTITCRASQDIRNDLGWYQQKPGKAPKRLIYAASSLQSG  
 35 VPSRFSGSGSGTEFTLTISSLQPEDFATYYCLQYNSYPLTFGGGTKVEIK

**SEQ ID NO: 93****SARS CoV S protein nucleotide sequence**

atgtttattttcttattatttcttactctcactagtggtagtgaccttgaccgggtgcacc  
 40 acttttgatgatgttcaagctcctaattacactcaacatactcatctatgaggggggtt  
 tactatcctgatgaaatttttagatcagacactcttatttaactcaggatttatttctt  
 ccattttattctaattgttacaggggttcatactattaatcatacgtttgacgaccctgtc  
 ataccttttaaggatggtatttttctgctgccacagagaaatcaaattgtgtccgtggt  
 tgggtttttggttctaccatgaacaacaagtcacagtcggtgattattattaacaattct  
 45 actaatgttggtatacagcatgtaactttgaattgtgtgacaacctttctttgtgtt

tctaaacccatgggtacacggacacatactatgatattcgataatgcatttaattgcact  
 ttcgagtacatactgatgccttttcgcttgatgtttcagaaaagtcaggtaattttaaa  
 cacttacgagagtttggtttaaaaataaagatgggtttctctatgtttataagggctat  
 caacctatagatgtagtctgatctaccttctggtttaacactttgaaacctatttt  
 5 aagttgcctcttggtattaacattacaaattttagagccattcttacagcctttcacct  
 gctcaagacacttggggcacgtcagctgcagcctattttgttggtatttaagccaact  
 acatttatgctcaagtatgatgaaaatggtacaatcacagatgctgttgattgttctcaa  
 aatccacttgctgaactcaaagctctgtaagagctttgagattgacaaaggaatttac  
 cagacctctaatttcagggtgttccctcaggagatgttgtagattccctaataattaca  
 10 aactgtgtccttttgagaggttttaagtactaaattcccttctgtctatgcatgg  
 gagaggaaaagaatttctaattgtgttgctgattactctgtgctctacaactcaacatct  
 tttcaacctttaagtctatggcgtttctgccactaagttgaatgatctttgcttctcc  
 aatgtctatgcagattcttttgtagtcaaggagatgatgtaagacaaatagcgccagga  
 caaactggtgttattgctgattataaattataaattgccagatgattcatgggtgtgtc  
 15 cttgcttggactaggaacattgatgctacttcaactggttaattataaattataaata  
 aggtatcttagacatggcaagcttaggccctttgagagagacatactaatgtgccttc  
 tctcctgatggcaaaccttgacccccacctgctcctaattgttattggccattaaatggt  
 tatggttttacaccactagtggcattggctaccaaccttacagagttgtagtactttct  
 tttgaacttttaaatgcaccggccacggtttgtggacaaaattatccactgaccttatt  
 20 aagaaccagtgtgtcaatttttaatttaaggactcactggtactggtgtgttaactcct  
 tcttcaaagagatttcaaccatttcaacaatttgccgtgatgtttctgatttactgat  
 tccgttcgagatcctaaaacatctgaaatatttagacattcaccttgccttttgggggt  
 gtaagtgttaattacacctggaacaaatgcttcatctgaagttgctgttctatatcaagat  
 gttactgcactgatgtttctacattaattcatgcagaacaaactcacaccagcttggcgc  
 25 atatattctactgaaacaaatgtattccagactcaagcaggctgtcttataggagctgag  
 catgtcgacacttcttagtgctgacattcctattggagctggcatttgtgctagtac  
 catacagtttcttactacgtagtagtagccaaaaatctatttggttatactatgtct  
 ttaggtgctgatagtcaattgcttactctaataacaccattgctatacctactaacttt  
 tcaatttagcattactacagaagtaatgcctgtttctatggctaaaacctccgtagattgt  
 30 aatatgtacatctgcggagattctactgaatgtgctaatttgcttctccaatatggtagc  
 ttttgcagacaactaaatcgtgcactctcaggtattgctgctgaacaggatcgcaacaca  
 cgtgaagtgttcgttcaagtcaacaaatgtacaaaacccaactttgaaagattttggt  
 ggttttaattttcacaaatattacctgacctctaaagccaactaagaggtctttatt  
 gaggacttgccttttaataaggtgacactcgtgatgctggcttcatgaagcaatatggc  
 35 gaatgcctagggtgatattaatgctagagatctcatttgtgcgcagaagttcaatggactt  
 acagtgttgccacctctgctcactgatgatattgctgcctacactgctgctctagt  
 agtggfactgccactgctggatggacatttgggtgctggcgtgctcttcaaatacctttt  
 gctatgcaaatggcatatagggtcaatggcattggagttacccaaaatgttctctatgag  
 aacaaaaacaaatcgccaaccaatttaacaaggcgattagtcaaattcaagaatcactt  
 40 acaacaacatcaactgcattgggcaagctgcaagacgttgtaaccagaatgctcaagca  
 ttaaacacacttgttaacaacttagctctaattttggtgcaatttcaagtgtgctaaat  
 gatattccttcgcgacttgataaagtcgagggcgaggtacaaattgacaggttaattaca  
 ggcagacttcaaagccttcaaacctatgtaacacaacaaactaatcagggtgctgaaatc  
 agggcttctgctaacttctgctgactaaaatgtctgagtgttcttggacaatcaaaa  
 45 agagttgacttttgcggaaagggtaccaccttatgcttctccacaagcagccccgcat  
 ggtgttcttctacatgtcacgtatgtgccatcccaggagaggaacttcaccacagcg  
 ccagcaatttgcataaggaagcatactccctcgtgaaggtgttttgtttaa  
 ggcacttcttgggttattacacaggaacttcttctccacaaataattactacagac

aatacattgtctcaggaaattgtgatgtcgttattggcatcattaacaacacagtttat  
 gatcctctgcaacctgagcttgactcattcaagaagagctggacaagtacttcaaaaat  
 catacatcaccagatgttgatcttggcgacatttcaggcattaacgcttctgtcgtcaac  
 attcaagaagaaattgaccgcctcaatgaggtcgctaaaaattaaatgaatcactcatc  
 5 gaccttcaagaattgggaaaatatgagcaatatattaaatggccttggtatgtttggctc  
 ggcttcattgctggactaattgccatcgatggttacaatcttgcttggatgact  
 agttgttgcaagtgcccaagggtgcatgctctgtggttcttgctgcaagttgatgag  
 gatgactctgagccagttctcaagggtgtcaaattacattacacataa

# 10 SEQ ID NO: 94

## SARS CoV S Protein amino acid sequence

MFIFLLFLTLTSGSDLDRCTTFDDVQAPNYTQHTSSMRGVYYPDEIFRSDTLYLTD  
 LFL  
 PFYSNVTGFHTINHTFDDPVIPFKDGIYFAATEKSNVVRGWVFGSTMNNKSQSVIIIN  
 15 NS  
 TNVVIRACNFELCDNPFFVVS KPMGTRTHTMIFDNAFNCTFEYISDAFSLDVSEKSG  
 NFK  
 HLREFVFKNKDGFLYVYKGYQPIDVVRDLPSGFNTLKPFIKLP LGINITNFRAILTA  
 SP  
 20 AQDTWGTSAAYFVGYLKPTTFMLKYDENGITITDAVDCSQNPLAELKCSVKSF  
 EID  
 KGIYQTSNFRVVP SGDVVRFPNITNLCPFGEVFNATKFPSVYAWERKRISNCVADYS  
 VLYNSTSFSTFKCYGVSATKLNDLCFSNVYADSFVVKGDDVRQIAPGQTGVIADYN  
 YKLPDDFMGCVLAWNTRNIDATSTGNVNYKYRYLRH GKLRPFERDISNVPFSPDG  
 KPCTPPAPNCYWPLNGYGFYTTSGIGYQPYRVVLSFELLNAPATVCGPKLSTDLIK  
 25 NQCVNFNFNGLTGTGVLTPSSKRFQPFQFGRDVSDFDTSVRDPKTSEILDISPC  
 SFG  
 GVSVITPGTNASSEVAVLYQDVNCTDVSTLIHAEQLTPAWRIYSTGNNVFQTQAGC  
 LIGAEHVDTSYECDIPGAGICASYHTVSSLRSTSQKSIVAYTMSLGADSSIAYSNNTI  
 AIPTNFSISITTEVMPVSMAKTSVDCNMYICGDSTECANLLLQYGSFCRQLNRALSGI  
 AAQDRNTREVFVQVKQMYKTPTLKDFFGFFNSQILPDPLKPTKRSFIEDLLFNKVT  
 30 LADAGFMKQYGECLGDINARDLCAQKFNGLTVLPLLTD DMIAAYTAALVSGTA  
 TAGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKQIANQFNKAISQIQESL  
 TTTSTALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQID  
 RLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLM  
 SFPQAAPHGVVFLHVITYVPSQERNFTTAPAICHEGKAYFPREGVVFVNGTSWFITQR  
 35 NFFSPQIITDNTFVSGNCDVVIGIINNTVYDPLQPELDSFKEELDKYFKNHTSPD  
 VD  
 LGDISGINASVVNIQEEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYVWLGFIA  
 GLIAIVMTILLCCMTSCCSCLKGACSCGSCCKFDEDDSEPV LKGVKLHYT

# SEQ ID NO: 95

## 40 SARS CoV S protein 12-261 amino acid sequence

SGSDLDRCTTFDDVQAPNYTQHTSSMRGVYYPDEIFRSDTLYLTDLFLPFYSNVT  
 GFHTINHTFDDPVIPFKDGIYFAATEKSNVVRGWVFGSTMNNKSQSVIIINNSTNVVI  
 RACNFELCDNPFFVVS KPMGTRTHTMIFDNAFNCTFEYISDAFSLDVSEKSGNFKHL

REFVFKNKDGFLYVYKGYQPIDVVRDLPSGFNTLKPIFKLPLGINITNFRAILTAFSP  
AQDTWGTSAAYFVGYLKPTT

**SEQ ID NO: 96****5 SARS CoV S Protein 318-510 amino acid sequence**

NITNLCPFGEVFNATKFPSVYAWERKRISNCVADYSVLYNSTSFSTFKCYGVSATKL  
NDLCFSNVYADSFVVKGDDVRQIAPGQTGVIADYNYKLPDDFMGCVLAWNTRNID  
ATSTGNYNYKYRYLRHGKLRPFERDISNVPFSPDGKPCPPAPNCYWPLNGYGFYT  
TSGIGYQPYRVVLSFELLNAPATV

10

**SEQ ID NO: 97****SARS CoV S Protein 15-680 amino acid sequence**

DLDRCTTFDDVQAPNYTQHTSSMRGVYYPDEIFRSDTLYLTQDLFLPFYSNVTGFH  
TINHTFDDPVIPFKDGIYFAATEKSNVVRGWVFGSTMNNKSQSVIIINNSTNVVIRAC  
15 NFELCDNPFFVVS KPMGTRTHMIFDNAFNCTFEYISDAFSLDVSEKSGNFKHLREF  
VFKNKDGFLYVYKGYQPIDVVRDLPSGFNTLKPIFKLPLGINITNFRAILTAFSPAQD  
TWGTSAAYFVGYLKPTTFMLKYDENGITITDAVDCSQNPLAELKCSVKSFEIDKGI  
YQTSNFRVVP SGDVVRFPNITNLCPFGEVFNATKFPSVYAWERKRISNCVADYSVL  
YNSTSFSTFKCYGVSATKLNDLCFSNVYADSFVVKGDDVRQIAPGQTGVIADYNYK  
20 LPDDFMGCVLAWNTRNIDATSTGNYNYKYRYLRHGKLRPFERDISNVPFSPDGKPC  
TPPAPNCYWPLNGYGFYTTS GIGYQPYRVVLSFELLNAPATVCGPKLSTD LIKNQ  
CVNFNFNGLTGTGVLTPSSKRFQPFQFGRDVSDFDTSVRDPKTSEILDISPCSFGGV  
SVITPGTNASSEVAVLYQDVNCTDVSTLIHAEQLTPAWRIYSTGNNVFQTQAGCLIG  
AEHVDTSYECDIPIGAGICASYHTVSSLRSTSQKSIVAYTMS

25

**SEQ ID NO: 98****SARS CoV S Protein 681-1255 amino acid sequence**

LGADSSIAYSNNTIAIPTNFSISITTEVMPVSMAKTSVDCNMYICGDSTECANLLLQY  
GSFCRQLNRALSGIAAEQDRNTREVFVQVKQMYKTPTLKDFGGFNFSQILPDPLKP  
30 TKRSFIEDLLFNKVT LADAGFMKQYGECLGDINARDLICAQKFNGLTVLPPLLTDD  
MIAAYTAALVSGTATAGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKQI  
ANQFNKAISQIQESLTTTSTALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDI

LSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQS  
KRVDFCGKGYHLMSFPQAAPHGVVFLHVTYVPSQERNFTTAPAICHEGKAYFPRE  
GVFVFNGTSWFITQRNFFSPQIITDNTFVSGNCDVVIGIINNTVYDPLQPELDSFKEE  
LDKYFKNHTSPDVDLGDISGINASVVNIQEEIDRLNEVAKNLNESLIDLQELGKYEQ  
5 YIKWPWYVWLGFIAGLIAIVMVTILLCCMTSCCCLKGACSCGSCCKFDEDDSEPV  
LKGVKLHYT



What is claimed is:

1. A neutralizing human monoclonal antibody or antigen-binding portion thereof that  
5 specifically binds to a region of human severe acute respiratory syndrome (SARS) Corona  
Virus (SARS-CoV) Spike (S) protein and blocks S protein binding to a receptor,  
wherein said region is selected from the group consisting of: amino acid residues 1-1255  
(SEQ ID NO: 94), a region that is at least 85% identical to SEQ ID NO: 94, amino acid  
residues 12-261 (SEQ ID NO: 95), a region that is at least 85% identical to SEQ ID NO: 95,  
10 amino acid residues 318-510 (SEQ ID NO: 96), a region that is at least 85% identical to  
SEQ ID NO: 96, amino acid residues 15-680 (SEQ ID NO: 97), and a region that is at least  
85% identical to SEQ ID NO: 97.
2. A human monoclonal antibody or antigen-binding portion that specifically binds to  
15 human severe acute respiratory syndrome (SARS) Corona Virus (SARS-CoV) S protein,  
wherein said antibody or antigen-binding portion neutralizes at least 50% of 200 times the  
tissue culture infectious dose (200xTCID<sub>50</sub>) of the virus at an antibody concentration of  
12.5 µg/ml or less.
- 20 3. The human monoclonal antibody or antigen-binding portion according to claim 1,  
wherein said antibody or antigen-binding portion comprises a heavy chain that utilizes a  
human VH 4-59 gene, a human VH 1-18 gene, a human VH 3-33 gene, or a human VH 1-2  
gene.
- 25 4. The human monoclonal antibody or an antigen-binding portion according to claim 1,  
wherein said antibody or antigen-binding portion comprises a light chain that utilizes a  
human VK A30 gene, a human VK L5 gene, or a human VK A1 gene.
5. A human monoclonal antibody or antigen-binding portion thereof that specifically  
30 binds SARS-CoV S protein comprising VL and VH domains that are at least 85% identical  
in amino acid sequence to the VL and VH domains, respectively, of a monoclonal antibody  
selected from the group consisting of: 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3,

2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2.

5 6. A human monoclonal antibody or antigen-binding portion thereof that specifically binds SARS-CoV S protein comprising:

(a) a heavy chain variable domain amino acid sequence that comprises the amino acid sequence of the heavy chain variable domain of an antibody selected from: 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 10 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2;

(b) a light chain variable domain amino acid sequence that comprises the amino acid sequence of the light chain variable domain of an antibody selected from: 1B5, 1G3, 2E8.1, 15 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2;

(c) a heavy chain variable domain of (a) and a light chain variable domain of (b); or

(d) heavy chain and light chain variable domain amino acid sequences comprising the heavy 20 chain and light chain variable domain amino acid sequences, respectively, from the same antibody selected from: 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2.

25 7. A monoclonal antibody or an antigen-binding portion thereof that specifically binds human SARS-CoV S protein comprising:

(a) a heavy chain variable domain amino acid sequence that comprises the heavy chain CDR1, CDR2 and CDR3 amino acid sequences of an antibody selected from: 1B5, 1G3, 30 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7,

5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2;

(b) a light chain variable domain amino acid sequence that comprises the light chain CDR1, CDR2 and CDR3 amino acid sequences of an antibody selected from: 1B5, 1G3, 2E8.1,

5 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2;

(c) a heavy chain variable domain of (a) and a light chain variable domain of (b); or

(d) the heavy chain variable domain and light chain variable domain of (c), comprising  
10 heavy chain and light chain CDR amino acid sequences from the same antibody selected from: 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2.

15

8. A monoclonal antibody or an antigen-binding portion thereof that specifically binds SARS-CoV S protein, wherein the antibody comprises FR1, FR2, FR3 and FR4 amino acid sequences from an antibody selected from: 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2.

20

9. A monoclonal antibody that specifically binds SARS-CoV S protein comprising:

(a) a heavy chain of an antibody selected from the group consisting of: 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2;

25

(b) a light chain of an antibody selected from the group consisting of: 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2; or

30

(c) a heavy chain and a light chain of the same antibody which is selected from the group consisting of: 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2.

10. A composition comprising at least one neutralizing human monoclonal antibody or antigen-binding portion thereof that specifically binds to a region of human SARS-CoV S protein,

10 wherein said region is selected from the group consisting of: amino acid residues 1-1255 (SEQ ID NO: 94), a region that is at least 85% identical to SEQ ID NO: 94, amino acid residues 12-261 (SEQ ID NO: 95), a region that is at least 85% identical to SEQ ID NO: 95, amino acid residues 318-510 (SEQ ID NO: 96), a region that is at least 85% identical to SEQ ID NO: 96, amino acid residues 15-680 (SEQ ID NO: 97), and a region that is at least 15 85% identical to SEQ ID NO: 97 and a pharmaceutically-acceptable carrier.

11. The composition according to claim 10, further comprising at least one additional therapeutic agent selected from the group consisting of:

(a) one or more antibodies or an antigen binding portion thereof, wherein said antibody is 20 from the group consisting of: 1B5, 1G3, 2E8.1, 2E8.2, 2B10.1, 2B10.2, 2B10.3, 2B10.4, 3A7, 3C7, 3F3, 3H12, 4E2, 4A10, 4D4.1, 4D4.2, 4D4.3, 4G2, 5E1.1.1, 5E1.1.2, 5E1.2, 5E1.3, 5E10.1, 5E10.2, 5E4, 5A5, 5A7, 5C12.1, 5C12.2, 5D1.1, 5D1.2, 5D1.3, 5D1.4, 5D1.5, 5D3, 5D6, 6B1, 6B5, 6B8, 6C1, and 6C2;

(b) one or more antibodies that specifically bind SARS-CoV S protein of a different SARS- 25 CoV strain;

(c) one or more SARS-CoV S protein neutralizing antibodies, wherein said antibodies do not bind SARS-CoV S protein;

(d) one or more agents that bind a SARS-CoV S protein receptor and blocks binding of S protein to the receptor; and

30 (e) one or more anti-viral agents.

12. The composition according to claim 10, wherein at least one additional SARS-CoV neutralizing human monoclonal antibody or antigen-binding portion thereof comprises at least two antibodies that specifically bind to different regions of human SARS-CoV S protein selected from the group consisting of: amino acid residues 1-1255 (SEQ ID NO: 94), a region that is at least 85% identical to SEQ ID NO: 94, amino acid residues 12-261 (SEQ ID NO: 95), a region that is at least 85% identical to SEQ ID NO: 95, amino acid residues 318-510 (SEQ ID NO: 96), a region that is at least 85% identical to SEQ ID NO: 96, amino acid residues 15-680 (SEQ ID NO: 97), and a region that is at least 85% identical to SEQ ID NO: 97.

13. An isolated cell line that produces (i) the antibody or antigen-binding portion according to claim 1; or (ii) the heavy chain or light chain of said antibody or antigen-binding portion.

14. An isolated nucleic acid molecule comprising a nucleotide sequence that encodes the heavy chain or an antigen-binding portion or the light chain or an antigen-binding portion thereof of an antibody according to claim 1.

15. A vector comprising the nucleic acid molecule according to claim 14, wherein the vector optionally comprises an expression control sequence operably linked to the nucleic acid molecule.

16. A host cell comprising the vector according to claim 15 or the nucleic acid molecule according to claim 14.

17. A non-human transgenic animal or transgenic plant comprising the nucleic acid according to claim 14, wherein the non-human transgenic animal or transgenic plant expresses said nucleic acid.

18. A method for isolating an antibody or antigen-binding portion thereof that specifically binds to human SARS-CoV S protein, comprising the step of isolating the antibody from the non-human transgenic animal or transgenic plant according to claim 17.

19. A method for producing a human monoclonal antibody according to claim 1 comprising the step of expressing the antibody in a host cell according to claim 16.

5 20. A method for decreasing S protein-mediated SARS-CoV binding to cells, the method comprising the step of contacting the S protein with the composition according to claim 10.

21. The method according to claim 20, wherein said cells express angiotensin  
10 converting enzyme 2 (Ace2).

22. A method for decreasing a SARS-CoV S protein-mediated activity, comprising contacting the S protein with the composition according to claim 10.

15 23. The method according to claim 22, wherein the SARS-CoV S protein-mediated activity is selected from: viral attachment to a cell, fusing of viral membrane with a cell, or combinations thereof.

24. The method according to claim 22, wherein the method is performed in a subject.  
20

25. A method for decreasing the SARS-CoV viral load in a subject in need thereof comprising the step of administering the composition according to claim 10.

26. A method for treating, preventing or alleviating the symptoms of a SARS-CoV-  
25 mediated disorder in a subject in need thereof, comprising the step of administering to said subject the composition according to claim 10.

27. The method according to claim 26, wherein the SARS-CoV-mediated disorder is severe acute respiratory syndrome (SARS).

30

28. A method for treating, preventing or alleviating the symptoms of a SARS-CoV-mediated disorder in a subject in need thereof, comprising the step of administering to said subject the composition according to claim 11.

- 5 29. A method for treating, preventing or alleviating the symptoms of a SARS-CoV-mediated disorder in a subject in need thereof, comprising the step of administering to said subject the composition according to claim 12.

Figure 1A

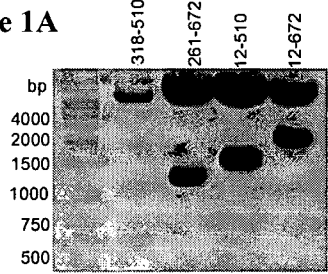
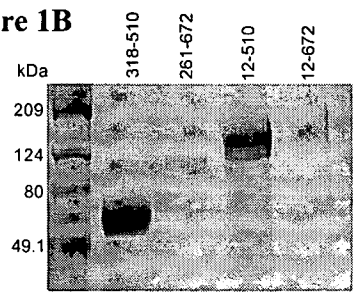
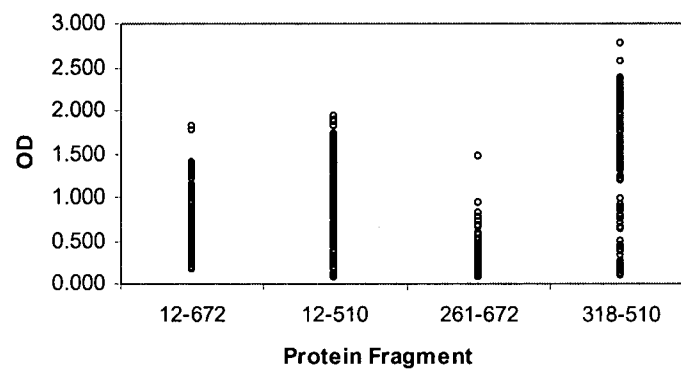


Figure 1B



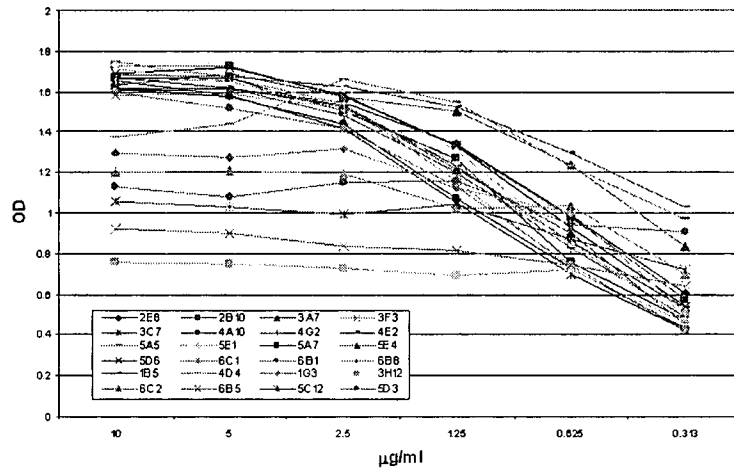


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**Figure 2**

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Figure 3

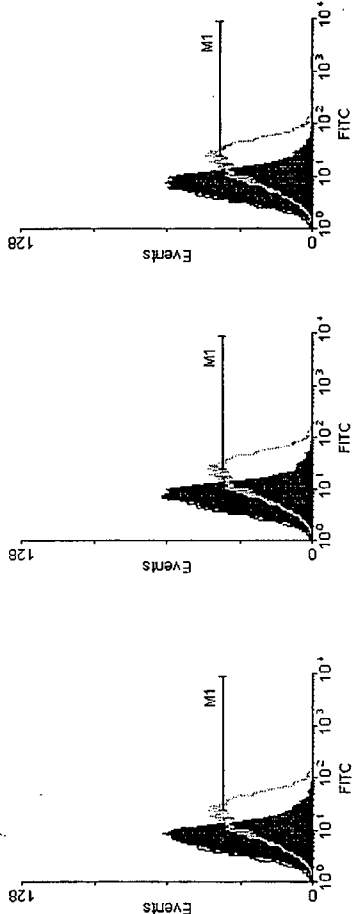
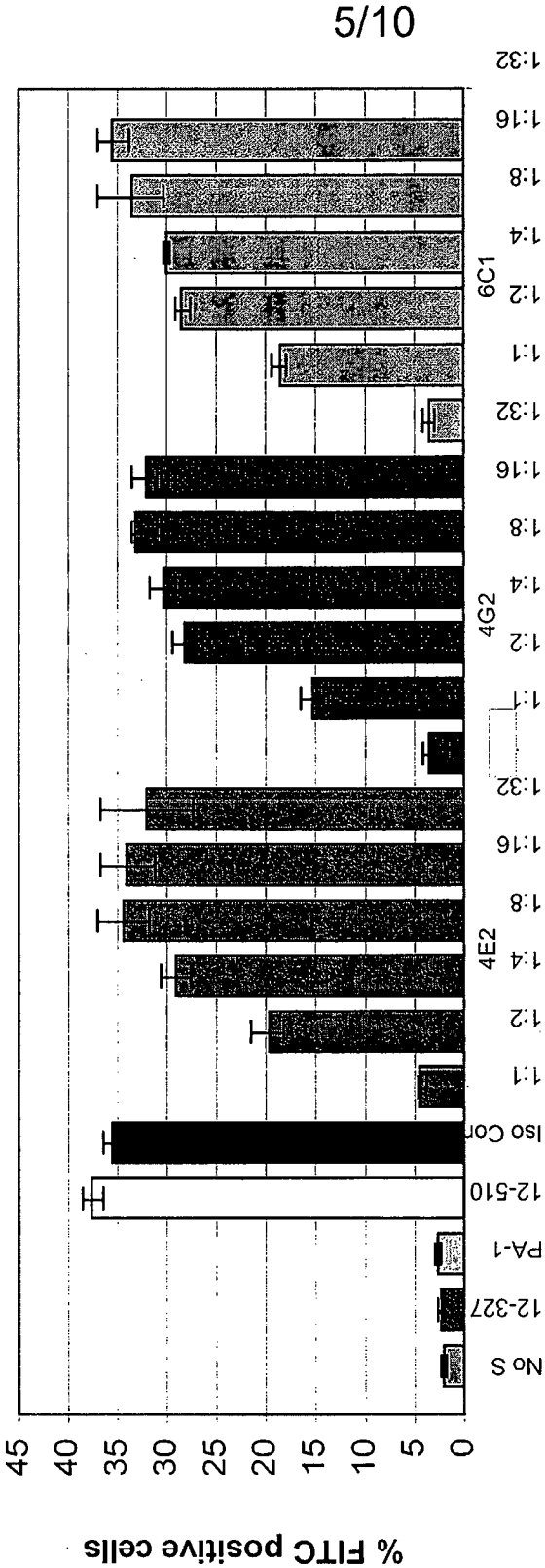


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Figure 4

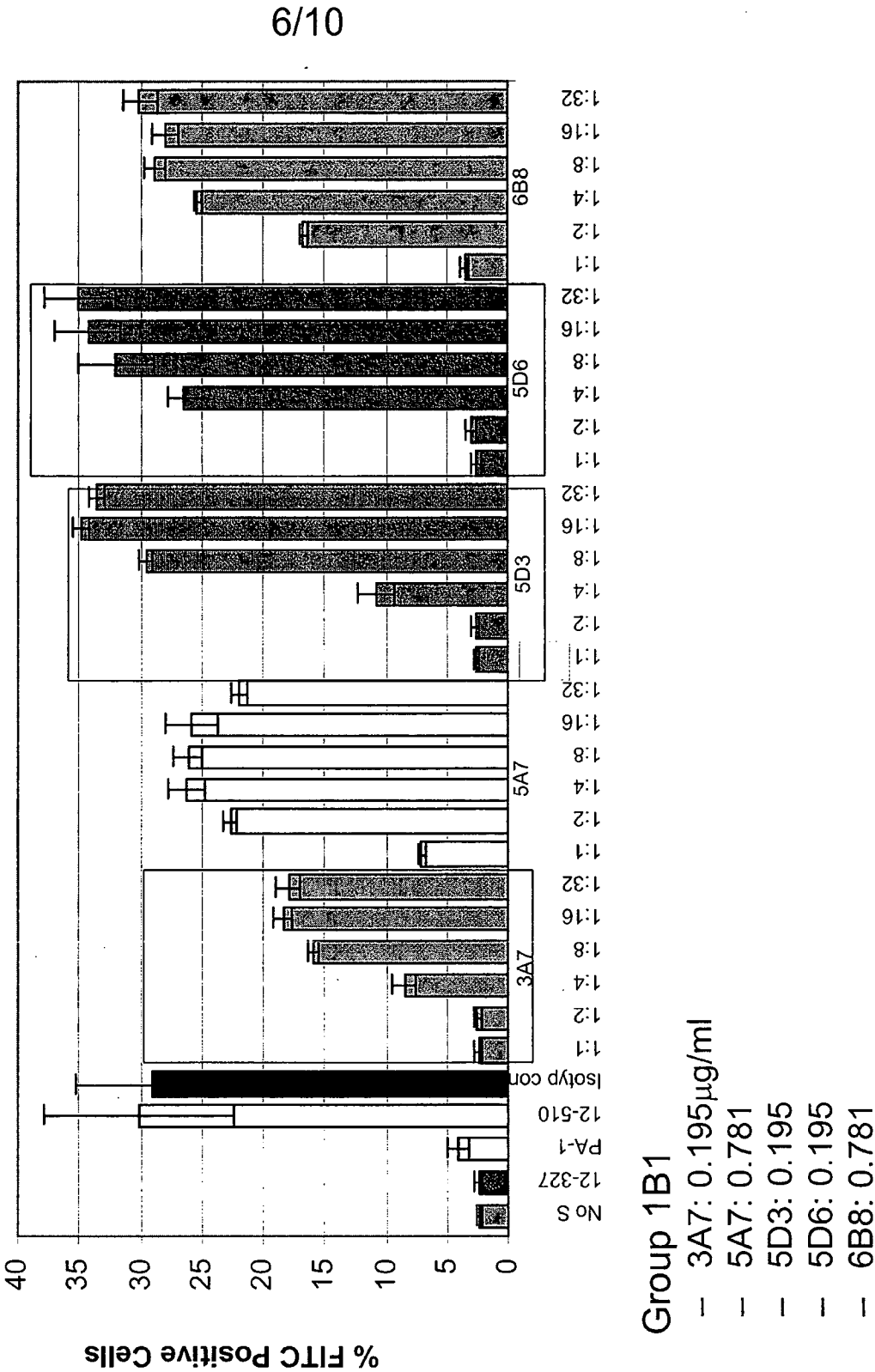
H Chain	CDR1	CDR2	CDR3	L chain	CDR1	CDR2	CDR3
<b>VH1-18, D1-26, JH4B</b>				<b>A1, JK2</b>			
Germline	GYTFTSYGHS	WISAYNGNTNYAQKLQG	GRYLDY	Germline	RSSQSLVYSDGNTYLN	KVSNWDS	
5D6	-----	-----	-----	4D4	-----R-----F--	-----	MQGTHWPPYVQ
5D3	-----	-----	-----	<b>A30, JK3</b>			
6B8	-----	-----	-----	Germline	RASQGIRNDLG	AASSLQS	LQHNSYPFT
3A7	-----	-----	-----	3H12	-----	-----	-----
5A7	-----	-----	-----	<b>A30, JK4</b>			
<b>VH1-2, N/A, JH4B</b>				Germline	RASQGIRNDLG	AASSLQS	LQHNSYPLT
Germline	GYTFTGYVMH	WINPNSGGTNYAQKFQG	GTYLIDY	5D6	-----D-----	-----	---Y-----
5E4	-----V-	-----	-R-----	5D3	-----D-----	-----	---Y-----
<b>VH1-2, D3-10, JH4B</b>				6B8	-----D-----	-----	---Y-----
Germline	GYTFTGYVMH	WINPNSGGTNYAQKFQG	GPHTFGSGSYFPDY	3A7	-----D-----	-----	---Y-----
3F3	-----	-----	-----	5A7	-----D-----	-----	---Y-----
6C2	-----	-----	-----	4A10	-----S-----	-----	Q-Y-----
6C1	-FP--D-----	--S-----	--S-----	5A5	-----S-----	-----	Q-Y-----
4G2	-FP--D-----	--S-----	--S-----	3F3	-----S-----	-----	Q-Y-----
4E2	-----	--S-----	--S-----	6C2	-----S-----	-----	Q-Y-----
5A5	-----	-----	-----	1B5	-----S-----	-----	Q-Y-----
4A10	-----	-----	-----	6C1	-----S-----	-----	Q-Y-----
6B5	-----	-----	-----	4G2	-----S-----	-----	Q-Y-----
<b>VH3-33, N/A, JH5B</b>				6B5	-----S-----	-----	Q-Y-----
Germline	GFTFSSYGMH	VIWYDGSNKYYADSVKG	GDFYWFDP	4E2	-----S-----	-----	Q-Y-----
1B5	-----	-----	-----	<b>A30, JK5</b>			
<b>VH3-33, D2-2, JH4B</b>				Germline	RASQGIRNDLG	AASSLQS	LQHNSYPIT
Germline	GFTFSSYGMH	VIWYDGSNKYYADSVKG	DPLGYCSSTSCSYFDY	5E4	-----	-----	---Y-----
6B1	-----[-	-----FH-----	-----	<b>L5, JK4</b>			
3C7	-----[-	-----F-----	-----	Germline	RASQGISSWLA	AASSLQS	QQANSF##T
<b>VH3-33, D4-17, JH4B</b>				6B1	-----N-----	-----	---N-PL-
Germline	GFTFSSYGMH	VIWYDGSNKYYADSVKG	GGDGERFDY	3C7	-----N-----	-----	---N-PL-
4D4	-----	-----	-----				
<b>VH4-59, D3-9, JH6B</b>							
Germline	GGSSYYWS	YIYYSGSTNYNPSLKS	DYDILTGYSNYGMDV				
3H12	D-----F-----	-----	-----				

Figure 5A



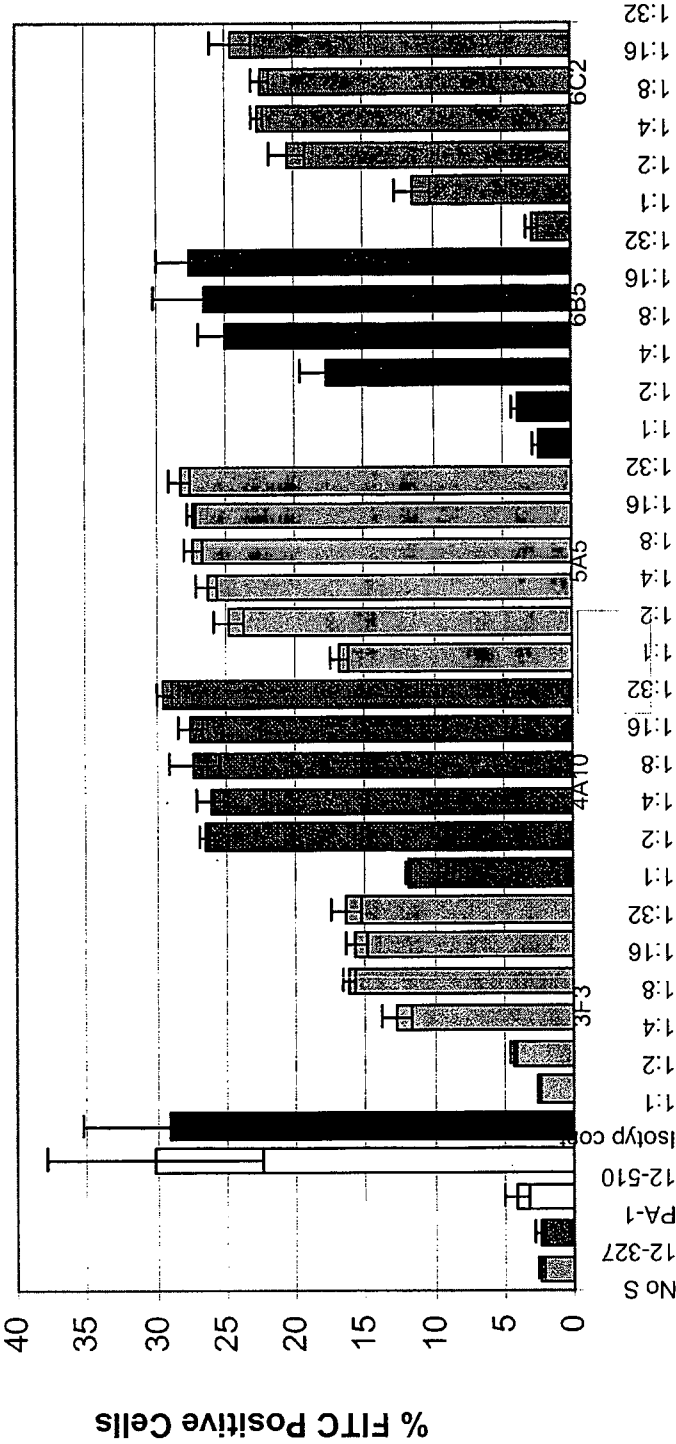
- Group 1A1 318-510
  - 4E2: 0.781 µg/ml
  - 4G2: 0.781 µg/ml
  - 6C1: 0.781 µg/ml

Figure 5B



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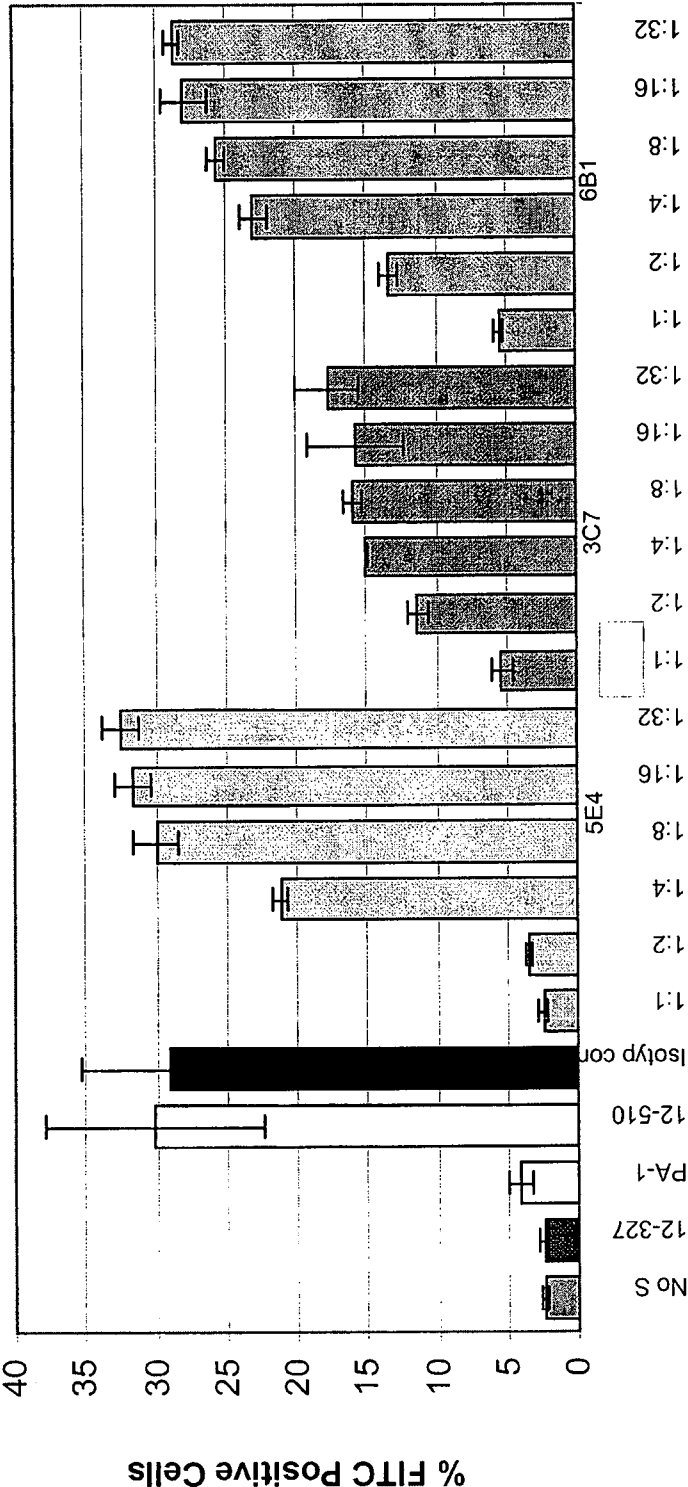
Figure 5C



- Group 1B2 mAbs
  - 3F3: 0.781 µg/ml
  - 4A10: 3.125
  - 5A5: 0.195
  - 6B5: 3.125
  - 6C2: 0.781

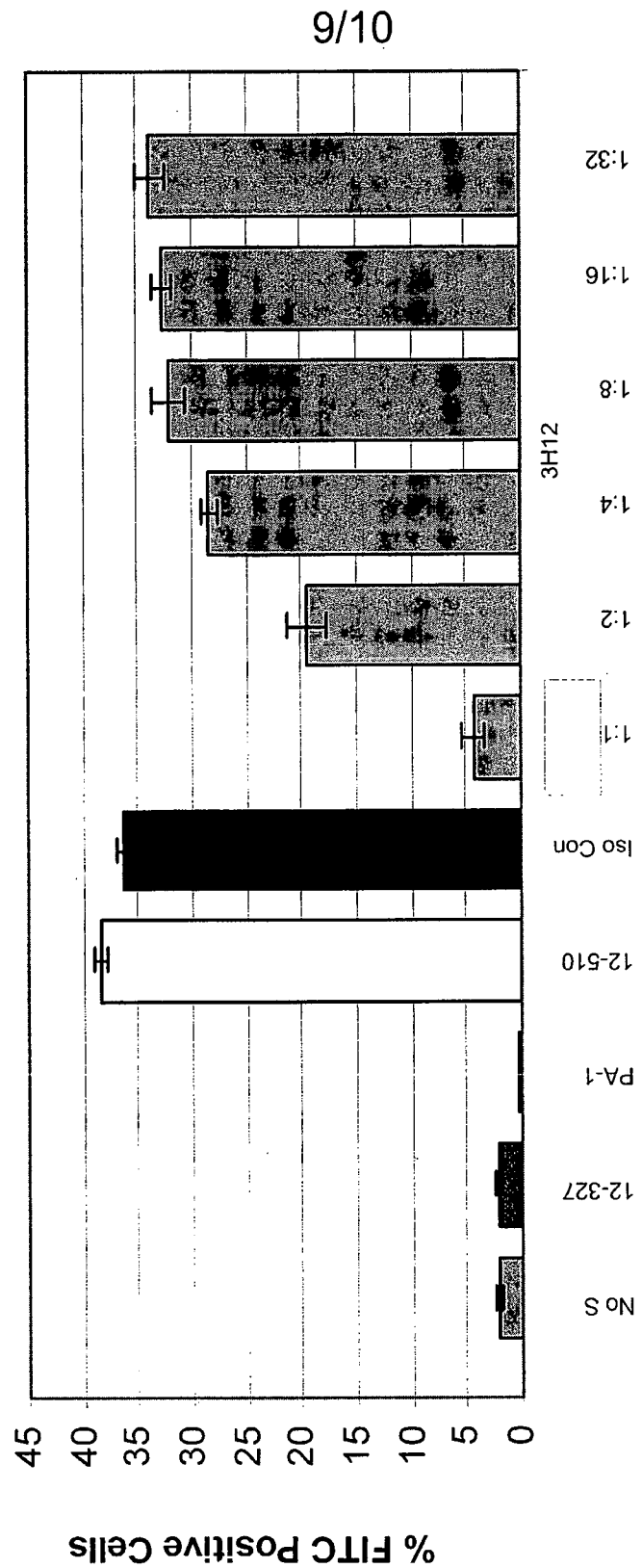
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Figure 5D

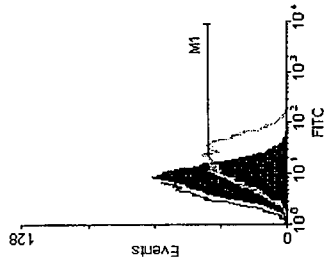


- Group 1B3 mAb
  - 5E4: 12.5
- Group 1B4 mAbs
  - 3C7: 12.5
  - 6B1: 3.125

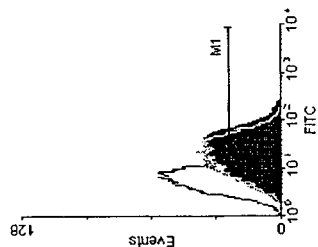
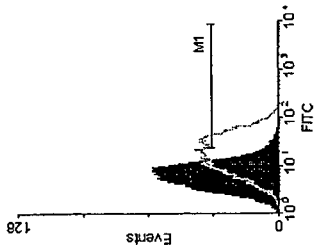
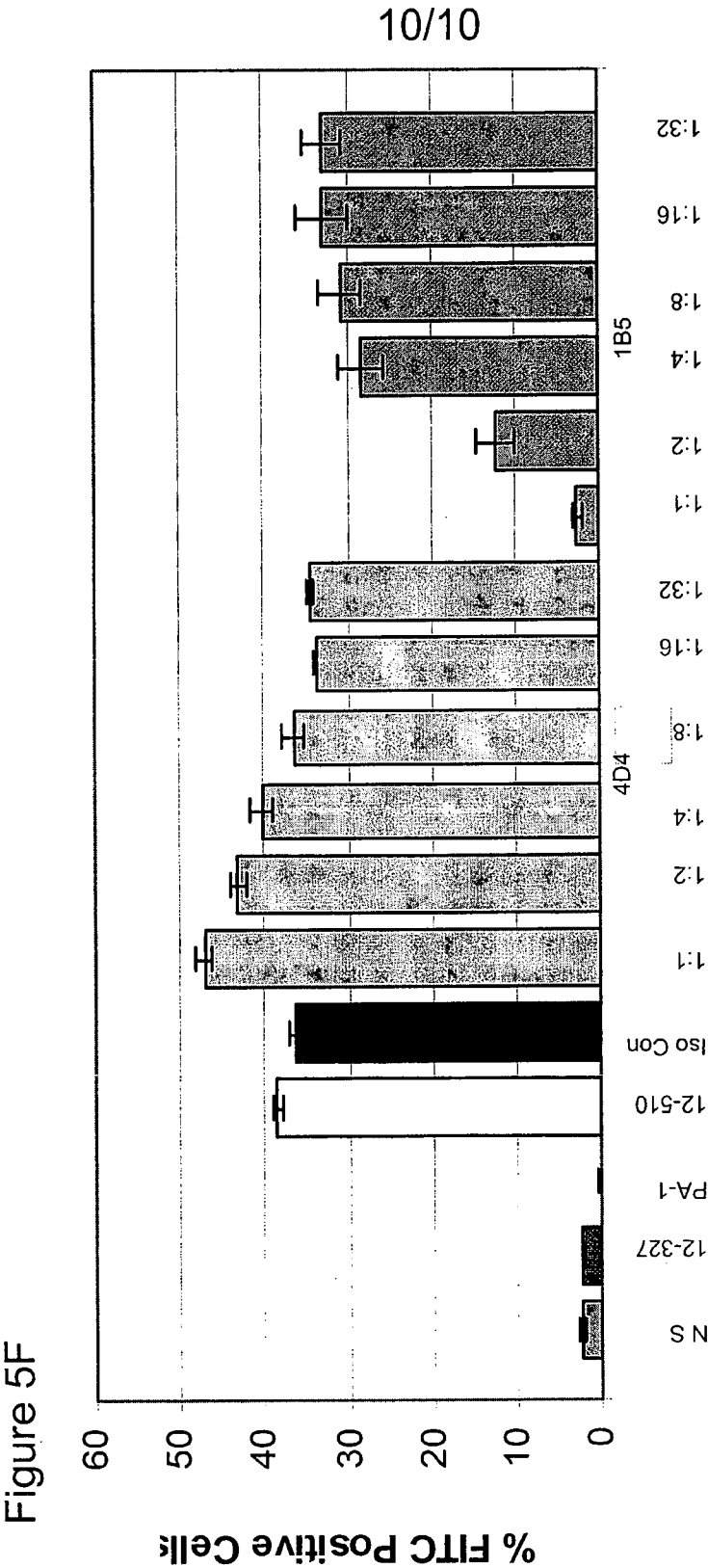
Figure 5E



- Group 1D mAb 318-510
  - 3H12: 3.125µg/ml







- Group 2B mAbs 12-261
- 2B1
  - 4D4: 12.5µg/ml
- 2B2
  - 1B5 : 0.195µg/ml